



IntechOpen

IntechOpen Series
Biochemistry, Volume 69

Mesenchymal Stem Cells

Biology, Therapeutics, and Beyond

*Edited by Rui Damásio Alvites
and Ana Colette Maurício*



Mesenchymal Stem Cells - Biology, Therapeutics, and Beyond

*Edited by Rui Damásio Alvites
and Ana Colette Maurício*

Published in London, United Kingdom

Mesenchymal Stem Cells – Biology, Therapeutics, and Beyond

<http://dx.doi.org/10.5772/intechopen.1008639>

Edited by Rui Damásio Alvites and Ana Colette Maurício

Contributors

Aleksei E. Bigildeev, Bingdong Sui, Chenxi Zheng, Cristiane Valverde Wenceslau, Gulcin Sahingoz Erdal, Hsuan-Ju Chen, Hsun-Ming Chang, Irina Kerkis, João Rafael Dias Pinto, Kai Zhang, Lei Zhang, Lucas Vinícius de Oliveira Ferreira, Lufan Yang, Nilgun Isiksacan, Rodrigo Pinheiro Araldi, Rogério Martins Amorim, Sha Zhang, Shengfeng Bai, Shirley O'Dea, Siyu Lan, Tamay Seda Tasci, Wen Qin, Yu Fu

© The Editor(s) and the Author(s) 2025

The rights of the editor(s) and the author(s) have been asserted in accordance with the Copyright, Designs and Patents Act 1988. All rights to the book as a whole are reserved by INTECHOPEN LIMITED. The book as a whole (compilation) cannot be reproduced, distributed or used for commercial or non-commercial purposes without INTECHOPEN LIMITED's written permission. Enquiries concerning the use of the book should be directed to INTECHOPEN LIMITED rights and permissions department (permissions@intechopen.com).

Violations are liable to prosecution under the governing Copyright Law.



Individual chapters of this publication are distributed under the terms of the Creative Commons Attribution 4.0 License which permits commercial use, distribution and reproduction of the individual chapters, provided the original author(s) and source publication are appropriately acknowledged. If so indicated, certain images may not be included under the Creative Commons license. In such cases users will need to obtain permission from the license holder to reproduce the material. More details and guidelines concerning content reuse and adaptation can be found at <http://www.intechopen.com/copyright-policy.html>.

Notice

Statements and opinions expressed in the chapters are those of the individual contributors and not necessarily those of the editors or publisher. No responsibility is accepted for the accuracy of information contained in the published chapters. The publisher assumes no responsibility for any damage or injury to persons or property arising out of the use of any materials, instructions, methods or ideas contained in the book.

First published in London, United Kingdom, 2025 by IntechOpen

IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 167-169 Great Portland Street, London, W1W 5PF, United Kingdom

For EU product safety concerns: IN TECH d.o.o., Prolaz Marije Krucifikse Kozulić 3, 51000 Rijeka, Croatia, info@intechopen.com or visit our website at intechopen.com.

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

Mesenchymal Stem Cells – Biology, Therapeutics, and Beyond

Edited by Rui Damásio Alvites and Ana Colette Maurício

p. cm.

This title is part of the Biochemistry Book Series, Volume 69

Topic: Cell and Molecular Biology

Series Editor: Ana Maria Carmona-Ribeiro

Topic Editor: Mary C. Maj

Associate Topic Editor: Felicia Ikolo

Print ISBN 978-1-83635-445-1

Online ISBN 978-1-83635-444-4

eBook (PDF) ISBN 978-1-83635-446-8

ISSN 2632-0983

If disposing of this product, please recycle the paper responsibly.

IntechOpen Book Series

Biochemistry

Volume 69

Aims and Scope of the Series

Biochemistry, the study of chemical transformations occurring within living organisms, impacts all of the life sciences, from molecular crystallography and genetics, to ecology, medicine and population biology. Biochemistry studies macromolecules - proteins, nucleic acids, carbohydrates and lipids –their building blocks, structures, functions and interactions. Much of biochemistry is devoted to enzymes, proteins that catalyze chemical reactions, enzyme structures, mechanisms of action and their roles within cells. Biochemistry also studies small signaling molecules, coenzymes, inhibitors, vitamins and hormones, which play roles in the life process. Biochemical experimentation, besides coopting the methods of classical chemistry, e.g., chromatography, adopted new techniques, e.g., X-ray diffraction, electron microscopy, NMR, radioisotopes, and developed sophisticated microbial genetic tools, e.g., auxotroph mutants and their revertants, fermentation, etc. More recently, biochemistry embraced the ‘big data’ omics systems. Initial biochemical studies have been exclusively analytic: dissecting, purifying and examining individual components of a biological system; in exemplary words of Efraim Racker, (1913 –1991) “Don’t waste clean thinking on dirty enzymes.” Today, however, biochemistry is becoming more agglomerative and comprehensive, setting out to integrate and describe fully a particular biological system. The ‘big data’ metabolomics can define the complement of small molecules, e.g., in a soil or biofilm sample; proteomics can distinguish all the proteins comprising e.g., serum; metagenomics can identify all the genes in a complex environment e.g., the bovine rumen. This Biochemistry Series will address both the current research on biomolecules, and the emerging trends with great promise.

Meet the Series Editor



Ana Maria Carmona-Ribeiro has been a full professor of Biochemistry at the University of São Paulo (USP), Brazil, since 2001. She founded the Biocolloids Laboratory at USP in 1993, and since then she has been developing novel important assemblies aimed at drug and vaccine delivery. Her background in Physics, Chemistry, Biology, and Pharmaceuticals has been useful for the development of biomolecular assemblies with potential for novel biomedical applications.

Meet the Volume Editor



Rui Damásio Alvites is the Coordinator of the Bachelor's Degree in Veterinary Nursing at the Northern Polytechnic Health Institute – Cooperative for Polytechnic and University Education (IPSN – CESPU). He serves as an Assistant Professor at the Polytechnic University Institute of Health Sciences and as an Adjunct Professor at the Northern Polytechnic Health Institute – Cooperative for Polytechnic and University Education (IUCS-IPSN – CESPU). He is also a Researcher at the Institute of Sciences, Technologies and Agroenvironment of the University of Porto (ICETA-UP), a Collaborating Researcher at The One Health Toxicology Research Unit (1H-TOXRUN), and an External Collaborator at the Institute of Biomedical Sciences Abel Salazar, University of Porto (ICBAS-UP). He holds a Ph.D. in Veterinary Sciences from the Abel Salazar Biomedical Sciences Institute of the University of Porto (2021) and a Master's in Veterinary Medicine from the University of Trás-os-Montes and Alto Douro (2015). His research focuses on Regenerative Medicine, Biomedical and Veterinary Sciences, particularly in cell culture with mesenchymal stem cells, biomaterials, and animal models for developing new regenerative therapies from a One Health perspective, with a special emphasis on regenerative therapies applied to peripheral nerve regeneration. Between 2017 and 2025, he participated in several research projects, supervised multiple Bachelor's, Master's, and Doctoral students, and received several awards and honors.



Ana Colette Maurício is a Full Professor at the Instituto de Ciências Biomédicas Abel Salazar of the University of Porto (ICBAS-U. Porto). She holds a degree in Veterinary Medicine (1995), a Ph.D. in Veterinary Sciences (1999, Faculdade de Medicina Veterinária da Universidade de Lisboa, FMV-UL), and Habilitation in Veterinary Sciences (2011, Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto, ICBAS-U. Porto). Her Ph.D. experimental work was carried out at the Instituto Gulbenkian de Ciência (IGC), Freiburg Medicine Faculty in Germany, and Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa (FCT-UNL). She is currently a Full Professor in the Veterinary Clinics Department (ICBAS-U. Porto), a Member of the Scientific Council and the Representatives Council (ICBAS-U. Porto), Director of the Veterinary Sciences Doctoral Programme (ICBAS-U. Porto), and Scientific Coordinator of the Regenerative Medicine/Experimental Surgery sub-unit (CECA-ICETA) and of the Thematic Line Comparative and Translational Medicine and Biotechnology within the AL4Animals – Associated Laboratory for Animal and Veterinary Sciences. For the past 15 years, she has coordinated a multidisciplinary research group in Experimental Surgery and Regenerative Medicine, developing biomaterials and cell-based therapies. Her research group works in this direction through a multidisciplinary team that includes veterinarians, engineers, medical doctors, and biologists. Through Experimental Surgery, this team plays a crucial role in developing new therapies and fostering close collaboration between biomaterials design, cellular system development, and clinical needs.

in both Human and Veterinary Medicine. The group has produced several significant publications in the field of Tissue Regeneration. In her laboratory, numerous Ph.D., Post-Doctoral, and Master's students from various countries have gained advanced expertise in the study of Tissue Regeneration. She supervises several Ph.D., Post-Doctoral, and Master's students (with 18 Ph.D. theses completed) and is the co-author of numerous scientific articles and book chapters. She has served as principal researcher or member of several national and international scientific projects, is the editor of six international scientific books, and holds five international patents from the University of Porto.

Contents

Preface	XIII
Section 1	
Biological Foundations and Advances in Cell Engineering	1
Chapter 1	3
Engineering Mesenchymal Stem Cells Using Electroporation <i>by Shirley O'Dea</i>	
Section 2	
Mechanisms of Action and Translational Approaches	27
Chapter 2	29
Mechanism of Action of NestaCell®: A Bioinformatics-Guided Translational Approach for Therapeutic Modulation in Huntington's Disease <i>by Rodrigo Pinheiro Araldi, Irina Kerkis, João Rafael Dias Pinto and Cristiane Valverde Wenceslau</i>	
Section 3	
Therapeutic Applications and Regenerative Medicine	65
Chapter 3	67
Mesenchymal Stem Cell Therapy and Its Clinical Application Potential <i>by Lei Zhang</i>	
Chapter 4	93
Mesenchymal Stem Cell-Based Therapeutic Strategies for Neurological Disorders in Dogs <i>by Rogério Martins Amorim and Lucas Vinícius de Oliveira Ferreira</i>	
Chapter 5	123
Stem Cell-Based Therapies for Refractory Thin Endometrium in <i>In vitro</i> Fertilization: Advances, Challenges, and Future Prospects <i>by Hsuan-Ju Chen and Hsun-Ming Chang</i>	

Chapter 6	141
Transplantation of MSCs for a Longstanding Engraftment and Maintenance of Bone Marrow Stroma	
<i>by Aleksei E. Bigildeev</i>	
Section 4	175
Extracellular Vesicles, Microenvironment, and Cancer	
Chapter 7	177
Mesenchymal Stem Cell-Derived Extracellular Vesicles in Therapeutic Use	
<i>by Yu Fu, Lufan Yang, Siyu Lan, Sha Zhang, Kai Zhang, Shengfeng Bai, Wen Qin, Chenxi Zheng and Bingdong Sui</i>	
Chapter 8	199
The Dual Role of Mesenchymal Stem Cells in Cancer: From Immune Modulation to Therapy Resistance	
<i>by Gulcin Sahingoz Erdal, Tamay Seda Tasci and Nilgun Isiksacan</i>	

Preface

Mesenchymal stem cells (MSCs) are among the most promising foundations of modern regenerative medicine. These cells possess an exceptional capacity for self-renewal, differentiation, and immune system modulation, playing a fundamental role in tissue repair methods, cell therapies, and the creation of advanced biotechnological products.

The growing scientific and clinical focus on MSCs highlights their therapeutic potential, as well as the diverse range of fields in which they are applied, including cell and molecular biology, tissue engineering, gene therapy, oncology, and neurosciences. An improved understanding of their operational mechanisms and interactions with the cellular microenvironment has driven the creation of new translational medicine strategies aimed at regenerating and modifying complex diseases.

The book *Mesenchymal Stem Cells – Biology, Therapeutics, and Beyond* brings together insights from researchers across different fields, exploring topics as genetic engineering and genome editing, clinical and preclinical uses in regenerative medicine, neuroprotection, immunomodulation, extracellular vesicle applications, and innovative perspectives on cell-based therapies and bioengineering. This variety of approaches demonstrates the multidisciplinary and constantly evolving nature of MSC research, as well as its growing influence on contemporary biomedicine.

This book aims to provide a comprehensive and current overview of the biology and therapeutic possibilities of MSCs, serving as a theoretical and practical resource for researchers, clinicians, and students eager to explore this rapidly growing field.

The editors would like to express their sincere gratitude to the IntechOpen editorial team for their assistance, as well as to all the authors who invested their effort and commitment in completing this volume.

Rui Damásio Alvites

CESPU – Advanced Polytechnic and University Cooperative, CRL,
Gandra, Porto, Portugal

Ana Colette Maurício

Department of Veterinary Clinics,
Instituto de Ciências Biomédicas Abel Salazar (ICBAS),
University of Porto (UP),
Porto, Portugal

Section 1

Biological Foundations and Advances in Cell Engineering

Engineering Mesenchymal Stem Cells Using Electroporation

Shirley O'Dea

Abstract

Despite an encouraging safety profile emerging from the large number of clinical trials carried out, only a relatively small number of MSC-based products have been approved globally to date. It is likely that the next generation of MSC cell therapy products will need to be engineered in order to enhance functionality and/or persistence in vivo and thus advance the field. Non-viral methods of cargo delivery, such as electroporation, are of interest for cell engineering as safety concerns remain with viral vectors. To ensure the success of future non-viral MSC engineering studies it will be critical for researchers to understand the principles and challenges associated with techniques such as electroporation. Here, the basic principles of electroporation are presented along with an discussion of critical parameters and the impact of these parameters on cell health and functionality. A review of the literature is also presented to assess the current state of the art in electroporation-mediated delivery to MSCs. This review highlights the diversity in the field and underscores the need for a standardised approach in terms of reporting of methods and selection of assays to assess cell viability and functionality.

Keywords: bone marrow, Cas9, cell therapy, CRISPR, gene editing, MSC, intracellular delivery, iPSC, non-viral, transfection

1. Introduction

Mesenchymal stem cells (MSCs) are a diverse subset of adult stem cells present in a variety of adult tissues. Bone marrow- (BM-) derived MSC have been most widely studied to date and other sources include adipose tissue, umbilical cord tissue (UCT) and induced pluripotent stem cells (iPSCs) [1]. MSCs are multipotent and are capable of both self-renewal and differentiation into mesodermal lineages such as osteocytes, adipocytes and chondrocytes [2]. In vivo, MSCs can secrete multiple bioactive molecules which promote cell survival and angiogenesis, decrease inflammation and enhance tissue repair in the surrounding microenvironment. These differentiation, immunoregulatory and tissue-protective properties of MSCs have attracted considerable interest in the field of translational medicine. Over 1500 clinical trials to date worldwide examining the therapeutic potential of MSCs as cell-based therapies for

conditions including heart disease, spinal cord injuries, stroke, Parkinson's disease, type-1 diabetes, graft-versus-host disease, autoimmune disease, inflammatory bowel disease and cancer [3].

However, despite an encouraging safety profile emerging from the large number of clinical trials carried out, only a relatively small number of MSC-based products have been approved globally to date [4]. In December 2024, Ryoncil (remestemcel-L-rknd), an allogeneic BM-derived MSC therapy indicated for the treatment of steroid-refractory acute graft-versus-host disease in paediatric patients, became the first U.S. FDA-approved MSC therapy [5]. Notwithstanding the large number of patients treated in trials, favourable pre-clinical outcomes have largely failed to translate into clear clinical benefits [4]. In hindsight it has become apparent that failure or inability to standardise key aspects of cell production and administration has been a significant confounding factor to success in the clinic. Deeper understanding of, and closer control over, areas including cell production methods, metabolic activity of the cells, dose and disease stage is required to improve translational outcomes [5]. In addition, cell engineering strategies to increase efficiency and specificity of MSC products have become the focus of much attention [6]. Furthermore, it has been proposed that use of allogenic cells (allo-MSC), rather than autologous cells which have been preferred to date, could help address these issues. However, while MSCs are considered immune privileged and several clinical studies have demonstrated the safety of allo-MSC, investigations in animal models and in patients have found low levels of engraftment and limited cell survival in vivo [7]. In addition to rejection, biological function of MSCs can be significantly reduced within a week of delivery [7]. Therefore, cell engineering approaches may also be required to support translation of allo-MSC into clinical studies.

A range of viral and non-viral methods have been used to introduce genes and other cargos into MSCs (reviewed in [6]). Electroporation, discussed in detail later here, is the most widely used 'physical' non-viral method. Other physical approaches reported for MSCs include sonoporation, which utilises ultrasonic waves to increase cell membrane permeability, and nanoparticles such as quantum dots, gold nanoparticles, carbon nanotubes and magnetic nano-beads which are used to bind and transfer material into cells by penetrating the cell membrane. Non-viral chemical methods include cationic lipid-based transfection, cationic polymers and cationic-based peptides. These chemicals form complexes with the cargo and interact with the cell membrane to transfer the cargo into the target cells. Non-viral methods generally have advantages compared with viral vectors such as low cost and safety. However, low efficiency and, notably, high cytotoxicity can be challenges.

In the last decade, advances in cell engineering technologies such as CAR-T and gene editing have stimulated the cell therapy field as a whole and generated renewed interest in MSC cell therapies. The possibility of genetically engineering MSCs to improve their therapeutic potential using approaches such as loading therapeutic agents onto/into MSCs, or modifying the homing or survival capabilities of the cells by genetic engineering, is being examined [2]. In particular, the CRISPR/Cas system has been advocated as an exciting opportunity for the next generation of MSC-based therapies [6, 8–10]. MSC are readily transducible with viruses and CRISPR/Cas9-mediated engineering has been achieved with lentiviral vectors [2, 11]. However, given the good safety profile of MSCs, non-viral delivery methods that could avoid risks of immunogenicity and carcinogenicity associated with viruses are considered attractive [12, 13]. Chemical-based methods have been used to deliver CRISPR/Cas

plasmids to MSCs [14–16]. However, the use of recombinant Cas9 protein and guide RNA complexes in the form of Cas9 ribonucleoproteins (RNPs) are viewed as preferable to plasmids due to lower rates of off-target effects [6].

Given the growing utilisation of electroporation as a non-viral transfection method in the CAR-T field, the purpose of this review is to discuss aspects of electroporation transfection processes that may warrant deeper consideration when applied to engineering of MSCs in order to support the ultimate success of future clinical studies. To this end, the mechanism of electroporation will be discussed briefly, with reference to several in depth review articles on the topic, and similarities and differences between common electroporation systems will be examined. The importance of considering potential effects of transfection methods on MSC viability and functionality will be highlighted. A review of the literature was carried out to determine whether a particular electroporation system and protocol has become standard in the field of MSC transfection to what extent studies have been carried out to assess MSC injury and functionality post-electroporation. It does not appear that a similar review has been carried out to date. While this was not an exhaustive search, it was comprehensive and is likely to be representative.

2. Therapeutic potential of MSCs and their derivatives

Several features of MSCs have stimulated interest in their therapeutic potential. These include their differentiation capacity, their production of a wide array of biologically active molecules and their ability to home to damaged tissues. Over the past decade there has been a paradigm shift in the view of the therapeutic potential of MSCs away from differentiation and in vivo cell replacement toward utilisation of the paracrine activity of these cells to stimulate innate tissue repair and modulate inflammation and immune responses [16]. MSCs synthesise cytokines, chemokines, hormones, growth factors and miRNAs that modulate cell behaviour and can protect damaged tissue and promote repair processes [17]. Initially, studies focused on delivery of whole MSCs to the target site in vivo in order to achieve a therapeutic benefit. It is now known that these active factors are primarily transported in vivo within MSC-derived extracellular vesicles (EVs) and this has driven the exploration of cell-free approaches that use these structures as delivery vehicles [18]. Notwithstanding this, cell-to-cell communication via gap junctions and tunnelling nanotubes is also an important mechanism by which MSCs transfer larger molecules, and even cell organelles such as mitochondria, to neighbouring cells, and the therapeutic potential of intact MSCs cell therapies remains under investigation [17].

The ability of MSCs to home to sites of tissue injury is also of interest for therapeutic applications. Injured or inflamed tissue release a chemokine gradient which directs MSC migration to the site via chemokine receptors on the MSC cell membrane [17]. MSCs then tether and transmigrate through the endothelium and basement membrane, to the injury site. Although the mechanisms of MSC homing and migration are not fully understood, these functions have been seconded for applications where the MSC membrane is being deployed as a drug coating material to enhance biocompatibility and tissue targeting [19].

The retention of MSC functionality is critical for the success of studies such as these and should be considered in light of the reported impacts of electroporation on cell viability and functionality as discussed below.

3. Mechanism of electroporation

Despite being widely used as a transfection method for several decades, the mechanism by which electroporation permeabilises the cell membrane and facilitates transfer of material into cells remains unclear. This is due in part to technical limitations with analysing sub-microsecond nano-scale alterations in the cell membrane [20]. Several review articles discuss theories on the mechanism of electroporation in depth [21–24]. In particular, Stewart et al. [25] provide a comprehensive overview of electroporation as an intracellular delivery method. Therefore, it is not intended to provide a detailed treatise here. Rather the aim is to summarise the current state of the art in so much as to provide context that may inform and advance the MSC therapeutics field. Firstly, the proposed mechanism by which the cell membrane is rendered permeable by electroporation will be summarised followed by a brief discussion of the proposed mechanisms by which molecules are transported into the permeabilised cell.

Amongst several theories on the mechanism, the aqueous pore formation theory is generally held as the best description of the electroporation process (reviewed in detail in [22]). This theory focuses on the fluid mosaic model which describes the cell membrane as a phospholipid bilayer in which proteins are embedded. The head of the phospholipid is a hydrophilic phosphate while the fatty acid tails are hydrophobic. Both inside and outside the cell, the cell membrane is surrounded by an aqueous environment containing electrolytes and various substances. As the membrane forms, phospholipids naturally arrange themselves into a double layer due to their hydrophilic and hydrophobic characteristics. In this structure, the phosphate heads of the phospholipids face outward toward the water on both sides, while the hydrophobic tails orient inward, away from the water, forming the inner part of the membrane. Due to a system of ion pumps and channels in the membrane, most cells maintain an electric potential difference, termed the resting transmembrane voltage (TMV), between the inner and outer side of the membrane. Eukaryotic cells generally exhibit a TMV between -40 and -70 mV [20].

The phospholipids in the cell membrane can move and rotate, giving the membrane fluidity and flexibility and allowing it to adapt to changes in cell shape and internal pressure. As a result of this fluidity, it is thought that, under normal conditions, local thermal fluctuations can cause the formation of random micropores in the cell membrane. The micropores are hydrophobic due to the phospholipid tail in the bilayer yet, despite being energetically unfavourable, a small number of water molecules may be able to penetrate the bilayer and enter the cell. These micropores are unstable and reseal within nanoseconds.

In the presence of a sufficiently strong external electric field such as that applied during electroporation, structural changes occur to the cell membrane and its constituent molecules which have relevance for the transfection process. When cells in solution are placed between plate electrodes and exposed to electric pulses, an electric field is established between the electrodes during each pulse and the TMV increases. Once the TMV reaches a sufficiently high, or ‘critical’, value, it is postulated that water molecules on either side of the cell membrane tend to orient their dipoles along the local electrical field created by the TMV, form intermolecular hydrogen bonds and increasingly ingress into the hydrophobic region of the bilayer, forming so-called ‘water-fingers’. These finally merge to form a water column that spans the bilayer [20, 23]. This in turn causes the polar head groups of neighbouring fatty acids to adjust

their orientation to face the water molecules, resulting in a hydrophilic pore through which more water can flow into the cell. The pore size increases and other polar molecules and ions can likewise enter through the permeable membrane.

The pores formed during electroporation are relatively stable, persisting from milliseconds to minutes, and will reclose spontaneously after the electric field is removed unless the duration or strength of the electric field has been excessive, in which case the permeability is irreversible [22]. Resealing of the pores can take several minutes and is faster at higher temperatures suggesting the involvement of enzymes [20, 23]. The cytoskeleton is also involved [23].

The membranes of intracellular organelles can also become permeabilised under certain conditions. Electroporation occurs primarily on the cell membrane when pulses with duration in the microsecond-millisecond range are used. When higher electric field strengths are used with pulses in the nanosecond range, mitochondrial and nuclear membranes may become electroporeabilised (**Figure 1**).

4. Mechanism of transmembrane molecular transport

Electroporation-mediated transport of molecules into cells is believed to occur via diffusion, electrophoresis or endocytosis, or a combination thereof, depending on the size and charge of the molecule and whether the electric pulse is in progress or has ceased. Small molecules may enter the electroporeabilised cell by diffusion across their concentration gradient, while charged small molecules will benefit from an added electrophoretic effect that can augment transfer into the cell. It has been reported that transport during the pulse is primarily electrophoretic while diffusion occurs after the pulse [23]. For these smaller molecules, microsecond-millisecond pulses of relatively low electric field strengths appear to be optimal for transport [23]. For larger macromolecules such as proteins and nucleic acids, larger pore sizes are required and nanosecond pulses with considerably higher field strengths are required to achieve transfection. Additionally, charge on the molecule plays a key role as electrophoretic forces are believed to be critical. Thus macromolecules such as DNA and RNA, which carry a negative charge, are typically efficiently transferred into cells by electroporation. However, transport mechanisms may differ between macromolecules. While siRNA can translocate during the pulse, DNA does not appear to enter the cell immediately and endocytosis may be the primary mechanism [23].

5. Electroporation and cell injury

As discussed by Stewart et al. [25], electroporation can be a highly efficient means of transporting molecules into cells but cell injury remains a challenge, particularly for cell therapy applications. Napotnik et al. [26] observe that reversible electroporation was developed in order to deliberately damage the cell membrane in a sub-critical manner. In addition to the desired influx of cargo, outflow of biomolecules from the cell will also occur during electroporation, which may affect the viability or function of the cell post-electroporeabilisation, although this may also apply to other membrane-disruption techniques. Several issues apply specifically to electroporation and their resolution is confounded by difficulties in measuring and controlling their

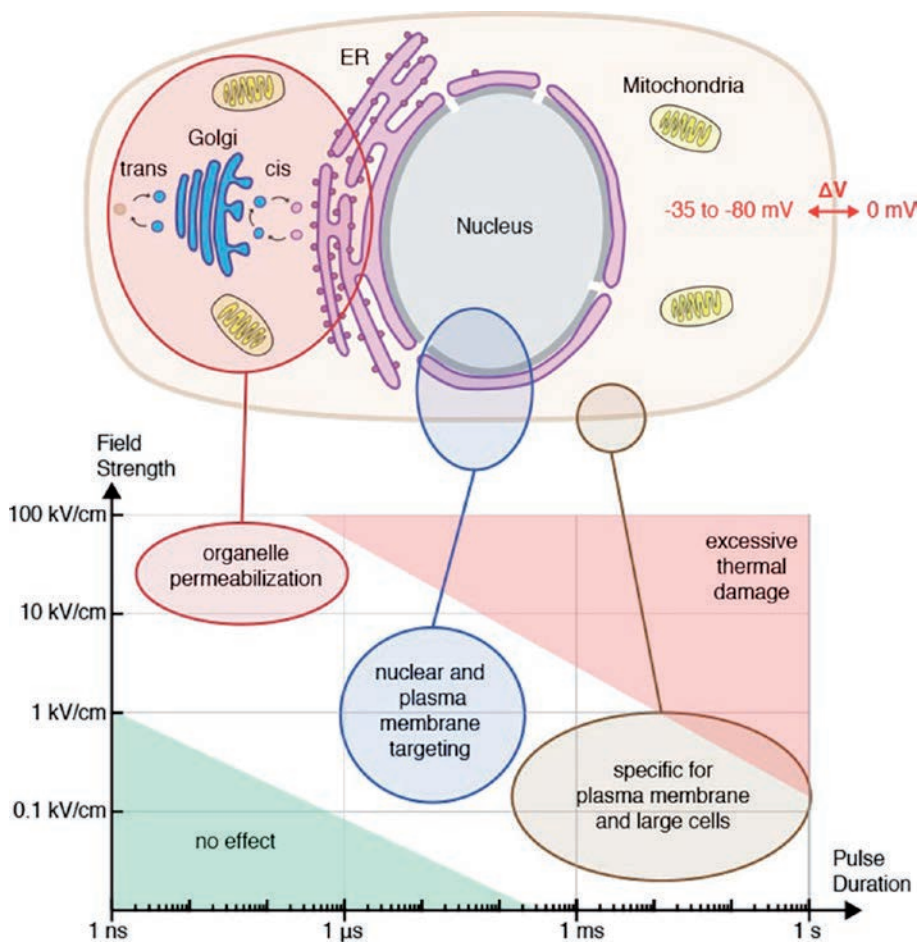


Figure 1. Relationship between the pulse strength-duration parameter space and subcellular targeting. High intensity short pulses are biased toward perturbing small membrane bound bodies like organelles, while milder, longer pulses are more specific for the plasma membrane and larger cells. At large field strengths and longer durations thermal damage due to heating becomes an issue, being also dependent on buffer conductivity. Figure and legend reproduced from ref. 25. Copyright 2018 with permission from American Chemical Society.

extent and impact in a given experiment. These issues include temperature increases caused by the electric current, metal contamination due to electrolysis of the electrodes, pH changes adjacent to the electrodes, non-uniformity of the electric field leading to heterogeneity in transfection rates and cell viability, and cell damage [25]. Electrically induced chemical changes to membrane lipids, such as peroxidation, and modulation of membrane proteins' functions, such as voltage gated channels, can also occur. The extent to which these processes occur has not been well-studied in any cell type including MSC. Other specific disruptions to normal cell physiology and health include influx of Ca^{2+} ions, disrupting intracellular calcium homeostasis and cell signalling and depleting intracellular ATP, production of reactive oxygen species and oxidation damage of unsaturated lipids, mitochondrial damage, DNA damage and protein damage [26]. Thus, electric pulses can have detrimental effects on many cellular molecules, structures and functions and it should be recognised that active cellular processes are required for cell recovery.

6. Electroporation systems and key parameters

The history of the development of electroporation devices and methods is well described elsewhere [21, 25]. In brief, for conventional electroporation, also known as bulk electroporation (BEP), the cell suspension and cargo are mixed together in a conducting buffer solution and transferred to a cuvette which contains two electrodes. The cuvette is placed into a device where it connects to a generator of high electric voltage where an electric field is generated. The pulse strength, pulse duration and number of pulses determine the coverage and size of pore formation respectively. Other key factors that influence pore formation include cell size, membrane composition and physiological condition as well as buffer solution composition and temperature [23]. If these parameters are not well understood and controlled, there is a high likelihood that the transfection process will fail due to either insufficient permeabilisation resulting in limited cargo transfer, or excessive pore formation resulting in cell death. Control of cargo dosage is also difficult to manage in the cuvette setup as volumes are relatively large.

Despite these issues, BEP remains widely used. Commonplace commercial systems include the the Neon® (Thermo Fisher Scientific), the Nucleofector™ (Lonza) and the Gene Pulser Xcell™ (Bio-Rad). The formats of these systems and others vary with respect to conformation of the cuvette/cassette/multi-well strip, buffer solution composition, optimal cell number and pulse regimes, meaning that process optimisation is required for a given application on each system. Several commercial providers have also made automated, higher throughput, GMP-aligned systems available to address the need to engineer larger numbers of cells for applications such as ex vivo cell therapy. A wide variety of academic-designed microscale and nanochannel electroporation platforms which attempt to address aforementioned issues have also been reported [21, 25].

As discussed below here, a review of the literature has found that the Neon and Nucleofector systems are by far the most commonly used electroporation instruments for transfecting MSCs (**Table 1**). Therefore, these systems will be discussed briefly. The Neon system was developed to address issues such as pH changes, electrolysis and extensive bubble formation associated with the fixed, plate-style electrodes used in cuvette electroporation formats [27]. Originally called ‘microporation’, a tip-type capillary electroporator was developed that integrated a wire type electrode that served to minimise the effective surface area of the electrode, resulting in enhanced transfection rate and reduced cell death (**Figure 2**). Currently, the Neon NxT is available in a single channel or eight channel format, according to the supplier’s website. The key electroporation parameters are programmable by the user and a small number of proprietary buffers are provided for different cell types, the compositions of which have not been made publicly available. The Nucleofection system has been reviewed in detail by Stewart et al. [25]. This system employs a cuvette format with parallel plate electrodes. Optimised, dual pulse settings and cell-type specific buffers appear to contribute to its efficacy in transfection studies. Several formats of the system have been developed including 24-, 96- and 384-well instruments as well as a closed large scale instrument. However, the electroporation parameters are preset into the instruments and are selected as codes, unlike other systems where the operator can select the specific pulse strength, pulse duration and number of pulses for an experiment. This prevents the operator from taking a rational, systematic approach to an optimisation study and can impede understanding of failed experiments. Nonetheless, the system is highly popular generally for electroporation studies, although based on the findings in this review, apparently less so for MSC studies.

Cell type	Investigation	Cargo	EP Device	EP parameters/ programmes reported	Post-EP treatment	Post EP MSC injury assays	Post-EP MSC functionality assays	Year	Reference
hAd-MSCs	Use of microport to deliver plasmid DNA to MSCs	Plasmid DNA	Microportor (subsequently known as Neon)	Optimisations: 900 V/20 ms/1 pulse; 900 V/20 ms/2 pulse; 1500 V/20 ms/1 pulse; 1500 V/20 ms/2 pulse; 1800 V/20 ms 1 pulse.	After electroporation, cells were distributed into 35-mm cellculture dishes and placed at 37°C in a 5% CO ₂ -humidified atmosphere	Cell cycle (flow cytometry); Cell death (lactate dehydrogenase release)	Osteogenic and adipogenic differentiation	2009	[28]
hAd-MSCs; hBM-MSCs; mBM-MSCs	Targeting of MSC to an injury site	Minicircle DNA	Neon	Optimisations for hAT ⁻ and hBM-MSCs: 990 V/40 ms/1 pulse; 1000 V/30 ms/3 pulse; 1300 V/30 ms/2 pulse; 1500 V/20 ms/1 pulse; 1500 V/20 ms/2 pulse. Optimisations for mBM-MSCs: 1500 V/30 ms/1 pulse	Cells were distributed into the wells of 12-well culture plates and placed in a 5% CO ₂ incubator at 37°C for 72 h	Viability (dye exclusion)	In vivo mouse model of homing and wound healing	2016	[29]
hUCB-MSCs	CRISPR RNP- mediated gene editing	Plasmid DNA	Neon	10 µl tips; 990 V/40 ms/1 pulse	Cells seeded in a 24-well plate containing pre- warmed medium	n.d.	n.d.	2017	[30]
CRISPR- Cas9 RNP									
hUCB-MSCs	CRISPR-Cpf1- mediated upregulation of targeted gene expression in MSCs	Plasmid DNA	Neon	Neon buffer R; 1600 V/20 ms/1 pulse	Cells seeded on 150 mm cell culture dish and incubated for 24 h	n.d.	Osteogenic differentiation	2020	[31]

Cell type	Investigation	Cargo	EP Device	EP parameters/ programmes reported	Post-EP treatment	Post EP MSC injury assays	Post-EP MSC functionality assays	Year	Reference
hAm-MSCs	TALEN-mediated genome editing of MSCs	Plasmid DNA	Neon	n.d.	n.d.	n.d.	Chondrogenic differentiation; in vivo attenuation of collagen-induced arthritis in mouse model	2021	[32]
hUCT-MSCs	Derivation of iPSCs	Plasmid DNA	Neon	1150 mV/30 ms/2 pulse; 1650 mV/30 ms/2 pulse	Cells plated onto Geltrex-coated 10 cm or 6-well plates for 24 h	n.d.	n.d.	2021	[33]
hAd-MSCs	Effect of AT-MSC secretome on breast cancer cells	Plasmid DNA	Neon	Neon buffer R; 1100 V/20 ms/1 pulse	n.d.	Viability (Muse cell analyser)	n.d.	2022	[34]
Rat BM-MSCs	Effect of MSC on experimental testicular torsion in rats	Plasmid DNA	Neon	n.d.	Cells incubated for 48 h in L-DMEM with 10% FBS with G-418 (200 µg/ml)	n.d.	n.d.	2022	[35]
hBM-MSCs	Nucleofection transfection	Plasmid DNA	Nucleofector	U-23; C-17	Cells were transferred to prewarmed fresh medium in 6-well plates	Viability (method n.d.)	Phenotypic markers; osteogenic and adipogenic differentiation; inhibition of T cell proliferation	2006	[36]

Cell type	Investigation	Cargo	EP Device	EP parameters/ programmes reported	Post-EP treatment	Post EP MSC injury assays	Post-EP MSC functionality assays	Year	Reference
hASCs	Development of buffers for nucleofection	Plasmid DNA	Nucleofector	A-20; T-20; T-30; X-01 (optimal); X-05; L-29; D-23	Cells were rinsed with 500 µL sterile culture medium, transferred to the well of a sterile 12-well plate and incubated at 37°C for 24 hours	n.d.	n.d.	2012	[37]
hAd-MSCs	Development of buffers for nucleofection	Plasmid DNA	Nucleofector II	U-023	Cells were gently resuspended in 1 ml pre-warmed medium and seeded in 12-well plates	Viability (Crystal violet staining)	n.d.	2017	[38]
hMSC-TERT (telomerized hMSC cell line)	Generation of inducible CRISPR cell lines	Plasmid DNA	Nucleofector 2B	U-23	Transfection was immediately followed by the addition of prewarmed culture medium and cells were then transferred into 6-well plates	n.d.	n.d.	2020	[39]
hUCB-MSCs	CRISPR Cas9- mediated generation of BDNF-secreting MSCs to treat Rett Syndrome	CRISPR- Cas9 RNP	Nucleofector 4D	n.d.	Cells were incubated at 37°C for 3 days to stabilise cell conditions	n.d.	Rett Syndrome mouse model	2021	[40]

Cell type	Investigation	Cargo	EP Device	EP parameters/ programmes reported	Post-EP treatment	Post EP MSC injury assays	Post-EP MSC functionality assays	Year	Reference
hAD-MSCs	Cell labelling method	Plasmid DNA	X-cell pulser BioRad	400–700 V/50– 200 µs/1 pulse square wave	n.d.	Growth retardation (colony forming assay); senescence (β-Galactosidase staining)	Phenotypic markers; osteogenic and adipogenic differentiation	2019	[41]
hWJ-MSCs	Differentiation of WJ-MSC into hepatocyte-like cells using miRNAs	miRNA; plasmid DNA	X-cell pulser BioRad	600 V/0.1 ms/2 pulse square wave	n.d.	n.d.	n.d.	2025	[42]
hBM-MSCs	Transfection by electroporation	Plasmid DNA	Multiporator (Eppendorf)	Optimisations: 300–700 V/50–125 µs (600 V/100 µs optimal)	Cells plated on 10 cm diameter tissue culture dishes in complete medium	Growth retardation (colony forming assay)	Osteogenic and adipogenic differentiation	2004	[43]
Rat BM-MSC-EVs	Engineering extracellular vesicles	miRNA	Multi- electroporator (Eppendorf)	750 V, 1000 V, 1250 V/20 ms	n.a.	n.a.	n.a.	2024	[44]
Ad-, UCB-, BM-MSCs	Homology- directed gene editing by CRISPR/ Cas9 using tube electroporation	Plasmid DNA; Protein; dsRNA; Cas9gRNA RNP	CTX-1500A LE (Celetrix)	DNA - hAd-MSC: 20 µl/440 V/30 ms/1 pulse; hUC-MSC: 120 µl/640 V/30 ms/1 pulse; hBM-MSC: 20 µl/440 V/30 ms/1 pulse	Cells immediately transferred back to warm medium	Viability (Trypan blue dye exclusion)	n.d.	2018	[45]

Cell type	Investigation	Cargo	EP Device	EP parameters/ programmes reported	Post-EP treatment	Post EP MSC injury assays	Post-EP MSC functionality assays	Year	Reference
				Protein - hAd-MSC: 20 µl/415 V/30 ms/1 pulse					
				dsRNA - hAd-MSC: 20 µl/415 V/30 ms/1 pulse					
				Cas9/gRNA RNP - hMSC: 120 µl/620 V/30 ms/1 pulse					
hAd-MSCs; hUCB-MSCs	Intracellular delivery of trehalose for cryopreservation	Trehalose	BTX Gemini	300 V/100 µs/8 pulse	n.d.	Viability (Trypan blue dye exclusion)	Stress-related gene expression; immunomodulatory gene expression	2021	[46]
iMSCs	Stem cell therapy for fibrodysplasia ossificans progressiva	Plasmid DNA	NEPA21 (Nepagene)	n.d.	n.d.	n.d.	n.d.	2024	[47]
hMSCs	Therapeutic protein expression in swine heart via mRNA-enhanced MSCs	mRNA	MaxCyte GT	OC-100 cuvette; OC-400 cuvette	Cells transferred to an incubator at 37°C and 5% CO ₂	Viability (Zombie Aqua staining)	n.d.	2024	[48]

Cell type	Investigation	Cargo	EP Device	EP parameters/ programmes reported	Post-EP treatment	Post EP MSC injury assays	Post-EP MSC functionality assays	Year	Reference
hAd-MSCs	Non-viral gene delivery	Plasmid DNA	Neon	Optimisations: 1000 V/20 ms/1 pulse; 1100 V/20 ms/1 pulse; 1200 V/20 ms/1 pulse; 1300 V /20 ms/1 pulse; 1400 V/20 ms/1 pulse; 1500 V/20 ms/1 pulse (optimal); 1600 V/20 ms/1 pulse	Cells were transferred to a tissue culture plate, where they were incubated at 37°C in a humidified CO ₂ incubator.	Viability (Countess cell counter)	Proliferation (trypan blue exclusion); osteogenic, adipogenic and chondrogenic differentiation	2014	[49]
hUCB-MSCs	Microporation for gene delivery	Plasmid DNA	Neon	1600 V/20 ms/1 pulse	Cells were incubated for 5 min at room temperature and transferred to the tissue culture dishes containing the growth medium	Viability (Countess cell counter)	Proliferation (trypan blue exclusion); osteogenic, adipogenic and chondrogenic differentiation	2010	[50]
hUCB-MSCs	Microporation for gene delivery	Plasmid DNA	Nucleofector	n.d.	Cells incubated in α -MEM/10% FBS without antibiotics for 24 hr	Viability (MTS assay)	Osteogenic and adipogenic differentiation; migration: BDNF- induced neural differentiation	n.d.	

Cell type	Investigation	Cargo	EP Device	EP parameters/ programmes reported	Post-EP treatment	Post EP MSC injury assays	Post-EP MSC functionality assays	Year	Reference
hUCB-MSCs	CRISPR RNP- mediated gene editing	Plasmid DNA	Neon	24 different sets of optimisation parameters listed, 1200 V/20 ms/2 pulse was optimal	Cells incubated in α -MEM/10% FBS without antibiotics for 24 hr	Viability (MTS assay)	n.d.	2024	[51]
					Cells incubated in α -MEM/10% FBS without antibiotics for 24 hr	Viability (MTS assay)	n.d.		
<i>Abbreviations: BM-MSC-EVs: extracellular vesicles derived from bone marrow-derived MSCs; EP: electroporation; hAd-MSCs: human adipose tissue-derived MSCs; hAm-MSCs: human amniotic MSCs; hASCs: human adipose stem cells; hBM-MSCs: human bone marrow-derived MSCs; hUCT-MSCs: human umbilical cord tissue-derived MSCs; hUCB-MSCs: human umbilical cord blood-derived MSCs; hWJ-MSCs: human Wharton's jelly-derived MSCs; iMSCs: MSCs derived from induced pluripotent stem cells; mBM-MSCs: mouse bone marrow-derived MSCs; ms: milliseconds; MSCs: mesenchymal stem cells; n.d.: not discussed; RNP: ribonucleoprotein; V: volts; μs: microseconds.</i>									

Table 1.
Summary of studies that used electroporation to transfect MSCs.

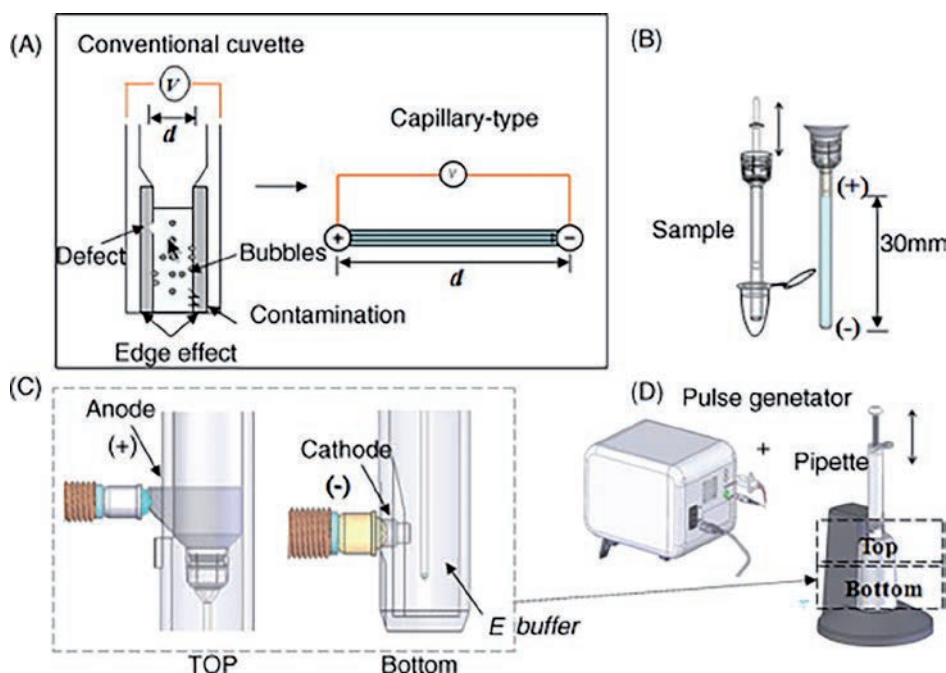


Figure 2. (A) Comparison of electrochemical side-effects due to the physical geometry of two parallel plate-type electrodes integrated in the cuvette system and a single wire-type electrode used in the capillary system; (B) schematic of a piston-driven capillary tip (diameter: 0.65 mm ; length: 30 mm); (C) the position of the anode and cathode in the capillary electroporation system; (D) schematic of the overall capillary electroporation system, which consisted of a high voltage pulse generator and a pipette station. Figure and legend reproduced from ref. 27. Copyright 2008 with permission from Elsevier.

7. Literature review of studies of electroporation-mediated transfection of MSCs

When the literature was examined for reports of electroporation-mediated transfection of MSCs it was found that most studies used human-derived primary MSCs, with adipose tissue-derived and umbilical cord blood derived-MSCs being the most common cells types (**Table 1**). Interestingly with respect to broader advances in cell therapy, one recent study reported the use of MSCs derived from induced pluripotent stem cells (iMSCs) and another used electroporation to engineer extracellular vesicles derived from MSCs [44, 47]. The aims of the published studies were diverse and included evaluations of electroporation and other non-viral delivery systems as well as engineering of MSCs for therapeutic applications.

Plasmid DNA was by far the most commonly used cargo. Some studies did not report the transfection efficiencies obtained or referred to data shown in figures without directly stating efficiency levels. The remaining body of studies used a wide range of assays to assess transgene expression and/or efficacy including direct or indirect flow cytometry, fluorescence or immunofluorescence, ELISA and Western blotting. Only half of the studies reported cell viability post-electroporation making interpretation of transfection efficiencies difficult. Therefore, efficiencies are not discussed here except for CRISPR Cas9 RNP studies which are of particular interest for cell engineering and are discussed in more detail later in this section.

As mentioned above, the literature review found that the Neon and Nucleofection systems have been the most widely used to date for MSC studies with a small number of studies using other systems (**Table 1**). Three of the studies carried out comparisons between at least two electroporation systems [49–51]. Most, but not all, studies provided some details of the electroporation parameters used (**Table 1**), and some also included parameters used during prior optimisation studies. Within the group of studies that used the Neon system, the cell type varied widely and there was no consistency evident in the electroporation parameters used. For Nucleofection, three studies reported using programme U23 even though the cell type was different in each study.

Experienced users of electroporation are aware that the handling of cells immediately post-electroporation has a significant impact on the viability of the cells. Factors such as temperature of culture medium, rest times and pipetting can affect cell recovery from the transfection process. Therefore, it was of particular interest to assess the extent to which these factors were reported. Most studies provided some level of information, such as type of culture medium and seeding vessel, but did not typically refer to cell handling steps per se (**Table 1**). A small number of papers mentioned the use of prewarmed culture medium [36, 38, 39, 45] and Chicaybam et al. [38] described ‘gently resuspending’ the cells. Otherwise, handling of the cells was not discussed.

Notably with respect to possible future translational studies, only half of the studies reported data analysing MSC viability or functionality post-electroporation. For cell viability, a range of dye exclusion or metabolic assays were employed. Lineage differentiation capability was the most common measure of functionality examined.

Despite its widespread use elsewhere, CRISPR Cas9 RNP studies in MSCs were fewer than expected. The earliest study, from Torres-Ruiz et al. in 2017, reported using the Neon system to deliver RNPs to induce translocations in human umbilical cord-derived MSCs [30]. Despite naming the Neon system in the Methods section of their paper, they used the term ‘nucleofection’ in the Results section which typically refers to the Nucleofection system, leading to some uncertainty, but their reference to tips and specific pulse parameters suggests the Neon system was indeed used. Translocation edit efficiencies of 1.0% were reported but neither cell viability nor functionality were discussed. Xu et al. used the Celetrix system to transfect RNPs and single stranded DNA oligonucleotides into human MSCs to achieve homology-directed repair [45]. They reported up to 80% knock out of β 2-microglobulin (B2M) in primary human MSCs but corresponding cytotoxicity levels are not discussed. Kim et al. used the Nucleofection 4D system to deliver RNPs to insert a brain-derived neurotrophic factor (*BDNF*) gene into human umbilical cord-derived MSCs to examine a therapeutic strategy to ameliorate Rett syndrome (RTT) symptoms [40]. The amount of BDNF secreted from transfected cells was 5.85 times higher than that of wild-type MSCs, as measured by ELISA. MSC viability or functionality assays were not discussed. When BDNF-MSCs were injected into a mouse model of RTT, several desirable therapeutic effects were observed. In the most comprehensive study, Han et al. (2024) compared the Neon and Nucleofector systems for delivery of RNPs to human umbilical cord-derived MSCs [51]. Though not explicitly stated in their report, it is assumed that they used the Neon system for their studies based on their early optimisation data. When targeting the B2M gene, mutation frequencies of 85.1% were reported with cell viability above 90%. B2M-knockout MSCs effectively suppressed CD8⁺ T-cell proliferation and results suggested that B2M-knockout augmented the priming effects of IFN- γ in upregulating immunomodulatory cytokines.

The relatively small number of studies reported to date suggests that success with electroporation-based delivery of CRISPR Cas9 RNPs in MSCs has been limited and has been either inefficient or toxic. The diversity of electroporation systems apparently used, the lack of clarity around which systems were actually used, and the variety of analytical assays used means that electroporation-mediated delivery of CRISPR-Cas9 for editing MSCs appears feasible but requires robust process development addressing critical process parameters as well as detailed reporting of procedures.

8. Conclusions

It is likely that the next generation of MSC cell therapy products will need to be engineered in order to enhance functionality and/or persistence in vivo and thus advance the field. In addition to having a better safety profile than viral vectors in terms of immunogenicity and carcinogenicity, other advantages of non-viral approaches include ease-of-use and flexibility. A wide range of non-viral methods have been used to delivery various cargos to MSCs including physical methods such as electroporation and sonotransfection and chemical methods such as cationic lipids, cationic polymers and nanoparticles [6]. However, low efficiency and toxicity challenges have generally hampered their success. The purpose of this study was to assess the current state of the art for electroporation-mediated delivery to MSCs.

Despite the expanded availability of electroporation systems and gene editing tools in recent years, the relatively low number of studies identified indicates that electroporation remains a challenging process in primary human MSCs, particularly for CRISPR Cas9-mediated editing (**Table 1**). In order for studies to be successfully optimised and reproduced, it is therefore imperative that investigators provide detailed protocols including the electroporation parameters used in their studies. The optimal handling of MSCs post-electroporation should be specifically studied and reported. Additionally, cell viability and functionality assays appropriate for the desired therapeutic effect should always be included in such studies. It would be helpful to the field if standardised assays could be agreed and recommend for this purpose.

Conflict of interest

Shirley O'Dea is a co-founder and shareholder of Avectas Ltd. which has developed a non-viral non-electroporation transfection system.

