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Recent Update on Mesenchymal Stem Cells

Edited by Khalid Ahmed Al-Anazi

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Meet the editor

Dr. Khalid Ahmed Al-Anazi is a consultant hemato-oncologist and was chairman of the Department of Adult Hematology and Hematopoietic Stem Cell Transplantation (HSCT), King Fahad Specialist Hospital (KFSH), Saudi Arabia, between August 2015 and November 2021. He trained in clinical hematology and HSCT at King's College Hospital, University of London, UK. He has 30 years of experience in adult clinical hematology and HSCT at Riyadh Armed Forces Hospital; King Faisal Specialist Hospital and Research Centre in Riyadh; King Khalid University Hospital (KKUH) and the College of Medicine, King Saud University in Riyadh; and KFSH in Dammam, Saudi Arabia. He established the adult HSCT program at KFSH in Dammam in 2010. He received the Best Teacher Award in the Department of Medicine, College of Medicine, and KKUH in Riyadh in 2014. He has more than 150 publications including retrospective studies, review articles, book chapters, and electronic books to his credit. He is a reviewer for twenty-four international medical journals and an associate editor of twenty-six medical journals. He is the editor-in-chief of four international medical journals in cell biology, Regenerative medicine, and stem cell therapy.

Contents

Preface

Since the first description of mesenchymal stem cells (MSCs) by Alexander Friedenstein and colleagues in 1966, stem cells have witnessed remarkable progress not only in their manufacture and preparation but also in their clinical utilization to treat several medical and surgical illnesses. Many scientists and experts in the field believe that, in the near future, MSCs may reshape the field of medical therapeutics and may eventually become potentially curative for several intractable medical conditions.

This book, which contains several chapters covering various aspects of MSCs, was written by scientists with expertise in MSCs. I would like to thank the authors for their valuable contributions as well as Publishing Manager Ms. Nina Miocevic at IntechOpen for her dedication and great efforts that ultimately made this book project a reality.

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Section 1

Sources and Functions of Mesenchymal Stem Cells

Chapter 1

Clinical Relevance of Mesenchymal Stromal Cells from Various Sources: Insights into Transcriptome Analysis for Identifying Inherent Potential

Dana M. Alhattab, Salwa Alshehri and Fatima Jamali

Abstract

This book chapter provides an in-depth overview of the clinical relevance of mesenchymal stem cells (MSCs) derived from various sources, highlighting the importance of whole transcriptome analysis in revealing their inherent potential. The chapter delves into different sources of MSCs, such as bone marrow, adipose tissue, umbilical cord, and placenta, and compares their respective properties and capabilities. Additionally, it explores the latest advancements in whole transcriptome analysis, including RNA sequencing and microarray analysis, and their applications in MSC research. The aim is to provide a comprehensive understanding of how high-end technologies, such as whole transcriptome analysis, can aid in identifying the inherent potential of cells for therapeutic applications. It will also discuss how such gene expression approaches helped identify the inherent potential of specific MSC sources, tailoring their use towards specific clinical applications, including immune tolerance and modulation, osteogenesis, and chondrogenesis. Additionally, it highlights the importance of extracellular vesicles derived from MSCs. This knowledge will be beneficial for researchers and clinicians working towards developing MSC-based treatments for regenerative medicine and cellular therapy.

Keywords: mesenchymal stromal cells, transcriptome analysis, RNA-seq, inherent potential, immune modulation, osteogenesis, chondrogenesis, exosomes

1. Introduction

Mesenchymal stromal cells (MSCs), also referred to as mesenchymal stem cells, are multipotent stromal cells with self-renewal capacity and multilineage differentiation potential [1]. There has been an increasing interest in MSCs in recent years due to their unique properties, including long-term proliferation, multilineage differentiation potential, and immunomodulatory capabilities [1]. These properties make MSCs a promising candidate for use in regenerative medicine and cellular therapy.

The discovery of MSCs dates back to 1976, when Friedenstein et al. identified a group of non-hematopoietic, plastic-adherent, fibroblast-like cells that could be extracted from the bone marrow (BM). Since then, MSCs have been identified in various tissues, including adipose tissue (AT), umbilical cord (UC), and amniotic fluid [2, 3]. It is, however, essential to note that MSCs are not homogenous in their properties and capabilities. The source from which they are derived significantly determines specific cellular characteristics. MSCs from different tissues exhibit distinctive properties that may influence their potential clinical applications [4, 5]. Several studies have been conducted that compare MSCs from various sources [6–8]. For instance, Hsieh et al. performed a side-by-side functional comparison between UC-MSCs and BM-MSCs and found that BM-MSCs can be easily differentiated into osteocytes compared to UC-MSCs, which showed delayed and insufficient differentiation into osteocytes [8]. Another study by Karahuseyinoglu et al. identified that UC-MSCs possess higher chondrogenic differentiation capacity compared to BM-MSCs [9]. Therefore, it is critical to understand the inherent potential of MSCs from various sources before they can be used for clinical applications.

Whole transcriptome analysis is a powerful tool that enables researchers to gain a deep understanding of the gene expression patterns and regulatory networks that govern the properties and functions of MSCs. This cutting-edge approach allows for a comprehensive exploration of the molecular mechanisms that underlie the unique characteristics of MSCs. To this end, various studies have analyzed the global gene expression profile for different types of MSCs [10–12]. Some of these studies focused on evaluating the expression levels of pluripotency genes and embryonic stem cell (ESC) markers [13, 14]. Other studies investigated the expression of genes that have functional significance, such as genes involved in bone and cardiovascular development [13, 15]. Overall, whole transcriptome analysis is a valuable tool for gaining insights into the complex mechanisms that govern the behavior of MSCs.

This chapter will review the clinical relevance of MSCs derived from various sources, highlighting the importance of whole transcriptome analysis in unraveling their inherent potential. It will discuss the different sources of MSCs, including BM, AT, UC, and placenta (PL), and compare their properties and capabilities. Additionally, it will explore the latest developments in whole transcriptome analysis, including RNA sequencing and microarray analysis, and their applications in MSC research. The aim is to provide a comprehensive insight into high-end technologies, such as whole transcriptome analysis, and how they can aid in identifying the inherent potential of cells for therapeutic applications. This knowledge will be useful for researchers and clinicians working towards developing MSC-based treatments for regenerative medicine and cellular therapy. Furthermore, new strategies to enhance the therapeutic potential of MSCs are discussed.

2. Mesenchymal stem cell sources

While bone marrow-derived MSCs (BM-MSCs) have been extensively studied for the past four decades, the last decade has witnessed a shift towards exploring alternative sources of MSCs. This shift is driven by the search for cell sources that are easily accessible, noninvasive, and available in abundant quantity [16]. As a result, MSCs have been successfully isolated from diverse tissues, such as AT and perinatal tissues like the UC and PL [2, 3, 17]. Each source of MSCs has its advantages and limitations, and ongoing research aims to further understand their unique properties

and refine their applications in clinical settings. Additionally, studies are exploring other sources, such as synovial fluid, dental pulp, and more, expanding the potential repertoire of MSCs for therapeutic purposes. Besides the MSCs derived from the BM, other tissue sources of MSCs provide technical advantages. For instance, harvesting BM-MSCs involves an invasive procedure, and their expansion is imperative due to the restricted number of stem cells [18]. Conversely, adipose tissue-derived MSCs (AT-MSCs) are more accessible and produce a greater number of cells, simplifying the expansion process [19]. Such technical distinctions highlight the importance of tailoring protocols for isolating and expanding MSCs based on their specific tissue source.

2.1 Bone marrow-derived MSCs (BM-MSCs)

Traditionally considered the gold standard, BM-MSCs have been the focus of extensive research and clinical trials [20]. Isolation from the bone marrow involves a relatively invasive procedure, typically bone marrow aspiration from the iliac crest [21]. BM-MSCs exhibit robust self-renewal capacity and the ability to differentiate into various cell lineages, including osteoblasts, adipocytes, and chondrocytes [22]. Surface markers commonly associated with BM-MSCs include CD90, CD105, CD73, and CD44, while they lack the expression of hematopoietic markers such as CD34 and CD45 [23]. Research on the application of BM-MSCs has shown promising results in treating various diseases [24]. Nonetheless, discrepancies in results among studies can be ascribed to factors like donor variability, cell preparation procedures, and a deficiency in standardization before transplantation [25]. It is important to note that the expansion and cultivation process can vary based on specific protocols, and researchers continuously work on optimizing these procedures for the best outcomes in terms of cell yield, quality, and therapeutic efficacy.

2.2 Adipose tissue-derived MSCs (AT-MSCs)

AT has emerged as a rich and easily accessible source of MSCs. AT-MSCs can be isolated from fat tissue through a minimally invasive procedure, usually involving liposuction or fat removal during other surgical procedures. AT-MSCs share similarities with BM-MSCs in terms of their differentiation potential [26, 27]. However, they may offer advantages in terms of abundance and ease of isolation, making them an attractive alternative to regenerative therapies. As BM-MSCs, AT-MSCs express typical stem cell surface markers and possess the potential to differentiate into various lineages [23, 28]. Due to its accessibility and abundance, AT yields the isolation of stem cells at a quantity 500 times greater than those obtained from bone marrow [29]. AT-MSCs have been studied for their ability to enhance wound healing processes. The cells contribute to tissue regeneration by promoting the proliferation and migration of various cells involved in wound repair [30–32]. Other studies have investigated the potential of AT-MSCs in neural tissue repair. AT-MSCs may contribute to neuroprotection and neurodegeneration after injury [33]. They have also demonstrated a pro-angiogenic effect, promoting the formation of new blood vessels. Studies suggest that the paracrine factors released by AT-MSCs contribute to angiogenesis, which is crucial for tissue repair and regeneration [34]. Research and preclinical studies have explored the use of AT-MSCs in specific autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus. Clinical trials have been conducted or are ongoing to assess the safety and efficacy of AT-MSCs in treating

these conditions [35]. However, additional research is crucial to establish the optimal dosage, administration route, and safety parameters for clinical applications [36].

2.3 Umbilical cord-derived MSCs (UC-MSCs)

MSCs derived from the UC present a unique perinatal source with distinct properties. Isolation from the Wharton's jelly of the UC is considered noninvasive and ethically uncontentious. UC-MSCs exhibit multilineage differentiation potential, like BM-MSCs. Moreover, they possess immunomodulatory properties that make them particularly interesting for therapeutic applications, especially in the context of immune-related disorders [24, 37]. In 1991, McElreavey et al. [38] made a groundbreaking discovery by isolating fibroblast-like cells from the Wharton's Jelly (WJ) of the human UC. These cells are readily available after childbirth, providing a noninvasive and ethical source of MSCs. Obtaining a significant quantity of UC-MSCs through several passages and extensive ex vivo expansion is a simple process [39]. UC-MSCs are considered to be in a more primitive state compared to MSCs from other tissues, and they possess a high proliferative capacity [40]. In addition, UC-MSCs can be cryopreserved and stored for future use, allowing the creation of cell banks for potential therapeutic applications [41]. UC-MSCs display comparable surface phenotypes, adherence to plastic surfaces, and multipotency characteristics as MSCs obtained from other MSC tissue sources. UC-MSCs present versatile applications in both autologous and allogeneic contexts. Autologous use involves employing UC-MSCs for the same individuals they are sourced from. On the other hand, allogeneic use refers to the application of UC-MSCs across different individuals. Allogeneic UC-MSCs can be expanded and cryopreserved in cell banks, ready for use by patients in need. However, it is crucial to verify the health of the baby, who is the donor of UC-MSCs, through genomic or chromosomal tests, as their normal growth without health problems cannot be guaranteed in advance. Understanding the advantages and disadvantages is essential for each specific clinical application in both autologous and allogeneic scenarios [42].

2.4 Placenta-derived MSCs (PL-MSCs)

The PL plays a crucial role in fetal development by providing nutrition and supporting immunological tolerance. In recent times, stem cells derived from the PL, known as PL-MSCs, have gained attention as an alternative perinatal source of MSCs in regenerative medicine. PL-MSCs, originating from the fetus, exhibit the capacity for self-renewal and multipotency, possessing immunomodulatory properties, making them valuable for therapeutic applications [43]. Additionally, the PL harbors a substantial number of MSCs, and its utilization for research is not encumbered by the ethical concerns associated with human embryonic stem cells [44, 45].

2.5 Other sources of MSCs

2.5.1 Dental pulp-derived MSCs (DP-MSCs)

The dental pulp is the soft tissue at the core of a tooth, a highly vascularized connective tissue. It is enveloped by mineralized hard tissue and contains diverse cell types, including odontoblasts and undifferentiated progenitor cells [46]. Within this population of undifferentiated progenitors, MSCs are found to exhibit a high

proliferation rate and a high degree of multipotency [47–49]. Adherent colonies of spindle-shaped cells, known as Colony-Forming Units Fibroblast (CFU-F), are formed by human dental pulp-derived stem cells. The analysis of CFU-F reveals that human dental pulp harbors a more abundant population of MSCs compared to human bone marrow. Specifically, the CFU-F capacity of DPSCs is five times greater than that of BM-MSCs [50, 51]. DP-MSCs have shown great promise in dental applications. They held the potential for dental pulp regeneration [52] and dentin repair [53], and showed regenerative capacity for nerve repair [54].

2.5.2 Synovial fluid-derived MSCs (SF-MSCs)

The existence and attributes of MSCs in synovium specimens were initially documented by De Bari et al. [55], and subsequent studies have extensively explored this topic [56, 57]. MSCs derived from synovium specimens (SF-MSCs) exhibit superior proliferative capacity and chondrogenic potential compared to MSCs from other sources. Various studies have demonstrated the multilineage differentiation potential of SF-MSCs in humans [58, 59]. Recent findings indicate that the chondrogenic differentiation capacity of equine SF-derived MSCs is comparable to that of bone marrow MSCs [60, 61], confirming the stemness of these cells according to the criteria set by the International Society for Cellular Therapy [23].

3. Transcriptome analysis and identification of inherent potentials

MSCs are being actively used in clinical trials as potential cellular therapies for various clinical conditions, with over 1000 trials registered on ClinicalTrials.gov (http://www.clinicaltrials.gov). MSCs isolated from the bone marrow are the first to be used in clinical trials and are the most commonly studied MSC source in preclinical and clinical studies [62]. MSCs from other sources are also being used to treat different clinical conditions, including MSCs from AT, UC, and others. However, variations in the clinical outcomes of MSC treatments exist, and to date, there is no consensus on the most suitable MSC source to treat a specific disease [63]. The inconsistent clinical outcomes of MSC treatments can be attributed to the heterogeneous potency and functional variations of the MSCs and the lack of efficient assays for assessing their potency [7, 62].

MSCs are still identified by their expression of a specific subset of surface marker proteins, trilineage differentiation potential, and immunomodulatory potential [23, 64]. Although these criteria have served the research community for a long time, they do not represent the heterogeneity of the MSC population. The scientific community should benefit from advanced technologies, such as whole transcriptome analysis and single-cell RNA-seq technology, to explore MSC identity and function regarding their tissue origin and functional status.

These approaches could provide a comprehensive view of gene expression patterns in MSCs, enabling researchers to gain a deeper understanding of the inherent capabilities of MSCs, including their differentiation potential, immunomodulatory effects, and regenerative properties. In a prior study, we conducted a gene expression analysis of four types of MSCs, including MSCs derived from the BM, AT, UC, and PL [16]. Our analysis revealed a unique set of genes that are either up- or down-regulated in one type of MSC compared to all other stem cell types. Moreover,

we identified signature genes exclusively expressed in one type of MSC [16]. These findings clearly indicate intrinsic differences in the potential of MSCs from different sources.

Such information can be leveraged to develop more effective and targeted therapies for various conditions, such as osteoarthritis, cardiovascular disease, and neurological disorders. Moreover, whole transcriptome analysis can identify the specific genes and pathways involved in the biological processes of MSCs. Such an approach can refine the operational definition of MSCs and further our understanding of their native physiological function. Furthermore, this knowledge can inform the development of optimized culture conditions and clinical manufacturing protocols, leading to more consistent and effective MSC-based patient therapies. In the following sections, we will discuss how such gene expression approaches helped identify the inherent potential of specific MSC sources, tailoring their use towards specific clinical applications, including immune tolerance and modulation, osteogenesis, and chondrogenesis.

3.1 Immune tolerance and modulation

The modulation of the immune system and its tolerance is a complex process involving gene expression related to various biological functions. One of the critical properties of MSCs is their immune tolerance and survival mechanisms, which are essential for the success of stem cell transplantations in treating diseases [65]. MSCs typically express major histocompatibility (MHC) Class I antigens on their surface and not Class II; however, Class II antigens are upregulated by inflammatory agents [66]. Studies have shown that both autologous and allogenic MSCs prevent lymphocyte proliferation without causing apoptosis of T cells [67, 68]. MSCs release factors such as transforming growth factor β (TGF-β), interleukin 10 (IL-10), interleukin 6 (IL-6), and nitric oxide, which are known to affect immune cells [69, 70]. MSCs also affect the maturation of immune cells, increasing regulatory T cells (Treg), anti-inflammatory T helper 2 (TH2), and dendritic (DC2) cells [66, 71, 72]. Furthermore, they were found to induce M1 macrophages to adopt the anti-inflammatory M2 form and reduce IgG production from B cells [73]. Although the immunoregulatory potential of MSCs has been well established, the mechanisms underlying their actions are not yet fully understood [65]. Moreover, variations in the immunomodulatory capabilities among different types of MSCs are identified [6, 74]. These findings are manifested not only in in vitro studies but also in the outcomes of various clinical trials. For example, AT-MSCs were found to possess greater immunosuppressive capabilities than BM-MSCs in vitro and in vivo [75, 76].

Whole-gene expression analysis of MSCs from various sources can help identify the most suitable cells for clinical use by uncovering differences in their immunomodulatory potential. We previously conducted a comprehensive transcriptome analysis of MSCs from four sources to gauge their inherent potential [16]. We studied around 1400 immune modulation-related genes to understand if there were any differences in the MSCs' ability to modulate immune responses. Our findings indicate that toll-like receptor 4 (TLR-4) expression was highest in BM-MSCs and lowest in UC- and PL-MSCs. While TLR-4 is traditionally known for activating innate immune cells against pathogens, its activation in MSCs has also been discovered to facilitate interactions with the surrounding environment [77] and induce Treg cell activation, which counteracts the inflammatory aspect of several diseases [78]. We have identified a significant immune modulatory trend in BM-MSCs, which was emphasized

with increased expression of interleukin 7 (IL-7), an inhibitor of T-cell proliferation [79]. We also found an increase in CD200 expression, an immune player, in both BM- and UC-MSCs compared to other MSC types. In addition to its immune tolerance roles, CD200 inhibits the maturation of myeloid progenitors into inflammatory cells and suppresses the secretion of proinflammatory TNF-α in stimulated macrophages [80, 81]. On the other hand, CD274, a known immune-modulatory protein that plays a negative role in immune modulation, was found to be uniquely upregulated in UC- and PL-MSCs. Additionally, we found several proinflammatory pathways to be activated in other MSC types compared to BM-MSCs, such as interleukin 8 (IL-8) signaling, diabetes mellitus signaling, and TWEAK signaling. Our transcriptome analysis suggests that BM-MSCs may have a higher immune modulation potential than other MSC types.

Several studies have been conducted to evaluate the similarity and variability of gene expression in MSC samples [11, 82, 83]. In a study by Sun C et al., the transcriptomic variation of MSCs from different tissues, including PL, UC, and dental pulp, was investigated in relation to immunomodulatory function [10]. They found that among the genes that exhibited highly variable expression were genes involved in the differentiation process of MSCs and genes that regulated immunomodulation, such as CD274, C-C motif chemokine ligand 2 (CCL2), C-C motif chemokine ligand 5 (CCL5), IL6, colony-stimulating factor 3 (CSF3), and hepatocyte growth factor (HGF). Conversely, genes involved in other biological processes, such as metabolic processes, gene expression, RNA processes, and RNA binding, showed minimal gene expression changes. The study also examined the transcriptomic changes of INFγ-preconditioned MSCs to identify the molecular mechanisms behind the varying immunomodulatory potencies of different MSC samples. They found that different groups of MSCs use similar regulation networks in response to inflammatory stimulations, but gene expression variations within those networks result in differences in immunosuppressive capability. These gene expressions were found to vary greatly between MSC samples. Accordingly, the study identified a panel of these responsive genes that can be used to assess the immunosuppressive potency of MSCs.

Generally, whole transcriptome analysis studies enable investigation of the cause of immunomodulatory functional variation in different MSC types at the molecular level. Consequently, it can aid in establishing minimum clinical release criteria in our pursuit to identify the best MSC source with immunomodulatory potency.

3.2 Osteogenesis and chondrogenesis

In accordance with the guidelines set forth by the International Society for Cellular Therapy (ISCT), human MSCs are required to possess the ability to differentiate into three distinct lineages in vitro, namely osteoblasts, adipocytes, and chondroblasts [23]. While all MSCs from different tissue sources must be able to demonstrate osteogenic and chondrogenic differentiation capabilities, variations in their differentiation potential have been extensively studied and reported [8, 84–86]. These inherent differences have significant implications, particularly when deciding on the source of MSCs to be used in clinical trials for related medical conditions such as cartilage regeneration in patients with knee osteoarthritis. The selection of appropriate MSCs is crucial to ensuring successful outcomes in such clinical trials and the development of effective therapies.

Our previous study, which analyzed the global gene expression profile of MSCs from different sources, revealed that BM-MSCs and AT-MSCs exhibit a higher

expression of genes related to osteogenesis. We identified forkhead box C1 (FOXC1) and distal-less homeobox 5 (DLX5) among the upregulated genes in BM-MSCs. FOXC1 plays a crucial role in regulating initial osteoblast differentiation by directly regulating the expression of Msx2, a key regulator of early osteogenic events [87]. Similarly, DLX5 is a transcription factor that plays a role in later stages of osteogenic differentiation and is regulated by bone morphogenic protein 2 (BMP2) signaling. On the other hand, AT-MSCs showed upregulation in the bone morphogenetic protein receptor type 1B (BMPR1B) gene, which binds to BMP ligands and transduces BMP signaling [88]. These results suggest that both BM- and AT-MSCs have a higher capacity to differentiate into osteoblasts than PL-MSCs and UC-MSCs. Our findings are consistent with other studies that analyzed gene expression profiles of MSCs from various sources, which also demonstrated that BM-MSCs have better osteogenic potential than MSCs from other sources [8, 89]. It is worth noting that several studies have reported comparable osteogenic differentiation capabilities of MSCs derived from different sources, such as BM, UC, or AT [90, 91]. However, in this discussion, we are primarily concerned with the inherent osteogenic potential that undoubtedly reflects the osteogenic differentiation capacity of MSCs, although it does not exclude the differentiation capacity of MSCs obtained from other sources.

A recent study conducted by Zhang et al. utilized advanced single-cell RNA sequencing to investigate the similarity and heterogeneity of BM-MSCs and MSCs derived from Wharton's Jelly [92]. The study revealed a unique gene expression profile for each MSC cell source and identified heterogeneity among MSCs from the same tissue source, with distinct subpopulations of cells being identified. These subpopulations were categorized according to their gene expression patterns. Notably, the study identified a multipotent progenitor subpopulation that had an expression signature enriched for trilineage differentiation, including osteogenic and chondrogenic differentiation. However, the study did not draw any conclusions about which cell source possesses a higher osteogenic or chondrogenic differentiation capability. This study provides valuable insights into the inherent cellular composition of MSCs from different sources. The unique gene expression profiles identified for each cell source highlight the potential differences in their therapeutic applications. Furthermore, the identification of a multipotent progenitor subpopulation with an expression signature enriched for trilineage differentiation is of particular interest for regenerative medicine applications.

The ability of MSCs to differentiate into chondrocytes has made cartilage repair a successful regenerative application of these cells. Among the several bone and cartilage disorders investigated in MSC-based clinical studies, knee osteoarthritis is one of the most extensively studied, with various clinical trials employing MSCs from different sources [93–95]. Most of these trials have utilized an autologous source of MSCs, either from the BM or AT, and have produced encouraging results, with one study progressing to the phase III stage [96]. Other sources, such as WJ, PL, and amniotic membrane/fluid, have also been explored [96]. Evaluating the inherent chondrogenic and survival potential of the MSC type before its use in clinical settings could minimize the risk of suboptimal cell sourcing, leading to improved patient outcomes.

We have conducted a whole transcriptome analysis of four tissue-specific MSCs and evaluated the expression level of 43 genes related to chondrogenesis [16]. Our results revealed that BM- and AT-MSCs exhibited the highest number of upregulated genes. In BM-MSCs, chondroitin Sulfate N Acetylgalactosaminyltransferase 1 (CSGalNAcT-1), chitinase-3-like 1 protein (CHI3L1), and mothers against decapentaplegic homolog 1 (SMAD1) were specifically upregulated. CSGalNAcT-1 plays a

crucial role in initiating cartilage chondroitin sulfate biosynthesis [97], while CHI3L1 is associated with proliferation and differentiation in osteogenic and chondrogenic cell lineages during fetal development and MSC differentiation [98]. SMAD1 is an essential regulator of Smad-dependent signal transduction pathways, which is vital for initiating chondrogenic differentiation [99]. In contrast, transforming growth factor beta-induced (TGFBI), transforming growth factor beta receptor 2 (TGFBR2), and short stature homeobox 2 (SHOX2) were found to be downregulated in all MSCs compared to AT-MSCs. Research has shown that transforming growth factor beta (TGFB) proteins are the most potent activators of chondrogenesis in human MSCs [100]. Inactivation of TGFBR2 in neural crest cells resulted in the aberrant formation of Meckel's cartilage and altered the development of the mandible [101]. SHOX2 has been shown to control chondrocyte maturation by regulating the expression of Runt-related transcription factor 1 (RUNX) genes through bone morphogenic protein 4 (BMP4) [102]. Therefore, our results propose that BM and AT-MSCs have higher chondrogenic differentiation potential than PL and UC-MSCs. Such findings provide a deeper understanding of the molecular mechanisms involved in chondrogenic differentiation and could potentially inform the development of new regenerative therapies for cartilage repair.

3.3 Extracellular vesicles of MSCs and exosome potential

Besides direct cell-to-cell contact, the therapeutic potential of MSCs is attributed mainly to their paracrine actions [103, 104]. These actions are primarily mediated by the secretion of extracellular vesicles (EVs), particularly exosomes. Exosomes are small EVs with an average size of ~100 nm that are released by the cells into the extracellular environment [105]. They are enriched with bioactive molecules, such as nucleic acids, proteins, and metabolites, with various important biological functions [106]. Exosomes play a crucial role in intercellular communication and can transfer important biomolecules to target cells, thereby modulating various physiological and pathological processes [107, 108]. Studies have demonstrated that EVs of MSCs can exert biological effects comparable to MSCs themselves [109, 110]. As such, the paracrine effects of MSCs through EV secretion have emerged as a promising avenue for developing novel cell-free therapies for multiple diseases.

The unique advantage of EV as an alternative therapy for MSCs stems from the fact that they are cell-free therapies and accordingly require less authority approval due to their superior safety profile and lower immunogenicity [111, 112]. In addition, the viability, longevity, and entrapment of MSCs in the lung microvasculature after implantation hinder their use [113–115], whereas EVs of MSCs do not follow these limits [103]. Although the therapeutic potential of MSCs-EV has been extensively studied in vivo, its applications in a clinical setting have been poorly investigated [111, 116]. Only a few clinical studies have demonstrated the effectiveness of their use [117–119].

Given the high potential of exosomes in modulating cellular behaviors and their crucial role in the paracrine action of MSCs, it is imperative to gain a deeper understanding of their function to develop effective exosome-based therapeutics. It has been observed that exosomes derived from different MSC types have varying effects, and different culture conditions also result in exosomes with greater therapeutic effects [103, 120]. Transcriptome analysis of exosome cargo can aid in elucidating key factors that govern their biological activities. Accordingly, such data could lead to the development of more effective therapies. For instance, Yao et al. investigated the effect of exosomes from BM-MSCs on pancreatic cancer cell characteristics [104].

They performed transcriptome sequencing on the BM-MSCs-derived exosomes at different stages and identified, for the first time, the role of circular RNA (circRNA) found in the BM-MSCs-derived exosomes in pancreatic cancer. The circRNA (circ_0030167) was found to be enriched and identified as the main effector molecule of the BM-MSCs-derived exosome. From there, they identified that circ-0030167 inhibited the malignant progression and stemness of pancreatic cancer via the miR-338-5p/wif1/wnt8/β-catenin axis.

Similarly, Liu W et al. employed a high-throughput sequencing approach to study miRNA expression profiling in exosomes derived from human UC-MSCs and murine compact bone MSCs (cm-MSCs) to explore their effects on acute graft versus host disease (aGVHD) [121]. Their findings revealed a high expression level of miRNA-223 and identified this miRNA's mechanistic action in attenuating aGVHD. In another study by Terunuma A et al., the transcriptomes of EV from DP-MSCS and AT-MSCs were analyzed and compared to the transcriptomes of MSCs for the same tissue types [121]. EVs obtained from DP-MSCs exhibit transcriptomic signatures associated with neurogenesis and neural retinal development, while those obtained from AT-MCSs exhibit signatures related to mitochondrial activity and skeletal system development. Notably, the transcriptomes of EV-derived MSCs closely resemble those of their parent MSCs, and genes associated with neurogenesis were found to be highly expressed in both DP-MSCs and their EVs. Conversely, AT-MSCs and their EVs were found to exhibit high expression of genes associated with angiogenesis, hair growth, and dermal matrices. These findings suggest that EVs derived from DP-MSCs may hold promise as a therapeutic target for neurodegenerative disorders and retinal diseases, while EVs-derived AT-MSCs may be useful in rejuvenating the musculoskeletal system and skin.

The transcriptome studies of EVs derived from MSCs have demonstrated the significance of advanced technologies. Specifically, the analysis of the exosome cargo transcriptome can offer valuable insights into the biological activities of exosomes and facilitate the development of effective exosome-based therapeutics. Further investigation into MSC-EVs from diverse tissue types may broaden the range of potential therapeutic targets and enhance our comprehension of the mechanisms underlying the therapeutic effects of MSC-EVs.

4. Strategies to improve the therapeutic outcome of MSCs

While the current clinical successes of MSC therapies are encouraging, reports of failure and minimum efficacy persist. These limitations may not necessarily stem from the potential of MSCs but rather from their suboptimal use. As evidenced by single-cell RNA-seq studies, MSCs are a heterogeneous group of populations with varying cellular characteristics and potential. Additional heterogeneity in the MSC population could be introduced during the manufacturing process (isolation and expansion) [122, 123]. Standardizing the therapeutic potency of MSCs is therefore crucial to ensuring reliable and reproducible results in clinical trials. This can be achieved by establishing consistent protocols for the selection, isolation, and expansion of MSCs, as well as developing protocols for assessing cell quality and potency prior to clinical application. Furthermore, the automation of the expansion process and large-scale production will result in consistent product quality, thereby eliminating variabilities related to the production process. Namely, isolating a homogenous population of cells with the desired potency characteristics and following standardized protocols for expansion can produce high-quality MSCs for therapeutic use.

Another strategy to enhance the therapeutic potential of MSCs is through priming or preconditioning with specific agents before clinical administration. Such a strategy entails priming (treating) MSCs with small molecules, growth factors, and other biological or chemical agents to boost their reparative and regenerative properties and immunomodulatory capabilities. Preconditioning can be viewed as a way of educating MSCs before clinical administration. The main aim is to circumvent problems of MSC proliferation and in vivo survival and to boost the cells' therapeutic potential. Depending on the intended clinical use of the MSCs, different agents may be used. For instance, the role of growth factors in promoting the survival of transplanted MSCs has been investigated. In cardiac therapy, preconditioning MSCs with TGF-α for 24 hours before transplantation into ischemic sites in a rat model of myocardial infarction increased cell survival [124]. Additionally, priming MSCs with IFNγ was found to enhance their immunomodulatory potential [92]. Alternatively, MCSs can be genetically engineered to induce the expression of genes with desired therapeutic outcomes, including growth factors, cytokines, enzymes, and microRNA, without interfering with cell differentiation and self-regeneration capabilities [125]. Various strategies can be employed for genetic modification to enhance the therapeutic benefits of MSCs while retaining their inherent properties [125].

Cells in vivo reside within three-dimensional (3D) niches, where cell-matrix and cell-cell interactions play a significant role in maintaining cellular characteristics. The current culture protocols for MSCs rely on two-dimensional (2D) expansion systems, which do not accurately recapitulate the native environment of the cells. Consequently, these 2D cultures induce cellular changes that might impact certain therapeutic characteristics of the cells. Differences in the transcriptome profile of MSCs in 2D and 3D cultures have been reported [126–128]. These differences encompass the expression levels of growth factors and cytokines, which play an essential role in the paracrine mechanism of MSCs' protection [127, 129]. Therefore, the employment of 3D culture, which closely mimics the native conditions, could be considered an alternative strategy to maintain the therapeutic potential of MSCs. Alternatively, 3D cultures of MSCs could be viewed as a route of cell administration, particularly if they support MSCs' proliferation and differentiation [130, 131].

5. Conclusions

The clinical relevance of MSCs from various sources cannot be overstated, as they have demonstrated great promise in treating a wide range of diseases and injuries. MSCs are a heterogeneous population of cells with varying inherent potential. Analyzing the whole transcriptome of MSCs offers a robust tool for identifying gene expression patterns and regulatory networks. Through this approach, researchers can gain insights into the molecular mechanisms that govern the properties and functions of MSCs. Identifying key gene expression signatures can help predict the therapeutic potential of MSCs and optimize their use in clinical settings. This high-end technology could help identify the optimal source of MSCs with the desired inherent potential for specific clinical applications. Furthermore, data obtained from analyses could aid in enhancing the efficacy of clinical applications of MSCs. Additionally, new strategies, such as potency testing, priming of MSCs, and the development of 3D biomimicry conditions, should be deployed to enhance the therapeutic outcomes of MSCs.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Wei X, Yang X, Han Z-P, Qu F-F, Shao L, Shi Y-F. Mesenchymal stem cells: A new trend for cell therapy. Acta Pharmacologica Sinica. 2013;**34**(6):747-754

[2] Francis MP, Sachs PC, Elmore LW, Holt SE. Isolating adipose-derived mesenchymal stem cells from lipoaspirate blood and saline fraction. Organogenesis. 2010;**6**(1):11-14

[3] De Coppi P, Bartsch G Jr, Siddiqui MM, Xu T, Santos CC, Perin L, et al. Isolation of amniotic stem cell lines with potential for therapy. Nature Biotechnology. 2007;**25**(1):100-106

[4] Prockop DJ, Oh JY. Medical therapies with adult stem/progenitor cells (MSCs): A backward journey from dramatic results in vivo to the cellular and molecular explanations. Journal of Cellular Biochemistry. 2012;**113**(5):1460-1469

[5] Aslam N, Abusharieh E, Abuarqoub D, Hattab D, Jafar H, Alshaer W, et al. An in vitro comparison of anti-tumoral potential of Wharton's jelly and bone marrow mesenchymal stem cells exhibited by cell cycle arrest in glioma cells (U87MG). Pathology and Oncology Research. 2021;**27**:584710

[6] Wegmeyer H, Bröske A-M, Leddin M, Kuentzer K, Nisslbeck AK, Hupfeld J, et al. Mesenchymal stromal cell characteristics vary depending on their origin. Stem Cells and Development. 2013;**22**(19):2606-2618

[7] Phinney DG. Functional heterogeneity of mesenchymal stem cells: Implications for cell therapy. Journal of Cellular Biochemistry. 2012;**113**(9):2806-2812

[8] Hsieh J-Y, Fu Y-S, Chang S-J, Tsuang Y-H, Wang H-W. Functional module analysis reveals differential osteogenic and stemness potentials in human mesenchymal stem cells from bone marrow and Wharton's jelly of umbilical cord. Stem Cells and Development. 2010;**19**(12):1895-1910

[9] Karahuseyinoglu S, Kocaefe C, Balci D, Erdemli E, Can A. Functional structure of adipocytes differentiated from human umbilical cord stroma-derived stem cells. Stem Cells. 2008;**26**(3):682-691

[10] Sun C, Zhang K, Yue J, Meng S, Zhang X. Deconstructing transcriptional variations and their effects on immunomodulatory function among human mesenchymal stromal cells. Stem Cell Research & Therapy. 2021;**12**(1):1-16

[11] Cho K-A, Park M, Kim Y-H, Woo S-Y, Ryu K-H. RNA sequencing reveals a transcriptomic portrait of human mesenchymal stem cells from bone marrow, adipose tissue, and palatine tonsils. Scientific Reports. 2017;**7**(1):17114

[12] Tsai MS, Hwang SM, Chen KD, Lee YS, Hsu LW, Chang YJ, et al. Functional network analysis of the transcriptomes of mesenchymal stem cells derived from amniotic fluid, amniotic membrane, cord blood, and bone marrow. Stem Cells. 2007;**25**(10):2511-2523

[13] Gao LR, Zhang NK, Ding QA, Chen HY, Hu X, Jiang S, et al. Common expression of stemness molecular markers and early cardiac transcription factors in human Wharton's jelly-derived mesenchymal stem cells and embryonic stem cells. Cell Transplantation. 2013;**22**(10):1883-1900

[14] Nekanti U, Rao VB, Bahirvani AG, Jan M, Totey S, Ta M. Long-term expansion and pluripotent marker array

analysis of Wharton's jelly-derived mesenchymal stem cells. Stem Cells and Development. 2010;**19**(1):117-130

[15] Yu S, Long J, Yu J, Du J, Ma P, Ma Y, et al. Analysis of differentiation potentials and gene expression profiles of mesenchymal stem cells derived from periodontal ligament and Wharton's jelly of the umbilical cord. Cells, Tissues, Organs. 2013;**197**(3):209-223

[16] Alhattab D, Jamali F, Ali D, Hammad H, Adwan S, Rahmeh R, et al. An insight into the whole transcriptome profile of four tissue-specific human mesenchymal stem cells. Regenerative Medicine. 2019;**14**(9):841-865

[17] Lee OK, Kuo TK, Chen W-M, Lee K-D, Hsieh S-L, Chen T-H. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood. 2004;**103**(5):1669-1675

[18] Lotfy A, El-Sherbiny YM, Cuthbert R, Jones E, Badawy A. Comparative study of biological characteristics of mesenchymal stem cells isolated from mouse bone marrow and peripheral blood. Biomedical Reports. 2019;**11**(4):165-170

[19] Bunnell BA, Flaat M, Gagliardi C, Patel B, Ripoll C. Adipose-derived stem cells: Isolation, expansion and differentiation. Methods. 2008;**45**(2):115-120

[20] Roberto BF, Pablo MO. Sources and clinical applications of mesenchymal stem cells: State-of-the-art review. Sultan Qaboos University Medical Journal. Aug 2018;**18**(3):e264-e277

[21] Berebichez-Fridman R, Gómez-García R, Granados-Montiel J, Berebichez-Fastlicht E, Olivos-Meza A, Granados J, et al. The holy grail of orthopedic surgery: Mesenchymal stem cells—Their current uses and potential applications. Stem Cells International. 2017;**2017**:2638305

[22] Williams AR, Hare JM. Mesenchymal stem cells: Biology, pathophysiology, translational findings, and therapeutic implications for cardiac disease. Circulation Research. 2011;**109**(8):923-940

[23] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. Cytotherapy. 2006;**8**(4):315-317

[24] Aslam N, Abusharieh E, Abuarqoub D, Ali D, Al-Hattab D, Wehaibi S, et al. Anti-oncogenic activities exhibited by paracrine factors of MSCs can be mediated by modulation of KITLG and DKK1 genes in glioma SCs in vitro. Molecular Therapy-Oncolytics. 2021;**20**:147-165

[25] Kramer J, Dazzi F, Dominici M, Schlenke P, Wagner W. Clinical perspectives of mesenchymal stem cells. Hindawi. 2012;**2012**:684827

[26] Yoshimura K, Suga H, Eto H. Adipose-derived stem/progenitor cells: Roles in adipose tissue remodeling and potential use for soft tissue augmentation. Regenerative Medicine. 2009;**4**:265-273

[27] Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Molecular Biology of the Cell. 2002;**13**(12):4279-4295

[28] Sober SA, Darmani H, Alhattab D, Awidi A. Flow cytometric characterization of cell surface markers to differentiate between fibroblasts and mesenchymal stem cells of different origin. Archives of Medical Science: AMS. 2023;**19**(5):1487

[29] Fan X-L, Zhang Y, Li X, Fu Q-L. Mechanisms underlying the protective effects of mesenchymal stem cell-based therapy. Cellular and Molecular Life Sciences. 2020;**77**:2771-2794

[30] Hassan WU, Greiser U, Wang W. Role of adipose-derived stem cells in wound healing. Wound Repair and Regeneration. 2014;**22**(3):313-325

[31] Shingyochi Y, Orbay H, Mizuno H. Adipose-derived stem cells for wound repair and regeneration. Expert Opinion on Biological Therapy. 2015;**15**:1285-1292

[32] Si Z, Wang X, Sun C, Kang Y, Xu J, Wang X, et al. Adipose-derived stem cells: Sources, potency, and implications for regenerative therapies. Biomedicine & Pharmacotherapy. 2019;**114**:108765

[33] Ribeiro TB, Duarte AS, Longhini ALF, Pradella F, Farias AS, Luzo AC, et al. Neuroprotection and immunomodulation by xenografted human mesenchymal stem cells following spinal cord ventral root avulsion. Scientific Reports. 2015;**5**(1):16167

[34] Krawczenko A, Klimczak A. Adipose tissue-derived mesenchymal stem/stromal cells and their contribution to angiogenic processes in tissue regeneration. International Journal of Molecular Sciences. 2022;**23**(5):2425

[35] Sabol RA, Bowles AC, Côté A, Wise R, Pashos N, Bunnell BA. Therapeutic potential of adipose stem cells. Cell Biology and Translational Medicine. 2018;**13**:15-25 Stem cells in development and disease

[36] Kim Y-J, Jeong J-H. Clinical application of adipose stem cells in plastic surgery. Journal of Korean Medical Science. 2014;**29**(4):462-467

[37] Minguell JJ. Umbilical cord-derived mesenchymal stem cells. In: Regenerative Medicine Using Pregnancy-Specific Biological Substances. London: Springer; 2010. pp. 249-253

[38] McElreavey KD, Irvine AI, Ennis KT, McLean WI. Isolation, culture and characterisation of fibroblast-like cells derived from the Wharton's jelly portion of human umbilical cord. Biochemical Society Transactions (Portland Press Ltd.). 1991;**19**(1):29S

[39] Sarugaser R, Lickorish D, Baksh D, Hosseini MM, Davies JE. Human umbilical cord perivascular (HUCPV) cells: A source of mesenchymal progenitors. Stem Cells. 2005;**23**(2):220-229

[40] Troyer DL, Weiss ML. Concise review: Wharton's jelly-derived cells are a primitive stromal cell population. Stem Cells. 2008;**26**(3):591-599

[41] Horie S, Gonzalez H, Brady J, Devaney J, Scully M, O'Toole D, et al. Fresh and cryopreserved human umbilical-cord-derived mesenchymal stromal cells attenuate injury and enhance resolution and repair following ventilation-induced lung injury. International Journal of Molecular Sciences. 2021;**22**(23):12842

[42] Nagamura-Inoue T, He H. Umbilical cord-derived mesenchymal stem cells: Their advantages and potential clinical utility. World Journal of Stem Cells. 2014;**6**(2):195

[43] Li CD, Zhang WY, Li HL, Jiang XX, Zhang Y, Tang PH, et al. Mesenchymal stem cells derived from human placenta suppress allogeneic umbilical cord blood lymphocyte proliferation. Cell Research. 2005;**15**(7):539-547

[44] Li C, Zhang W, Jiang X, Mao N. Human-placenta-derived mesenchymal stem cells inhibit proliferation and function of allogeneic immune cells. Cell and Tissue Research. 2007;**330**(3):437-446

[45] Atala A. Recent developments in tissue engineering and regenerative medicine. Current Opinion in Pediatrics. 2006;**18**(2):167-171

[46] Orban B. Oral Histology and Embryology. 2nd ed. St. Louis, MO: CV Mosby; 1949. p. 75

[47] Kawashima N. Characterisation of dental pulp stem cells: A new horizon for tissue regeneration? Archives of Oral Biology. 2012;**57**(11):1439-1458

[48] Rodas-Junco BA, Villicana C. Dental pulp stem cells: Current advances in isolation, expansion and preservation. Tissue Engineering and Regenerative Medicine. 2017;**14**(4):333-347

[49] Yang X, Li L, Xiao L, Zhang D. Recycle the dental fairy's package: Overview of dental pulp stem cells. Stem Cell Research & Therapy. 2018;**9**:1-8

[50] Sonoyama W, Liu Y, Fang D, Yamaza T, Seo B-M, Zhang C, et al. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One. 2006;**1**(1):e79

[51] Makino Y, Yamaza H, Akiyama K, Ma L, Hoshino Y, Nonaka K, et al. Immune therapeutic potential of stem cells from human supernumerary teeth. Journal of Dental Research. 2013;**92**(7):609-615

[52] Itoh Y, Sasaki J, Hashimoto M, Katata C, Hayashi M, Imazato S. Pulp regeneration by 3-dimensional dental pulp stem cell constructs. Journal of Dental Research. 2018;**97**(10):1137-1143

[53] Li K, O'Dwyer R, Yang F, Cymerman J, Li J, Feldman JD, et al. Enhancement of acellular biomineralization, dental pulp stem cell migration, and differentiation by hybrid fibrin gelatin scaffolds. Dental Materials. 2023;**39**(3):305-319

[54] Saez DM, Sasaki RT, Martins DO, Chacur M, Kerkis I, da Silva MCP. Rat facial nerve regeneration with human immature dental pulp stem cells. Cell Transplantation. 2019;**28**(12):1573-1584

[55] De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis and Rheumatism. 2001;**44**(8):1928-1942

[56] Imanishi Y, Miyagawa S, Kitagawa-Sakakida S, Taketani S, Sekiya N, Sawa Y. Impact of synovial membrane-derived stem cell transplantation in a rat model of myocardial infarction. Journal of Artificial Organs. 2009;**12**:187-193

[57] Chen K, Man C, Zhang B, Hu J, Zhu S-S. Effect of in vitro chondrogenic differentiation of autologous mesenchymal stem cells on cartilage and subchondral cancellous bone repair in osteoarthritis of temporomandibular joint. International Journal of Oral and Maxillofacial Surgery. 2013;**42**(2):240-248

[58] Koyama N, Okubo Y, Nakao K, Osawa K, Fujimura K, Bessho K. Pluripotency of mesenchymal cells derived from synovial fluid in patients with temporomandibular joint disorder. Life Sciences. 2011;**89**(19-20):741-747

[59] Matsukura Y, Muneta T, Tsuji K, Koga H, Sekiya I. Mesenchymal stem cells in synovial fluid increase after meniscus injury. Clinical Orthopaedics and Related Research. 2014;**472**:1357-1364

[60] Lee W-J, Hah Y-S, Ock S-A, Lee J-H, Jeon R-H, Park J-S, et al. Cell source-dependent in vivo immunosuppressive properties of mesenchymal stem cells derived from the bone marrow and synovial fluid of minipigs. Experimental Cell Research. 2015;**333**(2):273-288

[61] Ando W, Kutcher JJ, Krawetz R, Sen A, Nakamura N, Frank CB, et al. Clonal analysis of synovial fluid stem cells to characterize and identify stable mesenchymal stromal cell/mesenchymal progenitor cell phenotypes in a porcine model: A cell source with enhanced commitment to the chondrogenic lineage. Cytotherapy. 2014;**16**(6):776-788

[62] Levy O, Kuai R, Siren EM, Bhere D, Milton Y, Nissar N, et al. Shattering barriers toward clinically meaningful MSC therapies. Science. Advances. 2020;**6**(30):eaba6884

[63] Krampera M, Le Blanc K. Mesenchymal stromal cells: Putative microenvironmental modulators become cell therapy. Cell Stem Cell. 2021;**28**(10):1708-1725

[64] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;**284**(5411):143-147

[65] Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. Nature Reviews Nephrology. 2018;**14**(8):493-507

[66] Pittenger MF, Discher DE, Péault BM, Phinney DG, Hare JM, Caplan AI. Mesenchymal stem cell perspective: Cell biology to clinical progress. NPJ Regenerative Medicine. 2019;**4**(1):22

[67] Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, Simonetti DW, et al. T cell responses to allogeneic human mesenchymal stem cells: Immunogenicity, tolerance, and suppression. Journal of Biomedical Science. 2005;**12**:47-57

[68] Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood, The Journal of the American Society of Hematology. 2002;**99**(10):3838-3843

[69] Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005;**105**(4):1815-1822

[70] Nasef A, Chapel A, Mazurier C, Bouchet S, Lopez M, Mathieu N, et al. Identification of IL-10 and TGF-β transcripts involved in the inhibition of T-lymphocyte proliferation during cell contact with human mesenchymal stem cells. Gene Expression The Journal of Liver Research. 2006;**13**(4-5):217-226

[71] Ma S, Xie N, Li W, Yuan B, Shi Y, Wang Y. Immunobiology of mesenchymal stem cells. Cell Death & Differentiation. 2014;**21**(2):216-225

[72] Akiyama K, Chen C, Wang D, Xu X, Qu C, Yamaza T, et al. Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. Cell Stem Cell. 2012;**10**(5):544-555

[73] Le Blanc K, Mougiakakos D. Multipotent mesenchymal stromal cells and the innate immune system. Nature Reviews Immunology. 2012;**12**(5):383-396

[74] Neofytou E, Deuse T, Beygui RE, Schrepfer S. Mesenchymal stromal cell therapy: Different sources exhibit different immunobiological properties. Transplantation. 2015;**99**(6):1113-1118

[75] Melief SM, Zwaginga JJ, Fibbe WE, Roelofs H. Adipose tissue-derived multipotent stromal cells have a higher immunomodulatory capacity than their

bone marrow-derived counterparts. Stem Cells Translational Medicine. 2013;**2**(6):455-463

[76] Ivanova-Todorova E, Bochev I, Mourdjeva M, Dimitrov R, Bukarev D, Kyurkchiev S, et al. Adipose tissue-derived mesenchymal stem cells are more potent suppressors of dendritic cells differentiation compared to bone marrow-derived mesenchymal stem cells. Immunology Letters. 2009;**126**(1-2):37-42

[77] Ghosh AK, Sinha D, Mukherjee S, Biswas R, Biswas T. LPS stimulates and Hsp70 down-regulates TLR4 to orchestrate differential cytokine response of culture-differentiated innate memory CD8+ T cells. Cytokine. 2015;**73**(1):44-52

[78] Rashedi I, Gómez-Aristizábal A, Wang X-H, Viswanathan S, Keating A. TLR3 or TLR4 activation enhances mesenchymal stromal cell-mediated Treg induction via notch signaling. Stem Cells. 2017;**35**(1):265-275

[79] Nemoto Y, Kanai T, Takahara M, Oshima S, Nakamura T, Okamoto R, et al. Bone marrow-mesenchymal stem cells are a major source of interleukin-7 and sustain colitis by forming the niche for colitogenic CD4 memory T cells. Gut. 2013;**62**(8):1142-1152

[80] Amouzegar A, Mittal SK, Sahu A, Sahu SK, Chauhan SK. Mesenchymal stem cells modulate differentiation of myeloid progenitor cells during inflammation. Stem Cells. 2017;**35**(6):1532-1541

[81] Pietilä M, Lehtonen S, Tuovinen E, Lähteenmäki K, Laitinen S, Leskelä H-V, et al. CD200 positive human mesenchymal stem cells suppress TNF-alpha secretion from CD200 receptor positive macrophage-like cells. PLoS One. 2012;**7**(2):e31671

[82] Roson-Burgo B, Sanchez-Guijo F, Del Cañizo C, De Las RJ. Transcriptomic portrait of human mesenchymal stromal/stem cells isolated from bone marrow and placenta. BMC Genomics. 2014;**15**(1):1-18

[83] Jansen BJ, Gilissen C, Roelofs H, Schaap-Oziemlak A, Veltman JA, Raymakers RA, et al. Functional differences between mesenchymal stem cell populations are reflected by their transcriptome. Stem Cells and Development. 2010;**19**(4):481-490

[84] Bellavia M, Altomare R, Cacciabaudo F, Santoro A, Allegra A, Concetta Gioviale M, et al. Towards an ideal source of mesenchymal stem cell isolation for possible therapeutic application in regenerative medicine. Biomedical Papers. 2014;**158**(3):356-360

[85] Secunda R, Vennila R, Mohanashankar A, Rajasundari M, Jeswanth S, Surendran R. Isolation, expansion and characterisation of mesenchymal stem cells from human bone marrow, adipose tissue, umbilical cord blood and matrix: A comparative study. Cytotechnology. 2015;**67**(5):793-807

[86] Fong C-Y, Subramanian A, Gauthaman K, Venugopal J, Biswas A, Ramakrishna S, et al. Human umbilical cord Wharton's jelly stem cells undergo enhanced chondrogenic differentiation when grown on nanofibrous scaffolds and in a sequential two-stage culture medium environment. Stem Cell Reviews and Reports. 2012;**8**:195-209

[87] Mirzayans F, Lavy R, Penner-Chea J, Berry FB. Initiation of early osteoblast differentiation events through the direct transcriptional regulation of Msx2 by FOXC1. PLoS One. 2012;**7**(11):e49095

[88] Wu M, Chen G, Li Y-P. TGF-β and BMP signaling in osteoblast, skeletal development, and bone formation, homeostasis and disease. Bone Research. 2016;**4**(1):1-21

[89] Mohamed-Ahmed S, Fristad I, Lie SA, Suliman S, Mustafa K, Vindenes H, et al. Adipose-derived and bone marrow mesenchymal stem cells: A donor-matched comparison. Stem Cell Research & Therapy. 2018;**9**:1-15

[90] Rebelatto CK, Aguiar A, Moretao M, Senegaglia A, Hansen P, Barchiki F, et al. Dissimilar differentiation of mesenchymal stem cells from bone marrow, umbilical cord blood, and adipose tissue. Experimental Biology and Medicine. 2008;**233**(7):901-913

[91] Jo CH, Yoon PW, Kim H, Kang KS, Yoon KS. Comparative evaluation of in vivo osteogenic differentiation of fetal and adult mesenchymal stem cell in rat critical-sized femoral defect model. Cell and Tissue Research. 2013;**353**(1):41-52

[92] Zhang C, Han X, Liu J, Chen L, Lei Y, Chen K, et al. Single-cell transcriptomic analysis reveals the cellular heterogeneity of mesenchymal stem cells. Genomics, Proteomics & Bioinformatics. 2022;**20**(1):70-86

[93] Lee W-S, Kim HJ, Kim K-I, Kim GB, Jin W. Intra-articular injection of autologous adipose tissue-derived mesenchymal stem cells for the treatment of knee osteoarthritis: A phase IIb, randomized, placebo-controlled clinical trial. Stem Cells Translational Medicine. 2019;**8**(6):504-511

[94] Al-Najar M, Khalil H, Al-Ajlouni J, Al-Antary E, Hamdan M, Rahmeh R, et al. Intra-articular injection of expanded autologous bone marrow mesenchymal cells in moderate and severe knee osteoarthritis is safe: A phase I/II study. Journal of Orthopaedic Surgery and Research. 2017;**12**:1-6

[95] Samara O, Jafar H, Hamdan M, Al-Ta'mari A, Rahmeh R, Hourani B, et al. Ultrasound-guided intra-articular injection of expanded umbilical cord mesenchymal stem cells in knee osteoarthritis: A safety/efficacy study with MRI data. Regenerative Medicine. 2022;**17**(5):299-312

[96] Negoro T, Takagaki Y, Okura H, Matsuyama A. Trends in clinical trials for articular cartilage repair by cell therapy. NPJ Regenerative Medicine. 2018;**3**(1):17

[97] Sakai K, Kimata K, Sato T, Gotoh M, Narimatsu H, Shinomiya K, et al. Chondroitin sulfate N-acetylgalactosaminyltransferase-1 plays a critical role in chondroitin sulfate synthesis in cartilage. Journal of Biological Chemistry. 2007;**282**(6):4152-4161

[98] Hoover DJ, Zhu V, Chen R, Briley K, Rameshwar P, Cohen S, et al. Expression of the chitinase family glycoprotein YKL-40 in undifferentiated, differentiated and trans-differentiated mesenchymal stem cells. PLoS One. 2013;**8**(5):e62491

[99] Cleary MA, van Osch GJM, Brama PA, Hellingman CA, Narcisi R. FGF, TGFβ and Wnt crosstalk: Embryonic to in vitro cartilage development from mesenchymal stem cells. Journal of Tissue Engineering and Regenerative Medicine. 2015;**9**(4):332-342

[100] Green JD, Tollemar V, Dougherty M, Yan Z, Yin L, Ye J, et al. Multifaceted signaling regulators of chondrogenesis: Implications in cartilage regeneration and tissue engineering. Genes & Diseases. 2015;**2**(4):307-327

[101] Oka K, Oka S, Sasaki T, Ito Y, Bringas P Jr, Nonaka K, et al. The role of TGF-β

signaling in regulating chondrogenesis and osteogenesis during mandibular development. Developmental Biology. 2007;**303**(1):391-404

[102] Yu L, Liu H, Yan M, Yang J, Long F, Muneoka K, et al. Shox2 is required for chondrocyte proliferation and maturation in proximal limb skeleton. Developmental Biology. 2007;**306**(2):549-559

[103] Han Y, Yang J, Fang J, Zhou Y, Candi E, Wang J, et al. The secretion profile of mesenchymal stem cells and potential applications in treating human diseases. Signal Transduction and Targeted Therapy. 2022;**7**(1):92

[104] Yao X, Mao Y, Wu D, Zhu Y, Lu J, Huang Y, et al. Exosomal circ_0030167 derived from BM-MSCs inhibits the invasion, migration, proliferation and stemness of pancreatic cancer cells by sponging miR-338-5p and targeting the Wif1/Wnt8/β-catenin axis. Cancer Letters. 2021;**512**:38-50

[105] Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;**367**(6478):eaau6977

[106] McAndrews KM, Kalluri R. Mechanisms associated with biogenesis of exosomes in cancer. Molecular Cancer. 2019;**18**(1):52

[107] Emanueli C, Shearn AI, Angelini GD, Sahoo S. Exosomes and exosomal miRNAs in cardiovascular protection and repair. Vascular Pharmacology. 2015;**71**:24-30

[108] Liao FL, Tan L, Liu H, Wang JJ, Ma XT, Zhao B, et al. Hematopoietic stem cell-derived exosomes promote hematopoietic differentiation of mouse embryonic stem cells in vitro via inhibiting the miR126/Notch1 pathway. Acta Pharmacologica Sinica. 2018;**39**(4):552-560

[109] Nawaz M, Fatima F, Vallabhaneni KC, Penfornis P, Valadi H, Ekström K, et al. Extracellular vesicles: Evolving factors in stem cell biology. Stem Cells International. 2016;**2016**:1073140

[110] Shao L, Zhang Y, Lan B, Wang J, Zhang Z, Zhang L, et al. MiRNA-sequence indicates that mesenchymal stem cells and exosomes have similar mechanism to enhance cardiac repair. BioMed Research International. 2017;**2017**:4150705

[111] Gowen A, Shahjin F, Chand S, Odegaard KE, Yelamanchili SV. Mesenchymal stem cell-derived extracellular vesicles: Challenges in clinical applications. Frontiers in Cell and Developmental Biology. 2020;**8**:149

[112] Yeo RWY, Lai RC, Zhang B, Tan SS, Yin Y, Teh BJ, et al. Mesenchymal stem cell: An efficient mass producer of exosomes for drug delivery. Advanced Drug Delivery Reviews. 2013;**65**(3):336-341

[113] Wang S, Guo L, Ge J, Yu L, Cai T, Tian R, et al. Excess integrins cause lung entrapment of mesenchymal stem cells. Stem Cells. 2015;**33**(11):3315-3326

[114] Badillo AT, Beggs KJ, Javazon EH, Tebbets JC, Flake AW. Murine bone marrow stromal progenitor cells elicit an in vivo cellular and humoral alloimmune response. Biology of Blood and Marrow Transplantation. 2007;**13**(4):412-422

[115] Tran C, Damaser MS. Stem cells as drug delivery methods: Application of stem cell secretome for regeneration. Advanced Drug Delivery Reviews. 2015;**82**:1-11

[116] Moghadasi S, Elveny M, Rahman HS, Suksatan W, Jalil AT, Abdelbasset WK, et al. A paradigm shift in cell-free

approach: The emerging role of MSCs-derived exosomes in regenerative medicine. Journal of Translational Medicine. 2021;**19**(1):1-21

[117] Nassar W, El-Ansary M, Sabry D, Mostafa MA, Fayad T, Kotb E, et al. Umbilical cord mesenchymal stem cells derived extracellular vesicles can safely ameliorate the progression of chronic kidney diseases. Biomaterials Research. 2016;**20**(1):1-11

[118] Sun Y, Shi H, Yin S, Ji C, Zhang X, Zhang B, et al. Human mesenchymal stem cell derived exosomes alleviate type 2 diabetes mellitus by reversing peripheral insulin resistance and relieving β-cell destruction. ACS Nano. 2018;**12**(8):7613-7628

[119] Kordelas L, Rebmann V, Ludwig A, Radtke S, Ruesing J, Doeppner T, et al. MSC-derived exosomes: A novel tool to treat therapy-refractory graft-versus-host disease. Leukemia. 2014;**28**(4):970-973

[120] Liu W, Li L, Rong Y, Qian D, Chen J, Zhou Z, et al. Hypoxic mesenchymal stem cell-derived exosomes promote bone fracture healing by the transfer of miR-126. Acta Biomaterialia. 2020;**103**:196-212

[121] Liu W, Zhou N, Liu Y, Zhang W, Li X, Wang Y, et al. Mesenchymal stem cell exosome-derived miR-223 alleviates acute graft-versus-host disease via reducing the migration of donor T cells. Stem Cell Research & Therapy. 2021;**12**:1-16

[122] Juneja SC, Viswanathan S, Ganguly M, Veillette C. A simplified method for the aspiration of bone marrow from patients undergoing hip and knee joint replacement for isolating mesenchymal stem cells and in vitro chondrogenesis. Bone Marrow Research. 2016;**2016**:3152065

[123] De Wolf C, Van De Bovenkamp M, Hoefnagel M. Regulatory perspective on in vitro potency assays for human mesenchymal stromal cells used in immunotherapy. Cytotherapy. 2017;**19**(7):784-797

[124] Herrmann JL, Wang Y, Abarbanell AM, Weil BR, Tan J, Meldrum DR. Preconditioning mesenchymal stem cells with transforming growth factor-alpha improves mesenchymal stem cell-mediated cardioprotection. Shock. 2010;**33**(1):24-30

[125] Damasceno PKF, De Santana TA, Santos GC, Orge ID, Silva DN, Albuquerque JF, et al. Genetic engineering as a strategy to improve the therapeutic efficacy of mesenchymal stem/stromal cells in regenerative medicine. Frontiers in Cell and Developmental Biology. 2020;**8**:737

[126] Kim B-C, Kwack KH, Chun J, Lee J-H. Comparative transcriptome analysis of human adipose-derived stem cells undergoing osteogenesis in 2D and 3D culture conditions. International Journal of Molecular Sciences. 2021;**22**(15):7939

[127] Alhattab DM, Isaioglou I, Alshehri S, Khan ZN, Susapto HH, Li Y, et al. Fabrication of a three-dimensional bone marrow niche-like acute myeloid Leukemia disease model by an automated and controlled process using a robotic multicellular bioprinting system. Biomaterials Research. 2023;**27**(1):111

[128] Gallo A, Cuscino N, Contino F, Bulati M, Pampalone M, Amico G, et al. Changes in the transcriptome profiles of human amnion-derived mesenchymal stromal/stem cells induced by three-dimensional culture: A potential priming strategy to improve their properties. International Journal of Molecular Sciences. 2022;**23**(2):863

[129] Chang C, Yan J, Yao Z, Zhang C, Li X, Mao HQ. Effects of mesenchymal stem cell-derived paracrine signals and their delivery strategies. Advanced Healthcare Materials. 2021;**10**(7):2001689

[130] Alhattab DM, Khan Z, Alshehri S, Susapto HH, Hauser CA. 3D bioprinting of ultrashort self-assembling peptides to engineer scaffolds with different matrix stiffness for chondrogenesis. International Journal of Bioprinting. 2023;**9**(4):719

[131] Alshehri S, Susapto HH, Hauser CA. Scaffolds from self-assembling tetrapeptides support 3D spreading, osteogenic differentiation, and angiogenesis of mesenchymal stem cells. Biomacromolecules. 2021;**22**(5):2094-2106

Chapter 2

A Closer Look at Mesenchymal Stem Cells (MSCs), Their Potential and Function as Game-Changers of Modern Medicine

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Abstract

Mesenchymal stem cells (MSCs) have garnered significant attention in modern medicine as a potentially effective therapeutic intervention, owing to their distinctive characteristics, including the ability to self-renew, exhibit multipotency, elicit immunomodulatory effects, and promote tissue repair. MSCs are being studied extensively for their potential use in a wide range of clinical applications, including regenerative medicine, immunotherapy, and tissue engineering. In this chapter, we provide a comprehensive overview of the biology, potential, and function of MSCs, highlighting their role in modulating the immune system, promoting tissue repair, and restoring homeostasis in various disease conditions. We also discuss the challenges and limitations associated with MSC-based therapies, including issues related to their isolation, expansion, and delivery. Further research is needed to fully understand the mechanisms underlying MSCs' therapeutic effects and to optimize their clinical application. Nevertheless, MSCs hold great promise as game-changers in modern medicine, and their potential to revolutionize the field of regenerative medicine and immunotherapy cannot be overlooked.

Keywords: mesenchymal stem cells, regenerative medicine, tissue repair, multipotency, immunotherapy

1. Introduction

In the 1960s, scientists led by Friedenstein initially identified a population of fibroblast-like cells in bone marrow tissue that had the unique ability to renew themselves. Ever since this landmark discovery, these cells, now known as mesenchymal stem/stromal cells, have been the target of extensive research efforts due to their intriguing characteristics [1]. MSCs represent a subset of adult stem cells distinguished by their potential to develop into different cell lineages, their self-propagating capacity, and immunoregulatory functions [2]. Their proven capacity to mature into

cell types like osteocytes, chondrocytes, and adipocytes makes them highly valuable for promoting tissue repair and regeneration. Further research has shown that MSCs secrete a variety of biomolecules like growth factors, cytokines, and extracellular vehicles (EVs) involved in healing processes [3].

MSCs show great potential in the regenerative medicine field due to their unique traits and therapeutic capabilities. These cells, found in tissues like bone marrow (BM), fat, umbilical cord (UC), and dental pulp, hold significant promise for addressing many diseases and injuries [2]. Scholars have extensively investigated MSC isolation and expansion methods to benefit from their therapeutic properties. Researchers have created various isolation techniques like density centrifugation, adherence-based selection, and fluorescence-activated sorting [4]. These approaches help capture a population enriched with MSCs, which can then be grown in culture. Standardizing isolation protocols and characterization standards is crucial to make results across labs and clinical applications consistent and comparable. One remarkable MSC characteristic is immunomodulation. MSCs can alter immune responses by regulating various immune cell activities. They suppress the proliferation and functions of T cells, B cells, and natural killer (NK) cells, while promoting regulatory T cell (Treg) expansion. This immunosuppressive ability benefits autoimmune disorders, graft versus host disease (GVHD), and organ transplant treatment by curbing excessive immunity and fostering tolerance [5].

The therapeutic applications of MSCs and their derivatives like exosomes span a wide range of medical disciplines [6]. In orthopedics, MSCs have shown promise in regenerating bone and cartilage tissues, offering potential alternatives to traditional approaches such as joint replacements [7]. In cardiovascular medicine, MSCs have been investigated for their ability to improve cardiac function and promote blood vessel formation, holding the potential for treating heart failure and ischemic conditions [8]. Additionally, MSC-based therapies have shown encouraging results in neurological disorders, autoimmune diseases, and tissue injuries, demonstrating their versatility and potential to revolutionize patient care [9]. Due to these key characteristics, MSCs hold tremendous promise for regenerative medicine applications.