Author details

Shirley O'Dea
Maynooth University, Maynooth, Ireland

*Address all correspondence to: shirley.odea@mu.ie

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Thompson M, Mei SHJ, Wolfe D, Champagne J, Fergusson D, Stewart DJ, et al. Cell therapy with intravascular administration of mesenchymal stem cells continues to appear safe: An updated systematic review and meta-analysis. *EClinicalMedicine*. 2020;**19**:100249. DOI: 10.1016/j.eclinm.2019.100249
- [2] Park JS, Suryaprakash S, Lao YH, Leong KW. Engineering mesenchymal stem cells for regenerative medicine and drug delivery. *Methods*. 2015;**84**:3-16. DOI: 10.1016/j.jymeth.2015.03.002
- [3] Lu W, Alickson J. Mesenchymal stromal cell therapy: Progress to date and future outlook. *Molecular Therapy*. 2025;**33**:2679-2688. DOI: 10.1016/j.ymthe.2025.02.003
- [4] 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**:22. DOI: 10.1038/s41536-019-0083-6
- [5] Giebel B. A milestone for the therapeutic EV field: FDA approves Ryoncil, an allogeneic bone marrow-derived mesenchymal stromal cell therapy. *Extracellular Vesicles and Circulating Nucleic Acids*. 2025;**6**(1):183-190. DOI: 10.20517/evcna.2025.02. PMID: 40206802; PMCID: PMC11977348
- [6] Oggu GS, Sasikumar S, Reddy N, Ella KKR, Rao CM, Bokara KK. Gene delivery approaches for mesenchymal stem cell therapy: Strategies to increase efficiency and specificity. *Stem Cell Reviews and Reports*. 2017;**13**(6):725-740. DOI: 10.1007/s12015-017-9760-2
- [7] Li Y, Jin M, Guo D, Shen S, Kaining L, Pan R, et al. Unveiling the immunogenicity of allogeneic mesenchymal stromal cells: Challenges and strategies for enhanced therapeutic efficacy. *Biomedicine & Pharmacotherapy*. 2024;**180**:117537. DOI: 10.1016/j.biopha.2024.117537
- [8] Golchin A, Shams F, Karami F. Advancing mesenchymal stem cell therapy with CRISPR/Cas9 for clinical trial studies. In: *Advances in Experimental Medicine and Biology*. New York, NY: Springer; 2019
- [9] Liu S. Mesenchymal stem cell engineering. *Methods in Molecular Biology*. 2018;**1868**:145-150. DOI: 10.1007/978-1-4939-8802-0_15
- [10] Gerace D, Martiniello-Wilks R, Nassif NT, Lal S, Steptoe R, Simpson AM. CRISPR-targeted genome editing of mesenchymal stem cell-derived therapies for type 1 diabetes: A path to clinical success? *Stem Cell Research & Therapy*. 2017;**8**(1):62. DOI: 10.1186/s13287-017-0511-8
- [11] Sun S, Xiao J, Huo J, Geng Z, Ma K, Sun X, et al. Targeting ectodysplasin promotor by CRISPR/dCas9-effector effectively induces the reprogramming of human bone marrow-derived mesenchymal stem cells into sweat gland-like cells. *Stem Cell Research & Therapy*. 2018;**9**(1):8. DOI: 10.1186/s13287-017-0758-0
- [12] Wang W, Xu X, Li Z, Lendlein A, Ma N. Genetic engineering of mesenchymal stem cells by non-viral gene delivery. *Clinical Hemorheology and Microcirculation*. 2014;**58**(1):19-48. DOI: 10.3233/CH-141883
- [13] Hamann A, Nguyen A, Pannier AK. Nucleic acid delivery to mesenchymal

- stem cells: A review of non-viral methods and applications. *Journal of Biological Engineering*. 2019;**18**(13):7. DOI: 10.1186/s13036-019-0140-0
- [14] Keshavarz S, Nassiri SM, Siavashi V, Alimi NS. Regulation of plasticity and biological features of endothelial progenitor cells by MSC-derived SDF-1. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*. 2019;**1866**(2):296-304. DOI: 10.1016/j.bbamcr.2018.11.013
- [15] Meng X, Zheng M, Yu M, Bai W, Zuo L, Bu X, et al. Transplantation of CRISPRa system engineered IL10-overexpressing bone marrow-derived mesenchymal stem cells for the treatment of myocardial infarction in diabetic mice. *Journal of Biological Engineering*. 2019;**13**:49. DOI: 10.1186/s13036-019-0163-6
- [16] Calvo-Asensio I, Dillon ET, Lowndes NF, Ceredig R. The transcription factor Hif-1 enhances the radio-resistance of mouse MSCs. *Frontiers in Physiology*. 2018;**9**:439. DOI: 10.3389/fphys.2018.00439
- [17] Bagno LL, Salerno AG, Balkan W, Hare JM. Mechanism of action of mesenchymal stem cells (MSCs): Impact of delivery method. *Expert Opinion on Biological Therapy*. 2022;**22**(4):449-463. DOI: 10.1080/14712598.2022.2016695
- [18] Chen P, Wang F, Ling B, Zhu Y, Lin H, Huang J, et al. Mesenchymal stem cell-derived extracellular vesicles: Emerging therapies for neurodegenerative diseases. *International Journal of Nanomedicine*. 2025;**20**:8547-8565. DOI: 10.2147/IJN.S526945
- [19] Fan L, Wei A, Ggao Z, Mu X. Current progress of mesenchymal stem cell membrane-camouflaged nanoparticles for targeted therapy. *Biomedicine & Pharmacotherapy*. 2023;**161**(29):114451. DOI: 10.1016/j.biopha.2023.114451
- [20] Kotnik T, Rems L, Tarek M, Miklavcic D. Membrane electroporation and electroporomeabilization: Mechanisms and models. *Annual Review of Biophysics*. 2019;**48**:63-91. DOI: 10.1146/annurev-biophys-052118-115451
- [21] Shi J, Ma Y, Zhu J, Chen Y, Sun Y, Yao Y, et al. A review on electroporation-based intracellular delivery. *Molecules*. 2018;**23**:3044. DOI: 10.3390/molecules23113044
- [22] Wang F, Lin S, Yu Z, Wang Y, Zhang D, Cao C, et al. Recent advances in microfluidic-based electroporation techniques for cell membranes. *Lab on a Chip*. 2022;**22**:2624-2646. DOI: 10.1039/D2LC00122E
- [23] Rems L, Miklavcic D. Tutorial: Electroporation of cells in complex materials and tissue. *Journal of Applied Physics*. 2016;**119**:201101. DOI: 10.1063/1.4949264
- [24] Campelo SN, Huand P, Buie CR, Davalos RV. Recent advancements in electroporation technologies: From bench to bedside. *Annual Review of Biomedical Engineering*. 2023;**25**:77-100. DOI: 10.1146/annurev-bioeng-110220-023800
- [25] Stewart MP, Langer R, Jensen KF. Intracellular delivery by membrane disruption: Mechanisms, strategies, and concepts. *Chemical Reviews*. 2018;**118**(16):7409-7531. DOI: 10.1021/acs.chemrev.7b00678
- [26] Napotnik TB, Polajzer T, Miklavcic D. Cell death due to electroporation – A review. *Biochemistry*. 2021;**141**:107871. DOI: 10.1016/j.bioelechem.2021.107871

- [27] Kim JA, Cho K, Shin MS, Lee WG, Jung N, Chung C, et al. A novel electroporation method using a capillary and wire-type electrode. *Biosensors and Bioelectronics*. 2008;**23**:1353-1360. DOI: 10.1016/j.bios.2007.12.009
- [28] Wang Y, Ho M, Chang J, Chu H, Lai S, Wang G. Microporation is a valuable transfection method for gene expression in human adipose tissue-derived stem cells. *Molecular Therapy*. 2009;**17**(2):302-308. DOI: 10.1038/mt.2008.267
- [29] Mun J, Shin K, Kwon O, Lim Y, Oh D. Minicircle microporation-based non-viral gene delivery improved the targeting of mesenchymal stem cells to an injury site. *Biomaterials*. 2016;**101**:310-320. DOI: 10.1016/j.biomaterials.2016.05.057
- [30] Torres-Ruiz R, Martinez-Lage M, Martin MC, Garcia A, Bueno C, Castano J, et al. Efficient recreation of t(11;22) EWSR1-FLI1(+) in human stem cells using CRISPR/Cas9. *Stem Cell Reports*. 2017;**8**(5):1408-1420. DOI: 10.1016/j.stemcr.2017.04.014
- [31] Choi J, Bae T, Byambasuren N, Park S, Jo CH, Kim D, et al. CRISPR-Cpf1 activation of endogenous BMP4 gene for osteogenic differentiation of umbilical-cord-derived mesenchymal stem cells. *Molecular Therapy - Methods & Clinical Development*. 2020;**17**:309-316. DOI: 10.1016/j.omtm.2019.12.010
- [32] Chae D, Han S, Lee M, Kim S. Genome edited Sirt1-overexpressing human Mesenchymal stem cells exhibit therapeutic effects in treating collagen-induced arthritis. *Molecules and Cells*. 2021;**44**(4):245-253. DOI: 10.14348/molcells.2021.0037
- [33] Mohamed A, Chow T, Whiteley J, Fantin A, Sorra K, Hicks R, et al. Umbilical cord tissue as a source of young cells for the derivation of induced pluripotent stem cells using non-integrating episomal vectors and feeder-free conditions. *Cells*. 2020;**10**(1):49. DOI: 10.3390/cells10010049
- [34] Salkin H, Yay A, Gokdemir NS, Gonen ZB, Ozdamar S, Yakan B. TGF-B1-over-expressed adipose stem cells-derived secretome exhibits CD44 suppressor and anti-cancer properties via antagonistic effects against SMAD4 in breast cancer cells. *American Journal of Stem Cells*. 2022;**11**(5):64-78
- [35] Erturk A, Demir S, Gunal YD, Zengin M, Cinar M, Yidiz D, et al. The impact of bone marrow-derived mesenchymal stem cells on experimental testicular torsion in rats. *Turkish Journal of Medical Sciences*. 2022;**52**(2):522-523. DOI: 10.55730/1300-0144.5339
- [36] Aluigi M, Fogli M, Curti A, Isidori A, Gruppioni E, Chiodoni C, et al. Nucleofection is an efficient nonviral transfection technique for human bone marrow-derived mesenchymal stem cells. *Stem Cells*. 2006;**24**(2):454-461. DOI: 10.1634/stemcells.2005-0198
- [37] Flanagan M, Gimble JM, Yu G, Xia X, Bunnell BA, Li S. Competitive DNA transfection formulation via electroporation for human adipose stem cells and mesenchymal stem cells. *Biological Procedures Online*. 2012;**14**(1):7. DOI: 10.1186/1480-9222-14-7
- [38] Chicaybam L, Barcelos C, Peixoto B, Carneiro M, Limia CG, Redondo P, et al. An efficient electroporation protocol for the genetic modification of mammalian cells. *Frontiers in Bioengineering and Biotechnology*. 2017;**23**(4):99. DOI: 10.3389/fbioe.2016.00099
- [39] Chen L, Shi K, Qiu W, Aagaard L, Kassem M. Generation of inducible

CRISPRi and CRISPRa human stromal/stem cell lines for controlled target gene transcription during lineage differentiation. *Stem Cells International*. 2020;**2020**:1-11. DOI: 10.1155/2020/8857344

[40] Kim HJ, Bayarsaikhan D, Lee J, Bayarsaikhan G, Lee B. Brain-derived neurotrophic factor secreting human mesenchymal stem cells improve outcomes in rett syndrome mouse models. *Frontiers in Neuroscience*. 2021;**15**:725398. DOI: 10.3389/fnins.2021.725398

[41] von der Haar K, Jonczyk R, Lavrentieva A, Weyand B, Vogt P, Jochums A, et al. Electroporation: A sustainable and cell biology preserving cell labeling method for adipogenous mesenchymal stem cells. *BioResearch Open Access*. 2019;**8**(1):32-44. DOI: 10.1089/biores.2019.0001

[42] Afshari A, Azarpira N, Pakbaz S. Differentiation of Wharton's jelly-derived mesenchymal stromal cells into hepatocyte-like cells using a refined method. *BMC. Molecular and Cellular Biology*. 2025;**26**:9. DOI: 10.1186/s12860-025-00534-y

[43] Peister A, Mellad JA, Wang M, Tucker HA, Prockop DJ. Stable transfection of MSCs by electroporation. *Gene Therapy*. 2004;**11**(2):224-228. DOI: 10.1038/sj.gt.3302163

[44] Peng D, Liu T, Lu H, Zhang L, Chen H, Huang Y, et al. Intranasal delivery of engineered extracellular vesicles loaded with miR-206-3p antagomir ameliorates Alzheimer's disease phenotypes. *Theranostics*. 2024;**14**(19):7623-7644. DOI: 10.7150/thno.103596

[45] Xu X, Gao D, Wang P, Chen J, Ruan J, Xu J, et al. Efficient homology-directed

gene editing by CRISPR/Cas9 in human stem and primary cells using tube electroporation. *Scientific Reports*. 2018;**8**(1):1-11. DOI: 10.1038/s41598-018-30227-w

[46] Dovgan B, Miklavcic D, Knezevic M, Zupan J, Barlic A. Intracellular delivery of trehalose renders mesenchymal stromal cells viable and immunomodulatory competent after cryopreservation. *Cytotechnology*. 2021;**73**(3):391-411. DOI: 10.1007/s10616-021-00465-4

[47] Gao P, Inada Y, Hotta A, Sakurai H, Ikeya M. iMSC-mediated delivery of ACVR2B-Fc fusion protein reduces heterotopic ossification in a mouse model of fibrodysplasia ossificans progressiva. *Stem Cell Research & Therapy*. 2024;**15**(1):1-14. DOI: 10.1186/s13287-024-03691-7

[48] Al-Saadi J, Walden M, Sandell M, Sohlmer J, Grankvist R, Friberger I, et al. Endovascular transplantation of mRNA-enhanced mesenchymal stromal cells results in superior therapeutic protein expression in swine heart. *Molecular Therapy - Methods & Clinical Development*. 2024;**32**(2):101225. DOI: 10.1016/j.omtm.2024.101225

[49] Halim NSSA, Fakiruddin KS, Ali SA, Yahaya BH. A comparative study of non-viral gene delivery techniques to human adipose-derived mesenchymal stem cell. *International Journal of Molecular Sciences*. 2014;**15**(9):15044-15060. DOI: 10.3390/ijms150915044

[50] Lim JY, Park SH, Jeong CH, Oh JH, Kim SM, Ryu CH, et al. Microporation is a valuable transfection method for efficient gene delivery into human umbilical cord blood-derived mesenchymal stem cells. *BMC Biotechnology*. 2010;**10**(1):38. DOI: 10.1186/1472-6750-10-38

[51] Han AR, Shin HR, Kwon J, Lee SB, Lee SE, Kim E, et al. Highly efficient genome editing via CRISPR-Cas9 ribonucleoprotein (RNP) delivery in mesenchymal stem cells. *BMB Reports*. 2024;57(1):60-65. DOI: 10.5483/BMBRep.2023-0113

Section 2

Mechanisms of Action and Translational Approaches

Mechanism of Action of NestaCell®: A Bioinformatics-Guided Translational Approach for Therapeutic Modulation in Huntington's Disease

*Rodrigo Pinheiro Araldi, Irina Kerkis, João Rafael Dias Pinto
and Cristiane Valverde Wenceslau*

Abstract

Translational bioinformatics integrates computational tools with biomedical data to uncover novel therapeutic strategies for complex diseases, such as Huntington's disease (HD). In this chapter, we highlight the utility of BDASeq®, an AI-enhanced transcriptomic analytics platform, as a powerful tool for identifying previously unrecognized druggable targets and supporting therapeutic innovation. By performing the largest transcriptomic meta-analysis of post-mortem HD brain samples to date (n = 353), BDASeq® identified 4095 differentially expressed genes (DEGs), including 394 putative therapeutic targets. Of these, 187 downregulated genes (DRGs) are naturally expressed by NestaCell®, an advanced cellular therapy product (ACTP) derived from human dental pulp stem cells (hDPSCs). Comparative transcriptomic analysis further revealed that 112 of these DRGs are encapsulated in extracellular vesicles (EVs) secreted by hDPSCs, termed NestaExo. Functional enrichment analysis demonstrated that these transcripts are involved in biological processes such as neurogenesis, synaptic plasticity, cytoskeletal organization, and nervous system development, suggesting a mechanism of action (MOA) for NestaCell® based on both BDNF-mediated signaling and EV-mediated delivery of therapeutic mRNAs. Independent in vitro studies using an ALS model confirmed the neuroregenerative potential of NestaExo, showing dose-dependent increases in neurite and axon length. Clinical trials have also demonstrated that NestaCell® is safe and well tolerated in patients with HD. Altogether, this case study illustrates how BDASeq® and translational bioinformatics can inform the development of disease-modifying therapies, positioning ACTPs like NestaCell® as a promising alternative to conventional drugs remain limited to symptomatic relief in neurodegenerative disorders.

Keywords: translational bioinformatics, BDASeq®, NestaCell®, RNA-Seq, extracellular vesicles (EVs)

1. Introduction

1.1 Huntington's disease: A rare neurodegenerative disorder orphan of approved disease-modifier drugs

Huntington's disease (HD, OMIM 143100) is a rare, fatal autosomal-dominant monogenic neurodegenerative disorder characterized by progressive motor dysfunction, neuropsychiatric disturbances, and cognitive decline [1, 2].

The disease is caused by an abnormal expansion of cytosine-adenine-guanosine (CAG) trinucleotide repeats in the first exon of *HTT* gene. While less than >36 CAG repeats are considered non-pathogenic, expansions beyond this threshold (>36 repeats) lead to disease onset [3–5]. Despite considerable clinical heterogeneity, HD typically progresses through three well-defined stages: early, middle, and late.

In the early stage, patients may present with subtle involuntary movements such as chorea, clumsiness, or impaired coordination. Psychiatric manifestations commonly include depression, irritability, anxiety, apathy, or obsessive-compulsive behaviors. Cognitive impairments often involve difficulties with multitasking, reduced processing speed, and challenges in planning or organizing tasks [6].

During the middle stage, choreic movements become more pronounced, particularly in the limbs and facial muscles, while voluntary motor functions such as ambulation and speech—progressively decline. Psychiatric symptoms may intensify, including mood swings, impulsivity, manic episodes, and severe depression. Disinhibition and irritability increasingly affect social interactions, and cognitive deficits in memory, reasoning, and problem solving become more apparent [6].

In the late stage, chorea typically diminishes, giving way to rigidity and bradykinesia. Swallowing difficulties and severe motor impairments necessitate full-time care. Neuropsychiatric features such as profound apathy, social withdrawal, psychosis, delusions are common, and severe dementia leads to marked impairments in communication, memory, and comprehension [6].

In the general population, the wild-type *HTT* alleles usually contain fewer than 36 CAG repeats, typically ranging from 10 to 26 [7]. Approximately 6% of healthy individuals carry intermediate alleles (IA) with 27–35 repeats. Although these IAs do not cause overt clinical symptoms, they exhibit instability during germline transmission and may expand into the pathogenic range in subsequent generations [8–10]. The prevalence of IAs significantly across different populations, from 0.45% in Thailand to 8.7% in Brazil [8]. This high prevalence, along with Brazil's notable genetic diversity, make the country a strategically important setting for clinical trials of investigational products (IP) targeting HD, such as diversity enhances the generalizability and translational potential of trial outcomes.

Individuals carrying the mutated *HTT* allele (mHTT), defined by the expansion of more than 36 CAG repeats, are at risk of developing HD. The pathogenic threshold is further categorized by penetrance: alleles with 36–39 CAG repeats are associated with reduced penetrance, suggesting that some carriers may remain asymptomatic throughout life, while alleles with 40 or more repeats demonstrate full penetrance and are invariably associated with clinical manifestation of the disease [11].

HTT gene encodes huntingtin (HTT), a soluble α -helical protein with a molecular weight of approximately 348-kDa, comprising 3144 amino acids [12]. Although HTT is ubiquitously expressed throughout the body, its highest concentration is found in the central nervous system (CNS), where it plays key roles in neurodevelopment and

maintaining of neuronal function [5, 13]. HTT interacts with over 200 other proteins and is involved in numerous cellular and biological processes essential for neuronal health, including vesicular transport and recycling, endocytosis, endosomal trafficking, autophagy, and transcription regulation, cell cycle control, and energy metabolism—all of which are crucial for proper synaptic activity [5, 7, 14].

Pathogenic CAG expansion results in the elongation of the polyglutamine (polyQ) tract within the HTT protein, reducing its solubility and promoting intracellular aggregates of mHTT. These aggregates predominantly form in GABAergic medium spiny neurons (MSNs) of the striatum [5, 15]. MSNs, which account for 85–95% of striatal neurons, are essential for motor coordination and higher-order cognitive functions such as reward processing and decision-making [16].

Initially, the accumulation of insoluble mHTT aggregates was thought to be the primary driver of neurodegeneration. However, emerging evidence suggests that smaller, toxic N-terminal mHTT fragments may be more pathogenic, while larger aggregates could play a protective role. The precise contribution of mHTT aggregates remains under investigation. Notably, N-terminal fragments have been shown to impair mitochondrial axonal transport even in the absence of visible inclusions, indicating that neurotoxicity may occur early in the disease process [7, 17–21].

Since the discovery of mHTT in 1993, a wide array of pathogenic mechanisms has been linked to HD, including mitochondrial dysfunction [2, 22], metal ion dyshomeostasis [23–25], cholesterol imbalance [26–28], oxidative stress [5, 29, 30], excitotoxicity [31, 32], impaired proteostasis [33, 34], and neuroinflammation [35, 36]. Despite decades of research, no approved therapies can alter the underlying neurodegenerative process in HD, underscoring the urgent need for novel disease-modifying approaches.

To date, only three pharmacological agents have been approved for the treatment of HD: tetrabenazine (TBZ, *Xenazine®*), deutetabenazine (DEU, *Austedo®*), and valbenazine (VBZ, *Ingrezza®*). These drugs are exclusively indicated for the management of chorea—a hallmark motor symptom predominantly observed during the early stages of HD but typically absent in later stages due to neurodegeneration [37–40].

Despite their symptomatic benefit, particularly in reducing involuntary movements, none of these agents offers neuroprotective effects capable of delaying or halting disease progression. Furthermore, TBZ and DEU are frequently associated with a high incidence of both common and serious adverse events (AEs) that can significantly diminish patients' quality of life. These AEs include sedation, somnolence, and fatigue, which may aggravate the functional impairments associated with HD. More concerning, however, are severe psychiatric AEs, such as depression, anxiety, and dyskinesia—conditions that often necessitate treatment discontinuation [37–45]. Among these, depression is particularly noteworthy, affecting approximately 19% of TBZ-treated individuals in clinical trial [42], and is strongly associated with increased risk of suicide [41]. As a result, patients undergoing treatment with these agents require continuous psychiatric evaluation and monitoring throughout the treatment course [46].

In addition to safety concerns aside, TBZ's presents pharmacokinetics limitations. Its short half-life and rapid clearance of active metabolites lead to fluctuating plasma concentration, requiring administration three times daily to maintain symptom control [39]. These fluctuations are believed to contribute to CNS-related AEs, including drowsiness, insomnia, and anxiety [39].

To mitigate these limitations, DEU—a deuterated form of TBZ—was developed. The substitution of hydrogen atoms with deuterium, a stable, non-toxic isotope, slows the drug's metabolism, extending its half-life and leading to more stable plasma concentrations. This pharmacokinetic enhancement allows for less frequent dosing, improved therapeutic consistency, and a potentially better safety profile compared to TBZ [37–39].

Although no head-to-head clinical trials directly compare TBZ and DEU, meta-analysis is feasible due to the methodological similarities between the first-HD study (DEU) and the TETRA-HD studies (TBZ) [41]. Both agents showed significant reductions in chorea and total motor scores as measured by the Unified Huntington's Disease Rating Scale–Total Motor Score (UHDRS-TMS) compared to placebo. DEU was associated with fewer adverse events than TBZ, although its therapeutic effects remained confined to chorea control and did not influence disease progression [41].

Comparable results were observed in the KINECKT-HD trial evaluating VBZ, which is also presumed to exert its effects through vesicular monoamine transporter 2 (VMAT2) inhibition, although its precise mechanism of action in HD remains incompletely understood [38]. Like its predecessors, VBZ demonstrated limited efficacy restricted to the reduction of chorea [38].

In the absence of approved disease-modifying therapies, the current standard of care for HD remains palliative, focusing on symptom management using antipsychotics, antidepressants, and mood stabilizers to control motor, cognitive, and neuropsychiatric manifestations [47–49]. In addition to its clinical and therapeutic challenges, HD presents significant ethical and psychological considerations related to predictive testing and genetic counseling, especially in populations with a high prevalence of intermediate alleles.

Given the multifactorial pathogenesis of HD and the limitations of existing pharmacotherapies, cell-based interventions—such as advanced therapy medicinal products (ATMPs)—have emerged as promising candidates for disease modification.

2. Advanced therapy medicinal products (ATMPs): New avenues for HD treatment

Mesenchymal stromal/stem cells (MSCs) have emerged as pivotal agents in regenerative medicine and cell therapy, owing to their multifaceted therapeutic mechanisms and broad clinical potential. First identified by Alexander Friedenstein in the 1960s as plastic-adherent progenitor cells within bone marrow capable of differentiating into bone and cartilage lineages [50–53], MSCs were formally named by Arnold Caplan in 1991 [54]. Since then, advances in immunophenotyping and functional assays have refined their characterization. The International Society for Cell and Gene Therapy (ISCT) defines MSCs by several key characteristics. These include adherence to plastic under standard culture conditions, expression of the surface markers CD105, CD90, and CD73 in at least 95% of cells, and low expression ($\leq 2\%$) of hematopoietic and immune markers such as CD45, CD34, CD14/CD11b, CD19/CD79 α , and HLA-DR. Additionally, MSCs must demonstrate the ability to differentiate in vitro into osteoblasts, chondrocytes, and adipocytes [55].

Building upon this, a recent Delphi-driven consensus study involving international experts further refined the definition of MSCs and developed comprehensive reporting guidelines for MSC-based clinical trials, emphasizing transparency and

reproducibility by standardizing critical quality attributes such as cell marker expression, tissue origin, stemness, and MSC fitness, viability, and immune compatibility parameters [2025].

MSCs can be classified as endogenous or exogenous depending on their origin. Endogenous MSCs are naturally present within the body’s tissues—such as bone marrow, adipose tissue, placenta, and umbilical cord—and contribute to normal tissue repair and maintenance. In contrast, exogenous MSCs are derived from external sources and introduced into the body for therapeutic purposes [56]. These exogenous MSCs can be autologous, meaning they originate from the same individual receiving the treatment, or allogeneic, sourced from a different donor. Both autologous and allogeneic MSCs offer distinct advantages and challenges regarding immunogenicity, availability, and therapeutic potential. **Table 1** summarizes the key biological and therapeutic differences between endogenous and exogenous MSCs, which may be either autologous (self-derived) or allogeneic (donor-derived). The comparison highlights differences in origin, immunogenicity, availability, expansion potential, therapeutic mechanisms, and safety considerations relevant to clinical application.

The therapeutic potential of MSCs is closely linked to their origin, with endogenous and exogenous sources presenting distinct advantages and limitations. Endogenous MSCs support tissue repair through natural physiological mechanisms; however, their regenerative capacity may be compromised in aged or diseased individuals due to cellular senescence or disrupted microenvironment.

From this point forward, the discussion will focus on exogenous MSCs and their therapeutic potential in clinical applications. Due to their accessibility, scalability, and adaptability to manipulation, exogenous MSCs have become a cornerstone of

Feature	Endogenous MSCs	Exogenous MSCs
Origin	Naturally present in patient’s own tissues	Derived externally, introduced into patient
Types	—	Autologous (self-derived) or Allogeneic (donor)
Immunogenicity	Low (self)	Autologous: low; Allogeneic: low to moderate
Availability	Limited by patient’s health and tissue source	Scalable, off-the-shelf possible
Expansion	Limited proliferative capacity in vivo	Can be expanded in culture to large numbers
Therapeutic potential	Personalized, may be impaired in disease/age	Enhanced by culture, possible genetic modification
Risk of immune rejection	Minimal	Low for autologous; potentially higher for allogeneic
Paracrine effects	Natural, dependent on patient condition	Can be enhanced or modified in vitro
Homing ability	Intrinsic and physiologically regulated	Variable; can be preconditioned or engineered
Safety concerns	Minimal contamination risk	Risks related to culture conditions and donor compatibility

Table 1.
Comparison of endogenous and exogenous MSCs.

advanced cell-based therapies. Their use allows for the development of standardized products that can be manufactured under Good Manufacturing Practice (GMP) conditions, ensuring reproducibility and quality control. Moreover, exogenous and allogeneic MSCs can be sourced from healthy donors, circumventing issues related to cellular aging or disease-associated dysfunction often seen in endogenous and exogenous autologous populations. Through careful selection, priming, or genetic modification, exogenous MSCs can be tailored to enhance their immunomodulatory properties, secretion of trophic factors, and regenerative capabilities. These features make them particularly attractive for treating a wide range of conditions, including inflammatory, autoimmune, neurodegenerative, and fibrotic diseases.

The therapeutic efficacy of MSCs is primarily attributed to their pleiotropic mechanisms of action. While their capacity to differentiate *in vitro* under appropriate stimuli into mesodermal lineages suggests a contribution to tissue regeneration, a growing body of evidence indicates that mesenchymal stromal/stem cells (MSCs) exert most of their clinical benefits through paracrine signaling. They secrete a rich milieu of bioactive factors including cytokines, chemokines, growth factors, and extracellular vesicles (EVs) such as exosomes, which collectively modulate the local microenvironment [56]. This secretome promotes tissue repair by stimulating resident progenitor cells, enhancing angiogenesis, and attenuating fibrosis.

A particularly critical feature of MSCs is their potent immunomodulatory capacity. MSCs interact dynamically with various components of the immune system, suppressing activation and proliferation of T cells, B cells, natural killer (NK) cells, and dendritic cells. They secrete anti-inflammatory cytokines such as interleukin-10 (IL-10), transforming growth factor-beta (TGF- β), and prostaglandin E2 (PGE2), which promote the expansion of regulatory T cells (Tregs) and shift macrophage polarization from pro-inflammatory M1 to anti-inflammatory M2 phenotypes. This broad immunoregulatory effect not only dampens excessive immune responses but also creates an environment conducive to tissue healing and regeneration [56–58]. The ability of MSCs to modulate both innate and adaptive immunity underpins their therapeutic potential in autoimmune diseases, graft-versus-host disease, and other inflammatory conditions.

Importantly, the immunomodulatory and regenerative functions of MSCs can be significantly enhanced through a process called priming or licensing. Priming is a process that involves exposing MSCs to specific stimuli—such as inflammatory cytokines or environmental cues—to enhance their immunomodulatory and regenerative functions. This “activation” boosts the cells’ ability to secrete therapeutic factors and respond more effectively in damaged or diseased tissues. Thus, priming involves exposing MSCs to specific inflammatory cytokines—such as interferon-gamma (IFN- γ), tumor necrosis factor-alpha (TNF- α), or interleukin-1 beta (IL-1 β)—which activates intracellular signaling pathways that boost the production of immunosuppressive molecules, increase secretion of trophic factors, and enhance homing and survival capabilities. This activation “licenses” MSCs to exert stronger anti-inflammatory and tissue-protective effects; tailoring their response to the pathological microenvironment, they encounter post-transplantation. Primed MSCs demonstrate augmented capacity to induce regulatory immune cells, inhibit effector immune responses, and promote tissue repair, thereby improving therapeutic outcomes in preclinical models of inflammatory and neurodegenerative diseases [56, 57].

Moreover, MSCs exhibit notable neuroprotective properties, making them promising candidates for treating neurodegenerative diseases and central nervous system (CNS) injuries. MSC-secreted neurotrophic factors such as brain-derived

neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), and nerve growth factor (NGF) support neuronal survival, promote neurogenesis, and facilitate synaptic plasticity. Their immunomodulatory effects within the CNS help mitigate neuroinflammation, a common pathological feature in disorders like Parkinson's disease (PD), Alzheimer's disease (AD), and Multiple sclerosis (MS) [5, 59, 60]. Additionally, MSC-derived extracellular vesicles have demonstrated the capacity to cross the blood-brain barrier (BBB), delivering bioactive molecules that protect neurons and modulate microglial activation, further amplifying neuroprotective outcomes.

MSCs also demonstrate homing abilities, migrating toward sites of injury or inflammation in response to chemotactic signals, which facilitates targeted therapeutic action. Their low immunogenicity, attributable to limited expression of major histocompatibility complex (MHC) class II and co-stimulatory molecules, supports their use in allogeneic settings with minimal risk of rejection [56, 57].

Clinically, MSCs have been evaluated in numerous trials for a range of indications including graft-versus-host disease, osteoarthritis, myocardial infarction, autoimmune disorders, and neurodegenerative diseases. Advances in bioprocessing, genetic modification, and formulation strategies—such as encapsulation and scaffold integration—are enhancing MSC survival, potency, and delivery efficiency, accelerating their transition into approved ATMPs [57, 59, 61].

Given that the manufacturing process defines the final MSC product, a comprehensive description of the entire production process—including culture conditions—is essential. Each culture parameter (e.g., oxygen concentration, medium composition, serum source) can profoundly influence MSC potency, functional properties, and viability. Consequently, detailed reporting of these parameters is critical to enable reproducibility and meaningful comparison between studies.

Moreover, biological adjuvants such as serum or human platelet lysate may affect immune compatibility and host responses, with potential implications for patient safety. Thorough documentation of these biological supplements and any observed adverse clinical effects is therefore crucial to guide the development of safer MSC-based therapies for clinical application.

In summary, MSCs represent a versatile and promising platform in modern regenerative medicine. Their combined regenerative, immunomodulatory, and neuroprotective activities address the multifactorial pathophysiology of complex diseases, offering hope where traditional pharmacotherapies have fallen short. The recent consensus-driven refinement of their definition and standardized clinical reporting guidelines significantly advances the field by improving reproducibility and facilitating regulatory approval, thus positioning MSC-based therapies as cornerstone components of next-generation ATMPs [62–64].

3. Cell signaling and communication as mediators of ACTP's mechanism of action

Although the therapeutic potential of MSCs has been widely demonstrated in numerous systematic reviews and meta-analyses [5, 57, 59, 65, 66], their precise mechanism of action (MOA) remains incompletely understood.

Cumulative evidence shows that, following intravenous transplantation, MSCs (i) migrate and (ii) transmigrate through the endothelium to sites of injury/inflammation, (iii) adhere to target tissues (homing) [60, 67], and (iv) differentiate into

specialized cell types [56, 61, 68–70]. This led to the initial hypothesis that their therapeutic action effects were primarily due to the replacement of damaged or dead cells [69, 71].

However, a study involving glycerol-induced kidney injury demonstrated that transplanted MSCs remained at the lesion site for only a few days, indicating that the therapeutic effects might not rely on cell replacement alone [72]. Supporting this, further studies revealed that only 0.1–2.7% of transplanted MSCs successfully attach to target tissues [50, 51, 58] and merely 1–2% survive beyond one week following systemic administration [52–54, 71, 73, 74]. These findings challenge the notion that MSC-mediated tissue regeneration is primarily driven by cell differentiation and integration.

In 1996, Haynesworth et al. [75] reported that MSCs secrete a range of growth factors and cytokines that act in a paracrine manner on receptor cells, leading to the formulation of paracrine signaling hypothesis. This was later confirmed by Takahashi et al. [55] who demonstrated MSC-conditioned medium enhanced capillary density and cardiac function, thereby mitigating damage from acute myocardial infarction in rats. Since then, the therapeutic activity of MSCs has been largely attributed to their paracrine effects [54, 68, 69, 76–80].

A wide range of bioactive molecules—including cytokines, growth factors, lipids, nucleic acids, ions, and metabolites – influence intercellular signaling and communication pathway and mediates these effects [71, 81, 82]. These molecules can exist in free form or be encapsulated within extracellular vesicles (EVs) secreted by cells [83]. However, free biomolecules are more susceptible to degradation by hydrolyzed or oxidized, whereas EV—encapsulated molecules exhibit greater stability and bioavailability [59, 84].

As a result, EVs have emerged as key components of a novel and complementary therapeutic approach known as cell-free therapy [59, 68, 70, 84–86]. EVs comprise a heterogeneous group of particles initially classified based on their origin, size, and content into: (i) microvesicles (0.1–1 nm), (ii) apoptotic bodies (50–2000 nm), and (iii) exosomes (30–200 nm) [56, 86–89]. Among these, exosomes have garnered particular attention for their selective packaging and delivery of biomolecules involved in intercellular communication.

Originally identified by Harding and Stahl [90] and once dismissed as “cellular debris,” exosomes are now recognized as functional nanocarriers of coding and non-coding RNAs, proteins, lipids, and metabolites. Their ability to transfer genetic and molecular information to recipient cells has significantly increased interest in their biotechnological applications, as illustrated in **Figure 1** [86, 87, 91–94]. Furthermore, due to their small size (<200 nm), exosomes can efficiently cross biological barriers, including the blood-brain barrier, meningeal lymphatic vessels, and choroid plexus capillaries, thereby reaching multiple brain regions [95, 96].

Because EVs are secreted by cells, they inherently carry bioactive molecules that reflect the physiological state of their cells of origin. Exosomes possess complex machinery that facilitates the selective incorporation of bioactive molecules into their lumen. This characteristic establishes them as natural, highly selective nanocarriers and key mediators of intercellular communication [87–89, 97]. Consequently, exosomes play a fundamental role in mediating the signaling effects of MSCs and are now considered integral to their MOA.

In summary, the MOA of MSCs appears to occur through two distinct yet complementary pathways: (1) cell signaling, mediated by the paracrine action of bioactive molecules that bind to surface receptors on recipient cells, and (2) cell communication, involving the transfer of biomolecules via extracellular vesicles, particularly exosomes.

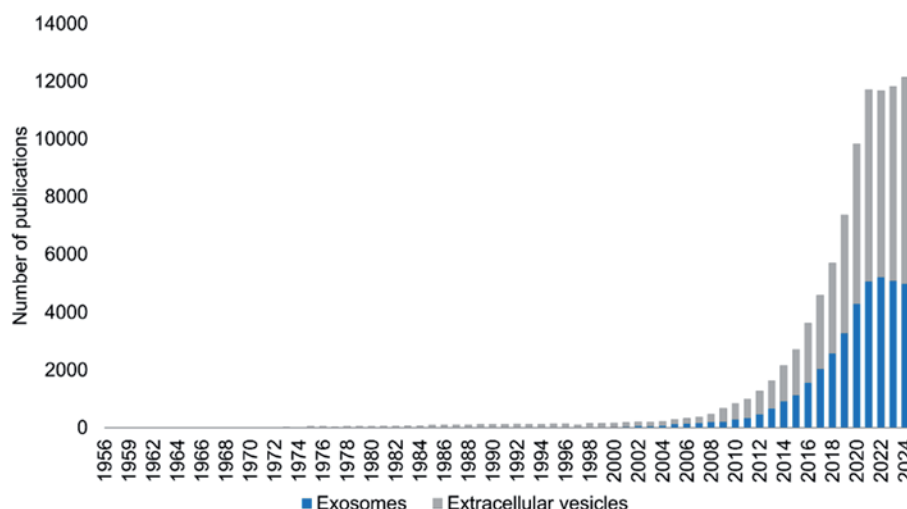


Figure 1. Number of publications on EVs from 1956 to January 22, 2025. The figure illustrates the increasing scientific interest in EVs over time, with a marked rise in publications beginning around 2009. Among the various types of EVs, exosomes have emerged as the most extensively studied subtype, particularly from 2015 onward. Publication trends were analyzed using data retrieved from PubMed.

4. The promises of NestaCell® for HD treatment

Although MSCs can be isolated from different tissues, they retain transcriptional signatures reflective of their tissue of origin, which may influence their functional properties [91, 98, 99]. Consequently, the therapeutic efficacy of MSCs may vary not only by source of tissues but also by donors, as recently demonstrated by Araldi et al. [92]. In this context, human dental pulp stem cells (hDPSCs) have emerged as particularly promising candidates for use as ATMPs in the treatment of neurodegenerative diseases.

Due to their ectomesenchymal origin from the neural crest, hDPSCs naturally express genes commonly found in the CNS, such as including *Nestin*, brain derived neurotrophic factor (*BDNF*), glial cell-derived neurotrophic factor (*GDNF*), nerve growth factor (*NGF*), and vascular endothelial growth factor (*VEGF*) [91, 93, 94]. This intrinsic gene expression profile endows hDPSCs with enhanced neuroprotective and neuroregenerative capacities compared to other typical MSCs [93, 100–103]. As such, these cells have garnered increasing biotechnological interest for the treatment of neurodegenerative disorders [104] such as HD [105], PD [106], AD [107] and amyotrophic lateral sclerosis (ALS) [90, 108, 109].

Phenotypically, hDPSCs fulfill the criteria established by the International Society for Cellular Therapy (ISCT) for defining MSCs [91, 95, 96, 110], and are therefore often described as MSC-like cells. While this classification may provide reassurance regarding the safety profile of hDPSCs—especially in light of extensive clinical and preclinical evidence supporting the safety of MSC-based ATMPs over the past two decades [111], the term “MSC-like” may also be misleading. It implies a MOA based primarily on cellular replacement through differentiation, in line with the original stromal/stem cell concept. However, this narrow classification underestimates the unique therapeutic potential of hDPSCs, which extends beyond the paradigms typically associated with MSCs.

Moreover, the continued reliance on surface marker profiles proposed by Dominici et al. in 2006 [112] for defining MSC identity has recently been challenged. ISCT now recommends that MSCs and MSC-like cells, including hDPSCs, be recognized as a distinct class of therapeutic cells, emphasizing their functional capabilities over phenotype alone.

Importantly, hDPSCs also exhibit superior immunomodulatory properties compared to other MSC types—a characteristic attributed to their native role within the dental pulp, an environment equipped to respond to cariogenic pathogens and contribute to dentin repair [113–115]. This immunomodulatory capacity is particularly relevant for the treatment of neurodegenerative diseases, in which progressive neuronal loss is closely linked to chronic neuroinflammation triggered by oxidative stress and the accumulation of misfolded protein aggregates [116–118].

From a practical standpoint, hDPSCs offer several advantages in terms of cell sourcing and production. They can be isolated from non-invasively exfoliated deciduous (baby) teeth [119, 120]. Moreover, being derived from young tissue, the cells exhibit reduced susceptibility to senescence and possess a higher proliferative capacity *in vitro* compared to MSCs isolated from adult tissues [94, 119]. These properties facilitate large-scale expansion and standardization of hDPSC-based therapies, making them highly suitable for clinical applications [121].

4.1 The development and unique properties of NestaCell® for HD therapy

Building on the unique therapeutic potential of hDPSCs, the Genetics Laboratory of Butantan Institute developed a proprietary and innovative technology for their isolation [122] and large-scale expansion [104]. This technology was patented and subsequently licensed to the Brazilian's biopharmaceutical company Cellavita Scientific Research LTD, which now manufactured hDPSCs under GMP conditions. These cells as passage 5 (P5) constitute the active pharmaceutical ingredient of NestaCell®, the investigational hDPSC-based ATMP, authorized for clinical use in humans, as registered on Clinical.Trial.gov (NCT02728115, available on <https://clinicaltrials.gov/study/NCT02728115>).

NestaCell® is a cryopreserved cell suspension of hDPSCs isolated from dental pulp of healthy donors aged 6–12 years. Their ectomesenchymal origin and derivation from a young tissue confer superior therapeutic characteristics when compared to MSC from adult cell sources [56, 61, 69], including enhanced proliferative capacity and reduced senescence.

Through bioinformatics and data analytics approaches, the molecular signature of hDPSC comprising NestaCell® was characterized revealing the expression of 375 unique genes not shared with other therapeutic cell types derived from bone marrow, adipose tissue, liver, menstrual blood, or vertebral tissue [92]. Functional enrichment analyses combined with *in vitro* studies (Pharma Intelligence) demonstrated that these genes confer distinct neuroprotective, neuroregenerative, and synaptogenic properties to NestaCell®, supporting its investigation as a potential therapy for HD [123].

Further supporting this therapeutic rationale, hDPSCs within the NestaCell® product were shown to express *nestin* in over 70% of the cell population and to secrete more than 1 ng/mL of BDNF.

Nestin is a type IV intermediate filament protein that plays central role in CNS development and axon growth [124–131]. Predominantly expressed in neural progenitor cells during early neurodevelopment, nestin is considered a hallmark of cellular

“stemness” in CNS [124–131]. It contributes to the structural integrity of proliferating and migrating cells, supporting processes such as proliferation, differentiation, and cytoskeletal reorganization. In the context of axonal development, nestin influences microtubule and actin filament dynamics, promoting neurite extension, axonal branching, and neuronal connectivity [124–131]. Moreover, nestin is re-expressed in response to CNS injury, where it participates in regeneration and repair [132]. Although its expression decreases upon full cellular differentiation, nestin persists in some adult tissues, including hDPSCs, particularly in niches with neurogenic or reparative functions [133].

BDNF, on the other hand, is a critical neurotrophin essential for striatal homeostasis [134, 135]. In HD, MSNs—which constitutes approximately 95% of striatal neurons—are selectively vulnerable. In HD-positive individuals, BDNF levels are significantly reduced due to impaired transcription caused by mutant huntingtin (mHTT) [136], as well as defective axonal transport of BDNF from cortical neurons to the striatum [137]. This depletion is strongly implicated in HD neuropathology, as BDNF is indispensable for MSN survival, neurogenesis, neuronal migration (via activation of CREB and PAX6), and cholesterol metabolism—processes all disrupted in HD [138–142].

Consequently, BDNF restoration has been proposed as a therapeutic strategy for HD [143, 144]. This is because nonclinical studies showed that BDNF level restoration ameliorated HD phenotype in animal models [136, 145]. Although recombinant BDNF could emerge as a potential candidate for HD treatment, its inability to cross the BBB limits the BDNF delivery to the striatum [146], requiring complex delivery methods, such as intrastrially administration. However, even when intrastrially administrated, therapeutic benefits of recombinant BDNF is limited to neuronal function improvement, with no confirmed benefit for the MSN survival [143, 144], underscoring its limited therapeutic effect as a monotherapy. The lack of effects on neuronal survival has been attributed to conformational differences between recombinant and native BDNF that reduce the neurotrophin biological activity [147].

Further supporting the therapeutic rationale for NestaCell®, it has been demonstrated that native BDNF, secreted by genetically modified MSCs, exhibits significantly higher neuroprotective activity—even at lower concentrations (0.23–0.70 ng/mL)—compared to recombinant BDNF administered at doses exceeding 10 ng/mL [148, 149]. This superior efficacy is likely due to conformational and post-translational differences that favor the biological functionality of the native form.

Given that the active component of NestaCell®—hDPSCs—naturally express and secrete native BDNF, this product emerges as a potential ATMP for HD. Additionally, nonclinical biodistribution studies have demonstrated that NestaCell® when administrated intravenously can cross the BBB [150]. Supporting these findings, the independent proof of concept study confirmed not only BBB penetration, but also selective engraftment of hDPSC in brain regions critically affected by HD pathology, including striatum, cerebral cortex, caudate and subventricular zone [151, 152].

Importantly, this preclinical model—based on male rats exposed to 3-nitropropionic acid (3-NPA), a well-established chemical model for HD—showed that administration of NestaCell® restored the expression of BDNF, DARPP-32, and dopamine receptor D2 (D2R) in the striatum and cerebral cortex [153, 154]. These molecular changes are highly relevant, as DARPP-32 and D2R are established biomarkers of medium spiny neuron (MSN) function, which is profoundly disrupted in HD. Collectively, these results indicate that NestaCell® exerts neuroprotective and neuroregenerative effects, at least in part, by restoring the expression of BDNF and MSN-specific biomarkers.

Given that BDNF is a signaling molecule with pleiotropic functions in the CNS, its endogenous secretion by hDPSCs suggests that BDNF is likely an important component of NestaCell®'s MOA. Supporting this, systems-level analyses using Pharma Intelligence tools revealed that hDPSCs—the active component of NestaCell®—exhibit nearly twice the signaling and intercellular communication potential compared to conventional MSCs [123, 155]. While BDNF may represent a key effector in this process, these findings indicate the involvement of multiple yet unidentified molecular mediators, consistent with the expected complexity of ATMPs.

Nevertheless, identifying the full spectrum of molecules contributing to the MOA of NestaCell® remains a significant challenge. This is particularly true in the context of HD, for which, as of 2025, no validated therapeutic targets or robust biomarkers have been universally accepted. Traditional target discovery pipelines are not only labour-intensive but also poorly suited for multifactorial diseases like HD, where the pathophysiology involves a wide array of interacting cellular and molecular processes. Therefore, comprehensive and integrative approaches will be essential to fully elucidate the therapeutic mechanisms underpinning NestaCell®'s clinical effects.

5. Reveling potential druggable targets and biomarkers for HD using BDASeq®

Traditionally employed by pharmaceutical companies, multi-Omics tools are critical for the identification of differentially expressed genes (DEGs) that may serve as potential druggable target and/or biomarkers to address synthetic drug design using computer-aided drug design (CADD). CADD has transformed pharmaceutical research by using computational methods for drug discovery and development. It enables the prediction of drug behavior, evaluation of interactions with biological targets, and optimizes pharmacokinetic properties before experimental synthesis and testing [156, 157].

CADD encompasses two principal strategies: (i) Structure-based drug design (SBDD), which uses the three-dimensional structure of a biological target (e.g., a protein) to design molecules capable of binding to it effectively; (ii) ligand-based drug design (LBDD), which relies on knowledge of existing molecules that interact with a target to predict new compounds with similar properties [156, 157]. These approaches significantly accelerate drug discovery by reducing both the time and cost associated with traditional experimental methods. However, the success of is highly contingent on the prior identification of viable biological targets—namely, druggable genes—thereby underscoring the importance of integrative bioinformatics tools such as RNA sequencing (RNA-Seq) in the context of HD research.

Over the past decade, RNA-Seq has emerged as a fundamental methodology for identifying DEGs and, therefore, druggable targets and/or biomarkers [123, 158, 159]. The standard RNA-Seq workflow begins with RNA isolation, followed by mRNA enrichment or rRNA depletion. For pharmaceutical purposes, mRNA enrichment is typically favored, as it prioritizes protein-coding transcripts. The enriched mRNAs are then reverse transcribed into cDNA and sequenced using high-throughput platforms such as Illumina™ at a depth of 10 to 100 million reads per sample. Sequencing data undergo quality control to assess metrics Phred score, GC content, and fragment length distribution. After quality filtering, reads are mapped to a reference genome to determine gene expression. These reads are then quantified, normalized, and subjected to statistical analysis to identify DEGs [160, 161].

Nevertheless, the reliability of RNA-Seq data is strongly influenced of biological replicates included in the study [161, 162]. Research indicates that a minimum of four samples is required to achieve sufficient statistical power. Insufficient sample sizes may hinder the detection of druggable genes, particularly in complex diseases such as HD, where phenotypic heterogeneity can obscure key transcriptomic signals [162, 163].

To date, four independent transcriptomic studies - analyzing a total of 268 *post-mortem* samples from the cerebral cortex (Brodmann areas 4 and 9) of individuals with HD (cases) and neurologically healthy subjects (controls), have been conducted with the aim of identifying potential druggable targets and biomarkers for the disease [132, 164–166]. Despite these efforts, the studies did not yield DEGs suitable for supporting computer-aided drug design (CADD), nor did they identify reliable blood-based biomarkers.

However, a reanalysis of one of these studies, for which sequencing datasets are publicly available in the Sequence Read Archive (SRA) database, demonstrated that the absence of age-matched controls (at time of death) significantly compromised DEG discovery by increasing both type I and II errors [133]. In addition to this limitation, the relatively small sample sizes analyzed in these studies likely contributed to the failure to identify robust druggable targets, particularly given the high degree of clinical and molecular heterogeneity characteristic of HD [132, 164–166].

To overcome these limitations, and with the support of the IHA and the ABH, BioDecision Analytics™ conducted the largest exploratory transcriptomic study on HD to date. This study identified and curated 353 RNA-Seq datasets from BA4 and BA9, including samples from HD-positive individuals (cases, $n = 146$) and neurologically healthy controls ($n = 207$) available in the SRA database.

Moreover, technical aspects of DEA, including statistical remodeling, normalization strategies, and correction for multiple hypothesis testing—play a critical role in the reliability of target identification [167–169]. To address these challenges and improve the precision of druggable target and biomarker discovery, we developed BDASeq®, a customized algorithm designed to simultaneously control type I and type II errors, thereby enhancing the robustness and accuracy of transcriptomic analysis in HD research [138, 170].

BDASeq® is an integrative, analytics-based platform specifically designed to facilitate identification of therapeutic targets through transcriptomic profiling [138, 139]. At the core of its architecture lies the Recursive Method Combination (RMC) framework, an innovative ensemble strategy that aggregates the results of eight most widely used DEA algorithms—DESeq2 [169], edgeR [140], limma-Voom [141], NOISeq [142], EBSeq [171], dearseq [172], Wilcoxon [173] e Firth logistic regression [174]. This ensemble approach mitigates method-specific biases and enhances the robustness and reproducibility of gene detection across heterogeneous datasets. In addition to DEA integration, BDASeq® incorporates artificial intelligence (AI) algorithms that combine transcriptomic signatures with clinical metadata, enabling the prioritization of candidate targets for CADD.

Using BDASeq®, we conducted the most comprehensive transcriptomic meta-analysis in HD to date, reanalyzing 353 RNA-Seq samples from 12 independent BioProjects (PRJNA271929, PRJNA316625, PRJNA670925, PRJEB59710, PRJNA253002, PRJNA557205, PRJNA602538, PRJNA531456, PRJNA398545, PRJNA729761, PRJNA388174 e PRJNA755746) deposited in the NCBI Sequence Read Archive, as described in Dias-Pinto et al. (**Figure 2**) [175].

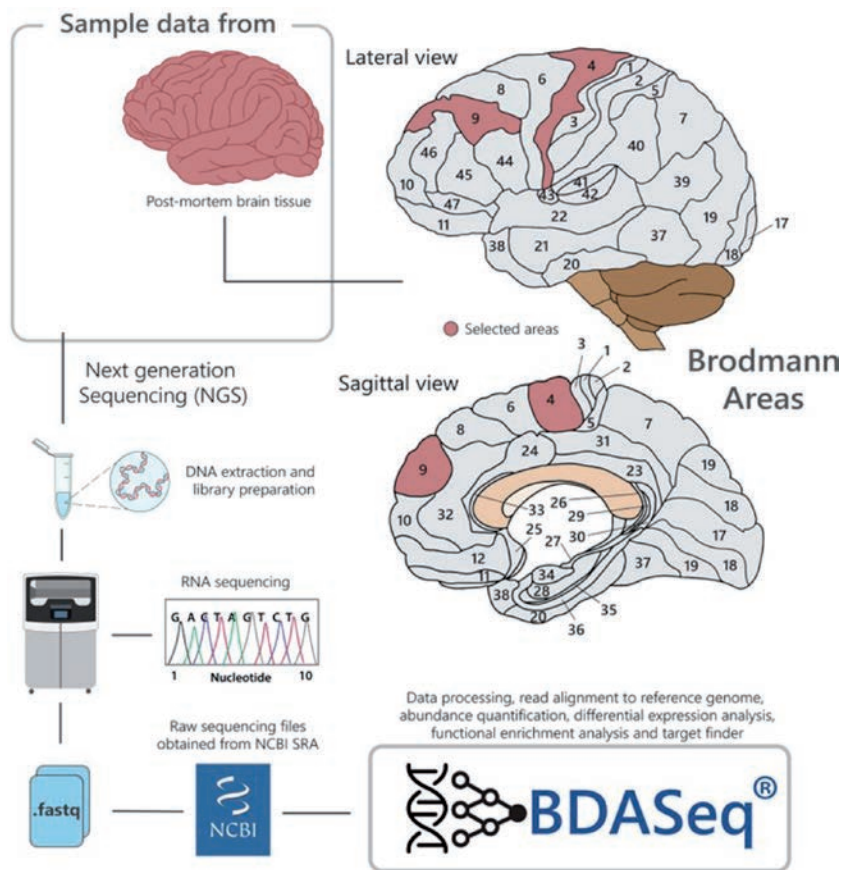


Figure 2. Schematic illustration of design study. Eligible brain (Brodman areas (BA) 4 and 9) and blood BioSamples (FASTQ files) from HD-positive (cases) and neurologically normal controls were downloaded from SRA database and processed using BDASeq®. The FASTQ files underwent quality control analysis, sequence alignment/mapping, and read counting. Subsequently, read counts were analyzed for differential expression using RMC algorithm. Differentially expressed genes were then integrated with clinical-pathological features to identify potential therapeutic targets or biomarkers using AI. Figure adapted from Dias-Pinto et al. [148].

The study considered as eligible BioSamples from humans carrying *mHTT* gene (cases) confirmed by genetic diagnostic (declared in BioProject metadata, cases) or not carrying *mHTT* gene, which cause of death was unrelated to any neurodegenerative disorder (controls). To avoid case-control bias, control samples without age matching with cases were excluded using the MatchIt package in R [149], implemented into the BDASeq® platform (propensity score matching (PSM) algorithm). FASTQ files of eligible BioSamples, including age-matched control samples, were downloaded from the SRA database using SRA Toolkit. Pre-eligible BioSamples were subjected to quality control analysis using FastQC [176] and MultiQC [153]. Samples selected for this study met the following criteria: a Phred score above 30, fragment length of 100–150 bp, and %GC of 50% ± 4, according to the recommendations by Conesa et al. [162]. Eligible BioSamples were then aligned with the human reference genome GRCh38 (release 108) using STAR [154]. Mapped reads were counted with featureCounts [155]. This process resulted in a count matrix, which serves as input for the next step differential expression analysis (DEA). Due to the combination

of different studies, with different strategies, the batch effects from the raw count matrix were removed by using CombatSeq algorithm [177]. Bioinformatic tools used for data downloading and processing are implemented in the first layer of BDASeq® (data processing). Outliers samples were filtered out using the combination of unsupervised algorithm with clustering analysis. Genes were then classified as zero (ZCG), low (LCG), equally (EEG), non-relevant (NRG), up- (UGR) or downregulated gene (DRG), as previously proposed by us [160]. Bioinformatic and statistics tools used for DEA and RMC algorithm are part of the second layer of BDASeq® (differential expression). Subsequently, the most relevant genes (UGRs and DRGs) and their association with clinical features of Huntington's Disease (HD)—including CAG repeat number, age at onset, Vonsattel grade (indicative of brain degeneration), and lifespan—were identified using supervised classification algorithms coupled with feature selection techniques [43]. This feature selection step combined with feature (i.e., gene) importance measurements were integrated into the third layer of BDASeq® (Gene Target Search) [175, 178]. A summary description of BDASeq® is shown in **Figure 3**.

This large-scale integrative analysis led to the identification of 4095 DEGs and 394 putative druggable targets associated with HD pathogenesis (**Figure 4**) [175]. These findings, which were recognized at the MENA Congress for Rare Disease (Abu Dhabi, UAE, 16–19 May 2024) [138, 139, 178], open new avenues for therapeutic development—particularly through drug reposition strategies aimed at accelerating the transition from target discovery to clinical application.

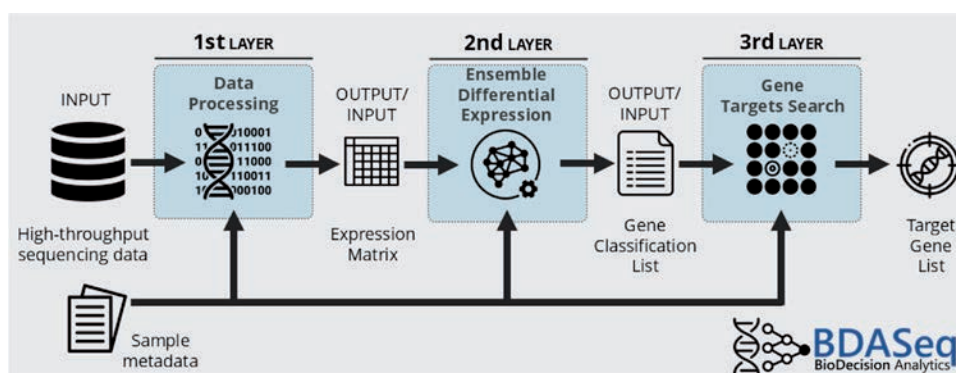


Figure 3. Schematic model of BDASeq® tool. High-throughput sequencing files/data (FASTQ) are imported into the tool using the SRA Toolkit. To minimize case-control bias, control samples are selected using the Propensity Score Matching (PSM) algorithm. Pre-selected BioSamples from controls and cases are then subjected to quality control analysis using FastQC. Samples that pass quality control are aligned to a reference genome using the STAR aligner and mapped reads are quantified using the featureCounts, generating a count matrix that serves as the input to the next step - differential expression analysis. In the differential expression analysis phase, eight different methods (DESeq2, edgeR, limma-Voom, NOISeq, EBSeq, deaRseq, Wilcoxon e Firth logistic regression) are applied individually. Results are then combined using the disruptive Recursive Method Combination (RMC) algorithm, which simultaneously reduces type I and type II errors, thus minimizing analytical bias. The process also filters out undesired samples through outlier-detection techniques. Following this integrative analysis, genes are classified into six categories: zero-count genes (ZCG), low-count genes (LCG), equally expressed genes (EEG), non-relevant (NRG), up- (UGR) or downregulated gene (DRG). Finally, in the target gene search layer, artificial intelligence (AI) algorithms are employed to both identify putative target as well as retrieve URGs and DRGs associated with clinical-pathological features of interest (based on the sample metadata variables). BDASeq® is proprietary intellectual property of BioDecision Analytics Ltd., registered in Brazilian National Institute of Industrial Property (INPI, Brazil, BR512025001097-4). Figure obtained from Dias-Pinto et al. [148].