2. Biology and characteristics of MSCs

The International Society for Cellular Therapy (ISCT) defines human MSCs as fibroblast-like, plastic-adherent cells that express surface markers CD73, CD90 and CD105. At the same time, they lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR. According to ISCT guidelines, the ability to differentiate into osteoblasts, adipocytes, and chondroblasts when cultured in vitro is another key characteristic used to identify MSCs [10]. However, self-renewal – the process by which a single stem cell divides to produce at least one new identical stem cell – is a fundamental property of all stem cells. The ability to proliferate and generate clonal progeny through successive cell divisions while maintaining the potential to differentiate is crucial for stem cell characterization and relies on self-renewal capability [11]. Importantly, it should be noted that the expression of surface markers on MSCs may fluctuate depending on the specific tissue they are derived from, in vitro expansion protocols, and the culture conditions used. Careful phenotypic analysis is therefore required to accurately characterize MSC populations [2]. For example, the surface marker CD105, also known as endoglin and a component of the TGF-β receptor

complex, has been shown to be expressed on mesenchymal stem cells derived from various tissues but with differing intensities. Research findings indicate that over 50% of liver-derived MSCs are positive for CD105, whereas less than 10% of MSCs from the amniotic membrane (AM-MSCs) and UC (UC-MSCs) express this antigen. Additionally, liver-derived MSCs were reported to have a low-level expression (5–10%) of CD45, a lineage marker that the majority of MSCs typically lack [12]. In a separate study comparing mesenchymal stem cells derived from BM (BM-MSCs) to those obtained from menstrual blood (referred to as MenSCs), distinct expression profiles were observed between the two populations. The research found that although both BM-MSCs and MenSCs lacked surface markers associated with pluripotency, namely SSEA-3, SSEA-4, and TRA-1-60, MenSCs demonstrated high levels of expression of HLA-ABC and CD49α [13]. So, proper identification of MSCs necessitates controlling for variables that can potentially impact marker expression. In terms of origin, MSCs have been identified in multiple tissues throughout the body, with sources comprising BM, adipose tissue, UC, and dental pulp, among others. Of these, MSCs derived from BM have undergone significant research and are viewed as the archetypal MSC population. However, it is important to recognize that MSCs from diverse tissues can demonstrate differences in their attributes and responses. Studies have highlighted variations in the properties of MSCs depending on their exact anatomical source. For example, a comparative study investigated the properties of MSCs isolated from BM, adipose tissue (AD-MSCs), placenta (P-MSCs), and cord blood (CB-MSCs). While all MSC types exhibited similarities in proliferation capacity, clonogenic potential (ability to form colonies), and surface marker expression, BM-MSCs and AD-MSCs demonstrated stronger osteogenic differentiation ability as determined by von Kossa staining. BM-MSCs and AD-MSCs also expressed higher, more consistent levels of the DLX-5 gene known to regulate osteogenesis [14]. Additionally, the research reported that CB-MSCs and P-MSCs lacked osteogenic potential and exhibited poor adipogenic differentiation as well [14]. Similar findings were reported in another study that compared the in vivo bone-forming capacity of MSCs derived from different tissues. Although BM-MSCs successfully generated bone tissue under the experimental conditions, AD-MSCs, UC-MSCs, and skin-derived MSCs did not exhibit the same ability to produce bone [15]. The results mirrored previous research indicating that while MSCs share a multilineage potential profile, their precise anatomical source can influence the extent of osteogenic differentiation, with BM-MSCs demonstrating superior skeletal lineage commitment ability compared to MSCs from adipose tissue, UC, and skin in this experimental setup [15].

Additionally, MSCs appear as spindle-shaped cells when observed under a microscope. They possess elongated and slender cytoplasmic extensions, enabling them to interact with their microenvironment and neighboring cells. This morphology allows MSCs to migrate to sites of injury or inflammation efficiently and participate in tissue repair processes [16].

3. Functional properties of MSCs

MSCs have an array of traits supporting their usefulness in regenerative medicine. Their immunomodulatory influences, capacity for tissue restoration and regeneration, the potential to evolve into numerous cell types, and role in regulating the microenvironment collectively underlie their therapeutic power.

3.1 Immunomodulatory effects of MSCs

The immunomodulatory function of MSCs involves complex interactions between cell-cell contact mechanisms and soluble factor secretion to regulate immune homeostasis. Both modes of action work synergistically to influence immune cell behavior. Their impact on immune signaling pathways is another key feature of MSCs in immunomodulatory function [17], which is explained with more detail below:

3.1.1 Cell-to-cell contact mechanisms

Direct contact between MSCs and immune cells allows interaction through cell surface molecules. MSC expression of indoleamine 2,3-dioxygenase (IDO) depletes the amino acid tryptophan locally, which inhibits T cell proliferation and activation while promoting Treg cell development [18]. Upregulation of programmed death ligand 1(PD-L1) engages with programmed cell death protein 1 (PD-1) on T cells, hindering their activation and inducing apoptosis. Fas ligand (FasL) expression carried out apoptosis of activated T cells through the Fas receptor, diminishing T cell responses [19]. Additionally, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) augmentation enables outcompeting co-stimulatory molecules on antigen-presenting cells (APCS) for binding, interrupting signals necessary for T cell activation [20]. Collectively, these membrane-bound pathways facilitate MSC modulation of multiple localized immune cell subsets.

3.1.2 Soluble factor-mediated mechanisms

MSCs demonstrate potent immunomodulation by secreting a diverse array of soluble factors like cytokines, chemokines, growth factors and EVs. These factors act autonomously and on neighboring cells to shape immune responses. Transforming growth factor-beta (TGF-β) released from MSCs strongly suppresses immunity, affecting T cell activity, regulatory T cell development, and natural killer/dendritic cell function. Prostaglandin E2 (PGE2) similarly governs T cell behavior and Treg amplification while transitioning the cytokine profile to anti-inflammation [21]. Interleukin-10 (IL-10) blocks proinflammatory cytokine generation and bolsters Treg cell's function. Hepatocyte growth factor (HGF) impacts T cell proliferation/dendritic cells and enables repair [22]. EVs such as exosomes transport molecules like TGF-β, PGE2, and IL-10 between MSCs and immune cells to regulate recipient responses. Collectively, these secreted components downscale excessive immune activation through coordinated suppression of proinflammatory signaling and augmentation of regulatory networks [23].

3.1.3 Modulation of immune signaling pathways

MSCs wield robust immunoregulation by targeting major networks governing immune initiation and inflammation. Precisely adjusting these pathways optimizes immune balance:

- Nuclear factor-kappa β (NF-κβ) signaling coordinates proinflammatory reactions. MSCs curb and dampens inflammatory triggers, shielding tissues from collateral harm [24].

- The Janus kinase-signal transducer/activator of transcription (JAK-STAT) cascade strengthens cytokine and growth factor signaling, impacting immune effectors. MSCs suppress JAK-STAT transmission, soothing inflammatory mediator synthesis, and immune cell behavior globally [25, 26].

- Toll-like receptor (TLR) engagement alerts cells to microbe detection, kicking off innate activation. MSCs deter TLR relay, easing downstream proinflammatory cytokine induction. This directly confronts inflammatory triggers at multiple receptors [27].

- Notch intercellular communication decides cell identity crucial for immune cell development. MSCs interact with Notch receptors on immune targets, steering differentiation toward regulatory phenotypes through guided differentiation [28].

- Additional influenced pathways include Wnt/β-catenin signaling hindered by MSCs. This pathway promotes survival but also proinflammatory cytokine synthesis when awry. Via multiple membrane and exosome proteins, MSCs achieve these functional suppressions [29].

Yet, studies showed MSC groups vary quantitatively in immune regulation strength and evasion depending on the source. For example, a study contrasting bone marrow and fat tissue MSCs found fat MSCs exerted stronger, less immunogenic effects. When exposed to low interferon-gamma (IFN-γ), fat MSCs made higher IDO versus bone marrow MSCs. Activated fat MSCs also exhibited lower HLA-DR and higher CD55 [30]. These enabled more efficient immune bypasses. Following activation, fat MSCs better halted T cell proliferation than bone marrow MSCs. In contrast, bone marrow MSCs suppressed natural killer cell proliferation more [30]. Another study assessed BM, fat, and Wharton's jelly MSC (WJ-MSC) immunomodulation – a co-culture combined each MSC type with immune cells, where fat MSCs most potently hindered proliferation. Additionally, WJ-MSCs uniquely failed to curb activated B cell and NK cell multiplication [1].

3.2 Tissue repair and regenerative potential of MSCs

Tissue damage and degenerative diseases are major health challenges worldwide, necessitating the development of innovative therapeutic strategies. MSCs, found in various tissues, hold great promise for tissue repair and regeneration [31]. In this section, we will discuss the role of MSCs in tissue repair in the context of different diseases.

3.2.1 Musculoskeletal disorders

Research shows MSCs demonstrate excellent potential for treating musculoskeletal disorders like osteoarthritis, tendon injuries, and bone breaks. In osteoarthritis, MSCs can mature into cartilage cells and promote repair. Additionally, their immunomodulation helps reduce inflammation and pain from joint degeneration [32]. For bone defects, MSCs aid regeneration by maturing into bone-forming cells and boosting the recruitment of other formative cells. They also play a pivotal role in remodeling the extracellular matrix (ECM), which is vital for repairing such conditions. MSCs make

and secrete ECM constituents such as collagens, proteoglycans, and glycosaminoglycans. By synthesizing and depositing these ECM components, MSCs fortify repaired musculoskeletal tissue structure and functionality [7].

3.2.2 Cardiovascular disorders

Research shows MSC-based therapies hold promise for heart conditions like heart attack and clogged arteries. MSCs encourage new blood vessel growth by releasing an array of pro-angiogenic factors including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), and hepatocyte growth factor [8]. These stimulate endothelial cell proliferation, migration, and tube formation, vital steps in blood vessel formation that aid angiogenesis in cardiovascular disease. MSCs may also decrease scar tissue and improve heart function by secreting anti-fibrotic factors. They secrete substances inhibiting extracellular matrix component formation, especially collagen, contributing to scars. These include HGF and Matrix metalloproteinases (MMPs) that can degrade excess collagen plus TGF-β blockers [33]. Additionally, MSCs can govern fibroblast and highly contractile myofibroblast behavior, which contributes to scar tightening. Released factors inhibit fibroblast activation, proliferation, and transitioning to myofibroblasts. This activity regulates scar formation. MSCs can also mature into endothelial and smooth muscle cells, assisting cardiac repair [34].

3.2.3 Neurological disorders

Neurological issues like stroke, spinal cord injury, and neurodegenerative diseases have limited treatment due to the nervous system's low regenerative capacity. MSCs can develop into neural cells such as neurons, astrocytes, and oligodendrocytes. Administered to the central nervous system (CNS), MSCs integrate damaged areas, replacing lost or nonfunctional cells to promote repair and regrowth. They secrete various neurotrophic factors including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and glial cell line-derived neurotrophic factor (GDNF) supporting existing neuron survival and growth [35]. These stimulate brain angiogenesis and new neural connections. MSC-secreted neurotrophins enhance neuronal survival and functional recovery. MSCs also possess immunomodulation, curbing inflammatory responses in the central nervous system by inhibiting microglia and T cell activation/proliferation and cytokine production. Reducing neuroinflammation favors neural recovery while decreasing secondary neuron damage. Moreover, MSCs activate resident brain stem and progenitor cells, aiding their maturation into functional neurons or other neural cells to boost endogenous repair mechanisms, contributing to neuronal recovery and tissue restoration [36].

By governing glial cell behavior and transitioning them to a more protective phenotype, MSCs assist inflammatory regulation, lessen glial scar formation, and build a supportive environment for neuronal survival and repair. These properties make them attractive candidates for treating neurological disorders and spinal cord injuries [37].

3.2.4 Autoimmune disorders

MSCs have a powerful ability to modulate immune responses, making them beneficial for treating conditions caused by abnormal immune activation like GVHD, COVID-19, and inflammatory bowel disease (IBD). MSCs can dampen

disproportionate immune reactions, balance proinflammatory and anti-inflammatory cytokine levels, and spur tissue healing by adjusting the local microenvironment [38]. MSCs have the adaptability to evolve into various cell types, including mesodermal derivatives such as fat, cartilage, and bone cells, plus non-mesodermal varieties including nerve and liver cells. In autoimmune diseases where tissue is harmed, MSCs can mature into specific cell types needed for repair and regrowth [39]. For example, in an autoimmune arthritis, MSCs can become cartilage cells to further mend cartilage. In autoimmune liver conditions, MSCs can evolve into liver cells to facilitate organ regrowth [40].

Table 1 shows a summary of the therapeutic application of MSCs in the mentioned diseases in some clinical trials.

Disease's category	Disease	Trial phase	Sample size	MSC source	MSC dose	Main outcome	Ref
	Osteoarthritis	Phase I/II	15	BM-MSCs	$40.9 \times 10^6 \pm 0.4 \times 10^6$	Significant improvement in parameters including bodily pain, physical role, and physical functioning	[41]
Musculoskeletal disorders	Bone defects	phase IIa	18	BM-MSCs	$15 \pm 4.5 \times 10^6$	Significant clinical improvement in patients.	[42]
	Lateral epicondylosis	Pilot study	6	AD-MSCs	10^6 or 10^7 cells	Significant decrease in patients' VAS & increase in elbow performance scores	[43]
	Severe ischemic heart failure	Phase II	60	BM-MSCs	NM	Significant improvement in myocardial function	[34]
Cardiovascular diseases	Myocardial infarction	Phase II	116	WJ-MSC	6×10^6 cells	Significant improvement in LVEF, and the myocardial viability (PET) and perfusion within the infarcted territory (SPECT) was observed.	[44]
	Ataxia	NM	24	UC-MSCs	1×10^6 cell/kg BW	Improvement in patient's movement and quality of life with delay in desease progression.	[45]

Disease's category	Disease	Trial phase	Sample size	MSC source	MSC dose	Main outcome	Ref
Neurological disorders	ALS	Phase I/II	15	BM-MSCs	1×10^6 cell/kg BW	Improvement in patient's status, ALS-FRS & FVC	[36]
	MS	Phase I/II	160	BM-MSCs	1–2 $\times 10^6$ cell/kg BW	MSC transplantation was safe and well tolerable by patients and showed improvement in EDSS score.	[46]
	MSA	Phase I/II	24	AD-MSCs	Two doses (1×10^7 and 1×10^8 cell/kg BW)	Significant decrease in the rate of disease progression (UMSARS)	[47]
	Refractory luminal Crohn's disease	Phase I	10	BM-MSCs	1–2 $\times 10^6$ cell/kg BW	Significant decrease in patient's CDAI	[48]
Autoimmune diseases	COVID-19	Phase I	20	UC-MSCs	1×10^6 Cell/kg BW	Improvement in patient's oxygenation and significant decrease in CRP and inflammatory cytokines	[49, 50]
	GVHD	NM	11	BM-MSCs	NM	Significant decrease in TH17 cells, but increase in Treg cells and a shift toward TH2-cell responses	[51]

Abbreviations: MSC, Mesenchymal stem cells; BM-MSCs, Bone Marrow derived Mesenchymal stem cells; AD-MSCs, Adipose-derived Mesenchymal stem cells; WJ-MSC, Wharton's jelly-derived mesenchymal stem cells; VAS, patients' visual analog scale; UC-MSCs, Umbilical cord-derived Mesenchymal stem cells; LVEF, Left Ventricular Ejection Fraction; ALS, Amyotrophic Lateral Sclerosis; MSA, Multiple System Atrophy; UMSARS, Unified Multiple System Atrophy Rating Scale; MS, Multiple sclerosis; EDSS, Expanded Disability Status Scale; GVHD, Graft-versus-host disease; CRP, C-reactive protein; CDAI, Crohn's disease activity index; TH17, T helper 17; Treg, T regulatory cell; NM, Not Mentioned.

Table 1.
Therapeutic application of MSCs in various disorders.

4. MSCs and exosomes

MSCs have been extensively studied in clinical trials due to their diverse roles including tissue repair, anti-inflammation, immunosuppression, and neuroprotection. Initially, it was believed that MSCs traveled to injury sites, differentiated, and replaced damaged cells. However, later research showed MSC engraftment and

differentiation at injuries is brief and limited [52, 53]. Current research suggests MSCs mainly act through secreting trophic factors. Some propose exosomes mediate MSC cell communication as they are abundantly secreted by MSCs [54].

Exosomes are tiny lipid bilayer vesicles formed through endocytosis and budding from late endosomes, released when multivesicular bodies fuse with the plasma membrane. They release from various cells including plasma, breast milk, serum, and cerebral fluid (CSF) under inflammation, disease, or immune imbalance [6, 55]. Exosomes have potential to treat infections like COVID-19, cancers, and neurodegeneration supported by evidence. Originally discovered in the 1980s studying sheep blood cell maturation, exosomes garnered attention as key communicators [56]. Though believed to dispose of proteins, exosomes stimulate immunity in vivo and in vitro, secreting from nearly all body cells found in fluids affecting lung, kidney, and liver functions [57, 58].

Exosomes uniquely carry a diverse cargo of lipids, proteins, DNAs, and RNAs specific to their cellular origins, regulating immunity and communication, unlike other extracellular vesicles. Exosomes are characterized by membrane proteins, such as Alix, TSG101, Rab5, Rab27a, Rab27b, and multiple tetraspanins (CD37, CD63, CD81, and CD82), distinguishing them from apoptotic bodies [59]. Exosomal mRNA or miRNA transported from donor cells may alter recipient cell fates. For example, brain cancer or regulatory immune cell exosomes impacted recipient cells [59, 60]. **Figure 1** schematically illustrates MSC exosome structures, contents, and target cells.

MSC exosomes are considered MSCs' paracrine effectors with comparable functions to MSCs. For instance, both alleviated neuroprotection and endotoxin-induced lung injury [61]. MSC exosomes contained miR-16 downregulating VEGF similarly to MSCs [62]. MSC exosomes express MSC antigens and adhesion molecules such as CD29, CD44, and CD73. Moreover, like MSCs, MSC exosomes contain genetic information for signaling pathways [63, 64].

5. Challenges and limitations in MSC therapy

There are currently some obstacles tied to using stem cells for medical functions because the way transplanted cells act is not fully decoded. Still, comprehending stem cell traits, behavior, and mechanisms more profoundly could facilitate customized treatments for many sicknesses. Before meshing stem cells with recipient tissues for lifelong gains, certain crucial points demand prudent consideration. More knowledge about stem cell properties may eventually alleviate the challenges of applying them therapeutically, leading to improved prognosis and quality of life [65].

5.1 Heterogeneity and inherent differential potency of MSCs

MSCs used for medical purposes can originate from either the patient (autologous) or a donor (allogeneic). These cells inhabit complex biological systems in the body and have diverse subpopulations. Ignoring this heterogeneity is a primary reason cell-based therapies have inconsistent results. Stem cells intrinsically proliferate indefinitely while maintaining an undifferentiated state when environmental conditions permit. Thus, appropriate signaling from adjacent cell types and local microenvironments is required for proper functioning. Variations in how cells signal to each other or respond to their surroundings can alter functional pathways. A thorough understanding of diversity across cell populations and their characteristics is essential for

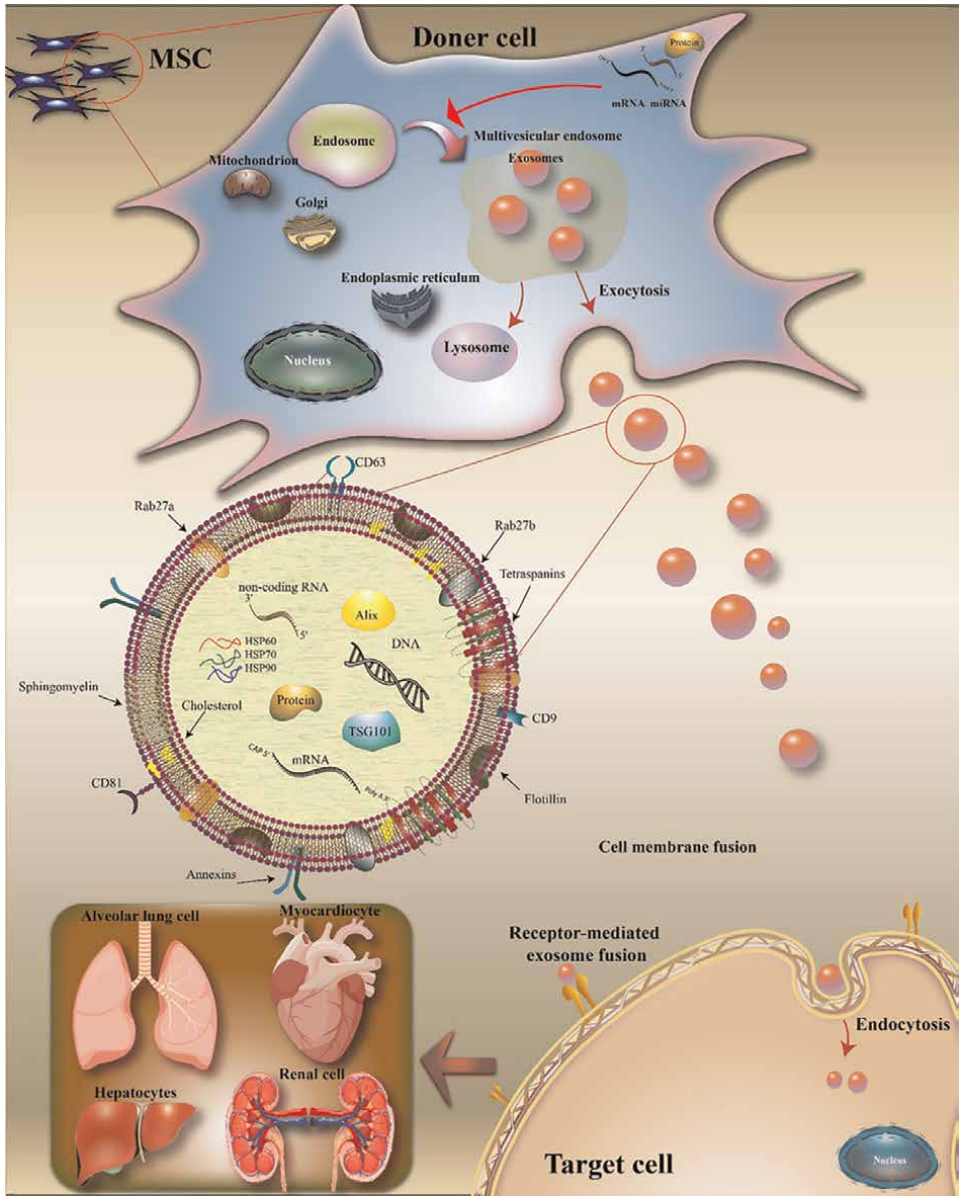

Figure 1.
Biogenesis, release, and uptake of exosomes (the figure is created using Adobe illustrator 2019).

developing well-designed clinical trials. As such, fully considering a stem cell's innate properties when selecting candidates for specific therapies is crucial [66, 67].

5.2 Heterogeneity of disease progression

Carefully reviewing disease worsening over time is pivotal for cell therapy success and demands serious attention. Such review informs customized remedies for enduring impacts. A thorough understanding of illness details, especially for degenerative

conditions, allows individual care. Induced pluripotent stem cells (iPSCs) offer game-changing ways to model human sicknesses, particularly genetic ones, by developing iPSCs from uncommon and prevalent disease patients. This provides invaluable disease modeling and medication development, letting progression study and disorder remedies. This process enhances comprehension of underlying molecular drivers as most maladies contain numerous intertwined subsets rather than single illnesses. Initially, stem cell use may concentrate on an affliction, then an individual, potentially ushering a more targeted therapy approach. However, early efforts involve trial and error that markedly improves over time [65, 68].

5.3 Homing and targeted MSC delivery

Stem cells prove highly effective in cell therapy due to sensing their surroundings via cytokine receptors, permitting migration toward damaged tissues or tumors by chemokine gradient trails. This innate navigation aids targeted delivery for enhanced treatment. Beyond natural locating traits, induced direction strategies developed encourage further precise transportation. Methods alter cells through membrane receptor swaps, lipid particles conveying customized cells, viral vectors transmitting genes, or antibody/peptide-hooked particles delivering targets. Still, just a small fraction embeds at wished spots, and engraftment relies on administration technique. Therefore, transportation optimization can emerge from route suitability consideration, physiological forces concentrating cells, preconditioning, and transgenes actuating homing expertly. Additional efforts are required to enhance the understanding and utilization of stem cell manipulation and targeted tissue environments in order to effectively leverage these techniques, either independently or in combination, to provide optimal healthcare outcomes [65, 69, 70].

5.4 Complexity of mechanism of action of cell therapy

When exploring new treatments, a key challenge lies in understanding intricate biochemical and physiological events during application. Animal models often study such processes pre-human use. Cell therapies proved workable clinically, relying on safe, reproducible advantages. Basically, therapy forestalls or reverses sickness worsening. Both approaches overlap and differ at the technique level for cell-based remedies, necessitating customizing to meet illness-specific demands. Identifying diverse biotherapeutics such as anti-death, immune modulation, anti-scarring, pro-vessel growth, cell-cell signaling chemotaxis, local stem/progenitor growth, and diversification aspect discharge is daunting. Defining biochemical pathways and intermolecular machinery involved in cell therapy is critical to grasping stem cell behavior/roles better, a compelling cell therapy platform necessity to heighten predictive abilities. Further exploration embracing cell intricacy centers on understanding underlying behavior/roles [65, 71, 72].

6. Future directions

MSC therapies hold tremendous promise for regenerative medicine, but their complete potential can be realized by implementing various techniques and approaches to reduce adverse effects and improve treatment outcomes. One strategy is the genetic engineering of MSCs, which permits precise modifications to their

genetic makeup in order to enhance their therapeutic properties. By introducing specific genes into MSCs, it is possible to improve their survival, ability to differentiate into desired cell types, promote tissue repair, modulate the immune response, or secrete therapeutic factors. This genetic engineering can be achieved through methods such as viral vectors, non-viral vectors, or genome editing tools like CRISPR-Cas9, ensuring targeted and efficient modification of MSCs [73, 74].

Furthermore, the utilization of MSC-derived products, such as exosomes and EVs, has emerged as a promising approach to augment the therapeutic effects of MSC therapies. These vesicles contain a diverse array of bioactive molecules, including proteins, nucleic acids, and signaling molecules that can exert potent therapeutic effects by facilitating intercellular communication, promoting tissue regeneration, and reducing inflammation [75]. To boost the efficacy of MSC-derived products, various techniques can be employed, including optimization of isolation procedures, standardization of production protocols, and characterization of cargo contents. Additionally, engineering MSCs to enhance the secretion of specific exosomes or EVs with desired therapeutic cargo can further enhance their therapeutic potential.

Besides, to mitigate adverse effects associated with MSC therapies, careful consideration should be given to factors such as cell dose, route of administration, and patient selection. Optimizing the dose of MSCs administered ensures that the therapeutic effects are maximized while avoiding potential complications. Choosing the appropriate route of administration, whether it be systemic, local, or targeted delivery, can enhance the homing and engraftment of MSCs to the desired tissue, improving treatment efficacy. Moreover, thorough patient evaluation and selection based on disease characteristics, immune compatibility, and pre-existing conditions can help minimize adverse reactions and maximize the safety and effectiveness of MSC therapies. Continued research and advancements in these areas will undoubtedly contribute to unlocking the full therapeutic potential of MSC-based therapies and pave the way for transformative regenerative medicine.

7. Conclusion

In summary, MSCs possess unique properties making them highly beneficial for therapeutic applications. Their ability to self-renew, differentiate into various cell types, and modulate immunological responses provide MSCs with regenerative capabilities across multiple organ systems. Extensive research over the past decades has revealed the therapeutic potential of MSCs and their exosomes for treating a wide range of medical conditions. As the introduction described, MSCs have shown promise in orthopedic applications like bone and cartilage repair. They have also been studied for their cardiovascular benefits in improving heart function and promoting blood vessel growth. Perhaps most importantly, MSC therapies have demonstrated encouraging results in central nervous system disorders as well as autoimmune and inflammatory conditions.

Looking ahead, further research is still needed to optimize isolation and culture methods to standardize MSC production. Additional pre-clinical and clinical studies are also required to fully characterize MSC engraftment, bio distribution, and mechanisms of action *in vivo*. Despite these remaining challenges, MSCs represent a promising tool for regenerative medicine. Their multipotency and immunomodulatory properties provide a versatile cell-based therapeutic approach with potential

applications across many areas of human disease. With continued advances in basic and translational research, MSC therapies may transform patient care and management of conditions currently lacking effective medical solutions.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Yoo KH, Jang IK, Lee MW, Kim HE, Yang MS, Eom Y, et al. Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. Cellular Immunology. 2009;**259**(2):150-156

[2] Farkhad NK, Mahmoudi A, Mahdipour E. How similar are human mesenchymal stem cells derived from different origins? A review of comparative studies. Current Stem Cell Research & Therapy. 2021;**16**(8): 980-993

[3] Han Y, Yang J, Fang J, Zhou Y, Candi E, Wang J, et al. The secretion profile of mesenchymal stem cells and potential applications in treating human diseases. Signal Transduction and Targeted Therapy. 2022;**7**(1):92

[4] Harichandan A, Bühring HJ. Prospective isolation of human MSC. Best Practice & Research Clinical Haematology. 2011;**24**(1): 25-36

[5] Song N, Scholtemeijer M, Shah K. Mesenchymal stem cell immunomodulation: Mechanisms and therapeutic potential. Trends in Pharmacological Sciences. 2020;**41**(9): 653-664

[6] Farkhad NK, Mahmoudi A, Mahdipour E. Regenerative therapy by using mesenchymal stem cells-derived exosomes in COVID-19 treatment. The potential role and underlying mechanisms. Regenerative Therapy. 2022;**20**:61-71

[7] Lin H, Sohn J, Shen H, Langhans MT, Tuan RS. Bone marrow mesenchymal stem cells: Aging and tissue engineering applications to enhance bone healing. Biomaterials. 2019;**203**:96-110

[8] Oliveira MS, Saldanha-Araujo F, Goes AM, Costa FF, de Carvalho JL. Stem cells in cardiovascular diseases: Turning bad days into good ones. Drug Discovery Today. 2017;**22**(11): 1730-1739

[9] Qu M, Xing F, Xing N. Mesenchymal stem cells for the treatment of cognitive impairment caused by neurological diseases. Biotechnology Letters. 2022; **44**(8):903-916

[10] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;**8**(4):315-317

[11] Viswanathan S, Shi Y, Galipeau J, Krampera M, Leblanc K, Martin I, et al. Mesenchymal stem versus stromal cells: International Society for Cell & gene therapy (ISCT®) mesenchymal stromal cell committee position statement on nomenclature. Cytotherapy. 2019; **21**(10):1019-1024

[12] Schmelzer E, McKeel DT, Gerlach JC. Characterization of human mesenchymal stem cells from different tissues and their membrane encasement for prospective transplantation therapies. BioMed Research International. 2019;**2019**:6376271

[13] Alcayaga-Miranda F, Cuenca J, Luz-Crawford P, Aguila-Díaz C, Fernandez A, Figueroa FE, et al. Characterization of menstrual stem cells: Angiogenic effect, migration and hematopoietic stem cell support in comparison with bone marrow mesenchymal stem cells. Stem Cell Research & Therapy. 2015;**6**(1):32

[14] Heo JS, Choi Y, Kim HS, Kim HO. Comparison of molecular profiles of human mesenchymal stem cells derived from bone marrow, umbilical cord blood, placenta and adipose tissue. International Journal of Molecular Medicine. 2016;**37**(1):115-125

[15] Reinisch A, Etchart N, Thomas D, Hofmann NA, Fruehwirth M, Sinha S, et al. Epigenetic and in vivo comparison of diverse MSC sources reveals an endochondral signature for human hematopoietic niche formation. Blood, The Journal of the American Society of Hematology. 2015;**125**(2): 249-260

[16] Bobis S, Jarocha D, Majka M. Mesenchymal stem cells: Characteristics and clinical applications. Folia Histochemica et Cytobiologica. 2006; **44**(4):215-230

[17] Herrero C, Pérez-Simón J. Immunomodulatory effect of mesenchymal stem cells. Brazilian Journal of Medical and Biological Research. 2010;**43**:425-430

[18] Farkhad NK, Reihani H, Moghadam AA, Moghadam AB, Tavakol-Afshari J. Are mesenchymal stem cells able to manage cytokine storm in COVID-19 patients? A review of recent studies. Regenerative Therapy. 2021;**18**:152-160

[19] Atsuta I, Liu S, Miura Y, Akiyama K, Chen C, An Y, et al. Mesenchymal stem cells inhibit multiple myeloma cells via the Fas/Fas ligand pathway. Stem Cell Research & Therapy. 2013;**4**:1-14

[20] Gaber T, Schönbeck K, Hoff H, Tran CL, Strehl C, Lang A, et al. CTLA-4 mediates inhibitory function of mesenchymal stem/stromal cells. International Journal of Molecular Sciences. 2018;**19**(8):2312

[21] Bassi ÊJ, de Almeida DC, Moraes-Vieira PMM, Câmara NOS. Exploring the role of soluble factors associated with immune regulatory properties of mesenchymal stem cells. Stem Cell Reviews and Reports. 2012;**8**:329-342

[22] Lee RH, Oh JY, Choi H, Bazhanov N. Therapeutic factors secreted by mesenchymal stromal cells and tissue repair. Journal of Cellular Biochemistry. 2011;**112**(11):3073-3078

[23] Huang Y, Wu Q, Tam PKH. Immunomodulatory mechanisms of mesenchymal stem cells and their potential clinical applications. International Journal of Molecular Sciences. 2022;**23**(17):10023

[24] Castro-Manrreza ME, Montesinos JJ. Immunoregulation by mesenchymal stem cells: Biological aspects and clinical applications. Journal of Immunology Research. 2015;**2015**:1-21

[25] Wang L, Deng Z, Sun Y, Zhao Y, Li Y, Yang M, et al. The study on the regulation of Th cells by mesenchymal stem cells through the JAK-STAT signaling pathway to protect naturally aged sepsis model rats. Frontiers in Immunology. 2022;**13**:820685

[26] Pokrywczynska M, Rasmus M, Jundzill A, Balcerczyk D, Adamowicz J, Warda K, et al. Mesenchymal stromal cells modulate the molecular pattern of healing process in tissue-engineered urinary bladder: The microarray data. Stem Cell Research & Therapy. 2019; **10**(1):1-17

[27] Romieu-Mourez R, François M, Boivin M-N, Bouchentouf M, Spaner DE, Galipeau J. Cytokine modulation of TLR expression and activation in mesenchymal stromal cells leads to a proinflammatory phenotype. The

Journal of Immunology. 2009;**182**(12): 7963-7973

[28] Fu D, Yao Q, Qi X. The role of Notch signaling pathway in bone immune regulation of osteogenic differentiation of macrophages and mesenchymal stem cells. 2023:1-20

[29] Elbaz EM, Helmy HS, El-Sahar AE, Saad MA, Sayed RH. Lercanidipine boosts the efficacy of mesenchymal stem cell therapy in 3-NP-induced Huntington's disease model rats via modulation of the calcium/calcineurin/ NFATc4 and Wnt/β-catenin signalling pathways. Neurochemistry International. 2019;**131**:104548

[30] Ménard C, Dulong J, Roulois D, Hébraud B, Verdière L, Pangault C, et al. Integrated transcriptomic, phenotypic, and functional study reveals tissue-specific immune properties of mesenchymal stromal cells. Stem Cells. 2020;**38**(1):146-159

[31] Patel DM, Shah J, Srivastava AS. Therapeutic potential of mesenchymal stem cells in regenerative medicine. Stem Cells International. 2013;**2013**:1-16

[32] Xiang X-N, Zhu S-Y, He H-C, Yu X, Xu Y, He C-Q. Mesenchymal stromal cell-based therapy for cartilage regeneration in knee osteoarthritis. Stem Cell Research & Therapy. 2022;**13**(1): 1-20

[33] Kasper G, Glaeser JD, Geissler S, Ode A, Tuischer J, Matziolis G, et al. Matrix metalloprotease activity is an essential link between mechanical stimulus and mesenchymal stem cell behavior. Stem Cells. 2007;**25**(8): 1985-1994

[34] Mathiasen AB, Qayyum AA, Jørgensen E, Helqvist S, Fischer-Nielsen A, Kofoed KF, et al. Bone marrow-derived mesenchymal stromal cell treatment in patients with severe ischaemic heart failure: A randomized placebo-controlled trial (MSC-HF trial). European Heart Journal. 2015;**36**(27): 1744-1753

[35] Najafi S, Najafi P, Farkhad NK, Torshizi GH, Darban RA, Boroumand AR, et al. Mesenchymal stem cell therapy in amyotrophic lateral sclerosis (ALS) patients: A comprehensive review of disease information and future perspectives. Iranian Journal of Basic Medical Sciences. 2023;**26**(8):872

[36] Tavakol-Afshari J, Boroumand AR, Farkhad NK, Moghadam AA, Sahab-Negah S, Gorji A. Safety and efficacy of bone marrow derived-mesenchymal stem cells transplantation in patients with amyotrophic lateral sclerosis. Regenerative Therapy. 2021;**18**: 268-274

[37] Li M, Chen H, Zhu M. Mesenchymal stem cells for regenerative medicine in central nervous system. Frontiers in Neuroscience. 2022;**16**:1068114

[38] Jasim SA, Yumashev AV, Abdelbasset WK, Margiana R, Markov A, Suksatan W, et al. Shining the light on clinical application of mesenchymal stem cell therapy in autoimmune diseases. Stem Cell Research & Therapy. 2022;**13**(1):1-15

[39] Wang Y, Fang J, Liu B, Shao C, Shi Y. Reciprocal regulation of mesenchymal stem cells and immune responses. Cell Stem Cell. 2022;**29**(11): 1515-1530

[40] Liu J, Gao J, Liang Z, Gao C, Niu Q, Wu F, et al. Mesenchymal stem cells and their microenvironment. Stem Cell Research & Therapy. 2022;**13**(1):429

[41] Soler R, Orozco L, Munar A, Huguet M, López R, Vives J, et al. Final results of a phase I–II trial using ex vivo expanded autologous mesenchymal stromal cells for the treatment of osteoarthritis of the knee confirming safety and suggesting cartilage regeneration. The Knee. 2016;**23**(4): 647-654

[42] Šponer P, Filip S, Kučera T, Brtková J, Urban K, Palička V, et al. Utilizing autologous multipotent mesenchymal stromal cells and-Tricalcium phosphate scaffold in human bone defects: A prospective, controlled feasibility trial. BioMed Research International. 2016;**2016**:1-13

[43] Lee SY, Kim W, Lim C, Chung SG. Treatment of lateral epicondylosis by using allogeneic adipose-derived mesenchymal stem cells: A pilot study. Stem Cells. 2015;**33**(10):2995-3005

[44] Gao LR, Chen Y, Zhang NK, Yang XL, Liu HL, Wang ZG, et al. Intracoronary infusion of Wharton's jelly-derived mesenchymal stem cells in acute myocardial infarction: Double-blind, randomized controlled trial. BMC Medicine. 2015;**13**:1-15

[45] Dongmei H, Jing L, Mei X, Ling Z, Hongmin Y, Zhidong W, et al. Clinical analysis of the treatment of spinocerebellar ataxia and multiple system atrophy-cerebellar type with umbilical cord mesenchymal stromal cells. Cytotherapy. 2011;**13**(8):913-917

[46] Uccelli A, Laroni A, Brundin L, Clanet M, Fernandez O, Nabavi SM, et al. MEsenchymal StEm cells for multiple sclerosis (MESEMS): A randomized, double blind, cross-over phase I/II clinical trial with autologous mesenchymal stem cells for the therapy of multiple sclerosis. Trials. 2019;**20**(1): 1-13

[47] Singer W, Dietz AB, Zeller AD, Gehrking TL, Schmelzer JD, Schmeichel AM, et al. Intrathecal administration of autologous mesenchymal stem cells in multiple system atrophy. Neurology. 2019;**93**(1): e77-e87

[48] Duijvestein M, Vos ACW, Roelofs H, Wildenberg ME, Wendrich BB, Verspaget HW, et al. Autologous bone marrow-derived mesenchymal stromal cell treatment for refractory luminal Crohn's disease: Results of a phase I study. Gut. 2010;**59**(12):1662-1669

[49] Kaffash Farkhad N, Sedaghat A, Reihani H, Adhami Moghadam A, Bagheri Moghadam A, Khadem Ghaebi N, et al. Mesenchymal stromal cell therapy for COVID-19-induced ARDS patients: A successful phase 1, control-placebo group, clinical trial. Stem Cell Research & Therapy. 2022; **13**(1):1-13

[50] Farkhad NK, Sedaghat A, Reihani H, Moghadam AA, Moghadam AB, Ghaebi NK, et al. Specific clinical and immunological changes following mesenchymal stem cell transplantation in COVID-19–induced acute respiratory distress syndrome patients: A phase-I clinical trial. Iranian Journal of Allergy, Asthma and Immunology. 2022;**21**:1-17

[51] Jitschin R, Mougiakakos D, Von Bahr L, Völkl S, Moll G, Ringden O, et al. Alterations in the cellular immune compartment of patients treated with third-party mesenchymal stromal cells following allogeneic hematopoietic stem cell transplantation. Stem Cells. 2013; **31**(8):1715-1725

[52] Nikfarjam S, Rezaie J, Zolbanin NM, Jafari R. Mesenchymal stem cell derived-exosomes: A modern approach in translational medicine. Journal of Translational Medicine. 2020;**18**(1):1-21

[53] Moghadam MS, Azimian H, Afshari JT, Toossi MTB, Farkhad NK, Aghaee-Bakhtiari SH. Chromosomal instability in various generations of human mesenchymal stem cells following the therapeutic radiation doses. 2023;**2023**:1-22

[54] Elahi FM, Farwell DG, Nolta JA, Anderson JD. Preclinical translation of exosomes derived from mesenchymal stem/stromal cells. Stem Cells. 2020; **38**(1):15-21

[55] Pegtel DM, Gould SJ. Exosomes. Annual Review of Biochemistry. 2019; **88**:487-514

[56] Vlassov AV, Magdaleno S, Setterquist R, Conrad R. Exosomes: Current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochimica et Biophysica Acta (BBA)-General Subjects. 2012;**1820**(7):940-948

[57] Gao K, Jin J, Huang C, Li J, Luo H, Li L, et al. Exosomes derived from septic mouse serum modulate immune responses via exosome-associated cytokines. Frontiers in Immunology. 2019;**10**:1560

[58] Zhang X, Sheng Y, Li B, Wang Q, Liu X, Han J. Ovarian cancer derived PKR1 positive exosomes promote angiogenesis by promoting migration and tube formation in vitro. Cell Biochemistry and Function. 2021;**39**(2): 308-316

[59] Li Q, Wang Z, Xing H, Wang Y, Guo Y. Exosomes derived from miR-188-3p-modified adipose-derived mesenchymal stem cells protect Parkinson's disease. Molecular Therapy-Nucleic Acids. 2021;**23**:1334-1344

[60] Okoye IS, Coomes SM, Pelly VS, Czieso S, Papayannopoulos V, Tolmachova T, et al. MicroRNA-containing T-regulatory-cell-derived exosomes suppress pathogenic T helper 1 cells. Immunity. 2014;**41**(1):89-103

[61] Chen J, Li C, Liang Z, Li C, Li Y, Zhao Z, et al. Human mesenchymal stromal cells small extracellular vesicles attenuate sepsis-induced acute lung injury in a mouse model: The role of oxidative stress and the mitogen-activated protein kinase/nuclear factor kappa B pathway. Cytotherapy. 2021; **23**(10):918-930

[62] Lee J-K, Park S-R, Jung B-K, Jeon Y-K, Lee Y-S, Kim M-K, et al. Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating VEGF expression in breast cancer cells. PLoS One. 2013;**8**(12): e84256

[63] Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. International Journal of Molecular Sciences. 2014;**15**(3):4142-4157

[64] Eirin A, Riester SM, Zhu X-Y, Tang H, Evans JM, O'Brien D, et al. MicroRNA and mRNA cargo of extracellular vesicles from porcine adipose tissue-derived mesenchymal stem cells. Gene. 2014;**551**(1):55-64

[65] Srijaya TC, Ramasamy TS, Kasim NHA. Advancing stem cell therapy from bench to bedside: Lessons from drug therapies. Journal of Translational Medicine. 2014;**12**:1-17

[66] Hope K, Bhatia M. Clonal interrogation of stem cells. Nature Methods. 2011;**8**(Suppl 4):S36-S40

[67] Cahan P, Daley GQ. Origins and implications of pluripotent stem cell variability and heterogeneity. Nature Reviews Molecular Cell Biology. 2013; **14**(6):357-368

[68] Ebben JD, Zorniak M, Clark PA, Kuo JS. Introduction to induced pluripotent stem cells: Advancing the potential for personalized medicine. World Neurosurgery. 2011;**76**(3–4): 270-275

[69] Komarova S, Roth J, Alvarez R, Curiel DT, Pereboeva L. Targeting of mesenchymal stem cells to ovarian tumors via an artificial receptor. Journal of Ovarian Research. 2010;**3**(1): 1-14

[70] Ansboro S, Greiser U, Barry F, Murphy M. Strategies for improved targeting of therapeutic cells: Implications for tissue repair. European Cells & Materials. 2012;**23**:310-318

[71] Laflamme MA, Zbinden S, Epstein SE, Murry CE. Cell-based therapy for myocardial ischemia and infarction: Pathophysiological mechanisms. Annual Review of Pathology and Mechanical Diseases. 2007;**2**:307-339

[72] Drukker M, Tang C, Ardehali R, Rinkevich Y, Seita J, Lee AS, et al. Isolation of primitive endoderm, mesoderm, vascular endothelial and trophoblast progenitors from human pluripotent stem cells. Nature Biotechnology. 2012;**30**(6):531-542

[73] Nowakowski A, Walczak P, Lukomska B, Janowski M. Genetic engineering of mesenchymal stem cells to induce their migration and survival. Stem Cells International. 2016;**2016**:1-10

[74] Damasceno PKF, De Santana TA, Santos GC, Orge ID, Silva DN, Albuquerque JF, et al. Genetic engineering as a strategy to improve the therapeutic efficacy of mesenchymal stem/stromal cells in regenerative medicine. Frontiers in Cell and Developmental Biology. 2020;**8**:737

[75] Bian D, Wu Y, Song G, Azizi R, Zamani A. The application of mesenchymal stromal cells (MSCs) and their derivative exosome in skin wound healing: A comprehensive review. Stem Cell Research & Therapy. 2022;**13**(1):24

Chapter 3

Immunomodulation of Antiviral Response by Mesenchymal Stromal Cells (MSCs)

Sterlin Raj and Harish C. Chandramoorthy

Abstract

Mesenchymal stromal cells (MSCs) are known for their immunomodulatory properties, and their role in antiviral response is poorly understood. The susceptibility of the MSCs to viral infection or viral tropism toward MSCs can be emanated from few available literature evidences. What makes MSCs special is the ability to sustain infection and reciprocate through immune intermediates like antimicrobial peptides, cytokines, and secretomes. However, care has to be taken to understand that MSCs can transmit viral infections and are known for their vulnerability to many microorganisms in general. In the recent past, after deadly infections like Ebola, Zika, and HIV, COVID-19 had posed a great threat, where stem cell transplantation was a suggestive therapeutic model in some cases due to the cytokine storm and other additional biochemical, molecular, and transcriptional factors associated with the pathology. This is true in many other common viral infections at large. In this chapter, the role of MSCs in combating viral infections as well as their susceptibility pattern are discussed. Further, the role of MSCs in immunomodulation and their antiviral factors cannot be delineated in understanding the immunological mechanisms preventing tissue damages associated with viral infection.

Keywords: MSCs, immunomodulation, mesenchymal stromal cells, secretomes, antiviral response

1. Introduction

Among the numerous forms of infectious diseases worldwide, viral infections have been the biggest known threat in the recent years [1]. The spread of a new coronavirus (COVID-19) caused by severe acute respiratory syndrome coronavirus (SARS-CoV-2) affected more than 110 million people worldwide. It has prompted the scientific world to prevent and treat by developing of new vaccination or through antiviral drugs [2]. Mesenchymal stromal cells (MSCs) constitute a heterogeneous population of immunoregulatory stem cells that are known to be highly regenerative and antiviral immune response [3]. They replicate vigorously in culture plates and maintain their biological properties. MSCs were isolated from many tissues around the body such as the placenta, adipose tissues and bone marrow. Besides their nature, MSCs have wide

clinical usage that includes inflammatory diseases, myocardial infarction, degenerative disorders, and pneumonia [4]. Nevertheless, MSCs have good antiviral properties and are involved in the treatment of viral infection in the last few years. Immediately after viral invasion, damaged associated molecular patterns (DAMPs) and/or pathogen-associated molecular patterns (PAMPs) induce pro-inflammatory (MSC1) phenotypes in MSCs [5] and regulate circulatory immune cells involved in antiviral response. The International Society of Cell Therapy (ISCT) has established universal criteria for MSC definition. Therefore, MSCs must display plastic-adherence capacity; fibroblastic spindle shape morphology in standard culture media; surface expression of CD90, CD73, and CD105 and absence of CD11b, CD34, CD45, and HLA-DR; and *in vitro* differentiation potential of osteogenesis and adipogenesis [6], thus providing authenticity.

MSCs are known to interact with various types of immune cells such as dendritic cells (DCs), macrophages, Natural Killer cells (NK) B-lymphocytes, CD4+, T helper cells, and cytotoxic T lymphocytes (CTLs) [5]. MSCs are known to inhibit NK cell and T-cell proliferation and reduce the differentiation of B cells to antibody-secreting plasma cells [7]. MSC-sourced interferons (IFNs) modulate the cytotoxic properties of NK cells, and CTLs enhance antigen-presenting properties of DCs. Macrophages and B cells contribute to the effective removal of virus-infected cells [8]. However, the activities of MSCs change due to influence of the local microenvironment, which leads to complexity in understanding the MSC-mediated immune response. Due to their potential immunomodulation and systemic inflammatory responses, MSCs are in a large number of clinical and experimental studies, exploring a wide area of new approach in the treatment of viral diseases [9]. In this book chapter, we summarized the current knowledge on MSC-dependent cellular mechanisms that are involved in the elimination of viruses, modulation of immune responses, and repair and regeneration of tissue damages caused by viral pathogens, and the efficacy of clinical practices about MSC-mediated therapy was put forth and evaluated.

2. Susceptibility of viral infection to MSCs

MSCs are prone to DNA (Deoxyribonucleic acid) or RNA (Ribonucleic acid) viruses both *in vitro* and *in vivo* [10–12]. Numerous biological factors facilitate the virus entry, and the most common one is the functional cell surface receptors. Receptors as active adhesive molecules provide structural bonding that are further inclined with other molecules that strengthen the attachment. For instance, MSCs express I-CAM1 for transmigration and immunomodulation. Several genera of viruses were known to infect MSCs are Herpes Simplex-1 (HSV-1), Varicella Zoster Virus (VZV), and Cytomegalovirus (CMV). HSV-1 infects the MSCs through heparin sulfate receptor [13]; however, Epstein Barr Virus (EBV) and Human Herpes Virus – 6, 7, and 8 (HHV-6, 7, & 8) do not infect MSCs but facilitate the passage of virus entry to the other cells [14]. Various factors play a crucial role in determining the viral tropism such as antiviral signaling of cytokines, intracellular host factors that favor DNA/RNA synthesis, and cell activation. Outcomes of viral infection to different MSCs were studied. Although MSCs are susceptible to viral infections, not all types are readily attacked. For instance, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have shown resistance to viral infection. HIV-1 was showed to infect the differential cells of MSCs but showed no infection to undifferentiated MSCs [15]. In some cases, however, RNA infected MSCs initiate immediate antiviral response

through pro-inflammatory cytokine production. This is because most of the RNA viruses are more likely to cause an acute infection than a DNA virus [16]. Most of the RNA viruses have compact genome inside a protein packing. These factors could hire prompt initiation and conformational changes necessary during host-cell entry [17]. Chikungunya virus (CHIKV) has shown to reduce MSC's osteogenic differentiation, thereby limiting their potential in regenerative medicine [18].

The parvovirus B19 is a DNA virus (single-stranded) that infects bone marrow BM-MSCs of human with upregulation of pro-inflammatory cytokine gene expression, such as IL-6 and TNF-α [19]. Cytokine-induced immunomodulatory function was lost when MSCs were infected with CMV and no longer inhibited microbial growth [20]. Also, the US11 protein used by CMV for immune invasion downregulated MHC class I expression of human MSCs, making them vulnerable to NK cell-mediated lysis [21]. A similar effect was obtained when horse MSCs was infected with equid herpesvirus-1 (EHV-1). The ability of viruses that invade and infect a cell varies between host types and is species-dependent. For instance, different MSCs of two closely related species human and murine produce distinct varied immunomodulatory mediators, and this indeed regulates the chance to limit or enhance the viral replication is an important concern. Indoleamine-2,3-dioxygenase (IDO) is known to be a primary mediator for viral replication in human MSCs, and it was found to have no effect in murine MSCs [22].

3. Antiviral response of MSCs

Despite the virus permissiveness, evidence has emerged that MSCs can mitigate viral infection *via* upregulation of their antiviral mechanisms. Through their intrinsic upregulation of IFN-stimulated genes (ISG), the gene that blocks viral replication, MSCs are more resistant to viral infection than their more differentiated forms [23]. Moreover, studies on gene silencing ISG like p21/CDKN1A and IFITM3 expression have resulted in increased chances of virus attack by MSCs [7]. Several studies have shown the expression of antiviral activation of MSCs in the presence of Coxsackievirus B3 [13]. Another method of triggering antiviral mechanism in MSCs is through miRNAs. The release of miRNAs by MSCs illustrated vigorous antiviral activity that could inhibit Hepatitis C virus infection [24]. Influenza virus-induced *in vivo* murine studies produced acute lung injury that were reduced and restored after MSC administration [25, 26]. In another murine model study of gammaherpesvirus-68 (MHV-68) infection, MSCs showed anti-herpesviral properties mediated through a cytosolic DNA pathway that was activated. However, the molecular switches of MSCs to inhibit viral propagation *in vivo* and their mechanisms require further investigation.

4. Effect of MSCs on cell-based immune response

Besides their intrinsic inhibition to viruses, MSCs could modify the antiviral response of immune cells as they normally do with antiviral defenses. However, these interactions influence both innate and adaptive immune components *via* NK cells and T-cells response. Insight studies into MSCs with NK-cells and T-cells show high complexity to understand. Certain cultured MSCs were marked and killed by NK cells upon their activation. IFN-γ primed MSCs mimic their exposure to the inflammatory environment but upregulate MHC class I (major histocompatibility complex)

expression and avoid NK-cell mediated destruction [27]. MSCs when targeted by NK cells could identify and alter NK cells' phenotype, which leads to inhibition of NK-cell proliferation, decreased cytokine production, and dampened cell differentiation *in vitro*. These effects were regulated by mediators IDO and prostaglandin E2 (PGE2) that downregulate NK-cell surface receptors [28]. With CD8 + T-cells, MSCs inhibit T-cell proliferation, releasing transforming growth factor beta (TGF-β) and hepatocyte growth factor (HGF). Expression of these growth factors reduces cyclin D2, causing proliferation arrest in G0G-1 phase of cell cycle [29]. However, in this, MSCs do not hinder CD8 + T cells, so their ability could be retained to lyse the targeted cells even in the presence of MSCs [30]. However, in addition, MSCs did not affect the CD8 + T-cell function in the context of viral infection caused by EBV (Ebstein-Barr virus) and CMV (Cytomegalo virus) [30]. A different study showed MSCs to inhibit proliferation of CD8 + T-cells upon performing a gentle pulse to T-cells with CMV phosphoprotein and influenza matrix protein antigen for a time of 2 h. IFN- γ derived from MSCs has a role of offsetting the immunosuppressive effect of MSCs and is involved to take part in partial cytotoxic responses during viral infection [31]. MSCs are likely dependent to alter immune cell response based on the specific host and its inflammatory parameters. Certain contradictory findings stated MSCs could not suppress T-cell responses. With varying environmental parameters, MSCs respond differently and are distinctly plastic that are not intrinsic, but activated by several combined cytokines, IFN- γ with TNFα, or IL-1β [32]. Therefore, MSCs associated immune cell response differs under various pathological conditions.

5. Engineered MSC-EVs as therapeutic vehicles

Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) represent potential cell-free alternative to stem cell therapy but are also rapidly emerging as a novel therapeutic platform particularly in the form of engineered EVs (EEVs) tailored to target a broad range of clinical indications. Several biodistribution studies using labeled dyes for EV uptake into animal models proved the accumulation of 70% dyes in the liver and spleen [33, 34]. However, the engineered EVs facilitate the distribution of therapeutic molecules into other organs and were considered by some biopharma companies "hard to treat disease," for example, cancer [35]. One method conferring this is to alter selected cell-surface-proteins. One route is to produce a fusion protein that inserts its tail into the EV membrane and the head binds to a projecting receptor (**Figure 1**) [37]. Modified EEVs could be accomplished by favoring selected cell surface protein alteration that confers target capabilities. One such copy was used to target neuron cells in the brain after systemic injection. The fusion protein was created between EV membrane protein Lam2b and rabies viral glycoprotein (RVG) peptide, which binds the acetylcholine receptor on the brain cells [36]. Tian et al. [38] have shown that cultured proteins from cell cultures can also be incorporated onto EVs for target delivery. A recent study has showed that fusion proteins that bind the phosphatidylserine onto EVs to ischemic brain tissues after systemic injection relieving inflammation. Similar approaches were also used to treat tumor cells in a mouse model of glioblastoma [39]. EEVs with protein receptors on their surface have been developed as decoys to capture target molecules such as the pro-inflammatory cytokine IL-6 as a potential therapeutic for chronic inflammatory diseases [40].

MSC-EVs are known to lodge therapeutic small molecules that promote regeneration in damaged tissues by lowering inflammation and inhibit apoptosis. MSCs have

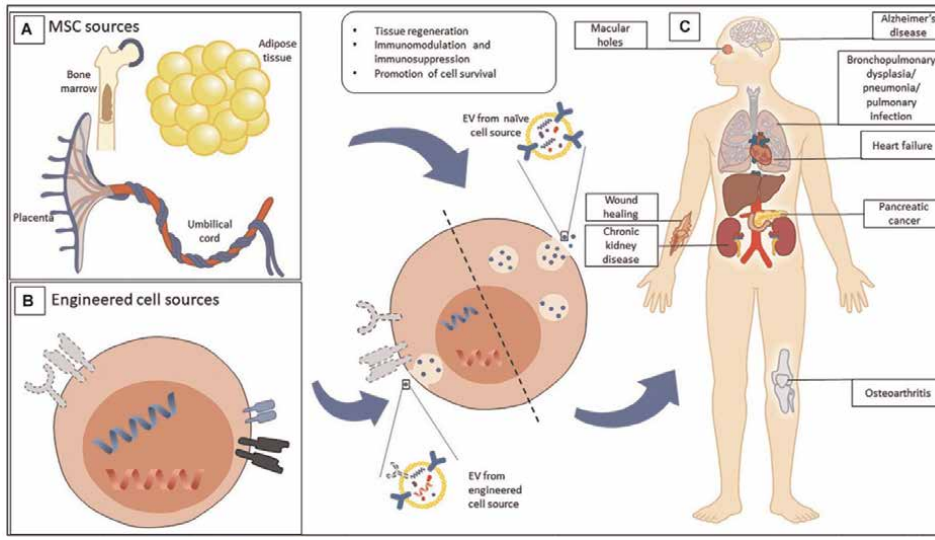

Figure 1.
Mesenchymal stromal cells (MSCs) EVs show therapeutic potential in a wide scale. (A) MSC-EVs had multiple isolation sources that include birth-associated tissues, adipose tissues, and bone marrow tissues. (B) MSCs are known to alter and import properties of demand for treatment. (C) EVs of both natural and engineered sources explored a wide range of clinical indicators. Adopted from: [36].

been recently engineered to enhance bone regeneration [41]. Another characteristic of EVs is to transfer non-immunogenic capsules that appeared to be ideal as drug vehicles, such as for tumor cells. This idea of EVs fastens new strategies to produce therapeutic cargo. These methods could be associated with the above incubation with drug delivery or transfecting of cells to allow specific small molecules into EVs. EV loading can also be facilitated after EV isolation from cell culture. Techniques including freeze-thawing, sonication, electroporation, osmotic shock, and saponin permeabilization have been adopted to temporarily disrupt the EV membrane sufficient for the uptake of therapeutic cargo.

6. Antiviral properties of MSCs

MSCs typically are virus-resistant cells compared to their more differentiated cell types. Such an ability was obtained by MSCs *via* IFN-stimulated genes (ISG), thereby resisting viruses to pass over cell membrane and blocking mRNA transcription, nuclear imports of mRNA, translation, and viral assembly and release [7, 42, 43]. PMAIP1, ISG15, IFI6, IFITM, SAT1, p21/CDKN1A, SERPINE1, and CCL2 are ISGs known to express during various viral infections such as dengue, Ebola, SARS, and influenza, that limits viral proliferation inside the cells [43]. The effects of ISGs were studied by silencing them. For instance, silencing p21/CDKNIA expression results in MSC susceptible to chikungunya virus infection, whereas silencing IFITM3 results, MSCs prone to yellow fever and Zika virus infection. A list of ISGs constitutively expressed by human MSCs was arrayed: IFITM1, IFI6, CCL2, ISG15, SAT1, PMAIP1, and nonconstitutive ISGs includes such as MT1G, CD74, SERPING1, IFNAR2, and MT1X. Besides those of original ISGs by MSCs, the upregulation of nonconstitutive ISGs represents adjustment ability in enhancing antiviral capacity. This feature was

notably beneficial in the context of respiratory tract infections [5]. Similarly, in another case, the IDO-expressing MSCs primed with IFN-γ *in vitro* reduced HIV-1/2 virion yield. The authors hypothesized that this effect might be related to tryptophan depletion, which limits emergent viral protein biosynthesis [25].

Indoleamine 2,3 dioxygenase (IDO) nutrient deprivation is a useful antiviral MSC strategy, and this effect has been observed against measles virus, cytomegalovirus, herpes simplex virus-1, and HBV [25, 44]. IDO seems to be a fundamental antiviral molecule of the MSCs. Another antiviral mechanism by MSCs is the production of noncoding miRNAs with antiviral activity by targeting viral replication. For instance, the antiviral activity of MSCs against hepatitis C virus (HCV) is conferred by Let-7f, miR-145, miR-199a, and miR-221 in derived extracellular vesicles (EVs) [44].

7. MSC-based therapies for COVID-19

The outbreak of the pandemic novel coronavirus (COVID-19) during 2019 drastically increased the number of patients and mortality rate worldwide [45]. Although the vaccine has been developed, rapid mutations made the virus more complex, and therefore, effective treatment measures were challenging. To date, the benefits of MSC-based viral therapy working on the basis of its immunomodulation, antiviral, anti-apoptotic, anti-infective, and angiogenic properties are promising [46]. While using a cellular product, it cannot suppress patients when dealing with the infection or must not make it susceptible to other infections [46]. MSCs could overcome this scenario because of their ability to fight against virus infection with immunomodulatory and regenerative abilities [13, 47]. As a concern of available resources, the preclinical models of acute lung injury, acute respiratory distress syndrome (ARDS), viral hepatitis, human immunodeficiency virus (HIV) infection, and viral pneumonia have been evaluated in the last years [48–50]. To date, there are no standard protocols designed that improve the therapeutic potential of MSCs to target the viral attack [51, 52]. Therefore, much safety has ensured on MSC-based therapy on all clinical trials with small patient groups. Some efforts were made only to boost the antimicrobial activity of MSCs. Hypoxia priming of MSCs increases microvesicles to release growth factors, upregulate chemokine-receptors, and decrease cellular senescence and thus import therapeutic efficiency [52].

COVID-19 represents public health emergencies that urge the need of alternative therapy [29, 53–55]. Almost every COVID-19 victim experienced severe lung complications such as ARDS were observed only in some limited cases. SARS-CoV-2 is thought to be a major disease complication with COVID-19 with rise in mortality [56]. At this point, MSC-based therapy line would be plausible because of the easy biological properties of the MSCs that can be easily expanded when intravenously infused. Owing to their remarkable immunomodulatory and regenerative abilities, MSCs could attenuate the cytokine and prevent progression to ARDS and protect victims from multiple organ failure in severe COVID-19 condition [56]. As an advantage, the intravenously infused MSCs are trapped into the lungs, and this in fact marks beneficial as the lungs were the primary organ targets of SARS-CoV-2 [56, 57].

Attention has to be ensured that MSCs are not transfused during the initial period of viral infection. When wrongly used, the immunosuppression of MSCs hinders physiological and replication inflammation that are much essential to control viral infection [58]. Several other challenges have to be taken into context when MSCs are allowed in therapy line. The time and dosage of MSCs during administration must be

calculated; if exceeded the exacerbated immunosuppression may have negative effect on the victim [56]. When administrated to large cohort groups, it must be ensured that the design plan is accurate and significant. Besides all of them, a good quality standard in the trials under appropriate regulatory supervision must be followed and has to be reported periodically in a complete and transparent manner. Ethical guidelines provided by the World Health Organization (WHO) for using cell-based therapy in clinical trials must be appropriate while using MSCs for COVID-19. Both ethical and moral aspects should be followed when performing the clinical trials during non-pandemic situation.

The critical pathological features of COVID-19 hospitalization are acute lung injuries (ALI) and ARDS characterized by immunopathological complications. Any treatment that hastens COVID-19 recovery would be in a substantial demand, and so, MSC therapeutics would be an ideal approach to handle the situation of COVID-19 symptoms due to their potential antiviral properties [59]. MSCs release various cellular components such as keratinocyte growth factor, prostaglandin E2, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, and IL-13 to facilitate phagocytosis and to further activate alveolar macrophages, alter the cytokine secretion profile of dendritic cell subsets, and decrease the limit of interferon γ from NK-cells. Tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase is found to suppress T-cell proliferation, and this could even change the cytokine profile of T-cells. Furthermore, the proliferation and differentiation of B cells were notably impaired by MSCs as well. However, these effects were restored by the MSCs, and all these mentioned functions might also be worked out by MSCs in COVID-19 infections also. One among the potential outbursts of COVID-19 is the elevated levels of inflammatory cytokines due to overstimulation of immune cells resulting in a cytokine storm that eventually damaged the tissues and organs, especially the lungs. On the other hand, systematic treatment measures using MSCs have been increasing and a complete idea on their effect is still lacking. In this book chapter, the meta-analysis of COVID-19 retrieved from various sources is discussed.

8. Meta-analysis and systematic review

This review protocol was planned through a module in which the eligible criteria were defined according to the PICOS (population intervention, comparison, outcomes, and study) format. The target study population was all COVID-19 laboratories that confirmed patients received MSC treatment with an age group of 18 and above regardless of the gender. The disease severity from moderate to severe cases was involved. The experimental group was administrated with non-modified form of MSCs from BM and PTs (e.g., placenta, umbilical cord). Studied that used MSCs derived from embryonic stem cells (ESCs) and pluripotent stem cells (IPSC) were excluded. Genetically modified MSCs were also excluded. The reports were compared with the non-MSCs receiving group. The outcomes were represented such as groups with mortality and adverse effects (AEs), reduced inflammatory reactions based on the schematic markers, and third category of improvement in pulmonary function and oxygenation. Including the experimental outcomes, the article publications were retrieved from 6 databases (**Figure 2**) for analysis in this review that includes terms and limitations (**Table 1**).

Randomized controlled trial (RCT) was conducted for quality assessment with the incidence of mortality, AEs, and serious adverse effects (SAEs). The levels of CRP and

IL-6 were assessed with the intention to treat the disease in an effective way. Meta-analysis on pulmonary function was not performed due to insufficient data. Of all the 100 articles retrieved, many articles failed in full-text retrieval, so 8 articles were selected finally for data extraction. **Table 2** shows the characteristics of review, which includes the details of treatment used. The umbilical cord-derived MSCs were intravenously infused into the COVID-19 patients. The outcomes of the study that included the mortality occurrence and AEs, elevation in the inflammatory responses, and improvement in patient pulmonary were recorded and tabulated (**Table 3**). It is noteworthy that patients in the MSCs-treated group were reported with less mortality when compared with those in the control group [60, 61]. Various levels of inflammatory responses were studied by researchers. The inflammatory markers CRP and procalcitonin (PCT) and certain pro-inflammatory cytokines like IL-6, IL-2, tumor necrosis factor alpha (TNF-α), and anti-inflammatory cytokine (IL-10) were compared in the meta-analysis. Those with MSCs treated cases notably decreased in the cytokines were observed [62]. Studies led by Adas and co-workers [63] reported increased levels of anti-inflammatory cytokine IL-10. Similarly, another study showed higher expression of IL-10 in MSC-treated patients [61]. However, CT score of MSC-applicable groups showed improvement in the lung clearance, the number of lobes involved, and ground-glass opacity (GGO) [64–66].