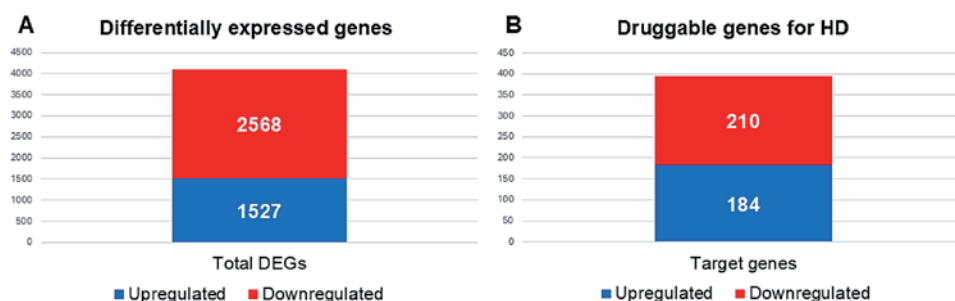


Figure 4.

Number of protein-coding genes (mRNAs) identified as DEGs (A), and corresponding therapeutic targets identified in post-mortem brain tissues (Brodmann areas 4 and 9) of individuals with HD (B). The analysis revealed 4095 DEGs, comprising 2568 DRGs and 1527 URGs. Among these, 394 mRNAs were classified as putative therapeutic targets for HD, including druggable genes and/or biomarkers. Of these targets, 210 were downregulated and 184 were upregulated, underscoring the disease's complex transcriptomic landscape and highlighting promising candidates for future therapeutic development.

By integrating CADD and pharmacogenomic data derived from Drug Bank, we observed that several top-ranked druggable genes identified through BDASeq® could be targeted by valproic acid (VA), also known as valproate. VA has been investigated as a therapeutic option for HD, particularly for the management of psychiatric symptoms such as aggressiveness and agitation [181]. Some clinical studies suggest that VA may alleviate these symptoms, thereby enabling better disease management without necessitating increased doses of neuroleptic agents, which are often associated with undesirable motor and psychiatric side effects [179]. Additionally, VA has demonstrated dose-dependent efficacy in improving myoclonic hyperkinesia – a rare but debilitating symptom observed in some HD patients [180].

The therapeutic activity of VA is primarily attributed to its capacity to enhance GABAergic neurotransmission, thereby stabilizing neuronal excitability. Moreover, VA exerts multiple neuroprotective effects through mechanisms such as inhibition of histone deacetylase, modulation of voltage-gates ion channels, and reduction of glutamatergic transmission. These combined actions may underline its potential utility in the treatment of neurodegenerative disorders like HD [181]. However, despite these pharmacological advantages, VA is associated with a range of adverse effects, including nausea, vomiting, tremors, cognitive impairments, extrapyramidal symptoms, hepatotoxicity, pancreatitis, and teratogenicity [181].

While these findings underscore the potential of AI-driven transcriptomic platforms as BDASeq®, in accelerating drug repurposing for neurodegenerative diseases, they also highlight the inherent limitation of synthetic pharmacological agents. Current treatments—including tetrabenazine, deutetrabenazine, and valbenazine—primarily offer symptomatic relief, particularly in the management of chorea, and are not expected to modify the natural course of HD.

In contrast, preclinical studies have demonstrated that NestaCell® product exert both neuroprotective and neuroregenerative effects in HD animal models [120, 151, 152, 182]. Clinical data further support its therapeutic potential: intravenous administration of NestaCell® has been shown to be safe and well tolerated in patients with HD, as evidenced by a first-in-human clinical trial using an advanced cellular therapy product (ACTP) for HD (Phase I study, SAVE-DH; NCT02728115). Moreover, results from both Phase I and II trials (ADORE-DH; NCT03252535) indicated that administration of 1–2 million hDPSCs/kg was associated with improvements in total

motor score (TMS), while the higher dose (2 million cells/kg) also led to a significant improvement in total functional capacity (TFC). Notably, some patients in advanced disease stages demonstrated functional recovery, transitioning from late to middle stage or from middle to early stage, as documented in the statistical regulatory report.

Despite the growing body of evidence supporting its clinical benefits, the MOA of NestaCell®, as well as other ACTPs, remains incompletely understood. Although it has been extensively demonstrated that the active component of NestaCell® naturally secretes BDNF—a molecule capable of mediating neuroprotective signaling—this alone does not fully account for the observed improvements in TFC in clinical studies, nor the neuroregenerative effects reported in preclinical models.

6. Reveling the MOA of NestaCell® using translational bioinformatics

Translational bioinformatics lies at the intersection of computational science and biomedical research, offering powerful robust methodologies for elucidate the MOA of both conventional drugs and ACTPs. By integrating diverse datasets—including multi-omics profiles, clinical trial data and molecular interaction work—this discipline enables systematic characterization of the biological pathways modulated by therapeutic interventions.

Bioinformatics-driven network analyses can uncover previously unrecognized mechanisms by mapping functional interactions among genes, proteins and metabolites. Such integrative approaches not only facilitate drug repurpose but also support the identification of predictive biomarkers, enabling stratification of patients based on likely therapeutic response. As translational bioinformatics continues to advance, its incorporation into precision medicine is poised to transform the development, testing, and clinical application of both pharmacologic and cell-based therapies—ultimately enabling more targeted and effective treatments across a spectrum of diseases.

In this context, BDASeq® has been employed to perform high-resolution transcriptomic profiling in both HD patient samples [138, 139, 160], and the active component of NestaCell® product [92]. Moreover, BDASeq® identified 394 putative druggable targets in HD that, if modified therapeutically, may alter the natural progression of the disease [138, 139, 160].

To explore the MOA of NestaCell®, we conducted a comparative analysis between the downregulated genes (DRGs) identified in 146 post-mortem brain samples from HD-positive individuals and the transcriptomic signature of NestaCell®'s active component. This analysis revealed that NestaCell® naturally expresses 187 of the 1527 DRGs, including 44 of the 184 druggable targets identified by BDASeq® (**Figure 5**). These findings suggest that NestaCell® may exert its therapeutic effects, at least in part, by replenishing or reactivating critical molecular pathways suppressed in HD, providing a mechanistic rationale for its observed neuroprotective and functional benefits in both preclinical and clinical settings.

Using functional enrichment analysis, we further demonstrated that the transcripts expressed by hDPSCs (NestaCell®) might exert therapeutic effect upon transfer to recipient cells. Specifically, these transcripts have the potential to: (i) enhance synaptic plasticity by stimulating axon guidance pathway, (ii) promote cholesterol metabolism, which is known to be dysregulated in HD, (iii) provide antioxidant protection, (iv) confer neuroprotective affect, and (v) support neurorregeneration by modulating multiple molecular pathways, as illustrated in **Figure 6**.

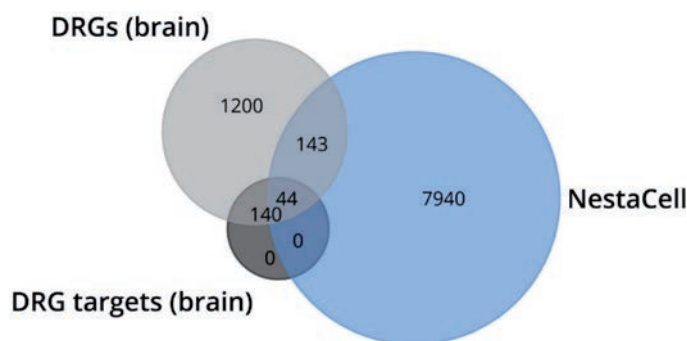


Figure 5.

Venn diagram comparing the 1527 downregulated genes (DRGs) identified in the brain of individuals with HD (DRGs brain), including the 184 druggable DRG targets identified by BDASeq® (DRG targets (brain)), with the transcriptomic profile of NestaCell®. The results indicate that NestaCell® naturally expresses 187 of the 1527 DRGs, including 44 of the 184 DRG targets considered therapeutically relevant due to their direct involvement in HD pathophysiology. Diagram generated using FunRich software v3.1.4.

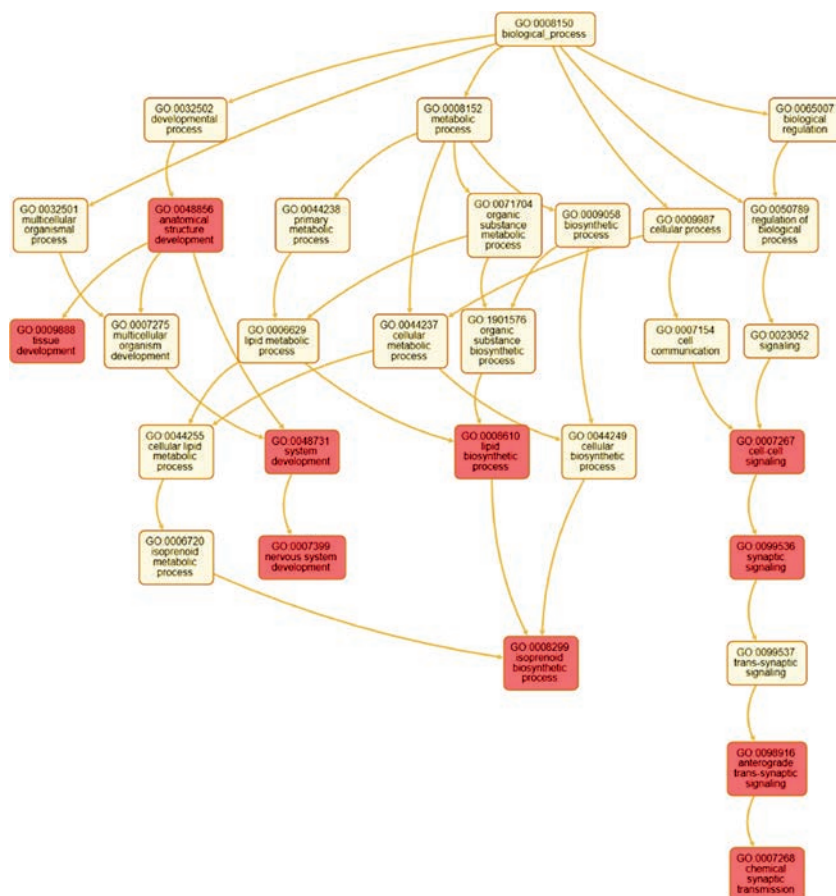


Figure 6.

Functional enrichment analysis of biological pathways using GO reveals that the 187 downregulated genes (DRGs)—, which are naturally expressed by the NestaCell® product—are significantly associated with biological processes related to neuroregeneration and cellular communication. Analysis was performed using the WebGestalt—Network Topology-based Analysis tool in conjunction with the FunMap database.

However, for these biomolecules to exert therapeutic effects, they must be effectively delivered to recipient cells via intercellular communication mechanism. As discussed in Section 3, EVs play a pivotal role in this process, acting as natural carriers for the transfer of bioactive molecules.

To investigate whether EVs derived from hDPSC can mediate the delivery of therapeutic transcripts, we isolated EVs from the conditioned culture medium of hDPSCs at passage five (NestaCell® product) using innovative technology developed and patented by Kerkis and Araldi (US patent number 63/484,440). These EVs, hereafter referred to as NestaExo, were subjected to RNA sequencing for transcriptomic profile.

Subsequently, the transcriptomic profiles of NestaExo, and NestaCell®, were comparatively analyzed in relation to the DRGs identified by BDASeq® in the HD-positive individuals. Bioinformatics and Data Analytics tools employed for this comparative analysis.

As anticipated, NestaExo was found to contain 5930 protein-coding genes (mRNAs) out of the 8127 mRNAs naturally expressed by hDPSCs comprising the NestaCell® (**Figure 5**). Importantly, 112 from 187 DRGs expressed by hDPSCs and identified as putative druggable targets for HD were also detected in NestaExo, supporting the hypothesis that hDPSC-derived EVs serve as vehicles for the delivery of therapeutically relevant molecules (NestaExo, **Figure 7**).

Based on these findings, the 112 genes identified within NestaExo were subjected to functional enrichment analysis to assess their potential biological roles. Remarkably, the results revealed that the mRNAs encapsulated within NestaExo, once delivered to recipient cells, are associated with pathways involved in neurorregeneration, including neuronal differentiation and generation, as well as synaptic plasticity. These transcripts contribute to critical processes such as cytoskeletal remodeling, microtubule dynamics, and synapse organization, underscoring their therapeutic relevance in the context of HD (**Figure 8**).

Further supporting the neuroregenerative potential of these biomolecules and the role of EVs in the MOA of the NestaCell® product, an independent study was conducted in collaboration with Tel Aviv University using an in vitro model of

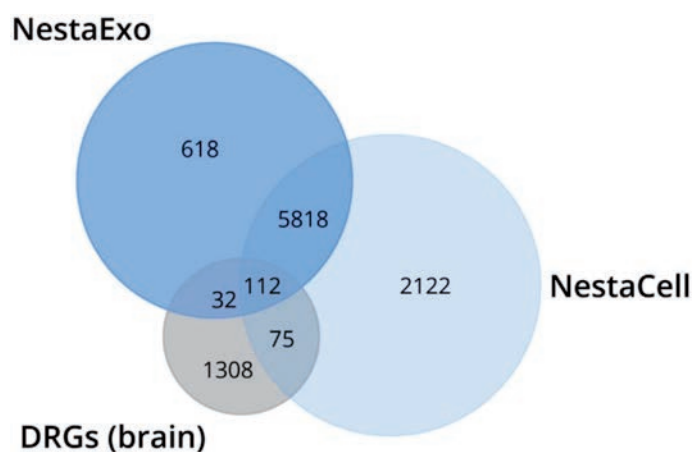


Figure 7.
 Venn diagram illustrating the overlap between the 187 DRGs identified in the brains of individuals with HD and the transcriptomic profiles of the NestaCell® product and its EVs (NestaExo). Of these 187 DRGs, 112 are not only expressed by NestaCell® but are also encapsulated within NestaExo, supporting their potential role in intercellular delivery of therapeutic transcripts. Diagram generated using FunRich software v3.1.4.

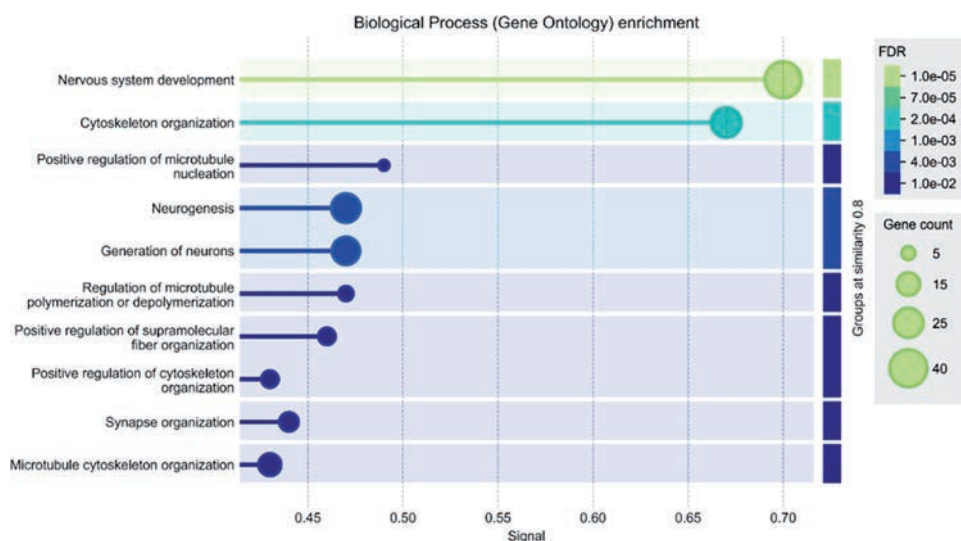


Figure 8. Results of GO-based functional enrichment analysis of biological processes associated with the 112 gene products expressed by NestaCell® and transferred via NestaExo. The analysis reveals that the synergistic action of these gene products may promote neuroprotective and neuroregenerative effects by supporting nervous system development, neurogenesis, and neuronal differentiation, as well as enhancing synaptic organization and neuronal plasticity. Results were obtained using the STRING webservice. FDR—false discovery rate (adjusted p-value).

ALS—another neurodegenerative disorder. In this study, treatment with NestaExo at a concentration of 50 µg/mL, corresponding to the EV yield from approximately 1 million hDPSCs/kg, resulted in significant promotion of cell body growth and a dose-dependent increase in axon and neurite length [123]. These findings further substantiate the therapeutic relevance of hDPSC-derived EVs in mediating neurorregeneration.

7. Conclusions

Altogether, these findings provide compelling evidence that BDASeq® serves as a powerful analytical platform—not only for uncovering previously unidentified druggable targets but also for advancing the field of translational bioinformatics. The case study presented in this chapter highlights the utility of translational bioinformatics in revealing novel therapeutic strategies for currently incurable diseases such as HD.

In this context, ACTPs emerge as one of the most promising therapeutic approaches. Unlike synthetic drugs, which are often limited to symptom management by targeting a narrow set of molecular pathways, ACTPs offer the potential to modulate complex disease networks through multimodal mechanisms of action.

Among ACTPs, NestaCell®—under investigation for HD treatment for over a decade [151]—represents a particularly promising candidate. As demonstrated through integrative bioinformatics analyses, NestaCell® exerts synergistic therapeutic effects by combining BDNF-mediated signaling with extracellular vesicle (EV)-mediated intercellular communication. Notably, NestaCell® delivers at least 112 mRNA transcripts, recognized as druggable targets, that are downregulated in the

brains of HD-positive individuals. These findings, together with the demonstrated clinical safety of NestaCell® in early-phase trials, provide strong support for its continued development as a disease-modifying therapy for HD.

Conflict of interest

The authors declare no conflict of interest.

Author details

Rodrigo Pinheiro Araldi^{1,2}, Irina Kerkis^{3*}, João Rafael Dias Pinto^{1,2}
and Cristiane Valverde Wenceslau⁴

1 Post-graduation Program in Structural and Function Biology, Paulista School of Medicine, Federal University of São Paulo (UNIFESP), São Paulo, Brazil

2 BioDecision Analytics, São Paulo, Brazil

3 Genetics Laboratory, Butantan Institute, São Paulo, Brazil

4 Cellavita Scientific Research, Valinhos, Brazil

*Address all correspondence to: irina.kerkis@butantan.gov.br

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Medina A, Mahjoub Y, Shaver L, Pringsheim T. Prevalence and incidence of Huntington's disease: An updated systematic review and meta-analysis. *Movement Disorders*. 2022;**37**(12):2327-2335
- [2] Jurcau A, Jurcau C. Mitochondria in Huntington's disease: Implications in pathogenesis and mitochondrial-targeted therapeutic strategies. *Neural Regeneration Research*. 2023;**18**(7):1472
- [3] Gatto EM, Rojas NG, Persi G, Etcheverry JL, Cesarini ME, Perandones C. Huntington disease: Advances in the understanding of its mechanisms. *Clinical Parkinsonism & Related Disorders*. 2020;**3**:100056
- [4] Rodríguez-Santana I, Mestre T, Squitieri F, Willock R, Arnesen A, Clarke A, et al. Economic burden of Huntington disease in Europe and the USA: Results from the Huntington's disease burden of illness study. *European Journal of Neurology*. 2023;**30**(4):1109-1117
- [5] 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*. London, UK: InTech; 2022. pp. 1-27
- [6] Saudou F, Humbert S. The biology of huntingtin. *Neuron*. 2016;**89**(5):910-926
- [7] Arning L, Nguyen HP. Huntington disease update: New insights into the role of repeat instability in disease pathogenesis. *Medizinische Genetik*. 2022;**33**(4):293-300
- [8] Ruiz de Sabando A, Ciosi M, Galbete A, Cumming SA, Álvarez V, Martinez-Descals A, et al. Somatic CAG repeat instability in intermediate alleles of the HTT gene and its potential association with a clinical phenotype. *European Journal of Human Genetics*. 2024;**32**(7):770-778
- [9] Semaka A, Kay C, Doty C, Collins JA, Bijlsma EK, Richards F, et al. CAG size-specific risk estimates for intermediate allele repeat instability in Huntington disease. *Journal of Medical Genetics*. 2013;**50**(10):696-703
- [10] Martí-Martínez S, Valor LM. A glimpse of molecular biomarkers in Huntington's disease. *International Journal of Molecular Sciences*. 2022;**23**(10):5411
- [11] Alcalá-Vida R, Seguin J, Lotz C, Molitor AM, Irastorza-Azcarate I, Awada A, et al. Age-related and disease locus-specific mechanisms contribute to early remodelling of chromatin structure in Huntington's disease mice. *Nature Communications*. 2021;**12**(1):364
- [12] Guo Q, Huang B, Cheng J, Seefelder M, Engler T, Pfeifer G, et al. The cryo-electron microscopy structure of huntingtin. *Nature*. 2018;**555**(7694):117-120
- [13] Imarisio S, Carmichael J, Korolchuk V, Chen CW, Saiki S, Rose C, et al. Huntington's disease: From pathology and genetics to potential therapies. *Biochemical Journal*. 2008;**412**(2):191-209
- [14] Barron JC, Hurley EP, Parsons MP. Huntingtin and the synapse. *Frontiers in Cellular Neuroscience*. 2021;**15**:689332
- [15] 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*. 2022;**11**(10):1664
- [16] Lorenzetti V, Cousijn J. Cannabis use disorders and brain morphology. In: *Neuropathology of Drug Addictions and Substance Misuse*. London: Elsevier; 2016. pp. 773-785
- [17] Ordway JM, Tallaksen-Greene S, Gutekunst CA, Bernstein EM, Cearley JA, Wiener HW, et al. Ectopically expressed CAG repeats cause intranuclear inclusions and a progressive late onset neurological phenotype in the mouse. *Cell*. 1997;**91**(6):753-763
- [18] Arrasate M, Finkbeiner S. Protein aggregates in Huntington's disease. *Experimental Neurology*. 2012;**238**(1):1-11
- [19] Ratovitski T, Gucek M, Jiang H, Chighladze E, Waldron E, D'Ambola J, et al. Mutant huntingtin N-terminal fragments of specific size mediate aggregation and toxicity in neuronal cells. *Journal of Biological Chemistry*. 2009;**284**(16):10855-10867
- [20] Marcellin D, Abramowski D, Young D, Richter J, Weiss A, Marcel A, et al. Fragments of HdhQ150 mutant huntingtin form a soluble oligomer Pool that declines with aggregate deposition upon aging. *PLoS One*. 2012;**7**(9):e44457
- [21] Lunkes A, Lindenberg KS, L B-Häiem, Weber C, Devys D, Landwehrmeyer GB, et al. Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions. *Molecular Cell*. 2002;**10**(2):259-269
- [22] Quintanilla RA, Johnson GVW. Role of mitochondrial dysfunction in the pathogenesis of Huntington's disease. *Brain Research Bulletin*. 2009;**80**(4-5):242-247
- [23] Rosas HD, Chen YI, Doros G, Salat DH, Kuei CN, Kwong KK, et al. Alterations in brain transition metals in Huntington disease. *Archives of Neurology [Internet]*. 2012;**69**(7):887-893. Available from: <http://archneur.jamanetwork.com/article.aspx?doi=10.1001/archneurol.2011.2945>
- [24] Pflazer AC, Yan Y, Kang H, Totten M, Silverman J, Bowman AB, et al. Alterations in metal homeostasis occur prior to canonical markers in Huntington disease. *Scientific Reports*. 2022;**12**(1):10373. Available from: <https://www.nature.com/articles/s41598-022-14169-y>
- [25] Hands SL, Mason R, Sajjad MU, Giorgini F, Wytenbach A. Metallothioneins and copper metabolism are candidate therapeutic targets in Huntington's disease. *Biochemical Society Transactions*. 2010;**38**(2):552-558. Available from: <https://portlandpress.com/biochemsoctrans/article/38/2/552/67087/Metallothioneins-and-copper-metabolism-are>
- [26] González-Guevara E, Cárdenas G, Pérez-Severiano F, Martínez-Lazcano JC. Dysregulated brain cholesterol metabolism is linked to neuroinflammation in Huntington's disease. *Movement Disorders*. 2020;**35**(7):1113-1127
- [27] Kacher R, Mounier C, Caboche J, Betuing S. Altered cholesterol homeostasis in Huntington's disease. *Frontiers in Aging Neuroscience*. 2022;**14**:797220
- [28] Phillips GR, Hancock SE, Brown SHJ, Jenner AM, Kreilaus F, Newell KA, et al. Cholesteryl ester levels are elevated in

- the caudate and putamen of Huntington's disease patients. *Scientific Reports*. 2020;**10**(1):20314
- [29] Abramov AY, Potapova EV, Dremmin VV, Dunaev AV. Interaction of oxidative stress and misfolded proteins in the mechanism of neurodegeneration. *Life*. 2020;**10**(7):101
- [30] Zheng J, Winderickx J, Franssens V, Liu B. A mitochondria-associated oxidative stress perspective on Huntington's disease. *Frontiers in Molecular Neuroscience*. 2018;**11**
- [31] Estrada Sánchez AM, Mejía-Toiber J, Massieu L. Excitotoxic neuronal death and the pathogenesis of Huntington's disease. *Archives of Medical Research*. 2008;**39**(3):265-276
- [32] Glaser T, Silva JB, Juvenal G, Maiolini PN, Turrini N, Petiz LL, et al. Various facets of excitotoxicity. *Exploration of Neuroprotective Therapy*. 2022;**2**:36-64
- [33] Yablonska S, Ganesan V, Ferrando LM, Kim J, Pyzel A, Baranova OV, et al. Mutant huntingtin disrupts mitochondrial proteostasis by interacting with TIM23. *Proceedings of the National Academy of Sciences*. 2019;**116**(33):16593-16602
- [34] Soares TR, Reis SD, Pinho BR, Duchon MR, Oliveira JMA. Targeting the proteostasis network in Huntington's disease. *Ageing Research Reviews*. 2019;**49**:92-103
- [35] Young D, Mayer F, Vidotto N, Schweizer T, Berth R, Abramowski D, et al. Mutant huntingtin gene-dose impacts on aggregate deposition, DARPP32 expression and neuroinflammation in HdhQ150 mice. *PLoS One*. 2013;**8**(9):e75108
- [36] Crotti A, Benner C, Kerman BE, Gosselin D, Lagier-Tourenne C, Zuccato C, et al. Mutant huntingtin promotes autonomous microglia activation via myeloid lineage-determining factors. *Nature Neuroscience*. 2014;**17**(4):513-521
- [37] Claassen DO, Ayyagari R, Garcia-Horton V, Zhang S, Alexander J, Leo S. Real-world adherence to Tetrabenazine or deutetabenazine among patients with Huntington's disease: A retrospective database analysis. *Neurology and Therapy*. 2022;**11**(1):435-448
- [38] Rodrigues FB, Duarte GS, Costa J, Ferreira JJ, Wild EJ. Tetrabenazine versus deutetabenazine for Huntington's disease: Twins or distant cousins? *Movement Disorders Clinical Practice*. 2017;**4**(4):582-585. DOI: 10.1002/mdc3.12483
- [39] Frank S, Testa C, Edmondson MC, Goldstein J, Kayson E, Leavitt BR, et al. The safety of deutetabenazine for chorea in Huntington disease: An open-label extension study. *CNS Drugs*. 2022;**36**(11):1207-1216. DOI: 10.1007/s40263-022-00956-8
- [40] Login IS, Cronin MJ, MacLeod RM. Tetrabenazine has properties of a dopamine receptor antagonist. *Annals of Neurology*. 1982;**12**(3):257-262. DOI: 10.1002/ana.410120308
- [41] Claassen DO, Carroll B, De Boer LM, Wu E, Ayyagari R, Gandhi S, et al. Indirect tolerability comparison of deutetabenazine and tetrabenazine for Huntington disease. *Journal of Clinical Movement Disorders*. 2017;**4**(1):3. DOI: 10.1186/s40734-017-0051-5
- [42] Shahani L. Tetrabenazine and suicidal ideation. *The Journal of Neuropsychiatry and Clinical*

- Neurosciences. 2013;**25**(1):E30-E30.
 DOI: 10.1176/appi.neuropsych.12020033
- [43] Dalton MP, Cheng MH, Bahar I CJ. Structural mechanisms for VMAT2 inhibition by tetrabenazine. *Elife*. 2024;**22**:RP91973
- [44] Kaur N, Kumar P, Jamwal S, Deshmukh R, Gauttam V. Tetrabenazine: Spotlight on drug review. *Annals of Neurosciences*. 2016;**23**(3):176-185. Available from: <https://www.karger.com/Article/FullText/449184>
- [45] Claassen DO, Ayyagari R, Garcia-Horton V, Zhang S, Alexander J, Leo S. Real-world adherence to tetrabenazine or deutetrabenazine among patients with Huntington's disease: A retrospective database analysis. *Neurology and Therapy*. 2022;**11**(1):435-448. DOI: 10.1007/s40120-021-00309-5
- [46] Alothman D, Marshall CR, Tyrrell E, Lewis S, Card T, Fogarty A. Risk of mortality from suicide in patients with Huntington's disease is increased compared to the general population in England. *Journal of Neurology*. 2022;**269**(8):4436-4439. DOI: 10.1007/s00415-022-11085-z
- [47] Molnar MJ, Molnar V, Fedor M, Csehi R, Acsai K, Borsos B, et al. Improving mood and cognitive symptoms in Huntington's disease with cariprazine treatment. *Frontiers in Psychiatry*. 2022;**12**:825532
- [48] van Duijn E. Treatment of irritability in Huntington's disease. *Current Treatment Options in Neurology*. 2010;**12**(5):424-433
- [49] Andriessen RL, Oosterloo M, Hollands A, Linden DEJ, de Greef BTA, Leentjens AFG. Psychotropic medication use in Huntington's disease: A retrospective cohort study. *Parkinsonism & Related Disorders*. 2022;**105**:69-74
- [50] Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood*. 2003;**101**(8):2999-3001
- [51] Kristocheck M, Dias LD, Ghem C, Eibel B, Kalil RAK, Markoski MM. The homing of bone marrow stem cells is differentially activated in ischemic and valvular heart diseases and influenced by beta-blockers. *Journal of Translational Medicine*. 2018;**16**(1):133
- [52] Biancone L, Bruno S, Deregibus MC, Tetta C, Camussi G. Therapeutic potential of mesenchymal stem cell-derived microvesicles. *Nephrology Dialysis Transplantation*. 2012;**27**(8):3037-3042
- [53] 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*. 2012;**3**
- [54] 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
- [55] Takahashi M, Li TS, 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*. 2006;**291**(2):H886-H893

- [56] Araldi RP, Delvalle DA, da Costa VR, Alievi AL, Teixeira MR, Dias Pinto JR, et al. Exosomes as a nano-carrier for chemotherapeutics: A new era of oncology. *Cells*. 2023;**12**(17):2144
- [57] Cai Z, Liu D, Yang Y, Xie W, He M, Yu D, et al. The role and therapeutic potential of stem cells in skeletal muscle in sarcopenia. *Stem Cell Research & Therapy*. 2022;**13**(1):28
- [58] Chapel A, Bertho JM, Bensidhoum M, Fouillard L, Young RG, Frick J, et al. Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome. *The Journal of Gene Medicine*. 2003;**5**(12):1028-1038
- [59] 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*. 2020;**9**(12):2663
- [60] Guo M, Yin Z, Chen F, Lei P. Mesenchymal stem cell-derived exosome: A promising alternative in the therapy of Alzheimer's disease. *Alzheimer's Research & Therapy*. 2020;**12**(1):109
- [61] Evangelista A, Soares MBP, Villarreal CF. Cell-free therapy: A neuroregenerative approach to sensory neuropathy? *Neural Regeneration Research*. 2019;**14**(8):1383-1384
- [62] Renesme L, Cobey KD, Lalu MM, Bubela T, Chinnadurai R, De Vos J, et al. Delphi-driven consensus definition for mesenchymal stromal cells and clinical reporting guidelines for mesenchymal stromal cell-based therapeutics. *Cytotherapy*. 2025;**27**(2):146-168
- [63] Renesme L, Pierro M, Cobey KD, Mital R, Nangle K, Shorr R, et al. Definition and characteristics of mesenchymal stromal cells in preclinical and clinical studies: A scoping review. *Stem Cells Translational Medicine*. 2022;**11**(1):44-54
- [64] Renesme L, Cobey KD, Le M, Lalu MM, Thebaud B. Establishment of a consensus definition for mesenchymal stromal cells (MSC) and reporting guidelines for clinical trials of MSC therapy: A modified Delphi study protocol. *BMJ Open*. 2021;**11**(10):e054740
- [65] Hmadcha A, Martin-Montalvo A, Gauthier BR, Soria B, Capilla-Gonzalez V. Therapeutic potential of mesenchymal stem cells for cancer therapy. *Frontiers in Bioengineering and Biotechnology*. 2020;**8**
- [66] Archambault J, Moreira A, McDaniel D, Winter L, Sun L, Hornsby P. Therapeutic potential of mesenchymal stromal cells for hypoxic ischemic encephalopathy: A systematic review and meta-analysis of preclinical studies. *PLoS One*. 2017;**12**(12):e0189895
- [67] de SCF, de NP, Han SW, de LVC, de C CAC. Células-tronco mesenquimais: células ideais para a regeneração cardíaca? *Revista Brasileira de Cardiologia Invasiva*. 2010;**18**(3):344-353
- [68] 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
- [69] 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

- [70] Teixeira MR, Alievi AL, da Costa VR, Dias Pinto JR, Araldi RP. Therapeutic potential of extracellular vesicles in Parkinson's disease. *Exploration of. Neuroscience*. 2023;106-122
- [71] Gomzikova MO, Rizvanov AA. Current trends in regenerative medicine: From cell to cell-free therapy. *BioNanoScience*. 2017;7(1):240-245
- [72] Duffield JS, Park KM, Hsiao LL, 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*. 2005;115(7):1743-1755
- [73] 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*. 2001;169(1):12-20
- [74] 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*. 2009;5(1):54-63
- [75] Haynesworth SE, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: Effects of dexamethasone and IL-1 α . *Journal of Cellular Physiology*. 1996;166(3):585-592. DOI: 10.1002/(SICI)1097-4652(199603)166:3<585::AID-JCP13%3E3.0.CO
- [76] Joladarashi D, Kishore R. Mesenchymal stromal cell exosomes in cardiac repair. *Current Cardiology Reports*. 2022;24(4):405-417
- [77] 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
- [78] Wendt S, Goetzenich A, Goettsch C, Stoppe C, Bleilevens C, Kraemer S, et al. Evaluation of the cardioprotective potential of extracellular vesicles—A systematic review and meta-analysis. *Scientific Reports*. 2018;8(1):15702
- [79] Pishavar E, Trentini M, Zanotti F, Camponogara F, Tiengo E, Zanolla I, et al. Exosomes as neurological nanosized machines. *ACS Nanoscience Au*. 2022;2(4):284-296
- [80] Li Y, Fang B. Neural stem cell-derived extracellular vesicles: The light of central nervous system diseases. *Biomedicine & Pharmacotherapy*. 2023;165:115092
- [81] 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):23
- [82] Lu J, Zhang Y, Yang X, Zhao H. Harnessing exosomes as cutting-edge drug delivery systems for revolutionary osteoarthritis therapy. *Biomedicine & Pharmacotherapy*. 2023;165:115135
- [83] 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
- [84] 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*. 2021;10(10):2617
- [85] Bu H, He D, He X, Wang K. Exosomes: Isolation, analysis, and

applications in cancer detection and therapy. *Chembiochem*. 2019;**20**(4):451-461

[86] Maas SLN, Breakefield XO, Weaver AM. Extracellular vesicles: Unique intercellular delivery vehicles. *Trends in Cell Biology*. 2017;**27**(3):172-188

[87] Kou M, Huang L, Yang J, Chiang Z, Chen S, Liu J, et al. Mesenchymal stem cell-derived extracellular vesicles for immunomodulation and regeneration: A next generation therapeutic tool? *Cell Death & Disease*. 2022;**13**(7):580

[88] Pan Y, Wu W, Jiang X, Liu Y. Mesenchymal stem cell-derived exosomes in cardiovascular and cerebrovascular diseases: From mechanisms to therapy. *Biomedicine & Pharmacotherapy*. 2023;**163**:114817

[89] Jiao YR, Chen KX, Tang X, Tang YL, Yang HL, Yin YL, et al. Exosomes derived from mesenchymal stem cells in diabetes and diabetic complications. *Cell Death & Disease*. 2024;**15**(4):271

[90] Ueda T, Ito T, Inden M, Kurita H, Yamamoto A, Hozumi I. Stem cells from human exfoliated deciduous teeth-conditioned medium (SHED-CM) is a promising treatment for amyotrophic lateral sclerosis. *Frontiers in Pharmacology*. 2022;**13**

[91] Shamir C, Venugopal C, Dhanushkodi A. Dental pulp stem cells for treating neurodegenerative diseases. *Neural Regeneration Research*. 2015;**10**(12):1910-1911

[92] 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

[93] Karaöz E, Demircan PC, Sağlam Ö, Aksoy A, Kaymaz F, Duruksu G. Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow-derived mesenchymal stem cells. *Histochemistry and Cell Biology*. 2011;**136**(4):455

[94] Lan X, Sun Z, Chu C, Boltze J, Li S. Dental pulp stem cells: An attractive alternative for cell therapy in ischemic stroke. *Frontiers in Neurology*. 2019;**10**:1-10

[95] Monteiro BG, Serafim RC, Melo GB, Silva MCP, Lizier NF, Maranduba CMC, et al. Human immature dental pulp stem cells share key characteristic features with limbal stem cells. *Cell Proliferation*. 2009;**42**(5):587-594

[96] Suchánek J, Visek B, Soukup T, El-Din Mohamed SK, Ivancaková R, Mokřý J, et al. Stem cells from human exfoliated deciduous teeth--isolation, long term cultivation and phenotypical analysis. *Acta medica (Hradec Králové) / Universitas Carolina, Facultas Medica Hradec Králové*. 2010;**53**(2):93-99

[97] Liang W, Han B, Hai Y, Sun D, Yin P. Mechanism of action of mesenchymal stem cell-derived exosomes in the intervertebral disc degeneration treatment and bone repair and regeneration. *Frontiers in Cell and Development Biology*. 2022;**9**

[98] Brooke G, Tong H, Levesque JP, Atkinson K. Molecular trafficking mechanisms of multipotent mesenchymal stem cells derived from human bone marrow and placenta. *Stem Cells and Development*. 2008;**17**(5):929-940

[99] Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, et al. Human dental pulp-derived stem cells promote locomotor recovery after

complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. *Journal of Clinical Investigation*. 2012;**122**:80-90

[100] De Souza PV, Alves FBT, Costa Ayub CLS, De Miranda Soares MA, Gomes JR. Human immature dental pulp stem cells (hIDPSCs), their application to cell therapy and bioengineering: An analysis by systematic revision of the last decade of literature. *The Anatomical Record*. 2013;**296**(12):1923-1928

[101] Nuti N, Corallo C, Chan BMF, Ferrari M, Gerami-Naini B. Multipotent differentiation of human dental pulp stem cells: A literature review. *Stem Cell Reviews and Reports*. 2016;**12**:511-523

[102] Ullah M, Liu DD, Thakor AS. Mesenchymal stromal cell homing: Mechanisms and strategies for improvement. *iScience*. 2019;**15**:421-438

[103] Zayed M, Iohara K. Immunomodulation and regeneration properties of dental pulp stem cells: A potential therapy to treat coronavirus disease 2019. *Cell Transplantation*. 2020;**29**:096368972095208

[104] 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. DOI: 10.1371/journal.pone.0039885

[105] Eskandari N, Boroujeni ME, Abdollahifar MA, Piryaei A, Khodaghali F, Mirbehbahani SH, et al. Transplantation of human dental pulp stem cells compensates for striatal atrophy and modulates neuro-inflammation in 3-nitropropionic acid rat model of Huntington's disease. *Neuroscience Research*. 2021;**170**:133-144. Available from: <https://>

<linkinghub.elsevier.com/retrieve/pii/S0168010220305058>

[106] Xiao Z, Lei T, Liu Y, Yang Y, Bi W, Du H. The potential therapy with dental tissue-derived mesenchymal stem cells in Parkinson's disease. *Stem Cell Research & Therapy*. 2021;**12**(1):5

[107] Xiong W, Liu Y, Zhou H, Li J, Jing S, Jiang C, et al. Human dental pulp stem cells mitigate the neuropathology and cognitive decline via AKT-GSK3 β -Nrf2 pathways in Alzheimer's disease. *International Journal of Oral Science*. 2024;**16**(1):40

[108] Younes R, Issa Y, Jdaa N, Chouaib B, Brugiotti V, Challuau D, et al. The secretome of human dental pulp stem cells and its components GDF15 and HB-EGF protect amyotrophic lateral sclerosis motoneurons against death. *Biomedicine*. 2023;**11**(8):2152

[109] Jiang S, Guan X, Shi M, Zhang Y, Li X, Su Y, et al. Human dental pulp MSCs attenuated motor neuron dysfunction and prolonged lifespan in ALS murine model. *Brain & Heart*. 2024;**2**(4):3996

[110] de S SF, Ramos RN, de ADC, Bassi EJ, Gonzales RP, SPH M, 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

[111] Wang Y, Yi H, Song Y. The safety of MSC therapy over the past 15 years: A meta-analysis. *Stem Cell Research & Therapy*. 2021;**12**(1):545. DOI: 10.1186/s13287-021-02609-x

[112] 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. DOI: 10.1080/14653240600855905

[113] Sarfi S, Azaryan E, Naseri M. Immune system of dental pulp in inflamed and normal tissue. *DNA and Cell Biology*. 2024;**43**(8):369-386

[114] Farges JC, Alliot-Licht B, Renard E, Ducret M, Gaudin A, Smith AJ, et al. Dental pulp defence and repair mechanisms in dental caries. *Mediators of Inflammation*. 2015;**2015**(1). DOI: 10.1155/2015/230251

[115] Yumoto H, Hirao K, Hosokawa Y, Kuramoto H, Takegawa D, Nakanishi T, et al. The roles of odontoblasts in dental pulp innate immunity. *Japanese Dental Science Review [Internet]*. 2018;**54**(3):105-117. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1882761617300443>

[116] Gronthos S, Brahimi J, Li W, Fisher LW, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem cells. *Journal of Dental Research*. 2002;**81**:531-535

[117] 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*. 2021;**23**(4):1

[118] Pierdomenico L, Bonsi L, Calvitti M, Rondelli D, Arpinati M, Chirumbolo G, et al. Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. *Transplantation*. 2005;**80**(6):836-842

[119] 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

[120] Kerkis I, Valverde Wenceslau C, Pinheiro AR. Stem cells from dental pulp of deciduous teeth: Twenty years of experience. In: *Recent Update on Mesenchymal Stem Cells*. London, UK: IntechOpen; 2024. Available from: <https://www.intechopen.com/chapters/1171018>

[121] 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

[122] 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. Available from: <https://www.karger.com/Article/FullText/99617>

[123] Khatoon Z, Figler B, Zhang H, Cheng F. Introduction to RNA-Seq and its applications to drug discovery and development. *Drug Development Research*. 2014;**75**(5):324-330

[124] Bernal A, Arranz L. Nestin-expressing progenitor cells: Function, identity and therapeutic implications. *Cellular and Molecular Life Sciences*. 2018;**75**(12):2177-2195. DOI: 10.1007/s00018-018-2794-z

[125] Fukuda S, Kato F, Tozuka Y, Yamaguchi M, Miyamoto Y, Hisatsune T. Two distinct subpopulations of nestin-positive cells in adult mouse dentate gyrus. *The Journal of Neuroscience*.

2003;**23**(28):9357-9366. DOI: 10.1523/JNEUROSCI.23-28-09357.2003

[126] Chen LW, Hu HJ, Liu HL, Yung KKL, Chan YS. Identification of brain-derived neurotrophic factor in nestin-expressing astroglial cells in the neostriatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mice. *Neuroscience*. 2004;**126**(4):941-953. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0306452204003069>

[127] Rutka JT, Ivanchuk S, Mondal S, Taylor M, Sakai K, Dirks P, et al. Co-expression of nestin and vimentin intermediate filaments in invasive human astrocytoma cells. *International Journal of Developmental Neuroscience*. 1999;**17**(5-6):503-515. DOI: 10.1016/S0736-5748%2899%2900049-0

[128] Bott CJ, Johnson CG, Yap CC, Dwyer ND, Litwa KA, Winckler B. Nestin in immature embryonic neurons affects axon growth cone morphology and Semaphorin3a sensitivity. *Molecular Biology of the Cell*. 2019;**30**(10):1214-1229. DOI: 10.1091/mbc.E18-06-0361

[129] Krishnasamy S, Weng YC, Thammisetty SS, Phaneuf D, Lalancette-Hebert M, Kriz J. Molecular imaging of nestin in neuroinflammatory conditions reveals marked signal induction in activated microglia. *Journal of Neuroinflammation*. 2017;**14**(1):45. DOI: 10.1186/s12974-017-0816-7

[130] Wilhelmsson U, Lebkuechner I, Leke R, Marasek P, Yang X, Antfolk D, et al. Nestin regulates neurogenesis in mice through notch signaling from astrocytes to neural stem cells. *Cerebral Cortex*. 2019;**29**(10):4050-4066. DOI: 10/4050/5263979

[131] Suzuki S, Namiki J, Shibata S, Mastuzaki Y, Okano H. The neural stem/

progenitor cell marker nestin is expressed in proliferative endothelial cells, but not in mature vasculature. *Journal of Histochemistry & Cytochemistry* [Internet]. 2010;**58**(8):721-730. Available from: <http://journals.sagepub.com/doi/10.1369/jhc.2010.955609>

[132] Labadorf A, Hoss AG, Lagomarsino V, Latourelle JC, Hadzi TC, Bregu J, et al. Correction: RNA sequence analysis of human Huntington disease brain reveals an extensive increase in inflammatory and developmental gene expression. *PLoS One*. 2016;**11**(7):10-12

[133] Dias-Pinto JR, Faustinoni-Neto B, Sanches-Fernandes JM, Kerkis I, Araldi RP. How does the age of control individuals hinder the identification of target genes for Huntington's disease? *Frontiers in Genetics*. 2024;**15**

[134] Park H, Poo M, ming. Neurotrophin regulation of neural circuit development and function. *Nature Reviews. Neuroscience*. 2013;**14**(1):7-23

[135] Baydyuk M, Xu B. BDNF signaling and survival of striatal neurons. *Frontiers in Cellular Neuroscience*. 2014;**8**:254

[136] Zuccato C, Caettaneo E. Role of brain-derived neurotrophic factor in Huntington's disease. *Progress in Neurobiology*. 2007;**81**(5-6):294-330

[137] Gauthier LR, Charrin BC, Borrell-Pagès M, Dompierre JP, Rangone H, Cordelières FP, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell*. 2004;**118**(1):127-138

[138] Dias-Pinto JR, Faustinoni-Neto B, Araldi RP. BDASeq: A novel and powerful tool able to accurately discover

therapeutic targets for the treatment of rare diseases. In: MENA Rare Disease Congress. Abu Dhabi, UAE; 2024. pp. 5-5

[139] Araldi RP, Faustinoni-Neto B, Dias-Pinto JR. Novel pharmaceutical targets and prognostic biomarkers for Huntington's disease: New perspectives for the drug development. In: MENA Rare Disease Congress. Abu Dhabi; 2024. pp. 4-4

[140] Robinson MD, McCarthy DJ, Smyth GK. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010;**26**(1):139-140. Available from: <https://academic.oup.com/bioinformatics/article/26/1/139/182458>

[141] Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*. 2015;**43**(7):e47-e47

[142] Tarazona S, García F, Ferrer A, Dopazo J, Conesa A. NOIseq: A RNA-seq differential expression method robust for sequencing depth biases. *EMBnet Journal*. 2012;**17**(B):18. Available from: <http://journal.embnet.org/index.php/embnetjournal/article/view/265>

[143] Saylor AJ, McGinty JF. An intrastratial brain-derived neurotrophic factor infusion restores striatal gene expression in Bdnf heterozygous mice. *Brain Structure & Function*. 2010;**215**(2):97-104

[144] Sauer H, Fischer W, Nikkhah G, Wiegand SJ, Brundin P, Lindsay RM, et al. Brain-derived neurotrophic factor enhances function rather than survival of intrastratial dopamine cell-rich grafts. *Brain Research*. 1993;**626**(1-2):37-44

[145] Lu B, Pang PT, Woo NH. The yin and yang of neurotrophin action. *Nature Reviews. Neuroscience*. 2005;**6**(8):603-614

[146] Fujisawa M, Takeshita Y, Fujikawa S, Matsuo K, Okamoto M, Tamada M, et al. Exploring lipophilic compounds that induce BDNF secretion in astrocytes beyond the BBB using a new multi-cultured human in vitro BBB model. *Journal of Neuroimmunology*. 2022;**362**:577783

[147] Fraser S, Fleener C, Ogborne K, Soderstrom C. When close is not close enough: A comparison of endogenous and recombinant biomarker stability samples. *Bioanalysis*. 2015;**7**(11):1355-1360

[148] Dias Pinto JR, Faustinoni Neto B, Munhoz L, Kerkis I, Araldi RP. From brain to blood: Uncovering potential therapeutic targets and biomarkers for Huntington's disease using an integrative RNA-Seq analytical platform (BDASeq®). *Cells*. 2025;**14**(13)

[149] Ho DE, Imai K, King G, Stuart EA. Matching as nonparametric preprocessing for reducing model dependence in parametric causal inference. *Political Analysis*. 2007;**15**(3):199-236

[150] Valverde CW. Assessing the biodistribution of human immature dental pulp stem cells (NestaCell via intravenous Administration in Mice: A non-clinical investigation). *Journal of Clinical Case Reports Medical Images and Health Sciences*. 2024;**8**(4)

[151] Kerkis I, Wenceslau CV, Souza DM, Mambelli-Lisboa NC, Ynoue LH, Araldi RP, et al. Preclinical assessment of NestaCell® in Huntington's disease 3-NP rat model demonstrates restoration of BDNF, DARPP32, and D2R expression

following intravenous single versus multiple injections. *Cytotherapy*. 2022;**24**(10):S5. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1465324922008556>

[152] 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*. 2022;**11**(10):1664. Available from: <https://www.mdpi.com/2073-4409/11/10/1664>

[153] Ewels P, Magnusson M, Lundin S, Källér M. MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* [Internet]. 2016;**32**(19):3047-3048. DOI: 10.1093/bioinformatics/btw354

[154] Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013;**29**(1):15-21

[155] Liao Y, Smyth GK, Shi W. featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*. 2014;**30**(7):923-930. Available from: <https://academic.oup.com/bioinformatics/article/30/7/923/232889>

[156] Niazi SK, Mariam Z. Computer-aided drug design and drug discovery: A prospective analysis. *Pharmaceuticals*. 2023;**17**(1):22

[157] Mihai DP, Nitulescu GM. Computer-aided drug design and drug discovery. *Pharmaceuticals*. 2025;**18**(3):436

[158] Yang X, Kui L, Tang M, Li D, Wei K, Chen W, et al. High-throughput transcriptome profiling in drug and

biomarker discovery. *Frontiers in Genetics*. 2020;**11**

[159] Patel PH, Jha A, Chakraborty GS. Role of bioinformatics in drug design and discovery. 2023:1-33. DOI: 10.2174/1568026617666161116143440

[160] Dias-Pinto JR, Faustinoni-Neto B, Sanches-Fernandes JM, Kerkis I, Araldi RP. How does the age of control individuals hinder the identification of target genes for Huntington's disease? *Frontiers in Genetics*. 2024;**15**. DOI: 10.3389/fgene.2024.1377237/full

[161] Stark R, Grzelak M, Hadfield J. RNA sequencing: The teenage years. *Nature Reviews. Genetics*. 2019;**20**(11):631-656. Available from: <http://www.nature.com/articles/s41576-019-0150-2>

[162] 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

[163] Lamarre S, Frasse P, Zouine M, Labourdette D, Sainderichin E, Hu G, et al. Optimization of an RNA-Seq differential gene expression analysis depending on biological replicate number and library size. *Frontiers in Plant Science*. 2018;**9**. DOI: 10.3389/fpls.2018.00108/full

[164] Labadorf A, Hoss AG, Lagomarsino V, Latourelle JC, Hadzi TC, Bregu J, et al. RNA sequence analysis of human Huntington disease brain reveals an extensive increase in inflammatory and developmental gene expression. *PLoS One*. 2015;**10**(12):1-21

[165] Agus F, Crespo D, Myers RH, Labadorf A. The caudate nucleus undergoes dramatic and unique transcriptional changes in human prodromal Huntington's disease brain. *BMC Medical Genomics*. 2019;**12**(1):137

- [166] Lin L, Park JW, Ramachandran S, Zhang Y, Tseng YT, Shen S, et al. Transcriptome sequencing reveals aberrant alternative splicing in Huntington's disease. *Human Molecular Genetics*. 2016;**25**(16):3454-3466
- [167] Maza E. In Papyro comparison of TMM (edgeR), RLE (DESeq2), and MRN normalization methods for a simple two-conditions-without-replicates RNA-Seq experimental design. *Frontiers in Genetics* [Internet]. 2016;7. DOI: 10.3389/fgene.2016.00164/abstract
- [168] Zhao Y, Li MC, Konaté MM, Chen L, Das B, Karlovich C, et al. TPM, FPKM, or normalized counts? A comparative study of quantification measures for the analysis of RNA-seq data from the NCI patient-derived models repository. *Journal of Translational Medicine*. 2021;**19**(1):269. DOI: 10.1186/s12967-021-02936-w
- [169] Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*. 2014;**15**(12):1-21
- [170] Dias-Pinto JR, Faustinoni-Neto B, Araldi RP. Biomarker Discovery Algorithm (BDASeq): An Integrative Analytics-Based Tool Designed to Identify Potential Diagnostic and Therapeutic Targets for Diseases. *Brazil; BR512025001097-4*; 2025. pp. 1-1
- [171] Leng N, Dawson JA, Thomson JA, Ruotti V, Rissman AI, Smits BMG, et al. EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics*. 2013;**29**(8):1035-1043. Available from: <https://academic.oup.com/bioinformatics/article/29/8/1035/228913>
- [172] Gauthier M, Agniel D, Thiébaud R, Hejblum BP. Dearseq: A variance component score test for RNA-seq differential analysis that effectively controls the false discovery rate. *NAR Genomics and Bioinformatics*. 2020;**2**(4). DOI: 10.1093/nargab/lqaa093/5992116
- [173] Wilcoxon F. Individual comparisons by ranking methods. *Biometrics Bulletin*. 1945;**1**(6):80. DOI: 10.2307/3001968?orig=in=crossref
- [174] Firth D. Bias reduction of maximum likelihood estimates. *Biometrika* [Internet]. 1993;**80**(1):27-38. DOI: 10.1093/biomet/80.1.27
- [175] Dias-Pinto JR, Faustinoni-Neto B, Munhoz L, Kerkis I, Araldi RP. From brain to blood: Uncovering potential therapeutic targets and biomarkers for Huntington's disease using an integrative RNA-Seq analytical platform (BDASeq®). *Cells*. 2025;**14**(13):976
- [176] Andrews S. FastQC: A Quality Control Tool for High Throughput Sequence Data. Available from: <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>; 2010
- [177] Zhang Y, Parmigiani G, Johnson WE. ComBat-seq: Batch effect adjustment for RNA-seq count data. *NAR Genomics and Bioinformatics*. 2020;**2**(3). DOI: 10.1093/nargab/lqaa078/5909519
- [178] Araldi RP, Dias Pinto JR, Kerkis I. AI-enhanced transcriptomic discovery of druggable targets and repurposed therapies for Huntington's disease. *Brain Sciences*. 2025;**15**(8):865. Available from: <https://www.mdpi.com/2076-3425/15/8/865>
- [179] Unti E, Mazzucchi S, Kiferle L, Bonuccelli U, Ceravolo R. Q09 valproic acid for the treatment of aggressiveness in Huntington's disease: 1-year follow-up. *Journal of Neurology, Neurosurgery, and*

Psychiatry. 2012;**83**(Suppl. 1):
A57.2-A5A57

[180] Saft C, Lauter T, Kraus PH, Przuntek H, Andrich JE. Dose-dependent improvement of myoclonic hyperkinesia due to valproic acid in eight Huntington's disease patients: A case series. BMC Neurology. 2006;**6**(1):11

[181] Schmidt D. Adverse effects of valproate. Epilepsia. 1984;**25**:s1

[182] Kerkis I, Araldi R, Wenceslau C, Mendes T. Advances in cellular and cell-free therapy medicinal products for Huntigton's disease treatment. In: From Physiopatology to Treatment of Huntigton's Disease. London, UK: InTech; 2022. pp. 1-27. Available from: <https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics%0A>. DOI: 10.1016/j.colsurfa.2011.12.014

Section 3

Therapeutic Applications and Regenerative Medicine

Mesenchymal Stem Cell Therapy and Its Clinical Application Potential

Lei Zhang

Abstract

Mesenchymal stem cells (MSCs) have attracted extensive attention in the fields of regenerative medicine and cell therapy. This is primarily due to their multipotent differentiation abilities, immunomodulatory properties, and ability to secrete various repair-promoting factors. Over the past few years, MSCs have been applied in a broad range of clinical conditions in clinical trials, including neurodegenerative diseases, cardiovascular disorders, cancer treatment, pulmonary injuries, osteoporosis, and others, and have demonstrated promising therapeutic potential. In this chapter, we aim to synthesize the biological characteristics of MSCs, their preparation processes, mechanisms of action, and current clinical applications. This chapter focuses specifically on the molecular mechanisms underlying MSC therapy, the role of extracellular vesicles, genetic engineering strategies, and the challenges in clinical translation. By integrating recent clinical trial data and foundational research findings, we aim to provide a theoretical basis and technical support for the clinical application and future exploration of MSCs.