In this book review, it was observed that the efficacy of MSCs was determined based on the inflammatory markers and pulmonary function in the COVID-19 patients. Due to insufficient data on pulmonary function, the meta-analysis was not performed. Thus, for convenience, details of CPR and IL-6 were included in this meta-analysis to standardize the study results [61].

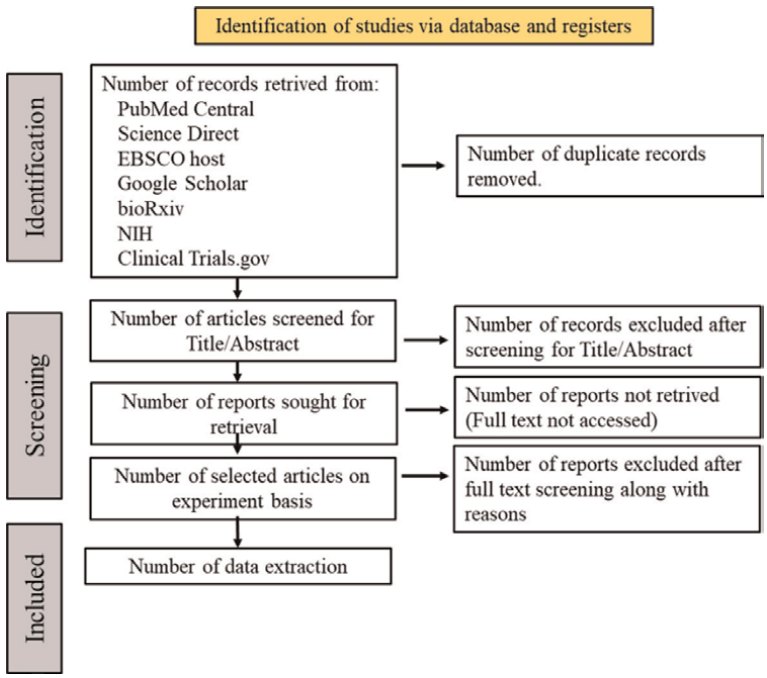

Figure 2.
PRISMA 2020 flow diagram shows data collection sources.

Databases	Search terms and limitations
PubMed Last searched: Nov 7, 2022	Search terms: (Mesenchymal stem cells OR Mesenchymal stromal cells OR MSC) AND (COVID-19 OR SARS-CoV-2) Filter: The search results were filtered for clinical trials and randomized controlled trials only
Science direct Last searched: Nov 7, 2022	Search terms: Mesenchymal stem cells OR Mesenchymal stromal cells or MSCs and COVID-19 or SARS-CoV-2 and cytokine storm OR cytokine release syndrome Filters: The year of publication was set from 2020 to 2022 to remove irrelevant articles The results were filtered to generate research articles and case reports only
EBSCO host Last searched: Nov 7, 2022	Search terms: Mesenchymal stem cells OR Mesenchymal stromal cells OR MSC) AND (COVID-19 OR SARS-CoV-2) AND (cytokine storm OR cytokine release syndrome) Filters: Searches by Boolean/Phrase was applied Results were filtered for full text English articles The terms were searched within full text of the articles Search for equivalent subjects was applied The year of publication was set from 2020 to 2022 to remove irrelevant articles The source type was filtered to academic journals only
Google scholar Last searched: Nov 15, 2022	Search items: Mesenchymal stem cells OR Mesenchymal stromal cells or MSCs and COVID-19 or SARS-CoV-2 and cytokine storm OR cytokine release syndrome Filters: The year of publication was set from 2020 to 2022 to remove irrelevant articles The results were filtered to generate research articles and case reports only
The Cochrane Library Last searched: Nov 7, 2022	Search terms: (Mesenchymal stem cells OR Mesenchymal stromal cells OR MSC) AND (COVID-19 OR SARS-CoV-2) *No filter applied
BioRxiv Last searched: Nov 15, 2022	Search items: Mesenchymal stem cells OR Mesenchymal stromal cells or MSCs and COVID-19 or SARS-CoV-2 and cytokine storm OR cytokine release syndrome Filters: The year of publication was set from 2020 to 2022 to remove irrelevant articles
ClinicalTrials.gov Last searched: Nov 7, 2022	Search items: Mesenchymal stem cells OR Mesenchymal stromal cells or MSCs and COVID-19 or SARS-CoV-2 and cytokine storm OR cytokine release syndrome Filters: The year of publication was set from 2020 to 2022 to remove irrelevant articles

** SARS-CoV, severe acute respiratory syndrome coronavirus 2.*

Table 1.
Search terms and limitations used in article searches.

Sources	Study types	Participants (n)		Mesenchymal stromal/stem cells			
	Experimental group		Control group	Sources	Passage	Dose	Delivery method
Adas et al.	Randomized, standard treatment-controlled trial, three parallel armed (two control arms)	Group 3 (critical illness): 10	Group 1 (moderate illness): 10 Group 2 (critical illness): 10	Wharton's Jelly	4	3×10^6 cells/kg in 150 ml of 0.9% NaCl (three infusions)	Intravenous infusion
Dilogo et al.	Multicentered, double-blind, randomized, placebo-controlled trial	20	20	Umbilical cord	5 or 6	1×10^6 cells/kg in 100 ml of 0.9% NaCl (single infusion)	Intravenous infusion
Lanzoni et al.	Phase I/IIa, double-blind, randomized, placebo-controlled trial	12	12	Umbilical cord	N/A	$100 \pm 20 \times 10^6$ cells/ infusion in 50 ml vehicle solution containing HSA and heparin (two infusions)	Intravenous infusion
Liang et al.	Case report	1	0	Umbilical cord	5	5×10^7 cells/infusion in 0.9% NaCl with 5% human albumin (three infusions)	Intravenous infusion
Monsel et al.	Multicentered, double-blind, randomized, placebo- controlled trial	21	24	Umbilical cord	4	1×10^6 cells/kg in 150 ml of 0.9% NaCl with 0.5% albumin (three infusions)	Intravenous infusion
Rebelatto et al.	Phase I/II, prospective, single-centered, randomized, double-blind, placebo-controlled clinical trial	11	6	Umbilical cord	3–5	5×10^5 cells/kg in 30 mL of vehicle solution containing saline solution, 5% anticoagulant citrate dextrose (ACD), and 20% albumin (three infusions)	Intravenous infusion
Shu et al.	Single-centered open-label, individually randomized, standard treatment- controlled trial	12	29	Umbilical cord	3–5	2×10^6 cells/kg in 100 ml of normal saline (single infusion)	Intravenous infusion
Zhu et al.	Case report	1	0	Umbilical cord	N/A	1×10^6 cells/kg in 100 ml of 0.9% NaCl (single infusion)	Intravenous infusion

MSC, mesenchymal stromal/stem cell; HAS, human serum albumin; N/A, not available.

Table 2.
Study characteristics comprising the design and details of MSC treatment.

Sources	MSC-treated					Control				
	Number of death events	Number of patients of with AEs patients with SAEs		Total Mortality rate (%)		Number of death events	Number of patients with AEs	Number of patients with SAEs	Total Mortality rate (%)	
Adas et al. Turkey	3	N/A	N/A	10	30	6	N/A	N/A	10	60
Dilogo et al. Indonesia	10	N/A	N/A	20	50	16	N/A	N/A	20	80
Lanzoni et al. United States	2	8	2	12	16.67	7	11	8	12	58.33
Liang et al. China *Case report	0	0	0	1	0	N/A	N/A	N/A	N/A	N/A
Monsel et al. France	5	18	6	21	23.81	4	18	6	24	16.67
Rebelatto et al. Brazil	5	N/A	N/A	11	45.45	1	N/A	N/A	6	16.67
Shu et al. China	0	N/A	N/A	12	0	3	N/A	N/A	29	10.34
Zhu et al. China *Case report	0	0	0	1	0	N/A	N/A	N/A	N/A	N/A

AE, adverse event; N/A, not available; MSC, Mesenchymal stromal/stem cell; SAE, serious adverse event.

Table 3.
Incidence of mortality and adverse events in MSC-treated and control groups.

9. Main pathways of MSCs immunomodulation

The immunomodulatory function of MSCs is versatile and is widely described here. The establishment of response is defined as the important regulatory work of MSCs and immune responses. The iNOS-NO axis induced cytokines that are involved to mediate the immunoregulation of rodents such as mouse, rat, and hamster, whereas IDO is preferably useful for mammalian species [67]. Upon activation by pro-inflammatory cytokines, the murine MSCs produce a high level of iNOS and NO. Inhibition of iNOS abolishes the mouse MSC-mediated antiproliferative effect on T cells [68]. SH2 domain-containing phosphatase-1 (SHP1) negatively modulates the iNOS expression in MSCs. High level of JAK1 and STAT3 phosphorylation shall occur to produce more iNOS and cyclooxygenase 2 (COX2) when SHI1 is deficient in MSCs that result in more immunosuppressive liver injury. NO may coordinate with phosphorylated STAT3 to increase PD-L1 expression in IL-17-stimulated MSCs. Thus, MSCs with pre-treated IL-17 acquire high potent immunosuppressive capacity with modulated mRNA stability through degrading ARE/poly(U)-binding/degradation factor 1 (AUF1). NO shall therefore be lost through oxidation. In order to be effective, T cells have to be attached in close proximity to MSCs by chemokines by adhesion

molecules such as ICAM-1 and VCAM-1. During tuberculosis progression, the pathogen recruits MSCs to the site and induces NO production, blunting T-cell responses and helping the bacterium to invade host immune responses [69]. Similar attempt was also noted with Coxsackievirus B3 (CVB3)-induced myocarditis, indicating MSCs to stimulate antiviral immunity to blunt T-cell activation in NO-dependent. Under inadequate stimulus or insufficient inflammation-exposure time, NO-mediated immunosuppression by MSCs is likely useful to enhance the effect. Ablation of iNOS expression in MSCs could still enhance immune response because chemokines attract immune cells and enhance immune response both *in vitro* and *in vivo* to suppress tumor cells as well [70]. The therapeutic effect of liver fibrosis was also mediated by MSCs by the expression of iNOS under inflammatory condition that produces cytokines but not NO without any pathological changes in the liver fibrotic mice [71]. In another experimental model of sclerosis, the iNOS−/− MSCs lost the capacity of exerting the anti-fibrotic effect. The tryptophan-IDO-kynurenine-aryl hydrocarbon axis IDO is a rate-limiting enzyme for degrading tryptophan (Trp) to N-formylkynurenine. The culture medium also resists the growth of T lymphocytes, a paracrine effect that depends on the expression of IDO. The IDO-mediated conversion of Trp into KYN induces apoptosis and cell cycle arrest in T cells. The catabolites of tryptophan such as KYN and picolinic acid also inhibit activated T cells and NK cells in the absence of Trp, but the addition of Trp restores allogenic T-cell proliferation. Human MSCs require IDO to promote monocytes into immunosuppressive macrophages. However, KYNA limited IL-10 production *via* the increase of intracellular cAMP in BM-derived macrophages and predicted poor prognosis in atherosclerosis [71]. When stimulated with IFNγ together with TNFα or IL-1, the MSCs express IDO and show immunosuppression in a STAT1-dependent manner. Overexpression of STAT1 results in T-cell suppression *in vitro*. This stimulation leads to a metabolic glycolysis shift. Once the MSCs are blocked by 2-Deoxy-d-glucose (2-DG) treatment, STAT1 binds to IFNγ which gets activated in the IDO1 promoter that eventually resulted in IDO upregulation and T-cell response inhibition [72]. An attempt to silence the IDO in human MSCs results in the acceleration of immune responses as MSCs facilitate PBMC stimulation in both low and high cell density levels. IDO and its metabolic components are highly essential as mediators for MSCs to enrich immune cells in varying environments as MSCs are also involved in the aging process. To note, hyperactivity of IDO-mediated tryptophan degradation would result in reduced other metabolic reaction pathways and so generate melatonin that serve as an antioxidant to reverse the aging phenomenon of the MSC itself [73]. KYN was found to accumulate over age in the plasma and in the bone tissues that results in vulnerable bone in mice and osteoporosis. It was reported that KYN inhibit autophagy and induce senescence in MSC *via* AhR signaling.

MSCs express Toll-like receptors (TLRs) to recognize pathogens including viruses [74, 75]. MSCs derived from adipose tissues express TLR2, TLR3, TLR4, and TLR9 through transcriptional and translational levels and provide TLR machinery to activate inflammatory NF- κB pathway and interferon factors, which are important for viral infection. MSC secretome helps the organism to repair damaged areas through secretions of proangiogenic, antiapoptotic, and antifibrotic factors. Due to severe inflammation and tissue destruction, the nutritional and oxygen depletion level is impaired that results in organic ischemia. Therefore, neo-vessels have to raise blood perfusion with maximized number of viable cells to finally restore tissue function and prevent tissue fibrosis [76]. Antiapoptotic factors secreted by MSC are ANG, ANGPT1, bFGF, CXCL12, EGF, ESM1, GF-1, IL-6, JAG1, LIF, MCP-1, MMP-1, PDGF, PIGF, PTN, STC1, TGF-β, and VEGF [77, 78].

10. MSCs attenuate COVID-19

One of the major problems that resulted during COVID-19 is multiple organ failure and death due to lung endothelial damage and activation of blood coagulation, which is also accompanied by cytokine storm. MSCs offer a promising innovative strategy for attenuating the cytokine storm and ultimately improving patient outcomes (**Figure 3**). Several methods of infusions (intravenous, intra-arterial, & direct) are in practice in which in the intravenous method, the MSCs get trapped into the inflamed lungs and exert immunomodulatory response directly by reacting with the epithelial and immune cells of the lungs. This response releases various mediators that ultimately reduce inflammation and protect epithelial cells in alveoli [79–81]. Administration through intratracheal in COVID-19 cases is also found to be conceivable and might work better. Recent studies have shown robust evidence with MSCs to treat lung injury and ARDS. Exposure of lung cells to MSCs results in reduced pro-inflammatory cytokines including IL-1α, IL-1β, IL-6, IFN-γ, and TNF-α and an increase in anti-inflammatory cytokines such as IL-4, IL-5, and IL-10, thus restoring

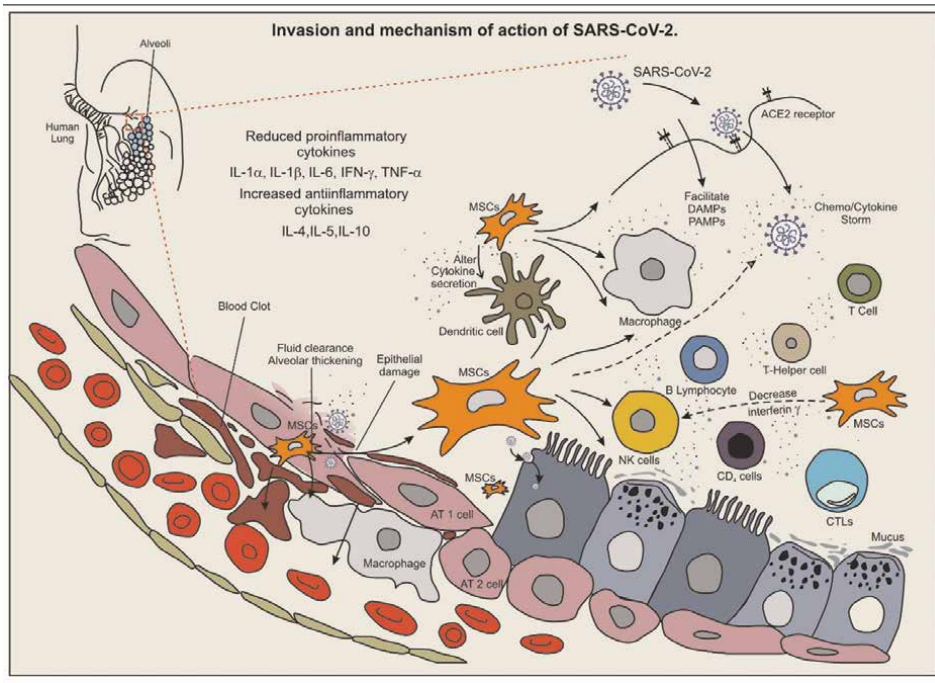

Figure 3.
Invasion and mechanism of action of SARS-CoV-2. The virus entry is facilitated through ACE2 cell surface receptor protein (angiotensin covering enzyme-II). Viral entry triggers the activation of immune response in dendritic cells, T-cells, B cells, macrophages, T-helper cells, CTLs, and NK cells. Chemokines/cytokine storm results in organ dysfunction and tissue damage in lung and alveolar cells. MSCs sources present in various body sites upon activation reduced damage by altering cytokine of dendritic cells and through decrease interferon-γ in NK-cells result relief outcome. Viral molecules facilitate cell rupture, fluid accumulation, alveolar thickening, and blood clot formation in AT1 and AT2 lung cells through damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), thus inducing MSC1 phenotype. Expressed interleukin IL-1α, IL-1β, and IL-6; interferon gamma (INF-γ) and TNF-α; and increase in anti-inflammatory cytokines such as IL-4, IL-5, and IL-10 help protect the lung cells; GM-CSF - granulocyte-macrophage Colony-stimulating factor, SARS-CoV-2 - severe acute respiratory syndrome Coronavirus-2, TNF - tumor necrosis factor, MSCs - mesenchymal stromal cells.

the fluid clearance of the lungs, thickening alveoli, increasing air space volume, and reducing inflammation markers [82]. MSCs can inhibit platelet activation through CD73 ectonucleotidase activity, which is one of key MSC membrane markers. Hence, MSCs might play a crucial role in dampening both inflammation and hypercoagulopathy status during SARS-CoV-2-related severe pneumonia [83]. MSCs isolated from different sources largely differ in their incompatibility of the expression of tissue factor (TF). For instance, bone marrow MSCs (BM-MSCs), demonstrate a high level TF expression with reduced hemocompatibility [81].

11. Anti-inflammation and immunomodulation COVID-19

Inflammatory modulation of MSCs is a key to the successful control of COVID-19. Corticosteroid therapy as it exerts potential anti-inflammatory effect was relatively restricted as it delays in virus clearance [84]. Thus, the urge of therapeutic interventions with anti-inflammatory effect was needed. MSCs are capable of reducing this risk, thereby protecting epithelial lung cells from undergoing death of COVID-19 [85]. Preclinical studies have showed MSCs could save acute alveolar injury in mouse model of ALI/ARDS [80, 86]. Once lodged into the lungs, MSCs secreted secretome (EVs) to exert anti-inflammatory effects. When incorporated into COIVD-19 patients, MSCs increased the peripheral lymphocytes with CRP levels decreased. Surprisingly, COVID-19 patients after MSC-based therapy displayed reduction in TNF-a level, a pro-inflammatory cytokine, and an increased anti-inflammatory mediator IL-10. The anti-inflammatory effect exerted by MSCs is a part of paracrine pathway. MSCs release anti-inflammatory cytokines and factors such as growth factor-b (TGFβ), vascular endothelial growth factor (HGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and brain-derived neurotrophic factor (BDNF). MSC-derived secretomes (EVs) contain specific peptides that are migrated to the injured site and have an effect on the treatment of subsequent pulmonary fibrosis. After invasion of SAR-CoV-2 in the host environment stimulated innate and subsequently adaptive immune response. Antigen presenting cells (APCs) drove the virus-derived compounds to T cells and triggered immune response. Though the antiviral response is much essential for the virus clearance, damages to the alveolar epithelium and lung endothelium have to be repaired [87]. During viral destruction, the immune system is highly activated with high levels of inflammatory cytokines worsening the lung injury and organ damage. MSCs with immunomodulatory property inhibit the innate immune response as well as adaptive responses [88]. MSCs therefore regulate the activity of T and B cells, macrophages, monocytes, dendritic cells, and NK cells through cell–cell communication and through secretome [80]. MSCs have been found to exert immunosuppressive functions *via* recruiting and promoting the generation of regulatory T cells (Tregs) from CD4 and CD8 T cells [89]. In the similar way, MSCs are capable of inducing cell cycle arrest in B cells with reduced antibody production. Furthermore, MSCs inhibit B cells by the induction of regulatory B cells. MSCs have an effect on innate immunity they suppress a group of major cells that are involved in innate immunity. They has also suppressed DCs maturation and therefore inhibit NK cells' cytotoxic activity and convert macrophages into regulatory macrophages with anti-inflammatory properties [90]. Aside from all these, MSCs escape from immune surveillance. This peculiar modulation renders MSCs a promising agent for the management of COVID-19.

12. MSC-based therapy of viral hepatitis

Experimental studies have documented the beneficial effect of attenuation of acute hepatitis and liver failure. MSCs were observed to suppress the activation of hepatotoxic IFN-γ and IL-17 in an NO- and IDO-dependent manner. This expression produces numerous cytokines and induces proliferation of FoxP3 and NKT cells. Additionally, MSCs promote hepatocyte proliferation and liver regeneration. The BM-derived MSCs helped survive 56 acute-on-chronic liver failure (ACLF) patients from hepatitis B virus. For this, MSCs were injected intravenously once in a week for 4 weeks. No infusion-related adverse effect was noted, which indicated the safety of MSCs in disease treatment for ACLF. As laboratory measurements, the total serum bilirubin for patients with End-Stage Liver Disease (MELD) score treated with MSCs was compared with a control group where normal treatment was acquired [91]. The infection was higher in the group of patients who received standard therapy than in the MSC-treated group. MSCs manage to suppress the activation of hepatotoxic immune cells without changing systemic immunosuppression and immunodeficiency [92]. Similar to this observation, a single stage umbilical cord-derived MSC was noted to improve the liver function of patients treated with HBV-ACLF. The improvement was ensured by hepatocyte-functioning markers including, albumin, alanine aminotransferase, aspartate aminotransferase, total bilirubin, prothrombin time (PT), and international normalized ratio (INR). A significant reduction in MELD score was noticed in patients after 4 weeks without causing side effects considering MSCs as an adjuvant therapy to treat HBV-ACLF. UC-MSC exosomes (UC-MSC-Exos) significantly improved the therapeutic efficacy of IFN-α, which are used as a standard therapy for patients with hepatitis C virus (HCV) infection. UC exosomes contain numerous immunosuppressive and miRNAs that bind to HCV RNA and prevent its replication.

13. Neutrophil extracellular traps (NETs) in COVID-19

During the severity of COVID-19 viral infection, networks of DNA-bound histone molecules wrapped around and detains the viral infection were observed. This sophisticated web-like structure called NETs through NETosis liberated by neutrophils upon activation was studied [93]. It was believed that NET production was triggered through fungal and bacterial infection. However, during COVID attack, it was reported that NETosis expression could defend viral diseases [94]. NETosis is known to be a cell-controlled process, but its mechanism still remains a mystery with some proven evidences stating that its expression begins in an ROS-independent manner [95]. Upon activation of neutrophils through viral infection, the nuclear envelop disintegrates, enabling the DNA to mix with granular proteins that are lines with effector proteins and peptidyl arginine deiminase type IV (PAD_4) activation [95]. Proteins including neutrophil elastase (NE) and myeloper oxidase (MPO) stimulate chromatin condensation and deteriorate histones [96]. In the presence of histone hypercitrullination, PAD_4 regulates decondensation and DNA-protein complexes are released extracellularly as NETs [97]. During chronic obstructive pulmonary disease and in ARDS patients, the levels of NETs were increased [98].

Nicolai et al. [99] and Skendros et al. [100] found the NET-related fibrin and platelet aggregation in COVID-19 patients and in SARS-CoV-2 infection alter the

disease severity. Similar studies conducted by Middleton et al. [101] found elevated NET formation with COVID-19-related ARDS. The release of NETs during viral infection seems to change the NE production that has changes in the macrophage role by cleavage of TLRs. In addition, cytokines TNF-α and IL-8 can lead to the increase in neutrophil release of NETs. In a study led by Veras *et al*., the potential detrimental function of NETs in 32 severe COVID-19 cases was found to have higher expression levels of NETs in tracheal aspiration and plasma with significant concentration of neutrophils naturally increasing the concentration of NETs [102].

Increased levels of cell-free DNA, myeloperoxidase-DNA (MPO-DNA) and citrullinated histone H3 (Cit-H3) were reported in the sera of COVID-19 patients [103]. Although the literature does not directly support the action mechanism of NETs with viral diseases, NETs found in sites of viral infection entrapping virus particles in a DNA web such that of SARS-CoV-2, influenza, COVID-19, and syncytial respiratory viral infection were reported [49, 50]. Therefore, treatments using the NETs to the new COVID-19 could reduce the disease severity caused by hyperinflammation [104], avoiding invasive mechanical ventilation and hence reduceing the mortality rate. Drugs such as gasdermin D [105], PAD4 [106], and NE [107] are known inhibitors that block molecules necessary for NET synthesis. Treatments on NETs in COVID-19 patients using various NET inhibitors are still in the development stage. Therefore, in the sense of COVID-19, treatments using NET-targets reduce the damage caused to lung cells and hyperinflammation, and research has to be considered further in various aspects to underline the neutrophil response mechanism and NETosis.

14. MSCs in the treatment of SARS-CoV-2

SARS-CoV-2-induced infection affects pneumocytes and ciliated cells of the lungs and results in alveolar injury and lung inflammation. In many cases of COVID-19 patients, the effective elimination of virus happens by activated alveolar macrophages, DCs, and T cells. Due to the overaccumulation of inflammatory cytokines and highly activated immune cells, cytokine storm occurs in some patients, making it complicated, which leads to life-threatening pneumonia, lung edema, and acute respiratory distress syndrome (ARDS) [85]. MSC-sourced hepatocyte growth factor (HGF), IL-10, and TGF-β act synergistically to induce alternatively activated anti-inflammatory (M2) phenotypes in alveolar macrophages. MSCs directly suppress the expansion of inflammatory IFN-γ producing Th1 and IL-17 in the injured lungs [47, 108]. MSCs, on the other hand, induce program death ligand (PDL) to induce apoptosis in over-activated T-cells. In addition, MSC-sourced TGF- β and HGF cause G1 cell cycle arrest, thereby suppressing the activation of JAk–Stat signaling pathway. Since MSCs effectively suppress immune response and provide additional oxygen supply to the injured lungs, investigations on the therapeutic potential of MSCs have proven to be significant. Within 48–96 h after MSC infusion in patients with SARS-CoV-2, the oxygen saturation level significantly increased and pneumonia-related symptoms of shortness of breath, cough, and fever started to disappear. It was confirmed by a computer tomography (CT) [109]. More importantly, MSCs prevented the influx of inflammatory immune cells in the patient's lungs, favored the expansion of anti-inflammatory cells, restored the function of liver and kidney, and prevented multiple organ dysfunctions.

15. Conclusion and future perspectives

Mesenchymal stem cells (MSCs) are considered to be a promising therapeutic (**Figure 1**) method of more severe viral infections like COVID-19. Due to their prominent mechanism in action (**Figure 2**) to various levels and demonstrated safety profile in the early phase studies, MSCs have been a major research focus in the recent years and in phase 2 clinical trials of COVID-19 pneumonia. In the context of well-studied differentiation potentials and immunomodulatory properties of MSCs are appealing to treat immunological disorders. Understanding more on the plasticity of MSC-mediated immunoregulation will help to guide the appropriate potential of MSCs. Another important thing to be considered is the pathophysiological role of MSCs in their original and inflammatory form. Although MSCs are involved to treat various immunological disorders, the role of tissue-resident in immunomodulation yet needs to be more investigated. Furthermore, new markers should be identified for specific inflammatory condition and MSC-based clinical protocols to be optimized so as to respond at different levels of disease progression.

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References

[1] Pandey A, Khan MK, Hamurcu M, Gezgin S. Natural plant products: A less focused aspect for the COVID-19 viral outbreak. Frontiers in Plant Science. 2020;**2020**(11):568890

[2] Harris G, Adalja A. ICU preparedness in pandemics: Lessons learned from the coronavirus disease outbreak. Current Opinion in Pulmonary Medicine. 2019; **27**:73-78

[3] Rocha JLM, de Oliveira WCF, Noronha NC, dos Santos NCD, Covas DT, Picanço-Castro V, et al. Mesenchymal stromal cells in viral infections: Implications for COVID-19. Stem Cell Reviews and Reports. 2021;**17**: 71-93

[4] Ahmadi AR, Chicco M, Huang J, Qi L, Burdick J, Williams GM, et al. Stem cells in burn wound healing: A systematic review of the literature. Burns. 2019;**45**: 1014-1023

[5] Gazdic M, Volarevic V, Arsenijevic N, Stojkovic M. Mesenchymal stem cells: A friend or foe in immune-mediated diseases. Stem Cell Reviews and Reports. 2015;**11**:280-287

[6] Martin I, Galipeau J, Kessler C, Le Blanc K, Dazzi F. Challenges for mesenchymal stromal cell therapies. Science Translational Medicine. 2019;**11** (eaat2189):1-3

[7] Khoury M, Cuenca J, Cruz FF, Figueroa FE, Rocco PRM, Weiss DJ. Current status of cell-based therapies for respiratory virus infections: Applicability to COVID-19. The European Respiratory Journal. 2020;**55**: 2000858. DOI: 10.1183/13993003.00858-2020

[8] Volarevic V, Gazdic M, Simovic Markovic B, Jovicic N, Djonov V, Arsenijevic N. Mesenchymal stem cell-derived factors: Immuno-modulatory effects and therapeutic potential. BioFactors. 2017;**43**:633-644

[9] Thanunchai M, Hongeng S, Thitithanyanont A. Mesenchymal stromal cells and viral infection. Stem Cells International. 2015;**2015**:860950

[10] Avanzi S, Leoni V, Rotola A, Alviano F, Solimando L, Lanzoni G, et al. Susceptibility of human placenta derived mesenchymal stromal/stem cells to human herpesviruses infection. PLoS One. 2013;**8**(8):e71412-e71412

[11] Behzadi Fard M, Kaviani S, Atashi A. Parvovirus B19 infection in human bone marrow mesenchymal stem cells affects gene expression of IL-6 and TNF-α and also affects hematopoietic stem cells differentiation. Indian Journal of Hematology and Blood Transfusion. 2019;**35**(4):765-772

[12] Arzi B, Kol A, Murphy B, Walker NJ, Wood JA, Clark K, et al. Feline foamy virus adversely affects feline mesenchymal stem cell culture and expansion: Implications for animal model development. Stem Cells and Development. 2015;**24**(7):814-823

[13] Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. Nature Reviews Nephrology. 2018;**14**: 493-507

[14] Naji A, Eitoku M, Favier B, Deschaseaux F, Rouas-Freiss N, Suganuma N. Biological functions of mesenchymal stem cells and clinical implications. Cellular and Molecular Life Sciences. 2019;**76**:3323-3348

[15] Leyendecker_Junior, A., Pinheiro, C., Amano, M, T., & Bueno, D. The use of human mesenchymal stem cells as therapeutic agents for the in vivo treatment of immune-related diseases: A systematic review. Frontiers in Immunology. 2018;**9**:2056. DOI: 10.3389/fimmu.2018.02056

[16] Vasandan AB, Jahnavi S, Shashank C, Prasad P, Kumar A, Jyothi Prasanna S. Human mesenchymal stem cells program macrophage plasticity by altering their metabolic status via a PGE_2-dependent mechanism. Scientific Reports. 2016;**6**:38308. DOI: 10.1038/srep38308

[17] Longhi S. Structural disorder in viral proteins. Protein and Peptide Letters. 2010;**17**(8):930-931

[18] Roy E, Shi W, Duan B, Reid SP. Chikungunya virus infection impairs osteogenic differentiation of bone marrow-derived mesenchymal stem cells. mSphere. 2020;**5**:e00347-e00320. DOI: 10.1128/mSphere.00347-20

[19] De La Garza-Rodea AS, Verweij MC, Boersma H, van der Velde-van DI, de Vries AAF, Hoeben RC, et al. Exploitation of herpesvirus immune evasion strategies to modify the immunogenicity of human mesenchymal stem cell transplants. PLoS One. 2011;**6**(1):e14493-e14493

[20] Meisel R, Brockers S, Heseler K, Degistirici O, Bulle H, Woite C, et al. Human but not murine multipotent mesenchymal stromal cells exhibit broad-spectrum antimicrobial effector function mediated by indoleamine 2,3-dioxygenase. Leukemia. 2011;**25**(4):648-654

[21] Meisel R, Heseler K, Nau J, Schmidt SK, Leineweber M, Pudelko S, et al. Cytomegalovirus infection impairs immunosuppressive and antimicrobial effector functions of human multipotent mesenchymal stromal cells. Mediators of Inflammation. 2014;**2014**:898630

[22] Wu X, Dao Thi VL, Huang Y, Billerbeck E, Saha D, Hoffmann H-H, et al. Intrinsic immunity shapes viral resistance of stem cells. Cell. 2018;**172**(3):423-438.e425

[23] Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood. 2004;**103**(12):4619-4621

[24] Li Y, Xu J, Shi W, Chen C, Shao Y, Zhu L, et al. Mesenchymal stromal cell treatment prevents H9N2 avian influenza virus-induced acute lung injury in mice. Stem Cell Research & Therapy. 2016;**7**(1):159

[25] Qian X, Xu C, Fang S, Zhao P, Wang Y, Liu H, et al. Exosomal MicroRNAs derived from umbilical mesenchymal stem cells inhibit hepatitis C virus infection. Stem Cells Translational Medicine. 2016;**5**(9):1190-1203

[26] Loy H, Kuok DIT, Hui KPY, Choi MHL, Yuen W, Nicholls JM, et al. Therapeutic implications of human umbilical cord mesenchymal stromal cells in attenuating influenza A(H5N1) virus-associated acute lung injury. The Journal of Infectious Diseases. 2019;**219**(2):186-196

[27] Spaggiari GM, Capobianco A, Becchetti S, Mingari MC, Moretta L. Mesenchymal stem cell-natural killer cell interactions: Evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation. Blood. 2006;**107**(4):1484-1490

[28] Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L. Mesenchymal stem cell inhibit natural killer–cell proliferation, cytotoxicity, and cytokine production: Role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood. 2008;**111**(3): 1327-1333

[29] Zhou M, Zhang X, Qu J. Coronavirus disease 2019 (COVID-19): A clinical update. Frontiers of Medicine. 2020;**14**: 126-135

[30] Karlsson H, Samarasinghe S, Ball LM, Sundberg B, Lankester AC, Dazzi F, et al. Mesenchymal stem cells exert differential effects on alloantigen and virus-specific T-cell responses. Blood. 2008;**112**(3):532-541

[31] Kang HS, Habib M, Chan J, Abavana C, Potian JA, Ponzio NM, et al. A paradoxical role for IFN-gamma in the immune properties of mesenchymal stem cells during viral challenge. Experimental Hematology. 2005;**33**(7): 796-803

[32] Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. 2008;**2**(2):141-150

[33] Wiklander OP, Nordin JZ, O'Loughlin A, Gustafsson Y, Corso G, Mäger I, et al. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. Journal of Extracellular Vesicles. 2015;**4**:26316

[34] Shelke GV, Lässer C, Gho YS, Lötvall J. Importance of exosomes depletion protocols to eliminate functional and RNA-containing extracellular vesicles from fetal bovine serum. Journal of Extracellular Vesicles. 2018;**3**:24783

[35] Lewis ND, Sia CL, Kirwin K, Haupt S, Mahimkar G, Zi T, et al. Exosome surface display of IL12 results in tumor-retained pharmacology with superior potency and limited systemic exposure compared with recombinant IL12. Molecular Cancer Therapeutics. 2021;**20**: 523-534

[36] Lydia AE, Yiqi S, Hai Fang Y, Corinne B, Samira L, Matthew JAW. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nature Biotechnology. 2011;**29**:341

[37] Johnson J, Shojaee M, Mitchell Crow J, Khanabdali R. Mesenchymal stromal cells to engineered extracellular vesicles. A new therapeutic paradigm. Frontiers in Cell and Developmental Biology. 2021; **9**:705676

[38] Tian T, Cao L, He C, Ye Q, Liang R, You W, et al. Targeted delivery of neural progenitor cell-derived extracellular vesicles for anti-inflammation after cerebral ischemia. Theranostics. 2021;**11**: 6507-6521

[39] Zhilan Y, Tao Z, Wenshan H, Honglin J, Cuiwei L, Zhe Y, et al. Methotrexate-loaded extracellular vesicles functionalized with therapeutic and targeted peptides for the treatment of glioblastoma multiforme. ACS Applied Materials & Interfaces. 2018;**10**: 12341

[40] Conceição M, Forcina L, Wiklander OPB, Gupta D, Nordin JZ, Vrellaku B, et al. Engineered extracellular vesicle decoy receptor-mediated modulation of the IL6 trans-signaling pathway in muscle. Biomaterials. 2021;**266**:120435

[41] Chun-Chieh H, Miya K, Yu L, Sajjad S, Iriarte-Diaz J, Lyndon FC, et al. Functionally engineered extracellular vesicles improve bone regeneration. Acta Biomaterialia. 2020;**109**:182

[42] Schoggins JW. Interferon-stimulated genes: What do they all do? Annual Review of Virology. 2019;**6**(1):567-584

[43] Kane M, Zang TM, Rihn SJ, Zhang F, Kueck T, Alim M, et al. Identification of interferon-stimulated genes with antiretroviral activity. Cell Host and Microbe. 2016;**20**(3):392-405

[44] Stebbing J, Phelan A, Griffin I, Tucker C, Oechsle O, Smith D, et al. COVID-19: Combining antiviral and anti-inflammatory treatments. The Lancet Infectious Diseases. 2020;**20**(4): 400-402

[45] Moll G, Drzeniek N, Kamhieh-Milz J, Geissler S, Volk HD, Reinke P. MSC therapies for COVID-19: Importance of patient coagulopathy, thromboprophylaxis, cell product quality and mode of delivery for treatment safety and efficacy. Frontiers in Immunology. 2020;**11**:1091

[46] Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: Pathological and therapeutic implications. Nature Immunology. 2014;**15**(11):1009-1016

[47] Harrell CR, Sadikot R, Pascual J, Fellabaum C, Jankovic MG, Jovicik Jovicic N, et al. Mesenchymal stem cell-based therapy of inflammatory lung diseases: Current understanding and future perspectives. Stem Cells International. 2019;**4236973**:1-15. DOI: 10.1155/2019/4236973

[48] Behnke J, Kremer S, Shahzad T, Chao C-M, Böttcher-Friebertshäuser E, Morty RE, et al. MSC based therapies—New perspectives for the injured lung. Journal of Clinical Medicine. 2020;**9**(3): 682

[49] Qin H, Zhao A. Mesenchymal stem cell therapy for acute respiratory distress syndrome: From basic to clinics. Protein & Cell. 2020;**11**(10):707-722. DOI: 10.1007/s13238-020-00738-2

[50] Kouroupis D, Sanjurjo-Rodriguez C, Jones E, Correa D. Mesenchymal stem cell functionalization for enhanced therapeutic applications. Tissue Engineering - Part B: Reviews. 2019;**25**: 55-77

[51] Noronha N, de Mizukami CA, Caliári-Oliveira C, Cominal JG, JLM R, Covas DT, et al. Correction to: Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. Stem Cell Research & Therapy. 2019;**10**(1):132

[52] Yen BL, Yen ML, Wang LT, Liu KJ, Sytwu HK. Current status of mesenchymal stem cell therapy for immune/inflammatory lung disorders: Gleaning insights for possible use in COVID-19. Stem Cells Translational Medicine. 2020;**9**:1162-1173. DOI: 10.1002/sctm.20-0186

[53] Zhao RC. Stem cell-based therapy for coronavirus disease 2019. Stem Cells and Development. 2020;**29**(11):679-681

[54] Yu F, Jia R, Tang Y, Liu J, Wei B. SARS-CoV-2 infection and stem cells: Interaction and intervention. Stem Cell Research. 2020;**46**:101859

[55] Du J, Li H, Lian J, Zhu X, Qiao L, Lin J. Stem cell therapy: A potential approach for treatment of influenza virus and coronavirus-induced acute lung injury. Stem Cell Research and Therapy. 2020;**11**:192

[56] Chen X, Shan Y, Wen Y, Sun J, Du H. Mesenchymal stem cell therapy in severe COVID-19: A retrospective study of short-term treatment efficacy and side effects. Journal of Infection. 2020;**116**: 1097-1100

[57] Golchin A, Seyedjafari E, Ardeshirylajimi A. Mesenchymal stem cell therapy for COVID-19: Present or future. Stem Cell Reviews and Reports. 2020;**16**:427-433

[58] World Health Organization. Managing Ethical Issues in Infectious Disease Outbreaks. Geneva, Switzerland: WHO; 2020. ISBN 978 9241549837, (NML classification: WA 105)

[59] Su J, Chen X, Huang Y, Li L, Li J, Cao K, et al. Phylogenetic distinction of iNOS and IDO function in mesenchymal stem cell-mediated immunosuppression in mammalian species. Cell Death and Differentiation. 2014;**21**:388-396. DOI: 10.1038/cdd.2013.149

[60] Monsel A, Hauw-Berlemont C, Mebarki M, Heming N, Mayaux J, Tchoumba ON, et al. Treatment of COVID-19-associated ARDS with mesenchymal stromal cells: A multicentre randomized double-blind trial. Critical Care. 2022;**26**(1):48. DOI: 10.1186/s13054-022-03930-4

[61] Carmen L, Kuniyoshi R, Alexandra C, Claudio S, Franck L, Regina D, et al. Safety and long-term improvement of mesenchymal stromal cell infusion in critically COVID-19 patients: A randomized clinical trial. Stem Cell Research & Therapy. 2022;**13**:122

[62] Hadisoebroto I, Dita D, Adhrie A, Erlina S, Triya B, Pompini D, et al. Umbilical cord mesenchymal stromal cells as critical COVID-19 adjuvant therapy: A randomized controlled trial. Stem Cells Translational Medicine. 2021;**10**(9):1279-1287. DOI: 10.1002/sctm.21-0046

[63] Adas G, Cukurova Z, Kart Y, Yilmaz K, Isiksacan R, Kasapoglu N, et al. The systematic effect of mesenchymal stem cell therapy in critical COVID-19 patients: A prospective double controlled trial. Cell Transplantation. 2021;**30**:1-14. DOI: 10.1177/09636897211024942

[64] Bing L, Junhui C, Tao L, Haiying W, Wenjie Y, Yanjiao L, et al. Clinical remission of a critically ill COVID-19 patient treated by human umbilical cord mesenchymal stem cells a case report. Medicine (Baltimore). 2020;**99**:e21429. DOI: 10.1097/MD.0000000000021429

[65] Lei S, Changming N, Ruyou L, Tingrong H, Yan W, Mao H, et al. Treatment of severe COVID-19 with human umbilical cord mesenchymal stem cells. Stem Cell Research & Therapy. 2020;**11**:361. DOI: 10.1186/s13287-020-01875-5

[66] Yue Z, Rongjia Z, Kun L, Xin L, Dezhong C, Dunyao B, et al. Human umbilical cord mesenchymal stem cells for adjuvant treatment of a critically ill COVID-19 patient: A case report. Infection and Drug Resistance. 2020;**28**(13):3295-3300

[67] Kazuya S, Katsutoshi O, Iekuni O, Akiko M, Keiko H, Tadashi N, et al. Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. Blood. 2007;**109**:228-234. DOI: 10.1182/blood-2006-02-002246

[68] Li W, Ren G, Huang Y, Su Y, Han Y, Li J, et al. Mesenchymal stem cells: A double-edged sword in regulating immune responses. Cell Death and Differentiation. 2012;**19**:1505-1513. DOI: 10.1038/cdd.2012.26

[69] Raghuvanshi S, Sharma P, Singh S, Van Kaer L, Das G. Mycobacterium tuberculosis evades host immunity by recruiting mesenchymal stem cells. Proceedings of the National Academy of

Sciences of the United States of America. 2010;**107**:21653-21658

[70] Metghalchi S, Padmapriya P, Tabassome S, Alain T, Soraya T, Ziad M. Indoleamine 2,3-dioxygenase fine-tunes immune homeostasis in atherosclerosis and colitis through repression of interleukin-10 production. Cell Metabolism. 2015;**22**:460-471. DOI: 10.1016/j.cmet.2015.07.004

[71] Chen X, Gan Y, Li W, Su J, Zhang Y, Huang Y, et al. The interaction between mesenchymal stem cells and steroids during inflammation. Cell Death and Disease. 2014;**5**:e1009. DOI: 10.1038/cddis.2013.537

[72] Jitschin R, Bottcher M, Saul D, Lukassen S, Bruns H, Loschinski R, et al. Inflammation-induced glycolytic switch controls suppressivity of mesenchymal stem cells via STAT1 glycosylation. Leukemia. 2019;**33**:1783-1796. DOI: 10.1038/s41375-018-0376-6

[73] Song N, Wakimoto H, Rossignoli F, Bhere D, Ciccocioppo R, Chen K-S, et al. Mesenchymal stem cell immunomodulation: In pursuit of controlling COVID-19 related cytokine storm. Stem Cells. 2021;**39**(6):707-722

[74] Luchetti F. Melatonin regulates mesenchymal stem cell differentiation: A review. Journal of Pineal Research. 2014; **56**:382-397

[75] Zhong Y-S, Lin N, Deng M-H, Zhang F-C, Tang Z-F, Xu R-Y. Deficient proliferation of bone marrow-derived mesenchymal stem cells in patients with chronic hepatitis b viral infections and cirrhosis of the liver. Digestive Diseases and Sciences. 2010;**55**(2):438-445

[76] Lin YP, Shaw M, Gregory V, et al. Avian-to-human transmission of H9N2 subtype influenza A viruses: Relationship between H9N2 and H5N1 human isolates. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**(17): 9654-9658

[77] Pak J, Chang J-J, Lee JH, Lee SH. Safety reporting on implantation of autologous adipose tissue-derived stem cells with platelet-rich plasma into human articular joints. BMC Musculoskeletal Disorders. 2013;**14**:337

[78] Blennow O, Fjaertoft G, Winiarski J, Ljungman P, Mattsson J, Remberger M. Varicella-zoster reactivation after allogeneic stem cell transplantation without routine prophylaxis—The incidence remains high. Biology of Blood and Marrow Transplantation. 2014;**20** (10):1646-1649

[79] Li Y, Chi Y, Bian Q, Wen T, Zhang W. Mesenchymal stem cells therapy for H9N2 avian influenza viruses induced acute lung injury in mice. In: Proceedings of the International Conference on Biomedical Engineering and Biotechnology (ICBEB'12). Macau, China: IEEE; 2012. pp. 1052-1055

[80] Ji F, Li L, Li Z, Jin Y, Liu W. Mesenchymal stem cells as a potential treatment for critically ill patients with coronavirus disease 2019. Stem Cells Translational Medicine. 2020;**9**: 813-814

[81] Johnson CL, Soeder Y, Dahlke MH. Concise review: Mesenchymal stromal cell-based approaches for the treatment of acute respiratory distress and sepsis syndromes. Stem Cells Translational Medicine. 2017;**6**(4):1141-1151

[82] Islam MN, Das SR, Emin MT, et al. Mitochondrial transfer from bone-marrow-derived stromal cells to pulmonary alveoli protects against acute lung injury. Nature Medicine. 2012;**18** (5):759-765

[83] Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;**8**(4):315-317

[84] Tatsumi K, Ohashi K, Matsubara Y, et al. Tissue factor triggers procoagulation in transplanted mesenchymal stem cells leading to thromboembolism. Biochemical and Biophysical Research Communications. 2013;**431**(2):203-209

[85] Lin BL, Chen JF, Qiu WH, Wang KW, Xie DY, Chen XY, et al. Allogeneic bone marrow-derived mesenchymal stromal cells for hepatitis B virus-related acute-on-chronic liver failure: A randomized controlled trial. Hepatology. 2017;**66**:209-219

[86] Zhang H, Baker A. Recombinant human ACE2: Acing out angiotensin II in ARDS therapy. Critical Care. 2017;**21**:305

[87] Zhang S, Jiang W, Ma L, Liu Y, Zhang X, Wang S. Nrf2 transfection enhances the efficacy of human amniotic mesenchymal stem cells to repair lung injury induced by lipopolysaccharide. Journal of Cellular Biochemistry. 2018; **119**:1627-1636

[88] Herold S, Becker C, Ridge KM, Budinger GR. Influenza virus induced lung injury: Pathogenesis and implications for treatment. The European Respiratory Journal. 2015;**45**: 1463-1478

[89] Hosseinikia R, Nikbakht MR, Moghaddam AA, Tajehmiri A, Hosseinikia M, Oubari F, et al. Molecular and cellular interactions of allogenic and autologus mesenchymal stem cells with innate and acquired immunity and their role in regenerative medicine. International Journal of Hematology-Oncology and Stem Cell Research. 2017; **11**:63-77

[90] Li N, Hua J. Interactions between mesenchymal stem cells and the immune system. Cellular and Molecular Life Sciences. 2017;**74**:2345-2360

[91] Rashedi I, Gómez-Aristizábal A, Wang XH, Viswanathan S, Keating A. TLR3 or TLR4 activation enhances mesenchymal stromal cell-mediated treg induction via notch signaling. Stem Cells. 2017;**35**:265-275

[92] Sava RI, Pepine CJ, March KL. Immune dysregulation in HFpEF: A target for mesenchymal stem/stromal cell therapy. Journal of Clinical Medicine. 2020;**9**:241

[93] Jenne CN, Wong CHY, Zemp FJ, McDonald B, Rahman MM, Forsyth PA, et al. Neutrophils recruited to sites of infection protect from virus challenge by releasing neutrophil extracellular traps. Cell Host and Microbe. 2013;**13**(2): 169-180

[94] Saitoh T, Komano J, Saitoh Y, et al. Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. Cell Host & Microbe. 2012;**12**(1):109-116

[95] Yousefi S, Stojkov D, Germic N, Simon D, Wang X, et al. Untangling "NETosis" from NETs. European Journal of Immunology. 2019;**49**(2):221-227. DOI: 10.1002/eji.201747053

[96] Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. The Journal of Cell Biology. 2010; **191**(3):677-691

[97] Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, et al. Novel cell

death program leads to neutrophil extracellular traps. The Journal of Cell Biology. 2007;**176**(2):231-241

[98] Mikacenic C, Moore R, Dmyterko V, West TE, Altemeier WA, et al. Neutrophil extracellular traps (NETs) are increased in the alveolar spaces of patients with ventilator-associated pneumonia. Critical Care. 2018;**22**(1): 358. DOI: 10.1186/s13054-018-2290-8

[99] Nicolai A, Leunig S, Brambs S, et al. Immunothrombotic dysregulation in COVID-19 pneumonia is associated with respiratory failure and coagulopathy. Circulation. 2020;**142**(12):1176-1189

[100] Skendros P, Mitsios A, Chrysanthopoulou A, Mastellos DC, Metallidis S, et al. Complement and tissue factor-enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. The Journal of Clinical Investigation. 2020; **130**(11):6151-6157. DOI: 10.1172/ JCI141374

[101] Middleton EA, He XY, Robert FD, Campbell A, David NG, et al. Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome. Blood. 2020;**136**(10):1169-1179

[102] Veras FP, Pontelli M, Silva C, Toller-Kewahisa JE, de Lima M, et al. SARS-CoV-2–triggered neutrophil extracellular traps mediate COVID-19 pathology. The Journal of Experimental Medicine. 2020;**217**(12):e20201129

[103] Zuo Y, Yalavarthi S, Shi H, Gockman K, Zuo M, Madison JA, et al. Neutrophil extracellular traps in COVID-19. JCI Insight. 2020;**5**(11):e138999

[104] Cicco S, Cicco G, Racanelli V, Vacca A. Neutrophil extracellular traps (NETs) and damage-associated molecular patterns (DAMPs): Two potential targets for COVID-19 treatment. Mediators of Inflammation. 2020;**2020**(7527953):25. DOI: 10.1155/ 2020/7527953

[105] Hu JJ, Liu X, Xia S, et al. FDA-approved disulfiram inhibits pyroptosis by blocking gasdermin D pore formation. Nature Immunology. 2020;**21** (7):736-745

[106] Yost CC, Schwertz H, Cody MJ, et al. Neonatal NETinhibitory factor and related peptides inhibit neutrophil extracellular trap formation. The Journal of Clinical Investigation. 2016;**126**(10): 3783-3798

[107] Tagami T, Tosa R, Omura M, Fukushima H, Kaneko T, et al. Effect of a selective neutrophil elastase inhibitor on mortality and ventilator-free days in patients with increased extravascular lung water: A post hoc analysis of the PiCCO pulmonary edema study. Journal of Intensive Care. 2014;**2**(1):67. DOI: 10.1186/s40560-014-0067-y

[108] Darif D, Hammi I, Kihel A, El Idrissi Saik I, Guessous F, Akarid K. The pro-inflammatory cytokines in COVID-19 pathogenesis: What goes wrong? Microbial Pathogenesis. 2021;**153**:104799

[109] Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, et al. Transplantation of ACE2- mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. Aging and Disease. 2020;**11**: 216-228

Section 2

Clinical and Therapeutic Applications of Mesenchymal Stem Cells

Chapter 4

Mesenchymal Stem Cell-Derived Exosomes as a New Possible Therapeutic Strategy for Parkinson's Disease

Zhongxia Zhang, Jing Kong and Shengjun An

Abstract

Mesenchymal stem cell-derived exosomes (MSC-Exos) are nano-sized extracellular vesicles that have low immunogenicity and the ability to transfer the effective substances enriched in stem cells freely and reported experimental studies have demonstrated MSC-Exos have effects on some diseases. As the second most predominant neurodegenerative disease worldwide, Parkinson's disease (PD) is characterized by severe progressive motor dysfunction caused by loss of dopaminergic neurons (DAn) and dopamine depletion. Since MSC-Exos serve as a beneficial promoter of neuroprotection and neurodifferentiation, in this article, we will summarize the application of MSC-Exos in PD treatment and the possible therapeutic mechanisms, especially the role of microRNAs included in MSC-Exos in the cellular and molecular basis of PD, and discuss the potential application prospects against PD.

Keywords: mesenchymal stem cells, exosomes, Parkinson's disease, blood–brain barrier, microRNA

1. Introduction

Parkinson's disease (PD), described in 1817 by James Parkinson, is an age-related neurodegenerative disorder. As older people in the world are growing, neurodegenerative diseases will become the second leading cause of death. Especially PD, with a prevalence of 1–2% [1, 2] among aging people [3], affects over 10 million people. Patients with PD have clinical features including motor symptoms, such as rigidity, resting tremor, bradykinesia, akinesia, postural and gait instability, and nonmotor symptoms, like sleep disorders, depression, dementia and peripheral injuries. The primary pathology of this disease is the degeneration of dopaminergic neurons (DAn) in the substantia nigra (SN), which is also associated with the Lewy bodies (LB; protein aggregates of α-synuclein) accumulation, and the decrease of dopamine production in the brain [4, 5]. Although there are some diagnostic test scales and some functional measurements like Positron emission tomography (PET) scan and MRI for PD, recently, the diagnosis of PD mainly still depends on clinical judgment

[6]. Therapies to increase DA levels mainly include deep brain stimulation (DBS) and pharmacological treatments based on DA substitutes such as Levodopa preparations, dopamine agonists, monoamine oxidase-B (MAO-B) and catechol-omethyltransferase (COMT) inhibitors [7, 8]. However, these treatments can only relieve symptoms in the early stages but have little effect on the progression of PD, that is to say, they cannot cure or prevent the process and even cause adverse reactions involving involuntary motor action that may affect the quality of their life.

Among all the stem cell-based treatments, mesenchymal stem cells (MSCs)-based therapy may be the most encouraging therapeutic strategy against PD. MSCs can be easily isolated from widespread sources throughout the body, including bone marrow, adipose tissue, umbilical cord Wharton's Jelly, peripheral blood, brain and dental pulp [9–12] and can also differentiate into at least osteoblasts, adipocytes and chondroblasts, with low immunogenicity and strong regeneration potential. Not only in experiments for rodent and nonhuman primate PD models but also in some clinical trials for mild to moderate PD patients [13], thanks to the secretion of neurotrophins, growth factors, and regulatory factors released [14–16], MSCs have been shown the great effects on the progression of PD. Recently, these effects have been ascribed to the products released into the extracellular milieu by MSCs, named extracellular vesicles (EVs). Exosomes, the subgroup with the smallest size of EVs [17], have been found in various body fluids or some tissues, affecting cell-to-cell communication [18] and effects of exosomes derived from MSCs on neurological disorders, especially neurodegenerative diseases, have been reported. Thus, in this chapter, we will review the therapeutic potential and the possible mechanisms of exosomes derived from MSCs in treating PD.

2. Exosomes

Exosomes were first discovered in maturing sheep reticulocytes as relatively uniform-sized particles in 1983 [19]. Later studies have shown that exosomes can be secreted by all cells in the body and most cell lines such as tumor cells, immune cells, neurons, stem cells, epithelial and endothelial cells [20], extracted from all body fluids involving blood, urine, cerebrospinal fluid, breast milk, amniotic and synovial fluid, ascites, pleural effusions and the cell culture supernatant [21, 22]. According to the criteria made by the International Society for Extracellular Vesicles (ISEV), exosomes are one type of EVs based on their size, origin and cargo. Therefore, they also meet the evaluation conditions of EVs [23, 24]: isolated from the conditioned cell culture or body fluids, have at least three different categories in the reparation-cytosolic proteins, transmembrane or lipid-bound extracellular proteins, verified by at least two different technologies in imaging and size measurements.

For the size, while the other two subtypes, microvesicles and apoptotic bodies, with a diameter of 50–1000 nm and 50–2000 nm, respectively, the exosomes have the smallest range of 30–150 nm [25]. For the origin, exosomes formed inside the multivesicular bodies (MVBs) and released by the fusion of MVBs to the plasma membrane, while the microvesicles formed directly by budding from the membrane and the apoptotic bodies are the products of apoptosis. For the cargo inside, exosomes carry a variety of molecules like oncoproteins, cytoskeleton protein, enzymes, hormones, lipids, steroids, sugars, signaling molecules, cytokines, growth factors and genetic material including small RNA, mRNA, microRNAs, long

noncoding RNA, ribosomal RNA (rRNA), transfer RNA (tRNA) and DNA fragments that are contributing to their functions [26, 27]. The contents of exosomes differ depending on the source types and physiological or pathological state of the donor cells.

Structurally, exosomes present a lipid, to be precise, a phospholipid bilayer membrane, which can keep the membrane stable [28]. Containing the internal proteins and nucleic acids mentioned above inside the exosomes, there are some membrane proteins and lipids on the membrane. The membrane proteins, containing membrane transport and fusion-related proteins (e.g., annexins, RABs, flotillins, ARFs and GTPases), antigen presentation-related proteins (MHC-I, MHC-II), adhesion molecules (MFGE8 and integrins), ESCRT complex (Alix, Tsg101), tetraspanins (CD9, CD63, CD81 and CD82) and other transmembrane proteins (e.g., PGRL, LAMP1, LAMP2 and TfR), participate in cell transporting, adhesion and mediate T cell activation [29–32]. The lipids includs phosphatidylserine, which has great flexibility and plays an important role in the budding and merging from the donor cell [33, 34], and ceramide, phosphatidic acid, diglycerides, ceramides are, which are not only contained in exosome biogenesis, but also in packaging and transporting the substances into the exosomes [35, 36], and other lipids like sphingomyelin, cholesterol, all of which are mainly involved in molecular signal transduction.

3. Treatment of MSCs in PD

Since PD is one of the progressive neurodegenerative disorders, there is not yet any curable therapeutic method for the progression [37]. More and more studies have shown the positive effects of MSCs on treating PD. Direct intracranial administration of MSCs-derived from bone marrow, adipose tissue, or umbilical cord with or without prior differentiation has provided improvement in motor function, striatal dopamine release and dopaminergic neuron survival in rodent models of PD [38]. Although there are still some concerns about the limited crossing of the blood–brain barrier, the effects of venous administration of MSCs on PD have been reported [38]. Our lab has shown that intranasal administration of MSCs-derived from the umblilical cord has similar effects on the MPTP-induced PD model mice through regulating intestinal microorganisms [39]. Moreover, positive effects of MSCs in human PD have also been reported. For example, in a study using autologous MSCs through intravenous and tandem (intranasal + intravenous) injections to 12 patients with PD, a decrease in the severity of motor and nonmotor symptoms (including depression, sleep quality, etc.) in the posttransplant period (1 and 3 months posttransplantation) have been found [40]. In a 12-month single-center open-label dose escalation phase 1 study, 20 subjects with mild or moderate PD accepted a single intravenous infusion of bone marrow-derived MSCs at doses of 1, 3, 6, or 10×10^6 per kilogram body weight, and they were evaluated at 3, 12, 24 and 52 weeks postinfusion. Results have shown that the infusion is safe, well tolerated, and not immunogenic in human PD, and the highest dose seems to be the most effective at 52 weeks [41]. However, there are still challenges with cell sources, the number of homing cells, functional and safety testing, manufacturing and storage, cell survival rate and immunomodulatory effects after implantation in vivo. While MSCs have demonstrated preclinical success in PD during clinical trials, some issues like the clinical-trial design, including cell dose, administration interval time and route of administration, are required to be improved [42, 43].

4. Potential of MSC-derived exosomes in PD

As reviewed above, MSCs do provide benefits to animals or patients with PD, and some reports have provided the idea that the substances released by MSCs may exert protective effects. Indeed, some researchers have started to focus on the use of MSC conditional medium or the MSC-derived secretome, both involving the exosomes, as a cell-free treatment for PD [44]. As previously mentioned, while exosomes are one subtype of EVs, which include the other two major subtypes, microvesicles and apoptotic bodies, the MSC-derived secretome was defined as the composition of EVs and some soluble factors [45, 46], and the MSC-derived conditioned medium (CM) includes all the soluble molecules and vesicular components derived from MSCs [47, 48].

It has been demonstrated that human MSCs conditioned medium could increase dopaminergic neurons and decrease the motor and histological symptoms in the transgenic PD model [49] and the similar effects in 6-OHDA PD model rats [50]. In research to compare the effects of BMSCs and their conditioned medium, for the PD model rats made by rotenone, both BMSCs and the medium had effects on the behavioral performances and histological characteristics, and the medium even had better effects than BMSCs [51]. Besides these, conditioned medium from human exfoliated deciduous teeth MSCs (SHED) [52], menstrual blood MSCs, and adipose MSCs [50, 53]. All showed the protective effects in PD rat model induced by rotenone, PD cell model treated by 1-methyl-4-phenylpyridinium (MPP+) and 6-OHDA-lesioned PD rats, respectively, which were related to the decreased neuroinflammation marked by Iba-1 and CD4 levels, induced oxidative stress, increased brain-derived neurotrophic factor (BDNF) and neurotrophin-3 expression.

In a study of the neuroprotective effects comparison between human bone marrow-derived MSC (hBM-MSCs) and secretome derived from them, for the 6-OHDA PD model, the secretome even has better effects on protecting dopaminergic neurons such as neuronal differentiation and survival than hBM-MSCs, and the proteomic analysis showed that there were some factors related to the ubiquitin-proteasome and histone systems [50]. Abnormal aggregation of α-synuclein, the biomarker of PD, could be degraded by matrix metalloproteinases (MMPs) that are contained in MSC-secretome both in vivo and in vitro PD models [54]. In addition, many neuroregulatory products, such as brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), pigment epithelium-derived factor (PEDF), DJ-1, and cystatin-C (Cys-C) contained in MSCs-secretome may be the mediators against PD [50, 55, 56].

As mentioned above, exosomes are included in the conditioned medium or the secretome and could be separated from the medium. Since the MSC-derived exosomes were first isolated from human MSCs-derived from embryonic stem cells (ESC) in 2010 [57], and the effects of the medium or the secretome have been proven, a number of studies have begun to explore their potential for various diseases, especially for the central neural systems diseases such as stroke [58], traumatic brain injury [59], spinal cord injury [60], Alzheimer's disease [61] and PD, which we highlight in this article. It has been found that in vitro, exosomes but not microvesicles, derived from human dental pulp stem cells from human exfoliated deciduous teeth (SHEDs) exerted their anti-apoptosis ability in the 6-OHDA-induced PD cell model, and the exosomes could reduce 80% of the dopamine neuron apoptosis [62]. After that, the same group reported that the EVs derived from SHEDs could improve motor symptoms and increase the expression of dopamine neurons of 6-OHDA-induced PD model rats in vivo [63].

In the previous study, our group found that the exosomes derived from human umbilical cord mesenchymal stem cells (hucMSCs) not only reduced the SH-SY5Y apoptosis induced by 6-OHDA in vitro but also relieved the motor disorder of PD rat model induced by 6-OHDA, increased the dopamine levels in striatum and the numbers of dopaminergic neurons in the substania nigra through the taken up of exosomes by SH-SY5Y cells in vitro and the neurons in vivo observed by the PKH labeling. The exosomes have played a role in increasing the autophagy ability of dopaminergic neurons [64]. Then, we further made an observation on the effect of exosome treatment in the different way in vivo and explored the anti-inflammatory effects. We found that the lateral ventricle transplantation of huc-MSCs-derived exosomes had the same effect as tail vein injection, and the exosomes could be absorbed by both dopaminergic neurons and microglia on the lesioned side of the brain in vivo and by the BV2 cells in vitro. The protection of dopaminergic neurons was perhaps mediated by inhibiting microglia activation. In vitro, exosomes reduced secretion of inflammation factors such as interleukin-1β and interleukin-18, prevented the pyroptosis-associated morphology of BV2 cells, and increased the cell viability of SH-SY5Y cells in the neuroinflammatory cell model system [65].

5. Mechanism of exosomes derived from MSCs in the treatment of PD

Compared with the MSC-based treatment and drug therapy, besides the advantages such as cell-free, rare side effects, no undesirable differentiation of transplanted cells, low immunogenicity, compatibility, long circulating half-lives, the most apparent advantage is the ability to cross the blood–brain barrier (BBB) [66]. For various neurological diseases, the most common barrier for the therapeutic stem cells or the drugs is the BBB, which is a selective semipermeable barrier maintaining the central nervous system homeostasis by preventing molecules larger than 400 Da [67–69]. Therefore, almost all the drug molecules, like recombinant proteins, peptides, antibodies and even genes, short interfering RNAs (siRNAs) cannot cross BBB [70]. Classical methods for the delivery of compounds to pass the BBB include invasive injection, changing the shape, size, surface charge, and ligands type, and using the transporters such as nanoparticles or liposomes or receptors that are highly expressed at the BBB surface, which are considered as the most popular way [71, 72].

Since the exosomes are nano-sized, protein-embedded and membrane-bound vesicles, they can cross the BBB well, not only from the bloodstream to the brain but also from the brain to the bloodstream, which has been proved that the exosomes derived from stem cells in the brain can be found in the peripheral blood [73]. The ways exosomes interact with the receiving cell described are as follows: 1) to adhere to the receiving cell surface and fuse with it, and release the inclusion into the cell, which probably causes the occurrence of some biological processes; 2) to associate with a cell surface protein G-coupled receptor and then induce signal cascade reactions; 3) through different transcytosis mediated by nonspecific/lipid raft or the receptor or by the macropinocytosis [74–76]; 4) in some pathological conditions like neurodegenerative diseases, BBB permeability may increase [77]. Among these routes, the key factors would be the surface markers that mediate the interaction of exosomes with receiving cells [74]. After passing BBB, exosomes may release the contents in the receiving cell cytoplasm and induce some related signal transduction, reach the receiving cell plasma membrane as the neoformed exosomes to the adjacent cell, or be degradated by lysosomes [78].

Another important mechanism is related to one of the most common contents of exosomes, miRNA. MiRNAs are a class of noncoding RNAs with a length between 21 and 25 nt. Through binding to the untranslated region (UTR) of mRNA and recruiting the RNA-induced silencing complex (RISC), miRNAs regulate the target genes expression by degradating mRNA or inhibiting the translation [79, 80]. Since miRNAs have been indicated as a potential tool for diagnostics and therapies of various diseases, massive miRNAs expression dysregulation has been shown in PD [81]. The most obvious biomarker of PD, a-synuclein, could be modulated by miR-433 [82], miR-16-1 [83], which binds to the fibroblast growth factor 20 (FGF 20) mRNA and the HSP70 mRNA, respectively, in addition with miR-153, miR-34b/c [84–86], miRNA-155 [87, 88], miR-7 [89], all of which could increase the a-synuclein level. Considering the related genes, LRRK2, PRKN and PARK7 genes, which play key roles in the pathogenic process of PD, such as formation of LB, mitochondria damage and oxidative stress damage, are correlated with miR-205, miR-34b/c [90], miR-494 and miR-4639-5P [91, 92] levels.

As a novel miRNAs carrier, exosomes derived from MSCs may deliver the miRNAs into the target cells of the brain crossing the BBB. It has been found that miR-133, which is an important factor in DAn development, presents in the exosomes derived from MSCs and could be transferred to the neuronal cells and promote neurite outgrowth [93]. In addition, miR-143, miR-21, miR-17, miR-18a, miR-19a/b, miR-20a and miR-90a that enriched in MSCs-derived exosomes, are able to modulate immune response, neurogenesis, axonal growth and neuronal death [94, 95]. On the basis of the effectiveness of exosomes derived from hucMSCs on PD models, our lab further examined the miRNAs included by high-throughput miRNA sequencing and identified 616 miRNAs (associated with 14,235 target genes) in exosomes. MiR-7, miR-125-5p, miR-122-5p, miR-126-3p and miR-199-3p were the most abundant miRNAs [65]. It has been shown that miR-7 can inhibit NLRP3 inflammasome activation and α-synuclein aggregation, attenuate the death of DAn in the MPTP-induced PD model mouse [89], mimics-miR-124 can promote behavioral improvements and neurogenesis in the 6-OHDA induced PD model mice [95], while the antago-miR-155 and antago-miR-126 [96] are useful to the PD therapy, since miR-155 could lead to neuroinflammation and miR-126 could result in the increased dopamine vulnerability. Despite limited research, and the cellular and molecular mechanisms in how they impact PD regulated by miRNAs are still not entirely clear, the beneficial effects of MSCs-derived exosomes have been demonstrated in the present findings, and new therapeutic approaches based on miRNAs may attract particular attention.

6. Conclusion

MSC-derived exosomes have been proposed as a new strategy for PD due to their characteristics. Their ability to cross BBB, secreting various factors into the receiving cells can improve symptoms and regulate vital biologic processes, such as inhibiting the neuroinflammation, reducing apoptosis, and regulating autophagy. Since miRNAs have gained an important status in PD researches recently for that they can not only affect the onset and the progression of PD, be served as the biomarkers of PD, but also be considered for the treatment of PD transported in the exosomes, therefore, as a natural carrier of miRNA and other effective substances, exosomes derived from MSCs will be a potential clinical therapy for PD patients. However, there are still some issues, like the selection of cell lines, development of isolation technique,

improvement of exosomes' targeting capability, mechanisms of how the miRNAs interact with target cells, exosomal cargo selection process, ways of taken up by cells should be overcome, and further investigations are needed to characterize all the bioactive molecules fully for better use in PD and other degenerative or central nervous system diseases.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Massano J, Bhatia KP. Clinical approach to Parkinson's disease: Features, diagnosis, and principles of management. Cold Spring Harbor Perspectives in Medicine. 2012;**2**:a008870. DOI: 10.1101/cshperspect.a008870

[2] Gao LL, Wu T. The study of brain functional connectivity in Parkinson's disease. Translational Neurodegeneration. 2016;**5**:18. DOI: 10.1186/s40035-016-0066-0

[3] Pringsheim T, Jette N, Frolkis A, Steeves TD. The prevalence of Parkinson's disease: A systematic review and meta-analysis. Movement Disorders. 2014;**29**:1583-1590. DOI: 10.1002/mds.25945

[4] Pagonabarraga J, Kulisevsky J, Strafella AP, Krack P. Apathy in Parkinson's disease: Clinical features, neural substrates, diagnosis, and treatment. Lancet Neurology. 2015;**14**(5):518-531. DOI: 10.1016/S1474-4422(15)00019-8

[5] Lees AJ, Hardy J, Revesz T. Parkinson's disease. Lancet. 2009;**373**(9680):2055-2066. DOI: 10.1016/S0140-6736(09)60492-X

[6] Tolosa E, Garrido A, Scholz SW, Poewe W. Challenges in the diagnosis of Parkinson's disease. Lancet Neurology. 2021;**20**(5):385-397. DOI: 10.1016/S1474-4422(21)00030-2

[7] Fox SH, Katzenschlager R, Lim SY, Barton B, de Bie RMA, Seppi K, et al. International Parkinson and movement disorder society evidence-based medicine review: Update on treatments for the motor symptoms of Parkinson's disease. Movement Disorders. 2018;**33**(8):1248-1266. DOI: 10.1002/mds.27372

[8] Armstrong MJ, Okun MS. Diagnosis and treatment of Parkinson disease: A review. Journal of the American Medical Association. 2020;**323**(6):548-560. DOI: 10.1001/jama.2019.22360

[9] Han Y, Li X, Zhang Y, Han Y, Chang F, Ding J. Mesenchymal stem cells for regenerative medicine. Cell. 2019;**8**:886. DOI: 10.3390/cells8080886

[10] Teixeira FG, Carvalho MM, Sousa N, Salgado AJ. Mesenchymal stem cells secretome: A new paradigm for central nervous system regeneration? Cellular and Molecular Life Sciences. 2013;**70**:3871-3882. DOI: 10.1007/s00018-013-1290-8

[11] Dominici M, Blanc KL, Mueller I, Slaper-Cortenbach I. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;**8**:315-317. DOI: 10.1080/14653240600855905

[12] Salgado AJ, Sousa JC, Costa BM, Pires AO, Mateus-Pinheiro A, Teixeira FG, et al. Mesenchymal stem cells secretome as a modulator of the neurogenic niche: Basic insights and therapeutic opportunities. Frontiers in Cellular Neuroscience. 2015;**9**:249. DOI: 10.3389/fncel.2015.00249

[13] Barbuti PA, Barker RA, Brundin P, Przedborski S, Papa SM, Kalia LV, et al. MDS scientific issues committee. Recent advances in the development of stem-cell-derived dopaminergic neuronal transplant therapies for Parkinson's disease. Movement Disorders.

2021;**36**(8):1772-1780. DOI: 10.1002/mds.28628

[14] Oliver Y, Clayton L, Joseph W. Exosomes derived from human primed mesenchymal stem cells induce mitosis and potentiate growth factor secretion. Stem Cells and Development. 2019;**28**(6):398-409. DOI: 10.1089/scd.2018.0200

[15] Lo Furno D, Mannino G, Giuffrida R. Functional role of mesenchymal stem cells in the treatment of chronic neurodegenerative diseases. Journal of Cellular Physiology. 2018;**233**:3982-3999. DOI: 10.1002/jcp.26192

[16] Joo HS, Suh JH, Lee HJ, Bang ES, Lee JM. Current knowledge and future perspectives on mesenchymal stem cell-derived exosomes as a new therapeutic agent. International Journal of Molecular Sciences. 2020;**21**:30727. DOI: 10.3390/ijms21030727

[17] Fayazi N, Sheykhhasan M, Soleimani AS, Najafi R. Stem cell-derived exosomes: A new strategy of neurodegenerative disease treatment. Molecular Neurobiology. 2021;**58**(7):3494-3514. DOI: 10.1007/s12035-021-02324-x

[18] Goetzl L, Merabova N, Darbinian N, Martirosyan D, Poletto E, Fugarolas K, et al. Diagnostic potential of neural exosome cargo as biomarkers for acute brain injury. Annals of Clinical Translational Neurology. 2018;**5**(1):4-10. DOI: 10.1002/acn3.499

[19] Harding C, Stahl P. Transferrin recycling in reticulocytes: pH and iron are important determinants of ligand binding and processing. Biochemical and Biophysical Research Communications. 1983;**113**:650-658. DOI: 10.1016/0006-291x(83)91776-x

[20] Budnik V, Ruiz-Cañada C, Wendler F. Extracellular vesicles round of communication in the nervous system. Nature Reviews. Neuroscience. 2016;**17**(3):160-172. DOI: 10.1038/nrn.2015.29

[21] Simpson RJ, Jensen SS, Lim JW. Proteomic profling of exosomes: Current perspectives. Proteomics. 2008;**8**(19):4083-4099. DOI: 10.1002/pmic.200800109

[22] Kalani A, Tyagi A, Tyagi N. Exosomes: Mediators of neurodegeneration, neuroprotection and therapeutics. Molecular Neurobiology. 2014;**49**(1):590-600. DOI: 10.1007/s12035-013-8544-1

[23] Lotvall J, Hill AF, Hochberg F, Buzas EI. Minimal experimental requirements for definition of extracellular vesicles and their functions: A position statement from the International Society for Extracellular Vesicles. Journal of Extracellular Vesicles. 2014;**3**:26913. DOI: 10.3402/jev.v3.26913

[24] Beer L, Mildner M, Ankersmit HJ. Cell secretome based drug substances in regenerative medicine: When regulatory affairs meet basic science. Annals of Translational Medicine. 2017;**5**:170. DOI: 10.21037/atm.2017.03.50

[25] Lener T, Gimona M, Aigner L, Borger V, Buzas E, Camussi G. Applying extracellular vesicles based therapeutics in clinical trials-An ISEV position paper. Journal of Extracellular Vesicles. 2015;**4**:30087. DOI: 10.3402/jev.v4.30087

[26] Vilaça-Faria H, Salgado AJ, Teixeira FG. Mesenchymal stem cells-derived exosomes: A new possible therapeutic strategy for Parkinson's disease. Cell. 2019;**8**:20118. DOI: 10.3390/cells8020118

[27] Beeraka NM, Doreswamy SH, Sadhu SP, Srinivasan A, Pragada RR, Madhunapantula SV, et al. The role of exosomes in stemness and neurodegenerative diseases-chemoresistant- cancer therapeutics and phytochemicals. International Journal of Molecular Sciences. 2020;**21**:186818. DOI: 10.3390/ijms21186818

[28] Subra C, Laulagnier K, Perret B, Record M. Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. Biochimie. 2007;**89**(2):205-212. DOI: 10.1016/j.biochi.2006.10.014

[29] Mathivanan S, Ji H, Simpson RJ. Exosomes: extracellular organelles important in intercellular communication. Journal of Proteomics. 2010;**73**(10):1907-1920. DOI: 10.1016/j.jprot.2010.06.006

[30] Frühbeis C, Fröhlich D, Kuo WP, Krämer-Albers EM. Extracellular vesicles as mediators of neuron-glia communication. Frontiers in Cellular Neuroscience. 2013;**7**(182):87. DOI: 10.3389/fncel.2013.00182

[31] Gho YS, Lee C. Emergent properties of extracellular vesicles: A holistic approach to decode the complexity of intercellular communication networks. Molecular BioSystems. 2017;**13**(7):1291-1296. DOI: 10.1039/c7mb00146k

[32] Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;**367**(6478):eaau6977. DOI: 10.1126/science.aau6977

[33] Subra C, Grand D, Laulagnier K, Stella A, Lambeau G, Paillasse M, et al. Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. Journal of Lipid Research. 2010;**51**(8):2105-2120. DOI: 10.1194/jlr.M003657

[34] Isaac R, Reis FCG, Ying W, Olefsky JM. Exosomes as mediators of intercellular crosstalk in metabolism. Cell Metabolism. 2021;**33**(9):1744-1762. DOI: 10.1016/j.cmet.2021.08.006

[35] Stahl PD, Barbieri MA. Multivesicular bodies and multivesicular endosomes: The "ins and outs" of endosomal traffic. Science Signaling. 2002;**2002**(141):pe32. DOI: 10.1126/stke.2002.141.pe32

[36] Zöller M. Exosomes in cancer disease. In: Cancer Gene Profiling. New York: Humana Press; 2016. pp. 111-149. DOI: 10.1126/stke.2002.141.pe32

[37] Mendes Filho D, Ribeiro P, Oliveira LF. Therapy with mesenchymal stem cells in Parkinson disease: History and perspectives. The Neurologist. 2018;**23**(4):141-147. DOI: 10.1097/NRL.0000000000000188

[38] Staff NP, Jones DT, Singer W. Mesenchymal stromal cell therapies for neurodegenerative diseases. Mayo Clinic Proceedings. 2019;**94**(5):892-905. DOI: 10.1016/j.mayocp.2019.01.001

[39] Sun Z, Gu P, Xu H, Zhao W, Zhou Y, Zhou L, et al. Human umbilical cord mesenchymal stem cells improve locomotor function in Parkinson's disease mouse model through regulating intestinal microorganisms. Frontiers in Cell and Development Biology. 2022;**9**:808905. DOI: 10.3389/fcell.2021.808905

[40] Boika A, Aleinikava N, Chyzhyk V, Zafranskaya M, Nizheharodava D, Ponomarev V. Mesenchymal stem cells in Parkinson's disease: Motor and nonmotor symptoms in the early posttransplant period. Surgical Neurology International.

2020;**11**:380. DOI: 10.25259/SNI_233_2020

[41] Schiess M, Suescun J, Doursout MF, Adams C, Green C, Saltarrelli JG, et al. Allogeneic bone marrow-derived mesenchymal stem cell safety in idiopathic Parkinson's disease. Movement Disorders. 2021;**36**(8):1825-1834. DOI: 10.1002/mds.28582

[42] Skidmore S, Barker RA. Challenges in the clinical advancement of cell therapies for Parkinson's disease. Nature Biomedical Engineering. 2023;**7**(4):370-386. DOI: 10.1038/s41551-022-00987-y

[43] Zhuo Y, Li X, He Z, Lu M. Pathological mechanisms of neuroimmune response and multitarget disease-modifying therapies of mesenchymal stem cells in Parkinson's disease. Stem Cell Research & Therapy. 2023;**14**(1):80. DOI: 10.1186/s13287-023-03280-0

[44] Joyce N, Annett G, Wirthlin L, Olson S, Bauer G, Nolta JA. Mesenchymal stem cells for the treatment of neurodegenerative disease. Regenerative Medicine. 2010;**5**(6):933-946. DOI: 10.2217/rme.10.72

[45] Ferreira JR, Teixeira GQ, Santos SG, Barbosa MA, Almeida-Porada G, Gonçalves RM. Mesenchymal stromal cell Secretome: Influencing therapeutic potential by cellular pre-conditioning. Frontiers in Immunology. 2018;**9**:2837. DOI: 10.3389/fimmu.2018.02837

[46] Phelps J, Sanati-Nezhad A, Ungrin M, Duncan NA, Sen A. Bioprocessing of mesenchymal stem cells and their derivatives: Toward cell-free therapeutics. Stem Cells International. 2018;**2018**:9415367. DOI: 10.1155/2018/9415367

[47] Sagaradze G, Grigorieva O, Nimiritsky P, Basalova N, Kalinina NI, Akopyan Z, et al. Conditioned medium from human mesenchymal stromal cells: Towards the clinical translation. International Journal of Molecular Sciences. 2019;**20**:1656. DOI: 10.3390/ijms20071656

[48] Pawitan JA. Prospect of stem cell conditioned medium in regenerative medicine. BioMed Research International. 2014;**2014**:965849. DOI: 10.1155/2014/965849

[49] Teixeira FG, Carvalho MM, Panchalingam KM. Impact of the secretome of human mesenchymal stem cells on brain structure and animal behavior in a rat model of Parkinson's disease. Stem Cells Translational Medicine. 2017;**6**(2):634-646. DOI: 10.5966/sctm.2016-0071

[50] Nakhaeifard M, Kashani MHG, Goudarzi I, Rezaei A. Conditioned medium protects dopaminergic neurons in parkinsonian rats. Cell Journal. 2018;**20**:348-3568. DOI: 10.22074/cellj.2018.5343

[51] Abdelwahab S, Elsebay SAG, Gaber MF, Hafez SMNA. Comparative study between bone marrow mesenchymal stem cell and their conditioned medium in the treatment of rat model of parkinsonism. Journal of Cellular Physiology. 2021;**236**(1):440-457. DOI: 10.1002/jcp.29872

[52] Chen YR, Lai PL, Chien Y, Lee PH, Lai YH, Ma HI, et al. Improvement of impaired motor functions by human dental exfoliated deciduous teeth stem cell-derived factors in a rat model of Parkinson's disease. International Journal of Molecular Sciences. 2020;**21**:3807. DOI: 10.3390/ijms21113807

[53] Li H, Yahaya BH, Ng WH, Yusoff NM, Lin J. Conditioned medium

of human menstrual blood-derived endometrial stem cells protects against MPP+-induced cytotoxicity in vitro. Frontiers in Molecular Neuroscience. 2019;**12**:80. DOI: 10.3389/fnmol.2019.00080

[54] Oh SH, Kim HN, Park HJ. The cleavage effect of mesenchymal stem cell and its derived matrix metalloproteinase-2 on extracellular α-synuclein aggregates in parkinsonian models. Stem Cells Translational Medicine. 2017;**6**(3):949-961. DOI: 10.5966/sctm.2016-0111

[55] Assunção-Silva RC. Exploiting the impact of the secretome of MSCs isolated from different tissue sources on neuronal differentiation and axonal growth. Biochimie. 2018;**155**:83-91. DOI: 10.1016/j.biochi.2018.07.026

[56] Pires AO, Pinheiro BM, Teixeira FG, Anjo SI, Samy SR. Unveiling the differences of secretome of human bone marrow mesenchymal stem cells, adipose tissuederived stem cells, and human umbilical cord perivascular cells: A proteomic analysis. Stem Cells and Development. 2016;**25**(14):1073-1083. DOI: 10.1089/scd.2016.0048

[57] Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. Stem Cell Research. 2010;**4**:214-222. DOI: 10.1016/j.scr.2009.12.003

[58] Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M. Systemic Administration of Exosomes Released from mesenchymal stromal cells promote functional recovery and neurovascular plasticity after stroke in rats. British Journal of Pharmacology. 2013;**33**:1711-1715. DOI: 10.1038/jcbfm.2013.152

[59] Zhang Y, Chopp M, Meng Y, Katakowski M, Xin H, Mahmood A, et al. Effect of exosomes derived from multipluripotent mesenchymal stromal cells on functional recovery and neurovascular plasticity in rats after traumatic brain injury. Journal of Neurosurgery. 2015;**122**:856-867. DOI: 10.3171/2014.11.JNS14770

[60] Vaccari JPDR, Brand F, Adamczak S, Lee SW, Pérez-Bárcena J, Wang MY, et al. Exosome-mediated inflammasome signaling after central nervous system injury. Journal of Neurochemistry. 2015;**136**:39-48. DOI: 10.1111/jnc.13036

[61] Reza-Zaldivar EE, Sapiéns MAH, Minjarez B, Gutiérrez-Mercado YK, Márquez-Aguirre AL, Canales-Aguirre A. Potential effects of MSC-derived exosomes in neuroplasticity in Alzheimer's disease. Frontiers in Cellular Neuroscience. 2018;**12**:317. DOI: 10.3389/fncel.2018.00317

[62] Jarmalavičiūtė A, Tunaitis V, Pivoraitė U, Venalis A, Pivoriūnas A. Exosomes from dental pulp stem cells rescue human dopaminergic neurons from 6-hydroxy-dopamine-induced apoptosis. Cytotherapy. 2015;**17**:932-939. DOI: 10.1016/j.jcyt.2014.07.013

[63] Narbute K, Piļipenko V, Pupure J. Intranasal administration of extracellular vesicles derived from human teeth stem cells improves motor symptoms and normalizes tyrosine hydroxylase expression in the substantia nigra and striatum of the 6-hydroxydopamine-treated rats. Stem Cells Translational Medicine. 2019;**8**(5):490-499. DOI: 10.1002/sctm.18-0162

[64] Chen HX, Liang FC, G P, Xu BL, Xu HJ, Wang WT, et al. Exosomes derived from mesenchymal stem cells repair a Parkinson's disease model by inducing autophagy. Cell Death & Disease. 2020;**11**:288. DOI: 10.1038/s41419-020-2473-5

[65] Zhang ZX, Zhou YJ, Gu P, Zhao W, Chen HX, Wu RY, et al. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate Parkinson's disease and neuronal damage through inhibition of microglia. Neural Regeneration Research. 2023;**18**(10):2291-2300. DOI: 10.4103/1673-5374.368300

[66] D'Anca M, Fenoglio C, Serpente M, Arosio B, Cesari M. Exosome determinants of physiological aging and age-related neurodegenerative diseases. Frontiers in Aging Neuroscience. 2019;**11**:232. DOI: 10.3389/fnagi.2019.00232

[67] Becker AJ, Mcculloch EA, Till JE. Cytologial demonstration of the clonal nature of spleen colonies derived from transplanted mouse arrow cells. Nature. 1963;**197**:452-454. DOI: 10.1038/197452a0

[68] Chen Y, Liu L. Modern methods for delivery of drugs across the blood–brain barrier. Advanced Drug Delivery Reviews. 2012;**64**(7):640-665. DOI: 10.1016/j.addr.2011.11.010

[69] Blanchette M, Fortin D. Blood-brain barrier disruption in the treatment of brain tumors. Methods in Molecular Biology. 2011;**686**:447-463. DOI: 10.1007/978-1-60761-938-3_23

[70] Jakubec M, Maple-Grødem J, Akbari S. Plasma-derived exosome-like vesicles are enriched in lyso-phospholipids and pass the blood-brain barrier. PLoS One. 2020;**15**(9):e0232442. DOI: 10.1371/journal.pone.0232442

[71] Saraiva C, Praça C, Ferreira R, Santos T, Ferreira L, Bernardino L. Nanoparticle-mediated brain drug delivery: Overcoming blood–brain barrier to treat neurodegenerative diseases. Journal of Controlled Release. 2016;**235**:34-47. DOI: 10.1016/j.jconrel.2016.05.044

[72] Gabathuler R. Approaches to transport therapeutic drugs across the blood–brain barrier to treat brain diseases. Neurobiology of Disease. 2010;**37**(1):48-57. DOI: 10.1016/j.nbd.2009.07.028

[73] Athauda D, Gulyani S, Karnati HK, Li Y, Tweedie D. Utility of neuronal-derived exosomes to examine molecular mechanisms that afect motor function in patients with Parkinson disease: A secondary analysis of the exenatide-PD trial. JAMA Neurology. 2019;**76**(4):420-429. DOI: 10.1001/jamaneurol.2018.4304

[74] Saint-Pol J, Gosselet F, Duban-Deweer S. Targeting and crossing the blood-brain barrier with extracellular vesicles. Cell. 2020;**9**(4):851. DOI: 10.3390/cells9040851

[75] Gonda A, Kabagwira J, Senthil GN, Wall NR. Internalization of exosomes through receptor-mediated endocytosis. Molecular Cancer Research. 2019;**17**(2):337-347. DOI: 10.1158/1541-7786.MCR-18-0891

[76] Chen CC, Liu L, Ma F, Wong CW, Guo XE, Chacko JV, et al. Elucidation of exosome migration across the blood-brain barrier model In vitro. Cellular and Molecular Bioengineering. 2016;**9**:509-529. DOI: 10.1007/s12195-016-0458-3

[77] Dozio V, Sanchez J-C. Characterisation of extracellular vesicle-subsets derived from brain endothelial cells and analysis of their protein cargo modulation after TNF exposure. Journal of Extracellular Vesicles. 2017;**6**(1):1302705. DOI: 10.1080/20013078.2017.1302705

[78] Morelli AE, Larregina AT, Shufesky WJ. Endocytosis, intracellular

sorting, and processing of exosomes by dendritic cells. Blood. 2004;**104**(10):3257-3266. DOI: 10.1182/blood-2004-03-0824

[79] Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nature Reviews. Genetics. 2010;**11**:597-610. DOI: 10.1038/nrg2843

[80] Brodersen P, Voinnet O. Revisiting the principles of microRNA target recognition and mode of action. Nature Reviews. Molecular Cell Biology. 2009;**10**:141-148. DOI: 10.1038/nrm2619

[81] Sonntag K-C. MicroRNAs and deregulated gene expression networks in neurodegeneration. Brain Research. 2010;**1338**:48-57. DOI: 10.1016/j.brainres.2010.03.106

[82] Wang G, van der Walt JM, Mayhew G, Li YJ, Zuchner S, Scott WK, et al. Variation in the miRNA-433 binding site of FGF20 confers risk for Parkinson disease by overexpression of alpha-synuclein. American Journal of Human Genetics. 2008;**82**:283-289. DOI: 10.1016/j.ajhg.2007.09.021

[83] Zhang Z, Cheng Y. miR-16-1 promotes the aberrant alpha-synuclein accumulation in parkinson disease via targeting heat shock protein 70. Scientific World Journal. 2014;**2014**:938348. DOI: 10.1155/2014/938348

[84] Doxakis E. Post-transcriptional regulation of alpha-synuclein expression by mir-7 and mir-153. The Journal of Biological Chemistry. 2010;**285**:12726-12734. DOI: 10.1074/jbc.M109.086827

[85] Fragkouli A, Doxakis E. miR-7 and miR-153 protect neurons against MPP(+)-induced cell death via upregulation of mTOR pathway. Frontiers in Cellular Neuroscience. 2014;**8**:182. DOI: 10.3389/fncel.2014.00182

[86] Kabaria S, Choi DC, Chaudhuri AD, Mouradian MM, Junn E. Inhibition of miR-34b and miR-34c enhances alpha-synuclein expression in Parkinson's disease. FEBS Letters. 2015;**589**:319-325. DOI: 10.1016/j.febslet.2014.12.014

[87] Ponomarev ED, Veremeyko T, Weiner HL. MicroRNAs are universal regulators of differentiation, activation, and polarization of microglia and macrophages in normal and diseased CNS. Glia. 2013;**61**:91-103. DOI: 10.1002/glia.22363

[88] Thome AD, Harms AS, Volpicelli-Daley LA, Standaert DG. microRNA-155 regulates AlphaSynuclein-induced inflammatory responses in models of Parkinson disease. The Journal of Neuroscience. 2016;**36**:2383-2390. DOI: 10.1523/JNEUROSCI.3900-15.2016

[89] Zhou Y, Lu M, Du RH, Qiao C, Jiang CY, Zhang KZ, et al. MicroRNA-7 targets Nodlike receptor protein 3 inflammasome to modulate neuroinflammation in the pathogenesis of Parkinson's disease. Molecular Neurodegeneration. 2016;**11**:28. DOI: 10.1186/s13024-016-0094-3

[90] Minones-Moyano E, Porta S, Escaramis G, Rabionet R, Iraola S, Kagerbauer B, et al. MicroRNA profiling of Parkinson's disease brains identifies early downregulation of miR-34b/c which modulate mitochondrial function. Human Molecular Genetics. 2011;**20**:3067-3078. DOI: 10.1093/hmg/ddr210

[91] Xiong R, Wang Z, Zhao Z, Li H, Chen W, Zhang B, et al. MicroRNA-494 reduces DJ-1 expression and exacerbates neurodegeneration. Neurobiology of Aging. 2014;**35**:705-714. DOI: 10.1016/j.neurobiolaging.2013.09.027

[92] Chen Y, Gao C, Sun Q, Pan H, Huang P, Ding J, et al. MicroRNA-4639 is a regulator of DJ-1 expression and a potential early diagnostic marker for Parkinson's disease. Frontiers in Aging Neuroscience. 2017;**9**:232. DOI: 10.3389/fnagi.2017.00232

[93] Xin H, Li Y, Buller B, Katakowski M, Zhang Y, Wang X, et al. Exosomemediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. Stem Cells. 2012;**30**:1556-1564. DOI: 10.1002/stem.1129

[94] Baglio SR, Rooijers K, Koppers-Lalic D, Verweij FJ, Perez Lanzon M, Zini N, et al. Human bone marrow- and adipose-mesenchymal stem cells secrete exosomes enriched in distinctive miRNA and tRNA species. Stem Cell Research & Therapy. 2015;**6**:127. DOI: 10.1186/s13287-015-0116-z

[95] Xin H, Katakowski M, Wang F, Qian JY, Liu XS, Ali MM, et al. MicroRNA cluster miR-17-92 cluster in exosomes enhance neuroplasticity and functional recovery after stroke in rats. Stroke. 2017;**48**:747-753. DOI: 10.1161/STROKEAHA.116.015204

[96] Kim W, Lee Y, McKenna ND, Yi M, Simunovic F, Wang Y, et al. miR-126 contributes to Parkinson's disease by dysregulating the insulin-like growth factor/phosphoinositide 3-kinase signaling. Neurobiology of Aging. 2014;**35**:1712-1721. DOI: 10.1016/j.neurobiolaging.2014.01.021

Chapter 5

Biology, Preclinical and Clinical Uses of Mesenchymal Dental Pulp Stem Cells

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Abstract

Dental pulp is a feasible source of stem cells that could be differentiated into osteoblast providing a therapeutic approach, which contribute to bone regeneration. Furthermore, as dental pulp stem cells originate from the neural crest, they have significant potential in regenerating neural tissues. To isolate dental pulp stem cells, it is not necessary to undergo an additional surgical procedure, they can be obtained from teeth that need to be extracted for specific reasons or naturally shed in children. Dental pulp stem cells have an expansive and clonogenic potential by culturing them in a high-security laboratory. As dental pulp stem cells do not express the Major Histocompatibility Complex, these cells can be used trough a universal donor in several clinical procedures. In this chapter, we present evidence about the role and the applications of DPSCs to regenerate bone as well as in clinic cases to treat neurological affections.

Keywords: dental pulp stem cells, MSCs, cell therapy, stroke, ischemia, spinal cord injury

1. Introduction

Dental pulp MSCs (DPSCs) exhibit immunomodulatory, anti-inflammatory, and antifibrotic properties. Thus, DPSCs are used in cell therapies for conditions of chronic inflammation such as autoimmune diseases, as well as for regenerating or replacing damaged cells or tissues [1–3].

Stem cells have the capacity for self-renewal, generating exact copies that can remain in quiescence or follow the differentiation pathway marked by the signaling pathways to which they are exposed. Stem cells are classified according to their potential for differentiation into multipotent; although they are embryologically

derived from the neural crest, they can induce the differentiation of neural progenitor cells into functional adult neurons through their secretome (e.g. Brain Derived Factor, Glial Cell Derived Factor) [1, 4]. DPSCs are located in the center of teeth called dental pulp. DPSCs can be differentiated into several cell types such as osteoblasts, chondrocytes, and muscle cells [5]. Thus, DPSCs are a promising source of stem cells that make then valuable for their use in regenerative medicine and tissue engineering applications [6].

2. Sources for obtaining MSCs from DPSCs

MSCs (MSCs) have been isolated from dental tissues such as dental pulp (DPSCs), periodontal ligament (PDLSC), deciduous teeth (SHED), apical papilla (SCAP), follicles (DFSC), and gingiva (GMSC) [3, 7, 8].

In 2000, Gronthos and cols. Carefully performed extractions from impacted third molars, reporting the first protocol to isolate, expand and cryopreserve DPSCs [9]. In this study, authors showed that adult dental pulps contain clonogenic cells, high proliferation, and tissue-regenerating capabilities. These properties allowed to define them as stem cells. Moreover, they demonstrated that a large number of cells can be obtained from a single tooth. It represented a potential clinical use for cell therapy in the future [9].

The main indications for third molar extraction are:

- Pericoronitis
- Orthodontic reasons
- Associated cystic or tumoral pathologies
- Damage due to resorption to the second molar [10].

Studies have shown that DPSCs can be isolated from human teeth without harming the tooth or the donor.

2.1 Obtaining from deciduous teeth

2.1.1 Exfoliation age

Planning is essential for the collection of dental pulp MSCs, because, it is necessary that the tooth have 1/3 of the root on the day of collection and that the root of the permanent tooth is partially formed and ready to be erupt.

3. Laboratory characteristics of INDEBIOC for manufacturing DPSCs

The MSCs expansion to perform the translation of preclinical into clinical-grade large-scale requires a precise standardization of the procedural parameters such as cell seeding density, culture medium, cultivation devices and laboratory infrastructure.

To ensure that MSCs are consistently produced and controlled according to high quality standards, the good manufacturing practice (GMP) should be employed to

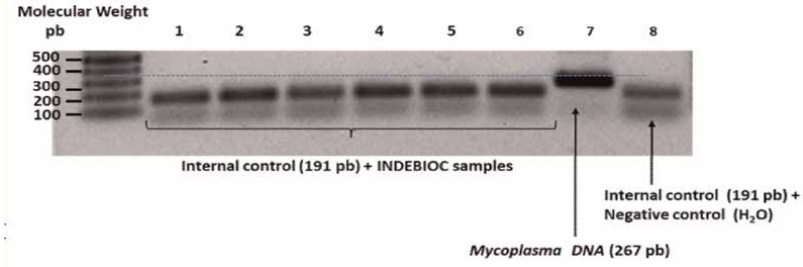

Figure 1.
Mycoplasma detection by PCR. Lane 1–6 correspond to samples obtained from the INDEBIOC laboratory with negative results, lane 7 corresponds to the positive control and lane 8 to the negative control.

produce clinical-grade stem cell products [1, 10–12]. In this way, the company INDEBIOC has a security 2 laboratory to isolate, expand and cryopreserve MSCs.

INDEBIOC performs high quality control procedures to ensure the identity, potency, purity, and safety of the biological products produced. This is maintained from the arrival of materials to the release of the final product.

Noticeably, the laboratory performs microbiological analysis (aerobic and anaerobic bacteria, viruses, fungi), specially for Mycoplasma to ensure the sterility of the biological products (**Figure 1**). All cells used in patients are previously tested to ensure the absence of tumor markers. Furthermore, every batch of cells is shipped to specialized companies for a Genetic Test to prove that those cells have a normal karyotype.

4. Isolation, expansion, culture, cryopreservation, thawing, and preparation of DPSCs for clinical using

To isolate cells from pulp tissue we collected molars by extraction or by waiting deciduous teeth exfoliation. We use the enzymatic dissociation (ED) method to digest the tissue and disaggregate the cells, which are then cultured in enriched medium. Cells placed in culture plates are maintained into the incubator at 37°C under saturated humidity, and 5% CO_2 conditions. At the moment that cells reach the 70% of confluence several passages are performed until we obtain the desired number of cells. Then, cells are cyoprotected using agents that maintain the integrity of the cells while the temperature is gradually reduced to be transferred to the liquid nitrogen tank, where they are stored until use (**Figure 2**) [13–16].

5. DPSCs characterization

To characterize mesenchymal cells it is needed that cells fulfill the following requirements conventionally established by global literature (**Figure 3**):

- Fusiform morphology.
- High clonogenicity and short duplication time.

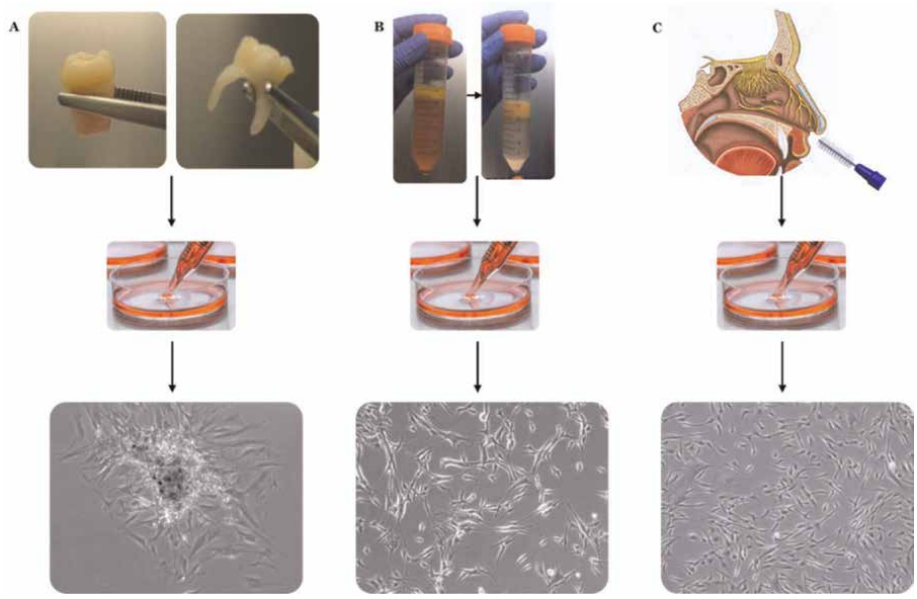

Figure 2.
Isolation of MSCs from different tissues: (A) dental pulp, (B) adipose tissue, (C) olfactory bulb. Secretome INDEBIOCs study.

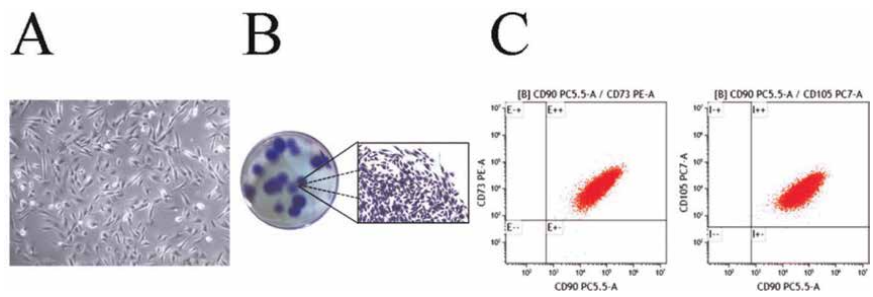

Figure 3.
Key points for the characterization of DPSC. (A) Representative microphotograph of spindle shaped morphology. (B) Representative image of clonogenecity. (C) Dot plot for the positive markers CD90, CD73, CD105.

- Positive (CD105, CD90, CD73) and negative (CD38 and HLA-DR) markers analyzed by flow cytometry.

- Differentiation potential to adipocytes, osteoblasts (alkaline phosphatase+ and osteocalcin+), and to chondrocytes.

6. Mechanisms of action and therapeutic potential of DPSCs

6.1 Safety

Thompson and cols. Reviewed 7473 clinical studies. From those, 55 studies with a total of 2696 patients evaluated fulfill the inclusion criteria indicating the side effects of the intravenous infusion of MSCs.

Figure 4.
Developed tumor with HeLa cells 11 × 40 mm. All HeLa mice were sacrificed later. No tumor growth was developed in the mice DPSCs mice.

MSCs as compared to controls were associated with an increased risk of fever, but not acute fever induced by infusion toxicity, infection, thrombotic/embolic events, tumor development nor death [17]. Unlike pluripotent stem cells, multipotent MSCs do not have a tumorigenic potential. However, considering Thompson's date, we performed a DPSCs tumorigenesis study in immunocompromised mice. We aimed to evaluate the tumorigenic capacity of MSCs derived from dental pulp in 21 immunodeficient mice of the FoxN1nu strain. For this, each mouse was inoculated with 3 million cells in each side of the body (6 million cells per mouse).

Freshly thawed DPSCs were administered in the study group, while HeLa cells were injected as the positive control (**Figure 4**). Furthermore, mice injected with saline physiologic solution were the negative control was used.

Malignant transformation of human MSCs has not been directly demonstrated, and attempts to induce a malignant phenotype by long-term ex vivo expansion have been unsuccessful [18, 19]. In contrast, the clinical applications that we have done using MSCs from our laboratory, we demonstrated that MSCs transplantations or infusions are safe.

6.2 Therapeutical mechanism of DPSCs

Years ago, biotechnologists and clinicians considered that the main therapeutic function of mesenchymal cells was to induce their differentiation into functional cells to regenerate organs. Today, we know that paracrine activity of the MSCs is a key mechanism in repairing mechanisms that involves the secretion of proteins/peptides and hormones that MSCs produce.

The secretome of MSCs include the secretion of growth factors, cytokines, and hormones. The MSCs secretome is involve in biological functions that include (1) immunomodulation, (2) anti-inflammation, (3) anti-fibrotic processes, (4) angiogenesis, and (5) induction of differentiation of progenitor cells from other organs (e.g. oligodendrocytes, cardiomyocytes, motor neurons).

Several reports show that MSCs are not constitutively inhibitory, but rather need to be activated by an inflammatory environment in the host for the induction of their immune-regulatory effect [20, 21].

Dra. Leblanc demonstrated for first time that MSCs have immunomodulatory properties [22]. To understand the immunomodulatory clinical potential, it is

necessary to understand the dynamic interaction between MSCs and the innate and adaptative immune response [23].

To demostrare the pharmacodynamics of MSCs in humans is difficult, but animal studies have shown that once infused, these cells will not integrate into tissues and organs, but will go through the followed described mechanisms to carry out their therapeutic functions. The presence of living MSCs in the lungs is temporal, within the first 24 h most of them die in the lungs while their secreted molecules are distributed to other sites, mainly the liver. Apoptosis is the primary mechanism of death of MSCs.

Cells that undergo apoptosis release the known "find me signals" that are directly or indirectly produced through the executioner caspases.

Once phagocytized, the second step is the identification of the apoptotic cell through "eat-me signals" by exposing phosphatidylserine on the plasma membrane of the cell [24].

Within the first hours of establishing, the inflammatory response molecules expressed by damaged tissues are recognized by innate effector cells. This triggers phagocytosis promoting the release of inflammatory mediators that initiate the innate immune response, mainly through the activation of phagocytic cells, including type 1 polarized pro-inflammatory macrophages [25].

6.3 MSCs and innate immunity

MSCs promote the formation of anti-inflammatory M2 macrophages by both, cell contact and MSCs-soluble factors secreted such as Prostaglandin E2 (PGE2) and catabolites of tryptophan activity including kynurenine and cyclooxygenase 2 (COX-2) [26, 27]. The polarization resulting from the effect of MSCs on M2 macrophages is related to the ability of MSCs to promote the emergence of regulatory T cells (Tregs) [28]. Tregs are a subset of T cells that play an important role in regulating the immune system and preventing autoimmunity. MSCs induce the differentiation of Tregs from naive T cells through several mechanisms, including the secretion of immunomodulatory factors such as IL-10 [29].

Moreover, MSCs might suppress the proliferation of T lymphocytes through the secretion of β-Transforming Growth Factor, Hepatocyte Growth Factor, Prostaglandin E2, and Indoleamine 2,3-dioxygenase (IDO). The release of these suppressive factors increases after MSCs are stimulated with Tumor necrosis factor (TNF)-alpha and Interferon (IFN)-gamma [30, 31].

Importantly, apoptosis of the MSCs induces receptor mediated immunomodulation. Apoptosis of MSCs is actively induced trough Perforin Dependent Apoptosis by cytotoxic cells; this process is essential to initiate the immunomodulation induced by MSC themselves [32]. Thus, to explore the fate of MSCs could help predict clinical responses.

7. DPSCs for bone reconstruction

Several reports show good results in the application of DPSCs in repairing bone defects. Interestingly, there were no major side effects in animal or human studies, proposing the use of this cells as safe and harmless for health [7, 33].

Bone defects are the result of trauma, resection of tumors, or surgical correction of congenital defects. The use of autologous bone increases donor site morbidity and can cause deformity. In addition, allogeneic grafts also imply risk for infection, disease transmission, and immune rejection [34, 35].

The existing techniques used for the reconstruction of bone deficiencies reflect both, the inadequacies of each method and the significant need to develop novel and improved approaches for bone regeneration.

The use of MSCs, and particularly those derived from teeth, have attracted much clinical and research attention [36].

The ideal strategy to promote bone formation would be the combination of a biomaterial scaffold with a cellular and molecular component that responds to environmental signals or the environment in which they are found. In this way, bone regeneration in critical defects is unpredictable if cells with osteoinductive and/or osteogenic properties are not used.

Bone defects, specially maxillary and mandibular defects of critical size (which do not regenerate on their own) require for their adequate reconstruction to regenerate an adequate volume of bone, both in height and thickness. In addition, it is needed to maintain the regenerated bone as a metabolically active bone [37, 38].

Three key elements are required for bone tissue engineering [39]:

1. Scaffold/matrix

2. Cells

3. Cell modulators and/or regulators

Three types of reconstruction methods are typically used to achieve these goals:

a. Free autografts, a procedure that requires a donor area with a good volume of bone and a recipient area with adequate vascularity and local cell activity.

b. Microvascularized bone grafts, which are a very expensive and time-consuming procedure with a big donor side.

c. Recombinant human morphogenic protein 2 carried in collagen sponge.

We demonstrate that the use of MSCs of dental origin provide adequate results in repairing critical defects, without the morbidity presented by the donor bed of free grafts, and without the high economic cost of microvascularized grafts or morphogenic protein. This, by combined an adequate carrier and osteoconductive materials plus microporosity and small particles that are quickly absorbed after fulfilling their osteoconduction and angioconduction functions.

7.1 Bone regeneration

Bone regeneration requires 3 biological processes: osteoconduction, osteoinduction, and osteogenesis. The activation of tyrosine kinase membrane receptors induce the osteoinductive activity of morphogenetic protein 2 tyrosine kinase membrane receptors activate intranuclear SMADS proteins, which are translocated to the nucleus and activate the bone differentiation gene in the chromosome 3 to differentiate MSCs into osteoblasts that begin to secrete collagen 1 which will be mineralized. This event produce osteopontin, osteonectin, alkaline phosphatase, osteocalcin, which induce bone mineralization.

7.1.1 Preclinical studies

7.1.1.1 Surgical creation of bone defects and bone mandibular regeneration in pig

Here, we present some cases treated with DPSCs for reconstructions of mandibular critical size bone defects (CSBD) in domestic pig's maxilla and mandible. We performed CSBD based in the extension of the defects, it is between 4 and 6 cm^3. In all cases we took out the periosteum that surrounded the defect to avoid the presence and activation of MSCs and pre-osteoblastic cells from this surrounding periosteum [40].

We performed a Control Defect—1100 mm^3 reconstructing it without cells (**Figure 5**). Furthermore, in each mandibular defect we used 6×10^6 human DPSCs/cm^3, cultivated in a collagen membrane as a scaffold in a clinical good manufacturing tissue practices settings (INDEBIOC's laboratory) and covering the reconstructed defects with a titanium mesh to avoid the collapse of the soft tissues over the grafted defects (**Figure 6**).

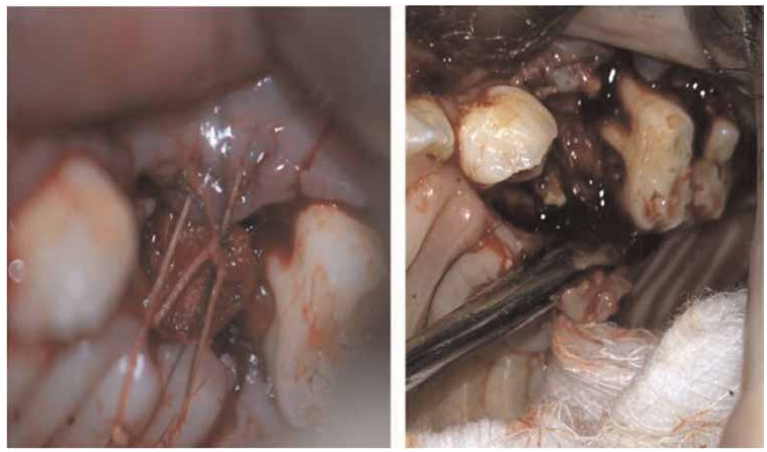

Figure 5.
Control defect (left side). Upper molar extraction and alveolar created defect treated with a collagen membrane without DPSC (right side). No bone regeneration 4 months later.

7.1.1.2 Periodontal regeneration in pig's periodontal induced disease

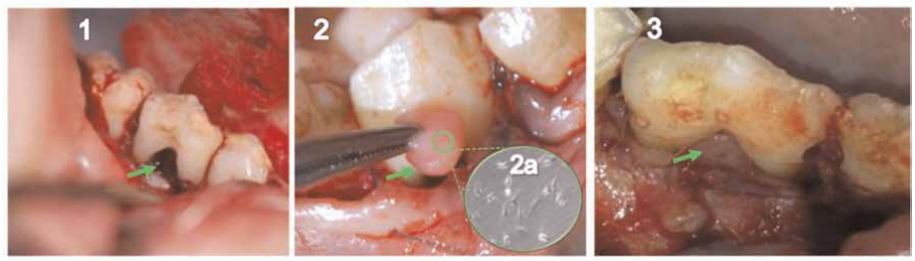

Figure 6.
New method of therapy for periodontal disease using stem cells in situ in pre-clinical studies. (1) Establishment of periodontal disease, (2) transplant human MSCs, (2a) human MSCs of dental origin, (3) regeneration (immature bone) after transplantation.

7.1.1.3 Critical size defects in pig's mandibles

Critical defect in pig mandible of 6 cm3 without periosteum did not heal spontaneously. Transplantation of 6 million green fluorescent protein-labeled DPSCs cultured on bovine collagen membrane + demineralized human cadaver bone as osteoconduction, contained by titanium mesh or lyophilized bone were plate to avoid collapse of the tissue engineered graft (**Figures 7–11**).

7.1.1.4 Created bone defects in the pig mandible mandibular body

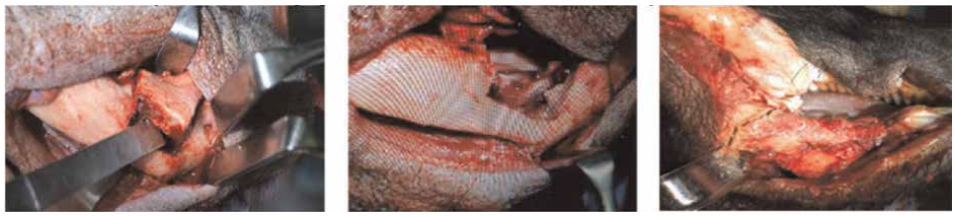

Figure 7.
Created critical bone size defect without periosteum and regenerated bone.

7.1.1.5 Created critical size bone defects in pig's mandibular angle

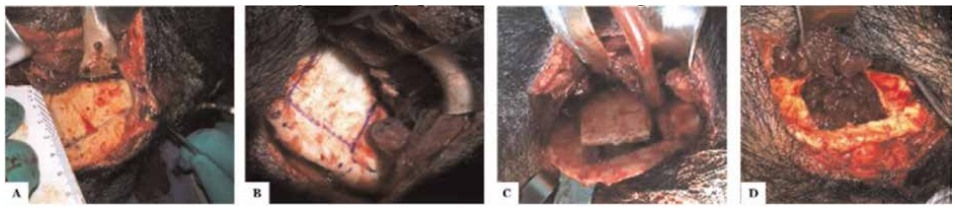

Figure 8.
Critical bone defects in pig's mandibular angle. (A) Complete size of the defect. (B) Periosteum removal from masseter muscle and periosteum from medial pterygoid muscle. (C) P full-thickness mandibular inferior alveolar artery and muscles without periosteum to avoid osteogenesis from these structures. (D) Critical size bone defect and pterygoid and masseter muscles without periosteum.

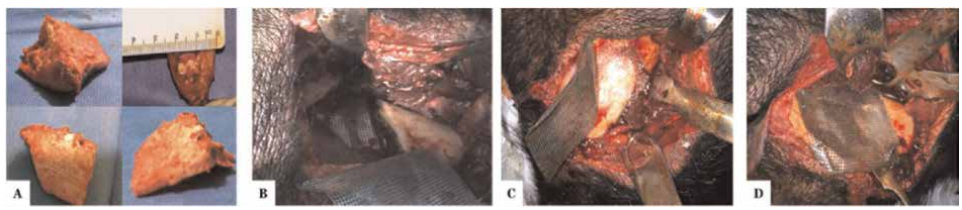

Figure 9.
Critical bone defects in pig's mandibular. (A) Full size of critical size defect. (B) Placement of lingual (internal) part of the titanium mesh. (C) Placement of the previously mentioned graft based on dental pulp MSCs. (D) Placement of the external side of titanium mesh.

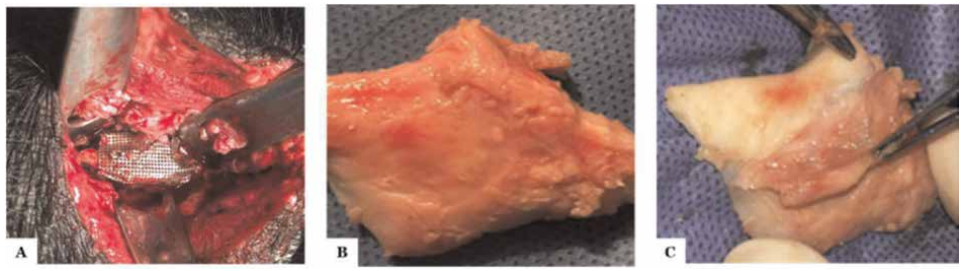

Figure 10.
(A) Re-approach to the grafted site 5 months after the bone and periosteum regeneration with DPSCs. (B) Cleaned sample obtained from the grafted mandible. Same size to the created defect. (C) Bone and periosteum thickness obtained from the DPSCs grafted site.

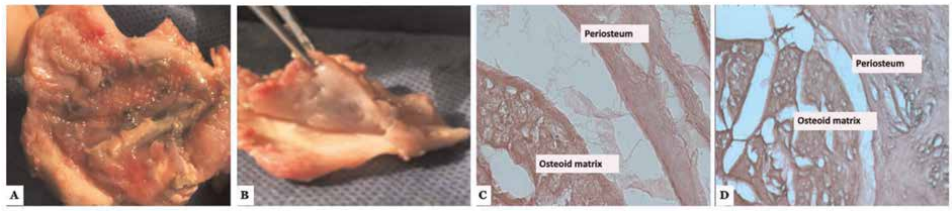

Figure 11.
(A) Titanium mesh impression which is observed in the periosteum of the DPSCs regenerated bone. (B) Normal macroscopic regenerated bone and periosteum with DPSCs. (C) and (D) Regenerated bone and periosteum histologic view that shows woven bone and some well-organized lamellas as mature bone.

8. DPSCs in human's maxillary and mandible bone defects

8.1 Case #1

This is a 58-year-old female patient who underwent titanium dental implant placement in the anterior maxilla 2 years ago. Despite significant maxillary bone atrophy, without any bone regeneration procedure, 4 implants were placed, which were not covered by bone. Fourlane of them were inserted into the nasal cavities. The patient developed multiple infectious processes. Moreover, the implants were mobile due the lack of bone and because they supported a dental prosthesis that was too large and heavy (**Figures 12** and **13**).

Two years later, the implants and 2 mobile teeth were removed, and a tissue-engineered graft was performed, as the patient refused the use of autologous grafts. We used 6 million DPSCs from dental pulp cultured in the INDEBIOC laboratory.

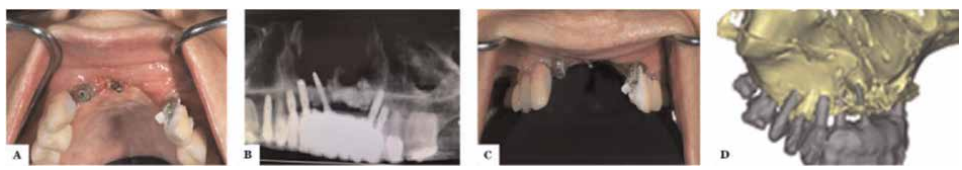

Figure 12.
(A) and (B) Clinical view of implants in an atrophic maxilla, with chronic inflammation, infection, and mobility. (C) and (D) Panoramic X-ray and CT scan that shows 5 nonintegrated implants, 2 of them inside the nose and very atrophic bone in the maxilla.

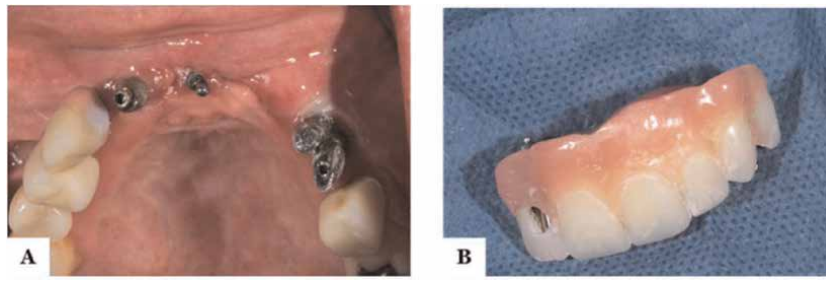

Figure 13.
(A) Infected nonintegrated titanium dental implants. (B) Unaesthetic nonintegrated implant supported denture.

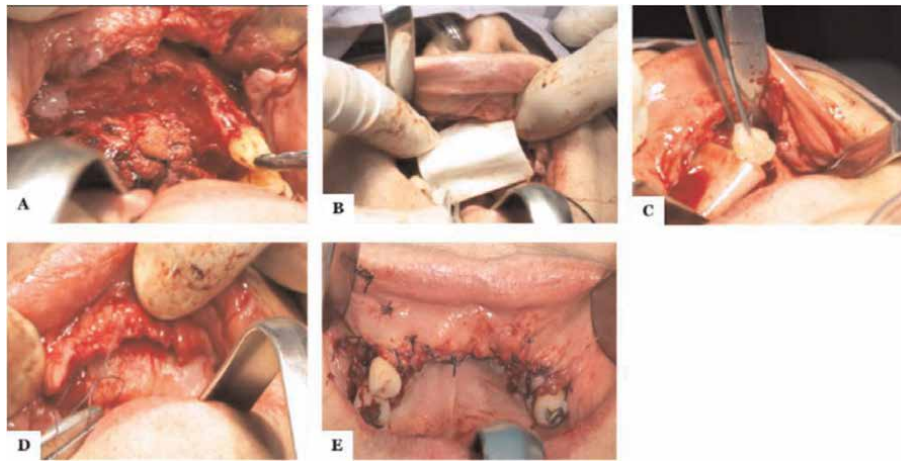

Figure 14.
(A) Uncovered vertical and vestibular-palatine critical bone defect. (B) Adapting, shaping height and thickness and securing the lyophilized bone sheet with 1.5 mm screws. (C) Grafting with DPSCs + RhBMP-2 + PRP + FDB into the space between the residual maxillary bone and the fixed bone sheet. (D) Fixed bone sheet covering the transplant of DPSCs + RhBMP-2 + PRP + FDB. (E) Suture covering the tissue-engineered graft while maintaining the height and thickness of the critical bone defect of the residual maxillary bone.

Cells were cultured on collagen membranes + recombinant human bone morphogenetic 2 protein (RhBMP2) + platelet-rich plasma (PRP) + dried and frozen bone (FDB). DPSCs + RhBMP-2 + PRP + FDB were covered and spaced in suitable height and thickness by fixed lyophilized bone sheets using screws (**Figures 14** and **15**).

8.2 CASE #2

In this case, a male 18 years old was underwent esthetic maxillomandibular osteotomy surgery. He exhibited post-surgical sequelae of maxillary necrosis. The entire anterior part of the maxilla became necrotic, resulting in the loss of bone, gum tissue, palatal mucosa, and 6 teeth. To face up this complex deformity with a critical size bone defect we combined 6 million cultured DPSCs $\times$ cm^3 on a collagen membrane + 2.4 mg of RhBMP2 + PRP + FDB. 2 years later, he got 4 dental titanium implants. To restore the patient's dental function and esthetic appearance for his age, an appropriate and esthetic fixed dental prosthesis was used (**Figures 16–19**).

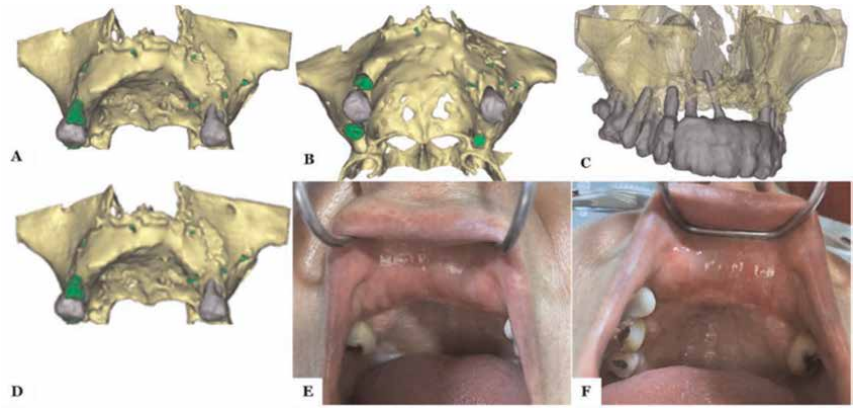

Figure 15.
(A) Frontal view. (B) Palatal view of the reconstructed maxilla with DPSCs + RhBMP-2 + PRP + FDB. (C) and (D) Comparative view of the height of the anterior maxillary bone reabsorbed by chronic infectious process before (C) and after (D) reconstruction with tissue-engineered. (E) Height and (F) thickness after transplant of DPSCs + RhBMP-2 + PRP + FDB covered with lyophilized bone sheets in a critical bone defect of the maxilla following the removal of chronically infected titanium dental implants.

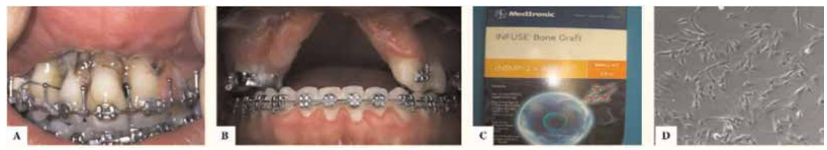

Figure 16.
(A) Male, 18 years old. Underwent esthetic maxillomandibular osteotomy surgery. Post-surgical sequelae of maxillary necrosis. (B) Necrotic loss of maxillary bone and 7 anterior teeth. (C) and (D) In this complex deformity due to bone, dental, gingival, and palatal mucosa loss, we combined 6 million cultured DPSCs/cm^3 on a collagen membrane + 2.4 mg of RhBMP2 + PRP + FDB.

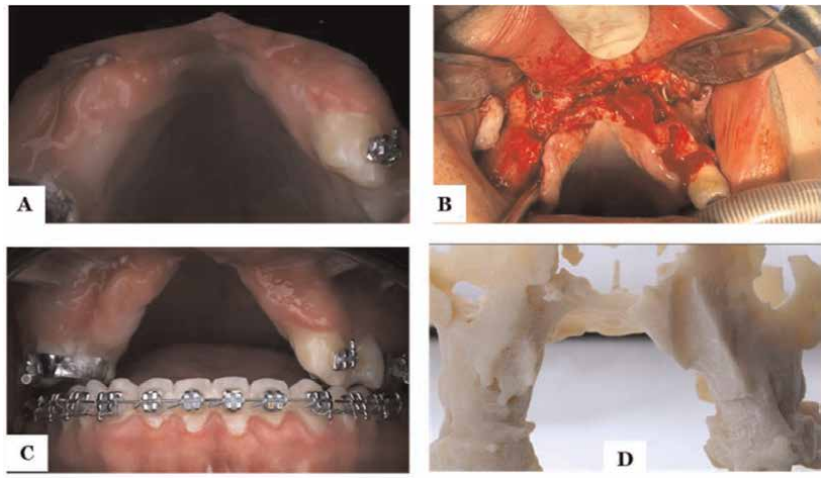

Figure 17.
(A) Pre-surgical image of the defect. (B) Image of the defect before reconstruction with DPSCs. (C) Critical size bone defect in the anterior maxilla. (D) 3D digital planning where we created a titanium mesh to prevent collapse of the DPSCs graft.

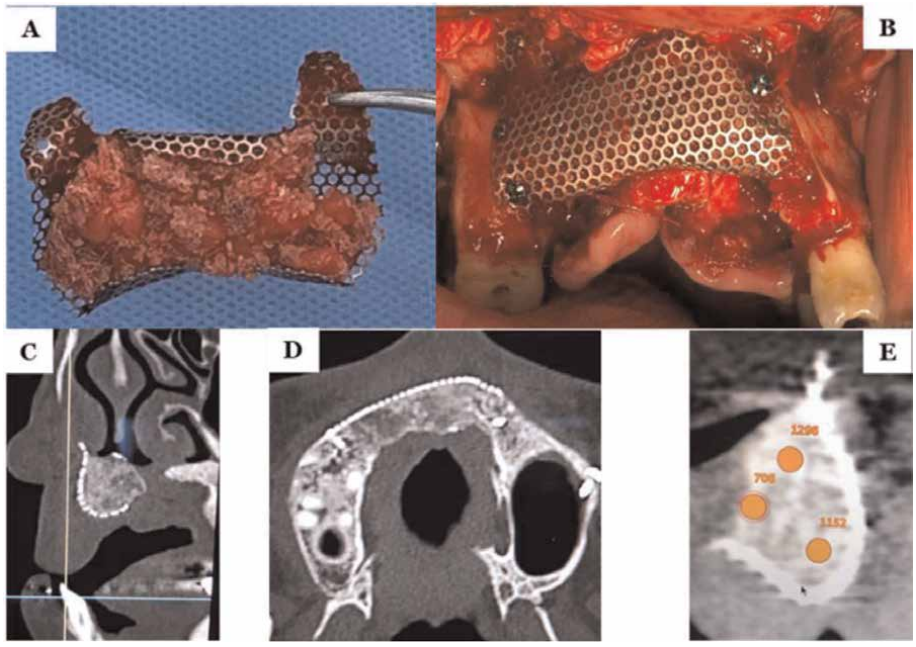

Figure 18.
(A) and (B) Pre molded titanium mesh covering and avoiding collapse of the engineered bone graft done with DPSCs + RhBMP + PRP + FDB. Post-surgical CT scan of the mesh providing height and thickness to the reconstruction. (C) Sagittal view, (D) coronal view, (E) bone density measurements, at 6 months.

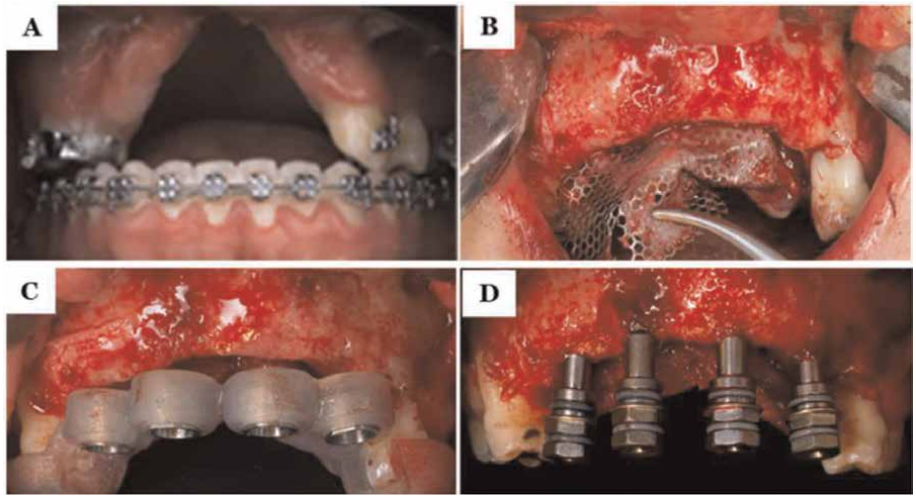

Figure 19.
(A) Post osteotomy maxillary necrosis defect. (B) Maxillary bone height and density obtained with DPSCs + Rh BMP-2 + PRP + FDB. Regenerated bone after 14 months. (C) Tissue engineered cortico-lamellar bone type. Excellent height and thickness for placement of titanium dental implants. (D) Implant surgical guide. 4 titanium dental implants in newly regenerated bone.

9. Case # 3 mandibular reconstruction with DPSCs

The following case is about a 18-year-old male who was diagnosed with mandibular ameloblastoma by incisional biopsy. We decided to perform an en bloc resection with 1 cm safety margins and placement of a reconstruction plate. The procedure was carried out under general anesthesia without complications. After one year without signs of recurrence, the planning for mandibular reconstruction under general anesthesia was initiated for future implant placement. At that time, it was decided to reconstruct using a block iliac crest graft, spongy iliac crest graft, and allograft as an osteoconductive material. The blocks were fixed to the preexisting reconstruction plate, and the aforementioned grafts were added.

The procedure was performed without complications. After this reconstruction, the COVID-19 pandemic favored that the patient stopped attending appointments but returned to us 2 years after the reconstruction surgery. Unfortunately, at that time, the graft had been resorbed by more than 50%, making it impossible to place dental implants on the new-formed bone. At this point, a second reconstruction was decided upon, in which BMP-2, allograft, and DPSCs were used. Under general anesthesia, the second reconstruction was successfully performed using the aforementioned materials. At 7 months post-reconstruction, guided dental implant placement is planned, which achieves an initial stability greater than 50 N. Currently, the patient is undergoing the necessary time for proper osteointegration to receive the implant-supported prostheses (**Figures 20–22**).

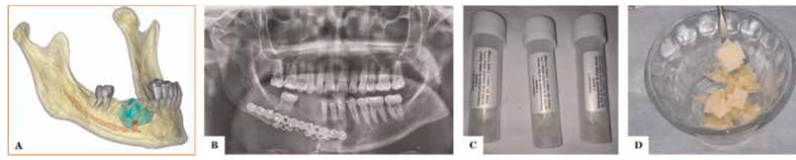

Figure 20.
(A) Mandibular 3D reconstruction with presence of mandibular ameloblastoma (green). (B) X-ray 2 years from iliac crest graft showing reabsorbed graft. (C) DPSCs in their vials for second reconstructive procedure. (D) Graft material with DPSCs (white scaffolds), allograft and BMP-2.