Keywords: mesenchymal stem cell therapy, immunomodulation, tissue repair, multipotent differentiation, stem cell exosomes, regenerative medicine

1. Introduction

Mesenchymal stem cells (MSCs) are a type of adult stem cell characterized by their unique ability to self-renew and differentiate into various cell types, including osteoblasts, adipocytes, and chondrocytes. They are primarily derived from various tissues, such as bone marrow, adipose tissue, and umbilical cord, and are recognized for their immunomodulatory properties and potential in regenerative medicine. MSCs play a crucial role in tissue repair and regeneration due to their capability to differentiate into other cell types and secrete a wide array of bioactive molecules, which influence the microenvironment, promote cell and tissue regeneration, and modulate the immune response [1]. This multifunctional nature of MSCs makes them a competitive choice in the development of novel therapeutic strategies for a variety of diseases, including degenerative conditions,

autoimmune disorders, and even complications arising from viral infections such as COVID-19 [2]. As of July 2025, ClinicalTrials.gov reports 1163 registered clinical trials focused on stem cell therapy (<http://www.clinicaltrials.gov>). These trials primarily address conditions such as central nervous system disorders, autoimmune diseases, graft-versus-host disease (GVHD), osteogenesis, and osteoarthritis, with the specific distribution illustrated in **Figure 1A**. The stem cell types utilized in these trials predominantly include bone marrow-derived MSCs, umbilical cord Wharton's jelly-derived MSCs, dental pulp-derived MSCs, and adipose tissue-derived MSCs, with the detailed breakdown shown in **Figure 1B**.

The significance of MSCs in regenerative medicine is underscored by their extensive applications in clinical trials aimed at treating a range of conditions. Their ability to migrate to sites of injury and exert paracrine effects enhances tissue repair and regeneration, making them ideal candidates for cell-based therapies. Recent studies have highlighted the therapeutic efficacy of MSCs in treating conditions such as ischemic heart disease, neurodegenerative disorders, and chronic inflammatory diseases [3, 4]. The versatility of MSCs not only aids in direct tissue regeneration but also provides a platform for developing advanced therapies that can address multiple pathological processes simultaneously [5].

Despite the promising potential of MSCs, the field faces significant challenges in translating these therapies from bench to bedside. One major hurdle is the heterogeneity of MSC populations, which can lead to inconsistent therapeutic outcomes. Variability in cell source, culture conditions, and donor characteristics can significantly impact the functional properties of MSCs [6]. Moreover, the standardization of manufacturing processes and the establishment of quality control measures are essential for ensuring the safety and efficacy of MSC therapies in clinical settings [7]. With the development of MSC applications, urgent action is needed to address these challenges and to unlock the full clinical potential.

This chapter systematically summarizes the current understanding of MSCs, their therapeutic mechanisms, and the technological advancements in their preparation and application. We aim to provide insights into the ongoing research efforts, clinical applications of MSCs in various diseases, the challenges faced in clinical translation, and future directions for research. By looking at the MSCs' characteristics and the barriers to their clinical application, we hope to contribute to the optimization of MSC-based therapies and to enhance patient outcomes in regenerative medicine.

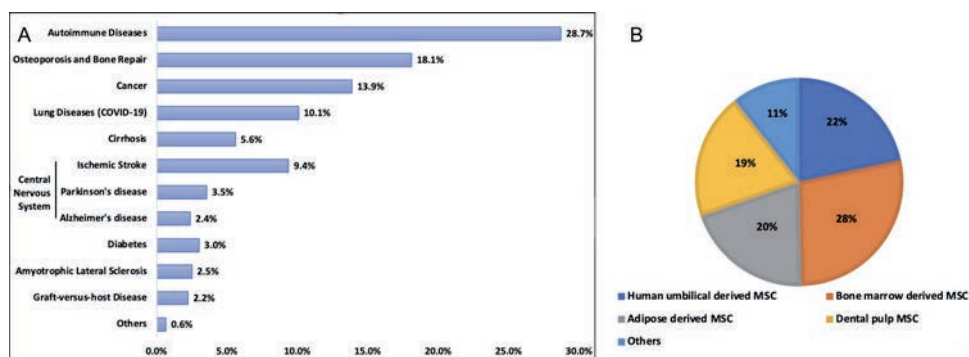


Figure 1. Overview of clinical stem cell therapy trials registered on ClinicalTrials.gov. A. Distribution of diseases targeted by stem cell therapies in clinical trials; B. Types of stem cells utilized in clinical therapeutic research.

2. Production and quality control of clinically applicable MSCs

2.1 GMP-compliant cell preparation techniques

The preparation of MSCs under Good Manufacturing Practice (GMP) standards is crucial for ensuring their safety and efficacy in clinical applications. MSCs isolated from the human placenta and umbilical cord show great promise. These tissues offer rich cellular content, allow for noninvasive acquisition, and present fewer ethical concerns. The process typically involves enzymatic digestion, where tissues are treated with collagenase or other proteolytic enzymes to liberate MSCs from their extracellular matrix. Following isolation, cells are cultured in media optimized for MSC growth, often supplemented with human serum or platelet lysate instead of fetal bovine serum to comply with GMP guidelines and minimize immunogenicity [2, 8]. Cryopreservation is another critical step in MSC preparation, allowing for the long-term storage of cells while maintaining their viability and functionality. The use of controlled-rate freezing and appropriate cryoprotectants, such as dimethyl sulfoxide (DMSO), ensures that MSCs retain their regenerative properties upon thawing. The establishment of a GMP-compliant production process also necessitates rigorous quality control measures, including assessments of cell viability, sterility, and differentiation potential, to ensure that the final product meets the required safety and efficacy standards for clinical use [9].

Although cultivation techniques for MSCs from various sources differ slightly, particularly in target cell yields and specific quality control metrics for cellular evaluation, a core set of standardized protocols ensures consistency. **Table 1** summarizes the essential workflow for culturing clinical-grade MSCs, detailing the fundamental procedures that support reliable and reproducible production across diverse MSC sources.

GMP aspect	Key protocols	Rationale	Regulatory alignment
Donor selection	• Screening for infectious diseases (HIV, Hepatitis B/C) <i>via</i> serological tests and nucleic acid amplification. • Evaluation of donor age (preference for younger donors) and health status (exclusion of chronic illnesses). • Genetic background assessment for potential impact on MSC functionality.	Ensures MSC sources are free from pathogens and possess optimal proliferative and differentiation potential.	Complies with FDA, EMA, and PMDA guidelines for donor eligibility and safety screening.
Isolation and purification	• Use of GMP-grade reagents (e.g., culture media, enzymes) and sterile equipment. • Standardized methods: density gradient centrifugation or plastic adherence (monitored for temperature, pH, and incubation time). • Validation of purity <i>via</i> flow cytometry (removal of contaminating cells).	Minimizes contamination risk and ensures consistent MSC phenotype and functionality.	Aligns with ISCT recommendations for standardized isolation protocols.

GMP aspect	Key protocols	Rationale	Regulatory alignment
Cell culture and expansion	- Culture in controlled environments (GMP-certified cleanrooms) with monitoring of oxygen tension, nutrient levels, and pH. - Limitation of passage number to prevent senescence (assessed <i>via</i> β-galactosidase staining). - Use of closed, single-use bioreactors to reduce cross-contamination.	Maintains MSC viability, potency, and genetic stability during expansion.	Adheres to EMA's emphasis on process quality and FDA's manufacturing control requirements.
Characterization	- Phenotypic analysis <i>via</i> flow cytometry ($\geq 95\%$ expression of CD73, CD90, CD105; $\leq 2\%$ expression of CD34, CD45). - Functional assays: trilineage differentiation (Alizarin Red for osteogenesis, Oil Red O for adipogenesis) and immunomodulatory tests (mixed lymphocyte reaction).	Verifies MSC identity, multipotency, and therapeutic efficacy.	Meets ISCT minimal criteria and FDA/EMA requirements for product characterization.
Safety testing	- Sterility testing: culture-based methods, membrane filtration, and rapid PCR for microbial contaminants. - Endotoxin detection <i>via</i> LAL assay (≤ 0.5 EU/mL). - Mycoplasma screening <i>via</i> PCR and culture-based assays. - Tumorigenicity assessment: Soft agar assay and nude mouse transplantation.	Prevents adverse reactions (infections, inflammation) and ensures non-tumorigenic properties.	Complies with FDA, EMA, and PMDA safety thresholds for cellular therapeutics.
Cryopreservation and storage	- Use of GMP-grade cryoprotectants (e.g., DMSO) and controlled-rate freezing. - Storage in validated cryogenic systems (-196°C in liquid nitrogen) with traceability of storage conditions. - Post-thaw viability testing ($\geq 70\%$ viable cells).	Preserves MSC functionality and ensures product stability during storage/distribution.	Aligns with ISCT guidelines for cryopreservation and the FDA's requirements for product traceability.
Documentation and traceability	- Detailed records of donor information, processing steps, test results, and batch history. - Implementation of batch release criteria based on predefined quality attributes.	Ensures accountability, reproducibility, and regulatory compliance.	Required by FDA, EMA, and PMDA for audit trails and batch consistency.

Table 1.
Summary of GMP protocols for clinical-grade MSC preparation.

2.2 Cell expansion and purification techniques

Optimizing the conditions for MSC expansion is essential to avoid heterogeneity and cellular senescence, which can compromise their therapeutic potential. Factors such as cell density, culture medium composition, and the presence of growth factors play pivotal roles in promoting optimal cell proliferation. Research has shown that the use of bioreactors for large-scale expansion can enhance the yield of MSCs while maintaining their functional characteristics [10]. Additionally, the implementation of purification techniques, such as magnetic-activated cell sorting (MACS) or fluorescence-activated cell sorting (FACS), allows for the isolation of high-purity MSC populations. These methods select cells that express specific surface markers, such as CD105, CD73, and CD90, while simultaneously lacking the expression of markers such as CD34 and CD45, which are typically expressed by hematopoietic stem cells. In this way, MSCs are enriched and purified. Quality control measures for assessing cell purity include flow cytometry for surface marker expression and functional assays to evaluate differentiation potential and immunomodulatory capabilities. Ensuring a high level of purity and activity is vital for the successful application of MSCs in regenerative medicine and therapeutic interventions [11].

2.3 Multi-omics analysis in MSC preparation

The integration of multi-omics approaches, including transcriptomics, proteomics, and metabolomics, has emerged as a powerful strategy to optimize MSC culture systems. Transcriptomic analysis enables the identification of gene expression profiles associated with MSC self-renewal and differentiation, providing insights into the molecular mechanisms underpinning cell behavior during culture [12]. Proteomic studies further elucidate the protein landscape of MSCs, revealing key signaling pathways that can be targeted to enhance cell function and therapeutic efficacy. Metabolomic profiling complements these analyses by assessing the metabolic state of MSCs, which can influence their proliferation and differentiation capabilities. For instance, the substitution of fetal bovine serum with platelet-rich plasma has been shown to improve MSC bioactivity and enhance their regenerative properties, aligning with GMP standards [13, 14]. By leveraging these multi-omics insights, researchers can refine MSC culture conditions and develop standardized protocols that ensure consistent quality and performance of MSCs for clinical applications. This comprehensive approach not only aids in the production of high-quality MSCs but also paves the way for personalized medicine strategies in regenerative therapies.

2.4 Quality control of MSCs

2.4.1 Clinical grade MSC quality requirements

Clinical-grade MSCs must meet stringent safety and efficacy requirements. For safety, sterility is critical, with regulatory bodies like the International Society for Cell and Gene Therapy (ISCT) and the European Medicines Agency (EMA) mandating rigorous testing *via* culture-based methods, membrane filtration, and rapid molecular techniques such as PCR. Endotoxin levels are strictly limited (≤ 0.5 EU/mL for injectables) and tested using the Limulus Amebocyte Lysate (LAL) assays, with

routine monitoring during production. Mycoplasma contamination, which can alter cell behavior, requires comprehensive screening *via* culture-based assays and PCR, as outlined in the European Pharmacopeia [15–17].

Regarding efficacy, viability is a key metric, with regulatory standards requiring at least 70% viability, assessed through methods such as trypan blue exclusion, flow cytometry, and metabolic assays. Proliferation capacity, evaluated *via* population doubling time and colony-forming unit (CFU) assays, is crucial for determining dosing and timing in therapies. Functional testing, including mixed lymphocyte reactions for immunomodulatory capacity and lineage-specific differentiation assays (osteogenic, adipogenic, chondrogenic), confirms therapeutic potential. These combined safety and efficacy measures ensure that MSCs are safe for clinical use and retain the functionality necessary for effective therapeutic applications [18–20].

2.4.2 Clinical grade MSC quality control in preparation

To meet the stringent quality control standards of MSC production, precise process control in MSC preparation is essential, encompassing mainly three stages: donor selection, isolation, and purification.

Donor selection is pivotal to ensure MSC safety and efficacy. Preference is given to younger donors due to their MSCs exhibiting superior proliferative capacity and differentiation potential, in contrast to older donors, whose MSCs often exhibit diminished functionality and increased senescence. Comprehensive health screenings are mandatory to exclude donors with chronic conditions that could impair cell quality to exclude those with chronic illnesses. Infectious disease screening for HIV, Hepatitis B, and Hepatitis C is required to prevent pathogen transmission *via* MSCs [21]. Additionally, genetic background is considered, as variations in genetic predisposition can influence MSC behavior and therapeutic responses, making it an essential component of donor evaluation [22].

In the isolation and purification of MSCs employ two primary techniques, density gradient centrifugation separates MSCs by density but may cause cell loss [23]. Alternatively, plastic adherence leverages MSCs' natural adherence to tissue culture surfaces, offering a simpler approach with high purity, though less effective for isolating specific subpopulations. Key parameters, such as temperature, pH, and incubation time, require strict monitoring to preserve cell viability and functionality. Consistency and reproducibility in these processes are upheld through robust quality control measures, including assessments of MSC identity, potency, and purity, to ensure the final MSC product meets clinical-grade quality standards for therapeutic applications [22, 24].

2.4.3 Criteria of clinical grade MSCs

For clinical applications, the final production of MSCs must meet stringent phenotypic and genetic criteria, validated through rigorous testing to ensure their safety, purity, and efficacy.

Phenotypic identity: $\geq 95\%$ of the cells must express CD73, CD90, CD44, and CD105, and $\leq 2\%$ may express CD45, CD34 or CD14, CD45, CD34, CD14, CD11b, CD79a, CD19, and HLA-DR, as defined by the ISCT. Flow cytometry is the primary tool for phenotypic quantification, requiring calibrated instruments, validated isotype and compensation controls, standardized sample handling, and qualified operators [25].

Multilineage potency: In lineage-specific induction media, the cells must demonstrate differentiation capacities into at least three other cell lineages, such as osteogenic, adipogenic, and chondrogenic lineages.

Genomic integrity: Metaphase karyotyping is used to exclude aneuploidy and structural rearrangements; STR profiling by polymerase chain reaction (PCR) and capillary electrophoresis confirms identity and detects drift during expansion; telomere length (qPCR or Southern blot) is measured to monitor replicative lifespan and avoid senescent batches [1, 26, 27].

Collectively, these assays, summarized with acceptance criteria in **Table 2**, ensure that clinical-grade MSCs are phenotypically uniform, genetically stable, and functionally competent.

Category	Item	Methods	Acceptance criteria
Cell dose	Viable cell count per dose	Hemocytometer counting automated cell counters (e.g., flow cytometry-based)	$\geq 5 \times 10^6$ viable cells/kg body weight
Morphology	Cellular morphology	Light microscopy observation	Typical fibroblast-like morphology; spindle-shaped, adherent to plastic surfaces
Vitality	Cell viability	Trypan blue exclusion assay, flow cytometry (propidium iodide staining), Cell Titer-Glo assay	$\geq 70\%$ viable cells
Purity	Residual contaminants	ELISA (for serum/cytokine residues), PCR (for exogenous agents)	No detectable residual serum/cytokine beyond acceptable limits
	Contaminating cells	Flow cytometry (detection of non-MSC markers)	$\leq 5\%$ of total cells are non-MSC populations
Phenotypes	Positive surface markers	Flow cytometry	$\geq 95\%$ expression of CD73, CD90, CD105, and CD44
	Negative surface markers	Flow cytometry	$\leq 2\%$ expression of CD34, CD45, CD14/CD11b, CD19, and HLA-DR
Colony-forming capacity	CFU-F (Colony-Forming Unit-Fibroblastic)	CFU-F assay (culture in semisolid media)	Formation of distinct, adherent colonies with fibroblastic morphology
Genetic stability	Chromosomal integrity	Karyotype analysis (metaphase chromosome staining)	Normal karyotype (no aneuploidy or structural chromosomal abnormalities)
	Cell identity verification	Short Tandem Repeat (STR) profiling	STR profile matches donor source; no cross-contamination with other cell lines
	Telomere length	Quantitative PCR, Southern blotting	Telomere length within the normal range for the cell source and passage number

Category	Item	Methods	Acceptance criteria
Safety	Sterility (bacteria/fungi)	Culture-based methods (incubation in growth media), membrane filtration, PCR	No growth of bacteria or fungi in culture; negative PCR results for microbial markers
	Mycoplasma contamination	Culture-based assays, PCR (detection of mycoplasma-specific DNA)	No mycoplasma detected
	Endotoxin levels	Limulus Amebocyte Lysate (LAL) assay (gel-clot or chromogenic format)	≤0.5 EU/mL (for injectable products)
Tumorigenicity	<i>In vitro</i> tumorigenic potential	Soft agar colony formation assay	No colony formation in soft agar
	<i>In vivo</i> tumorigenic potential	<i>In vivo</i> transplantation in nude mice	No tumor formation at the injection site within the observation period
Potency	Trilineage differentiation capacity	Osteogenic differentiation: Alizarin red staining (calcium nodules)	Positive staining for calcium nodules (osteogenesis)
		Adipogenic differentiation: Oil Red O staining (lipid droplets)	Positive staining for lipid droplets (adipogenesis)
		Chondrogenic differentiation: or Safranin O staining (cartilage matrix)	Positive staining for cartilage matrix (chondrogenesis)
	Immunomodulatory function	Mixed lymphocyte reaction (MLR), cytokine secretion assays (e.g., ELISA for IL-6, TGF-β)	Significant suppression of lymphocyte proliferation in MLR; detectable secretion of immunomodulatory cytokines

Table 2.
Testing methods and standards for endpoint MSCs quality control.

3. The mechanisms underlying MSC-based therapies

MSCs play a critical role in tissue regeneration through various mechanisms. Current mechanistic investigations focus mainly on three primary pathways: (1) direct cell replacement and tissue regeneration *via* MSC differentiation; (2) paracrine-mediated immunomodulation and trophic support through extracellular vesicles and soluble factors; and (3) mitochondrial transfer to rescue damaged cells.

3.1 Cell replacement and tissue regeneration

Research shows that MSCs have the ability to migrate and home to sites of injury, then differentiate into various cell types to promote tissue repair. The process begins

with the migration of MSCs to damaged tissues, often guided by chemokines and growth factors released from injured cells, which create a conducive microenvironment for stem cell homing. Once they arrive at the injury site, MSCs can differentiate into specialized cell types, such as osteoblasts, chondrocytes, and adipocytes, depending on the tissue context and the local microenvironment. This differentiation is crucial for restoring the structural integrity and function of the damaged tissue. Additionally, MSCs can engage in cell fusion with local cells, enhancing tissue repair through hybrid cell formation, which may contribute to the regenerative process. Furthermore, MSCs are involved in the remodeling of the extracellular matrix (ECM), which is essential for supporting new tissue formation and facilitating the migration and function of other cell types involved in healing. The interplay between MSCs and their microenvironment, including interactions with immune cells, is vital for effective tissue regeneration, as it helps to modulate inflammation and promote a favorable healing response [1, 28].

3.2 Secreted factors and paracrine effects

The therapeutic potential of MSCs extends beyond direct differentiation; their secretomes play a pivotal role in mediating tissue repair and modulating immune responses. MSCs secrete a variety of bioactive molecules, including growth factors, cytokines, and extracellular vehicles (EVs), which collectively contribute to their paracrine effects. These secreted factors can promote angiogenesis, reduce apoptosis, and modulate inflammation, thereby creating a favorable environment for tissue repair. For instance, factors such as hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) are known to enhance angiogenesis and tissue regeneration. Moreover, MSC-derived exosomes, which contain proteins, lipids, and RNA, have emerged as crucial mediators of intercellular communication, facilitating the transfer of bioactive molecules to target cells and enhancing their regenerative effects. The clinical application of MSC-derived exosomes is gaining traction, as they provide a cell-free therapeutic approach that overcomes some of the challenges posed by direct MSC transplantation, including low cell retention rates, potential tumorigenicity, and difficulty in penetrating the blood-brain barrier (BBB) for neurological diseases. The ability of these exosomes to modulate immune responses and promote tissue repair positions them as promising candidates for therapeutic interventions in various diseases, including those characterized by chronic inflammation and tissue degeneration [28, 29].

3.3 Mitochondrial transfer mechanisms

Recent studies have illuminated the role of mitochondrial transfer as a novel mechanism through which MSCs exert their therapeutic effects. Mitochondrial transfer involves the transfer of mitochondria from MSCs to recipient cells, which can significantly enhance the metabolic activity and survival of damaged cells. This process is particularly relevant in the context of tissue injury, where recipient cells may be compromised by oxidative stress and energy deficits. The transfer of healthy mitochondria can restore cellular function, improve ATP production, and reduce apoptosis in injured cells. The molecular mechanisms underlying mitochondrial transfer are complex and involve various signaling pathways and cellular interactions. For instance, the process may be facilitated by membrane fusion proteins and cytoskeletal elements that mediate the uptake of mitochondria by target cells. While the

therapeutic advantages of mitochondrial transfer are promising, there are also potential risks requiring further study: (1) The “two-way” nature of mitochondrial transfer between MSCs and damaged cells remains poorly understood, including when one direction dominates and how this directionality is initiated/regulated. (2) Studies that mostly focused on the transfer of mitochondria from MSCs to damaged cells, whether it is possible that there is a transfer from damaged cells to MSCs simultaneously, and how this decision is regulated. (3) The dual role of mitochondrial transfer is beneficial for damaged cells, but it is potentially harmful to MSCs (e.g., enhancing cancer cell aggressiveness), and the need to suppress it in such cases. Therefore, understanding the balance between the benefits and risks of mitochondrial transfer is crucial for optimizing MSC-based therapies in clinical settings [30, 31].

4. Applications of MSCs in neurological disorders

4.1 Ischemic stroke

MSCs have emerged as a promising therapeutic option for ischemic stroke, primarily due to their multifaceted roles in promoting neural regeneration, suppressing inflammation, and enhancing BBB repair. The regenerative potential of MSCs is largely attributed to their ability to secrete a variety of growth factors and cytokines that facilitate neuroprotection and repair following ischemic injury. For instance, studies have demonstrated that MSCs can promote angiogenesis, reduce apoptosis, and enhance neurogenesis, thereby contributing to functional recovery after stroke [30, 32, 33]. Furthermore, MSCs can modulate the inflammatory response, which is critical in the context of ischemic stroke, where excessive inflammation can exacerbate neuronal damage. Recent clinical trials have begun to evaluate the efficacy of MSC transplantation in patients with ischemic stroke, with preliminary results suggesting that MSC therapy may improve neurological outcomes and reduce disability [34, 35]. However, challenges remain regarding the optimal timing, dosage, and delivery methods for MSC therapy, as well as the need for standardized protocols to ensure safety and efficacy across diverse patient populations. Overall, the application of MSCs in ischemic stroke presents a promising method for enhancing recovery and improving patient outcomes, although further research is necessary to fully elucidate their mechanisms of action and optimize therapeutic strategies.

4.2 Neurodegenerative diseases

With the intensification of global population aging, the potential of MSCs in treating neurodegenerative diseases such as Parkinson’s disease (PD) and Alzheimer’s disease (AD) attracts increasing attention due to their unique properties, including their ability to differentiate into neuron-like cells and secrete neurotrophic factors. In the context of PD, MSCs have been shown to promote the survival of dopaminergic neurons and improve motor function in preclinical models [36, 37]. Mechanistically, MSCs may exert their therapeutic effects through paracrine signaling, where they release exosomes containing miRNAs and proteins that modulate inflammation and promote neuroprotection [38, 39]. Additionally, the combination of MSC transplantation with gene therapy strategies has emerged as a novel approach to enhance the therapeutic efficacy in neurodegenerative diseases. For example, genetically engineered MSCs that overexpress neurotrophic factors or anti-inflammatory cytokines

may provide synergistic benefits in restoring neuronal function and mitigating disease progression [40, 41]. A phase I/II clinical trial (NCT04388982) evaluating the safety and efficacy of intranasally administered allogeneic human adipose-derived MSC exosomes in patients with mild-to-moderate AD demonstrated cognitive improvement. Participants received the exosomes twice weekly for 12 weeks (total of 24 administrations). By week 12, cognitive function, measured by the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog), showed significant improvement compared to baseline levels. Notably, these improvements in ADAS-cog scores continued beyond the treatment period, decreasing further until week 36. While the trial found no significant differences in amyloid-beta or tau deposition, hippocampal volume atrophy was attenuated to some degree. The study concluded that intranasal administration of MSC-derived exosomes was safe and well-tolerated [42]. Despite the promising preclinical findings, the clinical translation of MSC therapies for neurodegenerative disorders faces several hurdles, including choice of cell sourcing, immunogenicity, and the need for rigorous clinical trials to establish the safety and efficacy therapy process. Leveraging MSC-derived exosomes to target and clear aging-related factors in mild AD appears to be a viable approach, given their capacity to cross the BBB and their senolytic therapeutic potential [43]. Nonetheless, the innovative strategies involving MSCs hold great promise for developing effective therapies for neurodegenerative diseases, potentially offering a novel therapeutic alternative for these debilitating conditions.

5. MSCs in clinical applications for lung diseases

5.1 Acute lung injury and pulmonary fibrosis

MSCs have garnered significant attention for their therapeutic potential in acute lung injury (ALI) and pulmonary fibrosis (PF), primarily due to their immunomodulatory and regenerative properties. MSCs can alleviate inflammation and inhibit the fibrotic process, which are critical in managing these conditions. For instance, in models of ALI, MSCs have been shown to reduce the levels of pro-inflammatory cytokines and promote the resolution of inflammation, thereby facilitating tissue repair [44]. Furthermore, in the context of PF, MSCs exert their effects through the secretion of various paracrine factors that can modulate fibroblast activity and extracellular matrix production, ultimately reducing collagen deposition and improving lung function [45]. A notable example of an MSC application is in the treatment of paraquat poisoning, which leads to severe lung injury. Recent studies have demonstrated that MSC therapy can significantly improve survival rates and lung histopathology in animal models of paraquat-induced lung damage, highlighting the potential for MSCs to serve as a therapeutic strategy in clinical settings [46]. It seems that MSCs alleviate lung damage by modulating NF- κ B and IL-17 signaling pathways in pulmonary cells [47]. Research studies underscore the promise of MSCs in mitigating the inflammatory response, phenotypic transformation of alveolar macrophages (AMs), and inhibiting the progression of fibrosis, making them a compelling option for treating ALI [48, 49].

5.2 COVID-19 and other infectious lung diseases

The COVID-19 pandemic has underscored the urgent need for effective therapies to manage severe respiratory complications associated with SARS-CoV-2 infection. MSCs

have emerged as a potential treatment modality due to their ability to modulate the immune response and promote tissue repair in the lungs. In COVID-19 patients, MSCs can help mitigate the cytokine storm—a hyper-inflammatory response that can lead to acute respiratory distress syndrome (ARDS) and subsequent lung damage. Clinical trials have shown that MSC therapy can improve lung function and reduce inflammatory markers in patients with severe COVID-19, suggesting that MSCs can play a critical role in controlling inflammation and facilitating recovery [50, 51]. Additionally, MSCs have been investigated for their efficacy in treating other infectious lung diseases, such as pneumonia and tuberculosis, where their immunomodulatory properties can help restore homeostasis in the lung microenvironment. The therapeutic effects of MSCs are attributed to their ability to secrete a variety of growth factors and cytokines that promote alveolar epithelial cell regeneration and inhibit apoptosis, thereby enhancing lung repair processes [52]. As ongoing clinical trials continue to evaluate the safety and efficacy of MSCs for treating COVID-19 and other infectious lung diseases, the results thus far indicate a promising avenue for therapeutic intervention, potentially transforming the management of these severe respiratory conditions.

6. MSCs in cancer treatment potential

6.1 Clinical trials involving MSCs in cancer treatment

Clinical trials have explored MSCs for cancer treatment, leveraging their tumor-homing ability, immune-modulatory capacity, and interaction with the tumor microenvironment. MSCs act as multifunctional agents: They deliver chemotherapeutics, oncolytic viruses, and gene therapies, while their interplay with the tumor microenvironment is dual-sided, secreting factors such as vascular epithelial growth factor (VEGF) and interleukin-6 (IL-6) to promote tumor growth and angiogenesis, yet also inhibiting tumors *via* immune modulation and apoptosis induction [53]. They influence T cell responses by suppressing cytotoxic T cells and fostering regulatory T cells, creating an immunosuppressive tumor microenvironment [54], and their exosomes carry miRNAs or proteins that further modulate tumor behavior, highlighting their complex role in therapy.

As drug delivery carriers, MSCs offer advantages: Their tumor tropism, mediated by the SDF-1/CXCR4 axis, enables targeted accumulation in the tumor microenvironment, reducing systemic side effects [55]. Loading strategies include nanoparticles and exosomes (biocompatible, barrier-crossing) to encapsulate drugs, viruses, or siRNA. Engineering MSCs to boost loading capacity and optimize release enhances their potential, making them a promising tool to improve treatment specificity and efficacy in cancer therapy.

In cancer clinical trials, MSCs have been applied exclusively as vectors for gene therapy, owing to their unique properties that enable enhanced targeted therapeutic delivery efficiency and their double-edged sword role within the tumor microenvironment. One of the primary advantages of using MSCs as gene therapy vectors is their inherent ability to home to tumor sites. This tumor tropism allows MSCs to deliver therapeutic genes directly to cancer cells while minimizing exposure to healthy tissues, thereby reducing side effects associated with conventional therapies. Recent studies have demonstrated that MSCs can be genetically modified to carry anticancer genes, such as those encoding tumor suppressor proteins or pro-apoptotic factors, which can induce apoptosis specifically in tumor cells. This targeted approach not only enhances the therapeutic efficacy but also mitigates the systemic toxicity

often seen with traditional chemotherapy [56, 57]. Furthermore, MSCs possess immunomodulatory properties that can reshape the tumor microenvironment. By secreting various cytokines and growth factors, MSCs can modulate the immune response, potentially converting an immunosuppressive environment into one that is more conducive to antitumor immunity [58]. This dual action of delivering therapeutic genes while simultaneously modulating the immune landscape presents a multifaceted strategy for combating cancer, making MSCs a vital component in the evolving landscape of cancer gene therapy [6, 59].

Currently, completed key clinical trials for cancer treatment are limited. The trial of MSC therapy for pancreatic cancer (NCT01494232) assessed the safety of gene-modified MSCs engineered to deliver therapeutic genes. The infusion was well-tolerated with no significant adverse events, establishing a crucial safety profile for using MSCs as gene therapy vehicles in this aggressive cancer and supporting future efficacy studies [60]. Another study (NCT02068794) used MSCs to deliver interferon- α to the ovarian cancer microenvironment. MSCs effectively delivered the payload, enhancing immune responses (improved immune markers) and demonstrating safety/tolerability. While tumor reduction was not universally achieved, the results support exploring MSCs to enhance immunotherapies [61].

Ongoing innovative trials include MSCs application in colorectal cancer (NCT03298763) and liver cancer (NCT02530047). In a colorectal cancer clinical trial, CRISPR technology is applied to genetically modify MSCs, aiming to enhance their anticancer properties for targeted tumor delivery. It will evaluate the safety and efficacy (tumor response) of these engineered cells, representing a significant advancement in personalized MSC therapies [62]. In liver cancer clinical trials, MSCs combined with PD-1 inhibitors aim to boost antitumor immune responses while potentially mitigating immune checkpoint inhibitor side effects *via* MSC immunomodulation. Preliminary data suggest the combination may improve outcomes by reactivating the immune system [61].

6.2 Combination strategies of MSCs and nanotechnology

The integration of nanotechnology with MSCs represents a novel and promising strategy for enhancing cancer treatment efficacy. Recent advancements have highlighted the potential of solid lipid nanoparticles (SLNs) combined with MSCs to improve drug delivery systems. SLNs can encapsulate chemotherapeutic agents and facilitate their targeted release at tumor sites when combined with MSCs, which naturally migrate toward these areas due to their homing capabilities. This synergistic approach not only improves the bioavailability of the drugs but also enhances the therapeutic index by reducing systemic exposure and associated side effects [63, 64]. Studies have shown that MSCs can be engineered to express specific receptors that can interact with SLNs, allowing for a more efficient uptake of therapeutic agents. Moreover, the safety and efficacy of these combined therapies have been rigorously evaluated, with findings indicating that the use of MSCs in conjunction with nanoparticles does not significantly increase the risk of tumorigenicity, a common concern with stem cell therapies [38, 65]. The dual evaluation of safety and therapeutic effectiveness is crucial, as it ensures that the innovative strategies not only provide a robust treatment option but also maintain patient safety, paving the way for clinical applications in oncology. This combination of MSCs and nanotechnology thus holds great promise for the future of targeted cancer therapies, offering a pathway toward more effective and less toxic treatment modalities.

7. Applications of MSCs in osteoporosis and bone repair

7.1 Mechanisms by which MSCs promote bone formation

MSCs have garnered significant attention for their potential to promote bone formation, particularly in conditions like osteoporosis. The mechanisms of MSCs influencing bone metabolism are multifaceted, primarily involving the modulation of signaling pathways that regulate osteoblast and osteoclast activity. Key signaling pathways, such as the Wnt/ β -catenin pathway, play a crucial role in osteogenic differentiation of MSCs. Activation of this pathway leads to the upregulation of osteogenic markers, thereby enhancing bone formation. Additionally, MSCs secrete various paracrine factors, including growth factors and cytokines, that further modulate the bone microenvironment. For instance, MSC-derived EVs have been shown to carry microRNAs and proteins that can inhibit osteoclastogenesis, thereby reducing bone resorption. Furthermore, the application of static magnetic fields (SMFs) has been shown to enhance the osteogenic differentiation of MSCs, providing a noninvasive method to promote bone formation. Studies indicate that SMFs can influence cellular signaling pathways, leading to increased expression of osteogenic markers and enhanced mineralization in MSCs. This dual approach of utilizing intrinsic MSC properties and external stimuli like SMFs presents a promising avenue for enhancing bone repair and addressing conditions such as osteoporosis [66, 67].

7.2 Current clinical applications and challenges

The clinical application of MSCs in treating osteoporosis and facilitating bone repair is rapidly evolving, with numerous studies exploring their efficacy in fracture healing and osteoporosis management. Recent clinical trials have demonstrated that MSC transplantation can significantly enhance fracture healing, reduce healing time, and improve overall bone density in osteoporotic patients. However, challenges remain that hinder the widespread adoption of MSC-based therapies. One major concern is the stability and safety of MSC treatments, particularly regarding the potential for tumorigenesis and immune rejection. The variability in MSC sources, preparation methods, and patient-specific factors can lead to inconsistent outcomes, complicating the standardization of MSC therapies. Moreover, the long-term effects of MSC transplantation are still not fully understood, necessitating rigorous clinical trials to assess their safety and efficacy over extended periods. The development of MSC-derived EVs as a cell-free alternative has emerged as a potential solution to some of these challenges, offering a therapeutic approach that minimizes the risks associated with live cell therapies. EVs have shown potential in promoting osteogenic differentiation and reducing inflammation, making them an attractive candidate for clinical applications in osteoporosis and bone repair [68, 69]. Future research should focus on optimizing MSC isolation and expansion protocols, enhancing targeting mechanisms for MSC delivery, and conducting large-scale clinical trials to validate the therapeutic potential of MSCs in bone-related disorders.

8. MSCs in reproductive medicine and other systemic diseases

8.1 Treatment of reproductive aging and infertility

MSCs have emerged as a promising therapeutic option in the field of reproductive medicine, particularly in addressing reproductive aging and infertility. The

regenerative potential of MSCs stems from their ability to home to damaged tissues, secrete paracrine factors, and modulate immune responses, thereby promoting ovarian function recovery. Recent studies have demonstrated that MSCs can enhance ovarian reserve and restore fertility in animal models of premature ovarian failure (POF) and other reproductive disorders. For instance, human umbilical cord-derived MSCs (hUC-MSCs) have shown significant efficacy in restoring ovarian function by reducing apoptosis in granulosa cells and enhancing follicular development, thereby improving hormone levels such as estradiol and progesterone [70, 71]. Clinical trials are beginning to reflect these findings, with preliminary data suggesting that MSC therapy can effectively improve reproductive outcomes in women suffering from various infertility issues, including those related to age and autoimmune conditions. The mechanisms underlying these benefits include the secretion of growth factors and cytokines that facilitate tissue repair and regeneration, as well as the immunomodulatory properties of MSCs that help create a favorable environment for follicle development [72, 73]. However, while the preclinical results are promising, further research is needed to fully elucidate the mechanisms at play and to establish the standardized protocols for MSC therapy in clinical settings.

8.2 Metabolic syndrome

The application of MSCs extends beyond reproductive medicine into the management of metabolic syndrome, where they exhibit significant therapeutic potential. Metabolic syndrome (MetS) represents a cluster of complex metabolic disorders characterized by insulin resistance, obesity, chronic low-grade inflammation, hypertension, and dyslipidemia, which significantly elevate the risk of cardiovascular diseases and type 2 diabetes [74]. MSCs are known to regulate metabolic disorders through their immunomodulatory effects and ability to differentiate into various cell types, which can help ameliorate insulin resistance, restore homeostasis in dysfunctional tissues [75]. In the context of metabolic syndrome, studies have shown that MSCs can improve insulin sensitivity and reduce inflammation by modulating cytokine profiles and enhancing the regenerative capacity of pancreatic cells [75, 76]. Currently, 35 clinical trials on MSC therapy for diabetes are registered, but no results have been published in academic literature.

8.3 Autoimmune diseases

Furthermore, in autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis, MSCs have been recognized for their ability to suppress over-immune responses and promote tissue repair [77, 78]. The mechanisms by which MSCs exert these effects include the secretion of extracellular vesicles rich in bioactive molecules that can influence immune cell behavior and promote tissue regeneration [79]. Comparative studies of autologous versus allogeneic MSC therapies have highlighted the safety and efficacy of both approaches, although challenges such as immune rejection and variability in patient responses remain [80].

The clinical application of MSCs in treating rheumatoid arthritis (RA) has garnered significant attention, particularly in Phase I and II clinical trials, such as NCT01547091. These trials have explored various administration routes, including intravenous (IV) and intra-articular (IA) injections, to evaluate their efficacy and safety. The findings indicate that both administration routes can be effective, but they yield varying results in terms of dosage effects and safety profiles. For instance,

studies have shown that IA injections may provide localized relief with fewer systemic side effects, while IV administration may offer broader immunomodulatory effects. The dose-response relationship remains a critical area of investigation, with some trials suggesting that higher doses of MSCs correlate with improved clinical outcomes, such as the American College of Rheumatology (ACR) 50 and ACR 70 response rates, which measure significant improvement in RA symptoms. Additionally, imaging studies have demonstrated joint structural improvements, including reduced synovial inflammation and erosion, further supporting the therapeutic potential of MSCs in RA management. However, safety data across these trials indicate that while serious adverse events are rare, they can occur, necessitating careful monitoring of patients receiving MSC therapy. Overall, the results from completed clinical trials underscore the promising role of MSCs in RA treatment but also highlight the need for standardized protocols and further research to optimize therapeutic strategies [81–83].

Overall, the use of MSCs in treating autoimmune diseases is a rapidly evolving field, with ongoing clinical trials aimed at optimizing their therapeutic applications and addressing safety concerns. As research progresses, MSCs may offer novel strategies for managing these complex and prevalent health issues.

9. MSCs treatment challenges and future directions

9.1 Heterogeneity and functional instability

The therapeutic application of MSCs is significantly complicated by their inherent heterogeneity and functional instability, which can be influenced by various factors, including the source of the cells, culture conditions, and individual patient differences. MSCs can be derived from multiple tissues, such as bone marrow, adipose tissue, and umbilical cord blood, each presenting distinct phenotypic and functional characteristics that can affect their therapeutic efficacy. For instance, the immunomodulatory properties of MSCs can vary based on their tissue of origin, which may influence their effectiveness in treating specific conditions. Additionally, the culture conditions, including the composition of the growth medium and the duration of expansion, can lead to alterations in MSC functionality, such as changes in their differentiation potential and secretory profiles [84]. Individual patient factors, such as age, health status, and underlying diseases, further contribute to variability in MSC responses, complicating treatment outcomes. Therefore, standardization of MSC production and rigorous quality control measures are essential to ensure consistent therapeutic effects. The establishment of GMP for MSCs is critical to address these challenges, as it would provide a framework for the production of high-quality cell products suitable for clinical applications [85]. This necessitates the development of robust protocols that encompass not only the isolation and expansion of MSCs but also their characterization and functional assessment prior to clinical use.

9.2 Safety concerns of MSCs

The potential for MSCs to induce tumor formation represents a major concern in their clinical application. While MSCs are generally considered to be non-tumorigenic, the possibility of tumorigenesis cannot be entirely eliminated. The presence of residual undifferentiated or partially differentiated cells within MSC preparations can bring risk of tumorigenesis, raising concerns about the long-term safety of MSC

therapies. Consequently, thorough long-term safety assessments are important to evaluate the risk of adverse events associated with MSC transplantation. To mitigate these risks, advancements in cell engineering technologies are being explored, such as genetic modification to enhance the immunomodulatory properties of MSCs or to eliminate tumorigenic potential [86]. Additionally, the use of MSC-derived EVs has emerged as a promising strategy to circumvent some of the immunogenicity issues associated with whole cell therapies. EVs retain many of the beneficial properties of MSCs while minimizing the risks associated with cell-based therapies, thus presenting a safer alternative for clinical applications [87].

9.3 Integration of cell engineering and emerging technologies

The integration of cell engineering techniques with emerging technologies holds great promise for enhancing the efficacy of MSC therapies. Innovations such as gene editing, particularly CRISPR/Cas9 technology, enable precise modifications of MSCs to improve their therapeutic potential, including enhancing their homing abilities to target tissues and modulating their secretory profiles [88, 89]. Furthermore, the application of nanotechnology in MSC therapies allows for the development of novel drug delivery systems that can enhance the bioavailability and targeting of therapeutic agents delivered by MSCs. For instance, the incorporation of MSCs with nanoparticles can facilitate the controlled release of therapeutic molecules, thereby improving treatment outcomes in various conditions, including cancer and neurodegenerative diseases [85]. Additionally, the exploration of biomaterials in conjunction with MSCs can create supportive microenvironments that promote cell survival and function, further enhancing the therapeutic efficacy of MSC-based treatments. The use of MSC-derived EVs as cell-free therapies represents another exciting frontier, as these vesicles can deliver bioactive molecules to target cells, thereby mimicking the paracrine effects of MSCs without the associated risks of cell therapy [40]. Collectively, these advancements underscore the potential of combining MSCs with cutting-edge technologies to address existing challenges and expand their clinical applications.

9.4 Clinical trial design and regulatory oversight

The design of clinical trials for MSC therapies must evolve to address the unique challenges associated with these complex biological products. There is a pressing need for multicenter, large-scale clinical trials that can provide robust data on the safety and efficacy of MSC treatments across diverse patient populations and conditions [90]. Such trials should incorporate standardized protocols for patient selection, treatment administration, and outcome assessment to ensure comparability and reproducibility of results. Furthermore, regulatory oversight plays a crucial role in the development and application of MSC therapies. The current landscape reveals that several guidelines have been proposed by international regulatory bodies, such as the Food and Drug Administration (FDA) in U.S.A., the European Medicines Agency (EMA), and the Chinese Pharmaceuticals and Medical Devices Agency (PMDA), the International Society for Cell and Gene Therapy (ISCT) and the World Health Organization (WHO) provide also essential guidelines that aim to standardize practices in the field of cell therapy, but there remains a significant gap in the implementation of these standards across different regions and clinical practices. The standards and regulations must adapt to the complexities of cell-based therapies, ensuring that safety and efficacy are thoroughly evaluated while also facilitating

timely access to innovative treatments for patients. The establishment of international standards for MSC production and application is essential to harmonize regulatory requirements across different regions, thereby promoting global collaboration in MSC research and clinical application [84]. As the field of regenerative medicine continues to advance, ongoing dialog between researchers, clinicians, regulatory bodies, and patient advocacy groups will be vital to navigate the challenges and opportunities presented by MSC therapies, ultimately leading to safer and more effective treatment options for patients.

10. Conclusions

In recent years, MSCs have emerged as a focal point of regenerative medicine, demonstrating remarkable potential in treating a variety of diseases due to their multifunctional capabilities and safety profile. As highlighted throughout this review, the clinical applications of MSCs have extended across diverse fields, including neurological disorders, pulmonary injuries, oncology, osteoporosis, and reproductive system diseases. This breadth of application underscores the versatility of MSCs, making them an invaluable resource in the therapy of multiple diseases.

The advancement of MSC-based therapies has been significantly influenced by the standardization of preparation processes, optimization of multi-omics technologies, and innovations in cell engineering. These factors are critical in enhancing the therapeutic efficacy of MSCs, allowing for more consistent and predictable outcomes in clinical applications. However, despite these advancements, the field is still immature with its challenges, such as heterogeneity among MSC populations, immune rejection responses, and safety concerns remain pressing issues that must be addressed through rigorous foundational and clinical research.

The majority of experts concur that integration of MSCs into clinical practice needs a balanced evaluation of diverse research findings and perspectives related to them. For instance, while some studies highlight the promising therapeutic effects of MSCs in certain conditions, others raise concerns regarding their safety and long-term efficacy. This dichotomy necessitates a careful evaluation of the existing literature, encouraging a dialog between researchers, clinicians, and regulatory bodies to reconcile these differing viewpoints.

Furthermore, the future of MSC therapy will evolve from advanced biotechnologies and stringent clinical trials into routine medical treatments. Novel techniques such as gene editing and bioreactor systems are revolutionizing the way MSCs are harvested, expanded, and differentiated, potentially overcoming some of the inherent challenges related to cell heterogeneity and immune response. Additionally, MSC therapies might be tailored to individual patient profiles according to the personalized medicine requirements, which could significantly enhance treatment outcomes and minimize adverse side effects.

To realize the full potential of MSC-based therapies in achieving the goals of precision regenerative medicine, it is imperative that researchers continue to explore the underlying mechanisms that govern MSC behavior and interaction within the host environment. This includes investigating the role of the immune system in modulating MSC function and identifying biomarkers that can predict patient responses to MSC therapies.

The collaboration between basic research and clinical application will be crucial moving forward. As we aim for a more translational approach, promoting

partnerships between academia, industry, and clinical practitioners will facilitate the sharing of knowledge and resources necessary for advancing MSC therapies into mainstream medicine.

In summary, the field of MSC research is at a pivotal moment, with both immense potential and substantial hurdles. By addressing the challenges of heterogeneity, immune rejection, and safety through innovative research and collaborative efforts, we can pave the way for the broader clinical application of MSC therapies. Ultimately, the integration of advanced biotechnological approaches and a commitment to rigorous clinical evaluation will be essential in achieving the ambitious goals of precision regenerative medicine and improving patient outcomes in all kinds of diseases.

Acknowledgements

The author would like to thank Prof. Yue Li-Min (West China Medical Center, Sichuan University in China) and Prof. Natalia De Isla (Biopôle of Nancy Medical College, Lorraine University in France) for their polishing of the language. This research was funded by the Central University Basic Scientific Research Project and the Medical-Engineering Integration Cultivation Special Project, grant number 2682025ZTPY056.

Conflict of interest

The authors declare no conflict of interest.

Author details

Lei Zhang
College of Medicine, Southwest Jiaotong University, Chengdu, China

*Address all correspondence to: zhanglei01@swjtu.edu.cn

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Jovic D, Yu Y, Wang D, Wang K, Li H, Xu F, et al. A brief overview of global trends in MSC-based cell therapy. *Stem Cell Reviews and Reports*. 2022;**18**(5):1525-1545
- [2] Costa-Ferro ZSM, Rocha GV, da Silva KN, Paredes BD, Loiola EC, Silva JD, et al. GMP-compliant extracellular vesicles derived from umbilical cord mesenchymal stromal cells: Manufacturing and pre-clinical evaluation in ARDS treatment. *Cytotherapy*. 2024;**26**(9):1013-1025
- [3] Nam YH, Kim JS, Yum Y, Yoon J, Song H, Kim HJ, et al. Application of mesenchymal stem cell-derived Schwann cell-like cells spared neuromuscular junctions and enhanced functional recovery after peripheral nerve injury. *Cells*. 2024;**13**(24):2137
- [4] Qu H, He S, He J, Wang C, Wang K, Deng C, et al. Potential future therapeutic application of mesenchymal stem cell-derived exosomes in ulcerative colitis. *Current Stem Cell Research & Therapy*. 2025;**20**(9):966-977
- [5] Akanyibah FA, He C, Cai P, Wang X, Wang Y, Mao F. Mechanism of cell death and its application in the repair of inflammatory bowel disease by mesenchymal stem cells. *Frontiers in Immunology*. 2025;**16**:1597462
- [6] Pharoun J, Berro J, Sobh J, Abou-Younes MM, Nasr L, Majed A, et al. Mesenchymal stem cells biological and biotechnological advances: Implications for clinical applications. *European Journal of Pharmacology*. 2024;**977**:176719
- [7] Chua JKE, Lim J, Foong LH, Mok CY, Tan HY, Tung XY, et al. Mesenchymal stem cell-derived extracellular vesicles: Progress and remaining hurdles in developing regulatory compliant quality control assays. *Advances in Experimental Medicine and Biology*. 2022;**1401**:191-211
- [8] Lechanteur C, Briquet A, Bettonville V, Baudoux E, Beguin Y. MSC manufacturing for academic clinical trials: From a clinical-grade to a full GMP-compliant process. *Cells*. 2021;**10**(6):1320
- [9] Chen T, Zhang S, Jin H, Fu X, Shang L, Lu Y, et al. Nonfreezing low temperature maintains the viability of menstrual blood-derived endometrial stem cells under oxygen-glucose deprivation through the sustained release of autophagy-produced energy. *Cell Transplantation*. 2022;**31**:9636897221086971
- [10] Jankovic MG, Stojkovic M, Bojic S, Jovicic N, Kovacevic MM, Ivošević Z, et al. Scaling up human mesenchymal stem cell manufacturing using bioreactors for clinical uses. *Current Research in Translational Medicine*. 2023;**71**(2):103393
- [11] Wu S, Wang Z, Wang Y, Guo M, Zhou M, Wang L, et al. Peptide-grafted microspheres for mesenchymal stem cell sorting and expansion by selective adhesion. *Frontiers in Bioengineering and Biotechnology*. 2022;**10**:873125
- [12] Yang Y, Zhang W, Wang X, Yang J, Cui Y, Song H, et al. A passage-dependent network for estimating the in vitro senescence of mesenchymal stromal/stem cells using microarray, bulk and single cell RNA sequencing. *Frontiers in Cell and Development Biology*. 2023;**11**:998666

- [13] Ma CY, Zhai Y, Li CT, Liu J, Xu X, Chen H, et al. Translating mesenchymal stem cell and their exosome research into GMP compliant advanced therapy products: Promises, problems and prospects. *Medicinal Research Reviews*. 2024;**44**(3):919-938
- [14] Ma YN, Hu X, Karako K, Song P, Tang W, Xia Y. Exploring the multiple therapeutic mechanisms and challenges of mesenchymal stem cell-derived exosomes in Alzheimer's disease. *Bioscience Trends*. 2024;**18**(5):413-430
- [15] Grau-Vorster M, Laitinen A, Nystedt J, Vives J. HLA-DR expression in clinical-grade bone marrow-derived multipotent mesenchymal stromal cells: A two-site study. *Stem Cell Research & Therapy*. 2019;**10**:164
- [16] Muñoz-Domínguez N, Carreras-Sánchez I, López-Fernández A, Vives J. Optimisation of processing methods to improve success in the derivation of human multipotent mesenchymal stromal cells from cryopreserved umbilical cord tissue fragments. *Cryobiology*. 2022;**108**:34-41
- [17] Oja S, Kaartinen T, Ahti M, Korhonen M, Laitinen A, Nystedt J. The utilization of freezing steps in mesenchymal stromal cell (MSC) manufacturing: Potential impact on quality and cell functionality attributes. *Frontiers in Immunology*. 2019;**10**:1627
- [18] Grau-Vorster M, Rodríguez L, del Mazo-Barbara A, Mirabel C, Blanco M, Codinach M, et al. Compliance with good manufacturing practice in the assessment of immunomodulation potential of clinical grade multipotent mesenchymal stromal cells derived from Wharton's jelly. *Cells*. 2019;**8**(5):484
- [19] López-Fernández A, García-Gragera V, Lecina M, Vives J. Identification of critical process parameters for expansion of clinical grade human Wharton's jelly-derived mesenchymal stromal cells in stirred-tank bioreactors. *Biotechnology Journal*. 2024;**19**(2):e2300381
- [20] López-Fernández A, Codinach M, Coca MI, Prat-Vidal C, Castaño J, Torrents S, et al. Comparability exercise of critical quality attributes of clinical-grade human mesenchymal stromal cells from the Wharton's jelly: Single-use stirred tank bioreactors versus planar culture systems. *Cytotherapy*. 2024;**26**(5):418-426
- [21] Zhang F, Zhang L, Yu H. Potential druggability of mesenchymal stem/stromal cell-derived exosomes. *Current Stem Cell Research & Therapy*. 2024;**19**(9):1195-1209
- [22] Nguyen VVT, Welsh JA, Tertel T, Choo A, van de Wakker SI, Defourny KAY, et al. Inter-laboratory multiplex bead-based surface protein profiling of MSC-derived EV preparations identifies MSC-EV surface marker signatures. *Journal of Extracellular Vesicles*. 2024;**13**(6):e12463
- [23] Yu H, Zhang F, He YC, Zhang LS. Quality by design strategy of human mesenchymal stem/stromal cell drug products for the treatment of knee osteoarthritis. *World Journal of Stem Cells*. 2025;**17**(5):106547
- [24] Gimona M, Brizzi MF, Choo ABH, Dominici M, Davidson SM, Grillari J, et al. Critical considerations for the development of potency tests for therapeutic applications of mesenchymal stromal cell-derived small extracellular vesicles. *Cytotherapy*. 2021;**23**(5):373-380
- [25] Brinkhof B, Zhang B, Cui Z, Ye H, Wang H. ALCAM (CD166) as a gene expression marker for

- human mesenchymal stromal cell characterisation. *Gene*. 2020;**763S**:100031
- [26] Nadeau S, Cheng A, Colmegna I, Rodier F. Quantifying senescence-associated phenotypes in primary multipotent mesenchymal stromal cell cultures. *Methods in Molecular Biology*. 2019;**2045**:93-105
- [27] Jaibaji M, Jaibaji R, Volpin A. Mesenchymal stem cells in the treatment of cartilage defects of the knee: A systematic review of the clinical outcomes. *The American Journal of Sports Medicine*. 2021;**49**(13):3716-3727
- [28] Xu Y, Zhou X, Wang X, Jin Y, Zhou L, Ye J. Progress of mesenchymal stem cells (MSCs) & MSC-exosomes combined with drugs intervention in liver fibrosis. *Biomedicine & Pharmacotherapy*. 2024;**176**:116848
- [29] Tetè G, D’Orto B, Nagni M, Agostinacchio M, Polizzi E, Agliardi E. Role of induced pluripotent stem cells (IPSCs) in bone tissue regeneration in dentistry: A narrative review. *Journal of Biological Regulators and Homeostatic Agents*. 2020;**34**(6 Suppl. 3):1-10
- [30] Han D, Zheng X, Wang X, Jin T, Cui L, Chen Z. Mesenchymal stem/stromal cell-mediated mitochondrial transfer and the therapeutic potential in treatment of neurological diseases. *Stem Cells International*. 2020;**2020**:8838046
- [31] Liu Q, Zhang X, Zhu T, Xu Z, Dong Y, Chen B. Mitochondrial transfer from mesenchymal stem cells: Mechanisms and functions. *Mitochondrion*. 2024;**79**:101950
- [32] Zhou L, Wang J, Huang J, Song X, Wu Y, Chen X, et al. The role of mesenchymal stem cell transplantation for ischemic stroke and recent research developments. *Frontiers in Neurology*. 2022;**13**:1000777
- [33] Ogata K, Moriyama M, Matsumura-Kawashima M, Kawado T, Yano A, Nakamura S. The therapeutic potential of secreted factors from dental pulp stem cells for various diseases. *Biomedicine*. 2022;**10**(5):1049
- [34] Zhou L, Zhu H, Bai X, Huang J, Chen Y, Wen J, et al. Potential mechanisms and therapeutic targets of mesenchymal stem cell transplantation for ischemic stroke. *Stem Cell Research & Therapy*. 2022;**13**:195
- [35] Zhu P, Tan H, Gao H, Wang J, Liu Y, Yang D, et al. Potential mechanism and perspectives of mesenchymal stem cell therapy for ischemic stroke: A review. *Global Medical Genetics*. 2024;**11**(4):278-284
- [36] Zhang X, Kuang Q, Xu J, Lin Q, Chi H, Yu D. MSC-based cell therapy in neurological diseases: A concise review of the literature in pre-clinical and clinical research. *Biomolecules*. 2024;**14**(5):538
- [37] Guo X, Tang L, Tang X. Current developments in cell replacement therapy for Parkinson’s disease. *Neuroscience*. 2021;**463**:370-382
- [38] Zhang X, Che X, Zhang S, Wang R, Li M, Jin Y, et al. Mesenchymal stem cell-derived extracellular vesicles for human diseases. *Extracellular Vesicles and Circulating Nucleic Acids*. 2024;**5**(1):64-82
- [39] Hedayat M, Ahmadi M, Shoaran M, Rezaie J. Therapeutic application of mesenchymal stem cells derived exosomes in neurodegenerative diseases: A focus on non-coding RNAs cargo, drug delivery potential, perspective. *Life Sciences*. 2023;**320**:121566