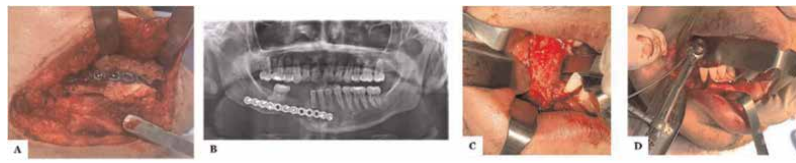

Figure 21.
(A) Newly formed bone 6 months after DPSC. (B) X-ray 6 months after reconstruction with DPSCs graft. (C) Grafted site with DPSCs. (D) Implant placement with adequate initial stability in new formed bone after DPSCs graft.

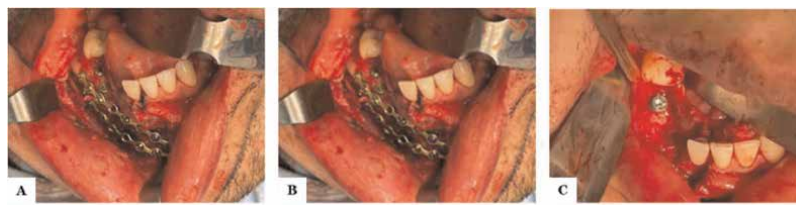

Figure 22.
Titanium dental implants placed on bone regenerated with DPSCs + Rh BMP-2 + PRP + FDB A, B and C.

10. Treatment of sequelae of cerebrovascular events in patients through different routes of administration

Considering that DPSCs have the same ectodermal origin as the central nervous system cells, we proposed to inject it into patients with spinal cord injury or stroke.

DPSCs have been also administrated in patients with neuropathic pain in the neck or lumbar region have been injected, from which any physical or organic injury has been ruled out. In this way, it leads us to treat the pain and being able to disguise any injury that compromises the patient's life.

10.1 Spinal cord injury

The following data report the treatment of the administration of 100 million mesenchymal cells in 3 patients with traumatic spinal cord injury that were evaluated 3 months later.

10.1.1 Patient 1

A 56 years old male with spinal cord injury due to fall at L3 level presented chronic injury with paraplegia. The patient was presented with anesthesia at L3 level and the rest of scan was negative. We administrated 100 million of DPSCs intrathecally, using the same cerebrospinal fluid of the patient as vehicle, extracting 10 cc and processing the cells to administer them by the same route without complications. He did not show any type of reaction to the application. Three months later, the patient reported and improvement in his bowel movements.

10.1.2 Patient 2

A 54 years old male suffered an automobile accident resulting in chronic cervical spinal cord injury with quadriplegia predominantly in the legs, sensory level in C4, with lack of sphincter control and unable to move his upper extremities with limited flexion and extension of the fingers. We administrated 100 million of DPSCs intrathecally, using the same cerebrospinal fluid of the patient as vehicle, extracting 10 cc and processing the cells to administer them by the same route without complications. He did not show any type of reaction to the application. Three months later there was no improvement.

10.1.3 Patient 3

A 20 years old patient male with traumatic thoracic spinal cord injury due to a fall into a ravine. The patient presented paraplegia and had to undergo surgery to place bars and screws for spinal stability. The patient presented sensory level in T5 and lack of sphincter control. We administrated 100 million of DPSCs intrathecally, using the same patient cerebrospinal fluid as vehicle, extracting 10 cc and processing the cells to administer them by the same route without complications. He did not show any type of reaction to the application. Three months later the patient did not report evident improvement. Six months later, the patient exhibited greater sensitivity of the legs in the form of non-specific patches. Eight months later, the patient had a decrease in the sensory level from T5 to L1, and he had an erection and a little sphincter control, with

slight movement of the feet and being able to feel the back, lower region and thus the incidence of bedsores was lower, since proprioception had improved. He missed his clinical evolution for a year.

10.2 Stroke

10.2.1 Patient 1

A 25-year-old male patient who, while in Australia for studies, presented a cerebral infarction without any type of recognized origin for it. The cerebral infraction leaving as a sequel right hemiparesis and language alterations. We administrated 25 million of DPSCs in saline solution intrathecally without complications. At follow-up there was no improvement. Three years later, 300 million cells were administered intracerebrally, administering the cells stereotaxically in the left posterior parietal region by a single application. There were no complications. The next day the movement of the right hand was significantly appreciated, less contracture and more movement. The walking was lighter and the patient was able to lift his leg higher. One month after treatment, the hand contracture returned. Despite the walking improved, language disturbances persisted. The patient has already been in office for four months and has been stable.

10.2.2 Patient 2

A 75-year-old male patient with left middle cerebral infarction with hemiplegia and language disorders was treated three months after his cerebral event. An angiography was performed and we administrated 100 million of DPSCs through the left middle cerebral artery and the overall increase in circulation was immediately appreciated in the control angiography at 15 minutes. Three months after its application, the patient has not had a noticeable improvement.

10.3 Subarachnoid hemorrhage

10.3.1 Patient 3

A 60-year-old female patient with subarachnoid hemorrhage in the frontal arteriovenous fistula, admitted to a coma, developed a subacute subdural hematoma during her hospitalization, which was operated on and drained adequately. Tracheostomy and gastrostomy were performed. It was totally dependent on the fan. At the third month, 100 million of DPSCs were administered intravenously without noticeable improvement. One week later, 100 million of DPSCs were administered intraarterially via angiography through the left middle cerebral artery. In the control cerebral angiography performed 10 min later, vasodilatation was observed, removing the vasospasm that it presented. A week after the transplant she was able to go home (**Table 1** and **Figure 23**).

10.3.2 Patient 4

This is the case of a 50-year-old male patient with subarachnoid hemorrhage due to bleeding that could not be identified angiographically. He was in a coma since

Neurological commitment	
Pre-transplant	**Post-transplant**
Patient who was already hospitalized for 3 months with tracheostomy and gastrostomy Two weeks before his discharge, 100 million DPSCs I.V. were applied. A week later, 100 millions MSC were applied by angiographic route in the posterior territory	No significant improvement in application I.V.
Not being able to speak	Started talking
Paralysis of the gaze (III, IV, and VI)	Onset with eye movements
Bifacial paralysis	Initiation with facial movements of oral predominance
Unable to eat or swallow (IX, X, XI and XII commited)	Began to eating and moving tongue
Paralysis of all four limbs	Started moving hands and legs
Dysautonomic imbalance	Began to normalizing
Other properties that the husband noticed	No hair loss, softer skin, more awake and other features more

Table 1.
Neurological compromise before and after transplantation of a 60-year-old female patient with subarachnoid hemorrhage from a frontal arteriovenous fistula treated with DPSC.

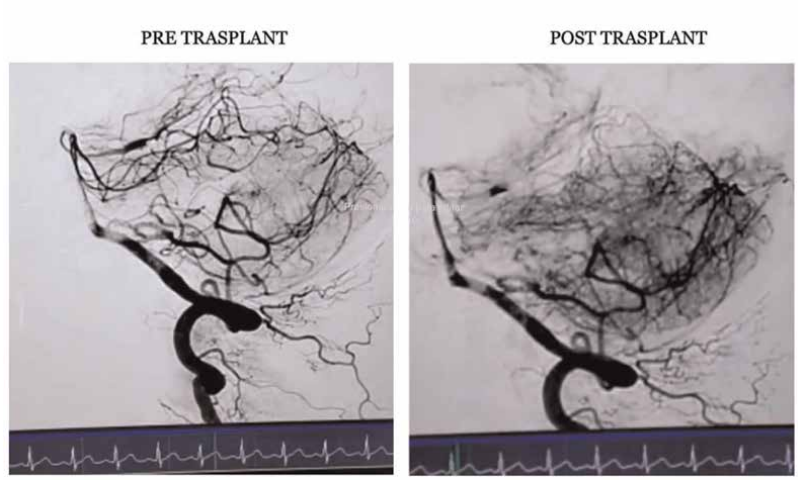

Figure 23.
Angiography before and after transplantation of a 60-year-old female patient with subarachnoid hemorrhage from a frontal arteriovenous fistula treated with DPSC.

admission development of hydrocephalus, performing referral. He presented a ventriculoperitoneal shunt, without being able to control exvacuo hydrocephalus due to neuronal damage. Three months later, he remained in a persistent vegetative state. We administrated 100 million of DPSCs by intraventricular route without complications, and without any type of rejection or dysautonomic reaction. He continued to depend on the ventilator and finally it was decided to withdraw the ventilator due to severe neurological damage and he died.

11. Conclusions

MSCs from dental pulp can be easily obtained and are highly clonogenic with multidifferentation and neuromuscular properties. Here, we presented a general view of the high quality methods used to isolate DPSCs and their subsequent culture and applications. In addition, we showed that DPSCs therapy for bone reconstruction.

Finally, we presented a series of clinical cases in which the administration of DPSCs was able to improve the quality life of patients who suffering of stroke or subarachnoid hemorrhage. All the present work is based in the fact that DPSCs and their secretome serve as critical immunomodulators that contribute with injury repair. Our clinical studies are promising in the use of DPSCs as a tool for treating bone disease, cerebrovascular events and spinal cord injury.

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Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

DPSCs	dental pulp MSCs
PDLSC	periodontal ligament MSCs
SHED	stem cells from human exfoliated deciduous teeth
SCAP	stem cells from apical papilla
DFSC	dental follicle stem cells
GMSC	gingival mesenchymal stem/progenitor cells
GMP	good manufacturing practice
INDEBIOC	Innovations and Development in Cellular Biotechnology
ED	enzymatic dissociation
MSCs	MSCs
PGE2	prostaglandin E2
COX-2	cyclooxygenase 2
Tregs	regulatory T cells
IDO	indoleamine 2,3-dioxygenase
TNF-alpha	tumor necrosis factor alpha
IL-10	Interleukin 10
SMADS	mothers against decapentaplegic protein
CSBD	critical size bone defects

rhBMP2	recombinant human bone morphogenetic 2 protein
PRP	platelet-rich plasma
FDB	dried and frozen bone
COVID-19	coronavirus disease
BMP-2	bone morphogenetic 2 protein

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References

[1] Vasanthan J, Gurusamy N, Rajasingh S, Sigamani V, Kirankumar S, Thomas EL, et al. Role of human MSCs in regenerative therapy. Cell. 2020; **10**(1):54. DOI: 10.3390/cells10010054

[2] Li P, Ou Q, Shi S, Shao C. Immunomodulatory properties of MSCs/dental stem cells and their therapeutic applications. Cellular & Molecular Immunology. 2023;**20**(6):558-569. DOI: 10.1038/s41423-023-00998-y

[3] Mishra VK, Shih H-H, Parveen F, Lenzen D, Ito E, Chan T-F, et al. Identifying the therapeutic significance of mesenchymal stem cells. Cell. 2020;**9** (5):1145. DOI: 10.3390/cells9051145

[4] Cevallos RR, Rodríguez-Martínez G, Gazarian K. Wnt/β-catenin/TCF pathway is a phase-dependent promoter of colony formation and mesendodermal differentiation during human somatic cell reprogramming. Stem Cells. 2018; **36**(5):683-695. DOI: 10.1002/stem.2788

[5] Sui B, Wu D, Xiang L, Fu Y, Kou X, Shi S. Dental pulp stem cells: From discovery to clinical application. Journal of Endodontia. 2020;**46**(9S):S46-S55. DOI: 10.1016/j.joen.2020.06.027

[6] Mahdavi-Jouibari F, Parseh B, Kazeminejad E, Khosravi A. Hopes and opportunities of stem cells from human exfoliated deciduous teeth (SHED) in cartilage tissue regeneration. Frontiers in Bioengineering and Biotechnology. 2023; **11**:1021024. DOI: 10.3389/fbioe.2023.1021024

[7] Roato I, Chinigò G, Genova T, Munaron L, Mussano F. Oral cavity as a source of mesenchymal stem cells useful for regenerative medicine in dentistry. Biomedicine. 2021;**9**(9):1085. DOI: 10.3390/biomedicines9091085

[8] Zhang W, Yelick PC. Tooth repair and regeneration: Potential of dental stem cells. Trends in Molecular Medicine. 2021;**27**(5):501-511. DOI: 10.1016/j.molmed.2021.02.005

[9] Yuan S-M, Yang X-T, Zhang S-Y, Tian W-D, Yang B. Therapeutic potential of dental pulp stem cells and their derivatives: Insights from basic research toward clinical applications. World Journal of Stem Cells. 2022;**14**(7): 435-452. DOI: 10.4252/wjsc.v14.i7.435

[10] Bastos VC, Gomez RS, Gomes CC. Revisiting the human dental follicle: From tooth development to its association with unerupted or impacted teeth and pathological changes. Developmental Dynamics. 2022;**251**(3): 408-423. DOI: 10.1002/dvdy.406

[11] Andriolo G, Provasi E, Brambilla A, Lo Cicero V, Soncin S, Barile L, et al. GMP-grade methods for cardiac progenitor cells: Cell bank production and quality control. Methods in Molecular Biology. 2021;**2286**:131-166. DOI: 10.1007/7651_2020_286

[12] Seet WT, Mat Afandi MA, Shamsuddin SA, Lokanathan Y, Ng MH, Maarof M. Current good manufacturing practice (cGMP) facility and production of stem cell. In: Stem Cell Production. Singapore: Springer Singapore; 2022. pp. 37-68

[13] Chen X, Huang J, Wu J, Hao J, Fu B, Wang Y, et al. Human mesenchymal stem cells. Cell Proliferation. 2022;**55**(4): e13141. DOI: 10.1111/cpr.13141

[14] Arora S, Cooper PR, Ratnayake JT, Friedlander LT, Rizwan SB, Seo B, et al. A critical review of in vitro research methodologies used to study

mineralization in human dental pulp cell cultures. International Endodontic Journal. 2022;**55**(Suppl 1(S1)):3-13. DOI: 10.1111/iej.13684

[15] Gross T, Dieterle MP, Vach K, Altenburger MJ, Hellwig E, Proksch S. Biomechanical modulation of dental pulp stem cell (DPSC) properties for soft tissue engineering. Bioengineering (Basel). 2023;**10**(3):323. DOI: 10.3390/bioengineering10030323

[16] Pilbauerova N, Schmidt J, Soukup T, Koberova Ivancakova R, Suchanek J. The effects of cryogenic storage on human dental pulp stem cells. International Journal of Molecular Sciences. 2021; **22**(9):4432. DOI: 10.3390/ijms22094432

[17] Thompson M, Mei SHJ, Wolfe D, Champagne J, Fergusson D, Stewart DJ, et al. Cell therapy with intravascular administration of mesenchymal stromal cells continues to appear safe: An updated systematic review and meta-analysis. EClinicalMedicine. 2020; **19**(100249):100249. DOI: 10.1016/j.eclinm.2019.100249

[18] Zamani H, Karami F, Mehdizadeh M, Baakhlag S, Zamani M. Long term culture of mesenchymal stem cells: No evidence of chromosomal instability. Asian Pacific Journal of Cancer Biology. 2022;**7**(4):349-353. Available from: http://www.waocp.com/journal/index.php/apjcb/article/view/985

[19] Liu P, Zhang Y, Ma Y, Tan S, Ren B, Liu S, et al. Application of dental pulp stem cells in oral maxillofacial tissue engineering. International Journal of Medical Sciences. 2022;**19**(2):310-320. DOI: 10.7150/ijms.68494

[20] Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. Stem Cell Research & Therapy. 2021;**12**(1):192. DOI: 10.1186/s13287-021-02265-1

[21] Bernardo ME, Fibbe WE. Mesenchymal stromal cells: Sensors and switchers of inflammation. Cell Stem Cell. 2013;**13**(4):392-402. DOI: 10.1016/j.stem.2013.09.006

[22] Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: A phase II study. Lancet. 2008;**371**(9624):1579-1586. DOI: 10.1016/s0140-6736(08)60690-x

[23] Kadri N, Amu S, Iacobaeus E, Boberg E, Le Blanc K. Current perspectives on mesenchymal stromal cell therapy for graft versus host disease. Cellular & Molecular Immunology. 2023; **20**(6):613-625. Available from: https://www.nature.com/articles/s41423-023-01022-z

[24] Li W, Liu Q, Shi J, Xu X, Xu J. The role of TNF-α in the fate regulation and functional reprogramming of mesenchymal stem cells in an inflammatory microenvironment. Frontiers in Immunology. 2023;**14**: 1074863. DOI: 10.3389/fimmu.2023.1074863

[25] Manneken JD, Currie PD. Macrophage-stem cell crosstalk: Regulation of the stem cell niche. Development [Internet]. 2023;**150**(8): dev201510. Available from: https://journals.biologists.com/dev/article-abstract/150/8/dev201510/307318

[26] Kulesza A, Paczek L, Burdzinska A. The role of COX-2 and PGE2 in the regulation of immunomodulation and other functions of mesenchymal stromal

cells. Biomedicine. 2023;**11**(2):445. Available from: https://pubmed.ncbi.nlm.nih.gov/36830980

[27] Jin L, Deng Z, Zhang J, Yang C, Liu J, Han W, et al. Mesenchymal stem cells promote type 2 macrophage polarization to ameliorate the myocardial injury caused by diabetic cardiomyopathy. Journal of Translational Medicine [Internet]. 2019;**17**(1):251. Available from: https://pubmed.ncbi.nlm.nih.gov/31382970/

[28] Giacomini C, Granéli C, Hicks R, Dazzi F. The critical role of apoptosis in mesenchymal stromal cell therapeutics and implications in homeostasis and normal tissue repair. Cellular & Molecular Immunology. 2023;**20**(6):570-582. Available from: https://www.nature.com/articles/s41423-023-01018-9

[29] Yudhawati R, Shimizu K. PGE2 produced by exogenous MSCs promotes immunoregulation in ARDS induced by highly pathogenic influenza a through activation of the wnt-β-catenin signaling pathway. International Journal of Molecular Sciences. 2023;**24**(8):7299. Available from: https://www.mdpi.com/1422-0067/24/8/7299

[30] Hisanaga M, Tsuchiya T, Watanabe H, Shimoyama K, Iwatake M, Tanoue Y, et al. Adipose-derived mesenchymal stem cells attenuate immune reactions against pig decellularized bronchi engrafted into rat tracheal defects. Organogenesis. 2023;**19**(1):2212582. DOI: 10.1080/15476278.2023.2212582

[31] Wu X, Jiang J, Gu Z, Zhang J, Chen Y, Liu X. Mesenchymal stromal cell therapies: Immunomodulatory properties and clinical progress. Stem Cell Research & Therapy. 2020;**11**(1):345. Available from: https://pubmed.ncbi.nlm.nih.gov/32771052/

[32] Li P, Ou Q, Shi S, Shao C. Immunomodulatory properties of mesenchymal stem cells/dental stem cells and their therapeutic applications. Cellular & Molecular Immunology. 2023;**20**(6):558-569. Available from: https://www.nature.com/articles/s41423-023-00998-y

[33] Alarcón-Apablaza J, Prieto R, Rojas M, Fuentes R. Potential of oral cavity stem cells for bone regeneration: A scoping review. Cells [Internet]. 2023;**12**(10):1392. Available from: https://www.mdpi.com/2073-4409/12/10/1392

[34] Chen R, Feng T, Cheng S, Chen M, Li Y, Yu Z, et al. Evaluating the defect targeting effects and osteogenesis promoting capacity of exosomes from 2D- and 3D-cultured human adipose-derived stem cells. Nano Today. 2023;**49**(101789):101789. Available from: https://www.sciencedirect.com/science/article/pii/S1748013223000385

[35] Gholamalizadeh A, Nazifkerdar S, Safdarian N, Ziaee AE, Mobedi H, Rahbarghazi R, et al. Critical elements in tissue engineering of craniofacial malformations. Regenerative Medicine. 2023;**18**(6):487-504. DOI: 10.2217/rme-2022-0128

[36] Ariano A, Posa F, Storlino G, Mori G. Molecules inducing dental stem cells differentiation and bone regeneration: State of the art. International Journal of Molecular Sciences. 2023;**24**(12):9897. Available from: https://www.mdpi.com/1422-0067/24/12/9897

[37] Rodriguez ADR, Chavez JAG, Rodriguez-Chavez JA, Curiel KM, Gonzalez RC, Gonzalez DEB. Colocación de implante en zona estética y regeneración de tejidos blandos utilizando técnica VISTA. Revista Odontológica Mexicana [Internet]. 2023;**26**(1):87-98. Available from: https://

www.medigraphic.com/cgi-bin/new/resumen.cgi?IDARTICULO=110536

[38] Banimohamad-Shotorbani B, Karkan SF, Rahbarghazi R, Mehdipour A, Jarolmasjed S, Saghati S, et al. Application of mesenchymal stem cell sheet for regeneration of craniomaxillofacial bone defects. Stem Cell Research & Therapy. 2023;**14**(1):68. DOI: 10.1186/s13287-023-03309-4

[39] Ramezanzade S, Aeinehvand M, Ziaei H, Khurshid Z, Keyhan SO, Fallahi HR, et al. Reconstruction of critical sized maxillofacial defects using composite allogeneic tissue engineering: Systematic review of current literature. Biomimetics (Basel). 2023;**8**(2):142. Available from: https://www.mdpi.com/2313-7673/8/2/142

[40] Zhao J, Zhou Y-H, Zhao Y-Q, Gao Z-R, Ouyang Z-Y, Ye Q, et al. Oral cavity-derived stem cells and preclinical models of jaw-bone defects for bone tissue engineering. Stem Cell Research & Therapy. 2023;**14**(1):39. DOI: 10.1186/s13287-023-03265-z

Chapter 6

Emerging Role and Therapeutic Application of Mesenchymal Stem Cell (MSC) and MSC-Derived Exosome in Coronavirus Disease-2019 (COVID-19) Infection

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Abstract

Over the past few years, the coronavirus disease-2019 (COVID-19) pandemic has infected billions of people worldwide. Most patients infected with COVID-19 present with fever, cough, headache, fatigue, and other clinical manifestations. For elderly patients or people with low immunity and underlying diseases, it is frequent to develop into severe or critical illness, which may even lead to multiple organ failure and death. Symptomatic treatment remains the most common treatment for patients with severe COVID-19 infection, whereas the effectiveness is limited. A large number of studies have shown that mesenchymal stem cells (MSCs) can inhibit viral growth, enhance tissue repair, and reduce inflammation, infection-induced cytokine storm, and multi-organ failure by secreting a variety of paracrine factors. In this paper, we summarized current relevant research, describe the mechanism of action and therapeutic effect of MSCs in patients with severe COVID-19 infection-related diseases, and discuss the therapeutic potential of MSCs and their exosome derivatives in patients with critical infections.

Keywords: COVID-19, SARS-CoV-2, CARDS, mesenchymal stem cell, exosome

1. Introduction

Expanding of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused a novel epidemic of coronavirus disease worldwide, which is named with

coronavirus disease 2019 (COVID-19) on account that it was firstly reported in 2019. SARS-CoV-2 belongs to the order Nidovirales of the β-coronavirus genus and is a class of enveloped single plus-stranded RNA viruses [1]. Humans of all ages are susceptible to SARS-CoV-2, while the virus can be transmitted via air, contact through nose, mouth, eye mucosa of infected patients, or inhalation of droplets of infected patients [2, 3]. The incubation period of SARS-CoV-2 virus is 3–14 days, then patients may present with typical symptoms, including fever, dry cough, dyspnea, fatigue, along with decreased white blood cell count, and obvious lesions in lung [4].

Invasion of SARS-CoV-2 is initiated with combination between viral S protein with the major receptor and angiotensin-converting enzyme 2 (ACE2) [5]. ACE2 is normally enriched in respiratory epithelial cells (e.g., type II alveolar epithelial cells) and capillary cells (e.g., endothelial cells), endowing these sites as major targets of the SARS-CoV-2. After initial recognition, activation of the fibrillin and decreased pH of endosomes trigger fusion of viral envelope to endosomal membrane, followed with entry of SARS-CoV-2 genetic material into cytosol to launch transcription and replication [6]. The newly synthesized RNA is subsequently transported to endoplasmic reticulum and Golgi apparatus, to be assembled with structural proteins before release as vesicles [7].

During the progress of SARS-CoV-2 infection, the abnormal activation and recruitment of immune cells promote release of a large number of cytokines, resulting in pulmonary inflammation, fibrosis, cell apoptosis, and alveolar fluid accumulation, eventually leading to respiratory failure and even multiple organ failure in severe patients [8]. Compared to healthy individuals, serum levels of inflammatory factors, such as interleukin (IL)-2, IL-7, granulocyte colony-stimulating factor (GCSF), monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor (TNF)-α in patients with COVID-19 admitted to ICU, were significantly elevated [9]. A stereotyped bronchiolar-alveolar pattern of lung remodeling was observed in lung tissue of patients with COVID-19, presented with symptoms including basal epithelial cell hyperplasia, mucinous differentiation, and immune activation [10]. Since the pathogenesis of severe COVID-19 is complicated and rapidly progressing, it is urgent to explore more effective therapeutic options in addition to active symptomatic treatment.

2. Introduction of mesenchymal stem cells (MSCs)

In 1966, Friedenstein et al. found that fibroblast-like cells obtained from mouse bone marrow would differentiate into bone cells when transplanted subcutaneously, which are named with mesenchymal stem cells (MSCs) [11]. In addition to be derived from bone marrow, MSCs can also be derived from other tissues such as adipose tissue, placenta, and umbilical cord [12]. MSCs are marked with self-renew property, manifested with regeneration, and multidirectional differentiation, as well as immune modulatory and anti-inflammatory function. Previous studies have shown that MSCs can exert anti-inflammatory effects by increasing the number of lymphocytes, enhancing antigen presentation of dendritic cells (DCs), and reducing the levels of pro-inflammatory cytokines (e.g., IL-6, IL-8, TNF-α, etc.). Additionally, MSCs can affect both innate and adaptive immune cells to play an immunomodulatory role [13]. Cytokines secreted by bone marrow MSCs, including IL-10, transforming growth factor (TGF)-β, and tryptophan catabolase indoleethylamine 2, 3-dioxygenase (IDO) can inhibit the overgrowth of T cells and alter the cytokine expression

profile of T cells [14]. Proliferation, differentiation, and chemotactic properties of B cells are also reported to be affected by MSCs [15]. MSCs can also affect immune homeostasis, regulate inflammatory processes, repair damaged cells by binding to cytokines, chemokines, and cell surface molecules, and repair vascular barrier [16].

Based on the function of repairment, MSCs have been widely explored for organ regeneration and tissue repair. In the past few years, safety and efficacy of MSC-based treatment have been preliminarily confirmed in neurogenesis and a variety of diseases such as traumatic injuries, neurogenesis and traumatic injury [17], osteosarcoma [18], type 1 diabetes [19], rheumatoid arthritis [20], acute liver failure [21], and acute kidney injury [22]. The therapeutic potential of stem cells and their derivatives in severe cases and sequelae of COVID-19 have been preliminarily demonstrated. Literature and clinical trials of MSC and its derivatives-based treatment in COVID-19 infection and sequelae were comprehensively summarized, as well as the molecular mechanisms involved.

3. Molecular mechanism of MSCs-based treatment of COVID-19

3.1 Antiviral effects of MSCs

During viral infection, innate immune response plays a central defensive role. Interferon (IFN) can be autogenously synthesized, inducing a batch of intracellular interferon-stimulated genes (ISGs) [23]. Products of those ISGs include non-constitutive ISG-expressed proteins such as metallothionein (MT)1X, MT1G, serpin family G member (SERPING)1, spermidine/spermine N1-acetyltransferase (SAT)1, IFNA receptor (IFNAR) [24, 25], and constitutive ISG express proteins such as IFNA inducible protein (IFI)6, IFN stimulated gene (ISG)15, and the C-C motif chemokine ligand (CCL)2 [26]. Those ISG-encoding proteins play a targeted inhibitory role in multiple stages of the viral infection cycle such as preventing SARS-CoV-2 cell membrane invagination process, mRNA transcription, genome amplification, protein translation, virus assembly, and release [25, 27, 28]. However, previous studies have revealed that coronaviruses (such as SARS-CoV-2 and MERS-CoV) can blunt IFN-mediated antiviral responses. For example, the nonstructural protein 2 (NSP2) of SARS-CoV-2 can directly interact with GIGYF2 protein. This interaction enhances the binding of GIGYF2 to 4EHP, which is the mRNA cap-binding protein, thereby repressing the translation of the IFN-β1 mRNA [29]. Meanwhile, cells in the target site of SARS-CoV-2 initial infection, including nasal epithelial cells, induced pluripotent stem cell-derived alveolar type 2 cells (iAT2), and cardiomyocytes (iCM), manifest with weak expression of IFN or OAS-RNase L [30]. In addition, MSCs stimulated with IFN-γ express IDO, which reduces the content of TRP in cells and is involved in the inhibition of virus synthesis [31].

3.2 Anti-inflammatory and immunomodulatory effects of MSCs

Cytokine storm syndrome (CSS) is considered as the major pathogenic mechanisms of respiratory failure and multi-organ damage in patients with COVID-19 [32]. A variety of plasma cytokines and numbers of immune cells are abnormally augmented in patients with severe COVID-19, which are involved in generation of cytokine storm. Along with the persistent infiltration of monocytes and macrophages, intensive inflammation leads to atrophy of the spleen, lymph nodes, and

lymphopenia, which is also accompanied by thrombosis and multi-organ dysfunction [33]. Under this condition, MSCs can be recruited to inflammatory sites by chemokines to play a systemic immunomodulatory function through direct contact and paracrine effects. The anti-inflammatory mediators released by MSCs are specific to different pathogens, mediated by corresponding pathogen-related receptors on the surface of MSCs [34]. Under viral infection, unmethylated viral DNA or viral RNA can activate the toll-like receptor-9 (TLR-9) and TLR-3 signaling pathways, respectively, inducing synthesis and secretion of numerous inflammatory factors [35]. In the early phase of infection, the production of these factors, such as type-I IFNs limits virus propagation; whereas uncontrolled increase of them leads to aberrant inflammation in the late phase of the infection, which is associated with poor clinical outcome [36].

Correspondingly, a variety of paracrine factors secreted by MSCs show mutual influence on immune cells and improvement of COVID-19 patients' condition. For example, indoleamine 2,3-dioxygenase, transforming growth factor-β (TGF-β), human leukocyte antigen (HLA), and prostaglandin E2 (PGE2) have been identified as the main effectors [37]. Growth factors, such as keratinocyte growth factor (KGF) and angiopoietin-1 (Ang1), can promote the recovery of the disrupted alveolar-capillary barrier during COVID-19 [38]. Beside, activated TLR-4 signaling is also reported to improve regeneration in alveolar epithelial cells during fibrotic status [39].

3.3 Repair effect of MSCs on damage cell/tissue

Aside from being effective in controlling viral replication, MSCs also show recover impact on injured organs during COVID-19. Generation of inflammatory factors induced by SARS-CoV-2, such as ILs and TNF as mentioned above, contributes to severe destruction of alveolar epithelial cells and cardiac tissue damage [40]. Patients suffer from long-term cardiopulmonary damage, presenting with continuous breathlessness, coughing, fatigue, and limited exercise ability [41]. The unique homing properties of MCSs enable them to migrate those damaged tissues through blood flow [42]. On the one hand, MCSs can repair both the histology and function of those damaged tissues. In a bleomycin-induced lung injury and fibrosis mice model, MSCs home to injury area and initiate epithelioid trans-differentiation, which significantly alleviated inflammation and collagen deposition in lung tissue [42]. Through scratch experiment and co-culture experiment, MSCs are firmed to ameliorate wound healing and protect primary small airway epithelial cells, by promoting the migration and proliferation of epithelial cells [43]. In addition, MSCs are found to transfer endothelial mitochondria to damaged alveolar epithelial cells, increase alveolar adenosine triphosphate (ATP) concentration, and reduce endotoxin-induced alveolar damage, thereby facilitate lung fluid clearance [44].

On the other hand, differentiation of MSCs is found to stimulate regeneration of those damaged areas and reconstruct the microenvironment in the case of pulmonary infection [45]. This is attributed to their tolerance to cytotoxic agents and the inhibition of signaling cascades in response to lung injury, as well as the release of growth factors, anti-inflammatory cytokines, extracellular vesicles, etc. [46]. Type-2 alveolar epithelial (AT-II) cells are generated by human induced pluripotent stem cells (hiPSCs), which are applied for SARS-CoV-2 infection and drug testing [47]. iPSCs-induced endothelial cells and pneumocytes can engraft in lungs of emphysematous mice and form functional lung units to ameliorate emphysema [48].

4. Clinical progress of treatment with MSCs in COVID-19 and severe complications

During the past years, MSCs and their released products have been proved to be effective in COVID-19 therapy, based on their immunomodulatory and repair capacities [49–51]. In a systematic review about stem cell-based therapy in ARDS of COVID-19 patients, CRP levels were found to be decreased in 14 of the 17 trials (82.3%). In addition, IL-6 levels were diminished in nine (52.9%) of seventeen studies [52]. Indicators involved in severe cases, including oxygen saturation and the PaO2/FIO2 ratio, are increased in 12 out of 14 (85.7%), while the lung picture on chest CT or radiography improved in 15 out of 17 studies (88.2%) [52]. MSCs based therapy showed obvious effect in reducing cytokine storm, recovering injured alveolar epithelial cells, and facilitating tissue repair by secreting anti-inflammatory cytokines and antifibrotic growth factors [53], suppressing excessive immune responses to protect the alveolar epithelial lining during acute respiratory distress syndrome [54, 55].

4.1 Cytokine release/storm syndrome (CRS/CSS)

CRS, known as CSS, is an abnormal systemic inflammatory response triggered by a variety of factors (infection, drugs, and other factors) [56]. In many patients infected with SARS-CoV-2, types of inflammatory cytokines are sharply stimulated and released into both the pulmonary and circulatory system, including IL-2, IL-7, IL-10, G-SCF, MCP-1, MIP-1α, and TNF-α [5, 9, 57, 58]. Clinical studies have found that CRS is much more common in critical patients with COVID-19, which is corresponded to poorer outcome or deterioration, such as ARDS and multiple organ failure [59, 60]. Patients with CRS presented with massive alveolar damage, interstitial inflammation, intra-alveolar edema, fibrin and collagen deposition, bronchiolitis, and leukocyte infiltration, contributing to progressive respiratory failure [61]. Glucocorticoids and immunosuppressive agents are recommended in the current treatment of CRS as they might increase the risk of side effects, such as osteonecrosis [56].

The immune modulatory and repair function of MSCs makes them as an ideal option toward rapidly developing CRS [59]. In a single-arm pilot study that was conducted in critical patients with COVID-19, transplantation of UC-MSCs increased oxyhemoglobin saturation, and improved cytokine storm without adverse reactions, demonstrating the safety and feasibility of UC-MSCs in the treatment of COVID-19-associated CRS [62]. The study performed by Zhinian Guo et al. also showed that injection of MSCs could restore oxygenation and down-regulate cytokine storm in COVID-19 patients without risk of side reactions. The average pressure ratio of arterial partial pressure of oxygen to the fraction of inspired oxygen (PaO2/FiO2) and lymphocyte count increased, and serum C-reactive protein, procalcitonin, D-dimer and IL-6 decreased after UC-MSCs infusion in 31 COVID-19 positive patients [63]. In another study, 210 severe/critical patients with COVID-19 were transplanted with 1–2 × 10^6/kg UC-MSCs, showing recovery of oxygenated SaO2 and remission of CRS [64]. The results of these clinical studies showed that MSCs treatment could effectively reduce the levels of IL-6 and other cytokines, which proved the preliminary potential of the treatment of COVID-19-related CRS.

4.2 Acute respiratory distress syndrome (ARDS)

ARDS is an acute respiratory disease with sustained serious lung injury, characterized with increased bilateral lung texture, blurred edges, and severe hypoxemia [65].

There are about 15–30% of hospitalized patients with COVID-19 will develop into COVID-19-related acute respiratory distress syndrome (CARDS), which is closely related to occurrence of CRS [66]. Development of ARDS is rapid, while ARDS patients have of lower survival rate and quality of life compared to COVID-19 patients with mild symptoms. Due to the persistent inflammation of the lung, the permeability of alveolar endothelial cells and epithelial cells increases, resulting in accumulation of pulmonary edema fluid [67, 68]. At present, the major therapeutic option is mechanical ventilation to improve oxygenation, which shows limited influence on patient mortality in related studies [68]. Current approved therapies, such as intravenous remdesivir and dexamethasone, have a modest effect on moderate to severe COVID-19 [69].

As evidenced by clinical results, MSCs-based therapy is effective in regulating the inflammatory process, repairing epithelial and endothelial cell damage, enhancing alveolar fluid clearance, and delaying the process of ARDS [70, 71]. Injection of MSCs derived from umbilical cord (UC-MSCs), placental (PL-MSCs), and bone marrow (BM-MSCs) have been proved to be safe and reliable in patients with COVID-19 induced ARDS, without showing any severe adverse reactions in relevant clinical trials. In the experiment conducted by Antoine Monsel et al., infusion with BM-MSCs significantly increased the survival rate of ARDS patients at both 28 days and 60 days (Day28, 100%:79.2%, $p = 0.025$; Day60, 100%:70.8%, $p = 0.0082$) [72, 73]. Compared with the patients in control group, the level of D-dimer and pulmonary microcirculation thrombosis was also reduced in patients treated with BM-MSCs, indicating alleviated ventilation disorder and restored coagulation [74]. Similar therapeutic efficacy was observed in patients under UC-MSCs treatment, performed as remodeled anti-inflammatory immunity and improved patient outcome. After being injected with UC-MSCs, levels of proinflammatory cytokines, such as IL-6, IFN-γ, TNF-α, and IL-17, were diminished, while levels of anti-inflammatory cytokines such as TGF-β, IL-1β, and IL-10 were increased [75, 76]. In the trial led by Seyed-Mohammad Reza Hashemian et al., lung opacity was significantly reduced after PL-MSCs treatment as determined by computed tomography (CT), and the recovery degree was better than that of the conventional treatment group [77].

4.3 Idiopathic pulmonary fibrosis (IPF)

IPF is a chronic, progressive pulmonary fibrosis disease, attributed to abnormal ECM formation in the alveolar epithelium and disrupted lung function. Accompanied with pandemic of COVID-19, the incidence and prevalence of IPF are increasing globally. IPF is characterized by interstitial fibrosis accompanied by reduced lung volume and hypoxemic respiratory failure [78]. IPF patients manifest with dry cough, fatigue, and dyspnea [79]. In patients with COVID-19-related ARDS, fibrosis can become one of the major long-term complications, with the incidence of persistent lung injury exceeding 30% within one year, and one-third of patients have fibrotic lung injury [80], even in asymptomatic patients with COVID-19 [81, 82]. The mechanism of pulmonary fibrosis caused by COVID-19 is that chronic inflammation leads to epithelial cell damage and fibroblast activation and proliferation, excessive deposition of collagen, and other extracellular matrix (ECM) components, resulting in destruction of normal lung structure and preventing the reconstruction of damaged alveolar epithelium [83]. The progression of pulmonary fibrosis compresses normal lung parenchyma and damages capillaries, leading to respiratory failure [84]. Current treatment of COVID-19-associated IPF mainly contains Pirfenidone and Nintedanib.

Pirfenidone can inhibit production of fibrosis-related proteins or ECM to reduce the aggregation of inflammatory cells, while Nintedanib targets on a variety of tyrosine kinases to block fibroblast proliferation. However, patients' tolerance of them is not satisfactory [85].

The testing of MSCs on IPF patients is positive. Animal experiments have shown that intravenously transplanted MSCs can accumulate in lung tissue, protecting alveolar epithelial cells and restoring the microenvironment in lung [77]. In bleomycin-induced pulmonary fibrotic mice, MSCs reduced the accumulation of collagen and matrix metalloproteinase through augmenting IL-1RA production [86]. IL-1RA is a cytokine that competitively binds with IL-1b, while IL-1b serves as the major inflammatory cytokines in pulmonary edema fluid of patients with ARDS [87]. Several clinical studies have proved that fibrosis-related proteins in the lung tissue are markedly lower in the MSCs treatment group than that of the control group (all $P < 0.05$) [88]. Forced vital capacity (FVC), carbon monoxide diffusing capacity (DLCO), and other indicators of pulmonary function are reduced in IPF patients [89]. In the clinical trial performed by Daniel C. Chambers et al., treatment with placenta-derived MSC prevents fibrotic progress in patients with COVID-19-associated IPF. Compared to baseline, there is no deterioration of FVC, DLCO, and radiological scoring at 6 months posttreatment [90, 91]. However, due to the limited number of clinical trials, the safety of MSCs in the treatment of IPF needs to be further verified.

5. MSCs-derived exosomes for COVID treatment

A large number of studies have shown that the therapeutic effect of MSCs in repair is mainly attributed to paracrine signals, including secreting extracellular vesicles (EVs), especially exosomes [92]. Exosomes are lipid bilayer vesicles with a typical diameter of 30 to 200 nm, packaged with biomolecules such as cytoskeletal proteins and signal transduction proteins, as well as a variety of nucleic acid components such as messenger RNA (mRNA), ribosomal RNA (rRNA), and microRNA (miRNA) [93]. As evidenced previously exosomes have the advantages in safety and expense over MSCs [94]. Beside, based on its carriage property and nano-size, exosomes can be used as an ideal loading vehicle for deliver functional components in COVID-19 patients.

A number of preclinical studies have shown that exosomes have good therapeutic effects in animal models of inflammatory diseases such as acute lung injury (ALI), ARDS [95], and IPF [93]. MSC-derived exosomes can improve indices of ALI and reduce total proteins in extravascular lung water and bronchoalveolar lavage in a KGF-mediated mechanism [96]. In a clinical study, 24 patients with severe COVID-19 were treated with exosomes derived from allogeneic BM-MSCs. At 72 hours after injection, their clinical condition was remised with restored oxygenation and downregulated CRS [97]. In the clinical trial performed by Meiping Chu et al., patients with COVID-19-associated pneumonia who received aerosolized MSCs-derived exosomes showed reduced CRP levels, improved lung lesion absorption [98]. In another clinical trial, seven patients with severe COVID-19-related pneumonia received nebulized human adipose (haMSC)-derived exosomes. All patients showed well tolerance to haMSC-Exos and with different degrees of resolution of pulmonary lesions [99]. Therefore, exosomes derived from MSCs may become a superior therapeutic tool for COVID-19 than MSCs [100]. Although exosomes have shown a good prospect in clinical experiments, the standard of isolating and verifying exosomes, especially obtained from different origins, must be established for quality ensurance.

6. The bottleneck of MSCs application in the treatment of severe COVID-19 patients

Despite the advantages described above for MSCs and exosomal products in COVID-19, many questions remain to be addressed before further application. First of all, the characterization of MSCs is a common problem of MSCs for clinical use. Lack of unified molecular markers makes it difficult to ensure the consistency and stability of MSCs [101]. Secondly, whether MSCs can be infected or hijacked by virus is also controversial. Although sequencing result demonstrated extremed low expression of ACE2 on MSCs, suggesting that MSCs are not suitable targets of SARS-CoV-2 virus [38]. However, there are also studies reported that in the inflammatory microenvironment of COVID-19, expression of ACE2 in MSCs can be abnormally stimulated by IFN expression, leading to an increased risk of viral infection and a detrimental effect on MSCs [102]. In addition, the survival rate of MSCs in damaged tissue areas and the low transplantation potential limit the effectiveness of MSCS in tissue repair [103]. Safety (potential tumorigenicity), cost in isolation, and storage restrict its application in patients infected with SARS-CoV-2 [104, 105].

7. Summary and prospect

Based on preclinical investigation and ongoing clinical trials (as summarized in **Table 1**), MSCs have shown great immune-modulatory ability in reforming unbalanced immune system, recovering lung tissue from immune damage or CRS, and improving the physical condition of patients with severe COVID-19. However, there are still limitations of MSCs or MSCs-derived exosome-based treatment in terms of lack of homogeneity markers and transplantation efficacy. Whether MSCs can be infected and damaged by viruses is still suspending. MSCs treatment could be considered as a potential candidate for patients with COVID-19, especially for cases suffering from severe symptoms, such as CARDS.

Trial ID	Source of MSCs	Phase	Numbers of patients	Doses and administration routes	Primary outcomes	Reference
NCT04313322	WJ-MSCs	1	5	Three doses of 1 X 10^6 cells/kg; IV	Improved clinical symptoms including fever, cough, and respiratory distress.	NA
NCT04252118	UC-MSCs	1	20	Three doses of 3 X 10^7 cells; IV	Safe and well tolerated.	[106]
NCT04355728	UC-MSCs	2	24	Two doses of 1 10^7 cells; IV	Safe and well tolerated. Improved oxygenation index and PEEP.	NA

Trial ID	Source of MSCs	Phase	Numbers of patients	Doses and administration routes	Primary outcomes	Reference
NCT04400032	UC-MSCs	1&2	15	Panel 1: Three doses of 25 X 10^6 cells; IV Panel 2: Three doses of 50 X 10^6 cells; IV Panel 3: Three doses of 90 X 10^6cells; IV	NA	NA
NCT04898088	MSCs	NA	30	Three doses; IV	NA	NA
NCT04625738	WJ-MSCs	2	30	day 0: 1 X 10^6 cells/kg; IV day 3: 0.5 X 10^6 cells/kg; IV day 5: 0.5 X 10^6 cells/kg; IV	NA	NA
NCT04399889	hCT-MSCs	1&2	12	Phase 1: Three doses of 1 X 10^6 cells/kg; IV Phase 2: One dose of 1 X 10^8 cells/kg; IV	Safe and well tolerated.	NA
NCT04333368	UC-MSCs	1&2	47	Three doses of 1 X 10^6 cells/kg; IV	NA	[107]
NCT04457609	UC-MSCs	1	40	One dose of 1 X 10^6 cells/kg; IV	NA	NA
NCT04269525	UC-MSCs	2	16	Four doses of 1 X 10^8 cells; IV	No adverse effects. Improved oxygenation index and lymphocyte count.	[108]
NCT04753476	Secretome-MSCs	2	48	1 cc every 12 hours for three days(Dosage not mentioned)	NA	NA
NCT04352803	AD-MSCs	1	20	5 X 10^6 cells/kg; IV	NA	NA
ChiCTR 2000029606	MB-MSCs	1	44	Three doses of 3 x10^7 cells; IV	Safe and well tolerated. Improved SpO2 and chest imaging.	[63]
NCT04416139	UC-MSCs	1	5	One dose of 1 x 10° cells /kg; IV	Improved PaO_2/FiO_2.	[63]

Trial ID	Source of MSCs	Phase	Numbers of patients	Doses and administration routes	Primary outcomes	Reference
NCT04392778	UC-MSCs	1&2	30	Three doses of 3 X 10^6 cells/ kg; IV	NA	NA
NCT04339660	UC-MSCs	2	58	One dose of 1 × 10^6 cells / kg; IV	Safe and well tolerated. Improved clinical symptoms, values of inflammatory parameters, and CT scan.	[63]
ChiCTR 2000029990	MSCs	1	10	1 X 10^6 cells/ kg; IV	Improved pulmonary function.	[106]
ChiCTR 2000031494	UC-MSCs	1	41	2 X 10^6 cells/ kg; IV	Clinical improvement.	[109]
NCT04288102	UC-MSCs	2	100	Three doses of 4 x10^7 cells; IV	Safe and well tolerated; Increased distance in 6MWD.	[110]
NA	UC-MSCs	1	9	A single dose of 1 × 10^6 cells or 5 × 10^6 cells or 1 × 10^7 cells;IV	Well tolerated; without serious adverse events.	[111]
NCT03042143	UC-MSCs	1	9	A single dose of 1 or 2 or 4 × 108; IV	Well tolerated. Adverse events included apyrexia, non-sustained ventricular tachycardia, and deranged liver function.	[112]
NA	BM-MSCs	Case report	2	A single dose of 2 × 106 cells/ kg; IV	Improved oxygenation and pulmonary compliance; reduced inflammation markers.	[113]
NA	UC-MSCs	1	31	1 × 10^6 cells/ kg; IV	No adverse events. Improved laboratory parameters.	[63]
NCT04276987	MSCs-derived exosomes	1	24	5 doses of 2 × 10^8 nanovesicles	Safe and well tolerated.	[114]

Trial ID	Source of MSCs	Phase	Numbers of patients	Doses and administration routes	Primary outcomes	Reference
NCT04491240	MSCs-derived exosomes	1&2		20 doses of $0.5–2 \times 10^{10}$ exosomes	No adverse effects.	NA

Abbreviations: MSCs: Mesenchymal stem cells, WJ-MSCs: Wharton's Jelly mesenchymal stem cells, UC-MSCs: Umbilical cord blood-derived mesenchymal stem cells, AD-MSCs: Adipose-derived mesenchymal stem cells, MB-MSCs: Menstrual blood-derived mesenchymal stem cells; hCT-MSCs: Human cord tissue mesenchymal stromal cells, BM-MSCs: Bone marrow-derived mesenchymal stem cells, MSCs-derived exosomes: Mesenchymal stem cells-derived exosomes. NA: Not available.

Table 1.
Summarization of clinical trials on COVID-19 patients treated with MSCs.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

[1] Shahrajabian MH, Sun W, Cheng Q. Product of natural evolution (SARS, MERS, and SARS-CoV-2); deadly diseases, from SARS to SARS-CoV-2. Human Vaccines & Immunotherapeutics. 2021;**17**:62-83

[2] Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: A study of a family cluster. Lancet. 2020;**395**:514-523

[3] Li H, Liu Z, Ge J. Scientific research progress of COVID-19/SARS-CoV-2 in the first five months. Journal of Cellular and Molecular Medicine. 2020;**24**:6558-6570

[4] Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): A review. JAMA. 2020;**324**:782-793

[5] Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. Journal of Autoimmunity. 2020;**109**:102433

[6] Schelker M, Mair CM, Jolmes F, Welke RW, Klipp E, Herrmann A, et al. Viral RNA degradation and diffusion act as a bottleneck for the influenza a virus infection efficiency. PLoS Computational Biology. 2016;**12**:e1005075

[7] Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020;**367**:1260-1263

[8] Rodriguez HC, Gupta M, Cavazos-Escobar E, El-Amin SF 3rd, Gupta A. Umbilical cord: An allogenic tissue for potential treatment of COVID-19. Human Cell. 2021;**34**:1-13

[9] Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;**395**:497-506

[10] Wu K, Zhang Y, Austin SR, Yin-Declue H, Byers DE, Crouch EC, et al. Lung Remodeling regions in Long-term coronavirus disease 2019 feature basal epithelial cell reprogramming. The American Journal of Pathology. 2023;**193**:680-689

[11] Abdelgawad M, Bakry NS, Farghali AA, Abdel-Latif A, Lotfy A. Mesenchymal stem cell-based therapy and exosomes in COVID-19: Current trends and prospects. Stem Cell Research & Therapy. 2021;**12**:469

[12] Askenase PW. COVID-19 therapy with mesenchymal stromal cells (MSC) and convalescent plasma must consider exosome involvement: Do the exosomes in convalescent plasma antagonize the weak immune antibodies? Journal of Extracellular Vesicles. 2020;**10**:e12004

[13] Basiri A, Mansouri F, Azari A, Ranjbarvan P, Zarein F, Heidari A, et al. Stem cell therapy potency in personalizing severe COVID-19 treatment. Stem Cell Reviews and Reports. 2021;**17**:193-213

[14] Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005;**105**:1815-1822

[15] Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, et al. Human mesenchymal stem cells

modulate B-cell functions. Blood. 2006;**107**:367-372

[16] Goolaerts A, Pellan-Randrianarison N, Larghero J, Vanneaux V, Uzunhan Y, Gille T, et al. Conditioned media from mesenchymal stromal cells restore sodium transport and preserve epithelial permeability in an in vitro model of acute alveolar injury. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2014;**306**:L975-L985

[17] Yang Y, Ye Y, Su X, He J, Bai W, He X. MSCs-derived exosomes and Neuroinflammation, neurogenesis and therapy of traumatic brain injury. Frontiers in Cellular Neuroscience. 2017;**11**:55

[18] Chang X, Ma Z, Zhu G, Lu Y, Yang J. New perspective into mesenchymal stem cells: Molecular mechanisms regulating osteosarcoma. Journal of Bone Oncology. 2021;**29**:100372

[19] Izadi M, Sadr Hashemi Nejad A, Moazenchi M, Masoumi S, Rabbani A, Kompani F, et al. Mesenchymal stem cell transplantation in newly diagnosed type-1 diabetes patients: A phase I/II randomized placebo-controlled clinical trial. Stem Cell Research & Therapy. 2022;**13**:264

[20] Kannan S, Viswanathan P, Gupta PK, Kolkundkar UK. Characteristics of pooled Wharton's jelly mesenchymal stromal cells (WJ-MSCs) and their potential role in rheumatoid arthritis treatment. Stem Cell Reviews and Reports. 2022;**18**:1851-1864

[21] Shokravi S, Borisov V, Zaman BA, Niazvand F, Hazrati R, Khah MM, et al. Mesenchymal stromal cells (MSCs) and their exosome in acute liver failure (ALF): A comprehensive review. Stem Cell Research & Therapy. 2022;**13**:192

[22] Ji C, Zhang J, Zhou Z, Shi H, Liu W, Sun F, et al. Platelet-rich plasma promotes MSCs exosomes paracrine to repair acute kidney injury via AKT/Rab27 pathway. American Journal of Translational Research. 2021;**13**:1445-1457

[23] Michalska A, Blaszczyk K, Wesoly J, Bluyssen HAR. A positive feedback amplifier circuit that regulates interferon (IFN)-stimulated gene expression and controls type I and type II IFN responses. Frontiers in Immunology. 2018;**9**:1135

[24] Lu Y, Michel HA, Wang PH, Smith GL. Manipulation of innate immune signaling pathways by SARS-CoV-2 non-structural proteins. Frontiers in Microbiology. 2022;**13**:1027015

[25] Martin-Sancho L, Lewinski MK, Pache L, Stoneham CA, Yin X, Becker ME, et al. Functional landscape of SARS-CoV-2 cellular restriction. Molecular Cell. 2021;**81**:2656-2668.e2658

[26] Holthaus D, Vasou A, Bamford CGG, Andrejeva J, Paulus C, Randall RE, et al. Direct antiviral activity of IFN-stimulated genes is responsible for resistance to paramyxoviruses in ISG15-deficient cells. Journal of Immunology. 2020;**205**:261-271

[27] Wong NA, Saier MH Jr. The SARS-coronavirus infection cycle: A survey of viral membrane proteins, their functional interactions and pathogenesis. International Journal of Molecular Sciences. 2021;**22**:1308-1371

[28] Gu W, Gan H, Ma Y, Xu L, Cheng ZJ, Li B, et al. The molecular mechanism of SARS-CoV-2 evading host antiviral innate immunity. Virology Journal. 2022;**19**:49

[29] Xu Z, Choi JH, Dai DL, Luo J, Ladak RJ, Li Q, et al. SARS-CoV-2

impairs interferon production via NSP2-induced repression of mRNA translation. In: Proceedings of the National Academy of Sciences of the United States of America. 2022;**119**:e2204539119

[30] Li Y, Renner DM, Comar CE, Whelan JN, Reyes HM, Cardenas-Diaz FL, et al. SARS-CoV-2 induces double-stranded RNA-mediated innate immune responses in respiratory epithelial-derived cells and cardiomyocytes. In: Proceedings of the National Academy of Sciences of the United States of America. 2021;**118**(16):e2022643118

[31] Rocha JLM, de Oliveira WCF, Noronha NC, Dos Santos NCD, Covas DT, Picanço-Castro V, et al. Mesenchymal stromal cells in viral infections: Implications for COVID-19. Stem Cell Reviews and Reports. 2021;**17**:71-93

[32] Tang Y, Liu J, Zhang D, Xu Z, Ji J, Wen C. Cytokine storm in COVID-19: The current evidence and treatment strategies. Frontiers in Immunology. 2020;**11**:1708

[33] Chen W, Pan JY. Anatomical and pathological observation and analysis of SARS and COVID-19: Microthrombosis is the Main cause of death. Biological Procedures Online. 2021;**23**:4

[34] Liotta F, Angeli R, Cosmi L, Filì L, Manuelli C, Frosali F, et al. Toll-like receptors 3 and 4 are expressed by human bone marrow-derived mesenchymal stem cells and can inhibit their T-cell modulatory activity by impairing notch signaling. Stem Cells. 2008;**26**:279-289

[35] Lai JH, Wang MY, Huang CY, Wu CH, Hung LF, Yang CY, et al. Infection with the dengue RNA virus activates TLR9 signaling in human dendritic cells. EMBO Reports. 2018;**19**(8):e46182

[36] Domizio JD, Gulen MF, Saidoune F, Thacker VV, Yatim A, Sharma K, et al. The cGAS-STING pathway drives type I IFN immunopathology in COVID-19. Nature. 2022;**603**:145-151

[37] Zhou Y, Yamamoto Y, Xiao Z, Ochiya T. The immunomodulatory functions of mesenchymal stromal/stem cells mediated via paracrine activity. Journal of Clinical Medicine. 2019;**8**(7):1025-1041

[38] Khoury M, Rocco PRM, Phinney DG, Krampera M, Martin I, Viswanathan S, et al. Cell-based therapies for coronavirus disease 2019: Proper clinical investigations are essential. Cytotherapy. 2020;**22**:602-605

[39] Liang J, Zhang Y, Xie T, Liu N, Chen H, Geng Y, et al. Hyaluronan and TLR4 promote surfactant-protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. Nature Medicine. 2016;**22**:1285-1293

[40] Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. Lancet. 2020;**395**:1054-1062

[41] D'Agnillo F, Walters KA, Xiao Y, Sheng ZM, Scherler K, Park J, et al. Lung epithelial and endothelial damage, loss of tissue repair, inhibition of fibrinolysis, and cellular senescence in fatal COVID-19. Science Translational Medicine. 2021;**13**:eabj7790

[42] Zhang LS, Yu Y, Yu H, Han ZC. Therapeutic prospects of mesenchymal stem/stromal cells in COVID-19 associated pulmonary diseases: From bench to bedside. World Journal of Stem Cells. 2021;**13**:1058-1071

[43] Gindele JA, Mang S, Pairet N, Christ I, Gantner F, Schymeinsky J, et al. Opposing effects of in vitro differentiated macrophages sub-type on epithelial wound healing. PLoS One. 2017;**12**:e0184386

[44] Hough RF, Islam MN, Gusarova GA, Jin G, Das S, Bhattacharya J. Endothelial mitochondria determine rapid barrier failure in chemical lung injury. JCI Insight. 2019;**4**(3):e124329

[45] Sagaradze GD, Basalova NA, Efimenko AY, Tkachuk VA. Mesenchymal stromal cells as critical contributors to tissue regeneration. Frontiers in Cell and Development Biology. 2020;**8**:576176

[46] Li J, Wang X, Li N, Jiang Y, Huang H, Wang T, et al. Feasibility of mesenchymal stem cell therapy for COVID-19: A mini review. Current Gene Therapy. 2020;**20**:285-288

[47] Tsuji K, Yamada S, Hirai K, Asakura H, Kanda Y. Development of alveolar and airway cells from human iPS cells: Toward SARS-CoV-2 research and drug toxicity testing. The Journal of Toxicological Sciences. 2021;**46**:425-435

[48] Altalhi W, Wu T, Wojtkiewicz GR, Jeffs S, Miki K, Ott HC. Intratracheally injected human-induced pluripotent stem cell-derived pneumocytes and endothelial cells engraft in the distal lung and ameliorate emphysema in a rat model. The Journal of Thoracic and Cardiovascular Surgery. 2023;**166**:e23-e37

[49] Kirkham AM, Monaghan M, Bailey AJM, Shorr R, Lalu MM, Fergusson DA, et al. Mesenchymal stem/stromal cell-based therapies for COVID-19: First iteration of a living systematic review and meta-analysis: MSCs and COVID-19. Cytotherapy. 2022;**24**:639-649

[50] Gorman E, Millar J, McAuley D, O'Kan C. Mesenchymal stromal cells for acute respiratory distress syndrome (ARDS), sepsis, and COVID-19 infection: Optimizing the therapeutic potential. Expert Review of Respiratory Medicine. 2021;**15**:301-324

[51] Kun L, Geng S-T, Tang S, Yang H, Xiong W, Fang X, et al. Clinical efficacy and mechanism of mesenchymal stromal cells in treatment of COVID-19. Stem Cell Research & Therapy. 2022;**13**:022-02743

[52] Zanirati G, Provenzi L, Libermann LL, Bizotto SC, Ghilardi IM, Marinowic DR, et al. Stem cell-based therapy for COVID-19 and ARDS: A systematic review. NPJ Regenerative Medicine. 2021;**6**:021-00181

[53] Rezakhani L, Kelishadrokhi AF, Soleimanizadeh A, Rahmati S. Mesenchymal stem cell (MSC)-derived exosomes as a cell-free therapy for patients infected with COVID-19: Real opportunities and range of promises. Chemistry and Physics of Lipids. 2021;**234**:105009

[54] Lin F, Ichim TE, Pingle S, Jone LD, Kesari S, Ashili S. Mesenchymal stem cells as living anti-inflammatory therapy for COVID-19 related. World Journal of Stem Cells. 2020;**12**:1067-1079

[55] Zimecki M, Actor JK, Kruzel ML. The potential for Lactoferrin to reduce SARS-CoV-2 induced cytokine storm. International Immunopharmacology. 2021;**95**:107571-107579

[56] Li Z, Niu S, Guo B, Gao T, Wang L, Wang Y, et al. Stem cell therapy for COVID-19, ARDS and pulmonary fibrosis. Cell Proliferation. 2020;**53**:e12939

[57] Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, Katze MG. Into the eye of the cytokine storm. Microbiology and Molecular Biology Reviews. 2012;**76**:16-32

[58] Sun Y, Zou Y, Wang H, Cui G, Yu Z, Ren Z. Immune response induced by novel coronavirus infection. Frontiers in Cellular and Infection Microbiology. 2022;**12**:988604

[59] Ye Q, Wang B, Mao J. The pathogenesis and treatment of the; cytokine Storm' in COVID-19. The Journal of Infection. 2020;**80**:607-613

[60] Uccelli A, de Rosbo NK. The immunomodulatory function of mesenchymal stem cells: Mode of action and pathways. Annals of the New York Academy of Sciences. 2015;**1351**:114-126

[61] Que Y, Hu C, Wan K, Hu P, Wang R, Luo J, et al. Cytokine release syndrome in COVID-19: A major mechanism of morbidity and mortality. International Reviews of Immunology. 2022;**41**:217-230

[62] Feng Y, Huang J, Wu J, Xu Y, Chen B, Jiang L, et al. Safety and feasibility of umbilical cord mesenchymal stem cells in patients with COVID-19 pneumonia: A pilot study. Cell Proliferation. 2020;**53**:e12947

[63] Guo Z, Chen Y, Luo X, He X, Zhang Y, Wang J. Administration of umbilical cord mesenchymal stem cells in patients with severe COVID-19 pneumonia. Critical Care. 2020;**24**:420

[64] Ercelen N O, Pekkoc-Uyanik KC, Alpaydin N, Gulay GR, Simsek M. Clinical experience on umbilical cord mesenchymal stem cell treatment in 210 severe and critical COVID-19 cases in Turkey. Stem Cell Reviews and Reports. 2021;**17**:1917-1925

[65] Meyer NJ, Gattinoni L, Calfee CS. Acute respiratory distress syndrome. Lancet. 2021;**398**:622-637

[66] Attaway AH, Scheraga RG, Bhimraj A, Biehl M, Hatipoğlu U. Severe covid-19 pneumonia: Pathogenesis and clinical management. BMJ. 2021;**372**:n436

[67] Huppert LA, Matthay MA, Ware LB. Pathogenesis of acute respiratory distress syndrome. Seminars in Respiratory and Critical Care Medicine. 2019;**40**:31-39

[68] Swenson KE, Ruoss SJ, Swenson ER. The pathophysiology and dangers of silent hypoxemia in COVID-19 lung injury. Annals of the American Thoracic Society. 2021;**18**:1098-1105

[69] Asselah T, Durantel D, Pasmant E, Lau G, Schinazi RF. COVID-19: Discovery, diagnostics and drug development. Journal of Hepatology. 2021;**74**:168-184

[70] Xiao K, Hou F, Huang X, Li B, Qian ZR, Xie L. Mesenchymal stem cells: Current clinical progress in ARDS and COVID-19. Stem Cell Research & Therapy. 2020;**11**:305

[71] Al-Khawaga S, Abdelalim EM. Potential application of mesenchymal stem cells and their exosomes in lung injury: An emerging therapeutic option for COVID-19 patients. Stem Cell Research & Therapy. 2020;**11**:437

[72] Monsel A, Hauw-Berlemont C, Mebarki M, Heming N, Mayaux J, Nguekap Tchoumba O, et al. Treatment of COVID-19-associated ARDS with mesenchymal stromal cells: A multicenter randomized double-blind trial. Critical Care. 2022;**26**:48

[73] Cheng H, Wang Y, Wang GQ. Organ-protective effect of angiotensin-converting enzyme 2 and its effect on the prognosis of COVID-19. Journal of Medical Virology. 2020;**92**:726-730

[74] Bonaventura A, Vecchie A, Dagna L, Martinod K, Dixon DL, Van Tassell BW,

et al. Endothelial dysfunction and immunothrombosis as key pathogenic mechanisms in COVID-19. Nature Reviews. Immunology. 2021;**21**:319-329

[75] Lanzoni G, Linetsky E, Correa D, Messinger Cayetano S, Alvarez RA, Kouroupis D, et al. Umbilical cord mesenchymal stem cells for COVID-19 acute respiratory distress syndrome: A double-blind, phase 1/2a, randomized controlled trial. Stem Cells Translational Medicine. 2021;**10**:660-673

[76] Dilogo IH, Aditianingsih D, Sugiarto A, Burhan E, Damayanti T, Sitompul PA, et al. Umbilical cord mesenchymal stromal cells as critical COVID-19 adjuvant therapy: A randomized controlled trial. Stem Cells Translational Medicine. 2021;**10**:1279-1287

[77] Hashemian SR, Aliannejad R, Zarrabi M, Soleimani M, Vosough M, Hosseini SE, et al. Mesenchymal stem cells derived from perinatal tissues for treatment of critically ill COVID-19-induced ARDS patients: A case series. Stem Cell Research & Therapy. 2021;**12**:91

[78] Lechowicz K, Drożdżal S, Machaj F, Rosik J, Szostak B, Zegan-Barańska M, et al. COVID-19: The potential treatment of pulmonary fibrosis associated with SARS-CoV-2 infection. Journal of Clinical Medicine. 2020;**9**(6):1917-1936

[79] Uzel FI, Iliaz S, Karatas F, Caglayan B. COVID-19 pneumonia and idiopathic pulmonary fibrosis: A novel combination. Turkish Thoracic Journal. 2020;**21**:451-453

[80] Hewlett JC, Kropski JA, Blackwell TS. Idiopathic pulmonary fibrosis: Epithelial-mesenchymal interactions and emerging therapeutic targets. Matrix Biology. 2018;**71-72**:112-127

[81] Rumende CM, Susanto EC, Sitorus TP. The Management of Pulmonary Fibrosis in COVID-19. Acta Medica Indonesiana. 2021;**53**:233-241

[82] Bazdyrev E, Rusina P, Panova M, Novikov F, Grishagin I, Nebolsin V. Lung fibrosis after COVID-19: Treatment prospects. Pharmaceuticals (Basel). 2021;**14**(8):807-821

[83] Sime PJ, O'Reilly KM. Fibrosis of the lung and other tissues: New concepts in pathogenesis and treatment. Clinical Immunology. 2001;**99**:308-319

[84] Razzaque MS, Taguchi T. Pulmonary fibrosis: Cellular and molecular events. Pathology International. 2003;**53**:133-145

[85] Spagnolo P, Kropski JA, Jones MG, Lee JS, Rossi G, Karampitsakos T, et al. Idiopathic pulmonary fibrosis: Disease mechanisms and drug development. Pharmacology & Therapeutics. 2021;**222**:107798

[86] Ortiz LA, Dutreil M, Fattman C, Pandey AC, Torres G, Go K, et al. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. In: Proceedings of the National Academy of Sciences of the United States of America. 2007;**104**:11002-11007

[87] Geiser T, Atabai K, Jarreau PH, Ware LB, Pugin J, Matthay MA. Pulmonary edema fluid from patients with acute lung injury augments in vitro alveolar epithelial repair by an IL-1beta-dependent mechanism. American Journal of Respiratory and Critical Care Medicine. 2001;**163**:1384-1388

[88] Zhao Y, Yan Z, Liu Y, Zhang Y, Shi J, Li J, et al. Effectivity of mesenchymal stem cells for bleomycin-induced pulmonary fibrosis: A systematic review

and implication for clinical application. Stem Cell Research & Therapy. 2021;**12**:470

[89] Ekbom E, Frithiof R, Öi E, Im L, Lipcsey M, Rubertsson S, et al. Impaired diffusing capacity for carbon monoxide is common in critically ill Covid-19 patients at four months post-discharge. Respiratory Medicine. 2021;**182**:106394

[90] Glassberg MK, Minkiewicz J, Toonkel RL, Simonet ES, Rubio GA, DiFede D, et al. Allogeneic human mesenchymal stem cells in patients with idiopathic pulmonary fibrosis via intravenous delivery (AETHER): A phase I safety clinical trial. Chest. 2017;**151**:971-981

[91] Chambers DC, Enever D, Ilic N, Sparks L, Whitelaw K, Ayres J, et al. A phase 1b study of placenta-derived mesenchymal stromal cells in patients with idiopathic pulmonary fibrosis. Respirology. 2014;**19**:1013-1018

[92] Joo HS, Suh JH, Lee HJ, Bang ES, Lee JM. Current knowledge and future perspectives on mesenchymal stem cell-derived exosomes as a new therapeutic agent. International Journal of Molecular Sciences. 2020;**21**:727-747

[93] Kiaie N, Ghanavati SPM, Miremadi SS, Hadipour A, Aghdam RM. Mesenchymal stem cell-derived exosomes for COVID-19 therapy, preclinical and clinical evidence. International Journal of Stem Cells. 2021;**14**:252-261

[94] Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: Biogenesis, biologic function and clinical potential. Cell & Bioscience. 2019;**9**:19

[95] Xia X, Yuan P, Liu Y, Wang Y, Cao W, Zheng JC. Emerging roles of extracellular vesicles in COVID-19, a double-edged sword? Immunology. 2021;**163**:416-430

[96] Zhu YG, Feng XM, Abbott J, Fang XH, Hao Q, Monsel A, et al. Human mesenchymal stem cell microvesicles for treatment of Escherichia coli endotoxin-induced acute lung injury in mice. Stem Cells. 2014;**32**:116-125

[97] Sengupta V, Sengupta S, Lazo A, Woods P, Nolan A, Bremer N. Exosomes derived from bone marrow mesenchymal stem cells as treatment for severe COVID-19. Stem Cells and Development. 2020;**29**:747-754

[98] Chu M, Wang H, Bian L, Huang J, Wu D, Zhang R, et al. Nebulization therapy with umbilical cord mesenchymal stem cell-derived exosomes for COVID-19 pneumonia. Stem Cell Reviews and Reports. 2022;**18**:2152-2163

[99] Zhu YG, Shi MM, Monsel A, Dai CX, Dong X, Shen H, et al. Nebulized exosomes derived from allogenic adipose tissue mesenchymal stromal cells in patients with severe COVID-19: A pilot study. Stem Cell Research & Therapy. 2022;**13**:220

[100] Cheng X, Jiang M, Long L, Meng J. Potential roles of mesenchymal stem cells and their exosomes in the treatment of COVID-19. Frontiers in Bioscience (Landmark Ed). 2021;**26**:948-961

[101] Brown C, McKee C, Bakshi S, Walker K, Hakman E, Halassy S, et al. Mesenchymal stem cells: Cell therapy and regeneration potential. Journal of Tissue Engineering and Regenerative Medicine. 2019;**13**:1738-1755

[102] Khoury M, Cuenca J, Cruz FF, Figueroa FE, Rocco PRM, Weiss DJ. Current status of cell-based therapies for respiratory virus infections: Applicability to COVID-19. The European Respiratory Journal. 2020;**55**(6):2000858-2000891

[103] Guo Y, Yu Y, Hu S, Chen Y, Shen Z. The therapeutic potential of

mesenchymal stem cells for cardiovascular diseases. Cell Death & Disease. 2020;**11**:349

[104] Schäfer R, Spohn G, Bechtel M, Bojkova D, Baer PC, Kuçi S, et al. Human mesenchymal stromal cells are resistant to SARS-CoV-2 infection under steady-state, inflammatory conditions and in the presence of SARS-CoV-2-infected cells. Stem Cell Reports. 2021;**16**:419-427

[105] Liang B, Xiang T, Wang H, Li Z, Quan X, Feng X, et al. Robust humoral and cellular immune responses in long-term convalescent COVID-19 individuals following one-dose SARS-CoV-2 inactivated vaccination. Frontiers in Immunology. 2022;**13**:966098

[106] Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, et al. Transplantation of ACE2- mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. Aging and Disease. 2020;**11**:216-228

[107] Monsel A, Hauw-Berlemont C, Mebarki M, Heming N, Mayaux J, Tchoumba ON, et al. Treatment of COVID-19-associated ARDS with mesenchymal stromal cells: A multicenter randomized double-blind trial. Critical Care. 2022;**26**:022-03930

[108] Feng Y, Huang J, Jianyuan W, Yan X, Chen B, Jiang L, et al. Safety and feasibility of umbilical cord mesenchymal stem cells in patients with COVID-19 pneumonia: A pilot study. Cell Proliferation. 2020;**53**:17

[109] Shu L, Niu C, Li R, Huang T, Wang Y, Huang M, et al. Treatment of severe COVID-19 with human umbilical cord mesenchymal stem cells. Stem Cell Research & Therapy. 2020;**11**:020-01875

[110] Shi L, Huang H, Lu X, Yan X, Jiang X, Xu R, et al. Effect of human umbilical cord-derived mesenchymal stem cells on lung damage in severe COVID-19 patients: A randomized, double-blind, placebo-controlled phase 2 trial. Signal Transduction and Targeted Therapy. 2021;**6**(1):58-66

[111] Yip H-K, Fang W-F, Li Y-C, Lee F-Y, Lee C-H, Pei S-N, et al. Human umbilical cord-derived mesenchymal stem cells for acute respiratory distress syndrome. Critical Care Medicine. 2020;**48**:e391-e399

[112] Gorman E, Shankar-Hari M, Hopkins P, Tunnicliffe WS, Perkins GD, Silversides J, et al. Repair of acute respiratory distress syndrome by stromal cell administration (REALIST) trial: A phase 1 trial. EClinicalMedicine. 2021;**41**:101167

[113] Zheng G, Huang L, Tong H, Shu Q, Yaoqin H, Ge M, et al. Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: A randomized, placebo-controlled pilot study. Respiratory Research. 2014;**15**:1465-9921

[114] Zhu Y-G, Shi M-M, Monsel A, Dai C-X, Dong X, Shen H, et al. Nebulized exosomes derived from allogenic adipose tissue mesenchymal stromal cells in patients with severe COVID-19: A pilot study. Stem Cell Research & Therapy. 2022;**13**:022-02900

Section 3

Mesenchymal Stem Cells in Tissue Engineering Pharma Intelligence and Research

Chapter 7

Mesenchymal Stem Cells and Tissue Engineering in Dentistry

Farah Shaikh, Neha Langade, Mahavish Khan, Sangeeta Muglikar and Nawar Zahra Ansari

Abstract

Conventional treatments rely on the removal of damaged or impaired tissues, followed by the use of restorative materials. However, the inability to regenerate a functional tooth complex simulating its original structure remains a major unmet treatment objective. Tissue engineering is an amalgamation of engineering and biological principles that aims to not just remove diseased tissue but also replicate and repair lost structures. This evolutionary concept draws from three key elements: cells, an extracellular matrix scaffold, and signaling molecules. Though tissue engineering has come a long way in regenerative medicine, its future in dentistry is promising too. Tissue engineering approaches in dentistry harbor the potential of inducing mesenchymal stem cells (MSCs) of dental origin to combine with biocompatible scaffold, and growth factors to create a three-dimensional environment for regeneration and repair of a fully functional tooth complex. This chapter summarizes the application of mesenchymal stem cells and tissue engineering in dentistry.

Keywords: tissue engineering, regenerative medicine, mesenchymal stem cells, scaffold, dentistry

1. Introduction

For a field as dynamic as medicine, the most significant challenge lies in regenerating or restoring missing or damaged organs or tissues. In the past few decades, miscellaneous regeneration techniques have been applied to rebuild tooth structure destroyed due to dental caries, pulpitis, fractures, and periodontal disorders. Conventional therapies work to remove harmed or impaired tissues and replace missing tissue with a variety of restorative substances. However, the capacity to repair the injured tissues still represents an unmet target. Therefore, the primary objective of regenerative dental medicine continues to be the predictable three-dimensional regeneration and repair of a healthy, functioning tooth complex that mimics its predisease structure [1].

Recently, it has been understood that the processes occurring during regeneration of a tissue mimic those occurring during the natural development of that tissue. This has led to the development of the concept of tissue engineering. Primarily used in the field of medical specialties for replacing vital structures damaged due to disease or trauma; the application of mesenchymal stem cells and tissue engineering in dentistry

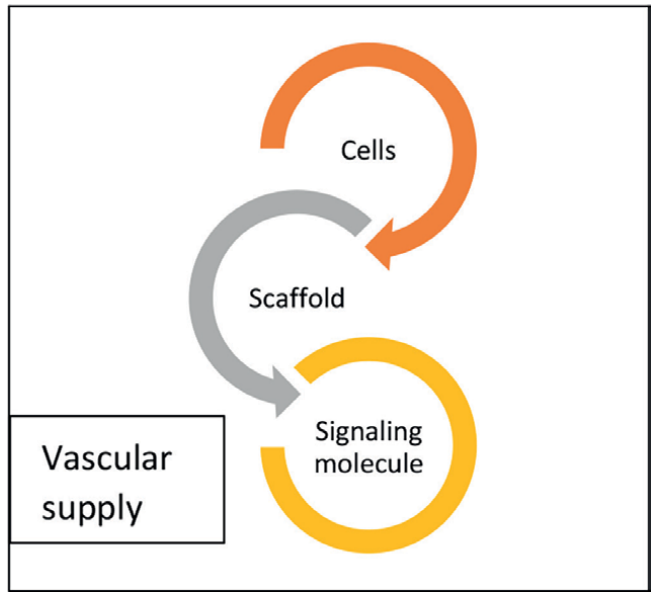

Figure 1.
Tissue engineering triad.

presents a potential solution to achieve predictable three-dimensional regeneration and repair of a fully functioning tooth complex [1, 2].

The field of tissue engineering was first described as "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ" by chemist R. Langer and surgeon J.P. Vacanti in the 1980s [3]. It is a science based on fundamental principles that involve the identification of appropriate cells with the ability to differentiate into specialized regenerative cells, certain signaling molecules required to induce cells to regenerate a tissue or organ, and a conductive scaffold with vascular networks to provide nutrition for tissue growth. In the last few years, medicine has begun to explore the possible applications of stem cells and tissue engineering toward the repair and regeneration of body structures.

Accordingly, tissue engineering triad combines three key elements (**Figure 1**) [4].

- Stem cells
- Scaffold or supporting matrix
- Signaling molecules

One of the most important factors in tissue engineering is the choice of scaffold and optimal stem cell population to employ.

2. Stem cells

Tissue regeneration requires specialized cells capable of synthesizing the extracellular matrix specific to each tissue. In this sense, stem cells have been extensively

used in regenerative medicine [5]. Stem cells are immature progenitor cells capable of both self-renewal and multi-lineage differentiation through mitosis into one or more types of specialized cells. They can be isolated from various sources, such as fetuses, embryos, or adult tissues, and their differentiation capability depends on the cell source.

Characteristics of stem cells [2]:

- Totipotent: these are embryonic cells and extra-embryonic cells, which can be differentiated into all cell types.
- Pluripotent: cells that can give rise to all the cell types that make up the body; except extra embryonic tissues such as placenta. For example, embryonic stem cells and induced pluripotent stem cells.
- Multipotent: these cells can develop into more than one cell type, which can give rise to tissues belonging to only one embryonic germ layer (ectoderm or mesoderm or endoderm), for example, cord blood stem cells and adult stem cells
- Clonogenicity: a stem cell is clonogenic as it can proliferate to form colony of cells.

Depending on the developmental stages of the tissues from which the stem cells are isolated, stem cells are broadly divided into embryonic stem cells and adult stem cells (**Figure 2**) [6].

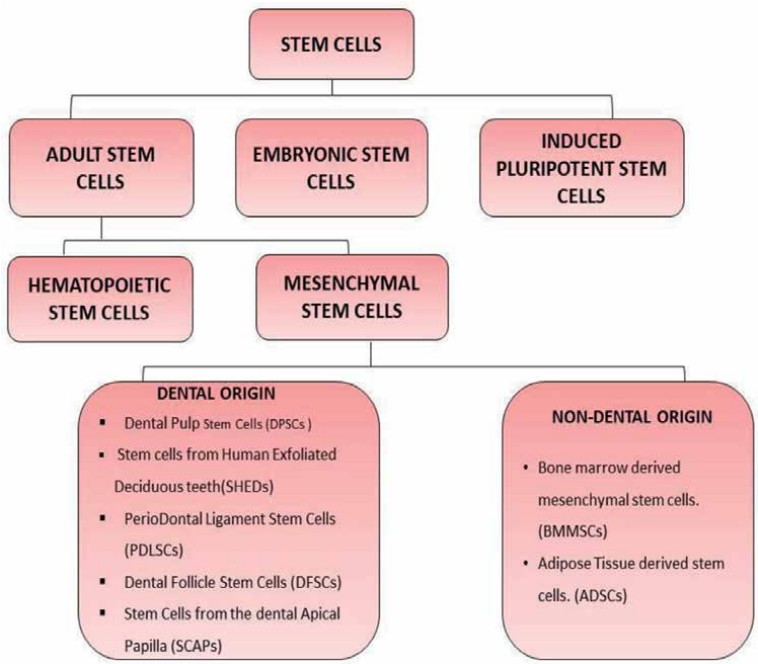

Figure 2.
Classification of stem cells.

2.1 Embryonic stem cells (ESCs)

Embryonic stem cells (ESCs) are derived from the cell of early-stage embryos, during the blastocyst stage. These cells are considered pluripotent type as they can differentiate into any cell type in the body [7]. ESCs have been the focus of much research due to their potential to treat a spectrum of diseases, including Parkinson's disease, diabetes, and heart disease. However, their use has been limited due to ethical concerns and the risk of teratoma formation and tumorigenicity.

2.2 Induced pluripotent stem cells (iPSCs)

Induced pluripotent stem cells (iPSCs) are obtained from somatic cells, such as skin cells, through the reprogramming of gene expression. These cells have similar properties to ESCs and can differentiate into any cell type in the body. iPSCs were first generated in 2006 and have since been used in disease modeling and drug screening, as well as in regenerative medicine. However, their use is still limited due to the risk of genetic abnormalities resulting from the reprogramming process [8].

2.3 Adult stem cells (ASCs)

Adult stem cells (ASCs) are multipotent stem cells and depending upon their origin, they are further classified into hemopoietic stem cells and mesenchymal stem cells.

2.3.1 Hematopoietic stem cells (HPCs)

An immature cell that gives rise to different types of blood cell types, including platelets, red blood cells, and white blood cells. Bone marrow and peripheral blood both contain hematopoietic stem cells. They are also known as blood stem cells. Many cancers (such as leukemia, lymphoma) and non-malignant conditions (such as sickle cell disease) are treated with HPCs in order to repair or rebuild the patient's hematopoietic system. This type of treatment is known as bone marrow or stem cell transplant [9].

2.3.2 Mesenchymal stem cells (MSCs)

Mesenchymal stem cells are nonhematopoietic and multipotent cells that can differentiate into an array of cell types comprising varieties of tissues. They were first identified in aspirates of adult bone marrow by Friedenstein in 1976 [10].

These cells demonstrate specific properties [11]:

1. MSCs have ability to adhere to plastic tissue-culture surfaces.

2. They have potential to differentiate into osteoblasts, adipocytes, chondrocytes, etc.

3. Immunoregulatory properties.

4. MSCs are positive for the surface antigens CD73, CD90, and CD105.

MSCs can be isolated from various sources, including bone marrow, blood vessels, skeletal muscle, umbilical cord, amniotic fluid, placenta, adipose tissue, and teeth.

Owing to their presence in teeth, and relatively easy availability of tooth-derived MSCs from deciduous and permanent teeth than other anatomical sites; dental mesenchymal stem cells are of great interest for research in the field of tissue engineering and regenerative medicine [12].

3. Mesenchymal stem cells of dental origin

MSCs are the main cell source. They are multipotent cells that can be cryopreserved safely, have immunosuppressive qualities, and express mesenchymal markers. Explant cultures or enzymatic digestion can be used to isolate dental mesenchymal stem cells (DMSCs). Autologous stem cells are great option since there is no chance of immunological rejection, less expensive, and they eliminate legal and ethical concerns.

Dental mesenchymal stem cells that have been isolated and grouped according to their position in the tooth are:

- Dental Pulp Stem Cells (DPSCs)
- Stem cells from Human Exfoliated Deciduous teeth (SHEDs)
- Periodontal Ligament Stem Cells (PDLSCs)
- Dental Follicle Stem Cells (DFSCs)
- Stem Cells from the dental Apical Papilla (SCAPs)

3.1 Dental pulp stem cells (DPSCs)

These are a common source of dental tissue-derived stem cells obtained from the pulp of permanent teeth. In 2000, Gronthos et al. were the first to spot MSCs in the dental pulp of teeth that are today referred to as DPSCs. He further studied the proliferation and differentiation capabilities of DPSCs and stated that they possess high plasticity and trans-differentiation potency of their population [13]. These cells possess the potential to differentiate into osteogenic, adipogenic, chondrogenic, and neural cells and show high expression of surface markers of MSCs. Due to their profound regeneration, differentiation, and proliferation capabilities, they can be induced *in vitro* to differentiate into cells of odontoblastic phenotype [14]. Dr. Irina Kerkis in 2006 reported discovery of Immature Dental Pulp Stem Cells (IDPSC), a pluripotent subpopulation of DPSC using dental pulp organ culture [15]. DPSCs can also be harvested from one cavity and applied to dentin regeneration in many teeth.

DPSCs could therefore be used as a generic allogenic source of MSCs.

3.2 MSCs from dental pulp of exfoliated deciduous teeth (SHED)

MSCs from dental pulp of exfoliated deciduous teeth (SHED) was first isolated in 2003 from pulp of human deciduous teeth [16]. *In vitro*, these cells may produce dentin, induce bone formation, and differentiate into various nondental mesenchymal cell descendants. SHED show enhanced population doublings, faster rates

of proliferation, *in vivo* osteoinductive potential, and the capacity to organize into sphere-like clusters. They cannot, however, repair whole dentin/pulp-like complexes *in vivo* like DPCSs. Dental stem cells may be useful for treating neurodegenerative illnesses and repairing damaged motor neurons because of their capacity to generate and release neurotrophic substances [17].

This multilineage potential makes SHEDs alternative source of dental stem cells.

3.3 MSCs from dental follicle (DFSCs)

The dental follicle, which encircles the developing tooth, contains a collection of dental mesenchyme stem cells that are essential for the growth of the alveolar bone, cementum, and periodontal ligament. In 2005, Morsczeck et al. isolated DFPCs from the dental follicle of human third molar teeth, and these cells were discovered to display the stem cell markers Notch and Nestin. DFSCs have the capacity to differentiate into osteoblasts, adipocytes, and nerve-like cells *in vitro* but could only produce cementum *in vivo*. A further investigation found that 4 weeks after DFSC implantation into mice, a new periodontal ligament had formed [18].

3.4 MSCs from the periodontal ligament (PDLSCS)

The periodontal ligament houses stem cells that self-renew and specialize to generate other tissues, such as cementum and alveolar bone, and it may be separated from the root of removed teeth [19]. *In vitro* differentiation of PDLSCs into adipocytes, osteoblasts, and chondrocytes is possible. Alkaline phosphatase, bone sialoprotein, osteocalcin, and TGF-receptor type I are among the cementoblastic/osteoblastic markers that are expressed by cultured PDLSCs, in addition to CD105, CD90, CD73, STRO-1, and CD146/MUC18 [20].

3.5 MSCs from apical papilla (SCAP)

In 2006, Sonoyama et al. identified apical part of dental papilla of human teeth as a unique source of MSCs, which can be considered for oral tissue regeneration. SCAP can be isolated from human third molars, thus making them easily accessible. Due to their greater ability to proliferate, they encourage the formation of roots and seem to be more effective in promoting tooth development than PDLSC. They create dentin and odontoblast-like cells. Additionally, they show adipogenic differentiation ability. Similar to DPSCs, SCAP exhibits stem cell markers (STRO-1, CD146, and CD34), but with substantially greater rates of proliferation and mineralization [21].

3.6 Gingiva-derived mesenchymal stem cells (GMSCs)

Zhang et al., in 2009, recognized human gingival tissue as a potential source of MSCs for tissue regeneration and therapy. Based on their quantity and accessibility, the GMSCs were examined. When isolated, they demonstrated greater proliferation potential than BM-MSCs [21]. *In vitro*, the GMSC successfully differentiated into mesoderm adipocytes and osteoblasts, as well as endodermal and neural ectodermal cells implying stem cell characteristics. Furthermore, the GMSCs were shown to have stem cell-specific cellular markers and a phenotype consistent with mesenchymal progenitor cells [22].

4. MSCs of nondental origin

The bone marrow microenvironment is the body's major MSC niche. MSCs are known to live in two distinct niches: endosteal and perivascular. The endosteal niche is considered to keep MSCs quiescent for a long time, whereas the perivascular niche is thought to keep MSCs proliferating and mediating circulation. BMMSCs and adipose-derived MSCs have been found in BM perivascular regions [23].

- Bone marrow mesenchymal stem cells (BMMSCs)
- Adipose tissue derived stem cells (ADSCs)

4.1 Bone marrow mesenchymal stem cells (BMMSCs)

Bone marrow mesenchymal stem cells (BMMSCs) are stem cells derived from the bone marrow. They can differentiate along a variety of mesenchymal lineages. BMMSCs have emerged as a unique option for the tissue engineering of teeth and may be used to produce both mesenchymal and epithelial cells [24]. Both BMSC and DPSC are capable of forming structures that resemble teeth or bones and have several traits in common with one another. In contrast to DPSC, BMSCs have a decreased odontogenic potential. After being implanted into damaged periodontal sites, BMSCs have the ability to develop alveolar bone, periodontal ligament, and cementum *in vivo*. In order to treat periodontal diseases, bone marrow offers an alternate source of MSC [25]. BMMSC harvesting results in poor cell numbers, discomfort, and morbidity.

4.2 Adipose-derived stromal cells (ADSCs)

ADSCs are a subset of pluripotent mesenchymal stem cells that are generated from fat. They are capable of multilineage differentiation, which includes adipogenesis, osteogenesis, and chondrogenesis. Due to their easy accessibility and efficiency in getting, through lipectomy and various esthetic and medical operations. ADSCs are the most often employed source of MSCs [26].

5. Scaffolds

The scaffold is a three-dimensional structure that serves as a template or framework for cell attachment, growth, and regeneration of new tissue. The purpose of a scaffold in tissue engineering is to provide a temporary framework for cells to attach and grow, and to mimic the natural extracellular matrix (ECM) that surrounds cells in the body [27].

The main functions of scaffolds in tissue engineering are:

- Structural support: Scaffolds provide a three-dimensional structure that can mimic the shape and mechanical properties of the target tissue. This support is critical for the survival and growth of the cells, and for the development of functional tissue.

- Cell attachment and proliferation: Scaffolds can provide a surface for cells to attach and proliferate. This attachment is vital for the formation of functional tissue, as it allows cells to interact and communicate with each other.

- Diffusion of nutrients and waste products: Scaffolds can facilitate the diffusion of nutrients and waste products throughout the developing tissue. This is important for the survival and function of the cells.

- Guided tissue regeneration: Scaffolds can be designed to promote the growth of specific types of tissue, such as bone, cartilage, or muscle. By controlling the properties of the scaffold, researchers can guide the development of the tissue to achieve a desired outcome [27].

Scaffolds can be made from a variety of materials such as natural polymers, synthetic polymers, metals, ceramics, or composites that are used to carry biologically active molecules to the site of regeneration [27, 28].

The ideal properties of scaffold are:

- It must be nontoxic

- It should be biocompatible, biodegradable, and highly cell adhesive

- It should be porous, to facilitate cell seeding

- It should have optimal physical and mechanical properties.

In tissue engineering, scaffolds have been fabricated using several natural and synthetic polymers.

5.1 Naturally derived scaffold materials

Natural polymers, including collagen, gelatin, chitosan, alginate, and hyaluronic acid, are used in tissue engineering applications [28].

- Fibrin: a crucial element of blood clots, is combined with thrombin to create an *in-situ* forming gel that serves as a framework for carrying different physiologically active molecules.

- Collagen: one of the most used scaffolding materials is collagen. Typically, gelatin and animal tissues are the sources of type I collagen.

- Chitosan: a cationic polymer generated from chitin is chitosan. Its scaffold creates an osteo-conductive hydrophilic surface, pointing to its potential utility in bone tissue creation. Brown algae are the source of the anionic polysaccharide known as alginate. When combined with divalent cations such as Ca.

- Hyaluronic acid: is a glycosaminoglycan made up of repeating disaccharide units that are nonsulfated. It contributes significantly to connective tissue and creates cross-linkable hydrogels with different modifications.

5.2 Synthetically derived scaffold materials

The commonly used chemical compounds to fabricate synthetic scaffolds include poly(a-hydroxyester) s, polyanhydrides, and polyorthoesters. Among these polymers, poly(a-hydroxy- ester)s such as polylactide (PLA), polyglycolide (PGA), and its copolymers are extensively used. These polymers are biocompatible, biodegradable, bioresorbable, and can be easily processed to form various 3-D structural Poly Lactic-co-Glycolic acid (PLGA) copolymers with controlled degradation matrices [29].

The behavior and mechanical characteristics of poly (lactide-co-glycolide) materials can be altered to meet specific needs. In order to accomplish effective tissue growth, they can be utilized to create nanofibrous scaffolds. The major drawback of these polymers is that when they break down, acidic byproducts may interfere with the regeneration process.

The extracellular matrix is composed of a complex meshwork of proteins and polysaccharides, which are produced by the resident cells in the tissue/organ. The extracellular matrix is generally composed of three categories of molecules: fibrous proteins (e.g., collagen, elastin, fibrillin, and fibulin), adhesive glycoproteins (e.g., laminin, fibronectin, tenasin, thrombospondin, and integrin), and glycosaminoglycans. More importantly, emerging studies suggest that the extracellular matrix can itself function as an inductive scaffold or modify a biomaterial-based scaffold for tissue and/or organ regeneration. This makes the extracellular matrix a critical element in the field of tissue engineering and regenerative medicine [28]. They are generally fabricated through decellularisation and other manufacturing processes. Extracellular matrix scaffold maintains initial geometry and flexibility, possesses a certain degree of mechanical strength, and comprises the main physical advantage of artificial scaffolds. Extracellular matrix scaffold, by obtaining an intact three-dimensional structure, also overcomes the drawback of synthetic scaffold [30].

Scaffolds can be fabricated using a variety of techniques, such as electrospinning, 3D printing, lyophilizing, phase separating, foaming, rapid prototyping, and microfabrication, and can be tailored to match the specific mechanical, chemical, and biological properties of the target tissue. For example, scaffolds for bone tissue engineering may be designed to have high stiffness and strength, while scaffolds for cartilage tissue engineering may be designed to have high flexibility and elasticity [31]. Therefore scaffold design is a critical aspect of tissue engineering, as the properties of the scaffold can significantly affect the behavior of the cells and the development of the tissue. Scaffold-based tissue engineering shows a great promise in a variety of applications, including bone and cartilage regeneration, skin tissue engineering, and organ transplantation. However, there are still many challenges to overcome in order to fully realize the potential of scaffold-based tissue engineering, including optimizing the design and fabrication of scaffolds, improving cell seeding and growth, and ensuring biocompatibility.

6. Signaling molecules

Tissue engineering involves the use of various signaling molecules to promote tissue regeneration and repair. These signaling molecules can be categorized into several groups based on their functions and roles in tissue regeneration.

6.1 Growth factors

Growth factors are a class of signaling molecules that plays a critical role in tissue engineering. They are naturally occurring proteins that are produced by cells and act on neighboring cells to stimulate or inhibit their activity. Growth factors can be incorporated into the scaffold material or delivered to the target site through a variety of methods, such as direct injection or controlled release from a biomaterial carrier to stimulate the growth and proliferation of stem cells, which are the building blocks of tissues. They can also promote the differentiation of stem cells into specific cell types, such as bone, cartilage, or muscle. By controlling the activity of stem cells, growth factors can help to regenerate damaged or diseased tissues.

There are many different types of growth factors that have been identified, in tissue engineering, among them are the bone morphogenetic proteins (BMP); fibroblast growth factor (FGF); interleukins; hedgehog proteins (HHS); tumor necrosis factor (TNF); and vascular endothelial growth factor (VEGF). Among these signaling molecules, the bone morphogenetic proteins (BMPs) are known for their ability to induce the formation of bone and cartilage and have been extensively studied and applied in dental regeneration (**Table 1**) [32–34].

6.2 Dental pulp-derived factors

Smith concluded from his findings that dentin matrix may be regarded as a powerful cocktail of bioactive molecules when released following tissue injury has

Name	Origin	Function
Bone morphogenetic proteins (BMPs) [32]	Demineralized bone matrix, MMSCs, and osteoblasts endothelial cells chondrocytes	Stimulates differentiation of mesenchymal stem cells into bone-forming cells, and promotes formation of bone and cartilage. BMP-2 and BMP-7 are commonly used.
Platelet-derived growth factor (PDGF)	Platelets, macrophages, keratinocytes, and endothelial cells	Potent mitogen and chemoattractant promote wound healing, and stimulate proliferation and migration of various cell types.
Transforming growth factor-α (TGF- α)	Platelets, macrophages, keratinocytes, and brain cells	Activates neutrophils, fibroblast mitogen, stimulates angiogenesis
Transforming growth factor-β (TGF- β)	Dentine matrix, platelets, osteoblasts, chondrocytes endothelial cells, fibroblasts	signal odontoblast differentiation, promotes wound healing, inhibits macrophage, and lymphocyte proliferation
Fibroblast growth factor (FGF)	MMSCs, osteoblasts, chondrocytes, endothelial cells	Bone and cartilage regeneration, nerve growth, proliferation, and angiogenesis.
Vascular endothelial growth factor (VEGF):	Platelets osteoblasts chondrocytes endothelial cells	Endothelial cell proliferation, angiogenesis
Insulin-like growth factor (IGF)	Osteoblasts, chondrocytes, endothelial cells	Osteoblasts proliferation, osteoclasts proliferation, and ECM synthesis

Table 1.
Growth factors in tissue engineering.

the potential to dramatically influence cellular events in the dentin-pulp complexes such as the group of angiogenic growth factors or any cytokines sequestered in the dentin matrix will be released due to caries demineralization and might contribute to the overall repair process. Dentin matrix proteins such as dentin sialoprotein (DSP) and dentin matrix protein 1 (DMP-1) are known to regulate odontoblast differentiation and dentin formation [35]. The research in tissue engineering is ongoing and new signaling molecules and strategies are continually being explored to improve the regeneration and repair of tissues.

7. Applications of tissue engineering

MSCs generated from dental tissue have demonstrated their multilineage differentiation capabilities and excellent therapeutic promise in oral and systemic disorders. Together with growth factors and/or scaffolds, dental MSCs make a highly effective interaction, which is of utmost importance in tissue engineering. It has shown great potential in the regeneration and restoration of damaged tissues or organs (**Figure 3**).

7.1 Application of tissue engineering in regenerative medicine

DPSCs have been found to have therapeutic benefits for myocardial infarction [36], cerebral ischemia [37], muscular dystrophy [38], and corneal reconstruction [39] due to their adaptability. These cells were discovered to exhibit

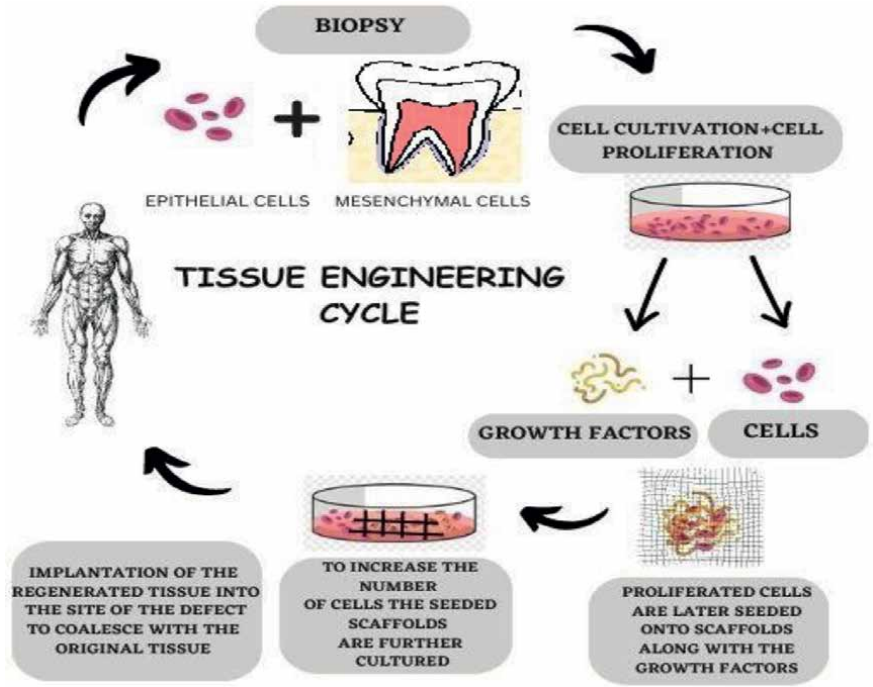

Figure 3.
Schematic presentation of tissue engineering.

neuron-specific markers in the damaged cortex, indicating that engrafted DPSC-derived cells may integrate into the host brain and may serve as a helpful source of neuro and gliogenesis *in vivo*. Furthermore, DPSC transplantation boosted neurogenesis and vasculogenesis in rats, showing that DPSCs might be a therapeutic option [40].

7.2 Application of tissue engineering in dentistry

Tissue engineering has the potential to revolutionize the way dental treatments are performed by providing new and improved approaches for repairing and replacing damaged or missing teeth. It is becoming a rapidly advancing field in the development of biomaterials that can mimic the natural structure and function of dental tissues. Thus, can benefit major dental branches like endodontics, oral surgery, and periodontics (**Figure 4**).

Potential applications in:

- Endodontics
- Periodontology
- Oral and maxillofacial

7.2.1 Endodontics

Tissue engineering can be applied in endodontics to regenerate dental pulp tissue, dentin, and other tissues that may have been damaged due to injury or disease. Here are some specific examples of how tissue engineering is being used in endodontics.

Regenerative endodontics [41] is a new treatment approach that aims at:

- Regeneration or restoration of lost or diseased dentinal tissue
- Revascularization of necrotic dental pulp

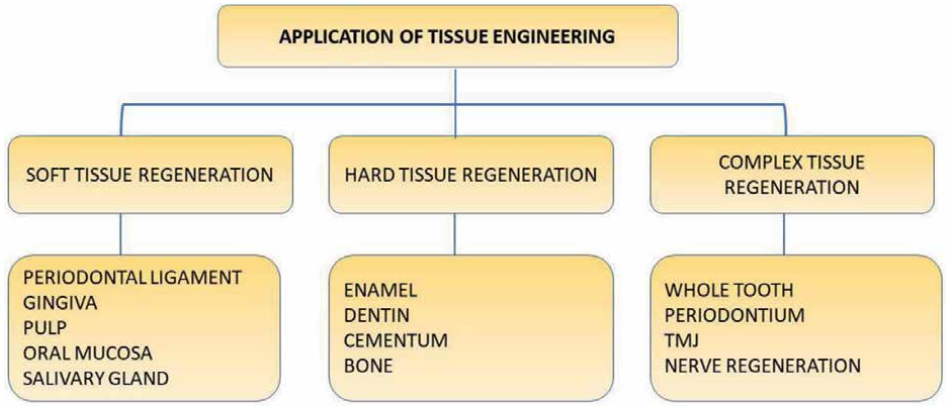

Figure 4.
Application of tissue engineering in dentistry.

7.2.1.1 Dentin pulp regeneration

One of the most promising applications of tissue engineering is the regeneration of dental pulp. Dental pulp is the soft tissue inside the tooth that contains nerves and blood vessels. Dental pulp is damaged due to decay or trauma, which can lead to infection and eventually tooth loss. Initially, for the regeneration of the dentin-pulp complex, various pulp capping materials (e.g., calcium hydroxide, mineral trioxide aggregates Biodentine) were used, which stimulates the pulp progenitor cells to differentiate into odontoblast-like cells or the secretion of TGF-b131, which plays an essential in angiogenesis, the recruitment of progenitor cells, cell differentiation, and ultimately mineralization of the damaged area. Tissue engineering techniques can be used to create a scaffold that mimics the structure of dental pulp and promotes the growth of new tissue. This approach has the potential to restore the function and vitality of damaged teeth [42].

Because of the size and confinement of the pulp within the root canal(s), cell treatment and/or injectable hydrogels were the most commonly used strategy for engineering the dentin-pulp complex. Encapsulated stem cells were also used for dentin-pulp regeneration such as Gelfoam-encapsulated dental stem cells encouraged dentin-pulp complex development in pulpless root canals of juvenile permanent incisors in beagles [43].

7.2.1.2 Revascularization of necrotic dental pulp

The term "revascularization" describes the occurrence of physiological tissue creation and regeneration that really took place [44, 45]. This might be explained by SCAPs continuing to function after the infection and causing this occurrence. It is also possible that the radiographic appearance of increased dentinal wall thickness is attributable to ingrowth of cementum, bone, or a dentin-like substance. This variation in cellular response is not surprising given that DPSCs can acquire odontogenic/osteogenic, chondrogenic, or adipogenic phenotypes based on their exposure to various combinations of growth factors and morphogens.

7.2.2 Oral and maxillofacial surgery

Oral and maxillofacial surgery is a surgical specialty that involve the diagnosis and treatment of diseases, injuries, and defects in the face, jaws, and oral cavity. Here are some of the potential applications of tissue engineering in oral and maxillofacial surgery:

7.2.2.1 Maxillary or mandibular reconstruction

The main causes of aberrant maxillofacial bone tissue in the maxillofacial region are the increasing loss of jaw bone tissue and periodontal tissue inflammation. By polarizing M2 macrophages, SHEDs may lessen periodontal inflammation. Encourage the regeneration of periodontal tissue [46]. Tissue engineering can be used to reconstruct large mandibular defects that result from trauma, cancer, or congenital malformations. This approach involves the use of biomaterial scaffolds that are seeded with bone-forming cells (DPSCs/SHEDs) and growth factors that promote bone regeneration. There have been a few clinical examples reported wherein large defects like mandibulectomy, treated with a titanium mesh cage, has been used to support the

HA-coated with signal proteins and in a case of segmental jaw deformity was repaired utilizing composite scaffolds (collagen-HA-tricalcium phosphate) and bone morphogenetic protein 2 (BMP2) [47]. The results of bone tissue engineering are encouraging and might help overcome the limitations of bone autografts and allografts.

7.2.2.2 Temporomandibular joint (TMJ) reconstruction

Tissue engineering can be very useful in repairing or regenerating a bony condyle or a fibrocartilagenous disc. Thomas et al. described the first *in vitro* creation of TMJ cartilage more than two decades ago, type I meshes were employed to culture chondrocyte-like cells *in vitro*. DPSCs have been utilized to differentiate chondrocytes to restore the cartilage [48]. Puelacher et al. attempted to tissue engineer the TMJ disc a few years later. Chondrocytes (Bovine articular) were planted on disc-shaped degradable and fibrous PGA and PLA scaffolds. After seven days, naked mice were implanted with chondrocyte-loaded scaffolds. Within three months, the creation of new cartilage and organic matrix revealed the prospect of TMJ disc tissue engineering [49].

7.2.2.3 Cleft palate repair

MSCs can differentiate into osteoblasts and are considered the best potential option for the repair of alveolar cleft palates, which are congenital defects that result from incomplete fusion of the palatal shelves during embryonic development. This approach involves the use of biomaterial scaffolds that are seeded with cells that can differentiate into the various tissues that make up the palate, as well as growth factors that promote tissue regeneration. BMP-2-aided bone regeneration has been reported for the reconstruction of the alveolar cleft [50].

7.2.2.4 Nerve regeneration

Tissue engineering can be used to regenerate nerves in the facial region, which is often necessary after facial trauma or surgery. Apart from their substantial neural differentiation potential, DPSCs produced from distinct cranial neural crest cell lineages express many factors that promote nerve and axon regeneration. DPSCs have been reported to express the neural crest cell markers CD271 and SOX10, which might be utilized to induce the development of Schwann cells, which are important in peripheral nerve repair [51].

7.2.2.5 Salivary gland regeneration

In Salivary gland bioengineering, the principal role of DPSCs is to regenerate the salivary stroma or mesenchymal-derived compartment, and these cells are one of the best choices for that purpose because the tooth mesenchyme and the Salivary gland mesenchyme share a common neural crest embryonic origin. The construction of three-dimensional salivary glandular tissue was attempted using biodegradable polymer scaffolds. Human salivary epithelial cells were seeded on scaffold and implanted into the mouse models. The constructed tissue can be implanted under the oral mucosa and secrete saliva directly into the oral cavity using engineered ducts. It has been proposed that implanted functioning salivary gland tissues may be a better therapy option for xerostomic patients due to their capacity to discharge saliva at a continual rate in more physiological ways [52].

7.2.3 Periodontics

Periodontics is a branch of dentistry concerned with the prevention, diagnosis, and treatment of diseases affecting tooth's supporting structures. Periodontium is a complex structure that includes two hard and two soft tissues: periodontal ligament, Gingiva, cementum, and bone.

7.2.3.1 Periodontal regeneration

Complete regeneration of the periodontium has always been challenging due to its complex structure. MSCs have the ability to repair new cementum, alveolar bone, and periodontal ligament. *In vitro* grown periodontal ligament cells were also effectively reimplanted into periodontal defects. PDLSCs and DFSCs have emerged as an additional cell source for periodontal regeneration treatment.

Masako Miura et al. in 2004 studied the possibility that human PDL includes stem cells capable of regenerating periodontal tissue. PDLSCs are isolated using single colony selection and other methods, then implanted into immunocompromised mice to test their ability to regenerate and repair tissue. PDLSCs developed into adipocytes, cementoblast-like cells, and collagen-forming cells under certain culture conditions [53]. In dog experiments, periodontal ligament cell sheets were employed to stimulate periodontal regeneration [54, 55]. Clinical experiments employing BM-MSCs and platelet-rich plasma (PRP) have shown successful periodontal regeneration [56].

7.2.3.2 Oral mucosa

The similarities between skin and oral mucosa resulted in the development of engineered oral mucosa, which followed the same protocol, starting with the development of an epithelial sheet. Oral keratinocytes were seeded on decellularised cadaveric human dermis (AlloDerm) [57] or a three-dimensional cell-seeded scaffold to create a composite oral mucosal equivalent. Thus, skin and mucosal replacements can be utilized interchangeably.

A review of recent developments in synthetic oral mucosa noted that the basic structure of the connective tissue component and the reconstituted basement membrane in such biomimetic models only allows for a simplistic representation of the native stromal microenvironment. As a result, the majority of researchers in the field of oral tissue engineering are shifting from the use of biomimetic models to more realistic approaches [58].

7.2.3.3 Gingival augmentation

Gingival augmentation involves the use of soft tissue grafts to increase the volume and thickness of the keratinized tissue around the teeth. Gingival epithelial sheets made from autologous gingival tissues were created and utilized to treat chronic desquamative gingivitis [59]. The results demonstrated that human-cultivated gingival epithelial sheets promoted gingival augmentation. GINTUIT, an allogeneic cellular product, was recently introduced. This product contains allogeneic cultivated keratinocytes and fibroblasts in bovine collagen. McGuire et al. in 2011 determined that the product was a safe and effective treatment for enhancing the keratinized gingival zone [60].

7.2.3.4 Bone regeneration

For bone augmentation, autogenous or allogeneic bones, as well as artificial materials, are used. However, there are several complications with these grafts, including donor site injury, the likelihood of infection or absorption, and ethical concerns. MSCs can be employed as an alternativeto autogenous bone transplants. DPSCs are an important source of osteoprogenitor cells, which play an important role in bone tissue plasticity processes. SHED and DPSC have osteoinductive properties, which allow them to develop into osteoblasts and stimulate bone formation [61]. The study was conducted to regenerate bone in a significant osseous defect with minimum invasiveness and good flexibility. To boost osteogenesis, they used platelet-rich plasma as an autologous scaffold with MSCs. Newly developed bone was discovered at 8 weeks [56].

8. Recent advancements in tissue engineering

8.1 Whole tooth regeneration

One of the most prevalent disorders is tooth loss, which can be caused by periodontal disease, caries, or trauma. Currently, dental implants are considered the best option for restoring the missing teeth and have achieved long-term success.

There have been several ways proposed for constructing complete biological teeth. Cell-tissue recombination and dental tissue engineering are the two main techniques utilized for tooth regeneration. Dental cell-tissue recombination techniques focus on mimicking natural tooth formation processes, in which, cultivated progenitor stem cell-tissue constructions are directly implanted in the defect site [62]. Young et al. claimed to have successfully regenerated the first-ever tooth structure with dentin and enamel using tooth buds from porcine third molars. Tooth bud cells were implanted in rats after being seeded onto biodegradable scaffolds. Within five to seven months, visible tooth structures (mature enamel with enamel organ, dentin with odontoblasts and pulp chamber, Hertwig's root sheet with cementoblasts) were regenerated. However, smaller tissues (4 mm2) were created, and tissue-engineered teeth did not conform to scaffold [63]. Duailibi et al. demonstrated that cultured 4-day post-natal rat tooth bud cells seeded onto PGA/PLLA and PLGA scaffolds, implanted, and grown in the mandible, could form organized bioengineered dental tissues such as dentin, enamel, pulp, and PDL. Bioengineered mandibular implant tooth tissues produced enamel and dentin proteins and exhibited morphological and histological similarities to naturally formed dentin and enamel. It could be concluded that *in vivo* whole tooth regeneration is achievable, but it is currently difficult to overcome translational barriers and test these methods on humans [64].

8.2 The bio-root regeneration

A bio-root was created by implanting preshaped root-like scaffolds along with mesenchymal stem cells into the alveolar bone to form a functional root with root-like structure, biomechanical properties, and elements similar to natural teeth, periodontal ligament-like tissue, and dentin-like matrix structure, and the ability to support post-crown prostheses [65].

SCAP and PDLSC are now being widely researched for bioroot engineering. Only dentin structure renewal was detected. As a result, regeneration of the whole tooth

structure was not completed in many cases. Instead of attempting to build a whole tooth, Sonoyama et al. in 2006 revealed that by combining SCAP with PDLSCs, they were able to generate a bioroot with periodontal ligament tissues. The autologous SCAP and PDLSCs were placed onto HA/TCP and gel foam scaffolds, respectively, then reimplanted into the sockets using a small swine model. Three months later, the bioroot established in the porcine jaw. The bioroot structure was made up of dentin that was randomly produced by the SCAP. The bioroot was surrounded by periodontal ligament tissue and appeared to have normal relationship with surrounding bone. However, the presence of residual HA in the newly regenerated dentin formed a structure different from that of normal dentin. This leads to a reduced mechanical strength of the bioroot, nearly two-thirds of a natural tooth [21].

9. Conclusion

MSCs have shown great potential for tissue engineering applications due to their ability to differentiate into various cell types, including osteoblasts, chondrocytes, and adipocytes. This capacity allows them to regenerate and repair damaged or lost tissues.

The use of MSCs in dentistry is still an active area of research, and, further research and clinical trials are needed to optimize their effectiveness and long-term outcomes. Regulatory approval and standardization of protocols are needed before widespread clinical application. Nonetheless, the combination of MSCs and tissue engineering approaches represents an exciting avenue for advancing dental treatments and regenerative therapies in the future by providing patient-specific therapy options that optimize function, esthetics, and patient care quality.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Dabra S, Chhina K, Soni N, Bhatnagar R. Tissue engineering in periodontal regeneration: A brief review. Dental Research Journal. 2012;**9**(6):671

[2] Rodríguez-Lozano FJ, Insausti CL, Iniesta F, Blanquer M, Meseguer L, Meseguer-Henarejos AB, et al. Mesenchymal dental stem cells in regenerative dentistry. Medicina oral, patologia oral y cirugia bucal. 2012;**17**(6):e1062

[3] Sándor GK. Tissue engineering: Propagating the wave of change. Annals of Maxillofacial Surgery. 2013;**3**(1):1-2

[4] Lynch SE, Castilla GR, Williams RC, Kiritsy CP, Howell TH, Reddy MS, et al. The effects of short term application of a combination of platelet-derived and insulin-like growth factors on periodontal wound healing. Journal of Periodontology. 1991;**62**(7):458-467

[5] Moro JD, Barcelos RC, Terra TG, Danesi CC. Tissue engineering perspectives in dentistry: Review of the literature. RGO-Revista Gaúcha de Odontologia. 2018;**66**:361-367

[6] Fortier LA. Stem cells: Classifications, controversies, and clinical applications. Veterinary Surgery. 2005;**34**(5):415-423

[7] Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature. 1981;**292**(5819):154-156

[8] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;**126**(4):663-676. DOI: 10.1016/j.cell.2006.07.024

[9] Jansen J, Hanks S, Thompson JM, Dugan MJ, Akard LP. Transplantation of hematopoietic stem cells from the peripheral blood. Journal of Cellular and Molecular Medicine. 2005;**9**(1):37-50

[10] Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of Guinea-pig bone marrow and spleen cells. Cell and Tissue Kinetics. 1970;**3**(4):393-403

[11] Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells.The International Society for Cellular Therapy position statement. Cytotherapy. 2006;**8**(4):315-317

[12] Tatullo M, Marrelli M, Paduano F. The regenerative medicine in oral and maxillofacial surgery: The most important innovations in the clinical application of mesenchymal stem cells. International Journal of Medical Sciences. 2015;**12**:72-77

[13] Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**:13625-13630

[14] Couble ML, Farges JC, Bleicher F, Perrat-Mabillon B, Boudeulle M, Magloire H. Odontoblast differentiation of human dental pulp cells in explant cultures. Calcified Tissue International. 2000;**66**(2):129-138. DOI: 10.1007/pl00005833

[15] Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira LV, et al. Isolation and characterization of a

population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells, Tissues, Organs. 2006;**184**(3-4):105-116. DOI: 10.1159/000099617

[16] Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: Stem cells from human exfoliated deciduous teeth. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(10):5807-5812. DOI: 10.1073/pnas.0937635100

[17] Govindasamy V, Abdullah AN, Ronald VS, Musa S, Aziz ZA, Zain RB, et al. Inherent differential propensity of dental pulp stem cells derived from human deciduous and permanent teeth. Journal of Endodontics. 2010;**36**(9):1504-1515

[18] Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, et al. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biology. 2005;**24**(2):155-165

[19] Isaka J, Ohazama A, Kobayashi M, Nagashima C, Takiguchi T, Kawasaki H, et al. Participation of periodontal ligament cells with regeneration of alveolar bone. Journal of Periodontology. 2001;**72**(3):314-323

[20] Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 2004;**364**(9429):149-155

[21] Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, et al. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One. 2006;**1**(1):e79

[22] Zhang Q, Shi S, Liu Y, Uyanne J, Shi Y, Shi S, et al. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. The Journal of Immunology. 2009;**183**(12):7787-7798

[23] Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. Journal of Bone and Mineral Research. 2003;**18**(4):696-704

[24] Peng L, Ye L, Zhou XD. Mesenchymal stem cells and tooth engineering. International Journal of Oral Science. 2009;**1**:6-12

[25] Kawaguchi H, Hirachi A, Hasegawa N, Iwata T, Hamaguchi H, Shiba H, et al. Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. Journal of Periodontology. 2004;**75**(9):1281-1287

[26] Zuk P. Adipose-derived stem cells in tissue regeneration: A review. International Scholarly Research Notices. 2013;**2013**:35

[27] Chan BP, Leong KW. Scaffolding in tissue engineering: General approaches and tissue-specific considerations. European Spine Journal. 2008;**17**(Suppl. 4):467-479

[28] Farzin A, Bahrami N, Mohamadnia A, Mousavi S, Gholami M, Ai J, et al. Scaffolds in dental tissue engineering: A review. Archives of Neuroscience. 2020;**7**(1):e97014

[29] Gunatillake PA, Adhikari R, Gadegaard N. Biodegradable synthetic polymers for tissue engineering. European Cells & Materials. 2003;**5**(1):1-6

[30] Yi S, Ding F, Gong L, Gu X. Extracellular matrix scaffolds for tissue

engineering and regenerative medicine. Current Stem Cell Research & Therapy. 2017;**12**(3):233-246

[31] Hutmacher DW. Scaffold design and fabrication technologies for engineering tissues—state of the art and future perspectives. Journal of Biomaterials Science. Polymer Edition. 2001;**12**(1):107-124

[32] Nakashima M, Reddi AH. The application of bone morphogenetic proteins to dental tissue engineering. Nature Biotechnology. 2003;**21**(9):1025-1032

[33] Orciani M, Fini M, Di Primio R, Mattioli-Belmonte M. Biofabrication and bone tissue regeneration: Cell source, approaches, and challenges. Frontiers in Bioengineering and Biotechnology. 2017;**5**:17

[34] Whitaker MJ, Quirk RA, Howdle SM, Shakesheff KM. Growth factor release from tissue engineering scaffolds. Journal of Pharmacy and Pharmacology. 2001;**53**(11):1427-1437

[35] Smith AJ, Scheven BA, Takahashi Y, Ferracane JL, Shelton RM, Cooper PR. Dentine as a bioactive extracellular matrix. Archives of Oral Biology. 2012;**57**(2):109-121

[36] Gandia C, Arminan A, Garcia-Verdugo JM, et al. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells. 2008;**26**(3):638-645

[37] Sugiyama M, Iohara K, Wakita H, et al. Dental pulp-derived CD31 (−)/CD146(−) side population stem/progenitor cells enhance recovery of focal cerebral ischemia in rats. Tissue Engineering Part A. 2011;**17**(9-10):1303-1311

[38] Kerkis I, Ambrosio CE, Kerkis A, et al. Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic? Journal of Translational Medicine. 2008;**6**:35

[39] Gomes JA, Geraldes Monteiro B, Melo GB, et al. Corneal reconstruction with tissue-engineered cell sheets composed of human immature dental pulp stem cells. Investigative Ophthalmology and Visual Science. 2010;**51**(3):1408-1414

[40] Kiraly M, Kadar K, Horvathy DB, et al. Integration of neuronally predifferentiated human dental pulp stem cells into rat brain in vivo. Neurochemistry International. 2011;**59**(3):371-381

[41] Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: A review of current status and a call for action. Journal of Endodontics. 2007;**33**:377-390

[42] Bogen G, Kim JS, Bakland LK. Direct pulp capping with mineral trioxide aggregate: An observational study. Journal of the American Dental Association. 2008;**139**:305-315

[43] Wang Y, Zhao Y, Jia W, Yang J, Ge L. Preliminary study on dental pulp stem cell-mediated pulp regeneration in canine immature permanent teeth. Journal of Endodontics. 2013;**39**:195-201

[44] Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dental Traumatology. 2001;**17**:185-187

[45] Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: New treatment protocol? Journal of Endodontics. 2004;**30**(4):196-200

[46] Gao X, Shen Z, Guan M, Huang Q, Chen L, Qin W, et al. Immunomodulatory role of stem cells from human exfoliated deciduous teeth on periodontal regeneration. Tissue Engineering. Part A. 2018;**24**(17-18):1341-1353

[47] Chao M, Donovan T, Sotelo C, Carstens MH. In situ osteogenesis of hemimandible with rhBMP-2 in a 9-year-old boy: Osteoinduction via stem cell concentration. The Journal of Craniofacial Surgery. 2006;**17**(3):405-412

[48] Thomas M, Grande D, Haug RH. Development of an in vitro temporomandibular joint cartilage analog. Journal of Oral and Maxillofacial Surgery. 1991;**49**(8):854-856 discussion 857

[49] Puelacher WC, Wisser J, Vacanti CA, Ferraro NF, Jaramillo D, Vacanti JP. Temporomandibular joint disc replacement made by tissue-engineered growth of cartilage. Journal of Oral and Maxillofacial Surgery. 1994;**52**(11):1172-1177 discussion 1177-8

[50] van Hout WM, Mink van der Molen AB, Breugem CC, Koole R, Van Cann EM. Reconstruction of the alveolar cleft: Can growth factor-aided tissue engineering replace autologous bone grafting? A literature review and systematic review of results obtained with bone morphogenetic protein-2. Clinical Oral Investigations. 2011;**15**(3):297-303

[51] Al-Zer H, Apel C, Heiland M, Friedrich RE, Jung O, Kroeger N, et al. Enrichment and Schwann cell differentiation of neural crest-derived dental pulp stem cells. In Vivo. 2015;**29**(3):319-326

[52] Joraku A, Sullivan CA, Yoo JJ, Atala A. Tissue engineering of functional salivary gland tissue. The Laryngoscope. 2005;**115**(2):244-248

[53] Miura M, Shi S. Human PDL contains stem cells that could be used to regenerate periodontal tissue. Lancet. 2004;**364**(9429):149-155

[54] Akizuki T, Oda S, Komaki M, Tsuchioka H, Kawakatsu N, Kikuchi A, et al. Application of periodontal ligament cell sheet for periodontal regeneration: A pilot study in beagle dogs. Journal of Periodontal Research. 2005;**40**:245-251

[55] Iwata T, Yamato M, Tsuchioka H, Takagi R, Mukobata S, Washio K, et al. Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. Biomaterials. 2009;**30**:2716-2723

[56] Yamada Y, Ueda M, Naiki T, Takahashi M, Hata K, Nagasaka T. Tissue Engineering. 2004;**10**(5-6):955-964

[57] Izumi K, Takacs G, Terashi H, Feinberg SE. Ex vivo development of a composite human oral mucosal equivalent. Journal of Oral and Maxillofacial Surgery. 1999;**57**(5):571-577 discussion 577-8

[58] Moharamzadeh K, Brook IM, Van Noort R, Scutt AM, Thornhill MH. Tissue-engineered oral mucosa: A review of the scientific literature. Journal of Dental Research. 2007;**86**(2):115-124

[59] Okuda K, Momose M, Murata M, Saito Y, lnoie M, Shinohara C, Wolff LF, Yoshie H. Treatment of chronic desquamative gingivitis using tissue-engineered human cultured gingival epithelial sheets: A case

report. The International Journal of Periodontics & Restorative Dentistry. 2004;**24**(2):119-125

[60] McGuire MK, Scheyer ET, Nevins ML, Neiva R, Cochran DL, Mellonig JT, et al. Living cellular construct for increasing the width of keratinized gingiva: Results from a randomized, within-patient, controlled trial. Journal of Periodontology. 2011;**82**(10):1414-1423

[61] Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: Recent advances and challenges. Critical Reviews in Biomedical Engineering. 2012;**40**(5):363-408. DOI: 10.1615/critrevbiomedeng.v40.i5.10

[62] Olaru M, Sachelarie L, Calin G. Hard dental tissues regeneration—Approaches and challenges. Materials. 2021;**14**(10):2558

[63] Young CS, Terada S, Vacanti JP, Honda M, Bartlett JD, Yelick PC. Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. Journal of Dental Research. 2002;**81**(10):695-700

[64] Duailibi SE, Duailibi MT, Zhang W, Asrican R, Vacanti JP, Yelick PC. Bioengineered dental tissues grown in the rat jaw. Journal of Dental Research. 2008;**87**(8):745-750

[65] Vijayakumar M, Arangannal P, Jeevarathan J, Amudha S. 'Bioroot'–A nascent paradigm In tooth regeneration. European Journal of Molecular & Clinical Medicine. 2020;**7**(2):6474-6481

Chapter 8

Stem Cells from Dental Pulp of Deciduous Teeth: Twenty Years of Experience

Irina Kerkis, Cristiane Valverde Wenceslau and Rodrigo Pinheiro Araldi

Abstract

Dental pulp stem cells (DPSCs) are a special mesenchymal stem cell (MSC) type. These cells can be isolated from the dental pulp (DP) of deciduous, adult, and wisdom teeth. Stem cells from milk/baby teeth fall naturally, representing an advantageous source of young stem cells. These cells are less studied than MSCs from bone marrow, adipose tissue, and umbilical cord. MSCs from these sources are currently widely used in clinical studies. However, obtaining significant quantities of DPSCs from one donor is still challenging, thus limiting their systemic application in patients, which requires doses starting from 5×10^5 per kg of weight and higher. In this chapter, we would like to share our experience of more than 20 years in the isolation and scaling up of DPSC from deciduous teeth. We will also provide information about their *in vitro* growth, differentiation, and therapeutic potential observed in animal models that mimic human diseases or injuries in preclinical studies. Finally, we will discuss our experience of DPSC production under good manufacturing practice conditions and their use in regulated clinical studies in Brazil for Huntington's disease.

Keywords: dental pulp stem cells, deciduous teeth, scaling up, preclinical studies, therapeutic potential

1. Introduction

The term "stem cells" was first proposed by Russian histologist Alexander Maksimov in 1908 to explain the ability of specific tissues, such as blood, to self-renew for the lifetime of an organism. Maksimov's unitary theory of hematopoiesis was based on stem cells and substantiated the concept of hematopoietic stem cells in various animal models [1]. Another Russian scientist, Alexander Friedenstein, studied the interactions between bone tissue and the blood system and proposed the idea of a hematopoietic microenvironment formed by populations of non-hematopoietic stromal stem cells or bone marrow-derived osteogenic precursors. He showed that these cells are adhesive fibroblast-like clonogenic cells and also showed high replicative ability and the possibility of differentiation into osteoblasts, chondrocytes, adipocytes, and stromal cells that support hematopoiesis [2–6].

Arnold Caplan, Professor of Biology and Director of the Skeletal Research Center at Case Western Reserve University, who was also the first to demonstrate the biotechnological and therapeutic potential of these cells, first suggested the term "mesenchymal stem cells" (MSCs). MSC means that the cells were isolated from mesoderm-bone marrow (BM) and can undergo *in vitro* differentiation into mesoderm derivatives. In 1991, Caplan wrote: "The study of these MSCs, whether isolated from embryos or adults, provides the basis for the emergence of a new therapeutic technology of self-cell repair. The isolation, mitotic expansion, and site-directed delivery of autologous stem cells can govern the rapid and specific repair of skeletal tissues" [7–9].

Mesenchymal stem cells were isolated from different tissues besides BM, such as adipose tissue, umbilical blood and cord, menstrual blood, placenta, DP, and other tissue sources. Moreover, these MSCs beyond mesoderm (BM-MSC) originated from extraembryonic tissue, such as the umbilical cord and placenta, and ectoderm as DP [10–14]. As demonstrated by Friedenstein and Caplan, all these MSCs are fibroblastic, adherent, and colony forming. Fibroblastic colony-forming units (CFU-F) were developed for plastic-adherent clonogenic BM-MSC, which grow in a monolayer and are self-renewing, and are used to characterize MSC from other sources. These cells can also differentiate from mesoderm derivatives *in vitro*, like BM-MSC [5–7].

Caplan and his colleagues were the first to discover cell-surface antigens such as CD105 (SH2) and CD73 (SH3/4) on human BM-MSC, which are detected by monoclonal antibodies [15]. Currently, these antibodies are recognized as principal markers for MSC identification and are part of the minimum criteria for defining MSC, along with CFU-F and differentiation assays [16].

Dental pulp stem cells (DPSCs) can be isolated from deciduous, adult, and wisdom teeth. Their discovery is more recent compared with BM-MSC. DPSC from adult DP for the first time was isolated in 2000, and 3 years later, these cells were isolated from human exfoliated deciduous teeth (SHED) [17]. These cells and their potential therapeutic application have been evaluated mainly in dentistry for a long time. Only recently, these cells and their potential use have attracted attention from other medical areas, for instance, for treating spinal cord injury, neurological and neurodegenerative diseases [18, 19].

Whether DPSC or MSC is still unknown, although they fit in the MSC definition proposed by the International Society for Cellular Therapy [16]. In this chapter, we would like to provide comprehensive, critical, and concise information about our group's experience with the isolation, characterization, nonclinical, clinical application, and scaling-up expansion to obtain significant quantities of DPSC from deciduous teeth, which can allow further possible commercialization of these cells.

2. Origin and function of MSC and DPSC

Figure 1 demonstrates the embryonic origin of tissues used for MSC isolation. This figure shows that BM-MSCs are originated from paraxial mesoderm [20], which gives rise to the axial skeleton. Adipose tissue-derived MSCs (AT-MSCs) [10] are derived from lateral plate mesoderm that gives rise to the appendicular skeleton and adipose tissue. DP is originated from neural crest (that gives rise to the craniofacial skeleton) and head mesenchyme (that gives rise to the bones, cartilages, muscles, tongue, craniofacial nerves, and teeth, and dental ectomesenchymal stem cells and connective

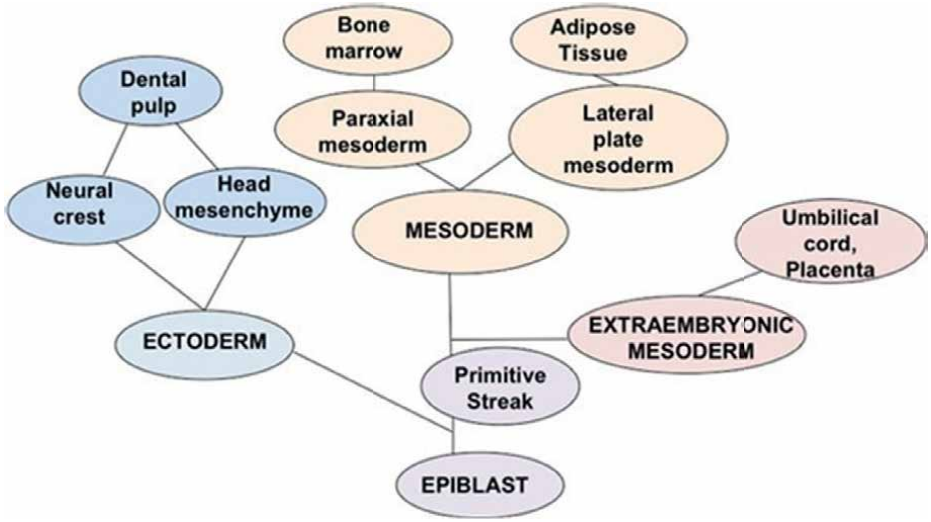

Figure 1.
Embryonic development ontology tree. This figure demonstrates the different embryonic origins of tissues, which can originate MSCs. MSC in the adult organism can be isolated from DP (ectoderm), bone marrow and adipose tissue (mesoderm), and umbilical cord and placenta (extraembryonic mesoderm).

tissues in the craniofacial complex) [14, 21, 22]. Umbilical cord and placenta-derived MSC are originated from extraembryonic mesoderm [23].

Mesenchymal stem cells, independently of origin, exert similar but not identical functions. Accumulation of scientific data about MSC showed that old ideas about replacing injured tissues with MSC have no basis anymore. *In situ* MSCs differ from isolated and cultured cells because the cultivation methods cannot reproduce *in vivo* microenvironments. Therefore, MSCs isolated and cultured *in vitro*, when transplanted back into the organism, show very limited differentiation capacity in mesodermal or another type of tissue without additional stimulus [24–26].

In recent years, the research has been directed toward a better understanding of the mechanisms of MSCs function. This includes the rescue and repair of injured organs and tissues. It has been discovered that MSCs, when transplanted into injured tissue, can be used mainly for paracrine activity involving the secretion of proteins/peptides and hormones by these cells [27–31].

2.1 Function of MSC and pericytes

Crisan et al. [32] demonstrated the link between MSC and perivascular cells, also called pericytes. His group compared the MSC and the pericytes' *in vivo* location and cell marker signatures of both. They demonstrated that cells with MSC markers also express markers associated with pericytes [32]. This raised the question of whether MSCs are pericytes. Pericytes, by definition, are mural cells surrounding blood vessels adjacent to endothelial cells. They control vascular branching morphogenesis, which is critical in maturation and maintenance. Pericytes are essential for the central nervous system (CNS) to form and regulate the blood-brain barrier (BBB). Pericyte deficiency occurs in the central nervous system (CNS) in diseases, such as multiple sclerosis, diabetic retinopathy, neonatal intraventricular hemorrhage, and neurodegenerative disorders [33, 34].