- [40] Zhang W, Uyemura R, Zhong K, Guo R, Zhong L. Current advances and future perspectives on mesenchymal stem cell-derived extracellular vesicles in Alzheimer's disease. *Aging and Disease*. 2024;**15**(5):2015-2027
- [41] Hu X, Ma YN, Peng J, Wang Z, Liang Y, Xia Y. Exosomes derived from olfactory mucosa mesenchymal stem cells attenuate cognitive impairment in a mouse model of Alzheimer's disease. *Bioscience Trends*. 2025;**19**(2):189-201
- [42] Xie X, Song Q, Dai C, Cui S, Tang R, Li S, et al. Clinical safety and efficacy of allogenic human adipose mesenchymal stromal cells-derived exosomes in patients with mild to moderate Alzheimer's disease: A phase I/II clinical trial. *General Psychiatry*. 2023;**36**(5):e101143
- [43] Gonzales MM, Garbarino VR, Kautz TF, Palavicini JP, Lopez-Cruzan M, Dehkordi SK, et al. Senolytic therapy in mild Alzheimer's disease: A phase 1 feasibility trial. *Nature Medicine*. 2023;**29**(10):2481-2488
- [44] Lai S, Guo Z. Stem cell therapies for chronic obstructive pulmonary disease: Mesenchymal stem cells as a promising treatment option. *Stem Cell Research & Therapy*. 2024;**15**:312
- [45] 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
- [46] Gong L, Wang X, Xu S, Liao F, Zhou M. Human amnion-derived MSCs alleviate acute lung injury and hinder pulmonary fibrosis caused by Paraquat in rats. *Oxidative Medicine and Cellular Longevity*. 2022;**2022**:3932070
- [47] Zhang L, Wang Y, Shen H, Zhao M. Combined signaling of NF-kappaB and IL-17 contributes to mesenchymal stem cells-mediated protection for Paraquat-induced acute lung injury. *BMC Pulmonary Medicine*. 2020;**20**:195
- [48] Zhu J, Zhou J, Feng B, Pan Q, Yang J, Lang G, et al. MSCs alleviate LPS-induced acute lung injury by inhibiting the proinflammatory function of macrophages in mouse lung organoid-macrophage model. *Cellular and Molecular Life Sciences*. 2024;**81**(1):124
- [49] Zhao R, Wang L, Wang T, Xian P, Wang H, Long Q. Inhalation of MSC-EVs is a noninvasive strategy for ameliorating acute lung injury. *Journal of Controlled Release*. 2022;**345**:214-230
- [50] Kançağı DD, Ovalı E. The therapeutic significance of mesenchymal stem cells in COVID-19 acute pulmonary respiratory disease. *Turkish Thoracic Journal*. 2022;**23**(5):355-363
- [51] Chen L, Qu J, Kalyani FS, Zhang Q, Fan L, Fang Y, et al. Mesenchymal stem cell-based treatments for COVID-19: Status and future perspectives for clinical applications. *Cellular and Molecular Life Sciences*. 2022;**79**(3):142
- [52] Fang Q, Wu W, Xiao Z, Zeng D, Liang R, Wang J, et al. Gingival-derived mesenchymal stem cells alleviate allergic asthma inflammation via HGF in animal models. *iScience*. 2024;**27**(5):109818
- [53] Zhao Y, Zhong X, Du F, Wu X, Li M, Wen Q, et al. The role of mesenchymal stem cells in cancer immunotherapy. *Current Stem Cell Research & Therapy*. 2023;**18**(8):1056-1068
- [54] Montazersaheb S, Fathi E, Mamandi A, Farahzadi R, Heidari HR. Mesenchymal stem cells and cancer stem cells: An overview of

tumor- mesenchymal stem cell interaction for therapeutic interventions. *Current Drug Targets*. 2022;**23**(1):60-71

[55] Ding W, Zhang K, Li Q, Xu L, Ma Y, Han F, et al. Advances in understanding the roles of mesenchymal stem cells in lung cancer. *Cellular Reprogramming*. 2023;**25**(1):20-31

[56] Xie M, Tao L, Zhang Z, Wang W. Mesenchymal stem cells mediated drug delivery in tumor-targeted therapy. *Current Drug Delivery*. 2021;**18**(7):876-891

[57] He W, Li Q, Lu Y, Ju D, Gu Y, Zhao K, et al. Cancer treatment evolution from traditional methods to stem cells and gene therapy. *Current Gene Therapy*. 2022;**22**(5):368-385

[58] Zhang L, Su XS, Ye JS, Wang YY, Guan Z, Yin YF. Bone marrow mesenchymal stem cells suppress metastatic tumor development in mouse by modulating immune system. *Stem Cell Research & Therapy*. 2015;**6**(1):45

[59] Wang L, Liu G, Zheng L, Long H, Liu Y. A new era of gene and cell therapy for cancer: A narrative review. *Annals of Translational Medicine*. 2023;**11**(9):321

[60] Ma Z, Hua J, Liu J, Zhang B, Wang W, Yu X, et al. Mesenchymal stromal cell-based targeted therapy pancreatic cancer: Progress and challenges. *International Journal of Molecular Sciences*. 2023;**24**(4):3559

[61] Tang J, Chen Y, Wang C, Xia Y, Yu T, Tang M, et al. The role of mesenchymal stem cells in cancer and prospects for their use in cancer therapeutics. *MedComm*. 2020;**5**(8):e663

[62] Yao H, Sun C, Wang C, Liu J, Li Y, Li L, et al. The double-edged

sword property of mesenchymal stem cell-derived exosomal microRNAs in colorectal cancer. *The Turkish Journal of Gastroenterology*. 2024;**35**(10):755-762

[63] Zhang W, Huang X. Stem cell membrane-camouflaged targeted delivery system in tumor. *Materials Today Bio*. 2022;**16**:100377

[64] Kamarehei F. The effects of combination therapy by solid lipid nanoparticle and dental stem cells on different degenerative diseases. *American Journal of Translational Research*. 2022;**14**(5):3327-3343

[65] Afkhami H, Mahmoudvand G, Fakouri A, Shadab A, Mahjoor M, Komeili MT. New insights in application of mesenchymal stem cells therapy in tumor microenvironment: Pros and cons. *Frontiers in Cell and Development Biology*. 2023;**11**:1255697

[66] Zhang G, Zhen C, Yang J, Zhang Z, Wu Y, Che J, et al. 1-2 T static magnetic field combined with ferumoxytol prevent unloading-induced bone loss by regulating iron metabolism in osteoclastogenesis. *Journal of Orthopaedic Translation*. 2022;**38**:126-140

[67] Yang YJ, Chen XE, Zhou XC, Liang FX. Mesenchymal stem cell-derived extracellular vesicles: A promising therapeutic strategy in diabetic osteoporosis. *World Journal of Diabetes*. 2024;**15**(12):2399-2403

[68] Huldani H, Abdalkareem Jasim S, Olegovich Bokov D, Abdelbasset WK, Nader Shalaby M, Thangavelu L, et al. Application of extracellular vesicles derived from mesenchymal stem cells as potential therapeutic tools in autoimmune and rheumatic diseases. *International Immunopharmacology*. 2022;**106**:108634

- [69] Torrecillas-Baena B, Pulido-Escribano V, Dorado G, Gálvez-Moreno MÁ, Camacho-Cardenosa M, Casado-Díaz A. Clinical potential of mesenchymal stem cell-derived exosomes in bone regeneration. *Journal of Clinical Medicine*. 2023;**12**(13):4385
- [70] Garg K, Zilate S. Umbilical cord-derived mesenchymal stem cells for the treatment of infertility due to premature ovarian failure. *Cureus*. 2022;**14**(10):e30529
- [71] Wei L, Bo L, Luo C, Yin N, Jiang W, Qian F, et al. Transplantation of human umbilical cord-derived mesenchymal stem cells improves age-related ovarian functional decline via regulating the local renin–angiotensin system on inflammation and oxidative stress. *Stem Cell Research & Therapy*. 2024;**15**:377
- [72] Ghajari G, Heydari A, Ghorbani M. Mesenchymal stem cell-based therapy and female infertility: Limitations and advances. *Current Stem Cell Research & Therapy*. 2023;**18**(3):322-338
- [73] Rizano A, Margiana R, Supardi S, Narulita P. Exploring the future potential of mesenchymal stem/stromal cells and their derivatives to support assisted reproductive technology for female infertility applications. *Human Cell*. 2023;**36**(5):1604-1619
- [74] Shimizu S. Association of detrusor underactivity with aging and metabolic syndrome: Suggestions from animal models. *Journal of Smooth Muscle Research*. 2024;**60**:23-30
- [75] Huang X, Liu Y, Li Z, Lerman LO. Mesenchymal stem/stromal cells therapy for metabolic syndrome: Potential clinical application? *Stem Cells*. 2023;**41**(10):893-906
- [76] Kotikalapudi N, Sampath SJP, Sukesh Narayan S, Nemani H, Mungamuri SK, et al. The promise(s) of mesenchymal stem cell therapy in averting preclinical diabetes: Lessons from in vivo and in vitro model systems. *Scientific Reports*. 2021;**11**:16983
- [77] Zare Moghaddam M, Mousavi MJ, Ghotloo S. Stem cell-based therapy for systemic lupus erythematosus. *Journal of Translational Autoimmunity*. 2024;**8**:100241
- [78] Lou S, Duan Y, Nie H, Cui X, Du J, Yao Y. Mesenchymal stem cells: Biological characteristics and application in disease therapy. *Biochimie*. 2021;**185**:9-21
- [79] Ahad Mir P, Hussain MS, Ahmad Khanday M, Mohi-Ud-Din R, Pottloo FH, Hasssan MR. Immunomodulatory roles of mesenchymal stem cell-derived extracellular vesicles: A promising therapeutic approach for autoimmune diseases. *Current Stem Cell Research & Therapy*. 2025;**20**(9):949-965
- [80] Asserson DB. Allogeneic mesenchymal stem cells after In vivo transplantation: A review. *Cellular Reprogramming*. 2023;**25**(6):264-276
- [81] Meng HY, Chen LQ, Chen LH. The inhibition by human MSCs-derived miRNA-124a overexpression exosomes in the proliferation and migration of rheumatoid arthritis-related fibroblast-like synoviocyte cell. *BMC Musculoskeletal Disorders*. 2020;**21**(1):150
- [82] Hwang JJ, Rim YA, Nam Y, Ju JH. Recent developments in clinical applications of mesenchymal stem cells in the treatment of rheumatoid arthritis and osteoarthritis. *Frontiers in Immunology*. 2021;**12**:631291
- [83] Bačenková D, Trebuňová M, Morochovič R, Dosedla E, Findrik Balogová A, Gašparová P, et al.

Interaction between mesenchymal stem cells and the immune system in rheumatoid arthritis. *Pharmaceuticals (Basel)*. 2022;**15**(8):941

cell-based treatment in autoimmune liver diseases: Underlying roles, advantages and challenges. *Therapeutic Advances in Chronic Disease*. 2021;**12**:2040622321993442

[84] Rasouli M, Naeimzadeh Y, Hashemi N, Hosseinzadeh S. Age-related alterations in mesenchymal stem cell function: Understanding mechanisms and seeking opportunities to bypass the cellular aging. *Current Stem Cell Research & Therapy*. 2024;**19**(1):15-32

[85] Bang OY, Kim JE. Stem cell-derived extracellular vesicle therapy for acute brain insults and neurodegenerative diseases. *BMB Reports*. 2022;**55**(1):20-29

[86] Jeung SY, An JH, Kim SS, Youn HY. Safety of gonadal tissue-derived mesenchymal stem cell therapy in geriatric dogs with chronic disease. *Animals (Basel)*. 2024;**14**(14):2134

[87] Park SY, Kim DS, Kim HM, Lee JK, Hwang DY, Kim TH, et al. Human mesenchymal stem cell-derived extracellular vesicles promote neural differentiation of neural progenitor cells. *International Journal of Molecular Sciences*. 2022;**23**(13):7047

[88] Basalova N, Illarionova M, Skryabina M, Vigovskiy M, Tolstoluzhinskaya A, Primak A, et al. Advances and obstacles in using CRISPR/Cas9 technology for non-coding RNA gene knockout in human mesenchymal stromal cells. *Noncoding RNA*. 2023;**9**(5):49

[89] Han AR, Shin HR, Kweon J, Lee SB, Lee SE, Kim EY, et al. Highly efficient genome editing via CRISPR-Cas9 ribonucleoprotein (RNP) delivery in mesenchymal stem cells. *BMB Reports*. 2024;**57**(1):60-65

[90] He C, Yang Y, Zheng K, Chen Y, Liu S, Li Y, et al. Mesenchymal stem

Mesenchymal Stem Cell-Based Therapeutic Strategies for Neurological Disorders in Dogs

Rogério Martins Amorim

and Lucas Vinícius de Oliveira Ferreira

Abstract

Neurological disorders in dogs pose a significant challenge in veterinary medicine, often leading to permanent deficits and reduced quality of life. Traditional therapeutic approaches frequently offer limited efficacy, especially in neuroimmune or degenerative conditions. In this context, mesenchymal stem cells (MSCs) have emerged as a promising therapeutic strategy due to their immunomodulatory, anti-inflammatory, neuroprotective, and neuroregenerative properties. This chapter provides a comprehensive review of MSC-based therapy for canine neurological disorders, with an emphasis on their application in meningoencephalomyelitis of unknown origin (MUO), peripheral nerve injuries, and spinal cord injury (SCI). Key aspects discussed include MSC sources, mechanisms of action, delivery routes, neurological improvement, functional recovery, and limitations. In addition, future directions and perspectives are explored to support the development of safe and effective MSC-based therapies in veterinary neurology.

Keywords: cell therapy, central nervous system, neuroprotection, peripheral nerve, regenerative medicine

1. Introduction

Neurological disorders in dogs represent a significant challenge in veterinary clinical practice due to the wide range of etiologies and the inherent complexity of the nervous system [1, 2]. Epidemiological data indicate that neurological disorders account for a significant proportion of veterinary cases, with one study reporting that 18.7% of all admitted French Bulldogs presented with neurological signs, and another showing that nearly 14% of all canine and feline referrals were diagnosed with a neurological disease [3, 4]. These conditions can have a substantial negative impact on the well-being of affected animals, often leading to prolonged clinical courses, functional impairments, and, in some cases, fatal outcomes [5, 6].

Conventional therapeutic approaches, such as glucocorticoid administration for meningoencephalomyelitis of unknown origin (MUO), which may be associated with side effects, and surgical decompression alone for spinal cord injury (SCI), may provide limited results in the management of neurological disorders [7, 8]. In this context, regenerative medicine has gained increasing attention in veterinary science, particularly the use of mesenchymal stem cells (MSCs), due to their immunomodulatory, anti-inflammatory, neuroprotective, and neuroregenerative properties [8–10].

Recent studies have explored the application of MSCs in a range of canine neurological disorders, such as SCI [11, 12], MUO [13], and peripheral nerve injuries (PNI) [14]. These investigations have demonstrated encouraging results, with reports of improved functional recovery and reduced lesion severity. Despite these advances, important challenges remain, including the standardization of treatment protocols, optimization of delivery routes, and the need for long-term safety and efficacy data.

In this context, this chapter provides a comprehensive overview of the use of MSCs in the treatment of neurological diseases in dogs, including cellular sources, mechanisms of action, administration strategies, clinical outcomes, and current limitations. Furthermore, future perspectives are discussed, with the aim of supporting the advancement of safe and effective MSC-based therapies in veterinary neurology.

2. MSCs

MSCs are progenitor cells characterized by high proliferative capacity, self-renewal ability, multilineage differentiation potential, and notable immunomodulatory, neuroprotective, and neuroregenerative properties [15, 16]. The International Society for Cellular Therapy has proposed minimal criteria for the identification and characterization of human MSCs *in vitro*. These include adherence to plastic under standard culture conditions; the potential to differentiate into mesodermal lineages, osteogenic, chondrogenic, and adipogenic; positive expression of surface markers CD73, CD90, and CD105; and lack of expression of hematopoietic and endothelial markers such as CD45, CD34, CD14 or CD11b, CD79 α or CD19, and HLA class II [17]. In canine MSCs, a similar surface antigen expression profile has been identified, including positive expression of CD29, CD44, CD90, and CD105, and lack of expression of CD34, CD45, CD146, CD11b, CD79 α , and MHC class II [18–21].

MSCs can be derived from various tissues, including adipose tissue, bone marrow, amniotic fluid, placenta, umbilical cord, muscle tissue, and peripheral blood [22]. Their therapeutic effects are primarily mediated through direct cell-to-cell interactions and the release of soluble factors and extracellular vesicles (EVs) *via* paracrine mechanisms [23, 24]. These cells secrete a wide range of bioactive molecules, including anti-inflammatory cytokines and neurotrophic factors, that modulate the immune response, reduce neuroinflammation, and promote neuronal survival and tissue repair [25, 26]. These mechanisms support the potential use of MSCs in the treatment of neurological disorders.

3. Meningoencephalomyelitis of unknown origin (MUO)

MUO encompasses a group of idiopathic, noninfectious inflammatory diseases affecting the central nervous system (CNS) of dogs [27]. It is characterized by several subtypes, granulomatous meningoencephalomyelitis (GME), necrotizing

meningoencephalitis (NME), and necrotizing leukoencephalitis (NLE), in which autoimmunity is regarded as the primary underlying mechanism [28]. GME presents in multifocal, focal, or ocular forms, affecting the forebrain, hindbrain, and spinal cord. Histologically, it is characterized by perivascular whorling mononuclear infiltrates in the white matter, meninges, and spinal cord, with acute lesions involving both gray and white matter and chronic lesions in the white matter. NME typically presents with focal or multifocal forebrain signs, with seizures being the most common clinical manifestation. Histologically, it is marked by asymmetric necrosis and cavitation affecting the cerebral cortex, subcortical white matter, and corona radiata, accompanied by mononuclear infiltrates, prominent astrogliosis, and leptomeningeal inflammation; lesions may also extend to the brainstem and cerebellum. NLE presents with focal or multifocal neurological signs involving the forebrain and hindbrain. Histologically, it is characterized by asymmetric necrosis and cavitation predominantly in the white matter, with mononuclear infiltration and marked reactive astrogliosis; the meninges are minimally affected [7]. A definitive diagnosis is only possible through histopathological examination. In England, MUO accounts for 47.5% of inflammatory CNS disorders in dogs [29].

MUO is clinically characterized by progressive focal or multifocal neurological signs associated with single or multiple/diffuse intra-axial lesions observed on T2-weighted magnetic resonance imaging of the CNS, cerebrospinal fluid showing inflammatory features, and exclusion of infectious agents [28, 29].

Glucocorticoids remain the cornerstone of initial treatment and, in the long term, are generally combined with other immunosuppressive agents [30]. However, corticosteroid use is frequently associated with severe side effects, including gastric ulceration, hepatopathy, alopecia, and iatrogenic hyperadrenocorticism [7].

Combination therapies involving additional immunosuppressive and immunomodulatory agents, such as cytarabine (cytosine arabinoside), azathioprine, lomustine, procarbazine, leflunomide, cyclosporin, and mycophenolate mofetil, have been employed to enhance remission rates and reduce corticosteroid dosages [30, 31]. Nonetheless, these drugs carry risks of myelosuppression, hepatotoxicity, gastrointestinal disturbances, hepatic dysfunction, and nephrotoxicity, among others [13, 30].

Considering that MUO is a fatal disease and that conventional treatments are often associated with severe adverse effects, there is an increasing demand for innovative therapeutic alternatives. Among these, MSC-based therapies have emerged as a promising approach.

3.1 Neuroinflammation and neurodegeneration mechanisms in MUO

The etiopathogenesis of MUO remains incompletely understood, but it is considered multifactorial and shares characteristics similar to multiple sclerosis (MS), including genetic susceptibility and immune-mediated processes that compromise CNS integrity [32].

Genomic analyses have identified genetic risk factors associated with histopathological subtypes of MUO in Pug [33] and Maltese dogs [34]. An immunohistochemical study has revealed that CD3⁺ T lymphocytes are the most abundant inflammatory cells in lesions of MUO subtypes, and CD163⁺ macrophages are predominantly expressed in GME [35]. Additionally, B lymphocytes have been identified in lesions of dogs with GME, NME, and NLE [35, 36], as well as in the CNS of patients with MS [37].

Cytokine expression profiles differ among MUO subtypes: dogs with NME exhibit elevated levels of interferon-gamma (IFN- γ), whereas those with GME display increased expression of interleukin-17 (IL-17) [38]. In GME, IL-17 is believed to drive macrophage and microglial activation. Similarly, in humans with MS, IL-17 produced by Th17 lymphocytes increases blood-brain barrier (BBB) disruption and facilitates T cell infiltration into the CNS [39].

Autoimmune responses in the CNS can arise due to failures in central or peripheral immune tolerance. These responses may be triggered by molecular mimicry, the presentation of self-antigens, or the exposure of previously sequestered antigens. As a result, autoreactive lymphocytes become activated in lymphoid organs, enter the bloodstream, and infiltrate the CNS by crossing the BBB [37, 40].

Immune imbalances within the microenvironment, such as elevated levels of IFN- γ , can promote macrophage polarization toward a classically activated macrophage (M1, pro-inflammatory) phenotype, shifting away from the alternatively activated macrophage (M2, anti-inflammatory) state [40]. Microglial cells undergo similar cytokine-driven phenotypic transitions. M1-polarized microglia enhance the release of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF- α), IL-6, and IL-1 β , as well as reactive oxygen species (ROS) and nitric oxide (NO), while reducing the production of neurotrophic factors [7]. Conversely, M2-polarized microglia secrete higher levels of anti-inflammatory cytokines, such as IL-10 and transforming growth factor beta (TGF- β), and neurotrophic factors, thereby dampening pro-inflammatory activity [7].

Chronic inflammation in the CNS promotes the release of neurotoxic factors, such as ROS, reactive nitrogen species, and pro-inflammatory cytokines. These molecules alter neuronal and axonal metabolism, leading to cytotoxicity, mitochondrial dysfunction, metabolic stress, and neurodegeneration [7, 37, 40].

Energy imbalance and demyelination lead to ion channel dysfunction and activation, which, together with glutamate accumulation, disrupt Ca²⁺/Na⁺ homeostasis and trigger the activation of lytic enzymes, thereby perpetuating neuroaxonal damage and resulting in oncotic cellular edema, apoptosis, and necrosis [41].

Experimental autoimmune encephalomyelitis (EAE) remains the most widely used experimental model for studying MS, as it mimics T cell-mediated responses against myelin antigens in laboratory animals [37]. Nonetheless, the pathological features elicited by EAE vary according to factors such as the myelin antigen used, the dose of peptide or adjuvant, and the species or strain of animal, resulting in a heterogeneous model whose applicability must be tailored to the specific research objective [42]. In this context, MUO in dogs serves as a natural model for MS due to similarities in immune mechanisms, pathological features, and spontaneous onset [43].

While these parallels support the use of MUO as a valuable natural model for MS, it is important to recognize interspecies differences that may impact translational interpretation. For instance, although both diseases exhibit neuroinflammation, MS involves infiltration of both B and T lymphocytes, whereas MUO is characterized predominantly by T cell infiltration [32]. Moreover, demyelination is more prominent in MS than in MUO [31]. Thus, while MUO provides an important spontaneous model that complements experimental systems, findings should be extrapolated to human MS with consideration of these biological distinctions.

3.2 MSCs as a treatment strategy for MUO

MSCs demonstrate significant therapeutic potential in autoimmune disorders, largely due to their diverse biological functions. These include the secretion of

paracrine factors, modulation of immune activity, and the capacity to migrate to sites of inflammation or tissue damage [44]. MSCs support neuroprotection mainly through the release of neurotrophic factors, which promote neuronal survival and contribute to remyelination processes within the CNS [26]. In addition, MSCs have been shown to enhance neurogenesis, promote astrocyte activation, stimulate angiogenesis, exert anti-apoptotic effects, and support synaptogenesis, all of which contribute to central nervous system repair and functional recovery (**Figure 1**) [26, 45].

Among these properties, the immunoregulatory role of MSCs is especially relevant, as it balances inflammatory processes through cytokine release that modulates local immune dynamics (**Figure 2**). One key mediator involved in this immunomodulation is prostaglandin E2 (PGE2), a lipid compound synthesized *via* the cyclooxygenase enzymes COX-1 and COX-2. PGE2 exerts pivotal effects by downregulating Th1 lymphocyte responses, suppressing macrophage and neutrophil effector functions, and promoting the accumulation of regulatory T cells (Tregs) [47, 48].

Natural killer (NK) cells play a key role in immune surveillance, but their activity can be tightly regulated by MSCs. Rather than simply responding to stimulatory cytokines like IL-2 and IL-15, which typically enhance NK cytotoxicity, these cells undergo functional suppression in the presence of MSCs. This suppression includes reduced proliferation, downregulation of key activation markers (such as NKG2D,

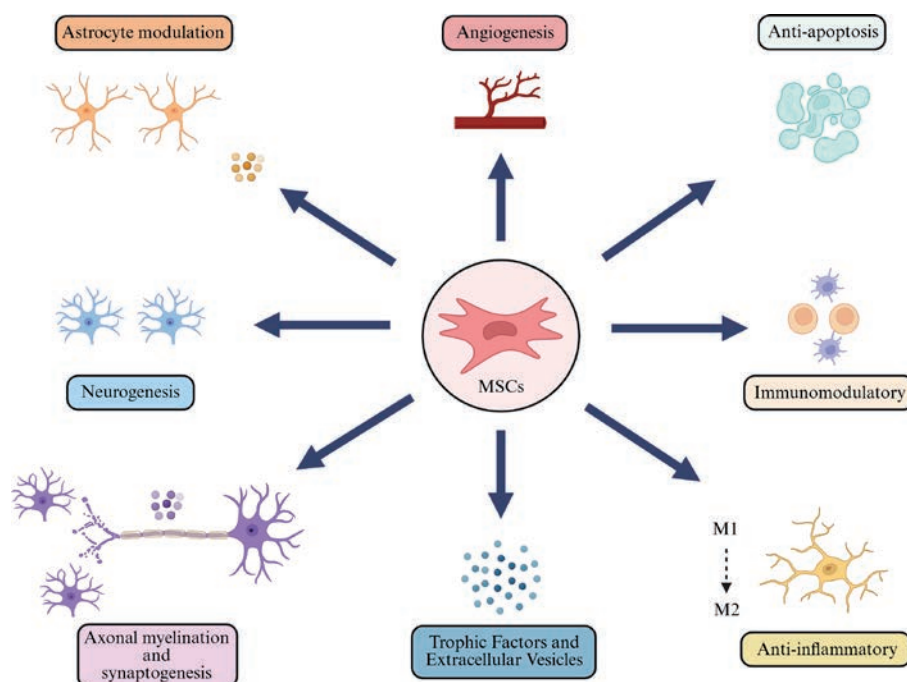


Figure 1. Mechanisms of action of mesenchymal stem cells (MSCs) in neurodegenerative diseases. MSCs exert therapeutic effects through the secretion of soluble factors and extracellular vesicles, as well as direct cell-to-cell interactions. These mechanisms contribute to neuroprotection and regeneration by promoting angiogenesis, synaptogenesis, axonal myelination, neurogenesis, astrocyte modulation, and a shift in microglia from a pro-inflammatory M1 phenotype to a protective M2 phenotype. Additionally, MSCs exhibit immunomodulatory and anti-apoptotic properties, limiting inflammation and supporting tissue repair in the central nervous system. Created in BioRender: Amorim, R. (2025) <https://BioRender.com/wn3f5og>.

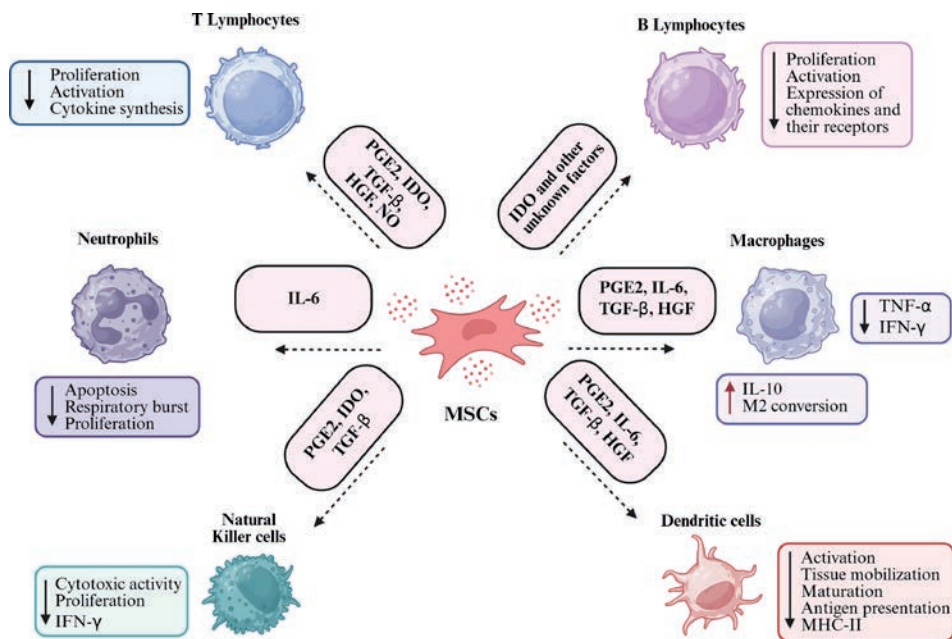


Figure 2.

Schematic representation of the immunomodulatory effects of mesenchymal stem cells (MSCs). MSCs modulate immune responses primarily through paracrine signaling via the release of soluble factors and extracellular vesicles, as well as through direct cell-to-cell interactions. They suppress T cell proliferation and activation, inhibit the cytotoxic functions of natural killer cells, modulate B cell activity, impair dendritic cell maturation and their capacity to activate T cells, and promote the polarization of macrophages from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype. HGF: Hepatocyte growth factor; IDO: Indoleamine 2,3-dioxygenase; IFN-γ: Interferon-gamma; IL-6: Interleukin 6; NO: Nitric oxide; PGE2: Prostaglandin E2; TGF-β: Transforming growth factor beta; TNF-α: Tumor necrosis factor alpha. The immunomodulatory effects of MSCs were adapted from both Andrzejewska et al. [44] and Dias et al. [46], with the list of soluble factors released by MSCs specifically based on Dias et al. [46]. Created in BioRender. Amorim, R. (2025) <https://BioRender.com/wn3jf5og>.

NKp30, and NKp44), and diminished secretion of inflammatory cytokines like IFN-γ [48]. In addition, the secretion of the anti-inflammatory cytokine IL-10 suppresses the production of pro-inflammatory mediators and contributes to the generation of regulatory T cells (Tregs) [49].

Beyond their effects on NK cells, MSCs exert broader immunomodulatory functions that are particularly relevant in neuroinflammatory contexts. They can regulate the activity of microglia by modulating the release of ROS and pro-inflammatory mediators [25]. In parallel, MSCs induce a shift in macrophage phenotype from the classically activated M1 type to the alternatively activated M2 type, promoting an anti-inflammatory environment and suppressing T lymphocyte proliferation [50]. Another crucial mechanism involves the inhibition of Th17 cell differentiation from naïve CD4⁺ T cells, a process observed in both human and canine MSCs [51, 52]. Given that Th17 cells are major producers of the pro-inflammatory cytokine IL-17, which plays a central role in the pathogenesis of MS and MUO, this pathway represents a key target for MSC-based therapies [38, 40, 53].

Studies using EAE have demonstrated that MSCs can suppress neuroimmune responses and promote local neuroregeneration [54–57]. Additionally, one study demonstrated the safety of autologous bone marrow-derived MSC transplantation in dogs and reported early improvements in their general and neurological conditions (Table 1) [13].

Cell	Culture protocols	Delivery	Models	Cell numbers	Outcome	Comments	Limitations	Ref.
Allogeneic AT-MSCs (murine) Xenogeneic AT-MSCs (human)	Murine MSCs: MesenCult +20% mouse MSC supplements + pen/strep Human MSCs: DMEM +10% FCS + glutamax +100 U/ml pen/strep	Intraperitoneal	EAE-induced mice	1×10^6	Significantly reduced clinical disease severity and decreased inflammation in the CNS	MSCs expanded in hypoxia. Murine AT-MSCs reduced EAE severity, partly by impairing dendritic cell and T cell function	Limited analysis after human AT-MSCs transplantation	[54]
Xenogeneic PMSCs (human)	DMEM +5% FBS + 20 ng/ml FGF + 20 ng/ml EGF + 1% pen/strep	Intravenous (IV)	EAE-induced mice	1×10^6	Improved motor function, reduced DNA damage in oligodendroglia, and increased myelination	Extracellular vesicles derived from PMSCs achieved similar clinical outcomes	EAE onset differed between male and female mice. Variation in disease onset was observed among animals	[55]
Xenogeneic AT-MSCs (human)	DMEM +10% FBS + 1% pen/strep	IV	EAE-induced mice	1×10^6	Improved immunomodulation, reduced demyelination, increased Olig2+ cells, and elevated MBP protein levels	AT-MSCs genetically modified to overexpress LIF and IFN- β	Sample size	[56]
Xenogeneic WJ-MSCs (human)	DMEM/F12 + 10% FBS + 1% pen/strep	Intracerebral ventricular injection	EAE-induced rats	1×10^5	Enhanced immunomodulation and neuroprotection, with increased astrocytic activation	Immunomodulatory and neuroprotective effects were observed with both WJ-MSCs and NPs; however, WJ-MSCs alone showed more significant potential	Sample size and short follow-up period	[57]

Cell	Culture protocols	Delivery	Models	Cell numbers	Outcome	Comments	Limitations	Ref.
Autologous BM-MSCs (canine)	DMEM + L-glutamine +4500 mg/L D-glucose, 25 mM HEPES without pyruvate +10% FBS + 1% pen/strep +1% glutamax + EGF + bFGF	Intrathecal (IT) and intra- arterial (IA); IT and IV	8 dogs with MUO	IT: 2×10^6 IA: 4×10^6 IV: 0.5×10^6	Improvements in cervical paraspinal hyperesthesia, behavior, proprioception, and postural reactions, and ataxia	All dogs relapsed after prednisone withdrawal and showed poor response to a second full-dose therapy prior to receiving MSC transplantation	Sample size, lack of a control group, and histopathological analysis of the CNS were performed in only one animal	[13]

AT-MSCs: Adipose tissue-derived mesenchymal stem cells; BM-MSCs: Bone marrow-derived mesenchymal stem cells; CNS: Central nervous system; DMEM: Dulbecco's modified Eagle's medium; EAE: Experimental autoimmune encephalomyelitis; EGF: Epidermal growth factor; FBS: Fetal bovine serum; FGF: Fibroblast growth factor; bFGF: Basic fibroblast growth factor; IA: Intra-arterial; IFN- β : Interferon-beta; IT: Intrathecal; IV: Intravenous; LIF: Leukemia inhibitory factor; MBP: Myelin basic protein; MSCs: Mesenchymal stem cells; MUO: Meningoencephalomyelitis of unknown origin; NPs: Neural precursors; olig2: Oligodendrocyte transcription factor 2; Pen: Penicillin; PMSCs: Placenta-derived mesenchymal stem cells; Strep: Streptomycin; WJ-MSCs: Wharton's Jelly-derived mesenchymal stem cells.

Table 1.
Use of mesenchymal stem cells in murine experimental autoimmune encephalomyelitis (EAE) models and in canine meningoencephalomyelitis of unknown origin (MUO).

One of the main mechanisms through which MSCs exert their immunomodulatory and neuroprotective effects is *via* the secretion of EVs. These MSC-derived EVs play a key role in intercellular communication by transferring bioactive molecules, such as microRNAs, mRNAs, proteins, and lipids, to target cells, thereby modulating immune responses, reducing neuroinflammation, and promoting neuroregeneration [58, 59]. EVs have been shown to stimulate M2 microglial polarization, suppress dendritic cell activation, upregulate soluble immunomodulatory factors such as TGF- β and PGE₂, and inhibit T and B cell proliferation [60].

In an EAE model, a high dose of EVs derived from placenta-derived mesenchymal stem cells (PMSCs) significantly improved motor function, reduced DNA damage in the oligodendroglia population, and enhanced spinal cord myelination [55]. Moreover, a comparative study in dogs with experimental MS demonstrated that intrathecal administration of MSC-derived exosomes led to clinical improvement, resolution of hyperintense lesions on magnetic resonance imaging, and histopathological evidence of remyelination [61].

While experimental studies using EAE models have demonstrated the immunomodulatory and neuroprotective effects of MSCs, their application in dogs with MUO remains poorly explored. This highlights the need for further research to determine safety, efficacy, and standardized treatment protocols for this specific condition.

4. PNI

PNI are common in dogs, typically resulting from trauma or surgical complications [62]. Traumatic PNI are relatively common in dogs, particularly involving the brachial plexus and sciatic nerve, usually resulting from excessive traction or abduction of the thoracic or pelvic limbs [63]. In the human population, peripheral nerve trauma affects approximately 13.9 individuals per 100,000 each year [64]. Overall, PNI impact millions of people worldwide annually [65].

4.1 Mechanisms of degeneration and regeneration in the PNI

Following PNI, Wallerian degeneration is initiated and characterized by the disintegration of the distal axonal segment and the removal of cellular and myelin debris that may inhibit regeneration, thus facilitating the establishment of a microenvironment that supports repair [66].

Between 12 and 24 hours after injury, the disorganization of axonal microtubules and cytoskeletal breakdown begin to occur [67]. The influx of calcium ions activates apoptotic signaling pathways, leading to neurofilament degradation and the accumulation of granular debris [68]. Schwann cells (SCs) begin to transdifferentiate into repair SCs, which support axonal regeneration by proliferating and releasing signaling molecules that promote the recruitment of macrophages and other immune cells to the injury site [69, 70]. Together, these cells are responsible for clearing the degenerating cellular debris, thereby creating a permissive environment for axonal regeneration.

In the proximal segment of the injured nerve, a limited retrograde degeneration occurs up to the next intact Ranvier node [67, 71]. Changes in this segment vary depending on the severity and location of the injury and may include apoptosis in lesions close to the neuronal cell body or, in some cases, reversible chromatolysis [71, 72].

Concomitantly with the degeneration process, axonal regeneration begins. This process depends on the survival of the neuronal cell body and SCs, as well as on the coordinated activity of neurotrophic factors, components of the extracellular matrix, and the inflammatory response [73, 74]. After injury, SCs proliferate, elongate, and align within their preserved basal lamina, forming Büngner bands, longitudinally oriented structures that guide and support the regrowth of axons [75]. In addition, SCs play a central role in this process by secreting various neurotrophic factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), neurotrophins NT-3 and NT-4/5, fibroblast growth factor 2 (FGF-2), neuregulins, and ciliary neurotrophic factor (CNTF) [68, 76, 77].

Although the peripheral nervous system possesses an intrinsic capacity for regeneration, this ability is often inadequate in long-gap nerve injuries due to several limiting factors. These include neuronal death resulting from axotomy and subsequent retrograde degeneration, the inability of regenerating axons to successfully cross the injury site, leading to atrophy of denervated target tissues, and the lack of accurate reinnervation caused by the random growth of axons along distal endoneurial tubes following complete nerve transection [78].

4.2 Therapeutic potential of MSCs in PNI

MSCs have emerged as promising candidates for the treatment of PNI due to their unique biological properties. The therapeutic effects of MSCs are achieved through multiple pathways, including the release of soluble factors and EVs, mitochondrial transfer, direct cell-cell interactions, and the stimulation of proliferation and differentiation of cells (**Figure 3**) [23].

A key mechanism by which MSCs promote nerve regeneration is the secretion of neurotrophic factors, such as NGF, BDNF, GDNF, CNTF, FGF-2, insulin-like growth factor (IGF), and hepatocyte growth factor (HGF) [79–81]. These molecules contribute to neuronal survival and enhance axonal outgrowth, thereby supporting neuroregeneration.

The immunomodulatory properties of MSCs also play a pivotal role. MSCs secrete anti-inflammatory cytokines and immunoregulatory molecules such as IL-10, TGF- β , PGE2, indoleamine 2,3-dioxygenase (IDO), NO, and IL-6 [82–85]. Moreover, MSCs can stimulate angiogenesis, mainly through the release of vascular endothelial growth factor (VEGF) [81], further supporting tissue regeneration. However, the immunomodulatory potential of MSCs may vary depending on the species and the tissue source from which the cells are derived [86, 87].

Under specific conditions, MSCs can transdifferentiate into Schwann-like cells (SLCs), thereby mimicking the functions of native SCs [88]. To the best of our knowledge, this approach has not yet been investigated in canine MSCs; however, several studies have demonstrated positive outcomes following the transplantation of SLCs in sheep [89, 90], rabbits [91], and rats [92].

The potential of MSC-based therapies has been demonstrated in dogs, where MSCs from different sources contributed to axonal regeneration and functional recovery. Furthermore, the incorporation of MSCs from dogs and humans into scaffolds has been shown to enhance recovery in nerve gap injuries in dogs (**Table 2**) [93, 95].

Although MSC-based therapies show promise in preclinical studies for treating PNI, their clinical use in dogs faces several obstacles. One key issue is the variability

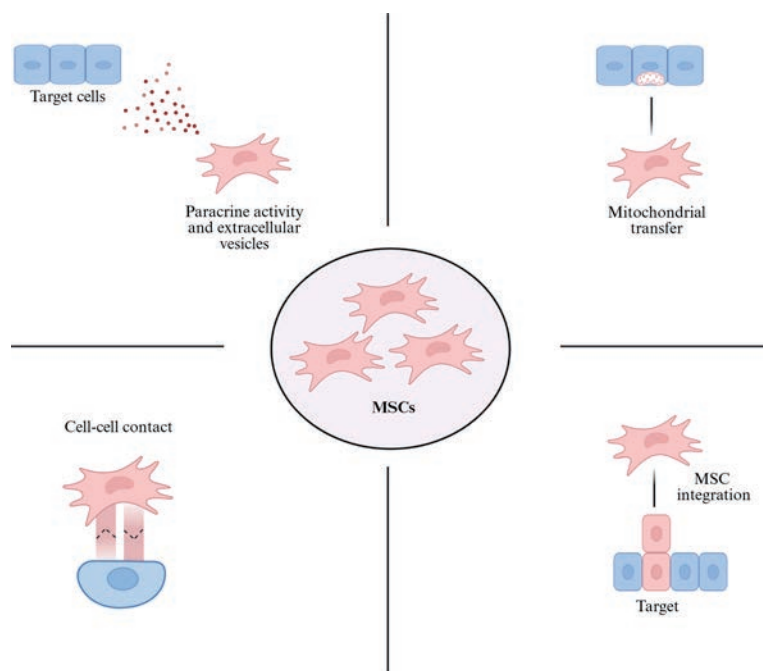


Figure 3.
 Illustration of the multifaceted mechanisms involved in MSC-mediated repair in nerve injury. Mesenchymal stem cells (MSCs) contribute to tissue regeneration through several complementary processes, including integration into damaged tissue, secretion of paracrine factors, release of extracellular vesicles, mitochondrial transfer, and direct cell-to-cell interactions. These mechanisms enable MSCs to tailor their therapeutic actions to the specific conditions of the local microenvironment. Created in BioRender. Amorim, R. (2025) <https://BioRender.com/wn3f50g>.

in MSC characteristics depending on their source, donor, and culture methods, which impacts their effectiveness. Moreover, the harsh environment at injury sites, including inflammation, may reduce MSC survival and integration. The absence of standardized guidelines for cell dose, delivery method, and treatment timing further complicates the consistency and scalability of these therapies. Furthermore, fundamental questions remain regarding the safety and efficacy of using allogeneic and xenogeneic MSCs for therapeutic purposes in animals. Additionally, more research is needed to confirm the long-term safety of MSC applications.

5. SCI

SCI represents severe neurological disorders that often result in permanent disability and a marked decline in quality of life for both humans and companion animals [96, 97]. Available data suggest that the yearly occurrence of SCI varies significantly, with estimates ranging from 12 to over 65 cases per million [98], resulting in a considerable socioeconomic impact due to both direct and indirect costs [99].

Although comprehensive epidemiological data in veterinary populations are limited, SCI is considered relatively common in clinical practice, especially in dogs. Canines frequently suffer from traumatic events such as vehicular accidents, but intervertebral disc disease (IVDD) is the most prevalent cause of spinal cord compression and remains the leading cause of paralysis in dogs [100, 101].

Cell	Culture protocols	Delivery/Conduit	Models	Cell numbers	Outcome	Comments	Limitations	Ref.
Autologous BM-MSCs (canine)	Iscove's modified Dulbecco's medium +10% FBS + antibiotics	Chitosan/PLGA (Chitosan nerve conduit (4.5 mm inner diameter) filled with ~1000 aligned PLGA fibers)	50-mm-long gaps in dog sciatic nerve	5×10^7	Improved nerve regeneration and functional recovery	Efficacy close to that of autografting	No detailed functional analysis, no gene expression of regeneration markers, and limited immunohistochemistry	[93]
Allogeneic BM-MSCs (canine)	Unknown	Local injection	Dog sciatic nerve transection and suture repair	5×10^6	Improvements in motor and sensory functions	Evaluation for 112 days	Sample size; lack of detailed motor and sensory functional analyses; no electroneuromyography or nerve regeneration marker assessment	[94]
Xenogeneic UC-MSCs (human)	Mesenchymal stem cell medium (ScienCell)	Longitudinally oriented- collagen conduit (Constructed by wrapping a collagen membrane around a cylindrical glass tube)	35-mm segment of the sciatic nerve surgically removed in dogs	1×10^6	Enhanced axonal regeneration and functional recovery	Nerve regeneration was less effective compared to autologous nerve grafts	Lack of detailed functional analysis and gene expression evaluation of nerve regeneration markers	[95]
Autologous BM-MSCs (canine)	α -MEM + 10% FBS +1 $\times$ nonessential amino acids +4 mM L-glutamine +100 U/ml pen +0.01 mg/ml strep	Vein conduit graft	10-mm-long segment of the facial nerve removed in dogs	1×10^6	Improved clinical recovery of facial nerve function	Similar clinical improvement in all groups during the first 3 weeks	Limited functional analysis, absence of electrophysiological and nerve regeneration marker assessments, and evaluation for 8 weeks	[14]

BM-MSCs: Bone marrow-derived mesenchymal stem cells; FBS: Fetal bovine serum; Pen: Penicillin; PLGA: Poly(lactic-co-glycolic acid); Strep: Streptomycin; UC-MSCs: Umbilical cord-derived mesenchymal stem cells; α -MEM: Minimum essential medium alpha.

Table 2.
Use of mesenchymal stem cells for treating peripheral nerve injuries in dogs.

Because of their anatomical and pathological similarities to human SCI, dogs have become important translational models for studying SCI pathophysiology and developing new therapeutic approaches. Definitive treatment options for SCI remain unavailable [102]. Among emerging treatments, MSCs stand out due to their well-documented anti-inflammatory, neuroprotective, and regenerative effects, positioning them as promising candidates for innovative therapies.

5.1 Mechanisms of spinal cord degeneration

SCI typically results from mechanical forces such as contusion, compression, or overstretching of the spinal cord. In more severe cases, complete transection may occur, particularly when associated with vertebral fractures. The pathophysiological response to SCI can be divided into two main phases: the primary injury and the secondary injury cascade [96].

The primary injury can be classified as either nontraumatic, caused by factors such as tumors, infections, or degenerative diseases, or traumatic, resulting from vertebral fractures or dislocations [103]. Mechanical damage to the spinal cord leads to immediate cell death and vascular injury, disrupting the blood-spinal cord barrier at the lesion site and triggering rapid infiltration of inflammatory cells into the affected area [96]. These alterations impair nerve conduction and axoplasmic transport, disrupt spinal cord pathways, and initiate the formation of a glial scar [104].

Following the initial injury, a complex secondary injury cascade ensues, significantly exacerbating tissue damage. This secondary phase encompasses vascular permeability changes, ionic imbalances, glutamate-mediated excitotoxicity, metabolic disturbances, a robust inflammatory response, and the establishment of a highly inhibitory microenvironment [103].

Together, these pathological processes lead to progressive degeneration of spinal cord tissue, including neuronal apoptosis and necrosis, demyelination, axonal disruption, cyst formation, and consolidation of the glial scar. The resulting environment poses substantial challenges to axonal regeneration and functional recovery.

5.2 Therapeutic potential of MSCs in SCI

Given the complex pathophysiology of SCI, developing effective therapeutic strategies that promote neuroregeneration while minimizing secondary damage is crucial. Current research focuses on several key objectives, including the replacement of lost neural cells, enhancement of neurotrophic factor secretion, modulation of intracellular signaling pathways, regulation of immune responses, neutralization of inhibitory factors that impede axonal growth, and provision of physical scaffolds to support and guide axonal regeneration.

In this context, MSCs have emerged as a highly promising therapeutic option due to their diverse properties, which include immunomodulation, neuroprotection, anti-apoptotic effects, promotion of angiogenesis, and reduction of glial scar formation [105].

Experimental studies investigating MSC therapy in traumatic SCI have been conducted across various animal models, including mice [106], rats [107], rabbits [108], and nonhuman primates [109]. In canines, clinical studies applying diverse MSC-based protocols to treat SCI caused by trauma or intervertebral disc disease have demonstrated variable but often positive neurological improvements (Table 3). These

Cell	Culture protocols	Delivery/Conduit	Models, preclinical and clinical trials	Cell numbers	Outcome	Comments	Limitations	Ref.
Allogeneic AT-MSCs (canine)	DMEM +10% FBS + 1% pen/strep	Local injection (post-hemilaminectomy)	Nine dogs with thoracolumbar intervertebral disc disease	1×10^7	Full recovery of neurological function was observed in 55.6% of the animals, while 22.2% showed clinical improvement (regained deep pain perception and ambulation, but still had mild ataxia)	In 22.2% of the cases, there was no successful improvement in the animals	Sample size and no histopathological analysis	[11]
Allogeneic AT-MSCs (canine)	DMEM low glucose +20% FBS + 2 mM L-glutamine +1% pen/strep	Local injection	Six dogs with chronic SCI	1×10^7	Three animals showed improved locomotion, and one of them was able to walk without support	No functional improvement was observed in two dogs following treatment	Sample size, absence of a control group, and no histopathological analysis	[12]
Allogeneic AT-MSCs (canine)	DMEM +10% FBS + 100 units/ml pen +100 µg/ml strep	Lumbar puncture	Nineteen paraplegic dogs with SCI	1.2×10^6	Improvements in motor recovery	The AT-MSCs + electrical stimulation group did not show better recovery than the AT-MSCs group or electrical stimulation alone	Sample size (only nine dogs received MSC only), no untreated control group, no histopathological analysis	[110]

Cell	Culture protocols	Delivery/Conduit	Models, preclinical and clinical trials	Cell numbers	Outcome	Comments	Limitations	Ref.
Allogeneic AT-MSCs (canine)	DMEM/F12 + 10% FBS + 1% antibiotics	Local injection (post-hemilaminectomy)	Eleven dogs with acute thoracolumbar disk disease and SCI	1×10^7	Improvements in locomotor recovery	AT-MSCs with decompression may speed locomotor recovery and shorten hospitalization vs. decompression alone	Sample size, no histopathological analysis	[111]
Allogeneic BM-MSCs (canine)	DMEM +15% FBS + 50 µg/ml gentamycin	Local injection	Dogs with SCI	1×10^6	Improvements in functional recovery	Administration of BM-MSCs every 15 days up to 45 days	Unspecified group size, short follow-up period, no histopathological analysis	[112]
BM-MSCs (canine)	DMEM low glucose +10% FBS + 4 mM L-glutamine	Gelatin sponge scaffold (Three-dimensional gelatin sponge scaffold with a 4 mm diameter and 5 mm length)	Transection of the spinal cord at T10 in dogs	5×10^5	Improvements were observed in the motor function of the paralyzed pelvic limbs and electrophysiological parameters	Co-culture with NT-3 Schwann cells induced TrkC BM-MSCs to differentiate into neuron-like cells	Sample size	[113]

Cell	Culture protocols	Delivery/Conduit	Models, preclinical and clinical trials	Cell numbers	Outcome	Comments	Limitations	Ref.
Allogeneic AF-MSCs (canine)	DMEM +10% FBS + 5 ng/ml FGF + 10 ng/ml EGF + 1% pen/strep	Local injection (postsurgical decompression)	Dog with acute SCI due to trauma	5×10^6	Improvement in the dog's hind limb movement and spinal cord regeneration	Pelvic limb function was evaluated weekly for 15 weeks to monitor recovery	Sample size and only one animal used as a positive control	[114]
AT-MSCs: Adipose tissue-derived mesenchymal stem cells; AF-MSCs: Amniotic fluid-derived mesenchymal stem cells; BM-MSCs: Bone marrow-derived mesenchymal stem cells; DMEM: Dulbecco's modified Eagle medium; EGF: Epidermal growth factor; FBS: Fetal bovine serum; FGF: Fibroblast growth factor; MSC: Mesenchymal stem cell; NT-3: Neurotrophin-3; Pen: Penicillin; SCI: Spinal cord injury; Shrep: Streptomycin.								

Table 3.
Use of mesenchymal stem cells for treating spinal cord injury in dogs.

encouraging results underscore the growing potential of MSC therapies as regenerative treatments for spinal cord injuries in both veterinary medicine and translational research.

Despite their therapeutic promise, the clinical application of MSCs for SCI faces several significant challenges. The hostile microenvironment at the injury site frequently leads to low cell survival and limited integration of transplanted MSCs into the damaged tissue. Moreover, persistent inhibitory factors in the lesion area may continue to hinder axonal regeneration, even in the presence of MSCs. Finally, concerns remain regarding long-term safety, possible adverse effects, and the lack of standardized protocols for cell preparation, delivery, and dosing, all of which complicate the translation of MSC therapy into routine clinical practice.

6. Future directions and perspectives

To advance safe and effective MSC-based therapies for neurological diseases in dogs, future research must prioritize the rigorous standardization of protocols encompassing cell isolation, culture conditions, expansion, and administration routes and dosages specifically for dogs. Harmonizing these parameters is essential to ensure reproducibility and optimize therapeutic potency.

In addition, comparative studies evaluating different MSC sources, as well as investigations into optimal delivery routes, are urgently needed to establish best practices tailored to canine models. Systematic exploration of these variables will help clarify which cell sources and administration methods maximize therapeutic efficacy and safety in different neurological conditions.

A comprehensive understanding of MSC behavior within the complex and often hostile injured microenvironment in dogs, including their interactions with resident neural cells, immune populations, and other components, is critical to maximizing regenerative outcomes and minimizing adverse effects.

Innovative strategies to enhance MSC survival, retention, and integration at lesion sites in canine nervous tissue hold significant promise. These include the use of biocompatible scaffolds and biomaterials that provide physical support and guidance cues for regenerating tissue, as well as modulation of the inflammatory microenvironment to create a more permissive milieu. Emerging approaches such as genetic modification or priming of MSCs to boost their neurotrophic factor secretion, immunomodulatory capacity, or resistance to oxidative stress also warrant exploration in canine models.

Crucially, well-designed, multicenter, and long-term clinical trials in dogs are required to systematically assess the efficacy, safety, optimal dosing regimens, delivery methods, and potential adverse effects of MSC-based therapies across a spectrum of canine neurological disorders. Such studies will establish evidence-based guidelines that can standardize clinical applications and improve outcomes for dogs.

Interdisciplinary collaboration in research among veterinary clinicians, bioengineers, and regulatory agencies will be essential to advance the clinical application of MSC therapies in veterinary medicine. Such collaborative efforts not only have the potential to improve the treatment of neurological diseases in dogs but also to generate valuable insights applicable to human regenerative medicine. To ensure the responsible and effective use of stem cell therapies in companion animals, the establishment of clear regulatory and ethical guidelines will be crucial.

To ensure the responsible and effective use of stem cell therapies in companion animals, the establishment of clear regulatory and ethical guidelines will be crucial. Key challenges include compliance with good manufacturing practices (GMP) for cell production to guarantee quality and safety, as well as navigating varying regional veterinary regulations. The choice between autologous and allogeneic MSC sources presents additional ethical and safety considerations, with allogeneic therapies raising concerns about immune rejection and autologous treatments involving individualized processing and cost.