Because of these findings, endogenous pericytes were widely associated with MSC, even though the cell fate plasticity of endogenous pericytes *in vivo* is unknown. In 2017, Guimarães-Camboa and colleagues [35], using lineage-tracing experiments of an inducible Tbx18-CreERT2 line, demonstrated that pericytes and vascular smooth muscle cells maintained their identity in aging and diverse pathological settings; however, they were not able to differentiate *in vivo* into other cell lineages. Therefore, not all MSCs are pericytes, mainly because large and small vessels are surrounded by perivascular cells, which exert different functions (e.g., brain macrophages-glial cells) and are not associated with the osteo-, chondro-, or adipogenic progeny of MSCs [36, 37].

The fact that MSC/pericytes secreted bioactive molecules expects their involvement in a regenerative microenvironment for a variety of injured adult tissues. These cells can limit the area of damage and induce a self-regulated endogenous regenerative response. In this context, the regenerative microenvironment was referred to as trophic, and MSCs play the role of valuable mediators for tissue repair and regeneration [38]. Therefore, the differentiation capacity of MSC was relegated to the background because the *in vivo* differentiation capacity of MSC after their intravenous or intra-arterial transplantation to the adult organism did not receive proper confirmation. Because of the latest findings, A. Caplan suggested denominating these cells used in multiple clinical studies focused mainly on their secretome potential as "Medicinal Signaling Cells," thus maintaining the abbreviation of MSC, however, reinforcing their therapeutic meaning [39, 40].

2.2 Dental pulp from deciduous teeth

Dental pulp is a soft, gelatinous, and non-mineralized oral tissue. A jelly-like core is composed of soft, loose connective tissue and vascular, lymphatic, and nervous cells found in each tooth's dental pulp cavity. DP is made up of Type I and Type III collagens; however, it lacks elastin fibers.

Dental pulp from deciduous teeth, discarded after exfoliation, represents a valuable source of young stem cells. The fact that the cells from deciduous teeth are young is significant for their therapeutic use. Evidence shows that MSCs function declines with age, thus limiting their therapeutic potential [38, 40–42].

The main difference in the biology of the DP isolated from deciduous and permanent teeth is that the lifetime of deciduous teeth is rather limited. Different from adult teeth, they start to grow during embryonic life, and their loss begins at the age of 6 years, starting with the central incisors and followed about a year later by the lateral incisors [43]. However, from a tiny piece of DP of deciduous teeth, significant quantities of young cells can be isolated [44].

3. DPSC isolation and characterization

As mentioned, one of the characteristics of MSCs is their adherence to plastic surfaces. Therefore, the principal method of MSC isolation is based on the ability of the MSCs to selectively adhere to plastic surfaces. The method of isolation and culturing of MSC is challenging and depends on the MSC method of preparations, which should account for variability in tissue sources, and donor-related issues, for instance, age, disease, gender, etc., culture media, and population doublings.

3.1 Explant culture

For the first time, we isolated human immature DP stem cells (hIDPSCs) from the DP of deciduous teeth using the explant method [44, 45]. This method is also denominated as organ culture and used for the *in vitro* growth of organs or part of organs containing the various tissue components, such as parenchyma and stroma, following their transfer to a nutrient medium. The explanted organ preserves its anatomical relationship and function as in tissue. It is essential that the DP is gently disrupted and requires careful handling. The media used for growing tissue culture are generally the same as those used for cell culture.

However, only recently, eleven years after our pioneering work [44], the explant method was recognized as more advantageous for MSCs isolation compared to enzymatic one. This method is gainful because first, it excludes the proteolysis step during cell isolation. Second, it provides the presence of small tissue pieces in the primary cell culture. Third, it removes lytic stress on cells and reduces *in vivo* to *in vitro* transition stress for the cells migrating out of tissue; and fourth, it provides supporting functions of extracellular matrix and released growth factors from tissue explant [46].

3.2 Isolation of human dental pulp stem cells (hDPSCs)

Using an explant method, the cells like MSC from other tissues can be isolated from DP, which is a very small organ; its length is approximately 4.04 mm, while the width is 1.0 mm. In deciduous teeth, a greater cellular density was observed in the coronal region, approximately 47.30 ± 14.71 cells/mm^2 [47]. However, this size allows an easy *in vitro* manipulation of DP tissue without microscopy. After the DP is placed into a culture dish in an appropriate culture medium and after a short period of approximately three days DP, it adheres to the plastic, and the cells start to outgrow from below DP, forming a circle of growing cells around DP (**Figure 2A**). After a week, the DP is transferred to the new culture dish. The adherent cells undergo enzymatic treatment and are transferred in a new culture flask for further growth and multiplication or frozen at passage 0 (P0) (**Figure 2B**). Thus, the first cycle of DP cell isolation is ended. Transferred DP continues growing and producing the cells that undergo enzymatic treatment and can be frozen or transferred in a culture flask for further growth and cell multiplication. Thus, the second, the cell isolation cycle, is ended, and so on. DP decreases in size following transfers and finally runs out, making further transfers impossible. The number of DP transfers-cycles of the cell's isolation is individual for each DP and can vary approximately from 5 to 15 or more cycles [48].

3.3 Characterization of the population of hIDPSC

After isolation, the cells present a fibroblast-like phenotype and adherent to the plastic. The term "immature" is due to the discovery that human immature DP stem cells (hIDPSCs) express embryonic stem cell markers, such as octamer-binding transcription factor 4 (Oct-4), Nanog, stage-specific embryo antigen 3 (SSEA-3), stage-specific embryo antigen 4 (SSEA-4), tumor resistance antigen 1–60 (TRA-1-60), and tumor resistance antigen 1–81 (TRA-1-81), at early passages and mainly in outgrowth culture (before enzymatic treatment) and under conditions described in Kerkis et al. [44]. These cells also express MSC markers, such as CD105, CD73 [16],

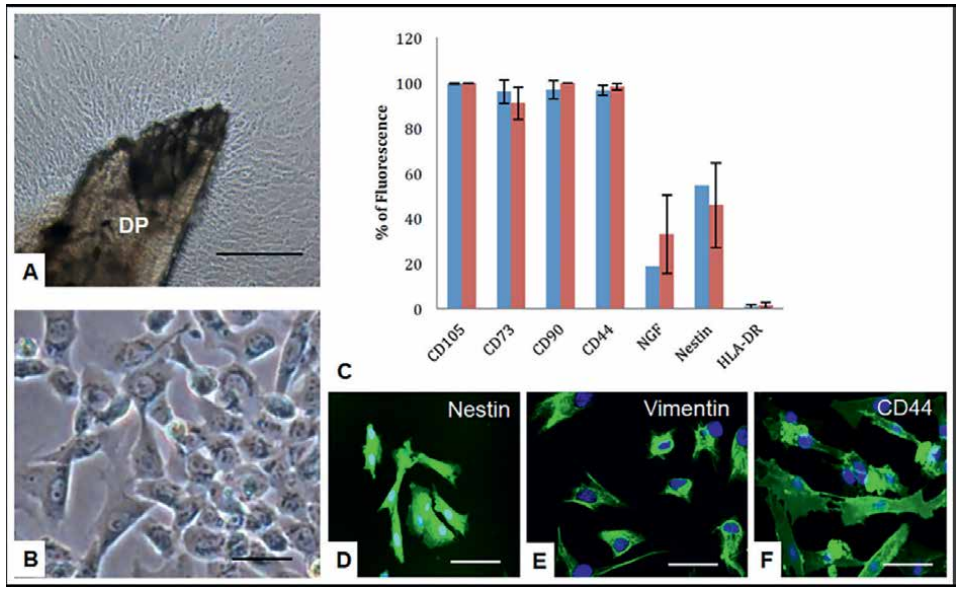

Figure 2.
Isolation and characterization of hIDPSC. A. The cells outgrowing from DP, passage 0. B. HIDPSC after passing (passage 1, P1). C. Flow cytometry demonstrates the expression of CD105, CD73, CD90, CD44, neurotrophic growth factor (NGF), nestin, and human leukocyte antigen HLA-DR isotype (major histocompatibility complex (MHC) class II cell-surface receptor). Immunofluorescence shows positive staining of hIDPSC to nestin (D), vimentin (E), and CD44 (cell-surface glycoprotein) (F). Azul—expression of studied markers after the first DP adhesion (P5), red—expression of studied markers after the first DP transfer (P5). Scale bar: (A, B) = 100 μm, (D–F) = 10 μm. A, B—light microscopy, phase contrast, D–F—epifluorescence.

and CD13, which is not a typical MSC marker, and they were negative for CD34, CD43, HLA-DR (**Figure 2C**), and CD45 ruling out the absence of contamination with hematopoietic and endothelial cells. The cells grow during at least 25 passages while maintaining the normal karyotype [44, 48]. Further investigation of the molecular phenotype of the cells demonstrated that they also express such markers as Nestin, vimentin, CD44, neurotrophic growth factor (NGF) (**Figure 2C–F**), CD29, CD146, CD31, alpha-fetoprotein (AFP), STRO-1, CD146, CD117 (c-KIT), and other markers [45].

4. *In vitro* differentiation and priming of hIDPSC

We also showed that hIDPSC, *in vitro*, could be stimulated to undergo uniform differentiation into smooth and skeletal muscles, cartilage, and bone under appropriate culture conditions and additional drugs that help to induce differentiation. They could also produce neurosphere-like structures and neuronal-like cells [44, 48]. Like MSCs, the hIDPSC can be prepared for the tissue-specific environment in which they will be transplanted, thus improving their survival and biological activity for a specific pathology. The modulation of the cell culture conditions, or the use of a biologically active scaffold, can improve cell function through a process known as preconditioning or priming [49]. Therefore, if the hIDPSC were preconditioned, they would be denominated "priming" hIDPSC.

4.1 Osteogenic and chondrogenic hIDPSC potential

The priming hIDPSC demonstrated the osteogenic and chondrogenic potential *in vitro* [44, 48] (**Figure 3A** and **B**). For this purpose, the cells were grown on biphasic calcium phosphate bioceramics that possess osteoconductive and osteoinductive properties and in a culture medium that induces osteogenic differentiation. As a result, the upregulation of two transcription factors already expressed in hIDPSC, SOX9 (Sex-Determining region Y-Type (SRY)-Box Transcription Factor 9) and RUNX2 (Runt-related transcription factor), was observed. The *RUNX2* gene is involved in developing and maintaining the teeth, bones, and cartilage, while SOX9 is essential for skeleton development and determining sex before birth. However, hIDPSC showed very low expression of peroxisome proliferator-activated receptor gamma (PPARy), which is extensively expressed in MSC from adipose tissue and less in bone marrow [50].

4.2 Neuronal *in vitro* differentiation

When treated with retinoic acid (RA)—a potential inductor of neuronal differentiation—hIDPSC exhibited morphology like that of neural cells. In addition to expressing neural proteins (Nestin, Beta-Tubulin, and anti-glial fibrillary acidic protein (GFAP)), hIDPSC presented an electrophysiological response for sodium and potassium and can trigger an action potential (**Figure 3C–F**). Curiously, the cells without RA treatment also express these markers and present an electrophysiological response. In addition, hIDPSCs were able to direct neural differentiation of embryonic stem cells in coculture assays [51, 52].

5. The immunomodulatory capacity of hIDPSC

One of the most essential characteristics of MSCs and their mechanism of action are immunomodulatory properties, which have critical clinical applications. Therefore, the hIDPSC's immunomodulatory potential has also been studied.

The immunomodulatory effects of hIDPSC on differentiation, maturation of dendritic cells (DCs) derived from monocytes (mo-DC), and their ability to activate T cells and check soluble factors released in coculture cells were analyzed. Peripheral blood mononuclear cells (PBMCs), T cells, and hIDPSCs were obtained from unrelated donors (n = 4). Monocytes were obtained by adhering and removing non-adherent cells from the PBMC cultivation. They were induced to differentiate into mo-DC by culture in the presence of interleukin 4 (IL-4) and granulocyte macrophage colony-stimulating factor (GM-CSF) for seven days. Lipopolysaccharide (LPS), added after five days of culture, was used to induce mo-DC maturation. Effects of hIDPSC were analyzed by flow cytometry after its addition to the cultures from day zero or after five days of culture at a ratio of 1:10. Monocytes derived from DC exposed to hIDPSC from day zero showed a reduction in mean fluorescence intensity (MFI) of the markers, such as blood dendritic cell antigen 1 (BDCA-1) (70%) and CD11c (32%), compared to the control. After activation by LPS, there was a decrease in MFI of CD40 levels (52%), CD80 (35%), CD83 (67%), and CD86 (50%) compared to the control. Mo-DC exposed to hIDPSC from day five showed no changes in the expression of markers. To assess the ability of mo-DC exposed

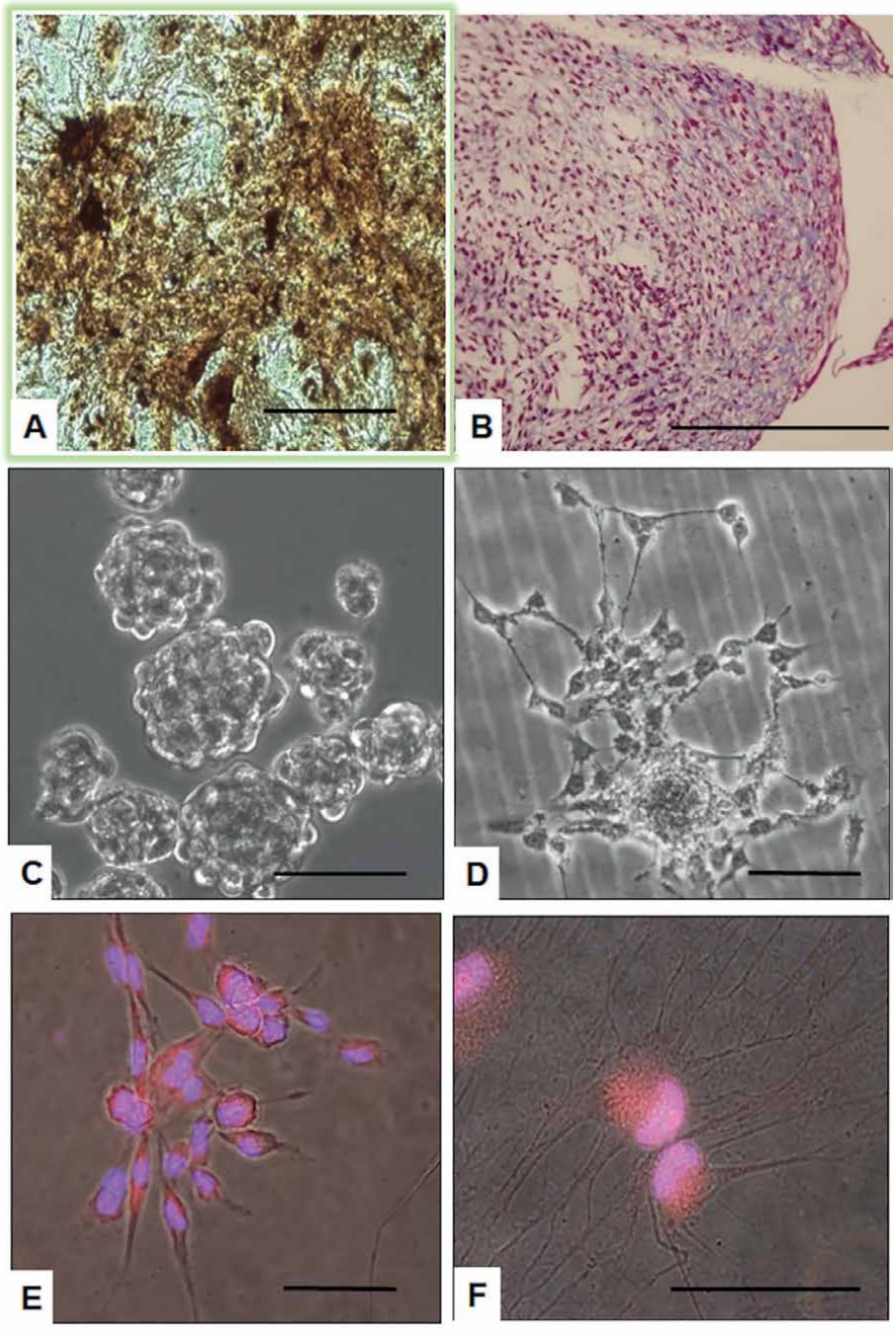

Figure 3.
Induced in vitro differentiation of hIDPSC. A. Neurosphere-like structures formed by hIDPSC suspension culture. B. Neurosphere-like structures adherent to plastic with neuron-like cells around. C. Rosette formed by neuron-like cells derived from hIDPSC positive for anti-Beta 3 tubulin antibody. D. Glial-like cells derived from hIDPSC positive for anti-glial fibrillary acidic protein (GFAP). E. Osteogenic differentiation of hIDPSC Von Kossa staining. F. Chondrogenic differentiation of hIDPSC Masson's trichrome staining. Scale bars: A, B = 100 μm; C = 50 μm; D–F = 20 μm.

to hIDPSC from day zero in activating T cell responses, mo-DC (HLA-DR +) were separated from hIDPSC (HLA-DR-) by magnetic beads and were cocultured (ratio 1:10) with T cells (carboxyfluorescein diacetate (CFSE)-labeled). After five days, cell proliferation was assessed by CFSE dilution. The proliferation of CD4+ T cells induced by mo-DC (exposed to hIDPSC) decreased by 63% for immature mo-DC (IDC) and 50% for mo-DC activated by LPS (mDC), compared to control non-cultivated with hIDPSC. Similarly, the proliferation of CD8+ T cells decreased by 40 and 26% by culture with immature dendritic cells (IDCs) and mature dendritic cells (mDCs), respectively, when cocultured with hIDPSC. There was an increase in the proportion of CD4 + FoxP3 + IL-10+ T cells and CD4 + FoxP3 + IFN-γ + T cells after cocultured with mDC previously cultivated with hIDPSC. The levels of the released soluble factors in cocultures demonstrate the immunomodulation of the studied cells. The levels of pro-inflammatory factors, such as interleukin 2 (IL-2), tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ), reduced while that of the anti-inflammatory factor IL-10 increased. These data showed that hIDPSC affects the differentiation of mo-DC, a phenomenon reflected in the reduction of markers of mo-DC maturation and a decreased ability of mo-DC to induce T cell proliferation. The anti-inflammatory balance of factors released to the medium supports observations [51–53].

6. *In vivo* biodiodistribution of hIDPSC in adult, embryonic, and fetal environment

Mesenchymal stem cells are one of the principal products of advanced cell therapies, which are of great interest due to their paracrine effect that provides a microenvironment improvement, and regenerative activity of endogenous cells in the injury site. The biodistribution of MSCs is an essential step in their characterization that is used to identify addressed migration and engraftment of MSC in injured sites. These properties are very important for defining MSC safety and efficacy after transplantation to human organisms. To understand MSC's therapeutic potential and differentiation capacities, biodistribution studies should be performed at different development stages.

6.1 Biodistribution in nude mice

After *in vivo* transplantation of these cells into immunocompromised mice, they showed dense engraftment in the lung, liver, kidney, and brain, which was evidenced by immunofluorescence and real time-polymerase chain reaction (real-time-PCR) analysis using specific primer for detection of human Gaucher disease region. However, this study did not prove whether these cells are intact and alive [44].

6.2 Biodistribution and fate of hIDPSC in developing mouse embryo

We injected the cells in mouse compacted morulae or early blastocysts to verify the biodistribution of hIDPSC in developing mouse embryos and their possible differentiation in the embryonic or fetal microenvironment [54]. Production of human/animal preterm chimeras is widely used to analyze mammalian cells' developmental potency in biomedical research [55–57].

We showed that hIDPSC presented biological compatibility with the mouse host embryo environment and could survive, proliferate, and contribute to the inner cell mass after introduction into early mouse embryos (n = 28), which achieved the hatching stage following 24 and 48 h in culture. This result demonstrates that hIDPSCs were not toxic for early stage embryo development. When transferred to foster mice (n = 5), these blastocysts with hIDPSC (n = 57) yielded embryos (n = 3) and fetuses (n = 6) (**Figure 4A–D**). The hIDPSC demonstrated robust engraftment in 11 d.p.c. (days post coitum) embryos in the nervous system in primary vesicles, telencephalon, mesencephalon, and rhombencephalon. Additionally, the hIDPSC engrafted in the ocular region on 18-d.p.c. hIDPSC showed comprehensive individual, organ, and tissue biodistribution in mouse fetuses. The cells were observed in different organs of the chimeras, such as the brain, liver, intestine, and muscles (**Figure 4E** and **F**). However, as expected, while fetal age increased the number of grafted hIDPSCs decreased. We verified whether hIDPSC would also be able to differentiate into tissue-specific cell types in the mouse environment. The contribution of hIDPSC in at least two types of tissues, muscles, and epithelial tissue, was confirmed. We showed that hIDPSC survived, proliferated, and differentiated in mice developing preterm chimeras [54].

Overall, these findings were significant for further exploration of the therapeutic potential of hIDPSC since the cells grafted into the growing fetus's brain and other tissues that form the animal head. Thus, this study suggested hIDPSC similarity with neural crest cells, which produce diverse cell lineages, such as melanocytes,

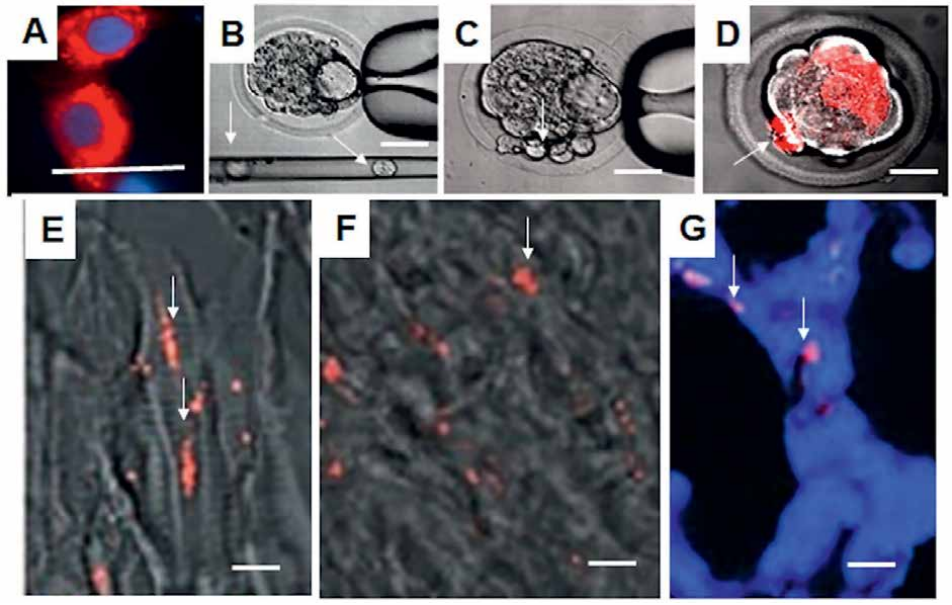

Figure 4.
Human immature dental pulp stem cell's biological compatibility with the mouse embryo environment. A. hIDPSC stained with Vybrant—cytoplasm, nucleus stained with 4′,6-diamidino-2-phenylindole (DAPI) (DNA stain). B. Mouse embryo and glass capillary with hIDPSC inside (white arrow). C. hIDPSC observed inside mouse embryo (white arrow). D. Epifluorescence + phase contrast showing Vybrant-stained hIDPSC (red, white arrow) aggregated with mouse blastocyst cells. Fluorescence in situ hybridization (FISH) analysis using human Y chromosome probe in (E) chimeric muscle fibers and in (F)—heart. G. It shows nuclear localization of Y chromosome signal (red), nuclei stained with DAPI (blue, epifluorescence). E–G—confocal microscopy. E and F—epifluorescence + DIC—digital interference contrast. G—epifluorescence. Scale bars: A–D = 20 μm; E–G = 10 μm.

craniofacial cartilage and bone, smooth muscle, peripheral and enteric neurons, and glia. Another important observation is that the cells could express human proteins in a tissue-specific manner under the environment clues provided by the fetuses.

6.3 Biodistribution of hIDPSC following *in utero* transplantation in canine model (*Canis lupus familiaris*)

Intrauterine stem cell transplantation (IUSCT) treats genetic, congenital, hematological, and immunological diseases. Basic research provides a model for studying the dynamics of migration, graft, and functional status of different types of stem cells. The cells can be transplanted in different moments of the gestational period, which can be divided into equivalent quarters. The choice of the cells and the quarter where the stem cells will be applied can influence cell behavior and transplantation results. Fetal and adult hematopoietic or bone marrow-derived MSCs were mainly used for IUSCT [58–60]. Our study aimed to evaluate the migration capacity, proliferation, and homing of IDPSCs after IUSCT during the third fetal period in dogs. All experimental procedures were approved by the Ethical Committee of the School of Veterinary Medicine and Animal Science of San Paulo University and were performed under appropriate anesthesia. Up to 1×10^6 undifferentiated green fluorescent protein (GFP)-positive human IDPSCs (GFP-hIDPSCs) were transplanted into five fetuses at 45 days of gestation. This transplantation occurred through laparotomy and intraperitoneal injection, guided by intra-operative ultrasound control. Five fetuses, which did not receive IDPSCs, were used as a control. Ultrasound analyses were performed daily before the collection of the fetuses. After seven days of ovarian hysterectomy, fetuses were collected; organs and tissues were isolated and fixed or cryopreserved. The biodistribution of GFP-IDPSCs within the organs and tissues was analyzed on cryosections (5 μm) under a confocal microscope. Homing of GFP-IDPSCs was observed in organs derived from three germ lines, endoderm, ectoderm, and mesoderm. GFP-IDPSCs were found in the intraglandular space and muscular mucosae in the stomach and the intestine. In the liver, these cells were observed in the hepatic parenchyma, in the heart within the myocardium, and in the brain within blood vessels in the cerebellum, specifically within Purkinje cells. Among the different organs, expressive homing was observed in the heart, spleen, and liver myocardium. The hIDPSCs were also found in the canine placenta, especially in blood vessels. These data were confirmed using anti-human nucleus antibody (immunohistochemistry), GFP-hIDPSC, and FISH analysis for the human chromosomes. Human IDPSC showed high migration and proliferation potential after IUSCT in dog fetuses. Therefore, hIDPSC demonstrated homing in fetal hematopoietic (placenta), epithelial (gastric glands), and perivascular stem cell niches [61].

7. hIDPSC local application and *in vivo* differentiation

Most *in vitro* cultured MSCs, after transplantation back into the *in vivo* environment, lost their proliferation and differentiation capacity. From experience with *in vitro* differentiation of MSC, we knew that induction culture media is necessary for differentiation. Therefore, *in vitro* cultured MSCs are not prepared for the *in vivo* setting, and they need to be primed using induction culture media, a different attached matrix, or any other stimulus able to prepare the cells for *in vivo* differentiation [66].

7.1 hIDPSC sheet transplantation in the damaged ocular surface in rabbits: unilateral limbal stem cell deficiency

The ability of hIDPSC to differentiate *in vivo* in epithelial cells [54] raised the investigation of their use in corneal epithelium reconstruction in cases of ocular surface injury.

Two distinct types of epithelial cells, conjunctival and corneal epithelia, compose the ocular surface. Corneal epithelial stem cells reside at the corneoscleral limbus, whose microenvironment is essential in maintaining their stemness. However, the limbal stem cells may be partially or depleted after limbus damage. Such stem cell deficiency results in corneal surface abnormalities that lead to "conjunctivalization" and corneal vascularization, producing an irregular and unstable epithelium and vision deficiency. Limbal stem cell transplantation is commonly used in patients with either uni- or bilateral total limbal stem cell deficiency (LSCD), which can be reproduced in rabbits. Commonly, the chemical burn in one eye of rabbits induces LSCD [62]. We verified hIDPSC *in vivo* differentiation capacity into the corneal epithelium using this model.

First, we verified whether hIDPSC expresses markers in common with LSCD, such as ABCG2 (adenosine triphosphate (ATP)-binding cassette subfamily G member 2), integrin beta 1, vimentin, p63, connexin 43, and cytokeratin 3/12 (K3/12). The expression of these markers was confirmed in undifferentiated hIDPSC, excluding K3/12, a marker of the differentiated corneal epithelium [63]. Next, we induced a rabbit model of unilateral burn eye—LSCD. We demonstrated that hIDPSC could reconstruct the eye surface after induction of LSCD in rabbits and transplantation of the priming hIDPSC sheet growing on the scaffold directly onto the exposed stromal bed. Morphological and immunohistochemical analysis using human-specific antibodies against limbal and corneal epithelium demonstrated corneal epithelium reconstruction, presenting an expression of human antibodies such as integrin beta 1 (ITGB1), cytokeratin 18 (CK18), and K3 [63].

Further investigation with a higher number of animals confirms that this study provided new histological electron microscopy and demonstrated the expression of corneal epithelium human's protein (ABCG2, CK18, p63, and K3 expression in a rabbit LSCD model) [64].

7.2 Reconstruction of significant cranial defects in rats using hIDPSC

Mesenchymal stem cells are critical in bone fracture repair by differentiating between bone-formation osteoblasts and cartilage-forming chondrocytes [65]. Kerkis and coworkers [44] showed remarkable *in vitro* osteogenic properties of hIDPSC. To evaluate the *in vivo* capacity of priming hIDPSC in bone healing, the large-sized cranial bone defects (5 × 8 mm) on each parietal region in non-immunosuppressed rats were analyzed. The left side (LS) was supplied with collagen membrane only and the right side (RS) with collagen membrane with growing priming hIDPSC. Bone formation was observed one month after surgery on both sides, but a more mature bone was present in the RS. Human DNA was PCR-amplified only at the RS, indicating the presence of human functional DNA. Our findings suggest that hIDPSC positively contributes to bone formation in the significant cranial defect in the rat [66].

8. hIDPSC and genetic disease animal model

It is known that adult stem cells can be used in treating genetic diseases. For instance, osteogenesis imperfecta (OI) is not a curable chronic disease. Off-the-shelf MSC appears as a candidate in a clinical therapy for OI. This is due to MSC's high osteogenic potency. Recently, preclinical and initial clinical data supported the use of MSC in treating OI [67]. We present here our preclinical study using DPSC to treat Duchenne muscular dystrophy (DMD) in dogs [68].

8.1 hIDPSC for muscle regeneration in golden retriever muscular dystrophy (GRMD) dogs: intramuscular and intra-arterial applications

Our previous study [44] also suggests using hIDPSC for muscle regeneration. Duchenne muscular dystrophy (DMD) is a lethal X-linked disease affecting newborn males. It is caused by mutations in a large gene located at Xp21 that encodes the muscle protein dystrophin. The closest model for human DMD is the golden retriever muscular dystrophy (GRMD) dog, which causes analogous skeletal and cardiac muscle disease and shows the absence of dystrophin. GRMD dogs are typically succumbing to the disease by around age 2.

In Kerkis and coauthors [68], transplantation of hIDPSC (2n = 46, XY) of four affected littermate GRMD (2 males and 2 females) aged 28–40 days by either arterial or muscular injections (6×10^7 cells per animal), and without using immunosuppression has been carried out. The females received one unique injection, whereas the males were treated with monthly injections, and one male received six intramuscular (biceps femoris) injections. In contrast, another subject received nine systemic injections of arterial (femoral artery) (**Figure 5A–C**). No signs of immune rejection were observed, and these results suggested that hIDPSC transplantation might be done without immunosuppression. Indeed, white blood cell counting did not present any critical changes in response to cell transplantation, and no lymphocyte infiltration was observed in muscle cells. A 1-year-old dog, which received nine systemic injections, showed a good performance with moderate scores, mainly in postural tone, standing up, crossing barriers, and hoping. At 26 months old, this dog still showed no decline. Our data suggested systemic multiple deliveries seemed more effective than local injections. Biopsies from the dog's muscles were obtained and checked by immunohistochemistry (dystrophin markers) and FISH using human antibodies and X and Y DNA probes. We showed that hIDPSC presented significant engraftment in GRMD dog muscles, although human dystrophin expression was modest and limited to several muscle fibers [68] (**Figure 5D–G**). Two years after we published our study on GRMD dogs, the dog that received nine systemic transplantations of hIDPSC was still alive (data not published).

Further studies confirmed our finding demonstrating that DMD mice and dog's phenotypes, such as pathological inflammation and motor dysfunction, can be significantly improved by repeated systemic injections of hIDPSC [69].

9. hIDPSC and neuroregenerative potential

Currently, the neuroregenerative potential of hIDPSC derived from young and adult teeth is widely explored and discussed in scientific literature. This is because

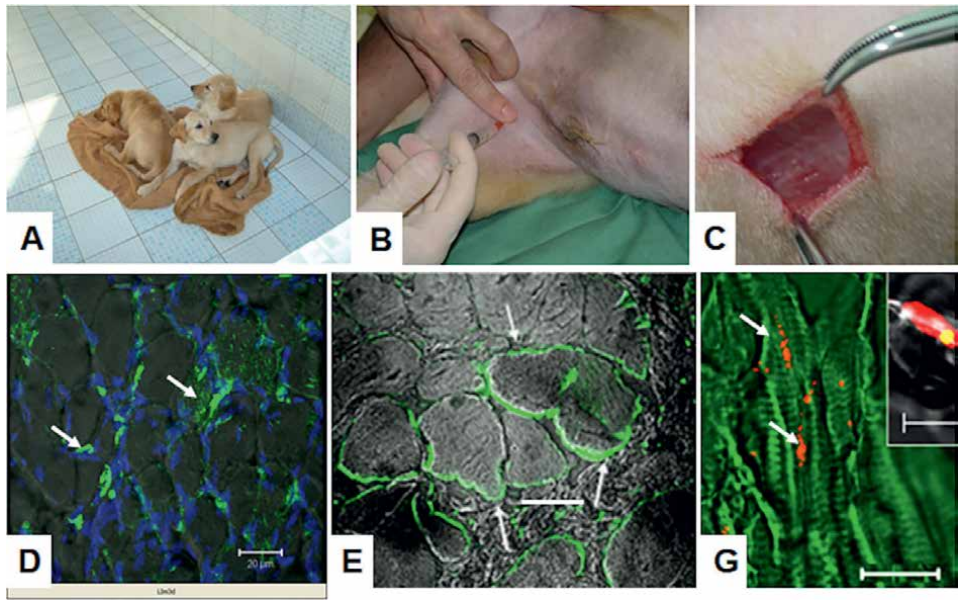

Figure 5.
Transplantation of hIDPSC to golden retriever muscular dystrophy (GRMD) dogs. A. Rare litter of GRMD dogs. B. Intra-arterial hIDPSC injection. C. Biceps femoralis biopsy. D. Chimeric human/canine muscle fibers present positive green, fluorescent immunostaining with anti-human nucleus antibody (white arrows). E. Positive immunostaining with anti-human dystrophin antibody, clone 2C6 (MANDYS106) in large dystrophic fibers (white arrows). G. FISH analysis of dystrophic male's muscles using specific human probe for chromosome: Y (red) and in inset X. Yellow, because merged images of propidium iodide (PI) (red)-stained nucleus and probe of chromosome X (green) are presented. D-G confocal microscopy: Epifluorescence + DIC. G—green artificial color. (D) Nucleus stained with DAPI (blue). Scale bars: A, E = 50 μm G = 10 μm.

hIDPSCs are a population affluent in cell proliferation and multipotency. After transplantation in animal models that mimic neurodegenerative disease, hIDPSCs showed neuron-protective effects. These protective effects are related to trophic factors released from hIDPSCs, which are neurotrophic and were effective for models of neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) [70–72].

9.1 hIDPSC delivery precision in a mouse model of compressive spinal cord injury: localized administration approach

A spinal cord injury (SCI) is damage to any part of the spinal cord or nerves at the end of the spinal canal. This injury causes temporary or permanent changes in spinal cord function. In the USA, 17,000 new SCIs occur yearly, while in Brazil, approximately 150,000 cases occur yearly. Among the factors that contribute to limited SCI recovery are the reduced ability of remyelination spared demyelinated axons, failure of axons to overcome the local expression of myelin-associated inhibitory molecules, a lack of neurotrophic factors to support axonal growth, and other factors [73–75].

We aimed to investigate the efficacy of hIDPSC after local transplantation in a compression lesion of the spinal cord induced in mice. Therefore, SCI was induced in C57/BL6 mice females via laminectomy at T9 and spinal cord compression with

a vascular clip for 1 min. The cells were transplanted 7 days or 28 days after the lesion. The recovery in a subacute or chronic phase of SCI was evaluated after cell therapy. We demonstrated that transplantation of hIDPSC into the site of the spinal cord lesion improved tissue organization in the injured site significantly. Larger areas of white-matter (WM) preservation were also evidenced only in groups treated with hIDPSC but not in control. In addition, in treated groups, the morphological ultrastructural analysis demonstrated large numbers of standard fibers, many axons being remyelinated by either Schwann cells or oligodendrocytes, and preserved neurons exhibiting intact synapses on their cell bodies. These effects were attributed to the statistically significant release of trophic factors observed in CSI animals treated with hIDPSC. This is because we showed in the present study that hIDPSCs express neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), nerve growth factor, beta polypeptide (NGF-b), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT-4/NT-5), as detected by reverse transcriptase-polymerase chain reaction (RT-PCR). Statistically significant differences in BDNF, NGFb, NT-3, and NT-4 expression in brain tissues between treated (increased) and control animals were observed. Before transplantation, the hIDPSCs labeled with cell tracer (red) were observed undestroyed and distributed in the white matter and demonstrated co-localization with anti-GFAP and anti-S-100 antibodies. Furthermore, we used the basic mobility scale (BMS) and the global mobility test to assess motor performance in animals with CSI treated with hIDPSC. Our data suggest that hIDPSC-treated groups showed better locomotor performance in both functional tests and exhibited higher speeds than the control groups, especially in the subacute animals that received cell transplantation seven days after injury. Therefore, we demonstrated the hIDPSC therapeutic potential in both subacute and chronic stages of a mouse compressive SCI, which suggests possible use of hIDPSC in human trials shortly [76].

9.2 hIDPSC in multiple sclerosis: clinical cases of canine distemper (hardpad disease), intravenous injection

Multiple sclerosis (MS) is a progressive disease affecting 2.8 million people worldwide without a definitive cure. The immune system attacks the brain and spinal cord tissues (central nervous system) and nerves by the own organism, causing MS. The etiology of MS is unclear. However, it may be a combination of genetic and environmental factors. Recently, it has been proposed that infection with the Epstein-Barr virus (EBV) may cause this disease [77].

In MS, the immune system attacks the protective sheath (myelin) that covers nerve fibers and causes communication problems between the brain and the rest of the body, thus causing permanent damage or deterioration of the nerve fibers. Canine distemper is an animal model for MS. Canine distemper is a viral disease of dogs, and this virus belongs to the paramyxovirus group. In approximately half of the cases, it is fatal. Impossible to cure, canine distemper is a severe viral illness that attacks a dog's body on all fronts. It can cause persistent infection of the dog's central nervous system, resulting in a progressive, multifocal demyelinating disease. Once an animal develops neurological symptoms of the disease, such as seizures or paralysis, its chances of surviving are slim, its quality of life is bound to worsen, and the animal dies [78].

Therefore, we conducted the study using hIDPSC in canine distemper following international animal care guidelines. The animals were enrolled in an experimental procedure after obtaining informed consent signed by the animals' owners. The treatment was also realized without cost to the owners, and animals received long-term follow-up. Eight dogs of variable breeds aged between 4 and 6 years and weighing 8–16 kg in canine distemper were diagnosed. They presented symptoms such as vomiting, diarrhea, and fever; others were enrolled. Before cell transplantation, these dogs received antibiotics to fight the cough and pneumonia, which did not provide any clinical amelioration. Following the dog's ability to fend off the effects of the virus, they acquired the symptoms of a neural form of this disease, such as paralysis of the hind paws or both hind and front paws.

We performed transplantation of previously cryopreserved hIDPSC (P3–P5) only after the effect of the virus was ended. The cells were thawed before transplantation and washed twice in prewarmed (37°C) sterile phosphate-buffered saline (PBS) following centrifugation for 5 min at 800 × g. At that moment, the viability of the cells was tested using trypan blue staining and was approximately 98% of live cells. The cell number used in each application was established following the dog weights and varied between 2×10^6 (8 kg) and 4×10^6 (16 kg). The cells were suspended in a mean volume of ±0.5 mL sterile physiological solution for subsequent intravenous injection. The dog received an injectable anesthetic, which dose was computed according to the weight of the dog and the sensitivity of certain breeds to the anesthetic. The dogs received single or multiple (no more than three) applications according to disease severity. Human IDPSCs were transplanted without any immunosuppressive protocol. None of the animals showed signs of immune rejection following single or numerous hIDPSC transplantations. Soon after the first hIDPSC application, all animals showed significant amelioration of symptoms of the neurological form of canine distemper. The animals, which were able only to crawl, started to half-rise, while those that were able to half-rise started to get up and stroll. Each animal had its healing dynamics, and those with stronger musculature demonstrated accelerated dynamics of amelioration. The dogs that presented more advanced clinical symptoms and could only crawl demonstrated significant amelioration after the third IDPSC application and even with difficulty, but these animals could walk. At a follow-up 4 months later, all animals recuperated their movement and even running capacities. At a follow-up 2 years later, the animals demonstrated that hIDPSC transplantation was safe and could maintain the physical performance of dogs, which were healthy. None of the animals demonstrated tumor formation, which supposedly can be caused by stem cell transplantation [79, 80].

9.3 hIDPSC in rat model of Huntington's disease

Huntington's disease (HD) is an inherited neurodegenerative disorder. It is caused by genetic mutation expansion of cytosine-adenine-guanine (CAG) repeats in the huntingtin (Htt) gene localized on chromosome 4. This rare disease is found in 0.4–5.7 cases per 100,000 people. The clinical symptoms appear in middle-aged or older people and present motor, cognitive, and behavioral changes. HD causes neuronal loss in the striatum and cortex. Mutant Htt protein forms neuronal intranuclear aggregates in medium-spiny neurons (MSNs). HD causes neuronal loss in the striatum and cortex. Mutant Htt protein forms neuronal intranuclear aggregates in

medium-spiny neurons. This neuronal population of HD-vulnerable γ-aminobutyric acid (GABA) neurons in the striatum constitutes a hallmark of degeneration in HD because these neurons die first.

Cumulative evidence has demonstrated that BDNF-expressing MSCs can confer neuroprotection, promoting functional recovery in rodent Huntington's disease (HD) models. To investigate the capability of BDNF-expressing hIDPSC to restore the BDNF, dopamine- and cAMP-regulated phosphoprotein, 32 kDa (DARPP32), and dopamine D2 receptor (D2R) expression, Wistar rats subjected to a subacute treatment with 3-nitropropionic acid (3-NP), a toxin that irreversibly inhibits the mitochondrial succinate dehydrogenase (SDH), leading to mitochondrial dysfunction and neurodegeneration of striatal cells, mimic the characteristics of HD. Although it is impossible to fully mimic the HD pathology since no genetically modified or chemically induced animal models exhibit the primary clinical signal of the disease, the chorea, the 3-NP has been successfully used to promote selective brain damage that inevitably leads to striatal neurons' (MSN) loss. Two doses of hIDPSC (single versus three consecutive injections) were injected intravenously. The cells demonstrated homing in the striatum, cortex, and subventricular zone. Thirty days after hIDPSC administration, the cells found in the brain still express hallmarks of undifferentiated MSC (**Figure 6**). Immunohistochemistry quantitative analysis revealed a significant increase in BDNF, DARPP32, and D2R positively stained cells in the striatum and cortex in the hIDPSC groups. The differences were more expressive in animals that received only one administration of hIDPSC. These data suggest that intravenous hIDPSC can restore the BDNF, DARPP32, and D2R expression, promoting neuroprotection and neurogenesis [81].

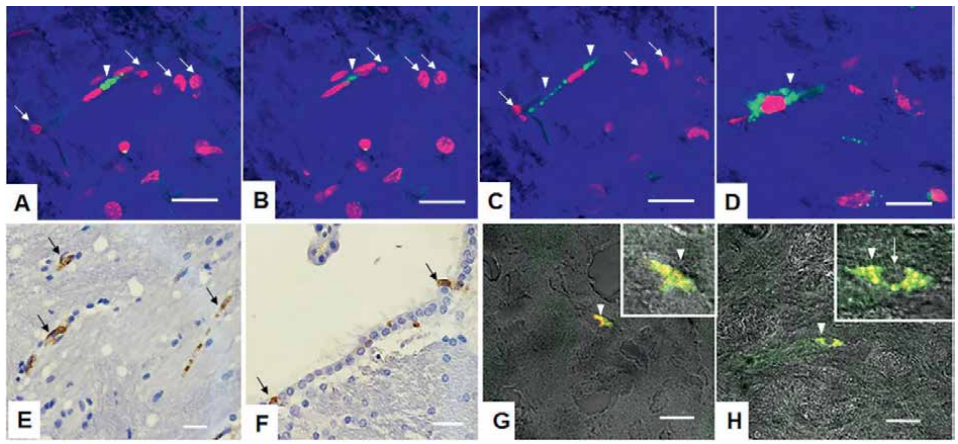

Figure 6.
Human immature dental pulp stem cell engraftment in mice brain after the treatment with 3-nitropropionic acid. A–C hIDPSCs stained with Vybrant (green) optical cuts demonstrate the polarization of hIDPSC, suggesting their migration within brain capillaries. White arrows indicate hIDPSC green cytoplasm is shown by white arrowheads, the same cells on A–C. D. hIDPSC fibroblastic morphology in brain parenchyma. hIDPSC engraftment observed in the striatum E and subventricular zone in F. Overlapping (yellow) of Vybrant-stained hIDPSC (red) with immunopositive staining for mesenchymal stem cell markers—CD105 (G) and CD73 (H) both in green. A–D, G, H—confocal microscopy: Epifluorescence + DIC. G—blue artificial color. (D) Nucleus stained with PI (red).

9.4 hIDPSC in rat facial nerve regeneration, local application

Facial nerve paralysis compromises muscles controlling smiling, blinking capacity, and other facial movements. It is also accompanied by disorders in speech and mastication, as well as esthetics. The successful facial nerve lesion regeneration depends on the Schwann cells' (SCs') support, which participates in the phagocytosis of axon and myelin debris and later in the myelination of newly formed axonal fibers, besides the production of neurotrophic factors that contribute to regularization and modulation of neuronal survival. We induced unilateral facial nerve crush in 70 Wistar rats (CG—control not injured group, G1—crushed group, G2—crushed + hIDPSC). The functional recovery was evaluated after 3, 7, 14, 21, and 42 postoperative days. The cells (5×10^5) were transplanted immediately after the crush injury. G2 exhibited statistically more significant values ($p < 0.05$) in nerve growth factor expression compared with CG and G1 at 7 days and showed complete functional recovery at 14 days, while G1 recovered after 42 days. Additionally, G2 presented histological improvement, evidencing better axonal and structural organization of the myelin sheath, and exhibited statistically higher values for the outer and inner perimeters and g-ratio (degree of myelination). At 42 days, both groups were close to the levels observed in the control group. Therefore, a single injection of hIDPSC accelerates facial nerve trunk regeneration [82].

10. Osteoarthritis and HIDPSC

Osteoarthritis (OA) affects millions of people worldwide. This common disease occurs because the protective cartilage cushions the ends of the bones and is gradually destroyed along the life, and it frequently affects hands, joints, knees, hips, and spine. These irreversible damages and diseased people suffer from joint pain and movement limitations. MSC can be injected into a joint helping to relieve pain, swelling, and loss of movement. They can also be used to make artificial cartilage in a laboratory, which can be transplanted into a joint helping to repair damaged bone, ligaments, and cartilage [83–85].

10.1 Cartilage regeneration observed after intra-articular administration of hIDPSC in experimental osteoarthritis rat model

Osteoarthritis is an incurable condition mainly affecting joints in your hands, knees, hips, and spine. Due to the complexity of the disease, we investigated the molecular and morphological effects after intra-articular injection of one single dose of hIDPSC (8×10^5) in the OA rat model. We also compared the effect of this treatment with the effect of diacerein and glucosamine-chondroitin drugs, which are known to relieve osteoarthritis symptoms. The cell therapy-treated group received the cells on day 14 after osteoarthritis induction, and euthanasia was performed after 60 days. The drug-treated groups were given 50 mg/kg of diacerein and 400/500 mg/kg of glucosamine-chondroitin starting on day 14 for 60 days. The morphological analysis and expression of SRY-Box Transcription Factor 5 (SOX-5), Indian hedgehog (IHH), matrix metalloproteinase-8 (MMP-8), matrix metalloproteinase-13 (MMP-13), and Type II collagen antibodies were statistically analyzed in lateral femoral condyle cartilage. We showed that after hIDPSC transplantation, structural reorganization of the tissues of lateral femoral condyles was observed, while the glucosamine-chondroitin sulfate provided

anti-inflammatory modulation. The diacerein treatment preserves the primordial cartilage of femoral condyles. In conclusion, we demonstrated that a single hIDPSC injection significantly improved cartilage regeneration in an OA rat model compared with the positive therapeutic effect of daily administered conventional drugs [86].

11. Aplastic anemia and hIDPSC

Aplastic anemia and myelodysplastic syndromes are rare disorders characterized by bone marrow failure, which lead to a significant reduction in the hematopoietic stem/progenitor cells (HSPCs), which results in defective mature blood cell production and peripheral pancytopenia [87, 88].

11.1 Hematoprotective role of hIDPSC for aplastic anemia and potentially for other hematopoietic failures

To induce the acquired aplastic anemia (AA), a mouse model subjected to total body irradiation (TBI) was used. After three consecutive hIDPSC (1×10^6 cells/animal) transplantations, we observed that the irradiated mice showed high BM cellularity, recovering the normal BM histology 62 days after cell transplantation (short-term treatment) when compared to irradiated mice treated with saline (placebo). Furthermore, we showed that at D182, the irradiated mice treated with hIDPSC demonstrated stable BM tissue improvement, as evidenced by histological studies. In contrast, BM of the irradiated and placebo-treated mice still presented a significant fat deposit. These data suggest that hIDPSC can stimulate BM tissue recovery and long-term hematoprotection. They (hIDPSCs) maintained fibroblast-like morphology and influence positively the expression of endogen Nestin, which is a marker of vascular structures in BM [44] and a selective marker of BM perivascular MSC that was downregulated after irradiation. In addition, the endogenous CD44+ cell increased in cell-treated mouse BM 180 days after hIDPSC transplantation. CD44 is essential for human hematopoietic regulation, including lymphocyte migration and activation, progenitor cell proliferation, and BM environment restoration. This result suggests that although hIDPSCs are of ectomesenchymal origin and differ from bone marrow MSCs, they accurately respond to BM microenvironment control, thus providing BM recovery [89].

12. Tumorigenic potenial of hIDPSC

Only the cell, which presents a modified genome leading to loss of control of cell growth, can produce a malignant mass tumor. Analyzing factors (proliferation rate, karyotype integrity, and mutations' presence) that may influence the tumorigenicity of hIDPSC to generate malignant tumors discards such a possibility. Moreover, no scientific evidence supports malignant tumor generation by the MSC [90].

Teratoma formation is essential in determining the pluripotency of any pluripotent cells, such as embryonic or induced pluripotent stem cells (ES and iPS cells). A consistent protocol for assessing the cells' teratoma-forming ability was established and used in studies [91]. This method is helpful for the biosafety analysis of other adult/mesenchymal stem cells (MSCs), such as those derived from DP of deciduous teeth, umbilical cord adipose tissue, and others. To form teratomas, the

normal pluripotent stem cells should have epithelial-like morphology and present gap junction, which helps to keep these cells connected. Functionally, gap junctions maintain cellular homeostasis by allowing communication between adjacent cells [92]. However, gap junctions also play an essential role in teratoma formation because they do not allow the migration of pluripotent cells from the local injection of the cells aiming to form teratoma. Furthermore, the enzymatically dissociated cells are used for teratoma formation. Therefore, the cells are co-injected with Matrigel, a solubilized basement membrane matrix used for pluripotent cell adherence to ensure cell mass formation. Co-injection of the pluripotent cells with Matrigel increased subcutaneous teratoma formation efficiency from 25 to 40% to 80–100% [93]. In early development, MSCs originated from pluripotent embryonic cells. Furthermore, they underwent epithelial-mesenchymal transition and became migratory cells. This argument discards the possibility of forming a teratoma by MSC because the cells should stay together to form a teratoma or tumor.

12.1 Experimental evidence demonstrates that hIDPSC does not produce teratomas

In Kerkis et al. [44], we reported that a few cells from the hIDPSC population express two of three pluripotent stem cell markers, such as octamer-binding transcription factor (Oct) 3/4 and Nanog. Therefore, hIDPSC can supposedly produce a teratoma. This capacity of hIDPSC was tested. For this purpose, BALB/c nude mice—females (Institute of Biomedical Sciences (ICB)/University of São Paulo (USP), Isogenic Mouse Vivarium) received an intraperitoneal injection of 1×10^6 hIDPSC. Animals (n = 15) were sacrificed 1, 2, and 3 months after injection by approved methods, and tissues of interest were freshly frozen to verify the presence of human cells. No teratoma or other type of tumor formation was observed. The human DNA sequence encoding the glucocerebrosidase (GBA) gene was detected by PCR in the sites of hIDPSC engraftment and found in the liver, spleen, brain, kidney, and other organs [44].

Furthermore, the capacity of hIDPSC to form teratomas was verified when hIDPSC was reprogrammed to induce pluripotent hIDPSCs using four Yamanaka factors [95]. Reprogrammed hIDPSC-induced pluripotent stem cells (iPSCs) acquired embryonic stem cell (ESC)-like morphology, expressed pluripotent markers, possessed stable, normal karyotypes, and demonstrated the ability to differentiate *in vitro* and *in vivo*. Their capacity to format teratomas has also been tested. In this study, no reprogrammed hIDPSCs were used as a control for hIDPSC-iPSC. Approximately 1×10^6 hIDPSC-iPSCs or hIDPSCs were suspended in PBS and injected into the right testicle or intramuscularly into the limb of nude female mice anesthetized with isoflurane. The injection sites were chosen based on reports of embryonic stem cell (ESC) teratoma formation assays [94]. As reported, teratomas were observed between 5 and 7 weeks after injection only in mice that received hIDPSC-iPSC but not in those receiving hIDPSC [95].

13. hIDPSCs display unique transcriptional signature

Since 2016, the hIDPSC isolation and cultivation technology [44, 48, 79], whose development started in 2000 by a research group led by Dr. Irina Kerkis within the Brazilian Butantan Institute, was licensed to Cellavita Pesquisas Científicas Ltda. Brazilian Startup Company in the middle of 2016. Based on this technology, an

advanced cell therapy investigational product was developed for treating neurodegenerative diseases, particularly Huntington's disease (HD). Currently, it comprises a hIDPSC suspension in sterile saline solution (0.9% NaCl), which expresses Nestin, a protein known for axon growth, and secretes high levels of brain-derived neurotrophic factor (BDNF). This revolutionary product is composed of cryopreserved hIDPSC, which after thawing corresponds to the active component of the NestaCell® product. Following intravenous (IV) administration, NestaCell® can migrate and home in the brain tissues. Because of the low expression of HLA-DR antigens, NestaCell® is safe for heterologous use without immune suppression. To provide further characterization and to demonstrate stability of three different batches (donors) of NestaCell® product, we performed the transcriptome analysis of these cells produced on a large scale using RNA-Seq. We used bioinformatics tools to obtain the list of differentially expressed genes (DEGs), which were next subjected to functional enrichment analysis. One of the most important findings is that the hIDPSC samples showed clustering with a less dimensional distance compared with other analyzed MSC samples. Although the hIDPSC shares at least 72% of transcripts with the other 137 MSC samples from different donors of adipocyte-derived (AD-MSC), bone marrow (BM-MSC), hepatocyte-derived (HD-MSC), menstrual blood (MB-MSC), umbilical cord (UC-MSC), and vertebral tissue (vMSC), they did not present overlapping with these samples. Additionally, we showed that NestaCell® uniquely expressed 375 genes that represent 4.61% (375/8128 genes) of the whole transcriptome of the product, and these genes mainly promote axon growth and guidance [96].

14. NestaCell® use in clinical regulatory studies in Huntington's disease and COVID-19

The neurogenic potential of hIDPSC was demonstrated in various animal models: spinal cord compressive injury model in mice, in Wistar rats that suffered unilateral injury due to crushing of the facial nerve, in dogs with neurological sequelae of distemper, and in a pharmaceutical model of HD [76, 80–82]. All these studies, to some extent, are related to HD pathologies. For instance, spinal cord compression injury induces brain white-matter (WM) impairment. HD also leads to WM degeneration, possibly due to an early breakdown in axon myelination [97]. The facial nerve regeneration model is also essential since uncontrollable movements of the face are between later symptoms of HD [98]. Therefore, all these studies justify the clinical investigation of NestaCell® product in HD.

Mesenchymal stem cell use in COVID-19 treatment appears helpful because of these cells' immunomodulatory, anti-inflammatory, and regenerative properties. It has been suggested that MSC can improve the survival rate of critically ill COVID-19 patients via inflammation control [99]. NestaCell also showed immunomodulatory potential *in vitro* [51–53], which made us believe that hIDPSC could improve clinical COVID-19 symptoms.

14.1 Clinical trials on intravenous administration of NestaCell® product in patients with Huntington's disease and COVID-19

NestaCell product was used in three clinical studies. The first-in-human, non-randomized, Phase I study entitled "SAVE" aimed to evaluate the safety and tolerability and preliminary evidence of the effectiveness of the NestaCell® after

intravenous administration in HD patients (ClinicalTrials.gov ID NCT02728115). This study started on October 16, 2017 and enrolled six patients who received three intravenous injections, two doses 1×10^6 and 2×10^6 cells/kg of body weight and followed for 5 years. The study showed that participants tolerated NestaCell® and that no treatment-related serious adverse events were observed at the end of 2 years and up to the present. Five of six patients presented improvement in the Unified Huntington's Disease Rating Scale (UHDRS) observed for six to nine months after the last administration [100]. The second is a Phase II study entitled "ADORE" (ClinicalTrials.gov ID NCT03252535), aimed at identifying the optimal NestaCell® dose for HD treatment. This is a prospective, Phase II, single-center, randomized, triple-blind, placebo-controlled study with two test doses of NestaCell® (= Cellavita HD) product. This study enrolled 35 patients who started on January 15, 2018. The patients were randomized in a 2:2:1 ratio for the groups G1: lower dose (1×10^6 cells/weight range), G2: higher dose (2×10^6 cells/weight range), or G3: placebo. The study confirmed that NestaCell® is safe for humans with no treatment-related adverse effects. The Phase II ADORE study was extended, and the patients from the control group started to receive the product since the patients from the experimental group demonstrated improvements in UHDRS (motor score). Thus, the extension study evaluates the long-term safety and efficacy of NestaCell® in patients who concluded the ADORE trial. The product NestaCell is also being evaluated to proceed with the Phase III study.

Clinical trial entitled "HOPE" (ClinicalTrials.gov ID NCT04315987) was conducted on COVID-19 hospitalized patients to evaluate the NestaCell® safety and preliminary efficacy. This Phase I/II prospective, randomized, double-blind, multicenter, placebo-controlled clinical trial enrolled the patients hospitalized with COVID-19 but not requiring mechanical ventilation. A total of 90 male and female patients received up to four intravenous injections of 20 million cells per injection (45 patients) or saline (45 patients) every other day (a maximum of 80 million cells per patient). NestaCell® was safe with no relevant treatment-related adverse effects [101]. However, there was no clinical or laboratory benefit in comparison with placebo.

14.2 NestaCell® do not engraft in preexisting malignant neoplasm

Although it is well stated that MSCs, including hIDPSCs, are safe in terms of tumorigenicity, there is a constant concern with the use of cell therapy in patients with preexisting malignancies. This is because several studies have reported that cancer naturally recruits stem cells (particularly bone marrow MSC), which can engraft within the tumor microenvironment (TME), contributing to cancer progression and metastasis, as previously revised by us in Costa et al. [102]. This concern is still higher for lung cancer. Due to the pulmonary first-passage effect associated with intravenous infusion (the most used route of MSC administration), there is a rising concern that these cells could be entrapped in the lungs and grafted in preexisting lung cancer [103]. Despite this concern, until 2021, no clinical report describes whether manufactured stem cells, when transplanted in patients, could engraft within TME, contributing to the oncogenic process.

In this regard, during the clinical trial of the NestaCell® product, a patient with Huntington's disease who attended all roll-in criteria established by the study protocol was subjected to the intravenous treatment with two cycles (one per year) of three doses of 1×10^6 cells/kg each, receiving a dose per month. However, a preexisting pulmonary nodule was identified during the trial screening. The patient was referred to a pulmonologist who considered the nodule to be a benign tumor and authorized

enrollment. One month after the last dose, it was observed as a nodule growth. For this reason, the nodule was biopsied through a bronchoscopy biopsy, which revealed a lung adenocarcinoma. The neoplasm was surgically excised, and the tumor was sectioned into six fragments (representing different areas). These fragments were subjected to RNA-Seq analysis. The transcriptome of each tumor section was compared with the transcriptomes of transplanted hIDPSCs (which correspond to the active component of the NestaCell® product). Using reduction dimension analysis (PCA or principal component analysis), we demonstrated no evidence of the hIDPSCs within the lung adenocarcinoma. This result suggests that transplanted manufactured hIDPSCs neither home nor graft within the pulmonary nodule [104], reinforcing that the cell therapy with the NestaCell® is safe even for patients with preexisting lung cancer.

15. Conclusions

The hIDPSC differential is the original method of isolation and culturing, which allows for obtaining significant quantities of cells from one DP. This method is based on DP explant culture and multiple DP transference to a new culture flask, avoiding excessive passages requiring enzymatic DP and cell treatment. This method drastically decreases the number of donors used for cell isolation and, consequently, genetic variability observed between donors during clinical studies. These cells were used in multiple nonclinical studies demonstrating safety and efficiency in animal models. As a result of technology transfer from academic to commercial areas through licensing, a new advanced cell therapy investigational product was created, which is currently under clinical investigation. Therefore, NestaCell® is an innovative, cryopreserved, off-the-shelf, allogenic cell therapy product, whose active ingredient is based on young, undifferentiated, early passage, hIDPSC, and release of high levels of brain-derived neurotrophic factor (BDNF) *in vitro* (minimum secretion level 1 ng/mL). It is believed that NestaCell® exerts its therapeutic effects via paracrine secretion of neurotrophic, growth, and anti-inflammatory factors in injured brain sites, like the striatum of HD patients.

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Conflict of interest

Dr. Prof. Irina Kerkis from Butantan Institute receives financial support from Cellavita Pesquisas Científicas Ltda, São Paulo, SP, Brazil.