Moreover, issues such as donor animal welfare, informed consent from pet owners, and transparent communication about the risks and benefits of MSC therapies must be thoroughly addressed. Expanding these discussions will enhance the practical relevance and guide stakeholders in advancing veterinary regenerative medicine responsibly.

7. Conclusions

The therapeutic potential of MSC-based approaches for neurological disorders is undeniably promising. Despite encouraging results, variability in outcomes highlights the influence of numerous biological and technical factors. As research advances, MSC therapies are expected to become key tools in addressing conditions where conventional treatments fall short. Achieving this, however, will depend on a deeper understanding of MSC biology, improved control over their differentiation pathways, and better insight into the mechanisms that affect their clinical performance. Such advancements will enable the development of more targeted, effective, and disease-specific treatment strategies for dogs.

Acknowledgements

The authors acknowledge the financial support from the São Paulo Research Foundation (FAPESP) through a doctoral scholarship (2024/19980-0).

Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

AF-MSCs	amniotic fluid-derived mesenchymal stem cells
AT-MSCs	adipose tissue-derived mesenchymal stem cells
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
BM-MSCs	bone marrow-derived mesenchymal stem cells
CNS	central nervous system
CNTF	ciliary neurotrophic factor
DMEM	Dulbecco's modified Eagle medium
EAE	experimental autoimmune encephalomyelitis
EGF	epidermal growth factor

EVs	extracellular vesicles
FBS	fetal bovine serum
FCS	fetal calf serum
FGF	fibroblast growth factor
FGF-2	fibroblast growth factor 2
bFGF	basic fibroblast growth factor
GDNF	glial cell-derived neurotrophic factor
GME	granulomatous meningoencephalomyelitis
GMP	good manufacturing practices
HGF	hepatocyte growth factor
IA	intra-arterial
IDO	indoleamine 2,3-dioxygenase
IFN- β	interferon-beta
IFN- γ	interferon-gamma
IGF	insulin-like growth factor
IL	interleukin
IT	intrathecal
IV	intravenous
IVDD	intervertebral disc disease
LIF	leukemia inhibitory factor
M1	classically activated, pro-inflammatory macrophages.
M2	alternatively activated, anti-inflammatory macrophages
MBP	myelin basic protein
MS	multiple sclerosis
MSCs	mesenchymal stem cells
MUO	meningoencephalomyelitis of unknown origin
NGF	nerve growth factor
NK	natural killer
NLE	necrotizing leukoencephalitis
NME	necrotizing meningoencephalitis
NO	nitric oxide
NPs	neural precursors
NT	neurotrophins
Olig2	oligodendrocyte transcription factor 2
Pen	penicillin
PGE2	prostaglandin E2
PLGA	poly(lactic-co-glycolic acid)
PMSCs	placenta-derived mesenchymal stem cells
PNI	peripheral nerve injuries
ROS	reactive oxygen species
SCs	Schwann cells
SCI	spinal cord injury
SLCs	Schwann-like cells
Strep	streptomycin
TGF- β	transforming growth factor beta
TNF- α	tumor necrosis factor alpha
UC-MSCs	umbilical cord-derived mesenchymal stem cells
VEGF	vascular endothelial growth factor
WJ-MSCs	Wharton's Jelly-derived mesenchymal stem cells
α -MEM	minimum essential medium alpha

Author details

Rogério Martins Amorim^{1,2*} and Lucas Vinícius de Oliveira Ferreira^{1,2}

1 Department of Veterinary Clinic, School of Veterinary Medicine and Animal Science, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

2 Center for Translational Research in Regenerative Medicine, Institute of Biotechnology, São Paulo State University, Botucatu, São Paulo, Brazil

*Address all correspondence to: rogerio.amorim@unesp.br

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Colbath AC, Goodrich L, Frye C, Dow S. Review of cellular therapies provides new insights into the potential treatment of diverse neurologic diseases in horses and dogs. *Journal of the American Veterinary Medical Association*. 2024;**262**:S121-S130. DOI: 10.2460/javma.23.12.0709
- [2] Espino L, Miño N. Common neurologic diseases in geriatric dogs. *Animals*. 2024;**14**:1753. DOI: 10.3390/ani14121753
- [3] Fluehmann G, Doherr MG, Jaggy A. Canine neurological diseases in a referral hospital population between 1989 and 2000 in Switzerland. *Journal of Small Animal Practice*. 2006;**47**:582-587. DOI: 10.1111/j.1748-5827.2006.00106.x
- [4] Mayousse V, Desquilbet L, Jeandel A, Blot S. Prevalence of neurological disorders in French bulldog: A retrospective study of 343 cases (2002-2016). *BMC Veterinary Research*. 2017;**13**:212. DOI: 10.1186/s12917-017-1132-2
- [5] Samsøe-Schmidt F, Berendt M, Miles JE. Chronic sequelae and owner burdens are common following canine intervertebral disc disease. *Veterinary Record*. 2025:e5371. DOI: 10.1002/vetr.5371
- [6] van Renen J, Kehl A, Buhmann G, Matiassek LA, Zablotski Y, Fischer A. Allele frequency of a genetic risk variant for necrotizing meningoencephalitis in pug dogs from Europe and association with the clinical phenotype. *Frontiers in Veterinary Science*. 2024;**11**:1407288. DOI: 10.3389/fvets.2024.1407288
- [7] Coates JR, Jeffery ND. Perspectives on Meningoencephalomyelitis of unknown origin. *Veterinary Clinics of North America: Small Animal Practice*. 2014;**4**:1157-1185. DOI: 10.1016/j.cvsm.2014.07.009
- [8] Picazo RA, Rojo C, Rodriguez-Quiros J, González-Gil A. Current advances in mesenchymal stem cell therapies applied to wounds and skin, eye, and neuromuscular diseases in companion animals. *Animals*. 2024;**14**:1363. DOI: 10.3390/ani14091363
- [9] Prišlin M, Vlahović D, Kostešić P, Ljolje I, Brnić D, Turk N, et al. An outstanding role of adipose tissue in canine stem cell therapy. *Animals*. 2022;**12**:1088. DOI: 10.3390/ani12091088
- [10] Baouche M, Ochota M, Locatelli Y, Mermillod P, Niżański W. Mesenchymal stem cells: Generalities and clinical significance in feline and canine medicine. *Animals*. 2023;**13**:1903. DOI: 10.3390/ani13121903
- [11] Kim Y, Lee SH, Kim WH, Kweon OK. Transplantation of adipose derived mesenchymal stem cells for acute thoracolumbar disc disease with no deep pain perception in dogs. *Journal of Veterinary Science*. 2016;**17**:123. DOI: 10.4142/jvs.2016.17.1.123
- [12] Escalhão CCM, Ramos IP, Hochman-Mendez C, Brunswick THK, Souza SAL, Gutfilen B, et al. Safety of allogeneic canine adipose tissue-derived mesenchymal stem cell Intraspinal transplantation in dogs with chronic spinal cord injury. *Stem Cells International*. 2017;**2017**:3053759. DOI: 10.1155/2017/3053759
- [13] Zeira O, Asiag N, Aralla M, Ghezzi E, Pettinari L, Martinelli L, et al. Adult autologous mesenchymal stem cells

for the treatment of suspected non-infectious inflammatory diseases of the canine central nervous system: Safety, feasibility and preliminary clinical findings. *Journal of Neuroinflammation*. 2015;12:181. DOI: 10.1186/s12974-015-0402-9

[14] Daradka M, Ismail Z, Irsheid M. Peripheral nerve regeneration: A comparative study of the effects of autologous bone marrow-derived mesenchymal stem cells, platelet-rich plasma and lateral saphenous vein graft as a conduit in a dog model. *Open Veterinary Journal*. 2021;11:686-694. DOI: 10.5455/OVJ.2021.v11.i4.20

[15] Babu S, Krishnan M, Panneerselvam A, Chinnaiyan M. A comprehensive review on therapeutic application of mesenchymal stem cells in neuroregeneration. *Life Science*. 2023;327:121785. DOI: 10.1016/j.lfs.2023.121785

[16] Issa EHB, Alghazo EM, Gharaibeh R, Momani NB, Taha DZ, Jaradat RJ, et al. Therapeutic potential and challenges of mesenchymal stem cells in neurological disorders: A concise analysis. *Journal of Neuropathology and Experimental Neurology*. 2025;84:668-679. DOI: 10.1093/jnen/nlaf021

[17] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, et al. 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

[18] Kisiel AH, McDuffee LA, Masaoud E, Bailey TR, Esparza Gonzalez BP, Nino-Fong R. Isolation, characterization, and in vitro proliferation of canine mesenchymal stem cells derived from bone marrow, adipose tissue, muscle,

and periosteum. *American Journal of Veterinary Research*. 2012;73:1305-1317. DOI: 10.2460/ajvr.73.8.1305

[19] Zhan XS, El-Ashram S, Luo DZ, Luo HN, Wang BY, Chen SF, et al. A comparative study of biological characteristics and transcriptome profiles of mesenchymal stem cells from different canine tissues. *International Journal of Molecular Science*. 2019;20:1485. DOI: 10.3390/ijms20061485

[20] Guest DJ, Dudhia J, Smith RKW, Roberts SJ, Conzemius M, Innes JF, et al. Position statement: Minimal criteria for reporting veterinary and animal medicine research for mesenchymal stromal/stem cells in orthopedic applications. *Frontiers in Veterinary Science*. 2022;9:817041. DOI: 10.3389/fvets.2022.817041

[21] Humenik F, Maloveska M, Hudakova N, Petrouskova P, Hornakova L, Domaniza M, et al. A comparative study of canine mesenchymal stem cells isolated from different sources. *Animals*. 2022;12:1502. DOI: 10.3390/ani12121502

[22] Kulus M, Sibiak R, Stefańska K, Zdun M, Wieczorkiewicz M, Piotrowska-Kempisty H, et al. Mesenchymal stem/stromal cells derived from human and animal perinatal tissues—Origins, characteristics, signaling pathways, and clinical trials. *Cells*. 2021;10:3278. DOI: 10.3390/cells10123278

[23] Dabrowska S, Andrzejewska A, Janowski M, Lukomska B. Immunomodulatory and regenerative effects of mesenchymal stem cells and extracellular vesicles: Therapeutic outlook for inflammatory and degenerative diseases. *Frontiers in Immunology*. 2021;11:591065. DOI: 10.3389/fimmu.2020.591065

- [24] Xue Z, Liao Y, Li Y. Effects of microenvironment and biological behavior on the paracrine function of stem cells. *Genes and Diseases*. 2024;**11**:135-147. DOI: 10.1016/j.gendis.2023.03.013
- [25] Skok M. Mesenchymal stem cells as a potential therapeutic tool to cure cognitive impairment caused by neuroinflammation. *World Journal of Stem Cells*. 2021;**13**:1072-1083. DOI: 10.4252/wjsc.v13.i8.1072
- [26] Gavasso S, Kråkenes T, Olsen H, Evjenth EC, Ytterdal M, Haugsøen JB, et al. The therapeutic mechanisms of mesenchymal stem cells in MS—A review focusing on neuroprotective properties. *International Journal of Molecular Science*. 2024;**25**:1365. DOI: 10.3390/ijms25031365
- [27] Beasley MJ, Shores A. Perspectives on pharmacologic strategies in the management of meningoencephalomyelitis of unknown origin in dogs. *Frontiers in Veterinary Science*. 2023;**10**:1167002. DOI: 10.3389/fvets.2023.1167002
- [28] Barber R, Downey KL. Treatment with Cytarabine at initiation of therapy with cyclosporine and glucocorticoids for dogs with meningoencephalomyelitis of unknown origin is not associated with improved outcomes. *Frontiers in Veterinary Science*. 2022;**9**:925774. DOI: 10.3389/fvets.2022.925774
- [29] Gonçalves R, De Decker S, Walmsley G, Butterfield S, Maddox TW. Inflammatory disease affecting the central nervous system in dogs: A retrospective study in England (2010-2019). *Frontiers in Veterinary Science*. 2022;**8**:819945. DOI: 10.3389/fvets.2021.819945
- [30] Cornelis I, Van Ham L, Gielen I, De Decker S, Bhatti SFM. Clinical presentation, diagnostic findings, prognostic factors, treatment and outcome in dogs with meningoencephalomyelitis of unknown origin: A review. *The Veterinary Journal*. 2019;**244**:37-44. DOI: 10.1016/j.tvjl.2018.12.007
- [31] Jeffery N, Granger N. New insights into the treatment of meningoencephalomyelitis of unknown origin since 2009: A review of 671 cases. *Frontiers in Veterinary Science*. 2023;**10**:1114798. DOI: 10.3389/fvets.2023.1114798
- [32] Prümmer JK, Stein VM, Marti E, Lutterotti A, Jelcic I, Schüpbach-Regula G, et al. Assessment of oligoclonal bands in cerebrospinal fluid and serum of dogs with meningoencephalitis of unknown origin. *PLoS One*. 2023;**18**:e0280864. DOI: 10.1371/journal.pone.0280864
- [33] Barber RM, Schatzberg SJ, Corneveaux JJ, Allen AN, Porter BF, Pruzin JJ, et al. Identification of risk loci for necrotizing meningoencephalitis in pug dogs. *Journal of Heredity*. 2011;**102**:S40-S46. DOI: 10.1093/jhered/esr048
- [34] Schrauwen I, Barber RM, Schatzberg SJ, Siniard AL, Corneveaux JJ, Porter BF, et al. Identification of novel genetic risk loci in Maltese dogs with necrotizing meningoencephalitis and evidence of a shared genetic risk across toy dog breeds. *PLoS One*. 2014;**9**:e112755. DOI: 10.1371/journal.pone.0112755
- [35] Park ES, Uchida K, Nakayama H. Comprehensive immunohistochemical studies on canine necrotizing meningoencephalitis (NME), necrotizing leukoencephalitis (NLE), and granulomatous meningoencephalomyelitis (GME).

Veterinary Pathology. 2012;**49**:682-692. DOI: 10.1177/0300985811429311

[36] Spitzbarth I, Schenk HC, Tipold A, Beineke A. Immunohistochemical characterization of inflammatory and glial responses in a case of necrotizing leucoencephalitis in a French bulldog. *Journal of Comparative Pathology*. 2010;**142**:235-241. DOI: 10.1016/j.jcpa.2009.08.158

[37] Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. *Nature Reviews Immunology*. 2015;**15**:545-558. DOI: 10.1038/nri3871

[38] Park ES, Uchida K, Nakayama H. Th1-, Th2-, and Th17-related cytokine and chemokine receptor mRNA and protein expression in the brain tissues, T cells, and macrophages of dogs with necrotizing and granulomatous meningoencephalitis. *Veterinary Pathology*. 2013;**50**:1127-1134. DOI: 10.1177/0300985813488957

[39] Balasa R, Barcutean L, Balasa A, Motataianu A, Roman-Filip C, Manu D. The action of TH17 cells on blood brain barrier in multiple sclerosis and experimental autoimmune encephalomyelitis. *Human Immunology*. 2020;**81**:237-243. DOI: 10.1016/j.humimm.2020.02.009

[40] Sospedra M, Martin R. Immunology of multiple sclerosis. *Seminars in Neurology*. 2016;**36**:115-127. DOI: 10.1055/s-0036-1579739

[41] Friese MA, Schattling B, Fugger L. Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. *Nature Reviews Neurology*. 2014;**10**:225-238. DOI: 10.1038/nrneurol.2014.37

[42] Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis

(EAE) as a model for multiple sclerosis (MS). *British Journal of Pharmacology*. 2011;**164**:1079-1106. DOI: 10.1111/j.1476-5381.2011.01302.x

[43] Jeffery ND, Barker AK, Alcott CJ, Levine JM, Meren I, Wengert J, et al. The Association of Specific Constituents of the fecal microbiota with immune-mediated brain disease in dogs. *PLoS One*. 2017;**12**:e0170589. DOI: 10.1371/journal.pone.0170589

[44] Andrzejewska A, Lukomska B, Janowski M. Concise review: Mesenchymal stem cells: From roots to boost. *Stem Cells*. 2019;**37**:855-864. DOI: 10.1002/stem.3016

[45] Ciervo Y, Ning K, Jun X, Shaw PJ, Mead RJ. Advances, challenges and future directions for stem cell therapy in amyotrophic lateral sclerosis. *Molecular Neurodegeneration*. 2017;**12**:85. DOI: 10.1186/s13024-017-0227-3

[46] Dias IE, Pinto PO, Barros LC, Viegas CA, Dias IR, Carvalho PP. Mesenchymal stem cells therapy in companion animals: Useful for immune-mediated diseases? *BMC Veterinary Research*. 2019;**15**:358. DOI: 10.1186/s12917-019-2087-2

[47] Jiang W, Xu J. Immune modulation by mesenchymal stem cells. *Cell Proliferation*. 2020;**53**:e12712. DOI: 10.1111/cpr.12712

[48] Huang Y, Wu Q, Tam PKH. Immunomodulatory mechanisms of mesenchymal stem cells and their potential clinical applications. *International Journal of Molecular Science*. 2022;**23**:10023. DOI: 10.3390/ijms231710023

[49] López-García L, Castro-Manrreza ME. TNF- α and IFN- γ participate in improving the

Immunoregulatory capacity of mesenchymal stem/stromal cells: Importance of cell–cell contact and extracellular vesicles. *International Journal of Molecular Science*. 2021;22:9531. DOI: 10.3390/ijms22179531

[50] Dehnavi S, Sadeghi M, Tavakol Afshari J, Mohammadi M. Interactions of mesenchymal stromal/stem cells and immune cells following MSC-based therapeutic approaches in rheumatoid arthritis. *Cellular Immunology*. 2023;393-394:104771. DOI: 10.1016/j.cellimm.2023.104771

[51] Ghannam S, Pène J, Torcy-Moquet G, Jorgensen C, Yssel H. Mesenchymal stem cells inhibit human Th17 cell differentiation and function and induce a T regulatory cell phenotype. *The Journal of Immunology*. 2010;185:302-312. DOI: 10.4049/jimmunol.09020

[52] Kol A, Walker NJ, Nordstrom M, Borjesson DL. Th17 pathway As a target for multipotent stromal cell therapy in dogs: Implications for translational research. *PLoS One*. 2016;11:e0148568. DOI: 10.1371/journal.pone.0148568

[53] Uchida K, Park E, Tsuboi M, Chambers JK, Nakayama H. Pathological and immunological features of canine necrotising meningoencephalitis and granulomatous meningoencephalitis. *The Veterinary Journal*. 2016;213:72-77. DOI: 10.1016/j.tvjl.2016.05.002

[54] Anderson P, Gonzalez-Rey E, O'Valle F, Martin F, Oliver FJ, Delgado M. Allogeneic adipose-derived mesenchymal stromal cells ameliorate experimental autoimmune encephalomyelitis by regulating self-reactive T cell responses and dendritic cell function. *Stem Cells International*. 2017;2017:2389753. DOI: 10.1155/2017/2389753

[55] Clark K, Zhang S, Barthe S, Kumar P, Pivetti C, Kreutzberg N, et al. Placental mesenchymal stem cell-derived extracellular vesicles promote myelin regeneration in an animal model of multiple sclerosis. *Cells*. 2019;8:1497. DOI: 10.3390/cells8121497

[56] Yousefi M, Nabipour A, Ganjalikhan H, Hakemi M, Ashja-Arvan M, Amirpour N, Salehi H. Transplantation of human adipose-derived stem cells overexpressing LIF/IFN- β promotes recovery in experimental autoimmune encephalomyelitis (EAE). *Scientific Reports*. 2022;12:17835. DOI: 10.1038/s41598-022-21850-9

[57] Perussolo MC, Mogharbel BF, Saçaki CS, da RNN, Irioda AC, de ONB, et al. Cellular therapy in experimental autoimmune encephalomyelitis as an adjuvant treatment to translate for multiple sclerosis. *International Journal of Molecular Science*. 2024;25:6996. DOI: 10.3390/ijms25136996

[58] Martin-Rufino JD, Espinosa-Lara N, Osugui L, Sanchez-Guijo F. Targeting the immune system with mesenchymal stromal cell-derived extracellular vesicles: What is the Cargo's mechanism of action? *Frontiers in Bioengineering and Biotechnology*. 2019;7:308. DOI: 10.3389/fbioe.2019.00308

[59] Kou M, Huang L, Yang J, Chiang Z, Chen S, Liu J, et al. Mesenchymal stem cell-derived extracellular vesicles for immunomodulation and regeneration: A next generation therapeutic tool? *Cell Death and Disease*. 2022;13:580. DOI: 10.1038/s41419-022-05034-x

[60] Gomzikova MO, James V, Rizvanov AA. Therapeutic application of mesenchymal stem cells derived extracellular vesicles for immunomodulation. *Frontiers*

in Immunology. 2019;**10**:2663.
DOI: 10.3389/fimmu.2019.02663

[61] Abdallah AN, Shamaa AA, El-Tookhy OS, Bahr MM. Comparison between stem cell therapy and stem cell derived exosomes on induced multiple sclerosis in dogs. BMC Veterinary Research. 2024;**20**:90. DOI: 10.1186/s12917-024-03920-4

[62] Dell’Apa D, Auletta L, Okonji S, Cauduro A, Dondi M, Opreni M, et al. Traumatic and iatrogenic sciatic nerve injury in 38 dogs and 10 cats: Clinical and electrodiagnostic findings. Journal of Veterinary Internal Medicine. 2024;**38**:1626-1638. DOI: 10.1111/jvim.17076

[63] Van Soens I, Struys MM, Polis IE, Bhatti SF, Van Meervenne SA, Martlé VA, et al. Magnetic stimulation of the radial nerve in dogs and cats with brachial plexus trauma: A report of 53 cases. The Veterinary Journal. 2009;**182**:108-113. DOI: 10.1016/j.tvjl.2008.05.007

[64] Asplund M, Nilsson M, Jacobsson A, von Holst H. Incidence of traumatic peripheral nerve injuries and amputations in Sweden between 1998 and 2006. Neuroepidemiology. 2009;**32**:217-228. DOI: 10.1159/000197900

[65] Liu Y, Zhang X, Xiao C, Liu B. Engineered hydrogels for peripheral nerve repair. Materials Today Bio. 2023;**20**:100668. DOI: 10.1016/j.mtbio.2023.100668

[66] Gu D, Xia Y, Ding Z, Qian J, Gu X, Bai H, et al. Inflammation in the peripheral nervous system after injury. Biomedicine. 2024;**12**:1256. DOI: 10.3390/biomedicines12061256

[67] Damásio Alvites R, Vieira Branquinho M, Rita Caseiro A, Santos

Pedrosa S, Lúcia Luís A, Geuna S, Varejão ASP, Maurício AC. Biomaterials and cellular Systems at the Forefront of peripheral nerve regeneration. In: Turker H, Benavides LG, Gallardo GR, Del Villar MM, editors. Peripheral Nerve Disorders and Treatment. 1st ed. IntechOpen. 2020. pp. 1-49. DOI: 10.5772/intechopen.87043

[68] Carroll SL, Worley SH. Wallerian degeneration. In: Stein J, Bennett D, Coen C, Dunbar R, Goodwin G, Husain M, et al., editors. The Curated Reference Collection in Neuroscience and Biobehavioral Psychology. Amsterdam: Elsevier Science Ltd.; 2016. pp. 485-491

[69] Nocera G, Jacob C. Mechanisms of Schwann cell plasticity involved in peripheral nerve repair after injury. Cellular and Molecular Life Sciences. 2020;**77**:3977-3989. DOI: 10.1007/s00018-020-03516-9

[70] Rhode SC, Beier JP, Ruhl T. Adipose tissue stem cells in peripheral nerve regeneration— *In vitro* and *in vivo*. Journal of Neuroscience Research. 2021;**99**:545-560. DOI: 10.1002/jnr.24738

[71] Menorca RMG, Fussell TS, Elfar JC. Nerve physiology. Hand Clinics. 2013;**29**:317-330. DOI: 10.1016/j.hcl.2013.04.002

[72] Rishal I, Fainzilber M. Axon–soma communication in neuronal injury. Nature Reviews Neuroscience. 2014;**15**:32-42. DOI: 10.1038/nrn3609

[73] Allodi I, Udina E, Navarro X. Specificity of peripheral nerve regeneration: Interactions at the axon level. Progress in Neurobiology. 2012;**98**:16-37. DOI: 10.1016/j.pneurobio.2012.05.005

[74] Scheib J, Höke A. Advances in peripheral nerve regeneration. Nature

Reviews Neurology. 2013;**9**:668-676.
 DOI: 10.1038/nrneurol.2013.227

[75] Jessen KR, Mirsky R. The success and failure of the Schwann cell response to nerve injury. *Frontiers in Cellular Neuroscience*. 2019;**13**:33. DOI: 10.3389/fncel.2019.00033

[76] Gonçalves NP, Mohseni S, El Soury M, Ulrichsen M, Richner M, Xiao J, et al. Peripheral nerve regeneration is independent from Schwann cell p75NTR expression. *Frontiers in Cellular Neuroscience*. 2019;**13**:235. DOI: 10.3389/fncel.2019.00235

[77] Hopf A, Schaefer DJ, Kalbermatten DF, Guzman R, Madduri S. Schwann cell-like cells: Origin and usability for repair and regeneration of the peripheral and central nervous system. *Cells*. 2020;**9**:1990. DOI: 10.3390/cells9091990

[78] Redolfi Riva E, Özkan M, Contreras E, Pawar S, Zinno C, Escarda-Castro E, et al. Beyond the limiting gap length: Peripheral nerve regeneration through implantable nerve guidance conduits. *Biomaterials Science*. 2024;**12**:1371-1404. DOI: 10.1039/d3bm01163a

[79] Caseiro AR, Pereira T, Ivanova G, Luís AL, Maurício AC. Neuromuscular regeneration: Perspective on the application of mesenchymal stem cells and their secretion products. *Stem Cells International*. 2016;**2016**:9756973. DOI: 10.1155/2016/9756973

[80] Rodríguez-Sánchez DN, Pinto GBA, Cartarozzi LP, de Oliveira ALR, Bovolato ALC, de Carvalho M, et al. 3D-printed nerve guidance conduits multi-functionalized with canine multipotent mesenchymal stromal cells promote neuroregeneration after sciatic

nerve injury in rats. *Stem Cell Research and Therapy*. 2021;**12**:303. DOI: 10.1186/s13287-021-02315-8

[81] Sumarwoto T, Suroto H, Mahyudin F, Utomo DN, Romaniyanto TD, Notobroto H, et al. Role of adipose mesenchymal stem cells and secretome in peripheral nerve regeneration. *Annals of Medicine and Surgery*. 2021;**67**:102482. DOI: 10.1016/j.amsu.2021.102482

[82] Carrade DD, Borjesson DL. Immunomodulation by mesenchymal stem cells in veterinary species. *Comparative Medicine*. 2013;**63**:207-217

[83] Ribeiro TB, Duarte ASS, Longhini ALF, Pradella F, Farias AS, Luzo ACM, et al. Neuroprotection and immunomodulation by xenografted human mesenchymal stem cells following spinal cord ventral root avulsion. *Scientific Reports*. 2015;**5**:16167. DOI: 10.1038/srep16167

[84] Kota DJ, Prabhakara KS, Toledano-Furman N, Bhattarai D, Chen Q, DiCarlo B, et al. Prostaglandin E2 indicates therapeutic efficacy of mesenchymal stem cells in experimental traumatic brain injury. *Stem Cells*. 2017;**35**:1416-1430. DOI: 10.1002/stem.2603

[85] Eleuteri S, Fierabracci A. Insights into the secretome of mesenchymal stem cells and its potential applications. *International Journal of Molecular Science*. 2019;**20**:4597. DOI: 10.3390/ijms20184597

[86] Carrade DD, Lame MW, Kent MS, Clark KC, Walker NJ, Borjesson DL. Comparative analysis of the immunomodulatory properties of equine adult-derived mesenchymal stem cells. *Cell Medicine*. 2012;**4**:1-12. DOI: 10.3727/215517912X647217

- [87] Shrestha M, Nguyen TT, Park J, Choi JU, Yook S, Jeong JH. Immunomodulation effect of mesenchymal stem cells in islet transplantation. *Biomedicine and Pharmacotherapy*. 2021;**142**:112042. DOI: 10.1016/j.biopha.2021.112042
- [88] Martins Amorim R, Vinícius de Oliveira Ferreira L. Schwann-like cells derived from mesenchymal stem cells: Their potential for peripheral nerve regeneration. In: Piccaluga PP, Visani G, Khattab SS, editors. *Stem Cell Transplantation*. 1st ed. IntechOpen; 2025. pp. 1-24. DOI: 10.5772/intechopen.1006506
- [89] Pedroza-Montoya FE, Tamez-Mata YA, Simental-Mendía M, Soto-Domínguez A, García-Pérez MM, Said-Fernández S, et al. Repair of ovine peripheral nerve injuries with xenogeneic human acellular sciatic nerves prerecellularized with allogeneic Schwann-like cells—An innovative and promising approach. *Regenerative Therapy*. 2022;**19**:131-143. DOI: 10.1016/j.reth.2022.01.009
- [90] Tamez-Mata Y, Pedroza-Montoya FE, Martínez-Rodríguez HG, García-Pérez MM, Ríos-Cantú AA, González-Flores JR, et al. Nerve gaps repaired with acellular nerve allografts recellularized with Schwann-like cells: Preclinical trial. *Journal of Plastic, Reconstructive and Aesthetic Surgery*. 2022;**75**:296-306. DOI: 10.1016/j.bjps.2021.05.066
- [91] Wang X, Luo E, Li Y, Hu J. Schwann-like mesenchymal stem cells within vein graft facilitate facial nerve regeneration and remyelination. *Brain Research*. 2011;**1383**:71-80. DOI: 10.1016/j.brainres.2011.01.098
- [92] Tawab SA, Omar SMM, Zeid AAA, Saba C. Role of adipose tissue-derived stem cells versus differentiated Schwann-like cells transplantation on the regeneration of crushed sciatic nerve in rats. A histological study. *International Journal of Stem Cell Research and Therapeutics*. 2018;**1**:1-10
- [93] Ding F, Wu J, Yang Y, Hu W, Zhu Q, Tang X, et al. Use of tissue-engineered nerve grafts consisting of a chitosan/poly(lactic- *co* -glycolic acid)-based scaffold included with bone marrow mesenchymal cells for bridging 50-mm dog sciatic nerve gaps. *Tissue Engineering Part A*. 2010;**16**:3779-3790. DOI: 10.1089/ten.TEA.2010.0299
- [94] Al-Timmemi H, Dhurgham H, Zaid A. Efficacy of bone marrow stromal cells implantation on regeneration of peripheral nerve injury in dogs. *American Journal of Research Communication*. 2014;**2**:115-130
- [95] Cui Y, Yao Y, Zhao Y, Xiao Z, Cao Z, Han S, et al. Functional collagen conduits combined with human mesenchymal stem cells promote regeneration after sciatic nerve transection in dogs. *Journal of Tissue Engineering and Regenerative Medicine*. 2018;**12**:1285-1296. DOI: 10.1002/term.2660
- [96] Mili B, Buragohain L, Ralte L, Chutia T, Gogoi A, Barman U. Application of mesenchymal stem cells for treating spinal cord injury in dogs: Mechanisms and their therapeutic efficacy. *Indian Journal of Animal Science*. 2022;**9**:806-813. DOI: 10.56093/ijans.v9i7.119081
- [97] Mili B, Choudhary OP. Advancements and mechanisms of stem cell-based therapies for spinal cord injury in animals. *International Journal of Surgery*. 2024;**110**:6182-6197. DOI: 10.1097/JS9.0000000000001074
- [98] Hamid R, Averbeck MA, Chiang H, Garcia A, Al Mousa RT, Oh SJ, et al.

Epidemiology and pathophysiology of neurogenic bladder after spinal cord injury. *World Journal of Urology*. 2018;**36**:1517-1527. DOI: 10.1007/s00345-018-2301-z

[99] Dadvand A, Yavari A, Teimourpour A, Farzad-Mohajeri S. Influential factors on stem cell therapy success in canine model of spinal cord injury: A systematic review and meta-analysis. *Brain Research*. 2024;**1839**:148997. DOI: 10.1016/j.brainres.2024.148997

[100] Jeffery ND, Smith PM, Lakatos A, Ibanez C, Ito D, Franklin RJM. Clinical canine spinal cord injury provides an opportunity to examine the issues in translating laboratory techniques into practical therapy. *Spinal Cord*. 2006;**44**:584-593. DOI: 10.1038/sj.sc.3101912

[101] Orlandin JR, da S GI, de F SLS, Fuertes Cagnim A, Casals JB, Carregaro AB, et al. Treatment of chronic spinal cord injury in dogs using amniotic membrane-derived stem cells: Preliminary results. *Stem cells. Cloning*. 2021;**14**:39-49. DOI: 10.2147/SCCAA.S324184

[102] Nakamoto Y, Nakamura T, Nakai R, Azuma T, Omori K. Transplantation of autologous bone marrow-derived mononuclear cells into cerebrospinal fluid in a canine model of spinal cord injury. *Regenerative Therapy*. 2023;**24**:574-581. DOI: 10.1016/j.reth.2023.10.003

[103] Lima R, Monteiro A, Salgado AJ, Monteiro S, Silva NA. Pathophysiology and therapeutic approaches for spinal cord injury. *International Journal of Molecular Science*. 2022;**23**:13833. DOI: 10.3390/ijms232213833

[104] Onose G, Anghelescu A, Muresanu DF, Padure L, Haras MA,

Chendreanu CO, et al. A review of published reports on neuroprotection in spinal cord injury. *Spinal Cord*. 2009;**47**:716-726. DOI: 10.1038/sc.2009.52

[105] Zeng CW. Multipotent mesenchymal stem cell-based therapies for spinal cord injury: Current Progress and future prospects. *Biology*. 2023;**12**:653. DOI: 10.3390/biology12050653

[106] Deng J, Li M, Meng F, Liu Z, Wang S, Zhang Y, et al. 3D spheroids of human placenta-derived mesenchymal stem cells attenuate spinal cord injury in mice. *Cell Death and Disease*. 2021;**12**:1096. DOI: 10.1038/s41419-021-04398-w

[107] Maeda Y, Otsuka T, Takeda M, Okazaki T, Shimizu K, Kuwabara M, et al. Transplantation of rat cranial bone-derived mesenchymal stem cells promotes functional recovery in rats with spinal cord injury. *Scientific Reports*. 2021;**11**:21907. DOI: 10.1038/s41598-021-01490-1

[108] Yang C, Wang G, Ma F, Yu B, Chen F, Yang J, et al. Repeated injections of human umbilical cord blood-derived mesenchymal stem cells significantly promotes functional recovery in rabbits with spinal cord injury of two noncontinuous segments. *Stem Cell Research and Therapy*. 2018;**9**:136. DOI: 10.1186/s13287-018-0879-0

[109] Deng YB, Liu XG, Liu ZG, Liu XL, Liu Y, Zhou GQ. Implantation of BM mesenchymal stem cells into injured spinal cord elicits de novo neurogenesis and functional recovery: Evidence from a study in rhesus monkeys. *Cytherapy*. 2006;**8**:210-214. DOI: 10.1080/14653240600760808

[110] Krueger E, Magri LMS, Botelho AS, Bach FS, Rebellato CLK, Fracaro L,

et al. Effects of low-intensity electrical stimulation and adipose derived stem cells transplantation on the time-domain analysis-based electromyographic signals in dogs with SCI. *Neuroscience Letters*. 2019;**696**:38-45. DOI: 10.1016/j.neulet.2018.12.004

[111] Bach FS, Rebelatto CLK, Fracaro L, Senegaglia AC, Fragoso FYI, Daga DR, et al. Comparison of the efficacy of surgical decompression alone and combined with canine adipose tissue-derived stem cell transplantation in dogs with acute thoracolumbar disk disease and spinal cord injury. *Frontiers in Veterinary Science*. 2019;**6**:383. DOI: 10.3389/fvets.2019.00383

[112] Bhat IA, Somal TB SA, Pandey S, Bharti MK, BSK P, MJA V, et al. An allogenic therapeutic strategy for canine spinal cord injury using mesenchymal stem cells. *Journal of Cellular Physiology*. 2019;**234**:2705-2718. DOI: 10.1002/jcp.27086

[113] Wu GH, Shi HJ, Che MT, Huang MY, Wei QS, Feng B, et al. Recovery of paralyzed limb motor function in canine with complete spinal cord injury following implantation of MSC-derived neural network tissue. *Biomaterials*. 2018;**181**:15-34. DOI: 10.1016/j.biomaterials.2018.07.010

[114] Kim EY, Kil TY, Kim MK. Case report: Amniotic fluid-derived mesenchymal stem cell treatment in a dog with a spinal cord injury. *Veterinary and Animal Science*. 2023;**22**:100318. DOI: 10.1016/j.vas.2023.100318

Stem Cell-Based Therapies for Refractory Thin Endometrium in *In vitro* Fertilization: Advances, Challenges, and Future Prospects

Hsuan-Ju Chen and Hsun-Ming Chang

Abstract

Refractory thin endometrium, characterized by a failure of the endometrial lining to achieve optimal thickness despite standard treatments, poses a significant barrier to successful embryo implantation and favorable reproductive outcomes during *in vitro* fertilization (IVF). In recent years, various regenerative therapies have been explored to address this condition. In recent years, regenerative therapies, particularly stem cell-based interventions, have garnered attention for their potential to address this condition. Clinical studies have reported encouraging outcomes using stem cells derived from various sources, including bone marrow, endometrial tissue, and adipose tissue. These therapies have been shown to promote endometrial regeneration, increase endometrial thickness, and enhance fertility outcomes, with intrauterine infusion and subendometrial injection being the primary delivery routes. Additionally, exosomes derived from stem cells have demonstrated regenerative potential in preclinical animal studies, especially when combined with biomaterial scaffolds. This emerging strategy presents a potential alternative treatment for patients with refractory thin endometrium. As a cell-free therapeutic approach, exosome-based therapy may offer significant advantages over traditional stem cell transplantation, such as reduced immunogenicity and a lower risk of tumorigenicity. This emerging strategy holds promise as an alternative treatment option for patients with refractory thin endometrium.

Keywords: thin endometrium, *in vitro* fertilization, regenerative therapy, stem cells, exosome

1. Introduction

In vitro fertilization (IVF) has become a cornerstone of assisted reproductive technology, comprising several essential steps: ovarian stimulation, oocyte retrieval, fertilization, and embryo transfer. Among these, the success of embryo transfer

largely depends on two critical factors: the quality of the embryo and the thickness of the endometrium [1]. Optimal endometrial thickness has been extensively studied, with most evidence indicating that a minimum threshold of 7 mm is associated with higher implantation and pregnancy rates [2, 3].

Clinically, multiple protocols are employed to prepare the endometrium for embryo transfer during IVF treatment, including natural cycle, modified natural cycle, mild stimulation cycle, and artificial (hormone replacement therapy) cycle. These approaches aim to enhance endometrial proliferation either by stimulating endogenous estrogen production (in natural and mild stimulation cycles) or through the administration of exogenous estrogen (in artificial cycles) [4]. Adjunct therapies, such as low-dose aspirin [5], sildenafil citrate [6], and pentoxifylline [7, 8], have also been investigated for their potential to improve endometrial receptivity by enhancing uterine blood flow.

Despite the availability of conventional treatments, a subset of patients fail to attain adequate endometrial thickness, referred to as a refractory thin endometrium. In the context of IVF, the reported prevalence of thin endometrium ranges from 0.7 to 2.5% [9]. Consequently, the prevalence of refractory thin endometrium is presumed to be even lower; however, definitive epidemiological data remain lacking. This condition remains a significant clinical challenge and has prompted interest in regenerative therapies as alternative interventions. Several regenerative strategies, including platelet-rich plasma, growth hormone, granulocyte colony-stimulating factor (G-CSF), and stem cell-based therapies, have been explored with varying degrees of success in clinical settings [10–12].

This article focuses on stem cell-based therapy, exploring its underlying mechanisms of action and current clinical evidence. Additionally, we discuss emerging research on stem cell-derived exosomes, which, although still in the preclinical stage, offer promising potential for tissue regeneration and present several advantages as a novel therapeutic option for patients with refractory thin endometrium.

1.1 Terminology and definition of the endometrial disorders

This section addresses key endometrial conditions, including refractory thin endometrium, atrophic endometrium, and Asherman syndrome. Currently, there is no universally accepted definition that clearly distinguishes refractory thin endometrium from atrophic endometrium. Therefore, we adopt the most commonly cited definitions in the literature and apply these standardized criteria consistently throughout the subsequent sections.

- *Refractory thin endometrium*: Typically defined as an endometrial thickness of less than 7 mm despite adequate estrogen treatment [13].
- *Atrophic endometrium*: Often considered a more severe form, characterized by an even thinner lining, generally measuring less than 5 mm [14, 15].
- *Asherman syndrome*: Characterized by intrauterine adhesions and the loss of normal endometrial function, most commonly resulting from mechanical trauma (e.g., dilation and curettage) or intrauterine infection. Clinical manifestations include intrauterine adhesions, abnormal menstrual patterns, and impaired reproductive outcomes [16, 17].

2. Stem cell therapy for refractory thin endometrium or Asherman syndrome

Stem cells are defined by their ability to self-renew and differentiate into various cell types. Based on their differentiation potential, they are classified into three main categories: totipotent, pluripotent, and multipotent stem cells. Totipotent stem cells, such as the zygote, can differentiate into both embryonic and extra-embryonic tissues. Pluripotent stem cells, exemplified by the inner cell mass of the blastocyst, can give rise to all three embryonic germ layers (ectoderm, mesoderm, and endoderm) but not to extra-embryonic tissues. In contrast, multipotent stem cells have a more limited differentiation capacity, restricted to specific cell lineages. Examples include mesenchymal stem cells (MSCs), hematopoietic stem cells, and endothelial stem cells [18, 19]. Among these, mesenchymal stem cells (derived from the mesoderm) play a central role in regenerative therapies due to their immunomodulatory properties, ability to differentiate into stromal and epithelial cells, and capacity to promote tissue repair through paracrine signaling [19]. MSCs can be isolated from various tissues, including bone marrow, adipose tissue, umbilical cord, and endometrium [20]. Their accessibility and versatility make them promising candidates for stem cell-based therapies targeting refractory thin endometrium and Asherman syndrome.

2.1 Mechanism of stem cell therapy

Several basic and clinical studies have investigated the underlying mechanisms by which stem cell therapy promotes endometrial regeneration. One key mechanism is the differentiation of transplanted stem cells into endometrial progenitor cells. Endometrial progenitor cells, also known as endometrial stem cells, are located in both the functional and basal layers of the endometrium and play a critical role in its cyclic regeneration.

Evidence suggests that bone marrow-derived stem cells (BMDSCs) can also serve as an exogenous source of such endometrial progenitor cells [21, 22]. For example, donor-derived endometrial cells have been identified in recipients following bone marrow transplantation, indicating that BMDSCs possess the ability to migrate to the endometrium and differentiate into epithelial and stromal cells [22]. In addition to cellular differentiation, the paracrine activity of stem cells plays a crucial role in tissue regeneration. Stem cells secrete a variety of bioactive molecules, such as growth factors (hepatocyte growth factor, insulin-like growth factor 1, and vascular endothelial growth factor), cytokines, and extracellular vesicles that contribute to immunomodulation, activation of resident progenitor cells, angiogenesis, and overall enhancement of the endometrial microenvironment [20, 23]. These paracrine effects contribute to tissue repair, cellular proliferation, and endometrial regeneration (**Figure 1**).

The therapeutic efficacy of stem cell therapy in promoting endometrial regeneration under IVF conditions has been demonstrated in several case reports and clinical studies (**Table 1**). Most participants in these studies presented with severe endometrial conditions and had not responded to conventional treatments, including refractory thin endometrium, atrophic endometrium, and Asherman syndrome.

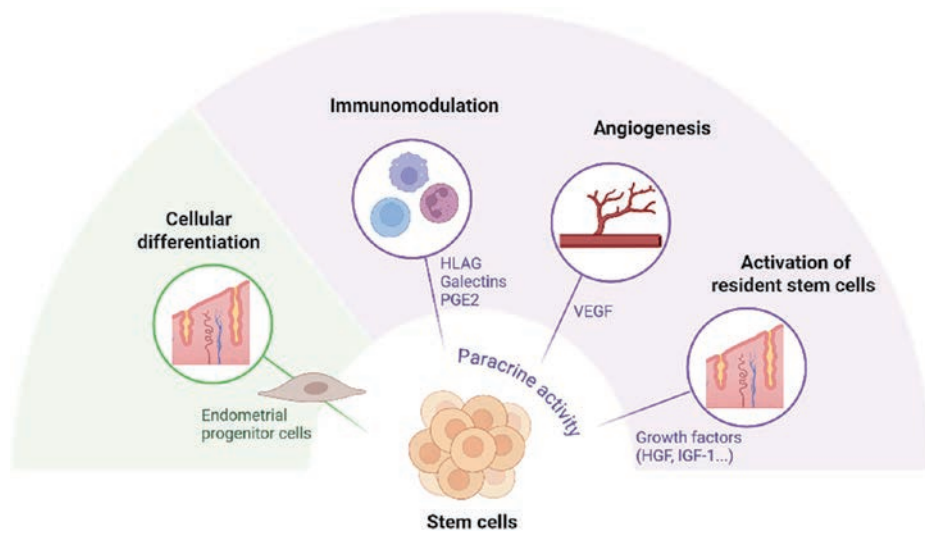


Figure 1.

Mechanisms of stem cell therapy in endometrial regeneration. Stem cell therapy promotes endometrial regeneration through two primary mechanisms: cellular differentiation and paracrine activity. Transplanted stem cells can directly differentiate into endometrial progenitor/stem cells and exert paracrine effects that facilitate immunomodulation, angiogenesis, and activation of resident stem cells. These actions collectively improve the endometrial microenvironment, thereby supporting tissue regeneration, repair, and cellular proliferation. Abbreviation: HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; VEGF, vascular endothelial growth factor; HLA-G, human leukocyte antigen-G; PGE2, prostaglandin E2. <https://BioRender.com/dg5bopz>

Study (year)	Study design	Objective (sample)	Administration route	EMT changes (mm)	Reproductive outcomes
Bone marrow-derived stem cells					
Nagori [24]	Case report	AS with AE (1)	Intrauterine infusion	3.6 → 7.1	Pregnancy <i>via</i> IVF, followed until 8 weeks
Singh [25]	Case series	AS (6)	Subendometrial injection	1.4 → 4.1 [*] (at 3 months)	5/6 resume of the menstrual cycle
Singh [14]	Cohort study	AS (12) AE (13)	Subendometrial injection	3.3 → 5.1 [*] (at 3 months)	6/7 resume of the menstrual cycle 11 ETs: 1 live birth
Santamaria [15]	Cohort study	AS (11) AE (5)	Spiral arterioles <i>via</i> intra-arterial catheterization	4.2 → 5.7 [*]	15/16 resume of the menstrual cycle 14 ETs: 7 pregnancies, 1 live birth
Endometrium-derived stem cells					
Sapozhak [26]	Case report	AE (1)	Subendometrial injection (hysteroscope)	2.0 → 6.3	ET (2 embryos): twin pregnancy, 1 take-home baby
Tersoglio [13]	Cohort study	Refractory thin EM with RIF (29)	Subendometrial injection	5.2 → 9.9 [*]	29 ETs CPR: 23/29 (79.31%) LBR: 10/22 (45.45%)

Study (year)	Study design	Objective (sample)	Administration route	EMT changes (mm)	Reproductive outcomes
Umbilical cord-derived stem cells					
Zhang [27]	Cohort study	AS with refractory thin EM (17)	Intrauterine placement of stem cell-coated scaffolds <i>via</i> a Foley catheter	4.1 → 5.9* (at 3 months)	22 ETs: 3 pregnancies, 2 live births
Menstrual blood-derived stem cells					
Tan [28]	Cohort study	AS (7)	Intrauterine infusion	3.3 → 6.7*	5 ETs: 2 pregnancies
Adipose tissue-derived stem cells					
Sudoma [29]	Cohort study	AE (25)	Subendometrial injection	20/25 increase in EMT	19 ETs: 11 pregnancies

*Significant difference.

EMT: endometrial thickness; AS: Asherman syndrome; EM: endometrium; IVF: in vitro fertilization; AE: Atrophic endometrium; ET: embryo transfer; RIF: recurrent implantation failure; CPR: clinical pregnancy rate; LBR: live birth rate.

Table 1.
 Summary of the clinical use of stem cell therapy in women with thin endometrium or Asherman syndrome.

2.2 Bone marrow stem cells (BMSCs)

As previously mentioned, the bone marrow is a recognized source of endometrial progenitor cells. BMSCs are among the most widely used and extensively studied types of stem cells in regenerative medicine [30].

2.2.1 Intrauterine infusion with BMSCs

In an animal study, heterologous BMSCs were infused into the uterine horns of rats with thin endometrium. The treated rats exhibited significantly increased endometrial thickness, enhanced cellular proliferation, and upregulation of receptivity markers. These improvements were attributed to both the differentiation potential and immunomodulatory properties of BMSCs [31].

The first reported human case of stem cell therapy for infertility caused by Asherman syndrome with associated atrophic endometrium was published in 2011. The patient, who had failed multiple frozen embryo transfer (FET) cycles despite hysteroscopic adhesiolysis and conventional therapies, received autologous bone marrow-derived stem cells. Endometrial angiogenic stem cells were isolated from the aspirated bone marrow, and 39 million cells—positive for CD9, CD44, and CD90 markers—were infused into the uterine cavity following endometrial curettage. Hormonal therapy was continued postinfusion. The patient's baseline endometrial thickness of 3.6 mm expanded to 7.1 mm on the day of donor oocyte embryo transfer, resulting in a clinical pregnancy [24]. This study highlighted the therapeutic potential of intrauterine stem cell infusion combined with mechanical endometrial stimulation.

2.2.2 Subendometrial zone injection with BMSCs

Beyond intrauterine infusion, another delivery method involves direct injection of autologous BMSCs into the subendometrial zone, the basal layer of the endometrium,

where resident stem cells reside. In the case series, 6 patients with Asherman syndrome were recruited; the isolated stem cells were injected into this zone under transvaginal ultrasound guidance using an ovum pickup needle [25]. The infused cells were isolated using the Ficoll density gradient separation method, in which diluted bone marrow was layered over a lymphocyte separation medium, followed by centrifugation to collect the mononuclear cell fraction. The harvested mononuclear cells were subsequently quantified based on CD34 expression, a marker of endothelial progenitor cells known for their roles in angiogenesis, immunomodulation, and anti-fibrotic activity [32]. Three months posttreatment, mean endometrial thickness significantly increased from 1.38 to 4.05 mm. However, further improvement plateaued by the 6th and 9th months. Notably, five out of six patients resumed their menstruation. In this series, genital tuberculosis was the predominant underlying cause of Asherman syndrome, which may have contributed to suboptimal posttreatment endometrial growth (less than 7 mm).

To further evaluate BMSCs therapy in patients with both Asherman syndrome and atrophic endometrium, the same research group conducted a prospective study using the same injection technique and similar stem cell isolation method. Similar growth patterns were observed, and 11 of 25 patients achieved the target endometrial thickness of ≥ 7 mm, allowing progression to IVF. Over a five-year follow-up period, three live births were reported, with one through IVF and two through spontaneous conception [14].

2.2.3 Spiral arterioles administration

Recognizing that the endometrial stem cell niche is located near the endothelial lining of spiral arterioles in the basal layer, researchers developed a novel technique for BMSC delivery *via* intra-arterial catheterization targeting these vessels. In this approach, stem cell mobilization was first induced with four doses of G-CSF, followed by the isolation of mononuclear cells from peripheral blood using a CobeSpectra separator, an apheresis system designed to separate blood components. Furthermore, CD133-positive cells, a marker of endothelial progenitor cells, were selected for administration with a mean cell count of 124.39 million cells [33]. In patients with an atrophic endometrium or Asherman syndrome, this method resulted in a marked improvement in endometrial thickness. Fifteen out of sixteen patients experienced the return of menstruation, although it was transient in some cases. Importantly, one patient achieved a live birth *via* IVF following treatment [15].

2.3 Endometrial stem cells (ESCs)

The endometrium consists of two layers: the functional layer and the basal layer. During each menstrual cycle, the functional layer undergoes dynamic changes, including proliferation, decidualization, and shedding. The basal layer serves as the structural foundation of the endometrium, contributing to tissue regeneration, vascular supply, and support. Stem cells residing in the basal layer are believed to account for the remarkable regenerative capacity of the endometrium [34]. Initially, endometrial stem cells (ESCs) were thought to reside exclusively within the endometrial tissue. However, accumulating evidence interestingly suggests that the bone marrow-derived stem cells may also contribute as an exogenous source [35].

A case report demonstrated the therapeutic potential of autologous stromal ESC transplantation in a woman with an atrophic endometrium and infertility.

Endometrial mesenchymal stem cells were isolated by enzymatic digestion from a biopsy of the endometrium (on day 4 of the menstrual cycle) and expanded to a total of 20 million cells. After sorting and confirming the immunophenotype of the stem cells, the cells were cryopreserved for later use. Following hysteroscopic removal of the intrauterine adhesion, the stem cells were sequentially administered into the sub-endometrial layer. The patient's endometrial thickness increased from 2 to 6.3 mm, and pregnancy was subsequently achieved *via* embryo transfer [26].

Similarly, a cohort reported encouraging reproductive outcomes in women with recurrent implantation failure and refractory thin endometrium following ESC transplantation. In this study, 2.5 to 3.6 million ESCs, diluted in autologous platelet-rich plasma, were injected into the subendometrial layer. The mean endometrial thickness improved significantly, and all participants underwent embryo transfer. Clinical pregnancy and live birth rates were 79% and 45.5%, respectively [13].

2.4 Other sources of stem cells

2.4.1 Umbilical cord mesenchymal stem cells (UCMSCs)

The umbilical cord, a typically discarded neonatal tissue, represents an ideal source of stem cells due to its high proliferative capacity, noninvasive collection process, and relatively high cell yield [27, 36]. A self-controlled prospective study explored the therapeutic efficacy of UCMSCs in patients with refractory thin endometrium (<5.5 mm) secondary to Asherman syndrome. Fresh umbilical cords were obtained from term deliveries. Following the removal of blood, vessels, and the outer membrane, the cords were minced into small fragments and cultured. The stem cell phenotype was confirmed using flow cytometry. A final suspension containing 10 million UCMSCs was applied to a collagen scaffold and transplanted into the uterine cavity *via* an inflated Foley catheter during two consecutive menstrual cycles. Posttreatment assessments revealed improved endometrial morphology and increased endometrial thickness. Fifteen out of 16 patients received embryo transfers, resulting in 2 live births [27].

2.4.2 Menstrual blood-derived stem cells (MenSCs)

In 2007, Meng et al. introduced a novel stem cell source derived from human menstrual fluid [37]. MenSCs share similar proliferative and differentiation potentials with mesenchymal stem cells from other sources while offering additional advantages such as a noninvasive collection process, low immunogenicity, and fewer ethical concerns. Although MenSCs share some characteristics with endometrial stem cells, they are not considered a direct subtype due to differences in their mechanisms of action and therapeutic effects [38]. In murine models, MenSCs have been shown to promote endometrial regeneration and improve fertility through activation of the AKT and ERK signaling pathways [39]. The AKT and ERK signaling pathways are well-characterized intracellular transduction cascades that have been extensively studied in the contexts of tumorigenesis and neural regeneration. These pathways play critical roles in regulating cellular growth, proliferation, and angiogenesis [39–41].

A cohort study evaluated the efficacy of autologous MenSCs in infertile women with severe Asherman syndrome. Menstrual blood was collected using a catheter rinsed with penicillin and streptomycin on the second day of the menstrual cycle. Following isolation and *in vitro* expansion for 14 days, the stem cells were infused

into the uterine cavity after endometrial scratching. All patients exhibited increased endometrial thickness. Among them, five underwent embryo transfer, resulting in two successful pregnancies [28].

2.4.3 Adipose tissue-derived stem cells (ADSCs)

Adipose tissue offers an alternative and accessible stem cell source, obtained through the minimally invasive procedure of liposuction. In one study, autologous adipose tissue was aspirated from the anterior abdominal wall. The stromal vascular fraction (SVF) was isolated by enzymatic digestion using collagenase I, followed by cell culture and selection. Stem cells expressing CD34, a marker associated with the vascular endothelial lineage, were identified and confirmed. After three courses of subendometrial injection of the stem cell suspension (1 million/ml), 19 out of 25 patients with a history of atrophic endometrium achieved an optimal endometrial thickness for embryo transfers. Ultimately, embryo transfer led to 11 successful pregnancies, corresponding to a 57.9% pregnancy rate [29].

2.5 Challenges

Despite the encouraging outcomes reported in case reports and small-scale studies, stem cell therapy for thin or atrophic endometrium and Asherman syndrome remains in the early stages of clinical application. The current evidence is primarily derived from case reports, case series, and cohort studies, which are limited by small sample sizes and methodological heterogeneity, particularly in terms of stem cell origin and routes of administration (**Table 1**). Although these preliminary studies suggest promising regenerative potential, there is a lack of large-scale, randomized controlled trials or comparative studies to robustly validate the efficacy and safety of this therapeutic approach. This limitation hampers the development of standardized clinical protocols.