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References

[1] Konstantinov IE. In search of Alexander A. Maximow: The man behind the unitarian theory of Hematopoiesis. Perspectives of Biology and Medicine. 2000;**43**:269-276

[2] Friedenstein AJ. Osteogenic stem cells in bone marrow. In: Heersche JNM, Kanis JA, editors. Bone and Mineral Research. Amsterdam: Elsevier; 1990. pp. 243-272

[3] Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: Revisiting history, concepts, and assays. Cell Stem Cell. 2008;**2**(4):313-319

[4] Friedenstein AJ, Chailakhjan RK, Lalykina KS. The development of fibroblast colonies in monolayer cultures of Guinea-pig bone marrow and spleen cells. Cell and Tissue Kinetics. 1970;**3**(4):393-403

[5] Owen M, Friedenstein AJ. Stromal stem cells: Marrow-derived osteogenic precursors. Ciba Foundation Symposium. 1988;**136**:42-60

[6] Friedenstein AJ, Chailakhyan RK, Gerasimov UV. Bone marrow osteogenic stem cells: In vitro cultivation and transplantation in diffusion chambers. Cell and Tissue Kinetics. 1987;**20**(3):263-272

[7] Caplan AI. Mesenchymal stem cells. Journal of Orthopaedic Research. 1991;**9**(5):641-650

[8] Bruder SP, Fink DJ, Caplan AI. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. Journal of Cellular Biochemistry. 1994;**56**(3):283-294

[9] Caplan AI. The mesengenic process. Clinics in Plastic Surgery. 1994;**21**(3):429-435

[10] Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Molecular Biology of the Cell. 2002;**13**(12):4279-4295

[11] Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood. 2004;**103**(5):1669-1675

[12] Beeravolu N, McKee C, Alamri A, Mikhael S, Brown C, Perez-Cruet M, et al. Isolation and characterization of mesenchymal stromal cells from human umbilical cord and fetal placenta. Journal of Visualized Experiments. 2017;**3**(122):55224

[13] Wagner W, Wein F, Seckinger A, Frankhauser M, Wirkner U, Krause U, et al. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Experimental Hematology. 2005;**33**(11):1402-1416

[14] Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**(25):13625-13630

[15] Haynesworth SE, Baber MA, Caplan AI. Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. Bone. 1992;**13**(1):69-80

[16] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society

for Cellular Therapy position statement. Cytotherapy. 2006;**8**(4):315-317

[17] Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: Stem cells from human exfoliated deciduous teeth. Proceedings of the National Academy of Sciences. 2003;**100**(10):5807-5812

[18] Ueda T, Inden M, Ito T, Kurita H, Hozumi I. Characteristics and therapeutic potential of dental pulp stem cells on neurodegenerative diseases. Frontiers in Neuroscience. 2020;**14**:532435

[19] Candelise N, Santilli F, Fabrizi J, Caissutti D, Spinello Z, Moliterni C, et al. The importance of stem cells isolated from human dental pulp and exfoliated deciduous teeth as therapeutic approach in nervous system pathologies. Cell. 2023;**12**(13):1686

[20] Caplan AI. Mesenchymal stem cells: The past, the present, the future. Cartilage. 2010;**1**(1):6-9

[21] Chai Y, Jiang X, Ito Y, Bringas P Jr, Han J, Rowitch DH, et al. Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development. 2000;**127**(8):1671-1679

[22] Solis-Castro OO, Rivolta MN, Boissonade FM. Neural crest-derived stem cells (NCSCs) obtained from dental-related stem cells (DRSCs): A literature review on current knowledge and directions toward translational applications. International Journal of Molecular Sciences. 2022;**23**(5):2714. DOI: 10.3390/ijms23052714

[23] Nagamura-Inoue T, He H. Umbilical cord-derived mesenchymal stem cells: Their advantages and potential clinical utility. World Journal of Stem Cells. 2014;**6**(2):195-202. DOI: 10.4252/wjsc.v6.i2.195

[24] Costa LA, Eiro N, Fraile M, Gonzalez LO, Saá J, Garcia-Portabella P, et al. Functional heterogeneity of mesenchymal stem cells from natural niches to culture conditions: Implications for further clinical uses. Cellular and Molecular Life Sciences. 2021;**78**(2):447-467. DOI: 10.1007/s00018-020-03600-0

[25] Czapla J, Matuszczak S, Kulik K, et al. The effect of culture media on large-scale expansion and characteristic of adipose tissue-derived mesenchymal stromal cells. Stem Cell Research & Therapy. 2019;**10**:235

[26] Quinn C, Flake AW. In vivo differentiation potential of mesenchymal stem cells: Prenatal and postnatal model systems. Transfusion Medicine and Hemotherapy. 2008;**35**(3):239-247. DOI: 10.1159/000129129

[27] Alvites R, Branquinho M, Sousa AC, Lopes B, Sousa P, Maurício AC. Mesenchymal stem/stromal cells and their paracrine activity-immunomodulation mechanisms and how to influence the therapeutic potential. Pharmaceutics. 2022;**14**(2):381. DOI: 10.3390/pharmaceutics14020381

[28] Merimi M, El-Majzoub R, Lagneaux L, Moussa Agha D, Bouhtit F, Meuleman N, et al. The therapeutic potential of mesenchymal stromal cells for regenerative medicine: Current knowledge and future understandings. Frontiers in Cell and Development Biology. 2021;**9**:661532. DOI: 10.3389/fcell.2021.661532

[29] Rasmusson I, Ringden O, Sundberg B, Le Blanc K. Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or

natural killer cells. Transplantation. 2003;**76**:1208-1213. DOI: 10.1097/01.TP.0000082540.43730.80

[30] Oliva AA, McClain-Moss L, Pena A, Drouillard A, Hare JM. Allogeneic mesenchymal stem cell therapy: A regenerative medicine approach to geroscience. Aging Medicine (Milton). 2019;**2**(3):142-146. DOI: 10.1002/agm2.12079

[31] Durand N, Zubair AC. Autologous versus allogeneic mesenchymal stem cell therapy: The pros and cons. Surgery. 2022;**171**(5):1440-1442. DOI: 10.1016/j.surg.2021.10.057

[32] Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell. 2008;**3**(3):301-313. DOI: 10.1016/j.stem.2008.07.003

[33] Winkler EA, Bell RD, Zlokovic BV. Central nervous system pericytes in health and disease. Nature Neuroscience. 2011;**14**(11):1398

[34] Girolamo F, Errede M, Bizzoca A, Virgintino D, Ribatti D. Central nervous system pericytes contribute to health and disease. Cell. 2022;**11**(10):1707

[35] Guimarães-Camboa N, Cattaneo P, Sun Y, Moore-Morris T, Gu Y, Dalton ND, et al. Pericytes of multiple organs do not behave as mesenchymal stem cells In vivo. Cell Stem Cell. 2017;**20**(3):345-359.e5. DOI: 10.1016/j.stem.2016.12.006

[36] Caplan AI. All MSCs are pericytes? Cell Stem Cell. 2008;**3**(3):229-230. DOI: 10.1016/j.stem.2008.08.008

[37] Caplan AI. New MSC: MSCs as pericytes are sentinels and gatekeepers. Journal of Orthopaedic Research. 2017;**35**(6):1151-1159. DOI: 10.1002/jor.23560

[38] Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. Journal of Cellular Physiology. 2007;**213**(2):341-347

[39] Caplan AI. Mesenchymal stem cells: Time to change the name! Stem Cells Translational Medicine. 2017;**6**(6):1445-1451. DOI: 10.1002/sctm.17-0051

[40] Hosseiniyan Khatibi SM, Kheyrolahzadeh K, Barzegari A, Rahbar Saadat Y, Zununi VS. Medicinal signaling cells: A potential antimicrobial drug store. Journal of Cellular Physiology. 2020;**235**(11):7731-7746. DOI: 10.1002/jcp.29728

[41] Zhang Y, Guo L, Han S, Chen L, Li C, Zhang Z, et al. Adult mesenchymal stem cell ageing interplays with depressed mitochondrial Ndufs6. Cell Death & Disease. 2020;**11**(12):1075

[42] Hung G, Ashvetiya T, Leszczynska A, Yang W, Hwang CW, Gerstenblith G, et al. Paracrine-mediated rejuvenation of aged mesenchymal stem cells is associated with downregulation of the autophagy-lysosomal pathway. NPJ Aging. 2022;**8**(1):10

[43] Arnold WH, Naumova LA, Goulioumis V. Pulp biology of deciduous and permanent teeth. In: Neuhaus KW, Lussi A, editors. Management of Dental Emergencies in Children and Adolescents. Wilay Online Library. Hoboken, New Jersey, United States: Wiley-Blackwell; 2019. Ch 1,2. pp. 12-13

[44] Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira LV, et al. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other

embryonic stem cell markers. Cells, Tissues, Organs. 2006;**184**(3-4):105-116

[45] Kerkis I, Caplan AI. Stem cells in dental pulp of deciduous teeth. Tissue Engineering. Part B, Reviews. 2012;**18**(2):129-138

[46] Hendijani F. Explant culture: An advantageous method for isolation of mesenchymal stem cells from human tissues. Cell Proliferation. 2017;**50**(2):e12334

[47] Guerrero-Jiménez M, Nic-Can GI, Castro-Linares N, Aguilar-Ayala FJ, Canul-Chan M, Rojas-Herrera RA, et al. In vitro histomorphometric comparison of dental pulp tissue in different teeth. PeerJ. 2019;7:e8212. DOI: 10.7717/peerj.8212

[48] Lizier NF, Kerkis A, Gomes CM, Hebling J, Oliveira CF, Caplan AI, et al. Scaling-up of dental pulp stem cells isolated from multiple niches. PLoS One. 2012;7(6):e39885

[49] Noronha NC, Mizukami A, Caliári-Oliveira C, Cominal JG, Rocha JLM, Covas DT, et al. Correction to: Priming approaches to improve the efficacy of mesenchymal stromal cell-based therapies. Stem Cell Research & Therapy. 2019;**10**(1):132

[50] Lobo SE, Glickman R, da Silva WN, Arinzeh TL, Kerkis I. Response of stem cells from different origins to biphasic calcium phosphate bioceramics. Cell and Tissue Research. 2015;**361**(2):477-495

[51] Pelegrino KO. Caracterização e diferenciação neural in vitro de células-tronco de polpa de dente decíduo humano [Dissertação de Mestrado]. São Paulo: Instituto de Biociências, Universidade de São Paulo; 2009. DOI: 10.11606/D.41.2009.tde-18092009-141614. Available from: www.teses.usp.br [Accessed: January 06, 2023]

[52] de Sá Silva F. Investigação da capacidade imunomoduladora de células-tronco imaturas de polpa dentária humana [tese]. São Paulo: Biotecnologia; 2013. DOI: 10.11606/T.87.2013. tde-05022013-090052

[53] Silva Fde S, Ramos RN, de Almeida DC, Bassi EJ, Gonzales RP, Miyagi SP, et al. Mesenchymal stem cells derived from human exfoliated deciduous teeth (SHEDs) induce immune modulatory profile in monocyte-derived dendritic cells. PLoS One. 2014;**9**(5):e98050

[54] Siqueira da Fonseca SA, Abdelmassih S, de Mello Cintra Lavagnolli T, Serafim RC, Clemente Santos EJ, Mota Mendes C, et al. Human immature dental pulp stem cells' contribution to developing mouse embryos: Production of human/mouse preterm chimaeras. Cell Proliferation. 2009;**42**(2):132-140

[55] Lensch MW, Schlaeger TM, Zon LI, Daley GQ. Teratoma formation assays with human embryonic stem cells: A rationale for one type of human-animal chimaera. Cell Stem Cell. 2007;**1**:253-258

[56] Yokoo T, Ohashi T, Shen JS, Sakurai K, Miyazaki Y, Utsunomiya Y, et al. Human mesenchymal stem cells in rodent whole-embryo culture are reprogrammed to contribute to kidney tissues. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:3296-3300

[57] Muotri AR, Nakashima K, Toni N, Sandçer VM, Gage FH. Development of functional human embryonic stem cell-derived neurons in mouse brain. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:18644-18648

[58] Guillot PV, Abass O, Bassett JH, Shefelbine SJ, Bou-Gharios G, Chan J,

et al. Intrauterine transplantation of human fetal mesenchymal stem cells from first-trimester blood repairs bone and reduces fractures in osteogenesis imperfecta mice. Blood. 2008;**111**(3):1717-1725. DOI: 10.1182/blood-2007-08-105809

[59] Chan JK, Götherström C. Prenatal transplantation of mesenchymal stem cells to treat osteogenesis imperfecta. Frontiers in Pharmacology. 2014;5:113459. DOI: 10.3389/fphar.2014.00223

[60] Huang J, Li Q, Yuan X, Liu Q, Zhang W, Li P. Intrauterine infusion of clinically graded human umbilical cord-derived mesenchymal stem cells for the treatment of poor healing after uterine injury: A phase I clinical trial. Stem Cell Research & Therapy. 2022;**13**(1):85. DOI: 10.1186/s13287-022-02756-

[61] Reginato AL. Estudo da biodistribuição de células tronco de polpa de dente decíduo humana (CTPDDh) após o transplante intra-uterino no modelo canino (Canis lupus familiares) [tese]. São Paulo: Faculdade de Medicina Veterinária e Zootecnia; 2012. DOI: 10.11606/T.10.2012.tde-07082013

[62] Dua HS, Azuara-Blanco A. Limbal stem cells of the corneal epithelium. Survey of Ophthalmology. 2000;**44**(5):415-425

[63] Monteiro BG, Serafim RC, Melo GB, Silva MC, Lizier NF, Maranduba CM, et al. Human immature dental pulp stem cells share key characteristic features with limbal stem cells. Cell Proliferation. 2009;**42**(5):587-594

[64] Gomes JA, Geraldes Monteiro B, Melo GB, Smith RL, Pereira C, da Silva M, et al. Corneal reconstruction with tissue-engineered cell sheets composed of human immature dental pulp stem cells. Investigative Ophthalmology & Visual Science. 2010;**51**(3):1408-1414

[65] Oryan A, Kamali A, Moshiri A, Baghaban EM. Role of mesenchymal stem cells in bone regenerative medicine: What is the evidence? Cells, Tissues, Organs. 2017;**204**(2):59-83

[66] de Mendonça CA, Bueno DF, Martins MT, Kerkis I, Kerkis A, Fanganiello RD, et al. Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. The Journal of Craniofacial Surgery. 2008;**19**(1):204-210

[67] Götherström C, Walther-Jallow L. Stem cell therapy as a treatment for osteogenesis imperfecta. Current Osteoporosis Reports. 2020;**18**(4):337-343. DOI: 10.1007/s11914-020-00594-3

[68] Kerkis I, Ambrosio CE, Kerkis A, Martins DS, Zucconi E, Fonseca SA, et al. Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic? Journal of Translational Medicine. 2008;**6**:35. DOI: 10.1186/1479-5876-6-35

[69] Nitahara-Kasahara Y, Kuraoka M, Guillermo PH, et al. Dental pulp stem cells can improve muscle dysfunction in animal models of Duchenne muscular dystrophy. Stem Cell Research & Therapy. 2021;**12**:78. DOI: 10.1186/s13287-020-02099-3

[70] Ueda T, Inden M, Ito T, Kurita H, Hozumi I. Characteristics and therapeutic potential of dental pulp stem cells on neurodegenerative diseases. Frontiers in Neuroscience. 2020;**14**:407. DOI: 10.3389/fnins.2020.00407

[71] Staniowski T. Therapeutic potential of dental pulp stem cells according to

different transplant types. Molecules. 2021;**26**(24):7423. DOI: 10.3390/molecules26247423

[72] Xiao Z, Lei T, Liu Y, et al. The potential therapy with dental tissue-derived mesenchymal stem cells in Parkinson's disease. Stem Cell Research & Therapy. 2021;**12**:5. DOI: 10.1186/s13287-020-01957-4

[73] Blesch A, Fischer I, Tuszynski MH. Gene therapy, neurotrophic factors and spinal cord regeneration. Handbook of Clinical Neurology. 2012;**109**:563-574. DOI: 10.1016/B978-0-444-52137-8.00035-8

[74] Hu X, Xu W, Ren Y, Wang Z, He X, Huang R, et al. Spinal cord injury: Molecular mechanisms and therapeutic interventions. Signal Transduction and Targeted Therapy. 2023;**8**(1):1-28. DOI: 10.1038/s41392-023-01477-6

[75] Tian T, Zhang S, Yang M. Recent progress and challenges in the treatment of spinal cord injury. Protein & Cell. 2023;**14**(9):635-652. DOI: 10.1093/procel/pwad003

[76] de Almeida FM, Marques SA, Ramalho Bdos S, Rodrigues RF, Cadilhe DV, Furtado D, et al. Human dental pulp cells: A new source of cell therapy in a mouse model of compressive spinal cord injury. Journal of Neurotrauma. 2011;**28**(9):1939-1949

[77] Bjornevik K, Cortese M, Healy BC, Kuhle J, Mina MJ, Leng Y, et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. Science. 2022;**375**(6578):296-301. DOI: 10.1126/science. abj8222

[78] Carvalho OV, Botelho CV, Ferreira CG, Scherer PO, Soares-Martins JA, Almeida MR, et al. Immunopathogenic and neurological mechanisms of canine distemper virus. Advances in Virology. 2012;**2012**:163860. DOI: 10.1155/2012/163860

[79] Kerkis I, Glozman S. Multifunctional immature dental pulp stem cells and therapeutic applications (US Patent # 9,790,468). IP USA BOOTH UDALL FULLER. 2014. Available from: https://patents.justia.com/patent/20200339954; https://patentcenter.uspto.gov/applications/14214016

[80] Barros MAD. Evaluation of allogenic transplantation of mesenchymal stem cells isolated from adipose tissue in dogs with neurological sequela caused by dumpster. 2017. Available from: https://www.oasisbr.ibict.br/vufind/Record/BRCRIS_10dd783a47b1ac25ebb41856df7b26c4

[81] Wenceslau CV, de Souza DM, Mambelli-Lisboa NC, Ynoue LH, Araldi RP, da Silva JM, et al. Restoration of BDNF, DARPP32, and D2R expression following intravenous infusion of human immature dental pulp stem cells in Huntington's disease 3-NP rat model. Cell. 2022;**11**(10):1664. DOI: 10.3390/cells11101664

[82] Saez DM, Sasaki RT, Martins DO, Chacur M, Kerkis I, da Silva MCP. Rat facial nerve regeneration with human immature dental pulp stem cells. Cell Transplantation. 2019;**28**(12):1573-1584

[83] Mancuso P, Raman S, Glynn A, Barry F, Murphy JM. Mesenchymal stem cell therapy for osteoarthritis: The critical role of the cell secretome. Frontiers in Bioengineering and Biotechnology. 2019;**7**:419383. DOI: 10.3389/fbioe.2019.00009

[84] Freitag J, Wickham J, Shah K, Tenen A. Real-world evidence of mesenchymal stem cell therapy in knee

osteoarthritis: A large prospective two-year case series. Regenerative Medicine. 2022;**17**(6):355-373. DOI: 10.2217/rme-2022-0002

[85] Lv Z, Cai X, Bian Y, Wei Z, Zhu W, Zhao X, et al. Advances in mesenchymal stem cell therapy for osteoarthritis: From preclinical and clinical perspectives. Bioengineering (Basel). 2023;**10**(2):195. DOI: 10.3390/bioengineering10020195

[86] Correa Maldonado D, Nicoliche T, Faber J, Kerkis I, Martinez Saez D, Tetsuo Sasaki R, et al. Intra-articular human deciduous dental pulp stem cell administration vs. pharmacological therapy in experimental osteoarthritis rat model. European Review for Medical and Pharmacological Sciences. 2021;**25**(9):3546-3556

[87] Gonzaga VF, Wenceslau CV, Lisboa GS, Frare EO, Kerkis I. Mesenchymal stem cell benefits observed in bone marrow failure and acquired aplastic anemia. Stem Cells International. 2017;**2017**:8076529. DOI: 10.1155/2017/8076529

[88] Gonzaga V, Policiquio B, Wenceslau C, et al. Alternative immune-mediated-based methods in the aplastic anemia treatment. In: Human Blood Group Systems and Haemoglobinopathies. London, UK: IntechOpen; 2021. DOI: 10.5772/intechopen.89090

[89] Gonzaga VF, Wenceslau CV, Vieira DP, Policiquio BO, Khalil C, Araldi RP, et al. Therapeutic potential of human immature dental pulp stem cells observed in mouse model for acquired aplastic anemia. Cell. 2022;**11**(14):2252. DOI: 10.3390/cells11142252

[90] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;**284**:143-147

[91] Gropp M, Shilo V, Vainer G, Gov M, Gil Y, Khaner H, et al. Standardization of the teratoma assay for analysis of pluripotency of human ES cells and biosafety of their differentiated progeny. PLoS One. 2012;**7**(9):e45532

[92] Houghton FD. Role of gap junctions during early embryo development. Reproduction. 2005;**129**(2):129-135

[93] Prokhorova TA, Harkness LM, Frandsen U, Ditzel N, Schrøder HD, Burns JS, et al. Teratoma formation by human embryonic stem cells is site dependent and enhanced by the presence of Matrigel. Stem Cells and Development. 2009;**18**(1):47-54

[94] Cooke MJ, Stojkovic M, Przyborski SA. Growth of teratomas derived from human pluripotent stem cells is influenced by the graft site. Stem Cells and Development. 2006;**15**(2):254-259

[95] Beltrão-Braga PC, Pignatari GC, Maiorka PC, Oliveira NA, Lizier NF, Wenceslau CV, et al. Feeder-free derivation of induced pluripotent stem cells from human immature dental pulp stem cells. Cell Transplantation. 2011;**20**(11-12):1707-1719

[96] Araldi RP, Viana M, Colozza-Gama GA, Dias Pinto JR, Ankol L, Wenceslau CV, et al. Unique transcriptional signatures observed in stem cells from the dental pulp of deciduous teeth produced on a large scale. Pharmacologia. 2023;**14**(1):72-95

[97] Bourbon-Teles J, Bells S, Jones DK, Coulthard E, Rosser A, Metzler-Baddeley C. Myelin breakdown in human Huntington's disease: Multi-modal evidence from diffusion

MRI and quantitative magnetization transfer. Neuroscience. 2019;**403**:79-92. DOI: 10.1016/j.neuroscience.2017.05.042

[98] Roos RA. Huntington's disease: A clinical review. Orphanet Journal of Rare Diseases. 2010;**5**:40. DOI: 10.1186/1750-1172-5-40

[99] Abu-El-Rub E, Khasawneh RR, Almahasneh F, Altaany Z, Bataineh N, Zegallai H, et al. Mesenchymal stem cells and COVID-19: What they do and what they can do. World Journal of Stem Cells. 2021;**13**(9):1318-1337. DOI: 10.4252/wjsc.v13.i9.1318

[100] Macedo J, Pagani E, Wenceslau C, Ferrara L, Kerkis I. A phase I clinical trial on intravenous administration of immature human dental pulp stem cells (NestaCell HDTM) to Huntington's DISEASE patients. Cytotherapy. 2021;**23**(4):1. DOI: 10.1016/j.jcyt.2021.02.008

[101] Araldi RP, Prezoto BC, Gonzaga V, Policiquio B, Mendes TB, D'Amélio F, et al. Advanced cell therapy with low tissue factor loaded product NestaCell® does not confer thrombogenic risk for critically ill COVID-19 heparin-treated patients. Biomedicine & Pharmacotherapy. 2022;**112920**:72-95. DOI: 10.1016/j.biopha.2022.112920

[102] Costa VR, Araldi RP, Vigerelli H, D'Amélio F, Mendes TB, Gonzaga V, et al. Exosomes in tumor microenvironment: From biology to clinical applications. Cell. 2021;**10**(10):2617

[103] Fischer UM, Harting MT, Jimenez F, Monzon-Posadas WO, Xue H, Savitz SI, et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: The pulmonary first-pass effect. Stem Cells and Development. 2009;**18**(5):683-692. DOI: 10.1089/scd.2008.0253

[104] Silva JM, Araldi RP, Colloza-Gama GA, Pagani E, Sid A, Valverde CW, et al. Human immature dental pulp stem cells did not graft into preexisting human lung adenocarcinoma. Case Reports in Oncology. 2021;**15**(1):413-422

Chapter 9

Pharma Intelligence Applied to the Research and Development of Cell and Cell-Free Therapy: A New Era of the Medicine

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Abstract

Cellular and cell-free therapies have provided novel therapeutic opportunities for treating various incurable diseases. This is because the mesenchymal stromal/stem cells (MSCs) produce a plethora of bioactive molecules able to target different biological pathways through extracellular vesicle-mediated paracrine mechanisms simultaneously. However, to share transcriptomic signatures with their origin tissue, it is expected that each MSC population has a unique molecular profiler. In this sense, to analyze the transcriptome of therapeutic cells, it is crucial to identify the molecular profiler of these cells to predict the potential clinical benefits promoted by these cells. Herein, we discuss the application of high-throughput RNA-sequencing (RNA-Seq) in the Pharma Intelligence Era, discussing and exemplifying how the combination of molecular biology with Analytics can revolutionize the Research, Development, & Innovation (RD&I) of advanced medicinal products.

Keywords: mesenchymal stem cell, extracellular vesicles, RNA-Seq, pharma intelligence, analytics

1. Introduction

Since their discovery, mesenchymal stromal/stem cells (MSCs) have been proposed as the best candidate for treating non-curable diseases [1–7].

Stem cells can be obtained from both embryonic and adult tissues. Embryonic stem cells (ESCs) are derived from the inner cell mass of the human blastocyst at the pre-implantation stage. However, hESC are pluripotent cells and can differentiate into all cell types, which compose adult organisms. Therefore, when transplanted in an animal model, these cells form teratomas—benign tumors that contain derivate of all three germ layers, the most typical ones being bone, cartilage, neuronal rosettes, and epithelium of the airways and gut [8]. By contrast, human adult stem cells (hASCs) comprise a population of undifferentiated precursor cells that naturally reside in all tissues and organs. However, hASCs show limited differentiating capability (multipotent cells), and they do

not confer any risk of forming teratomas. This is one of the reasons why hASCs are the most studied type of stem cell for therapeutic purposes.

The bone marrow hASCs are classified as stromal cells or mesenchymal stem cells (MSCs). Stromal cells are progenitors of the hematopoiesis-supporting stroma and are components of skeletal tissue such as bone, cartilage, and adipocytes. MSCs can be isolated from numerous adult and perinatal tissues, such as bone marrow (BM), umbilical cord (UC) vein, Wharton's jelly, adipose and placental tissues, menstrual blood (MB), liver, spleen, and dental pulp (DP) [2, 3, 9, 10]. Moreover, MSCs can be easily expanded *in vitro* and in accordance with Good Manufacturing Process (GMP) conditions, which is crucial to guarantee a large-scale production of therapeutic cells for therapeutic purposes.

For a long time, it has been suggested that the therapeutic properties of stem cells were based on the capacity of these cells to replace dead cells in injured tissues [3, 11]. However, studies showed that transplanted MSCs remain in the injury site for a few days and, subsequently, are not found in the tissue [11–13]. These results were later confirmed by numerous independent studies [10, 14, 15], which reported that less than 1% of transplanted MSCs survive for more than 1 week after systemic administration [16, 17]. Historically, these studies broke the paradigm that the therapeutic action of these cells is based on their capability to replace dead cells. Despite the therapeutic properties and the facilities to isolate and produce these cells, the mechanism of action (MoA) of the MSCs remains not completely understood.

Supporting this breaking paradigm, Takahashi et al. [18] showed that injection of MSC conditioned culture medium (CCM) increases the capillary density, reduces the damage size, and improves the cardiac function in murine models for heart attack. This result, combined with previous evidence that less than 1% of MSCs survive for more than 1 week after systemic administration, provided strong evidence that the therapeutic properties of MSCs are mediated by the "secretome" of these cells, which is composed of a plethora of bioactive molecules [2, 19, 20].

To date, several bioactive molecules have already been identified in the MSCs' secretome, such as chemokines cytokines, interleukins, growth factors, lipid steroids, nucleotides, nucleic acids, ions, and metabolites [2, 21, 22]. These molecules can be found in free form (when directly released into the extracellular space) or within extracellular vesicles (EVs), which are recognized as a key component of paracrine secretion [6, 22, 23].

The rediscovery of EVs and the discovery that these vesicles mediate cell-to-cell communication led to the conceptualization of the paracrine stimulation theory [11, 24]. This theory postulates that MSCs release a variety of bioactive molecules into the intercellular space, playing a key role in cross-talk communication between the cells and the surrounding tissues [1–3, 10, 25]. Thus, the MSCs' secretome is the key mediator of the regenerative action of these cells [6, 11, 21, 26]. For this reason, in 2017, Prof. Dr. Arnold Caplan, who isolated for the first time the MSC population for therapeutic purposes, proposed to rename these cells to signaling cells [27].

The paracrine stimulation theory not only brought novel horizons to elucidate the MoA of stem cells but also allowed us to explore the biotechnological potential of these EVs in a new therapeutic modality known as cell-free therapy [2, 6, 22, 28].

2. Cell-free therapy: novel horizons for the treatment of non-curable diseases

The discovery that the MSC conditioned culture medium (CCM) elicits therapeutic properties similar to the MSCs [18] aroused the interest in exploring the content

of this CCM as an alternative to cell therapy. However, CCM contains components such as bovine fetal serum, antibiotics, and pH indicators that limit the direct application of this medium for clinical purposes. For this reason, the CCM is discarded as a subproduct of the MSC manufacturing process. Thus, biopharmaceutical companies are discarding a valuable source of feedstock rich in bioactive molecules that could be explored for clinical purposes in cell-free therapy [6, 28].

Although the MSC-derived bioactive molecules could be found in a free form diluted in the CCM, generally, in this form these bioactive molecules are easily degraded or oxidized. By contrast, when released within EVs, these bioactive molecules are preserved, increasing the half-life of these molecules [6, 28]. Therefore, it is not surprising that cell-free therapy is based on the therapeutic use of these EVs, justifying the increased interest in these vesicles, as illustrated by the number of publications involving EVs shown in **Figure 1**.

Although the interest in these vesicles had emerged two decades ago (**Figure 1**), the EVs were discovered in the second half of the 1940s, when Chargaff, studying blood coagulation, observed small "membrane debris" sedimented at high-speed centrifugation of plasma supernatant [29, 30]. Twenty years later, after Chargaff's report, Peter Wolf confirmed the existence of corpuscles, "platelet dust," through electron microscopy [31]. However, the biological nature of these corpuscles remained unknown until 1974, when Nunez et al. [32] identified structures with a size under 1000 nm, called multivesicular bodies (MVBs), opening up the path in the identification of a subtype of EVs that later was called exosomes or small EVs (30–150 nm) [33].

The EVs comprise a heterogenous group of small cell-released particles [34], which are classified according to their size and biogenesis in exosomes (30–200 nm), microvesicles (150–1500 nm), and apoptotic bodies (1000–5000 nm) [6, 35, 36]. Among these EVs, exosomes are the most studied and useful type of EV for therapeutic purposes [6, 36]. For this reason, we will focus on this class of EV.

Exosomes are nanosized vesicles, surrounded by a phospholipid membrane, containing cholesterol, sphingomyelin, ceramide, lipid rafts, and evolutionarily conserved markers such as tetraspanins (CD9, CD63, and CD81), heat shock proteins (HSP60, HSP70, and HSP90), major histocompatibility component (MHC) classes I and II, Alix, TSG101, lactadherin, and lysosome-associated membrane glycoprotein 2 [6].

Exosomes constitutively originate from late endosomes, which are formed by inward budding of the limited multivesicular body (MVB) [6, 37, 38].

MVBs and late endosomes are a subset of specialized endosomal compartments rich in intraluminal vesicles (ILVs) [39]. During the ILV formation process, certain proteins are incorporated into the invaginating membrane, while the cytosolic components are engulfed and enclosed within the ILV [38, 40, 41].

Along the biogenesis, the endosomal sorting complex required for transport (ESCRT), which consists of ESCRT-0, -I, -II, -III, combined with the vacuolar protein sorting 4-vesicle trafficking 1 (VPS4-VTA1), and some accessory proteins such as the ALG-2-interacting protein X (ALIX) homodimer [42] promotes the sorting of specific biomolecules to the exosomes [43].

Upon maturation, the MVBs can be transported to the plasma membrane via the cytoskeletal and microtubule network and undergo exocytosis post-fusion with the cell surface, whereby the ILVs get secreted as exosomes [40, 44]. Alternatively, MVBs can also follow a degradation pathway either by direct fusion with lysosomes or by fusion with autophagosomes followed by lysosomes [39]. Although both the secretory and degradative MVB pathways coexist, the mechanism that influences these pathways remains unclear [39].

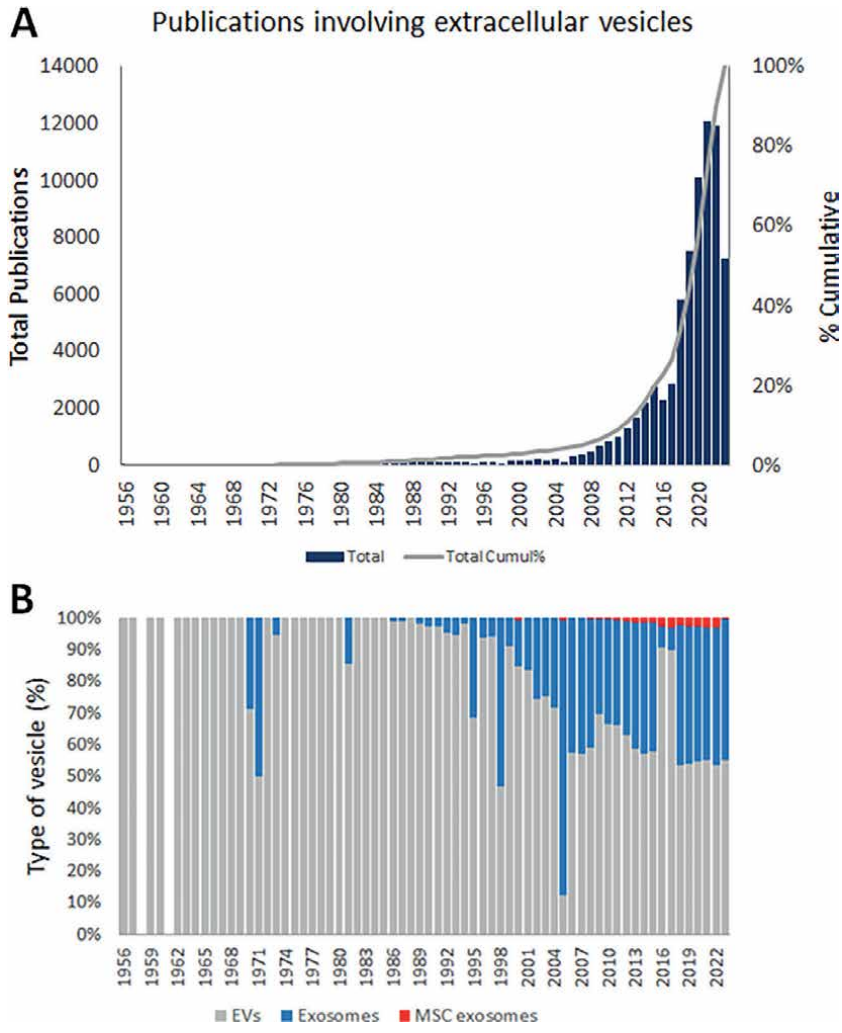

Figure 1.
Number of publications involving extracellular vesicles from 1956 to October 10, 2023 (A). Numbers show an increased interest in these vesicles, which has increased since 2008. Results also show an increased interest in exosomes from 2004 (B), a specific class of EV. However, the number of publications using exosomes derived from MSCs has been reduced since 2010 (B).

Once released into the extracellular space, exosomes interact with the extracellular matrix and recipient cells, serving as natural vehicles for the nanodelivery of nucleic acids, proteins, metabolites, and lipids [6, 36, 39].

Considering that all cell types naturally produce and secrete exosomes, all cell types can be considered a source of exosomes [6, 28]. However, for therapeutic purposes, the MSCs emerge as the most important source of therapeutic exosomes. This is because, as previously discussed, MSCs can be easily isolated and produced on a large scale. In addition, these cells naturally produce a plethora of bioactive molecules that can elicit therapeutic responses. Thus, considering that during the biogenesis, the ESCRT complex promotes the sorting of specific molecules that may be driven to the lumen of these nanosized vesicles, it is expected that the molecular cargo of exosomes changes according to their origin. Therefore, no prototype MSCs can serve

as a universal source of exosomes to treat all types of diseases. For this reason, knowing the molecular profiler (transcriptomic signature of MSCs) can help us to identify appropriate exosomes for the treatment of a set of diseases. In this regard, RNA-Seq emerges as a useful tool to identify the molecular profiler of these cells and their respective exosomes and, with this, to predict the clinical application of these cells and/or exosomes.

3. RNA-Seq analysis applied to identification of molecular profiler of ATMPs

The advent of high-throughput techniques presents a multitude of opportunities to the biopharmaceutical industry [45, 46]. Among these techniques, transcriptome, which includes RNA sequencing (RNA-Seq) and single-cell RNA sequencing (scRNA-Seq), stands out due to their capability to assess qualitative and quantitatively the gene expression levels, serving as important tools to characterize MSCs and MSC-derived exosomes with therapeutic interest. While RNA-Seq can be applied to characterize a cell or exosome population, scRNA-Seq is useful to analyze the heterogenicity of cell type in the MSC population. This chapter will focus on RNA-Seq since this technique is most useful to characterize MSC or MSC-derived exosome populations and predict their therapeutic properties.

Due to the complexity of the RNA-Seq data, several recommendations have been proposed to guarantee the quality of the results obtained with this technique [46–49]. In general, these recommendations are focused on experimental questions, such as the number of samples, sequencing depth, quality control, read alignment, and quantification of gene levels [47–49]. However, there is no single analysis pipeline to be used in all cases. In this sense, the multiple bioinformatic tools, especially those applied to assess the differential expression, make the RNA-Seq data analysis a laborious task, requiring basic knowledge of genetics, bioinformatics, and statistics [46].

Statistical analyses comprise a mandatory step to identify differentially expressed genes (DEGs), which can be used to characterize the molecular profile and predict the therapeutic potential of MSCs or MSC-derived exosomes. Although the statistical techniques required for these purposes are included in the packages used to analyze the RNA-Seq, the choice for inappropriate controls and/or the lack of the most powerful analytics techniques, particularly those based on artificial intelligence (AI), can negatively affect the power of the transcriptome to predict the therapeutic potential of cell and cell-free products.

However, the combined use of traditional statistical methods with Analytics tools to analyze Pharma Big Data such as those generated by the RNA-Seq has revolutionized both pharmaceutical and biopharmaceutical industries. The combination of these techniques applied to analyze (bio)pharmaceutical data led to the advent of Pharma Intelligence, which has revolutionized the research, development, & innovation (RD&I) in life sciences [46].

4. Pharma Intelligence applied to the identification of transcriptomic signature of different types of MSCs

Pharma Intelligence (PI) is a generic term employed to refer to the combined use of mathematics, statistics, Data Analytics (or Analytics), Business Intelligence (BI),

bioinformatics, and artificial intelligence (AI) to analyze and extract valuable information from big data produced in the multi-Omics Era [46].

Pharma Intelligence emerged as a need of the pharmaceutical sector that, over the last two decades, evidenced numerous disadvantages of the traditional empirical drug development model [46]. The traditional empirical drug development model is based on meeting regulatory requirements to guarantee the investigational product (IP) gets market access instead of providing the most useful details about the IP, such as the eventual side effects and even the mechanism of action (MoA), which could predict the drug repositioning, accelerating the RD&I in the pharmaceutical area [46]. For this reason, historical evidence of the traditional empirical drug development model not only increases the time to obtain the registry of new (bio)pharmaceutical products for the treatment of NCDs but also causes economic losses to the entire pharmaceutical supply chain. In this regard, technologies that could predict the eventual risk of the IP, and MoA are crucial to mitigate the failure rate of the IP during the clinical trials.

On the one hand, the advent of "Omics" technologies (genomics, transcriptome, proteome, and metabolome) revolutionized the RD&I in the (bio)pharmaceutical sector; on the other hand, these multi-Omics tools have contributed to the generation of high amount of data (Big data) that are not efficiently used by (bio)pharmaceutical industry to generate valuable insights [46, 47, 50, 51]. Moreover, due to the complex nature of the pharmaceutical data, which combine structured (tabular data) and non-structured data (image bank, sound, and video recording from clinical trial), the conventional statistical techniques are not sufficient to efficiently explore these datasets [46]. This occurs because standard statistical tests assume that a single hypothesis is tested. However, when multiple hypotheses are tested simultaneously, there is a higher probability of a type I error (false-positive)—an incorrect rejection of a true null hypothesis [46, 52]. However, PI has allowed us to overcome the challenges imposed by the Pharmaceutical Big Data Era, helping to select variables of interest, revealing unexpected correlations among these variables, and significantly reducing the time and cost of drug discovery [46, 52, 53].

In addition, the PI possibilities the reanalyze of datasets available on public data banks, such as the Sequence Read Archive (SRA) of the National Institute of Health (NIH, https://www.ncbi.nlm.nih.gov/sra), facilitating the RD&I of ATMPs.

5. Pharma intelligence applied to prediction of therapeutic potential of MSCs and MSC-derived exosomes

To share a transcriptomic signature with their origin tissue, each MSC population is expected to possess different molecular profiles. Thus, it is not surprising that there is no universal MSC that can confer therapeutic benefits for all diseases. For this reason, identifying the molecular profile (or transcriptomic signature) of each MSC population is crucial to predicting the therapeutic potential, as well as the mechanism of action (MoA) of these cells.

In this sense, the SRA can serve as a useful source of RNA-Seq datasets of MSCs and MSC-derived exosomes to predict the appropriate use of each MSC or MSC-derived exosome for target disease.

Generally, standard analysis of RNA-Seq, using conventional packages (DSeq2, NOISeq, edgeR, etc.) to assess the genes differentially expressed, provides noise results. This is because these packages are based on statistical tests that provide p-values. Thus, all gene that shows a p-value <0.05 is recognized as differentially

expressed. However, slight biological expression differences result in a significant p-value (p-value <0). Although commonly employed, analysis using this analytical approach can result in the identification of numerous genes/transcripts with low biological relevance. Alternatively, some bioinformaticians have calculated the log2 fold-chance (log2FC) and adopted a cutoff value that could justify a phenotypic difference (i.e., a biological response), assuming that this cutoff represents a statistically significant difference. However, using appropriate analytical strategies based on PI, it is possible to combine the log2FC to statistical tests to select genes of interest accurately for downstream prediction analyses.

This strategy was currently used to assess the transcriptional profile of human immature dental pulp stem cells (hIDPSCs) produced on a large scale [54]. In order to increase the predictor power of this method, the study compared the hIDPSCs transcriptional profile with the transcriptome signature of other MSCs obtained from adipose tissue (AD-MSC, BM-MSC (HD-MSC, hepatocyte-derived-MSC), menstrual blood (MB-MSC), UC-MSC and vertebral tissue (vMSC) [54]. For this, the study employed a dataset comprised of more than 130 samples from the SRA [54]. Results obtained by the PI showed that the hIDPSCs have a unique transcriptome when compared with other analyzed MSCs [54]. The study also identified that the hIDPSCs express 375 unique genes (**Table 1**), which are not expressed by other MSCs [54]. These unique genes were subjected to the biological functional enrichment analysis. Interestingly, the results of these analyses suggest that the hIDPSCs have a higher capacity to mediate cell communication when compared to other MSCs and that the genes exclusively expressed by the hIDPSCs regulate metabolic processes and neurogenesis [54]. To confirm experimentally these results, primary motor neurons from transgenic mice for amyotrophic lateral sclerosis (ALS) were treated with three different concentrations of exosomes isolated from the conditioned culture medium of these cells for up to 14 days. Results showed that the exosomes derived from the hIDPSCs were able to promote both neurite and axon guidance length in a concentration- and time-dependent manner [54]. These data provide evidence that the PI applied to the analysis of RNA-Seq of therapeutic cells has an accurate predictor power.

Using this same analytical approach (based on PI), herein we reanalyzed these data, showing that the MSCs share the expression of 5912 genes in common, which represents between 61.62% (MB-MSC) to 85.53% (BM-MSC) of the genes encoded by these cells (**Table 1**).

	Number of genes		
MSC population	**Total identified**	**Commonly expressed (%[1])**	**Exclusively expressed**
AD-MSC	7581	5912 (77.98%)	13
BM-MSC	6912	5912 (85.53%)	0
HD-MSC	7739	5912 (76.39%)	93
hIDPSC	8127	5912 (72.75%)	375
MB-MSC	9593	5912 (61.62%)	832
UC-MSC	7011	5912 (84.32%)	0
vMSC	7970	5912 (74.17%)	114

[1]*In relation to the total genes identified/MSC population.*

Table 1.
Genes commonly e exclusively expressed in each MSC population.

Aiming to predict the biological pathways in which these 5912 genes commonly expressed by all MSCs, it was employed the same functional enrichment technique used to predict the hIDPSCs therapeutic properties. Results showed that 98.34% (5814/5912) genes commonly expressed by all MSCs encode proteins, which are involved in (i) metabolic process (4246) and (ii) biological regulation (3877), (iii) response to stimulus (2811), (iv) cellular component organization (2557), and (v) cell communication (1968) (**Figure 2**). The high number of cell communication-related genes identified as commonly expressed by the MSCs reinforces the paracrine

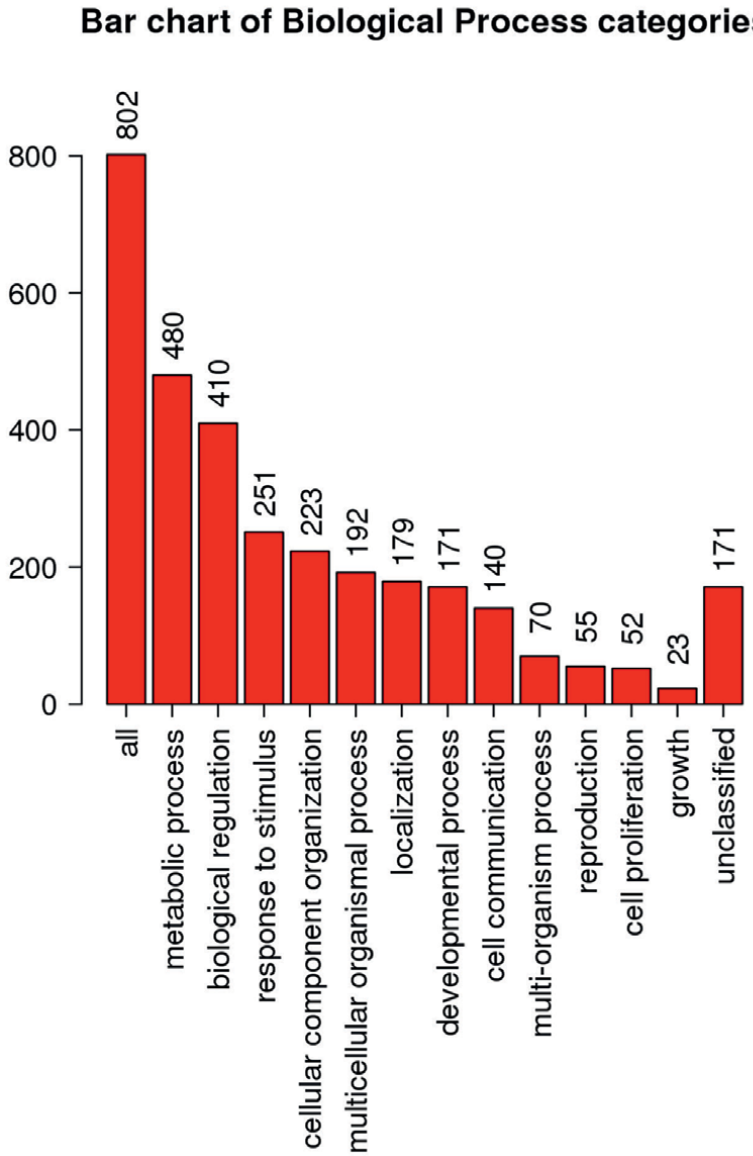

Figure 2.
Biological functional enrichment in terms of biological process of the 5814 genes commonly expressed by the MSC populations analyzed. Results were obtained using the WEB gestalt tool, available from: https://www.webgestalt.org/.

stimulation theory, suggesting that the therapeutic effects of MSCs can be mediated by the bioactive molecules naturally produced and secreted by these cells within EVs.

Biological function enrichment also showed that the genes commonly expressed by the MSCs are involved in (i) transcriptional and (ii) translational regulation, being associated with protein folding and polyubiquitination (**Table 2**). Interestingly, the products of these genes are also involved in (iii) mitochondrial regulation, (iv) cell survival, (v) angiogenesis, and (vi) vesicle-mediated transport (**Table 2**). These data show that MSCs have a complex mechanism of action since the proteins encoded by these cells can act synergically and simultaneously in multiple biological pathways, which are found deregulated in most diseases.

Using the same approach, we also analyzed the set of genes identified as exclusively expressed by the MB-MSCs, since this MSC population showed the highest number of unique genes, followed by the hIDPSCs (**Table 1**). Results showed that 96.39% of the genes exclusively expressed by the MB-MSCs (802/832) encode proteins, which are related to (i) metabolic process (480), (ii) biological regulation (410), (iii) response to stimulus 251), (iv) component organization (223), and other biological process, including (v) cell communication (**Figure 3**).

Biological function enrichment analyses of the genes exclusively expressed by the MB-MSCs showed that most of the products encoded by these genes bind to the DNA, acting as transcriptional factors, regulating cellular responses to DNA damages, or are involved in DNA replication, as part of the replication fork (**Table 3**).

Interestingly, the reanalyzing of the 375 genes exclusively expressed by the hIDPSCs showed that, although the unique genes found in hIDPSCs are involved in the same biological process regulated by the unique genes found in MB-MSCs (**Figure 3**), they govern distinct biological pathways (**Table 4**), conferring different therapeutic properties for these two populations of MSC. This is because the unique genes expressed by hIDPSCs are involved in neurogenesis and synapsis (**Table 4**), suggesting that these cells can be explored for the treatment of neurological disorders, such as neurodegenerative diseases.

Confirming the predictor power of the Pharma Intelligence applied to cell therapy, preclinical studies involving the intravenous administration of the hIDPSCs in rat models for Huntington's (HD, neurodegenerative disorder characterized by a progressive loss of medium spiny neurons (MSNs) in the striatum, causing motor, cognitive and neuropsychiatric disorders) provided evidence that these cells can cross the brain-blood barrier and engraft within the brain areas involved in the HD pathophysiology. Besides this, the study showed that the hIDPSCs administration increases the expression of MSN biomarkers, suggesting a neuroregenerative action [55]. In another independent study, using a rat model for Parkinson's disease, it was also observed that the intravenous administration of these cells ameliorated the motor function of these animals only 3 days after the cell transplantation [56]. Similar results were also demonstrated in phase I clinical trial for HD, which provided evidence that the transplantation of hIDPSCs can improve the motor function of patients with HD [57].

6. Perspectives of the use of pharma intelligence in cell and cell-free therapy

Although already commonly used by pharmaceutical companies to accelerate the RD&I in drug discovery, the complex nature of MSCs and/or MSC-derived exosomes still has limited the application of Pharma Intelligence as part of the characterization

	Enrichment result	FDR	p-value	Gene set size	Overlap
Geneontology (GO)	Regulation of catabolic process (GO:0009894)	0.000	0.000	875	510
	Positive regulation of catabolic process (GO:0031331)	0.000	0.000	348	204
	Vesicle-mediated transport (GO:0016192)	0.000	0.000	1942	899
	Translational initiation (GO:0006413)	0.000	0.000	192	154
	RNA binding (GO:0003723)	0.000	0.000	1603	1056
	RNA processing (GO:0006397)	0.000	0.000	487	337
	Protein targeting (GO:0006605)	0.000	0.000	412	264
	Protein folding (GO:0006457)	0.000	0.000	210	131
	Protein polyubiquitination (GO:0000209)	0.000	0.000	255	155
	Ubiquitin ligase complex (GO:0000151)	0.000	0.000	273	166
	Mitochondrial protein complex (GO:0098798)	0.000	0.000	266	167
Pathway (KEGG, Pnather, Reactome, Wikipathay)	Protein processing in endoplasmic reticulum (has04141)	0.000	0.000	165	125
	Ubiquitin-mediated proteolysis (hsa04120)	0.000	0.000	136	100
	Ubiquitin proteasome pathway (P00060)	0.000	0.000	44	37
	p38 MAPK pathway (P05918)	0.000	0.000	34	24
	RAS pathway (P04393)	0.000	0.000	70	48
	PI3 kinase pathway (P00048)	0.000	0.000	47	30
	PDGF signaling pathway (P00047)	0.000	0.000	125	72
	Integrin signaling pathway (P000340	0.000	0.000	166	95
	P53 pathway (P00059)	0.000	0.000	79	44
	Transcription regulation of TP53 (R-HAS-3700989)	0.000	0.000	365	221
	Vesicle-mediated transport (R-HAS-5653656)	0.000	0.000	667	397
	Cellular response to stress (R-HAS-2262752)	0.000	0.000	426	242
	PTEN regulation (R-HAS-6807070)	0.000	0.000	140	104
	mRNA processing (WP411)	0.000	0.000	126	108
	Electron transport chain (OXOPHOS system in mitochondria) (WP111)	0.000	0.000	103	71
	VEGFA-VEGFR2 signaling pathway (WP3888)	0.000	0.000	431	280
	EGF/EGFR signaling pathway (WP437)	0.000	0.000	162	109

FDR – false discovery ratio; GO – Geneontology; has – KEGG; P – Panther; R-HAS – Reactome; WP – Wikipathway.

Table 2.
Biological functional enrichment of the genes commonly by all analyzed MSC population.

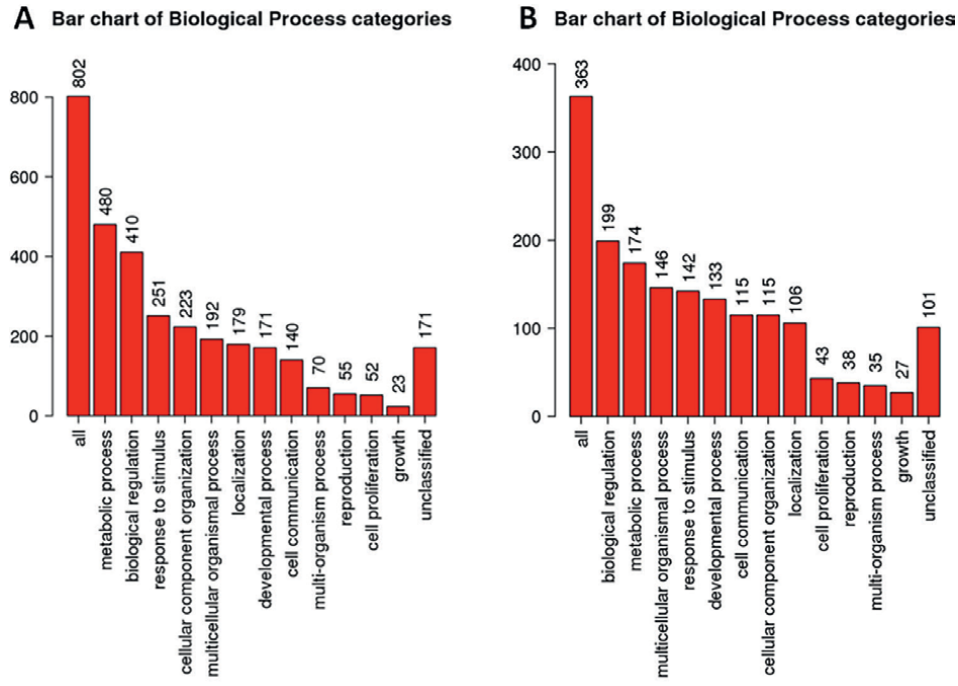

Figure 3.
Biological functional enrichment in terms of biological process of the genes exclusively expressed by the MB-MSCs (A) and hIDPSCs (B) results were obtained using the WEB gestalt tool, available from: https://www.webgestalt.org/.

	Enrichment result	FDR	p-value	Overlap
Geneontology (GO)	RNA modification (GO:0009541)	0.000	0.000	41
	ncRNA processing (GO:0034470)	0.000	0.000	38
	DNA repair (GO:0006281)	0.000	0.000	43
	Cellular response to DNA damage stimulus (GO:0006974)	0.000	0.000	62
	DNA replication (GO:0006260)	0.000	0.000	27
	Replication fork (GO:0005657)	0.034	0.000	10
	DNA-binding transcriptional factor activity (GO:0003700)	0.000	0.000	114
	Methyltransferase activity (GO:0008168)	0.000	0.000	27
Pathway	Chromosome maintenance (H-SRA-73886)	0.000	0.000	17
	Ubiquitin-mediated proteolysis (hsa04120)	0.000	0.000	100
	Mitotic spindle checkpoint (H-SRA-69618)	0.000	0.000	16
	Cell cycle (H-SRA-1640170)	0.000	0.000	49
	Gene expression (transcription) (H-SRA-1640170)	0.000	0.000	49

FDR – false discovery ratio; GO – Geneontology; R-HAS – Reactome.

Table 3.
Biological functional enrichment of the genes exclusively expressed by MB-MSC population.

	Enrichment results	FDR	p-value	Overlap
GO	Neurogenesis (GO:0022008)	0,000	0,000	51
	Neuron spine (GO:0044309)	0,007	0,000	10
	Dendritic spine (GO:0043197)	0,0000	0,007	10
	Neuron to neuron synapsis (GO:0098984)	0,019	0.000	13

FDR – false discovery ratio; GO – Geneontology.

Table 4.
Biological functional enrichment of the genes exclusively expressed by hIDPSC population.

and, as a tool to predict the therapeutic potential of cells and exosomes. However, fortunately, today, we already have specialists and facilities providing innovative solutions that combine mathematical, statistics, analytics, and bioinformatic tools to extract valuable information from multi-Omics, preclinical, and even clinical datasets. Although the number of specialists and companies dedicated to employing Pharma Intelligence in RD&I of cell and cell-free therapy remains reduced when compared to the number of companies offering services dedicated to drug discovery, PI is no longer a promise for the future of these therapies. PI is the New Era of advanced medical therapies.

Moreover, the capability of the algorithms to predict results that can drive the correct use of each MSC population or even each MSC-derived exosome has improved, particularly with the combined use of AI. Section 6 exemplifies how the appropriate use of PI can drive the correct use of MSC populations for clinical purposes.

However, the recent emergence of ChatGPT (which is a type of AI) and the idealization of the use of AI brought by science fiction (sci-fi) could lead to a false interpretation of the power of these techniques, creating an over-expectation about the PI.

In this sense, PI may be understood as a powerful predictor tool to characterize molecular cells and exosome candidates for clinical purposes that can bring many advantages to the R&D of cell and cell-free therapies. Thus, PI serves as a tool to facilitate decision-making, allowing us to identify the best application or predict the MoA of a specific MSC population or MSC-derived exosome.

On the one hand, the PI brings novel opportunities, including job offers. On the other hand, it requires qualified professionals who have expertise in life science (particularly with expertise in cell and cell-free therapy) and analytics. This is because the predictive value of any result obtained using PI depends on many factors, such as (i) the data quality, (ii) data organization and availability, advanced knowledge in (iii) programming, (iv) statistics, (v) bioinformatics, (vi) molecular and cellular biology, and in (vii) the pathophysiology of the target disease. Despite this complexity, which requires a multidisciplinary effort to extract valuable information from data, PI can be considered a revolutionary tool for the biopharmaceutical sector, accelerating the time to obtain the registry of an investigational product and reducing costs with non-necessary experiments needed to bring the proof of concept (POC) required for Regulatory Agencies.

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References

[1] Baek G, Choi H, Kim Y, Lee HC, Choi C. Mesenchymal stem cell-derived extracellular vesicles as therapeutics and as a drug delivery platform. Stem Cells Translational Medicine. 2019;**8**:880-886

[2] L. PK, Kandoi S, Misra R, Vijayalakshmi S, Rajagopal K, Verma RS. The mesenchymal stem cell secretome: A new paradigm towards cell-free therapeutic mode in regenerative medicine. Cytokine Growth Factor Review [Internet]. 2019;46(April):1-9. Available from: doi:10.1016/j.cytogfr.2019.04.002

[3] Cheng L, Zhang K, Wu S, Cui M, Xu T. Focus on mesenchymal stem cell-derived exosomes: Opportunities and challenges in cell-free therapy. Stem Cells International. 2017;**2017**:1-10

[4] Evangelista A, Soares MBP, Villarreal CF. Cell-free therapy: A neuroregenerative approach to sensory neuropathy? Neural Regeneration Research. 2019;**14**(8):1383-1384

[5] Bianco P, Robey O, Simmons P. Mesenchymal stem cells: Revisiting history, concepts and assays. Cell Stem Cell. 2008;**2**(4):313-319

[6] Araldi RP, D'Amelio F, Vigerelli H, de Melo TC, Kerkis I. Stem cell-derived exosomes as therapeutic approach for neurodegenerative disorders: From biology to biotechnology. Cells [Internet]. 2020;**9**(12):2663. Available from: https://www.mdpi.com/2073-4409/9/12/2663

[7] Kerkis I, Araldi R, Wenceslau C, Mendes T. Advances in cellular and cell-free therapy medicinal products for Huntington's disease treatment. In: From Physiopathology to Treatment of Huntington's Disease [Internet]. London, UK: IntechOpen; 2022. pp. 1-27. DOI: 10.1016/j.colsurfa.2011.12.014

[8] Calabrese EJ. Hormesis and embryonic stem cells. Chemico-Biological Interactions [Internet]. 2022;**352**:109783. Available from: https://linkinghub.elsevier.com/retrieve/pii/S000927972100421X

[9] Lee JY, Hong S-H. Hematopoietic stem cells and their roles in tissue regeneration. International Journal of Stem Cells [Internet]. 2020;**13**(1):1-12. Available from: http://www.ijstemcell.com/journal/view.html?doi=10.15283/ijsc19127

[10] Fujita Y, Kadota T, Araya J, Ochiya T, Kuwano K. Clinical application of mesenchymal stem cell-derived extracellular vesicle-based therapeutics for inflammatory lung diseases. Journal of Clinical Medicine. 2018;**7**(10):355

[11] Gomzikova MO, Rizvanov AA. Current trends in regenerative medicine: From cell to cell-free therapy. Bionanoscience [Internet]. 2017;**7**(1):240-245. DOI: 10.1007/s12668-016-0348-0

[12] Duffield JS, Park KM, Hsiao L-L, Kelley VR, Scadden DT, Ichimura T, et al. Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. Journal of Clinical Investigation [Internet]. 2005;**115**(7):1743-1755. Available from: http://www.jci.org/articles/view/22593

[13] Biancone L, Bruno S, Deregibus MC, Tetta C, Camussi G. Therapeutic potential of mesenchymal stem cell-derived microvesicles. Nephrology

Dialysis Transplantation [Internet]. 2012;**27**(8):3037-3042. Available from: https://academic.oup.com/ndt/article-lookup/doi/10.1093/ndt/gfs168

[14] Gao J, Dennis JE, Muzic RF, Lundberg M, Caplan AI. The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion. Cells Tissues Organs [Internet]. 2001;**169**(1):12-20. Available from: https://www.karger.com/Article/FullText/47856

[15] Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, et al. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell [Internet]. 2009 Jul;**5**(1):54-63. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1934590909002124

[16] Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, et al. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Frontiers in Immunology [Internet]. 2012;**3**:3. Available from: http://journal.frontiersin.org/article/10.3389/fimmu.2012.00297/abstract

[17] Song YS, Lee HJ, Doo SH, Lee SJ, Lim I, Chang K-T, et al. Mesenchymal stem cells overexpressing hepatocyte growth factor (HGF) inhibit collagen deposit and improve bladder function in rat model of bladder outlet obstruction. Cell Transplantion [Internet]. 2012 Aug;**21**(8):1641-1650. Available from: http://journals.sagepub.com/doi/full/10.3727/096368912X637488

[18] Takahashi M, Li T-S, Suzuki R, Kobayashi T, Ito H, Ikeda Y, et al. Cytokines produced by bone marrow cells can contribute to functional improvement of the infarcted heart by protecting cardiomyocytes from ischemic injury. American Journal of Physiology-Heart and Circulatory Physiology [Internet]. 2006;**291**(2):H886-H893. Available from: https://www.physiology.org/doi/10.1152/ajpheart.00142.2006

[19] Gurunathan S, Kang M-H, Jeyaraj M, Qasim M, Kim J-H. Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes. Cell. 2019;**8**(4):307

[20] Haraszti RA, Miller R, Stoppato M, Sere YY, Coles A, Didiot MC, et al. Exosomes produced from 3D cultures of MSCs by tangential flow filtration show higher yield and improved activity. Molecular Therapy [Internet]. 2018;**26**(12):2838-2847. DOI: 10.1016/j.ymthe.2018.09.015

[21] Haider KH, Aramini B. Mircrining the injured heart with stem cell-derived exosomes: An emerging strategy of cell-free therapy. Stem Cell Research & Therapy. 2020;**11**(1):1-12

[22] Teixeira MR, Alievi AL, da Costa VR, Dias Pinto JR, Araldi RP. Therapeutic potential of extracellular vesicles in Parkinson's disease. Exploration of Neuroscience [Internet]. 2023;**29**:106-122. Available from: https://www.explorationpub.com/Journals/en/Article/100616

[23] Han C, Sun X, Liu L, Jiang H, Shen Y, Xu X, et al. Exosomes and their therapeutic potentials of stem cells. Stem Cells International. 2016;**2016**:1-11

[24] Baraniak PR, McDevitt TC. Stem cell paracrine actions and tissue regeneration. Regenerative medicine [Internet]. 2010 Jan;**5**(1):121-143. Available from: https://www.futuremedicine.com/doi/10.2217/rme.09.74

[25] Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal stem cell secretome: Toward cell-free therapeutic strategies in regenerative medicine. International Journal of Molecular Sciences. 2017;**18**(9):1852

[26] Hu P, Yang Q, Wang Q, Shi C, Wang D, Armato U, et al. Mesenchymal stromal cells-exosomes: A promising cell-free therapeutic tool for wound healing and cutaneous regeneration. Burn Trauma. 2019;**7**:1-10

[27] Caplan AI. Mesenchymal stem cells: Time to change the name! Stem Cells Translational Medicine [Internet]. 2017;**6**(6):1445-1451. Available from: https://academic.oup.com/stcltm/article/6/6/1445/6448531

[28] da Costa VR, Araldi RP, Vigerelli H, D'Ámelio F, Mendes TB, Gonzaga V, et al. Exosomes in the tumor microenvironment: From biology to clinical applications. Cells [Internet]. 2021;**10**(10):2617. Available from: https://www.mdpi.com/2073-4409/10/10/2617

[29] Chargaff E. Cell structure and the problem of blood coagulation. Journal of Biological Chemistry [Internet]. 1945;**160**(1):351-359. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021925818431316

[30] Chargaff E, West R. The biological significant of the thromboplastic protein of blood. The Journal of Biological Chemistry. 1946;**166**(1):189-197

[31] Wolf P. The nature and significance of platelet products in human plasma. British Journal of Haematology [Internet]. 1967;**13**(3):269-288. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1365-2141.1967.tb08741.x

[32] Nunez EA, Wallis J, Gershon MD. Secretory processes in follicular cells of the bat thyroid. III. The occurrence of extracellular vesicles and colloid droplets during arousal from hibernation. American Journal of Anatomy [Internet]. 1974;**141**(2):179-201. Available from: https://onlinelibrary.wiley.com/doi/10.1002/aja.1001410203

[33] Palazzolo S, Canzonieri V, Rizzolio F. The history of small extracellular vesicles and their implication in cancer drug resistance. Frontiers in Oncology [Internet]. 2022;**24**:12. Available from: https://www.frontiersin.org/articles/10.3389/fonc.2022.948843/full

[34] Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV 2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. Journal of Extracellular Vesicles. 2018;**7**(1):1535750

[35] Sheta M, Taha EA, Lu Y, Eguchi T. Extracellular vesicles: New classification and tumor immunosuppression. Biology (Basel) [Internet]. 2023;**12**(1):110. Available from: https://www.mdpi.com/2079-7737/12/1/110

[36] Da Costa VR, Araldi RP, Vigerelli H, D'Ámelio F, Mendes TB, Gonzaga V, et al. Exosomes in the tumor microenvironment: From biology to clinical applications. Cell. 2021;**10**(10):1-27

[37] Sarko DK, McKinney CE. Exosomes: Origins and therapeutic potential for neurodegenerative disease. Frontiers in Neuroscience. 2017;**11**:1-7

[38] Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: Biogenesis, biologic function and clinical potential. Cell & Bioscience [Internet]. 2019;**9**(1):19. Available from:

https://cellandbioscience.biomedcentral.com/articles/10.1186/s13578-019-0282-2

[39] Gurung S, Perocheau D, Touramanidou L, Baruteau J. The exosome journey: From biogenesis to uptake and intracellular signalling. Cellular Communication Signalling [Internet]. 2021;**19**(1):1-19. Available from: https://biosignaling.biomedcentral.com/articles/10.1186/s12964-021-00730-1

[40] Han Q-F, Li W-J, Hu K-S, Gao J, Zhai W-L, Yang J-H, et al. Exosome biogenesis: Machinery, regulation, and therapeutic implications in cancer. Molecular Cancer [Internet]. 2022;**21**(1):207. Available from: https://molecular-cancer.biomedcentral.com/articles/10.1186/s12943-022-01671-0

[41] Bebelman MP, Smit MJ, Pegtel DM, Baglio SR. Biogenesis and function of extracellular vesicles in cancer. Pharmacology & Therapeutics [Internet]. 2018;**188**:1-11. DOI: 10.1016/j.pharmthera.2018.02.013

[42] Ju Y, Bai H, Ren L, Zhang L. The role of exosome and the ESCRT pathway on enveloped virus infection. International Journal of Molecular Sciences. 2021;**22**(16):9060

[43] Wang M, Yu F, Ding H, Wang Y, Li P, Wang K. Emerging function and clinical values of exosomal microRNAs in cancer. Molecular Therapy—Nucleic Acids [Internet]. 2019;**16**:791-804. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2162253119301209

[44] Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;**367**(6478):eaau6977

[45] Vamathevan J, Clark D, Czodrowski P, Dunham I, Ferran E, Lee G, et al. Applications of machine learning in drug discovery and development. Nature Reviews Drug Discovery [Internet]. 2019;**18**(6):463-477. Available from: http://www.nature.com/articles/s41573-019-0024-5

[46] Faustinoni-Neto B, Dias-Pinto JR, Araldi RP. Pharma data analytics (pharma intelligence): Opportunities and challenges in the multi-omics era of drug discovery and development. Pharmacologia. 2023;**14**(1):29-39

[47] Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, et al. A survey of best practices for RNA-seq data analysis. Genome Biology. 2016;**17**(1):1-19

[48] Tarazona S, Furió-Tarí P, Turrà D, Di PA, Nueda MJ, Ferrer A, et al. Data quality aware analysis of differential expression in RNA-seq with NOISeq R/bioc package. Nucleic Acids Research [Internet]. 2015;**43**(21):e140. Available from: https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkv711

[49] Tarazona S, García F, Ferrer A, Dopazo J, Conesa A. NOIseq: A RNA-seq differential expression method robust for sequencing depth biases. EMBnet Journal [Internet]. 2012;**17**(B):18. Available from: http://journal.embnet.org/index.php/embnetjournal/article/view/265

[50] Chung M, Bruno VM, Rasko DA, Cuomo CA, Muñoz JF, Livny J, et al. Best practices on the differential expression analysis of multi-species RNA-seq. Genome Biology [Internet]. 2021;**22**(1):121. Available from: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-021-02337-8

[51] Luecken MD, Theis FJ. Current best practices in single-cell RNA-seq analysis: A tutorial. Molecular Systems Biology [Internet]. 2019;**15**(6):e8746. Available

from: https://onlinelibrary.wiley.com/doi/10.15252/msb.20188746

[52] Hernandez I, Zhang Y. Using predictive analytics and big data to optimize pharmaceutical outcomes. American Journal of Health-System Pharmacy [Internet]. 2017;**74**(18):1494-1500. Available from: https://academic.oup.com/ajhp/article/74/18/1494/5102866

[53] Parikh S, Patel R, Khunt D, Chavda VP, Vora L. Data analytics and data visualization for the pharmaceutical industry. In: Bioinformatics Tools for Pharmaceutical Drug Product Development [Internet]. Beverly, MA, USA: Wiley; 2023. pp. 55-76. DOI: 10.1002/9781119865728.ch4

[54] Araldi RP, Viana M, Colozza-gama GA. Unique transcriptional signatures observed in stem cells from the dental pulp of deciduous teeth produced on a large scale. Pharmacologia. 2023;**14**(1):72-95

[55] Wenceslau CV, de Souza DM, Mambelli-Lisboa NC, Ynoue LH, Araldi RP, da Silva JM, et al. Restoration of BDNF, DARPP32, and D2R expression following intravenous infusion of human immature dental pulp stem cells in Huntington's disease 3-NP rat model. Cells [Internet]. 2022;**11**(10):1664. Available from: https://www.mdpi.com/2073-4409/11/10/1664

[56] Araldi RP, Ramos AT, Alievi AL, Policíquio B, Teixei MR, Mendes TB, et al. NestaCell® promotes motor, cognitive and neuropsychiatric functions amelioration and dopaminergic neurons restoration in a preclinical model of Parkinson's disease. Cytotherapy [Internet]. 2022;**24**(10):S3. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1465324922008490

[57] Macedo J, Pagani E, Wenceslau C, Ferrara L, Kerkis I. A phase I clinical trial on intravenous administration of immature human dental pulp stem cells (NestaCell) to Huntington's disease patients. Cytotherapy [Internet]. 2021;**23**(4):1. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1465324921000657

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This book is a comprehensive overview of the recent developments in the clinical and research fields of mesenchymal stem cells (MSCs). It is divided into three main sections that cover a wide range of topics including sources and functions of MSCs, their therapeutic applications, their role in tissue engineering, and the application of pharmacologic intelligence in research and development of MSCs.

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