Several critical challenges remain unresolved. First, the optimal source of stem cells, whether autologous or allogeneic and derived from the endometrium, bone marrow, umbilical cord, menstrual blood, or adipose tissue, has yet to be clearly defined. Each stem cell type differs in terms of yield, proliferative capacity, immunogenicity, and therapeutic mechanism, complicating the decision-making process in clinical practice. Second, the lack of consensus on specific cell surface markers for stem cell isolation and characterization hinders reproducibility and standardization across studies.

Moreover, various delivery methods have been employed, ranging from intra-uterine infusion and subendometrial injection to scaffold-based transplantation, yet no single route has been proven superior in terms of cell retention, engraftment, or therapeutic effect. Identifying the most effective administration strategy is crucial for improving clinical outcomes and minimizing procedural risks.

Another major challenge is the limited understanding of the long-term fate of transplanted stem cells, including their survival, differentiation potential, and risk of undesired effects such as fibrosis, ectopic tissue formation, or tumorigenicity. To ensure the safety of stem cell therapy, long-term follow-up data are essential but currently insufficient.

Finally, regulatory and ethical considerations also pose barriers to widespread clinical adoption. Regulatory frameworks for stem cell-based therapies differ widely

across countries and regions, creating uncertainty in clinical translation. For example, compliance with Good Manufacturing Practice (GMP) is required for clinical-grade stem cell production, yet many current protocols lack GMP-standard validation. Additionally, the use of allogeneic stem cells, particularly from perinatal sources, raises ethical questions regarding donor consent, commercialization, and long-term donor-recipient safety. Ensuring ethical sourcing, traceability, and oversight will be essential for maintaining public trust and regulatory approval. Harmonizing standards for cell processing, storage, transportation, and clinical use remains an unmet need in the field.

In summary, while stem cell therapy holds great promise for endometrial regeneration and fertility restoration, further high-quality research or comparative studies are urgently needed to establish standardized treatment and cell isolation protocols, ensure long-term safety, and define patient populations who would benefit most from this novel therapeutic approach.

3. Exosome derived from stem cells

In recent years, exosomes derived from stem cells have garnered growing attention in the field of regenerative medicine. Exosomes are small extracellular vesicles, typically 30–150 nm in diameter, enclosed by a single lipid bilayer and released by various cell types, including stem cells [42, 43]. Functioning as intercellular communication messengers, exosomes carry a diverse cargo of bioactive molecules, including microRNA, peptide hormones, proteins, and lipids [44]. Exosomes derived from stem cells exhibit therapeutic effects comparable to those of their parent cells. However, they offer additional advantages, including lower immunogenicity, reduced risk of tumor formation, and improved safety and stability, making them an attractive alternative to direct stem cell transplantation [42]. Currently, the use of stem cell-derived exosomes for the treatment of injured or atrophic endometrium is under active investigation in preclinical studies, primarily through *in vitro* experiments and animal models. These studies aim to elucidate the underlying mechanisms and evaluate the regenerative potential of exosomes in promoting endometrial repair. However, it is important to recognize the translational gap between these models and human clinical applications. Differences in endometrial structure, hormone responsiveness, immune interactions, and tissue remodeling capacity between species may affect therapeutic outcomes. Thus, while preliminary results are promising, further research in humans is needed to confirm their efficacy, determine optimal administration strategies, and assess long-term safety before clinical application can be realized.

3.1 Mechanism for endometrial repair and regeneration

3.1.1 Suppression of fibrotic processes

Several animal studies have shown that stem cell-derived exosomes facilitate endometrial regeneration by downregulating the TGF- β /Smad signaling pathway, a key regulator of tissue fibrosis [45]. In this pathway, TGF- β transmits signals through its receptor, leading to the activation of Smad proteins, which subsequently regulate gene transcription. The TGF- β /Smad axis plays a critical role in wound healing by promoting angiogenesis and remodeling of the extracellular matrix (ECM). This is achieved through the upregulation of vascular endothelial growth factor (VEGF) and

the enhancement of fibroblast-mediated production of collagen, matrix metalloproteinases (MMPs), fibronectin, and other ECM components [46].

In a rabbit model of endometrial injury, exosomes derived from BMSCs promoted endometrial repair and reversed epithelial-to-mesenchymal transition (EMT), primarily through the inhibition of the TGF- β /Smad signaling pathway [47]. Similarly, in a rat model of uterine injury, BMSC-derived exosomes transferred miR-340, which suppressed the expression of TGF- β and α -smooth muscle actin (α -SMA), two key mediators of fibrosis, thereby exerting an anti-fibrotic effect [48]. Another study revealed that miR-29a, delivered *via* BMSC-derived exosomes, downregulated α -SMA and Smad proteins, further supporting the anti-fibrotic role of exosome microRNAs [49]. Comparable findings were observed in studies using human umbilical cord mesenchymal stem cell (UCMSC)-derived exosomes, both *in vitro* and *in vivo*, demonstrating consistent suppression of fibrotic markers and improvement in endometrial histology [50]. Additionally, exosomes contribute to collagen remodeling and extracellular matrix degradation, which are critical for reversing fibrosis. In a rat model of Asherman syndrome, administration of uterine-derived mesenchymal stem cell exosomes enhanced endometrial proliferation and neovascularization. This was accompanied by elevated expression of matrix metalloproteinases (MMP-2 and MMP-9), enzymes essential for ECM degradation [51]. Another study identified miR-202-3p, delivered *via* UCMSC-derived exosomes, as a regulator of MMP11 expression, further promoting ECM remodeling in an injured rat uterus [52].

3.1.2 Downregulation of inflammatory responses

Stem cell-derived exosomes also play a crucial role in attenuating inflammation during endometrial regeneration. In an *in vitro* model using injured mouse endometrial epithelial cells, exosomes from human UCMSCs were shown to restore cell viability and reduce inflammation. This effect was attributed to the downregulation of proinflammatory cytokines, including IL-1 β , IL-6, and TNF- α . [53] Furthermore, exosomes exhibit immunomodulatory properties that contribute to tissue repair. In a rat model of endometrial injury, UCMSC-derived exosomes promoted regeneration and fertility restoration by modulating macrophage polarization. Specifically, the exosomes facilitated the transition to an M2 macrophage phenotype, which is associated with anti-inflammatory responses and increased production of anti-inflammatory cytokines [54].

3.2 The combination of exosomes and biomaterial scaffolds

In recent years, the integration of exosomes with biomaterial scaffolds has emerged as a promising strategy in regenerative medicine, including for the treatment of endometrial injury. Biomaterials such as hyaluronic acid, collagen, and hydrogels offer structural support with excellent biocompatibility, biodegradability, and tunable physical properties (**Figure 2**). Key properties that enhance exosome delivery include porosity, degradation rate, mechanical strength and elasticity, and surface chemistry. Porosity, which allows for nutrient and oxygen exchange and facilitates cell migration and vascular infiltration. Degradation rate, which can be engineered to match the healing timeline of the injured tissue, ensuring sustained exosome release. Mechanical strength and elasticity, which help maintain scaffold integrity and mimic the biomechanical properties of the uterine environment. Surface chemistry can be modified to enhance exosome loading, retention, and interaction with surrounding

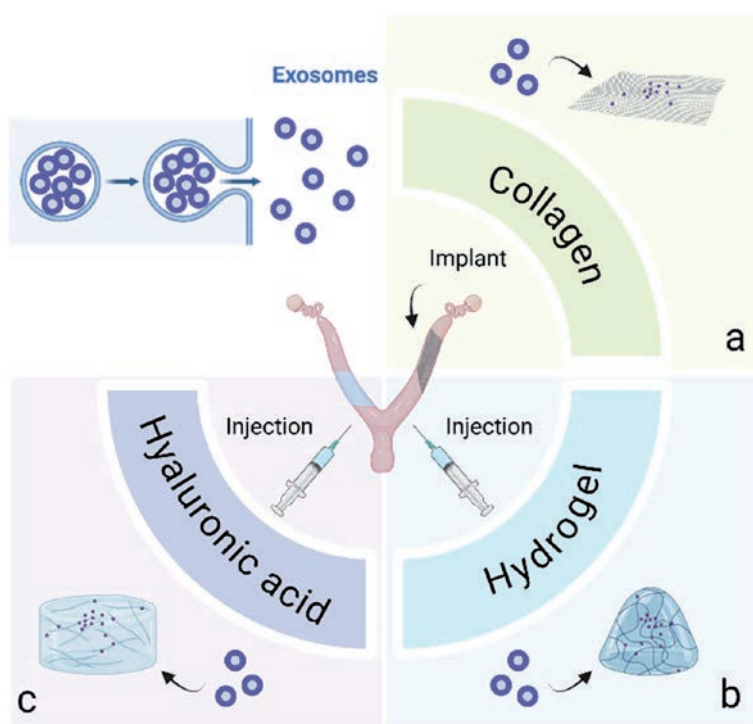


Figure 2. Strategies for exosome delivery using biomaterials in endometrial regeneration: (a) Exosome delivery to the endometrial surface using a collagen scaffold. (b) Intrauterine delivery of exosomes encapsulated within a hydrogel. (c) The intrauterine delivery of exosomes loaded and incorporated into a hyaluronic acid-based gel or mucus formulation with hyaluronic acid-based gel or mucus. <https://BioRender.com/xvnh93s>

tissues. When combined with stem cell-derived exosomes, these scaffolds serve as effective delivery systems with appropriate and stable degradation rates, enhancing exosome stability, sustained release, and localized retention at the injury site. This synergistic approach can significantly enhance cell migration, adhesion, proliferation, and tissue regeneration [43].

The following are representative examples of studies utilizing combinations of exosomes and biomaterial scaffolds for endometrial regeneration:

In a rat model of uterine injury, UCMSC-derived exosomes were delivered to the endometrial surface using a collagen scaffold. (**Figure 2a**) Both the exosome-only group and the collagen-exosome combination group showed enhanced endometrial regeneration and fertility restoration, demonstrating the scaffold's role in improving therapeutic delivery and efficacy [54].

Two additional animal studies investigated the therapeutic potential of hydrogel-based systems (**Figure 2b**). Lin et al. designed a silver ion (Ag^+)-infused hydrogel with antimicrobial properties, incorporating adipose-derived stem cell exosomes [55]. Liang et al. used an alginate-based hydrogel to encapsulate exosomes derived from mouse decidual stromal cells [56]. In both studies, intrauterine injection of the exosome-loaded hydrogels resulted in significant endometrial regeneration, angiogenesis, improved endometrial receptivity, and restoration of fertility [55, 56].

Moreover, hyaluronic acid (HA)-based biomaterials have also shown encouraging outcomes (**Figure 2c**). Zhang et al. employed an HA-based gel with

UCMSC-derived exosomes, leading to enhanced endometrial thickness and tissue repair [50]. Li et al. used an HA-based mucus formulation coated with exosomes derived from urine- and adipose-derived stem cells [57]. Posttreatment analyses revealed increased endometrial thickness and improved reproductive outcomes, which were attributed to the suppression of fibrosis and inflammatory responses [50, 57].

These findings support the potential of exosome-biomaterial systems as a promising strategy for treating intrauterine damage and enhancing reproductive success. Nevertheless, their translation into clinical practice remains contingent on addressing key challenges, including standardization of exosome isolation and characterization, scalable production, long-term safety evaluation, and confirmation of efficacy in human trials. Bridging this translational gap will require well-designed clinical studies that account for species-specific differences in endometrial physiology and regeneration capacity.

4. Conclusion

Preclinical and clinical studies collectively indicate that stem cell therapy holds considerable promise for endometrial regeneration, particularly in patients with thin endometria or intrauterine adhesions. Stem cells exert their therapeutic effects through a variety of mechanisms, including paracrine signaling, immunomodulation, anti-fibrotic actions, and the activation and differentiation of endogenous endometrial progenitor cells. To date, BMSCs and ESCs have been the most commonly utilized in clinical practice. However, increasing attention is being directed toward minimally invasive and ethically favorable alternatives, such as UCMSCs, MenSCs, and ADSCs. These sources offer advantages including ease of collection, high proliferative potential, and reduced ethical concerns.

Despite variability in stem cell isolation, culture, and transplantation methods, the majority of clinical case reports and small cohort studies have reported favorable outcomes, notably increased endometrial thickness, resumption of menstruation, and enhanced fertility outcomes, particularly in assisted reproductive technology settings. Nevertheless, the lack of large-scale randomized controlled trials, standardized cell dosages, and long-term safety data continues to limit the widespread clinical adoption of these therapies. In parallel, exosomes derived from stem cells have emerged as a compelling cell-free therapeutic alternative, capable of delivering similar regenerative signals with lower immunogenicity and tumorigenic risk. Animal studies have demonstrated their efficacy in reducing fibrosis, modulating inflammation, and promoting angiogenesis and endometrial repair. Furthermore, the integration of exosomes with biomaterial scaffolds, such as collagen, hydrogels, and hyaluronic acid-based systems, has further enhanced their therapeutic potential. These delivery platforms improve exosome retention, bioavailability, and targeted release, leading to more robust tissue regeneration and restoration of reproductive function in preclinical models.

Looking ahead, continued research is needed to optimize cell sourcing, define standardized protocols, and evaluate long-term outcomes and safety in human trials. Nonetheless, stem cell-based and exosome-based strategies collectively represent a transformative frontier in regenerative medicine for uterine disorders, offering new hope to women facing infertility due to endometrial damage.

Acknowledgements

This work was funded by the China Medical University Hospital Research Foundation (DMR-113-080). Additionally, the research received support from the National Science and Technology Council (NSTC 113-2314-B-039-055).

Conflict of interest

The authors declare no conflict of interest.

Author details

Hsuan-Ju Chen and Hsun-Ming Chang*
Department of Obstetrics and Gynecology, China Medical University Hospital,
Taichung, Taiwan

*Address all correspondence to: changobs@hotmail.com

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Taylor HS, Pal L, Seli E. Speroff's Clinical Gynecologic Endocrinology and Infertility, 9th edition. Philadelphia, PA: Lippincott Williams & Wilkins, a Wolters Kluwer business; 2020
- [2] Bozdag G, Esinler I, Yarali H. The impact of endometrial thickness and texture on intracytoplasmic sperm injection outcome. *The Journal of Reproductive Medicine*. 2009;**54**(5):303-311
- [3] Kasius A, Smit JG, Torrance HL, Eijkemans MJ, Mol BW, Opmeer BC, et al. Endometrial thickness and pregnancy rates after IVF: A systematic review and meta-analysis. *Human Reproduction Update*. 2014;**20**(4):530-541
- [4] Zhang Y, Fu X, Gao S, Gao S, Ma J, et al. Preparation of the endometrium for frozen embryo transfer: An update on clinical practices. *Reproductive Biology and Endocrinology*. 2023;**21**(1):52
- [5] Hsieh YY, Tsai HD, Chang CC, Lo HY, Chen CL. Low-dose aspirin for infertile women with thin endometrium receiving intrauterine insemination: A prospective, randomized study. *Journal of Assisted Reproduction and Genetics*. 2000;**17**(3):174-177
- [6] Li X, Luan T, Zhao C, Zhang M, Dong L, Su Y, et al. Effect of sildenafil citrate on treatment of infertility in women with a thin endometrium: A systematic review and meta-analysis. *The Journal of International Medical Research*. 2020;**48**(11):300060520969584
- [7] Acharya S, Yasmin E, Balen AH. The use of a combination of pentoxifylline and tocopherol in women with a thin endometrium undergoing assisted conception therapies--a report of 20 cases. *Human Fertility (Cambridge, England)*. 2009;**12**(4):198-203
- [8] Krief F, Simon C, Goldstein R, Ellenberg LP, Ledee N. Efficacy of tocopherol and pentoxifylline combined therapy for women undergoing assisted reproductive treatment with poor endometrial development: A retrospective cohort study on 143 patients. *Human Fertility (Cambridge, England)*. 2021;**24**(5):367-375
- [9] Liu KE, Hartman M, Hartman A. Management of thin endometrium in assisted reproduction: A clinical practice guideline from the Canadian fertility and andrology society. *Reproductive Biomedicine Online*. 2019;**39**(1):49-62
- [10] Mouanness M, Ali-Bynom S, Jackman J, Seckin S, Merhi Z. Use of intra-uterine injection of platelet-rich plasma (PRP) for endometrial receptivity and thickness: A literature review of the mechanisms of action. *Reproductive Sciences (Thousand Oaks, Calif)*. 2021;**28**(6):1659-1670
- [11] Shang Y, Wu M, He R, Ye Y, Sun X. Administration of growth hormone improves endometrial function in women undergoing in vitro fertilization: A systematic review and meta-analysis. *Human Reproduction Update*. 2022;**28**(6):838-857
- [12] Xie Y, Zhang T, Tian Z, Zhang J, Wang W, Zhang H, et al. Efficacy of intrauterine perfusion of granulocyte colony-stimulating factor (G-CSF) for infertile women with thin endometrium: A systematic review and meta-analysis. *American Journal of Reproductive Immunology*. 2017;**78**(2):e12701-e12710

- [13] Tersoglio AE, Tersoglio S, Salatino DR, Castro M, Gonzalez A, Hinojosa M, et al. Regenerative therapy by endometrial mesenchymal stem cells in thin endometrium with repeated implantation failure. A novel strategy. *JBRA Assisted Reproduction*. 2020;**24**(2):118-127
- [14] Singh N, Shekhar B, Mohanty S, Kumar S, Seth T, Girish B. Autologous bone marrow-derived stem cell therapy for Asherman's syndrome and endometrial atrophy: A 5-year follow-up study. *Journal of Human Reproductive Sciences*. 2020;**13**(1):31-37
- [15] Santamaria X, Cabanillas S, Cervelló I, Arbona C, Raga F, Ferro J, et al. Autologous cell therapy with CD133+ bone marrow-derived stem cells for refractory Asherman's syndrome and endometrial atrophy: A pilot cohort study. *Human Reproduction* (Oxford, England). 2016;**31**(5):1087-1096
- [16] Conforti A, Alviggi C, Mollo A, De Placido G, Magos A. The management of Asherman syndrome: A review of literature. *Reproductive Biology and Endocrinology: RB&E*. 2013;**11**:118
- [17] Yu D, Wong YM, Cheong Y, Xia E, Li TC. Asherman syndrome--one century later. *Fertility and Sterility*. 2008;**89**(4):759-779
- [18] Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: Past, present, and future. *Stem Cell Research & Therapy*. 2019;**10**(1):68
- [19] Hoang DM, Pham PT, Bach TQ, Ngo ATL, Nguyen QT, Phan TTK, et al. Stem cell-based therapy for human diseases. *Signal Transduction and Targeted Therapy*. 2022;**7**(1):272
- [20] Gharibeh N, Aghebati-Maleki L, Madani J, Pourakbari R, Yousefi M, Ahmadian HJ. Cell-based therapy in thin endometrium and Asherman syndrome. *Stem Cell Research & Therapy*. 2022;**13**(1):33
- [21] Du H, Taylor HS. Contribution of bone marrow-derived stem cells to endometrium and endometriosis. *Stem Cells*. 2007;**25**(8):2082-2086
- [22] Taylor HS. Endometrial cells derived from donor stem cells in bone marrow transplant recipients. *Journal of the American Medical Association*. 2004;**292**(1):81-85
- [23] Jimenez-Puerta GJ, Marchal JA, López-Ruiz E, Gálvez-Martín P. Role of mesenchymal stromal cells as therapeutic agents: Potential mechanisms of action and implications in their clinical use. *Journal of Clinical Medicine*. 2020;**9**(2)
- [24] Nagori CB, Panchal SY, Patel H. Endometrial regeneration using autologous adult stem cells followed by conception by in vitro fertilization in a patient of severe Asherman's syndrome. *Journal of Human Reproductive Sciences*. 2011;**4**(1):43-48
- [25] Singh N, Mohanty S, Seth T, Shankar M, Bhaskaran S, Dharmendra S. Autologous stem cell transplantation in refractory Asherman's syndrome: A novel cell based therapy. *Journal of Human Reproductive Sciences*. 2014;**7**(2):93-98
- [26] Sapozhak IM, Gubar OS, Rodnichenko AE, Zlatska AV. Application of autologous endometrial mesenchymal stromal/stem cells increases thin endometrium receptivity: A case report. *Journal of Medical Case Reports*. 2020;**14**(1):190
- [27] Zhang Y, Shi L, Lin X, Zhou F, Xin L, Xu W, et al. Unresponsive thin endometrium caused by Asherman syndrome treated with umbilical cord

mesenchymal stem cells on collagen scaffolds: A pilot study. *Stem Cell Research & Therapy*. 2021;**12**(1):420

[28] Tan J, Li P, Wang Q, Li Y, Li X, Zhao D, et al. Autologous menstrual blood-derived stromal cells transplantation for severe Asherman's syndrome. *Human Reproduction (Oxford, England)*. 2016;**31**(12):2723-2729

[29] Sudoma IPL, Kremenska Y, Goncharova Y. Application of autologous adipose-derived stem cells for thin endometrium treatment in patients with failed ART programs. *Journal of Stem Cell Therapy and Transplantation*. 2019;**3**:001-008

[30] Poliwoda S, Noor N, Downs E, Schaaf A, Cantwell A, Ganti L, et al. Stem cells: A comprehensive review of origins and emerging clinical roles in medical practice. *Orthopedic Reviews (Pavia)*. 2022;**14**(3):37498

[31] Zhao J, Zhang Q, Wang Y, Li Y. Uterine infusion with bone marrow mesenchymal stem cells improves endometrium thickness in a rat model of thin endometrium. *Reproductive Sciences (Thousand Oaks, Calif)*. 2015;**22**(2):181-188

[32] Hassanpour M, Salybekov AA, Kobayashi S, Asahara T. CD34 positive cells as endothelial progenitor cells in biology and medicine. *Frontiers in Cell and Development Biology*. 2023;**11**:1128134-1128145

[33] Veljković D, Nonković OŠ, Radonjić Z, Kuzmanović M, Zečević Ž. Bone marrow processing for transplantation using Cobe spectra cell separator. *Transfusion and Apheresis Science*. 2013;**48**(3):359-363

[34] Verdi J, Tan A, Shoaee-Hassani A, Seifalian AM. Endometrial stem cells

in regenerative medicine. *Journal of Biological Engineering*. 2014;**8**(1):20

[35] Hong IS. Endometrial stem/progenitor cells: Properties, origins, and functions. *Genes & Diseases*. 2023;**10**(3):931-947

[36] Bieback K, Brinkmann I. Mesenchymal stromal cells from human perinatal tissues: From biology to cell therapy. *World Journal of Stem Cells*. 2010;**2**(4):81-92

[37] Meng X, Ichim TE, Zhong J, Rogers A, Yin Z, Jackson J, et al. Endometrial regenerative cells: a novel stem cell population. *Journal of Translational Medicine*. 2007;**5**:57

[38] Chen L, Qu J, Cheng T, Chen X, Xiang C. Menstrual blood-derived stem cells: Toward therapeutic mechanisms, novel strategies, and future perspectives in the treatment of diseases. *Stem Cell Research & Therapy*. 2019;**10**(1):406

[39] Zhang Y, Lin X, Dai Y, Hu X, Zhu H, Jiang Y, et al. Endometrial stem cells repair injured endometrium and induce angiogenesis via AKT and ERK pathways. *Reproduction*. 2016;**152**(5):389-402

[40] Cao Z, Liao Q, Su M, Huang K, Jin J, Cao D. AKT and ERK dual inhibitors: The way forward? *Cancer Letters*. 2019;**459**:30-40

[41] Rhim J, Luo X, Gao D, Xu X, Zhou T, Li F, et al. Cell type-dependent Erk-Akt pathway crosstalk regulates the proliferation of fetal neural progenitor cells. *Scientific Reports*. 2016;**6**(1):26547

[42] Tan F, Li X, Wang Z, Li J, Shahzad K, Zheng J. Clinical applications of stem cell-derived exosomes. *Signal Transduction and Targeted Therapy*. 2024;**9**(1):17

- [43] Liu Y, Jia D, Li L, Wang M. Advances in Nanomedicine and biomaterials for endometrial regeneration: A comprehensive review. *International Journal of Nanomedicine*. 2024;**19**:8285-8308
- [44] Tabeeva G, Silachev D, Vishnyakova P, Asaturova A, Fatkhudinov T, Smetnik A, et al. The therapeutic potential of multipotent mesenchymal stromal cell-derived extracellular vesicles in endometrial regeneration. *International Journal of Molecular Sciences*. 2023;**24**(11):9431-9448
- [45] Hu H-H, Chen D-Q, Wang Y-N, Feng Y-L, Cao G, Vaziri ND, et al. New insights into TGF- β /Smad signaling in tissue fibrosis. *Chemico-Biological Interactions*. 2018;**292**:76-83
- [46] Deng Z, Fan T, Xiao C, Tian H, Zheng Y, Li C, et al. TGF- β signaling in health, disease and therapeutics. *Signal Transduction and Targeted Therapy*. 2024;**9**(1):61
- [47] Yao Y, Chen R, Wang G, Zhang Y, Liu F. Exosomes derived from mesenchymal stem cells reverse EMT via TGF- β 1/Smad pathway and promote repair of damaged endometrium. *Stem Cell Research & Therapy*. 2019;**10**(1):225
- [48] Xiao B, Zhu Y, Huang J, Wang T, Wang F, Sun S. Exosomal transfer of bone marrow mesenchymal stem cell-derived miR-340 attenuates endometrial fibrosis. *Biology Open*. 2019;**8**(5):39958-39966
- [49] Tan Q, Xia D, Ying X. miR-29a in exosomes from bone marrow mesenchymal stem cells inhibit fibrosis during endometrial repair of intrauterine adhesion. *International Journal of Stem Cells*. 2020;**13**(3):414-423
- [50] Zhang S, Wang D, Yang F, Shen Y, Li D, Deng X. Intrauterine injection of umbilical cord mesenchymal stem cell exosome gel significantly improves the pregnancy rate in thin endometrium rats. *Cell Transplantation*. 2022;**31**:9636897221133345
- [51] Saribas GS, Ozogul C, Tiryaki M, Alpaslan Pinarli F, Hamdemir KS. Effects of uterus derived mesenchymal stem cells and their exosomes on asherman's syndrome. *Acta Histochemica*. 2020;**122**(1):151465
- [52] Wang S, Liu T, Nan N, Lu C, Liang M, Wang S, et al. Exosomes from human umbilical cord mesenchymal stem cells facilitates injured endometrial restoring in early repair period through miR-202-3p mediating formation of ECM. *Stem Cell Reviews and Reports*. 2023;**19**(6):1954-1964
- [53] Liang L, Wang L, Zhou S, Li J, Meng L, Zhang H, et al. Exosomes derived from human umbilical cord mesenchymal stem cells repair injured endometrial epithelial cells. *Journal of Assisted Reproduction and Genetics*. 2020;**37**(2):395-403
- [54] Xin L, Lin X, Pan Y, Zheng X, Shi L, Zhang Y, et al. A collagen scaffold loaded with human umbilical cord-derived mesenchymal stem cells facilitates endometrial regeneration and restores fertility. *Acta Biomaterialia*. 2019;**92**:160-171
- [55] Lin J, Wang Z, Huang J, Tang S, Saiding Q, Zhu Q, et al. Microenvironment-protected exosome-hydrogel for facilitating endometrial regeneration, fertility restoration, and live birth of offspring. *Small (Weinheim an der Bergstrasse, Germany)*. 2021;**17**(11):e2007235
- [56] Liang Y, Shuai Q, Zhang X, Jin S, Guo Y, Yu Z, et al. Incorporation of

Decidual stromal cells derived exosomes in sodium alginate hydrogel as an innovative therapeutic strategy for advancing endometrial regeneration and reinstating fertility. *Advanced Healthcare Materials*. 2024;**13**(13):e2303674

[57] Li C, Tan X, Deng D, Kong C, Feng L, Wang W, et al. A dopamine-modified hyaluronic acid-based mucus carrying phytoestrogen and urinary exosome for thin endometrium repair. *Advanced Materials (Deerfield Beach, Fla)*. 2024;**36**(39):e2407750

Transplantation of MSCs for a Longstanding Engraftment and Maintenance of Bone Marrow Stroma

Aleksei E. Bigildeev

Viam supervadet vadens

Abstract

The modern concept of allogeneic hematopoietic stem cell transplantation (alloHSCT) implies the transfer of the donor's hematopoietic system exclusively, the essence of which is the infusion of hematopoietic stem cells (HSCs) in the form of a single-cell suspension into the body of the recipient, who is previously subjected to intensive high-dose chemotherapy in order to eradicate tumor cells and destroy his own hematopoiesis, including immune cells (to exclude immunological conflict). This concept does not include transplantation of BM stroma, because the results of early works dating back to the 70–80s of the twentieth century spoke in favor of the fact that mesenchymal stem cells (MSCs) and their progeny are not capable of transplantation. Today, there are strong reasons to state that such an assumption is wrong. Despite the low engraftment efficiency of MSCs demonstrated in the early studies, alternative targeted strategies could lead to successful MSC transplantation. In this chapter, the question of the possibility of BM stroma transplantation is considered, arguments in favor of the expediency of co-transplantation of MSCs and HSCs in the therapy of blood system diseases are given, and a new concept of co-transplantation of BM stroma and the hematopoietic system is proposed.

Keywords: bone marrow transplantation, MSCs, MSC transplantation, MSCs and HSCs co-transplantation, alternative concept of bone marrow transplantation, bone marrow stroma damage, MSCs stable engraftment

1. Introduction

Allogeneic BM transplantation (alloBMT) is widely used for the therapy of a whole range of malignant hematologic diseases, primarily acute leukemia and aggressive lymphomas, as well as other diseases of the blood system, such as myelodysplastic syndrome (MDS) and aplastic anemia (AA). It is often the only way to

achieve long-term remission in a patient or even cure the disease. There are several graft sources available for alloBMT, namely primary whole BM (BM), BM primed with granulocyte colony growth factor (G-CSF), mobilized CD34⁺ hematopoietic stem cells (HSCs), and umbilical cord blood HSCs. The current trend in hematology is that mobilized CD34⁺ cells are used progressively more often than other sources. These cells are mobilized from the donor's BM into the peripheral blood (PB) using several stimulants and collected by apheresis. Compared to the whole BM as a source of graft, this approach has a number of advantages, including low invasiveness for a donor, no traumatization of the donor, the ability to control the cellularity of the transplant by selecting the optimal protocols of mobilization and apheresis, and the ability to prepare for collection of the cells on an outpatient basis. At the same time, this approach excludes transplantation of the stromal component of BM. After infusion of donor CD34⁺ cells, the hematopoietic tissue of the BM and immune system is replaced by donor hematopoietic cells, but the stromal tissue of the BM remains the recipient's own. There are a number of fundamental problems associated with this approach, since the recipient's own stroma is allogeneic to the hematopoietic graft, partially damaged by previous chemotherapy, and its properties are altered by tumor cells, which may lead to ineffective reconstitution of hematopoiesis or poor graft function and even graft failure. Moreover, the preserved host stroma can support the survival of leukemia stem cells (LSCs), which are a source of relapses and refractory course of the disease. Thus, there are significant arguments in favor of transplanting not only hematopoietic but also stromal tissue of the BM from the same donor. This will reduce the incidence of allogeneic graft failures, relapses, and refractory course of the disease and, ultimately, will increase the long-term survival of patients or will lead to the cure of those diseases that are currently considered to be incurable. Successful BM stroma transplantation will also increase the treatment efficiency of diseases associated with bone, fat, and cartilage dysfunction, as early attempts to use mesenchymal stromal cells showed promising but short-term clinical responses [1–3].

Despite the high relevance of BM stroma transplantation, to date, among hematologists, there is an entrenched view that stroma transplantation is impossible. This opinion is based on the results of early studies dating back to the 70–80s of the twentieth century stating that mesenchymal stem cells (MSCs) and their progeny are not capable of transplantation, at least via intravenous injection in the form of a single-cell suspension. Today, there are strong reasons to state that such a view is wrong. In this chapter, the question of the possibility of BM stroma transplantation is considered, arguments in favor of the expediency of co-transplantation of MSCs and HSCs in the therapy of blood system diseases are given, and a new concept of co-transplantation of BM stroma and the hematopoietic system is proposed.

2. Early studies stating that BM stroma is not transplantable

There are several key seminal studies in which the question of BM stroma transplantability was considered, and the authors concluded that the answer is “no.”

Several studies on this subject are common in their design. They investigated donor chimerism in plastic-adherent stromal cell layers of long-term bone marrow cultures (LTBMCs) developed from the BM of patients with different hematologic malignancies after HLA-identical or compatible alloBMT from donors

of the opposite sex [4–6]. The authors stated that stromal cells within LTBMCS were exclusively of host origin. An analogous approach was implemented in a mouse model [7]. The authors did not detect donor cells in the stromal sublayer of LTBMCS derived from the BM of mice that had been previously irradiated and then transplanted with BM from donor mice whose genome contained the marker chromosome T6.

Attempts to clarify the transplantability of BM stroma were also made later, using other methods, in particular, *in situ* hybridization of a probe specific to the Y chromosome. It was used in trepan biopsies of the BM from patients who underwent alloBMT from a related HLA-identical donor of the opposite sex [8]. BM trepan biopsies were obtained from patients on days 3–203 after infusion of donor BM, fixed in formalin, decalcified, and hybridized with a Y-chromosome-specific DNA probe, and the probe was further visualized using two independent methods, and its presence in stromal and hematopoietic cells was assessed by optical microscopy. The advantage of this study is that the assessment of stromal cells' identity does not require *in vitro* cell culture under conditions far from physiologic, and it is possible to study cells in their natural anatomical environment, taking into account their morphology. The authors concluded that the cells of the stromal system (adipocytes, fibroblasts, osteoblasts, osteocytes, and endothelial cells) in BM grafts were of host origin. The authors hypothesized that engraftment of BM stromal cell precursors did not occur as a result of alloBMT, in which the source of cells is BM.

Thus, we state that in most of the studies published in the 70s and 80s of the twentieth century, both on animal models and in studies involving humans, it was shown that in BM transplantations, in which a single-cell suspension of total BM cells without separation into fractions of hematopoietic and non-hematopoietic cells was used as a source of graft, donor stromal cells were not detected either in the primary BM of recipients after alloBMT or in the stromal sublayers of LTBMCS obtained from such BM. Although these studies were conducted on a methodological level that corresponded to the level of technological development of that time, they had several methodological flaws, such as insufficient sensitivity and utilizing cultured cells instead of primary BM.

The given analysis of studies concerning MSC and BM stroma transplantation allows us to formulate several key theses, which are most often given as reasons why stroma transplantation is impossible:

- Nonspecific retention of MSCs in the lungs immediately after injection;
- Absence of MSCs in the allogeneic recipient early after injection;
- Absence of the mechanism of MSC homing to the BM;
- Loss of MSC functions due to cell dissociation;
- Absence of immune privileges of stromal cells in MSCs;

Further, we will consider experimental data on which these theses are based, and we will give data from modern research that show that the given statements are erroneous or, at least, can be questioned, and obstacles on the way to of MSC transplantation can be overcome.

3. Re-evaluation of basic statements about the non-transplantability of BM stroma

3.1 Nonspecific retention of MSCs in the lungs immediately after injection?

The statement that MSCs are nonspecifically retained in lung tissue is based on the studies in which BM mesenchymal cells were labeled with a radioisotope marker and then injected intravenously into the recipient, and their fate was monitored [9]. The authors observed that after intravenous injection of labeled cells, which in this study were cultured mesenchymal stromal cells (which we will abbreviate hereinafter as “MCs,” and not primary mesenchymal stem cells of BM existing *in vivo*, which we denote here as “MSCs”), they, through the small circle of blood circulation, got nonspecifically stuck in the lungs, where they were detected 1 hour after intravenous injection. During the first 24 hours after infusion, MCs remained in the lungs, and after 72 hours, they could not be detected in the recipient’s body. The same authors subsequently published a review [10], in which they noted that most of the studies that investigated the ability of stromal cells to transplant used *in vitro* cultured MCs, which at that time were called “MSCs,” as an object of observation, and that the low ability of such cells to transplant may be associated with changes in their size, morphology, and surface phenotype, including activation of adhesion molecule expression as a result of *in vitro* cultivation. Indeed, it is known that cultivation of BM stromal cells on plastic leads to the fact that over time, small round-shaped cells turn into large spindle-shaped cells [11]. In another study, cultured MCs have been shown to become as large as 19 μm in diameter [12]. Since this diameter is larger than the size of pulmonary microcapillaries (5–10 μm), it is not surprising that such cells are retained in the lungs after intravenous injection. Besides, in one of the early studies, it was experimentally proved that pre-culturing reduces the ability of “MSCs” to migrate through the basal membrane [13], which creates difficulties for the migration of these cells. Considerations about the ratio of the linear size of cells and the diameter of microcapillaries in the lungs are important when considering the ability of MSCs to overcome this barrier, in light of the fact that “very small embryonic-like stem cells” (VSELs) are known. These cells have been isolated from mouse BM as a population of Sca-1⁺lin[−]CD45[−] cells expressing SSEA-1, Oct-4, Nanog, and Rex-1 markers characteristic of pluripotent stem cells. VSELs constitute 0.02% of BM mononuclear cells and are characterized by a small size (2–4 μm), smaller than the diameter of pulmonary capillaries [14]. In addition, VSELs express CXCR4 and thus are potentially capable of homing to the BM. A wide range of differentiation of VSELs has been demonstrated: they are able to differentiate into osteoblasts, cardiomyocytes, neurons, astrocytes, oligodendrocytes, Schwann cells, and insulin-producing cells [14, 15]. Thus, mouse BM contains a relatively rare cell population with osteogenic differentiation capacity that may escape the “lung trap.” It is possible that the BM contains other cell subpopulations with similar properties. Although no direct analog of VSELs has been found in adult humans, VSELs in humans are hypothetical, but we cannot exclude the theoretical possibility of discovering cells with similar properties in the future. For example, a population of small stem cells with diameters of up to 5 μm resembling VSELs was sorted recently from human embryonic stem cell (hESC) cultures [16]. These VSEL-like cells expressed stem-cell-related markers (CD133, SSEA-4) and markers of germinal lineage (DDX4/VASA, PRDM14). These cells were comparable to similar populations of small stem cells sorted from cell cultures of normal ovaries and were the predominant cells in the ascites of recurrent ovarian cancer.

3.2 Absence of MSCs in the organism of an allogeneic recipient in the early terms after injection?

The idea that MSCs may not survive for a long time after administration is based on the data indicating that most MSCs become apoptotic after administration [17], as well as on the results of early studies on the biodistribution of MCs [9]. However, several groups of investigators have shown in different kinds of model animals that MCs can be detected for long periods of time (up to several months) in a wide range of tissues, including BM, after systemic administration, even despite prior *in vitro* cultivation [18–24]. In one of these studies, PCR analysis showed that few donor murine cells were present in recipient mice after 1 week, but after 1–5 months, donor cells accounted for 1.5–12% of cells in bone, cartilage, and lung, as well as in BM and spleen [18]. The results demonstrated that at least some of the mesenchymal progenitor cells of BM expanded in culture can not only avoid arrest in lung microvessels and repopulate other organs and tissues but also survive long term in bone, cartilage, and lung and, more importantly, retain their functionality, since the authors were able to show that donor cells represented 2.5% of the chondrocytes isolated from the xiphoid and articular cartilage of the recipient. Interestingly, in these studies on the biodistribution of MCs, their frequency of detection and concentration in BM were often higher than in other tissues. This indicates the propensity of these cells to repopulate BM, which is not surprising if one assumes that the cells have a tropism for the microenvironment from which they were extracted. Recently, a systematic review has been published on the issue of MCs biodistribution [25], which shows that the biodistribution of MCs depends on the route of administration, that after intravenous administration MCs are detected in a wide range of organs and tissues, and that MCs are able to accumulate in damaged organs and tissues. In the majority of studies discussed, it is shown that the BM and spleen are the preferred sites of MCs homing. At the same time, the authors of a number of papers note that the efficiency of MCs homing into the BM is low. It is shown that the ratio of donor stromal cells is estimated as 0.1% of BM nuclear cells [18, 21, 22]. This may be due to the fact that the cited studies performed infusion of pre-cultured cells, and cultivation may result in a reduced ability of cells to invade through the vascular endothelial layer, as shown [13]. In those studies, where it is stated that mesenchymal cells are absent in the recipient's organism after a short time, the sensitivity of the applied methods could be insufficient to observe rare cell subpopulations, which include MSCs (~0.01%–0.001% of nuclear BM cells).

It can be concluded that at intravenous injection of pre-expanded *in vitro* MCs, the majority of such cells are indeed retained in the lungs of the recipient for some time. However, this does not mean that rare cell subpopulations present in MC cultures, including cells resembling real MSCs by their properties, are also nonspecifically and irreversibly retained in the lung tissue. Low efficiency of homing, together with detection of cells in other organs and tissues, raised a question about the presence or absence of the mechanism of homing to the BM.

3.3 Lack of a mechanism for homing MSCs to BM?

An example of studies on which this statement is based is the article in which the arrest of MSCs in the lungs was shown [9]. In this study, the biodistribution of MSCs was monitored for 72 hours after intravenous injection of MSCs. At none of the investigated time points were injected MSCs detected in BM [9].

At the same time, by now there is a plenty of researches in which it is shown that intravenously injected stromal cells can, similarly to leukocytes, perform rolling and adhesion to vascular endothelial cells [26] and extravasate into various tissues, including BM, from the vascular channel through the basal membrane [27], although the number of cells migrating through the membrane is usually low [28]. In one of the already mentioned studies, the authors managed to detect eGFP-labeled cells in bones and BM of two recipients using PCR DNA ELISA analysis [19], and subsequently, the presence of MCs was detected in other organs and tissues, but using a more sensitive method [20]. The authors conclude that after intravenous infusion, MCs are predominantly localized in the BM, indicating the possibility of the existence of a mechanism of directed migration (homing) of these cells to the BM. Also, in another study, it was shown [29] that after intravenous injection of cells of stromal line GB1/6 to mice irradiated at doses of 3–8.5 Gy with additional irradiation of the right hind limb at a dose of 10–12.5 Gy, the injected cells were detected *in situ* in the hematopoietic sinuses of the recipient BM. Donor cells were undetectable 2 months after infusion in other organs—in the spleen, liver, and lungs. This indicates that intravenously infused stromal cells can infiltrate the vascular system of the BM and selectively accumulate in it [29].

Thus, both in animal models and in humans, it has been shown that after intravenous administration, stromal cells isolated from BM are capable of homing into their “native” BM niches, although with relatively low efficiency [28]. What limits the tropism and engraftment of MSCs in the BM remains not fully understood. There have been published papers showing the ability of cultured BM mesenchymal cells to migrate through the basal membrane and endothelial cell layer in model *in vitro* experiments and demonstrating the role of the metalloprotease MMP2, which is able to cleave type IV collagen (a major component of the basal membrane) in this process [27]. Interestingly, it is sufficient to culture primary BM-derived mesenchymal stromal cells for 24–48 hours for their ability to home to BM to be significantly reduced [13]. This information explains why, in many experiments on intravenous injection of multipotent mesenchymal stromal cells (MMSCs), it was not possible to observe significant homing of injected cells into the BM. At the time of publication of the described study, the mechanism of this phenomenon remained unknown, but much later it was found that MMSCs cultivation leads to a decrease in the expression of CXCR4 on their surface—a key molecule in the migration of various types of stem cells. The studies on the mechanisms of directed migration of MSCs and BM-MCs can be conditionally divided into those that show mobilization of stromal cells from BM into peripheral blood and those that study the mechanisms and pathways of migration of BM-MCs after their intravenous administration. It was noted that intravenously injected BM-MCs are able to accumulate in the sites of damage, hypoxia, and ischemia [30]. This ability of MMSCs was confirmed in myocardial infarction and ischemic brain injury [31, 32]. It has been hypothesized that proinflammatory cytokines, chemokines, including SDF-1 α , and hypoxia factors, such as HIF1 α , are involved in the mechanisms of MSCs migration to various tissues, and, in particular, to the BM, in which hypoxic conditions are maintained physiologically [30]. The following studies are devoted to this issue.

Among chemokines and their corresponding receptors, the SDF-1 α /CXCR4 axis is a thoroughly studied system [30, 33–37]. This axis is known to be central to the homing of HSCs to the BM when administered intravenously [38], regulating the migration and homing of CXCR4+ hematopoietic progenitor cells, pre-B lymphocytes, and T-lymphocytes [33–35, 39]. More recent evidence suggests that in addition to HSCs,

functional CXCR4 is expressed on the surface of various types of tissue-specific stem cells, such as neuronal stem cells [40], skeletal muscle satellite cells [41], liver oval cells [42], primordial germ cells [43], and other stem cell types including Muse cells and VSELs [44, 45].

There has been considerable evidence to suggest that the SDF-1 α /CXCR4 axis is important for the migration of mesenchymal stromal/stem cells isolated from various sources [36, 37, 46, 47] and, more importantly, determines their ability to home to BM after intravenous administration [48]. Interestingly, data from previous studies suggested that a small subpopulation of cultured MSCs/MMSCs/BM-MCs initially maintains CXCR4 expression on the cell surface [48, 49], but upon *ex vivo* expansion, expression gradually declines and becomes barely detectable after prolonged cultivation [50]. This phenomenon may partly explain the fact that many researchers have failed to observe efficient repopulation of intravenously injected MSCs/MMSCs/BM-MCs after their preliminary expansion *in vitro*.

Kyriakou et al. showed that despite low surface expression of CXCR4, human MSCs/MMSCs/BM-MCs migrated in response to SDF-1 α *in vitro*, and this was enhanced when CXCR4 was overexpressed in cells by lentiviral transduction [51]. It was also shown that artificial overexpression of CXCR4 in human adipose tissue stromal cells (hADSCs) helps to direct these cells to damaged tissues in response to SDF-1 α signaling [36]. Chen et al. observed the same correlation both *in vitro* and *in vivo* for CXCR4-transduced murine MSCs transplanted into irradiated mice [52]. Moreover, Bobis-Wozowicz described that transplantation of CXCR4-transduced human MMSCs isolated from adipose tissue into NOD/SCID mice resulted in increased engraftment to BM [53]. Interestingly, CXCR4 overexpression caused a significant increase in homing to the BM and spleen of animals that had previously received irradiation [51, 52]. This indicates that prior damage to the BM stroma may be a prerequisite or at least a factor contributing to the engraftment of intravenously injected mesenchymal progenitor cells in the recipient. It has been shown that irradiation of an animal with ionizing radiation or exposure to other DNA-toxic agents leads to increased expression of SDF-1 α in BM [54], and this may be one of the mechanisms behind the increased ability of MSCs to engraft in the BM of irradiated mice [55].

Enhanced survival and ability of MSCs to migrate *in vitro* in response to hypoxia and cultivation in the presence of SDF-1 α have been demonstrated [46, 56]. The ability of BM-MCs to migrate along the SDF1 α gradient was experimentally demonstrated in a mouse model of hypoxic-ischemic brain injury (HIB), and this effect was abrogated by the CXCR4 inhibitor (AMD3100) [30].

These prerequisites, namely the expression of CXCR4 in BM-MCs at basal level and its activation in response to hypoxia, the local increase in SDF1 α levels under hypoxic conditions, which can occur as a consequence of ischemization or injury, are also physiological in BM [57], together with the ability of BM-MCs to accumulate *in vivo* in ischemic and injured tissues, were the grounds to suggest that the SDF-1 α /CXCR4 axis is the key and determines the ability of homing to BM not only of HSCs but also MSCs or their progeny.

Thus, summarizing the presented data, we can conclude that the ability of MSCs to chemotaxis along gradients of SDF1 α is well documented. This is primarily shown for the migration of MSCs to injury sites. Among other things, it has been shown that CXCR4 expression is increased on the surface of MSCs in response to several chemokines, including SDF-1 α . Stromal cells have been shown to be able to migrate along the SDF-1 α gradient and penetrate the endothelial barrier in the BM from peripheral blood. At the same time, the efficiency of MSCs homing into BM is usually low, which

is associated by researchers with a low level of expression of receptors to chemokines on the surface of primary, uncultured MSCs; an even greater decrease of expression of these receptors during *in vitro* cultivation of MSCs/BM-MCs isolated from the organism; and heterogeneity of the BM-MCs population.

3.4 Loss of MSC functions due to cell dissociation?

One of the significant arguments that can be heard in favor of the view that transplantation of BM stroma is impossible is the notion that MSCs lose their functional abilities to regenerate stromal tissue after dissociation of intercellular contacts and transformation of BM into a single-cell suspension before intravenous administration to the recipient. The conclusion that stromal cells are not capable of transplantation in the form of a single-cell suspension was made by several independent, authoritative researchers in the 70–80s of the twentieth century who conducted experiments on animals. In similar studies by Ivanov-Smolensky and Friedenstein, BM explants from (CBA x C57BL/6)F1 mice were used to investigate this issue. F1 mice were lethally irradiated at a dose of 11 Gy and reconstituted with BM from CBA mice, and BM explants were obtained from the recipients. Colonies of fibroblasts grew from the explants, which, judging by isoantigens in the indirect immunofluorescence reaction with anti-C57BL/6 serum, were of recipient origin [58, 59]. The same conclusion about the impossibility of stroma transplantation was made by Chertkov [60, 61]. In 1983 the authors demonstrated that if BM is explanted in culture as a whole fragment without cell separation and then the stromal sublayer is collected and transplanted under the kidney capsule, an ectopic focus of hematopoiesis is formed in the implantation site, i.e., MSCs fulfill their function and build a full-fledged hematopoietic microenvironment [60]. However, if BM is transformed into a single-cell suspension before explanting into the culture, the cellular composition of the stromal sublayer of the culture is quite different, and, most importantly, when it is implanted under the kidney capsule of the recipient mouse kidney, a focus of ectopic hematopoiesis is not formed, i.e., the function of MSCs is lost due to the preliminary dissociation of cells. Then, 2 years later, following a largely similar scheme to the experiments of Ivanov-Smolensky et al. [59], Chertkov et al. studied the cells of the stromal sublayer of LTBMCS from the BM of radiation chimeras, which were obtained by irradiating mice with a lethal dose of radiation and reconstituting hematopoiesis by intravenous injection of donor syngeneic or allogeneic BM in the form of a single-cell suspension [61]. The origin of stromal cells in the LTBMCS of chimeric BM was studied using double fluorochrome staining with mouse lineage-specific antisera. It was shown that the plastic-adherent cells of the LTBMCS of allogeneic chimeras contained only recipient stromal progenitor cells. The authors concluded that stromal progenitor cells cannot be transplanted intravenously in mice.

It is interesting to note that J. Chertkov and A. Friedenstein published the results of their other experiments, in which the strength of the basis of the hypothesis put forward is questionable. Thus, for example, in the same issue of *Experimental Hematology*, I.L. Chertkov publishes another article [62], in which a similar experiment of forming the foci of ectopic hematopoiesis from a whole fragment of BM and from a single-cell suspension of BM, which was pelleted by centrifugation on a small fragment of a filter, was conducted. The difference between this study and the study described above by the same authors was that in the latter study, the stage of seeding the BM cells in culture was omitted. In this arrangement of the experiment, foci of ectopic hematopoiesis were formed in both cases. To the surprise of the authors, the

dissociation of BM cells not only failed to prevent the formation of foci, but other way round the proportion of donor cells in such foci was increased. Also, Friedenstein published a study in which it was shown that transplantation of fibroblasts in the form of a suspension under the kidney capsule led to the formation of elements of BM stroma, including bone, and to the presence of hematopoietic cells in the implants, which indicated that dissociation of cells in these experiments still did not lead to a complete loss of MSC function [63].

The statement that MSCs lose their functions after dissociation of intercellular contacts directly contradicts later studies, where it was shown that MSCs are able to migrate in the organism to the microenvironment of solid tumors and to the site of inflammation [64–66]. For example, MSCs have been shown to be selectively recruited to injured or inflamed tissues due to the release of multiple inflammatory factors by injured tissues [67]. This process, in particular, is essential for the resolution of infection and bone regeneration. The ability of MSCs to migrate is increased during acute inflammation, a process associated with the release of tumor necrosis factor (TNF) and activation of the NF- κ B pathway [68, 69]. It is known that MSCs migrate from the BM to the gastric cancer microenvironment [70]. Thus, MSCs or some of their descendants are able to move around the organism as separate cells, accumulate in inflamed or damaged tissues, and actively participate in regeneration processes. Consequently, dissociation of BM cells before their administration to the recipient does not necessarily mean the cessation or decrease of MSC functionality.

3.5 Lack of immune privileges for MSCs?

The question of immune privileges (IPs) of MSCs and other stromal progenitor cells has a long history of study. *In vitro* studies of immunomodulatory properties of MMSCs have shown that these cells are able to inhibit proliferation and activation of T-lymphocytes during cocultivation, express MHC class I at a low level, and do not express MHC class II on their surface [71–73]. It has also been shown that these cells and the extracellular vesicles and individual proteins and other molecules secreted by them are able to reduce the activation of immunologic responses in a number of autoimmune pathologies and in graft-versus-host disease after alloHSCT [74–76].

However, it was subsequently found that pre-cultured BM-MCs do not possess immune privileges, and their main part causes immune responses in the organism and is eliminated by the immune system in MHC-incompatible transplantation [77, 78]. However, even in the case of cultured cells, the possibility that among the total cell population, there is a rare population of cells still possessing IPs cannot be excluded. Indeed, compelling experimental evidence for IPs of BM-MCs *in vivo* has subsequently emerged and includes studies performed in mice, large primates, and humans. We will describe here only a few of them.

Long-term presence of donor BM stromal cells in the body of recipients after alloHSCT has been demonstrated: in the already mentioned study of the ability of BM-MCs to home to BM in primates, in addition to autoBMT, MHC-incompatible allogeneic combined infusion of HSCs and genetically labeled BM-MCs was performed [19]. It was shown that markers of donor MCs were detectable in the BM up to +76 days. The same authors published another study on primates, in which they injected autologous GFP-labeled MSCs into a baboon previously lethally irradiated and recovered by its own hematopoietic cells mobilized into PB before irradiation and a baboon that had not been irradiated or exposed to any cytotoxic therapy [20].

MSCs expressing immunogenic protein GFP persisted in the recipient's organism for several months (up to 21 months) without being eradicated by the immune system of the organism [20]. For human allogeneic MSCs, their long-term (for several months) presence in the BM of a recipient allogeneic to the donor after intraosseous injection of a suspension of donor MSCs has been demonstrated [79, 80].

The IPs of MSCs have proven to be so strong that they allow these cells to survive in a xenogeneic organism. One study demonstrated the presence of xenogeneic human MSCs in the BM of immunodeficient (nude) mice for several months after intravenous infusion [23]. In another study, the authors transplanted a well-characterized population of human MSCs to sheep embryos at the early stages of pregnancy, before and after the expected development of immunological competence [81]. In this xenogeneic system, human MCs engrafted and persisted in various tissues for 13 months after transplantation. The transplanted human cells underwent site-specific differentiation into chondrocytes, adipocytes, myocytes, cardiomyocytes, BM stromal cells, and thymus stroma. Long-term engraftment was unexpectedly recorded even when cells were transplanted after the development of immunocompetence.

Recently, it was shown that MSCs have pronounced IPs *in vivo* in adult mice: MSCs expressing immunogenic marker GFP survived and maintained their functionality for 6 weeks in immunologically complete adult mice [82, 83]. It is known that *in vitro* cultivation significantly changes the properties of cells extracted from the organism. For example, it has been shown that MMSCs cultivation leads to the induction of MHC expression, changes in the expression of genes encoding proinflammatory cytokines [73, 84], changes in the global DNA methylation and gene expression profile, and the occurrence of chromosomal rearrangements [85, 86]. As a consequence, it is legitimate to suggest that the immunological characteristics of cells *in vivo* and *in vitro* differ significantly.

Thus, it can be concluded that in mammalian BM, there are one or more distinct cell populations that have IPs and can be successfully transplanted to an MHC-compatible or incompatible recipient and survive in their body despite an active immune system.

If we think about stroma transplantation in patients as a component of alloBMT, we can assume that the probability of survival of these cells is much higher than in other settings, because at the moment of infusion of donor cells and for a certain period of time after it, the patient is in deep agranulocytosis, and the activity of the remaining circulating T-lymphocytes of the recipient is significantly reduced. Humoral immune response to MSCs and their differentiating progeny is also unlikely in such a situation. If combined transplantation of MSCs and HSCs is successful, the recipient becomes a "chimera:" his hematopoiesis and immune system become donor and, consequently, syngeneic with injected MCs, so there is no reason for a systemic immune response to injected donor MCs. However, local immune reactions presumably can be observed due to the preserved resident macrophages of the recipient residing in organs and tissues and residual circulating T-lymphocytes.

It can be concluded that for today there are reliable and numerous experimental confirmations that at intravenous injection of whole BM cell suspension containing true multipotent, not subjected to pre-cultivation MSCs, the latter are able to survive for a long time in the recipient's organism, migrate into BM using specialized molecular mechanisms, and perform their direct function—to participate in regeneration and maintenance of BM stromal tissue.

4. New concept of bone marrow transplantation that includes a change of both stromal and hematopoietic components to donor origin

At the same time, there were studies that testified in favor of the fact that transplantation of elements of the stromal component of BM is still possible. This was demonstrated by independent teams of researchers for small mammals (mice), large mammals (primates, dogs), and humans [13, 28, 29, 55, 87–91].

MSCs are an integral component of BM stroma and can be considered its key element. Such perception of MSCs is generally accepted because these cells are capable of *de novo* formation of a full-fledged hematopoietic territory with appropriate spatial structure and biochemical composition, containing all the necessary cellular elements of the stroma, including adipocytes and their precursors adipoblasts, osteocytes and their precursors osteoblasts, reticular cells, pericytes, and other types of stromal cells [92–95]. To date, a number of key properties of MSCs have been demonstrated, namely:

- the ability of donor allogeneic MSCs to survive in the recipient's body for a long period of time; and in the recipient's body [18–24];
- the ability of intravenously injected MSCs to repopulate the BM due to specialized molecular mechanisms [19, 27];
- the ability of MSCs to maintain their functionality (to differentiate in the osteogenic direction and proliferation) after their dissociation and intravenous injection in the form of a single-cell suspension into the recipient's body [55];
- immune privileges of MSCs, i.e., their ability to survive despite the recipient's immune system being activated against their antigens [82, 83];

This creates a fundamental basis for the development of protocols for effective MSC transplantation and BM stroma replacement in patients with hematologic diseases, including oncology, as well as in patients with skeletal diseases, including genetic diseases. Successful BM stroma transplantation also has the potential to improve the effectiveness of treatment of diseases associated with dysfunction of bone, fat, and cartilage tissue [1, 2, 92]. However, given the failure of numerous studies of BM stroma transplantation attempts described above, what might be the key to success?

Many researchers note the ability of MSCs to migrate to the areas of ischemic and injured tissues, but none of the reviewed studies, in particular in the field of hematology, proposes to increase the efficiency of homing and engraftment of MSCs into the recipient's BM during alloBMT by targeted and intentional damage of BM stromal tissue before infusion of donor BM. Meanwhile, it can be one of the reasonable tactics, since the function of MSCs is to regenerate the damaged tissue, first of all, the stromal tissue.

I present here a new concept of BM transplantation that includes transplantation of both MSCs and HSCs from the same donor, where transplantation means not only injection of cells but also their stable engraftment and participation in regeneration and maintenance of the corresponding tissue. The central idea of this concept is the necessity of sufficient damage to the BM stroma in the recipient before BM graft infusion. There are preclinical studies that suggest MSCs may constitute a powerful means of treating tissue lesions caused by ionizing radiation, either after accidental exposure to radioactivity or as a side effect of clinical radiotherapy. Animal studies and early

clinical experiences suggest a role for MSCs in the regeneration of these tissue lesions, both by differentiating into functional parenchymal cells and by creating a nurturing microenvironment for other cells [96]. We have our own experimental data demonstrating that prior severe damage to the recipient BM stroma is a necessary condition of effective and lasting engraftment of donor MSCs. However, more research is needed to fully reveal the regenerative potential of MSCs in the BM [97].

4.1 The strategy

This alternative concept consists of the following key steps/stages (**Figure 1**).

A hypothetical timeline for the four-step protocol is as follows. Eradication of BM stroma could be done after induction therapy and simultaneously with consolidation chemotherapy. Infusion of donor MSCs should be done in advance, for example, at day-14, in order to give the cells some time to home to the BM, engraft, and reconstitute BM stroma. Infusion of HSCs on day 0. Supportive and regenerative therapy for at least 3 months after HSCs infusion. This timeline is speculative and theoretical; a real-time course should be established in future experiments.

The first step is “Eradication of the recipient’s BM stroma.” The goal of this step is to eradicate the host’s MSCs in the BM and/or significantly damage the patient’s BM stroma. This is necessary so that the donor MSCs, which will be injected into the recipient at the next step, would get into the organism in which the function of the BM stroma is lost/damaged and, reacting to this, carry out their direct duty—to regenerate the lost/damaged tissue. A direct analogy can be made here with alloHSCT. This procedure is aimed not only at the elimination of tumor cells but also at almost complete destruction of the patient’s own hematopoiesis, including his immune system, as well as emptying hematopoietic niches in the BM. Destruction of the immune system and emptying of hematopoietic niches create favorable conditions for the introduced donor’s HSCs to engraft by populating empty niches without experiencing pressure from the recipient’s immunity, activating their regenerative program, and fully restoring hematopoiesis. It is likely that successful engraftment of donor HSCs and reconstitution of hematopoiesis is not in the least due to the fact that HSCs enter the body with the lost/damaged hematopoietic tissue and activate their regenerative function to replenish it. At this step, various strategies and approaches can be used, the detailed development of which is still to come. One such approach is non-specific stromal damage by radiation or polychemotherapy (PCT). Several studies that have demonstrated successful engraftment of the donor’s stroma have used high doses of radiation [29, 89], so in principle, this approach is feasible. However, it should be remembered that stromal cells are much more resistant to ionizing radiation [96, 98] than hematopoietic cells. This means that if ionizing radiation is considered as a factor capable of damaging MSCs and applied as TBI, it may require too high doses of radiation to effectively suppress the stromal component of BM. The side effects of using such doses may be acute radiation sickness, high cytotoxicity, and the

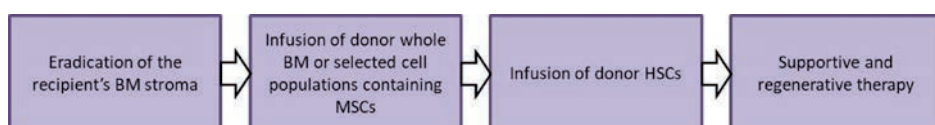


Figure 1.
The main steps of the alternative strategy for sequential co-transplantation of BM stroma and HSCs.

occurrence of secondary tumors. It is doubtful that in the case of TBI it will be possible to achieve doses that would, on the one hand, lead to destruction of BM stroma or at least MSCs within the stroma and, on the other hand, be acceptable in terms of toxicity/complications profile. Radiation therapy is actively developing, and methods of precision irradiation of cells with a precision of up to 1 millimeter are available today, which is a promising alternative to TBI. The development of 3D-4D conformal radiation therapy approaches, such as CyberKnife radiosurgery technology, which is used for radiosurgical treatment of solid tumors, primarily brain tumors and vascular malformations, has become more accessible nowadays. This technology allows irradiation of areas with an accuracy of less than a millimeter and, importantly, affords continuous tracking of the target position and correction of the course of irradiation when the target shifts with respiration or when the patient moves intentionally or not. This makes it possible to selectively and precisely apply ionizing radiation to all areas where BM is concentrated—bones of the skull, ribs, spine, pelvis, and epiphyses of limb bones. Another method of stromal eradication may be the administration of chemotherapeutic drugs that have a cytotoxic effect on stromal cells. It is difficult to think of any significant selectivity with respect to them, although it can be assumed that those drugs used for the treatment of malignant bone tumors and, in particular, osteosarcomas—tumors arising from osteoblasts—may be more effective here than other substances [99]. These drugs include cisplatin, doxorubicin, ifosfamide, etoposide, epirubicin, and methotrexate [100]. This list is certainly not exhaustive; additional information can be drawn from the Russian national clinical guidelines for the treatment of malignant bone tumors or the US National Comprehensive Cancer Network (NCCN) national clinical cancer guidelines [100]. Other chemotherapeutic agents with distinct effects on stromal cells can be selected in functional tests on stromal cell lines (e.g., OP-9) [101, 102], fibroblasts [103], or on primary cultures of BM-derived MMSCs, adipose tissue, or other sources [104], although it is most appropriate to test drugs specifically on primary, previously uncultured BM stromal cells, since this tissue is the therapeutic target in this case, and cultured MSCs isolated from different sources other than BM vary in their ability to proliferate, differentiate, migrate, and secrete extracellular matrix, which may result in different sensitivity to the drugs being tested [105], and cell culturing itself leads to changes in cell characteristics, as mentioned above. It is also worth noting that the tumor microenvironment (TME) is currently the focus of attention of oncologists and oncology researchers, and there are actively developing therapeutic approaches that target TME. Tumor-associated fibroblasts (CAFs) are one of the significant components of TME, and attempts are being made to targetedly eradicate these cells in order to improve the effectiveness of the treatment of primary and metastasizing solid tumors [106]. The most effective drugs, which were discovered in these studies, can be considered as promising candidates for the eradication of BM stromal cells within the proposed new approach to alloBMT and treatment of stromal tissue diseases. Another therapeutic tactic for stromal eradication may be targeting certain cell subpopulations, in particular, MSCs in BM or their differentiated progeny involved in niches for HSCs, such as osteoblasts. Such tactics require knowledge about the surface markers specific to these cells. Unfortunately, to date, there is no unambiguous opinion on the surface immunophenotype of BM MSCs: different researchers mention different surface markers and believe that cells expressing them satisfy the definition of MSCs. Some authors argue that MSCs are cells expressing *Lepr* and *Cxcl12* [107]. It has also been shown that the population of stem/progenitor mesenchymal cells in BM is significantly enriched in a subpopulation of CD271⁺ cells [108, 109].

Other researchers consider MSCs to be pericytes characterized by the expression of smooth muscle α -actin (Acta2) [107, 110]. Finally, CD45⁻Nes⁺ cells are considered MSCs [93]. They are exclusively located along BM arterioles and are a component of hematopoietic niches that regulate the quiescent state of HSCs [111]. The mentioned subpopulations can partially overlap with each other [111], but the ability to transfer hematopoietic activity *in vivo* has been clearly demonstrated so far only for Nes⁺ MSCs [93]. In addition, previously described Muse cells are characterized as SSEA3⁺ cells [112], and VSEL cells are described as Sca1⁺CXCR4⁺SSEA1⁺lin⁻CD45⁻ [14]. By definition, MSCs are capable of differentiating in at least three classical directions: osteogenic, adipogenic, and chondrogenic. This ability ensures the formation and maintenance of the basic cellular elements of the BM stroma. As a result of MSC differentiation in the osteogenic direction, osteoblast-osteocyte precursors are formed, the main function of which is the formation of new bone tissue due to active secretion of extracellular matrix substances. Some studies claim that osteoblasts are direct participants of niches for HSCs and their progeny in the BM [113–115]. Considering the important role of osteoblasts in HSC maintenance, a special term, “osteoblastic niche” of HSCs, was even introduced. However, it should be noted that in a number of papers, authors have disputed the importance of osteoblasts in HSC maintenance [116]. Osteoblasts are exclusively present in BM, lining the inner surface of bone. Osteoblasts are characterized by the expression of the surface membrane proteins CD10 [117], CD44 [118], CD46, CD55, and CD59 [119], as well as the recently discovered A7 antigen, which has strong specificity [120]. There is reason to suggest that osteoblasts are a favorable target for chemotherapy aimed at damaging the stroma as part of the preparatory step before its transplantation, because osteoblasts express a number of soluble extracellular matrix proteins, such as osteocalcin, osteopontin, procollagen, procollagen peptidase, and bone sialoprotein. The content of these proteins can be measured in peripheral blood, and their levels can serve to assess the efficiency of osteoblast destruction along with soluble forms of the osteoblast surface antigens described above. Moreover, since osteoblasts are present specifically in bones, while MSCs are distributed throughout the body, this makes the former a more specific target, and their destruction, presumably, may have fewer undesirable side effects on the body than the destruction of MSCs. Thus, targeted chemotherapy can be directed at osteoblasts either separately or in addition to targeted therapy directed at MSCs. The arsenal of methods for targeting specific cell types is currently quite wide and includes not only chemical agents but also various types of antibodies (monoclonal antibodies, bispecific antibodies, and mini-antibodies conjugated to a cytostatic agent) [121, 122], and T-lymphocytes or NK cells with a chimeric T cell receptor (CAR-T, CAR-NT cells, respectively) engineered to specifically recognize a particular target [123–126]. Such CAR-T or CAR-NT cells can be generated against a single target in a cell population selected for exposure. CAR-T and CAR-NT cells can be used in cocktails for a more efficient cytotoxic effect against a selected cell subpopulation [127].

Various combinations of nonspecific and targeted drugs directed at different types of target cells and at different antigens on the surface of these cells are possible. This part requires detailed development in preclinical studies.

Theoretically, such a strategy can radically transform the schemes of induction and consolidation therapy before alloBMT: such schemes and regimens can be developed that will be primarily directed not so much at hematopoietic, tumor, and immune cells but at BM stroma. No niches for HSCs—no hematopoiesis and no leukemia. At the same time, it is likely that PCT regimens will retain drugs aimed at reducing the

total mass of leukemia cells in the blood/BM in order to reduce the negative impact of these cells on the body. An additional option for promising regimens may be to affect T regulatory cell (Treg) populations in the recipient, as it is these cells, together with MSCs, that confer immune privileges to HSCs in the BM, keeping the latter dormant, and thus may contribute to the persistence of leukemia stem cells in the BM [128, 129]. On the other hand, Tregs may affect the manifestation and severity of GVHD [130], so a balance between benefit and risk must be maintained when targeting Tregs.

The second step of the proposed concept is “Preparing the foothold,” which is an infusion of donor whole BM cell suspension or selected subpopulations of MSCs. The aim of this stage is to repopulate the vacant BM territory with donor MSCs so that they begin to recreate the hematopoietic microenvironment in the BM and build niches for HSCs. It is important to emphasize that the aim of this stage is not to reconstitute hematopoiesis at the expense of donor hematopoietic cells. The efficiency of engraftment of donor HSCs and, as a consequence, donor chimerism of hematopoietic tissue will be low due to the fact that at this time point in the recipient's body, there will be significantly damaged or destroyed niches for them. Consequently, at this stage, not the whole BM but only its stromal component or separate stromal cell subpopulations, in particular, MSCs, if it is possible to isolate them in any way, are feasible to be infused. On the other hand, infusion of whole BM implies infusion of various subpopulations of immune cells, which may support the recipient's immunity for some time at the expense of granulocytes and memory lymphocytes.

The third stage is “Airborne.” This stage consists of the infusion of donor HSCs, as is currently done in alloHSCT. This (second) infusion should probably be performed some time (what time, it is to be determined) after the first one so that MSCs have time to build new niches for HSCs. This will allow the HSCs to inhabit the niches/territories recreated for them and engraft effectively. Probably due to the fact that the stroma recovery may not be complete or the efficiency of its transplantation will not be too high, it will be necessary to adjust the therapeutic doses of CD34⁺ cells administered to the recipient toward their increase.

The fourth stage is “Support/Supply Pathway Establishment.” Damaging the BM stroma can have serious negative consequences for the patient's body. The injected donor MSCs may not be able to cope effectively with the task of building a new hematopoietic territory throughout the body. They may need support. What kind of support is the best, should be subject of further research. It could be supportive infusions of CAR-MSCs, which have pronounced homing ability to BM and significant immune modulatory effects [131, 132], or agents that stimulate regeneration of nerves in the BM [133], or chemotherapeutic agents used for prophylaxis and treatment of GVHD.

At each of these stages, it is advisable to utilize factors that can increase the efficiency of BM stromal transplantation; their combined application can result in a significantly increased likelihood of high-quality, stable, complete BM stromal transplantation. These factors may include effective eradication of the recipient BM stroma. It has been shown that irradiation of an animal with ionizing radiation or exposure to another DNA-toxic agent, such as 5-fluorouracil (5-FU) or cyclophosphamide (Cy), leads to increased expression of SDF-1 in the BM [54]. The ability of BM-MCs to migrate along the SDF1a gradient was experimentally demonstrated [30]. Together with overexpression of CXCR4 on BM-MCs, it could facilitate the engraftment of injected cells [52]. Another factor that can increase the efficiency of MSC transplantation is the introduction of an increased amount of these cells. In those studies, in which it was possible to demonstrate long-term presence of donor stromal cells in the recipient's organism, the authors attributed the success to the

increased dose of injected cells [28, 55, 89, 90]. Another factor that is likely to be significant in the engraftment of stromal cells is the use of vasodilators. It has been shown that intravenous injection of rat BM-MCs after vasodilator administration increased the efficiency of accumulation of radioactive label embedded in the cells by 50% compared to a group that was not injected with a vasodilator [21]. Finally, after infusion of mesenchymal cells into the recipient, it is advisable to consider supportive therapy that will promote engraftment of the injected cells, their proliferation, and differentiation for complete reconstruction of the stromal component of the bone marrow. Many cytokines have a stimulatory effect on mesenchymal progenitor cells. For example, it has been shown that the basic fibroblast growth factor FGF2 stimulates the proliferation of BM-MCs and their differentiation into tenocytes [134]. Transforming growth factor beta (TGF β) takes part in adipogenic differentiation of MSCs [135]. Also, immunity factors have an important influence on MSCs. For example, it has been shown that interleukin-1 beta is a growth factor for stromal progenitor cells [136, 137], and tumor necrosis factor (TNF) and interferon gamma can influence the immunomodulatory properties of BM-MCs [138, 139]. It is reasonable to consider the possibility of supportive therapy aimed at the regeneration of BM stroma and hematopoiesis. Sensory neuronal signaling is known to modulate the functionality of MSCs and osteoclasts in BM [140]. It has been shown that active proliferation of tumor cells in BM in acute myeloid leukemia (AML) has a deleterious and persistent effect on the sympathetic neuronal component of HSC niches in human BM, which can lead to long-term hematologic dysfunction [133].

4.2 Risks of the proposed strategy

Despite the considerations presented about the potential benefits of BM stroma transplantation, it is necessary to evaluate all possible risks to the patient and to ensure that the safety and clinical efficacy of this procedure are appropriate. The risks associated with the proposed procedure of MSC transplantation after prior eradication therapy are highlighted in this chapter. Given the need for high-dose radiation therapy and/or potential intensification of induction and consolidation protocols to achieve profound damage to the stromal component of the BM, the risks of secondary tumors, a higher incidence of infectious complications, and associated mortality as well as some other risks should be evaluated [141–143].

4.2.1 Risks associated with damage to the BM stroma and failure of donor MSC engraftment

Potential transplantation protocols will require approaches that would result in significant damage and possibly even the eradication of the recipient's BM stroma. And if no engraftment of donor MSCs and regeneration of BM stroma follow, there will be no engraftment of hematopoietic tissue due to a lack of necessary niches for HSCs. This may result in extremely severe complications, long rehabilitation, and even a lethal outcome for the patient. There is also a risk of such undesirable phenomena as osteogenesis disorder with the formation of severe progressive osteoporosis (progressive bone loss). The risk of immune rejection of MSCs is another factor that should be addressed. To minimize these risks, *it is important to establish and develop new criteria for donor selection for MSCs and HSCs co-transplantation and methods that facilitate BM stroma regeneration* [131–133].

4.2.2 Risk associated with MSC-driven support of tumor cells

Tumor recurrence is one of the leading problems that arise after alloHSCT. Here, the risk of MSC application is connected with the fact that these donor cells can perform not only antitumor function and replace tumor-remodeled stroma with a healthy microenvironment but also get involved in the tumor microenvironment again, preserving and stimulating malignant cells [144–146]. Some effects exerted by MSCs may depend on the histological type of the tumor and its molecular characteristics, while others may be similar for different tumor types [147, 148]. For example, several studies have shown that MSCs exert antitumor effects on breast and lung cancer cell lines *in vitro* [149, 150] and on pancreatic tumors *in vivo* [151]. However, other studies demonstrate that, in contrast, MSCs promote proliferation of breast cancer and melanoma cells when co-cultured with tumor cell lines *in vitro* [152, 153]. They also enhance tumor growth when administered to mice with lung or prostate cancer [154, 155]. Most of the work in the field of investigating the effect of MSCs directly on tumor cells and on the progression and metastasis concerns solid tumors [148, 156, 157], but the risks and benefits of MSCs have also been investigated in the field of malignant hematologic diseases [143, 158–160]. In addition, MSCs isolated from the BM of a healthy donor can induce drug resistance of hematopoietic tumor cells, both cell lines and primary tumor cells, including CML, OML, and T- and B-ALL cells [161–167].

This determines an important principle of using MSCs in clinical practice during their transplantation as a part of the strategy for joint sequential transplantation of BM stroma and hematopoietic tissue: *MSCs should be used for the purpose of BM stroma transplantation, if possible, during complete remission of the underlying disease.*

4.2.3 Spontaneous transformation of stromal cells in culture

The possibility of spontaneous transformation of mesenchymal cells themselves cannot be discounted. Intense *ex vivo* expansion of any cell type can lead to malignant transformation through a selection of rapidly dividing cells, which increases the risk of accumulation of genetic and epigenetic alterations and may ultimately lead to spontaneous aberrant transformations. BM-MCs cultured *in vitro* are no exception, although data regarding the resistance of these cells to the accumulation of genetic abnormalities in culture and the probability of their spontaneous transformation are contradictory. Some studies claim that long-term cultivation does not reveal chromosomal abnormalities and telomerase activity up to 60 population doublings [168, 169], and does not lead to tumor formation when transplanted into immunodeficient mice [169, 170]. In other studies, the authors report that after prolonged *ex vivo* expansion, human mesenchymal stromal cells derived from adipose tissue or BM were prone to malignant transformation, had an increased proliferation rate, acquired an altered immune phenotype and cell morphology compared to typical BM-MCs, and carried cytogenetic abnormalities [171, 172].

This allows for the formulation of one more principle of MSC use: *for BM stroma transplantation, it is advisable to use primary, not pre-cultured MSCs or BM-MCs, minimally expanded in culture.*

4.2.4 Tumors from primary uncultured MSCs

It should also be kept in mind that tumors originating in the body from cells of mesenchymal nature are known, namely malignant tumors of bone, such as

osteosarcoma, Ewing's sarcoma, chondrosarcoma, fibrosarcoma of bone, and others [100]. These diseases are caused by different populations of BM stromal cells that are descendants of MSCs, such as osteoblasts in the case of osteosarcoma [173, 174]. The frequency of such tumors is relatively low, but still, before using MSCs and their derivatives for the purpose of their transplantation from a donor, it is necessary to have criteria that can confirm that normal, non-pathological cells are being injected. *At least, it is required to exclude diagnoses with involvement of donor mesenchymal cells in tumorigenesis, and it is better to test MSCs for genetic abnormalities associated with the risk of tumor transformation of these cells.*

4.2.5 Secondary tumors as a result of high-dose radiation therapy

It is known that radiation therapy carries the risk of developing not only acute radiation sickness but also secondary tumors [175–177]. In addition, mesenchymal cells and MSCs in particular are much more resistant to the effects of radiation than HSCs [98]. Therefore, the use of TBI to damage MSCs would be associated with high doses that would be necessary to achieve the desired effect, and this in turn is associated with high toxicity and the likelihood of secondary tumor development. Therefore, this approach appears to be ineffective. To overcome these difficulties and increase the efficacy and safety of MSC eradication in the recipient, *it is necessary to develop targeting and low-toxic ways to target MSCs and other stromal cell subpopulations of BM, such as 3D and 4D conformal radiation therapy, CAR-T, CAR-NK cells targeting MSCs, monoclonal antibodies, and their conjugates with cytotoxic drugs.*

4.2.6 MSCs as potential carriers of infections

Another aspect that should be considered when using MSCs in clinical practice is the possibility of their infection by various pathogens. It is known that part of MSCs *in vivo* are pericytes that are in close contact with blood vessels [178], making MSCs ideal guardians on the path of infection, and at the same time, targets for viruses. It has been shown that MSCs can be infected by various members of the Herpesviridae family, H5N1 avian influenza virus, Zika virus, and others [179]. These reservoirs are difficult to eliminate by the immune system, as BM MSCs have been shown to have immune privileges [83], which may be part of the immune-tolerant microenvironment of the BM [128, 157]. This allows for the formulation of one more principle of MSCs utilization: *before MSCs introduction into the recipient's organism, it is necessary to test the cells for pathogen infection.*

Summarizing the data presented in this section, it can be stated that the use of BM-MCs and MSCs in clinical practice is associated with a number of significant risks. These risks can be minimized by using primary MSCs that have not undergone *ex vivo* expansion in culture, as well as by using MSCs in complete remission of malignant disease of the blood system. An alternative strategy of BM transplantation involving combined sequential transplantation of MSCs and HSCs does not involve prior *ex vivo* culturing of MC-MSCs and therefore avoids the risks associated with the accumulation of genetic abnormalities in culture. Nevertheless, regardless of the preliminary cultivation, before transplantation of donor MSCs, they should be carefully tested for the presence of genetic abnormalities and infectious agents, even in the case of a healthy donor, in whose blood no infection is detected.

5. Conclusion

Science, and medical science in particular, is not only the expansion of knowledge about the world and, in particular, about the principles of functioning of the human body and other living beings, but also the narrowing of the realm of the impossible. What was considered impossible a few decades ago is now becoming not only achievable but even routine. Those diseases that were considered incurable are gradually losing their status. The successes of surgery, transplantology, and other branches of medicine are vivid examples of this. In my opinion, now it is time to raise the question of whether the combined transplantation of BM stromal and hematopoietic tissue for the purpose of therapy of malignant and non-malignant diseases of the blood system and skeletal diseases is really impossible. A lot of studies performed on various animal species and on humans show that all major fundamental obstacles for this are absent. For example, it was shown that MSCs are able to retain their functionality after intravenous injection in the form of single-cell suspension into the recipient's body, have a specialized molecular mechanism of homing into the BM, and are able not only to survive but also to divide and differentiate in the necessary stromal directions in the recipient's BM. The main problem at present remains the extremely low efficiency of engraftment of donor mesenchymal cells, and its solution will open the way for fundamentally new schemes of therapy of the abovementioned diseases. In my opinion, one of the possible solutions here is preliminary destruction or significant damage of the recipient's BM stroma, which is necessary to activate the regenerative potential of the injected stem cells, as well as the use of uncultured true MSCs or more universal stem cells for transplantation, the presence of which in the mammalian organisms has been recently shown. Obviously, this approach carries many potential risks. What is the risk of targeted destruction of stem cells that maintain stromal tissues? Or what are the consequences of the destruction of osteoblasts responsible for bone regeneration and maintenance of HSCs? To what extent will this aggravate the situation of a patient who has also had his own hematopoiesis and immune system destroyed as part of induction and consolidation therapy before alloBMT? Are there any immunological risks? These questions must be answered in many, many experimental animal studies. Some risks can be considered to be low. For example, since a patient is in deep agranulocytosis before infusion of donor cells of alloBMT and because MSCs have immune privileges, the probability that infused MSCs or their differentiated progeny will be rejected seems to be rather low. Some risks are indeed high, for example, the risk of low efficiency of MSCs engraftment to the BM. Although if one thinks about alloBMT, it could also be considered a wild idea: the deliberate destruction of the entire hematopoiesis along with the immune system. It would seem to be incompatible with life. Nevertheless, today tens of thousands of people all over the world undergo this procedure, and many take undoubted risks for the sake of a clear goal—to get rid of deadly cancer and no less dangerous other diseases. And it often succeeds! So, for all the potential risks and difficulties, the emerging benefits of the proposed approach to combined stromal and hematopoietic transplantation seem as attractive as the devastating consequences. It will reduce the incidence of BM graft failure, leukemia and lymphoma relapses, and refractory courses of these diseases, and thus help save many lives. There is a long road ahead, but the one who walks it will conquer it.

Acknowledgements

I would like to thank my teacher, Nina Drize, for the development of the ability to think critically, without which the birth of these ideas would not have been possible. I express my heartfelt gratitude to my colleagues who participated in writing this chapter, discussing the ideas presented here, searching for sources of information, and providing support while working on this text. My heartfelt thanks to my family and friends for their continuous support and understanding during the long work on the manuscript. The study was conducted under the state assignment of Lomonosov Moscow State University.

Author details

Aleksei E. Bigildeev^{1,2,3,4,5}

1 Laboratory of Epigenetic Regulation of Hematopoiesis, National Medical Research Center for Hematology, Moscow, Russia

2 I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia

3 Kurchatov Institute National Research Centre, Moscow, Russia

4 Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia

5 Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

*Address all correspondence to: bigildeev.ae@yandex.ru

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Horwitz EM, Prockop DJ, Gordon PL, Koo WWK, Fitzpatrick LA, Neel MD, et al. Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood*. 2001;**97**:1227-1231
- [2] Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, et al. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy*. 2005;**7**:393-395
- [3] Horwitz EM, Prockop DJ, Fitzpatrick LA, Koo WWK, Gordon PL, Neel M, et al. Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. *Nature Medicine*. 1999;**5**:309-313
- [4] Golde DW, Hocking WG, Quan SG, Sparkes RS, Gale RP. Origin of human bone marrow fibroblasts. *British Journal of Haematology*. 1980;**44**:183-187
- [5] Laver J, Jhanwar S, O'Reilly R, Castro-Malaspina H. Host origin of the human hematopoietic microenvironment following allogeneic bone marrow transplantation. *Blood*. 1987;**70**:1966-1968
- [6] Simmons PJ, Przepiorka D, Thomas ED, Torok-Storb B. Host origin of marrow stromal cells following allogeneic bone marrow transplantation. *Nature*. 1987;**328**:429-432
- [7] Bentley S, Knutsen T, Whang-Peng J. The origin of the hematopoietic microenvironment in continuous bone marrow culture. *Experimental Hematology*. 1982;**10**:367-372
- [8] Athanasou NA, Quinn J, Brenner MK, Prentice HG, Graham A, Taylor S, et al. Origin of marrow stromal cells and haemopoietic chimaerism following bone marrow transplantation determined by in situ hybridisation. *British Journal of Cancer*. 1990;**61**:385-389
- [9] 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*. 2012;**3**:1-8
- [10] Eggenhofer E, Luk F, Dahlke MH, Hoogduijn MJ. The life and fate of mesenchymal stem cells. *Frontiers in Immunology*. 2014;**5**:1-6
- [11] Colter DC, Class R, DiGirolamo CM, Prockop DJ. Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;**97**:3213-3218
- [12] Schrepfer S, Deuse T, Reichenspurner H, Fischbein MP, Robbins RC, Pelletier MP. Stem cell transplantation: The lung barrier. *Transplantation Proceedings*. 2007;**39**:573-576
- [13] Rombouts WJC, Ploemacher RE. Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture. *Leukemia*. 2003;**17**:160-170
- [14] Kucia M, Reza R, Campbell FR, Zuba-Surma E, Majka M, Ratajczak J, et al. A population of very small embryonic-like (VSEL) CXCR4(+) SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow. *Leukemia*. 2006;**20**:857-869
- [15] Abouzaripour M, Kashani IR, Pasbakhsh P, Atlasy N. Intravenous

transplantation of very small embryonic like stem cells in treatment of diabetes mellitus. *Avicenna Journal of Medical Biotechnology*. 2015;7:22

[16] Virant-klun I, Skerl P, Novakovic S, Vrtacnik-bokal E, Smrkolj S. Similar population of cd133+ and ddx4+ vsel-like stem cells sorted from human embryonic stem cell, ovarian, and ovarian cancer ascites cell cultures: The real embryonic stem cells? *Cells*. 11 Jul 2019;8(7):706. DOI: 10.3390/cells8070706

[17] Liu XB, Chen H, Chen HQ, Zhu MF, Hu XY, Wang YP, et al. Angiopoietin-1 preconditioning enhances survival and functional recovery of mesenchymal stem cell transplantation. *Journal of Zhejiang University Science B*. 2012;13:616-623

[18] Pereira RF, Halford KW, O'Hara MD, Leeper DB, Sokolov BP, Pollard MD, et al. Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. *Proceedings of the National Academy of Sciences of the United States of America*. 1995;92:4857

[19] Devine SM, Bartholomew AM, Mahmud N, Nelson M, Patil S, Hardy W, et al. Mesenchymal stem cells are capable of homing to the bone marrow of non-human primates following systemic infusion. *Experimental Hematology*. 2001;29:244-255

[20] Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R. Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood*. 2003;101:2999-3001

[21] 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*. 2001;169:12-20

[22] Erices AA, Allers CI, Conget PA, Rojas CV, Minguell JJ. Human cord blood-derived mesenchymal stem cells home and survive in the marrow of immunodeficient mice after systemic infusion. *Cell Transplantation*. 2003;12:555-561

[23] Allers C, Sierralta WD, Neubauer S, Rivera F, Minguell JJ, Conget PA. Dynamic of distribution of human bone marrow-derived mesenchymal stem cells after transplantation into adult unconditioned mice. *Transplantation*. 2004;78:503-508

[24] Bensidhoum M, Chapel A, Francois S, Demarquay C, Mazurier C, Fouillard L, et al. Homing of in vitro expanded Stro-1- or Stro-1+ human mesenchymal stem cells into the NOD/SCID mouse and their role in supporting human CD34 cell engraftment. *Blood*. 2004;103:3313-3319

[25] Sanchez-Diaz M, Quiñones-Vico MI, de la Torre RS, Montero-Vílchez T, Sierra-Sánchez A, Molina-Leyva A, et al. Biodistribution of mesenchymal stromal cells after administration in animal models and humans: A systematic review. *Journal of Clinical Medicine*. 29 Jun 2021;10(13):2925

[26] Rüster B, Göttig S, Ludwig RJ, Bistrrian R, Müller S, Seifried E, et al. Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells. *Blood*. 2006;108:3938-3944

[27] De Becker A, Van Hummelen P, Bakkus M, Broek IV, De Wever J, De Waele M, et al. Migration of culture-expanded human mesenchymal stem cells through bone marrow

endothelium is regulated by matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-3. *Haematologica*. 2007;**92**:440-449

[28] Cilloni D, Carlo-Stella C, Falzetti F, Sammarelli G, Regazzi E, Colla S, et al. Limited engraftment capacity of bone marrow-derived mesenchymal cells following T-cell-depleted hematopoietic stem cell transplantation. *Blood*. 2000;**96**:3637-3643

[29] Anklesaria P, Kase K, Glowacki J, Holland CA, Sakakeeny MA, Wright JA, et al. Engraftment of a clonal bone marrow stromal cell line in vivo stimulates hematopoietic recovery from total body irradiation. *Proceedings of the National Academy of Sciences of the United States of America*. 1987;**84**:7681-7685

[30] Yu Q, Liu L, Lin J, Wang Y, Xuan X, Guo Y, et al. SDF-1 α /CXCR4 axis mediates the migration of mesenchymal stem cells to the hypoxic-ischemic brain lesion in a rat model. *Cell Journal*. 2015;**16**:440-447

[31] Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: Feasibility, cell migration, and body distribution. *Circulation*. 2003;**108**:863-868

[32] Ji JF, He BP, Dheen ST, Tay SSW. Interactions of chemokines and chemokine receptors mediate the migration of mesenchymal stem cells to the impaired site in the brain after hypoglossal nerve injury. *Stem Cells*. 2004;**22**:415-427

[33] Aiuti A, Webb IJ, Bleul C, Springer T, Gutierrez-Ramos JC. The chemokine SDF-1 is a chemoattractant for human CD34⁺ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34⁺

progenitors to peripheral blood. *The Journal of Experimental Medicine*. 1997;**185**:111-120

[34] Ma Q, Jones D, Borghesani PR, Segal RA, Nagasawa T, Kismimoto T, et al. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America*. 1998;**95**:9448-9453

[35] Kim CH, Broxmeyer HE. Chemokines: Signal lamps for trafficking of T and B cells for development and effector function. *Journal of Leukocyte Biology*. 1999;**65**:6-15

[36] Cho HH, Kyoung KM, Seo MJ, Kim YJ, Bae YC, Jung JS. Overexpression of CXCR4 increases migration and proliferation of human adipose tissue stromal cells. *Stem Cells and Development*. 2006;**15**:853-864

[37] Wu Y, Zhao RCH. The role of chemokines in mesenchymal stem cell homing to myocardium. *Stem Cell Reviews and Reports*. 2012;**8**:243-250

[38] Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, et al. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science*. 1999;**283**:845-848

[39] Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa SI, Kitamura Y, et al. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature*. 1996;**382**:635-638

[40] Lazarini F, Tham TN, Casanova P, Arenzana-Seisdedos F, Dubois-Dalcq M. Role of the alpha-chemokine stromal cell-derived factor (SDF-1) in the developing and mature central nervous system. *Glia*. 2003;**42**:139-148

- [41] Ratajczak MZ, Majka M, Kucia M, Drukala J, Pietrzkowski Z, Peiper S, et al. Expression of functional CXCR4 by muscle satellite cells and secretion of SDF-1 by muscle-derived fibroblasts is associated with the presence of both muscle progenitors in bone marrow and hematopoietic stem/progenitor cells in muscles. *Stem Cells*. 2003;**21**:363-371
- [42] Hatch HM, Zheng D, Jorgensen ML, Petersen BE. SDF-1 α /CXCR4: A mechanism for hepatic oval cell activation and bone marrow stem cell recruitment to the injured liver of rats. *Cloning and Stem Cells*. 2002;**4**:339-351
- [43] Ara T, Nakamura Y, Egawa T, Sugiyama T, Abe K, Kishimoto T, et al. Impaired colonization of the gonads by primordial germ cells in mice lacking a chemokine, stromal cell-derived factor-1 (SDF-1). *Proceedings of the National Academy of Sciences of the United States of America*. 2003;**100**:5319-5323
- [44] Wojakowski W, Tendera M, Michałowska A, Majka M, Kucia M, Maślankiewicz K, et al. Mobilization of CD34/CXCR4+, CD34/CD117+, c-met+ stem cells, and mononuclear cells expressing early cardiac, muscle, and endothelial markers into peripheral blood in patients with acute myocardial infarction. *Circulation*. 2004;**110**:3213-3220
- [45] Iseki M, Kushida Y, Wakao S, Akimoto T, Mizuma M, Motoi F, et al. Muse cells, nontumorigenic pluripotent-like stem cells, have liver regeneration capacity through specific homing and cell replacement in a mouse model of liver fibrosis. *Cell Transplantation*. 2017;**26**:821-840
- [46] Liu X, Duan B, Cheng Z, Jia X, Mao L, Fu H, et al. SDF-1/CXCR4 axis modulates bone marrow mesenchymal stem cell apoptosis, migration and cytokine secretion. *Protein & Cell*. 2011;**2**:845-854
- [47] Cornelissen AS, Maijenburg MW, Nolte MA, Voermans C. Organ-specific migration of mesenchymal stromal cells: Who, when, where and why? *Immunology Letters*. 2015;**168**:159-169
- [48] Wynn RF, Hart CA, Corradi-Perini C, O'Neill L, Evans CA, Wraith JE, et al. A small proportion of mesenchymal stem cells strongly expresses functionally active CXCR4 receptor capable of promoting migration to bone marrow. *Blood*. 2004;**104**:2643-2645
- [49] Sordi V, Malosio ML, Marchesi F, Mercalli A, Melzi R, Giordano T, et al. Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. *Blood*. 2005;**106**:419-427
- [50] Honczarenko M, Le Y, Swierkowski M, Ghiran I, Glodek AM, Silberstein LE. Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors. *Stem Cells*. 2006;**24**:1030-1041
- [51] Kyriakou C, Rabin N, Pizzey A, Nathwani A, Yong K. Factors that influence short-term homing of human bone marrow-derived mesenchymal stem cells in a xenogeneic animal model. *Haematologica*. 2008;**93**:1457-1465
- [52] Chen W, Li M, Cheng H, Yan Z, Cao J, Pan B, et al. Overexpression of the mesenchymal stem cell Cxcr4 gene in irradiated mice increases the homing capacity of these cells. *Cell Biochemistry and Biophysics*. 2013;**67**:1181-1191
- [53] Bobis-Wozowicz S, Miekus K, Wybieralska E, Jarocho D, Zawisz A, Madeja Z, et al. Genetically modified

adipose tissue–derived mesenchymal stem cells overexpressing CXCR4 display increased motility, invasiveness, and homing to bone marrow of NOD/SCID mice. *Experimental Hematology*. 2011;**39**:686-696.e4

[54] Ponomaryov T, Peled A, Petit I, Taichman RS, Habler L, Sandbank J, et al. Induction of the chemokine stromal-derived factor-1 following DNA damage improves human stem cell function. *The Journal of Clinical Investigation*. 2000;**106**:1331-1339

[55] Piersma AH, Ploemacher RE, Brockbank KGM. Transplantation of bone marrow fibroblastoid stromal cells in mice via the intravenous route. *British Journal of Haematology*. 1983;**54**:285-290

[56] Meng SS, Xu XP, Chang W, Lu ZH, Huang LL, Xu JY, et al. LincRNA-p21 promotes mesenchymal stem cell migration capacity and survival through hypoxic preconditioning. *Stem Cell Research & Therapy*. 25 Oct 2018;**9**(1):280

[57] Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nature Medicine*. 2004;**10**:858-864

[58] Friedenstein A, Ivanov-Smolenski A, Chajlakjan R, Gorskaya U, Kuralesova A, Latzinik N, et al. Origin of bone marrow stromal mechanocytes in radiochimeras and heterotopic transplants. *Experimental Hematology*. 1978;**6**:440-444

[59] Ivanov-Smolenskiĭ AA, Chaĭlakhian RK, Gerasimov IV, Gorskaia IF, Kuralesova AI. Origin of stromal bone marrow mechanocytes according to the results of typing

them by isoantigens and chromosomal markers. *Ontogenez*. 1978;**9**:245-252

[60] Chertkov JL, Drize NJ, Gurevitch OA, Udalov GA. Hemopoietic stromal precursors in long-term culture of bone marrow: I. Precursor characteristics, kinetics in culture, and dependence on quality of donor hemopoietic cells in chimeras. *Experimental Hematology*. 1983;**11**:231-242

[61] Chertkov J, Drize N, Gurevitch O, Samoylova R. Origin of hemopoietic stromal progenitor cells in chimeras. *Experimental Hematology*. 1985;**13**:1217-1222

[62] Chertkov J, Drize N, Gurevitch O. Hemopoietic stromal precursors in long-term culture of bone marrow: II. Significance of initial packing for creating a hemopoietic microenvironment and maintaining stromal precursors in the culture. *Experimental Hematology*. 1983;**11**:243-248

[63] Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV. Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. *Transplantation*. 1974;**17**:331-340

[64] Ahn SY. The role of MSCs in the tumor microenvironment and tumor progression. *Anticancer Research*. 2020;**40**:3039-3047

[65] Gu F, Zhang K, Li J, Xie X, Wen Q, Sui Z, et al. Changes of migration, immunoregulation and osteogenic differentiation of mesenchymal stem cells in different stages of inflammation. *International Journal of Medical Sciences*. 2022;**19**:25-33

[66] Liu J, Gao J, Liang Z, Gao C, Niu Q, Wu F, et al. Mesenchymal stem cells

and their microenvironment. *Stem Cell Research & Therapy*. 20 Aug 2022;**13**(1):429

[67] Ponte AL, Marais E, Gallay N, Langonné A, Delorme B, Hérault O, et al. The in vitro migration capacity of human bone marrow mesenchymal stem cells: Comparison of chemokine and growth factor chemotactic activities. *Stem Cells*. 2007;**25**:1737-1745

[68] Yang N, Wang G, Hu C, Shi Y, Liao L, Shi S, et al. Tumor necrosis factor α suppresses the mesenchymal stem cell osteogenesis promoter miR-21 in estrogen deficiency-induced osteoporosis. *Journal of Bone and Mineral Research*. 2013;**28**:559-573

[69] Durand N, Russell A, Zubair AC. Effect of comedications and endotoxins on mesenchymal stem cell secretomes, migratory and immunomodulatory capacity. *Journal of Clinical Medicine*. 11 Apr 2019;**8**(4):497

[70] Li Y, Zhong X, Zhang Y, Lu X. Mesenchymal stem cells in gastric cancer: Vicious but hopeful. *Frontiers in Oncology*. 11 May 2021;**11**:617677

[71] Le Blanc K, Rasmusson I, Götherström C, Seidel C, Sundberg B, Sundin M, et al. Mesenchymal stem cells inhibit the expression of CD25 (interleukin-2 receptor) and CD38 on phytohaemagglutinin-activated lymphocytes. *Scandinavian Journal of Immunology*. 2004;**60**:307-315

[72] Pradier A, Passweg J, Villard J, Kindler V. Human bone marrow stromal cells and skin fibroblasts inhibit natural killer cell proliferation and cytotoxic activity. *Cell Transplantation*. 2011;**20**:681-691

[73] Petinati NA, Kapranov NM, Bigil'deev AE, Popova MD, Davydova YO, Gal'tseva IV, et al. Changing the properties of

multipotent mesenchymal stromal cells by IFN γ administration. *Bulletin of Experimental Biology and Medicine*. 2017;**163**:230-234

[74] López-García L, Castro-Manreza ME. Tnf- α and ifn- γ participate in improving the immunoregulatory capacity of mesenchymal stem/stromal cells: Importance of cell-cell contact and extracellular vesicles. *International Journal of Molecular Sciences*. 2 Sep 2021;**22**(17):9531

[75] Liu H, Li R, Liu T, Yang L, Yin G, Xie Q. Immunomodulatory effects of mesenchymal stem cells and mesenchymal stem cell-derived extracellular vesicles in rheumatoid arthritis. *Frontiers in Immunology*. 20 Aug 2020;**11**:1912

[76] Mendt M, Rezvani K, Shpall E. Mesenchymal stem cell-derived exosomes for clinical use. *Bone Marrow Transplantation*. 2019;**54**(54):789-792

[77] Berglund AK, Fortier LA, Antczak DF, Schnabel LV. Immunoprivileged no more: Measuring the immunogenicity of allogeneic adult mesenchymal stem cells. *Stem Cell Research & Therapy*. 22 Dec 2017;**8**(1):288

[78] Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: Immune evasive, not immune privileged. *Nature Biotechnology*. 2014;**32**:252-260

[79] Petinati N, Drize N, Sats N, Risinskaya N, Sudarikov A, Drovkov M, et al. Recovery of donor hematopoiesis after graft failure and second hematopoietic stem cell transplantation with intraosseous administration of mesenchymal stromal cells. *Stem Cells International*. 10 Apr 2018;**2018**:6495018

[80] Kuzmina LA, Petinati NA, Sats NV, Drize NJ, Risinskaya NV, Sudarikov AB,

et al. Long-term survival of donor bone marrow multipotent mesenchymal stromal cells implanted into the periosteum of patients with allogeneic graft failure. *International Journal of Hematology*. 2016;**104**:403-407

[81] Liechty KW, Mackenzie TC, Shaaban AF, Radu A, Moseley AMB, Deans R, et al. Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. *Nature Medicine*. 2000;**6**:1282-1286

[82] Karpenko D, Kapranov N, Bigildeev A. Nestin-GFP transgene labels immunoprivileged bone marrow mesenchymal stem cells in the model of ectopic foci formation. *Frontiers in Cell and Developmental Biology*. 5 Sep 2022;**10**:993056

[83] Karpenko D, Kapranov N, Bigildeev A. Strong Immune Privileges of MSC and Other Nes-GFP+ Progenitors in Bone Marrow of Transgenic Mice. *Immune Network*. Apr 2025;**25**:e20

[84] Petinati N, Kapranov N, Davydova Y, Bigildeev A, Pshenichnikova O, Karpenko D, et al. Immunophenotypic characteristics of multipotent mesenchymal stromal cells that affect the efficacy of their use in the prevention of acute graft vs host disease. *World Journal of Stem Cells*. 2020;**12**:1377-1395

[85] Metwally AM, Li H, Houghton J. Alterations of epigenetic regulators and P53 mutations in murine mesenchymal stem cell cultures: A possible mechanism of spontaneous transformation. *Cancer Biomarkers*. 2021;**32**:327-337

[86] Saidi N, Ghalavand M, Hashemzadeh MS, Dorostkar R, Mohammadi H, Mahdian-shakib A. Dynamic changes of epigenetic

signatures during chondrogenic and adipogenic differentiation of mesenchymal stem cells. *Biomedicine & Pharmacotherapy*. 2017;**89**:719-731

[87] Keating A, Singer JW, Killen PD, Striker GE, Salo AC, Sanders J, et al. Donor origin of the in vitro haematopoietic microenvironment after marrow transplantation in man. *Nature*. 1982;**298**:280-283

[88] Marshall MJ, Nisbet NW, Evans S. Donor origin of the in vitro hematopoietic microenvironment after marrow transplantation in mice. *Experientia*. 1984;**40**:385-386

[89] Sale G, Storb R. Bilateral diffuse pulmonary ectopic ossification after marrow allograft in a dog. Evidence for allotransplantation of hemopoietic and mesenchymal stem cells. *Experimental Hematology*. 1983;**11**:961-966

[90] Nilsson SK, Dooner MS, Weier HU, Frenkel B, Lian JB, Stein GS, et al. Cells capable of bone production engraft from whole bone marrow transplants in nonablated mice. *The Journal of Experimental Medicine*. 1999;**189**:729-734

[91] Goan SR, Junghahn I, Wissler M, Becker M, Aumann J, Just U, et al. Donor stromal cells from human blood engraft in NOD/SCID mice. *Blood*. 2000;**96**:3971-3978

[92] 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

[93] Méndez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, MacArthur BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature*. 2010;**466**:829-834

- [94] Kfoury Y, Scadden DT. Mesenchymal cell contributions to the stem cell niche. *Cell Stem Cell*. 2015;**16**:239-253
- [95] Chertkov JL, Gurevitch OA, Udalov GA. Role of bone marrow stroma in hemopoietic stem cell regulation. *Experimental Hematology*. 1980;**8**:770-778
- [96] Nicolay NH, Lopez Perez R, Debus J, Huber PE. Mesenchymal stem cells - A new hope for radiotherapy-induced tissue damage? *Cancer Letters*. 2015;**366**:133-140
- [97] Bigildeev AE, Bigildeev EA, Boulygina ES, Tsygankova SV, Gusakova MS, Illarionova OI. Successful bone marrow stroma transplantation is enabled by preliminary recipient's stromal compartment injury. *Scientific Reports*. 2025. DOI: 10.20944/preprints202502.1215.v1
- [98] Chertkov J, Gurevitch O. Radiosensitivity of progenitor cells of the hematopoietic microenvironment. *Radiation Research*. 1979;**79**:177-186
- [99] Valiev AK, Konev AA, Kurilchik AA, Machak GN, Musaev ER, Rogozhin DV, et al. Malignant bone tumors. *Malignant Tumors*. 2023;**13**:335-355
- [100] National Comprehensive Cancer Network. Bone Cancer (Version 2.2025). 2025. p. 2
- [101] Yoshida H, Hayashi SI, Kunisada T, Ogawa M, Nishikawa S, Okamura H, et al. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature*. 1990;**345**:442-444
- [102] Nakano T, Kodama H, Honjo T. Generation of lymphohematopoietic cells from embryonic stem cells in culture. *Science*. 1994;**265**:1098-1101
- [103] Globig P, Willumeit-Römer R, Martini F, Mazzoni E, Luthringer-Feyerabend BJC. Optimizing an osteosarcoma-fibroblast coculture model to study antitumoral activity of magnesium-based biomaterials. *International Journal of Molecular Sciences*. 2020;**21**:1-18
- [104] Minguell JJ, Erices A, Conget P. Mesenchymal stem cells. *Experimental Biology and Medicine* (Maywood, N.J.). 2001;**226**:507-520
- [105] Costela-ruiz VJ, Melguizo-rodríguez L, Bellotti C, Illescas-montes R, Stanco D, Arciola CR, et al. Different sources of mesenchymal stem cells for tissue regeneration: A guide to identifying the most favorable one in orthopedics and dentistry applications. *International Journal of Molecular Sciences*. 6 Jun 2022;**23**(11):6356
- [106] Yang D, Liu J, Qian H, Zhuang Q. Cancer-associated fibroblasts: From basic science to anticancer therapy. *Experimental & Molecular Medicine*. 2023;**55**:1322-1332
- [107] Baryawno N, Przybylski D, Kowalczyk MS, Kfoury Y, Severe N, Gustafsson K, et al. A cellular taxonomy of the bone marrow stroma in homeostasis and leukemia. *Cell*. 2019;**177**:1915-1932.e16
- [108] Li H, Ghazanfari R, Zacharaki D, Lim HC, Scheduling S. Isolation and characterization of primary bone marrow mesenchymal stromal cells. *Annals of the New York Academy of Sciences*. 2016;**1370**:109-118
- [109] Watson JT, Foo T, Wu J, Moed BR, Thorpe M, Schon L, et al. CD271 as a marker for mesenchymal stem cells in bone marrow versus umbilical cord blood. *Cells, Tissues, Organs*. 2013;**197**:496-504

- [110] Armulik A, Genové G, Betsholtz C. Pericytes: Developmental, physiological, and pathological perspectives, problems, and promises. *Developmental Cell*. 2011;**21**:193-215
- [111] Kunisaki Y, Bruns I, Scheiermann C, Ahmed J, Pinho S, Zhang D, et al. Arteriolar niches maintain haematopoietic stem cell quiescence. *Nature*. 2013;**502**:637
- [112] Wakao S, Kitada M, Kuroda Y, Shigemoto T, Matsuse D, Akashi H, et al. Multilineage-differentiating stress-enduring (muse) cells are a primary source of induced pluripotent stem cells in human fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America*. 2011;**108**:9875-9880
- [113] Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. *Nature*. 2014;**505**:327-334
- [114] Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature*. 2003;**425**:841-846
- [115] Coskun S, Hirschi KK. Establishment and regulation of the HSC niche: Roles of osteoblastic and vascular compartments. *Birth Defects Research. Part C, Embryo Today*. 2010;**90**:229-242
- [116] Sugiyama T, Omatsu Y, Nagasawa T. Niches for hematopoietic stem cells and immune cell progenitors. *International Immunology*. 2019;**31**:5-11
- [117] Reyes-Botella C, Montes MJ, Abadía-Molina AC, Vallecillo-Capilla MF, Ruiz C. CD10 expression in cultured human osteoblast-like cells. *Folia Biologica*. 1999;**45**:257-260
- [118] Reyes-Botella C, Montes M, Vallecillo-Capilla M, Olivares E, Ruiz C. Antigenic phenotype of cultured human osteoblast-like cells. *Cellular Physiology and Biochemistry*. 2002;**12**:359-364
- [119] Ignatius A, Schoengraf P, Kreja L, Liedert A, Recknagel S, Kandert S, et al. Complement C3a and C5a modulate osteoclast formation and inflammatory response of osteoblasts in synergism with IL-1 β . *Journal of Cellular Biochemistry*. 2011;**112**:2594-2605
- [120] Badawy T, Kyumoto-Nakamura Y, Uehara N, Zhang J, Sonoda S, Hiura H, et al. Osteoblast lineage-specific cell-surface antigen (A7) regulates osteoclast recruitment and calcification during bone remodeling. *Laboratory Investigation*. 2019;**99**:866-884
- [121] Brinkmann U, Kontermann RE. The making of bispecific antibodies. *MAbs*. 2017;**9**:182-212
- [122] Shim H. Bispecific antibodies and antibody-drug conjugates for cancer therapy: Technological considerations. *Biomolecules*. 26 Feb 2020;**10**(3):360
- [123] Gross G, Waks T, Eshhar Z. Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. *Proceedings of the National Academy of Sciences of the United States of America*. 1989;**86**:10024-10028
- [124] Irving BA, Weiss A. The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. *Cell*. 1991;**64**:891-901
- [125] Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced Leukemia. *Science Translational Medicine*. 2011;**3**:95ra73

- [126] Xie G, Dong H, Liang Y, Ham JD, Rizwan R, Chen J. CAR-NK cells: A promising cellular immunotherapy for cancer. *eBioMedicine*. Sep 2020;**59**:102975
- [127] Gourd E. CAR T-cell cocktail therapy for B-cell malignancies. *The Lancet Oncology*. 2019;**20**:e669
- [128] Hirata Y, Furuhashi K, Ishii H, Li HW, Pinho S, Ding L, et al. CD150 high bone marrow Tregs maintain hematopoietic stem cell quiescence and immune privilege via adenosine. *Cell Stem Cell*. 2018;**22**:445-453.e5
- [129] Riether C. Regulation of hematopoietic and leukemia stem cells by regulatory T cells. *Frontiers in Immunology*. 2 Nov 2022;**13**:1049301
- [130] Kasahara H, Kondo T, Nakatsukasa H, Chikuma S, Ito M, Ando M, et al. Generation of Allo-antigen-specific induced Treg stabilized by vitamin C treatment and its application for prevention of acute graft versus host disease model. *International Immunology*. 2017;**29**:457-469
- [131] Sirpilla O, Sakemura RL, Hefazi M, Kimball BL, Huynh TN, Siegler EL, et al. Chimeric antigen receptor design in human mesenchymal stromal cells (CAR-MSCs) mediates homing to target sites and downstream immunosuppression for immune disease treatment. *Transplantation and Cellular Therapy*. 2024;**30**:S11-S12
- [132] Yan L, Li J, Zhang C. The role of MSCs and CAR-MSCs in cellular immunotherapy. *Cell Communication and Signaling: CCS*. 2023;**211**(21):1-22
- [133] Kovtun I, von Bonin M, Ibneeva L, Frimmel J, Middeke JM, Kunadt D, et al. Profound sympathetic neuropathy in the bone marrow of patients with acute myeloid leukemia. *Leukemia*. 2024;**38**:393-397
- [134] Cai TY, Zhu W, Chen XS, Zhou SY, Jia LS, Sun YQ. Fibroblast growth factor 2 induces mesenchymal stem cells to differentiate into tenocytes through the MAPK pathway. *Molecular Medicine Reports*. 2013;**8**:1323-1328
- [135] Li SN, Wu JF. TGF- β /SMAD signaling regulation of mesenchymal stem cells in adipocyte commitment. *Stem Cell Research & Therapy*. 29 Jan 2020;**11**(1):41
- [136] Bigildeev AE, Zhironkina OA, Lubkova ON, Drize NJ. Interleukin-1 beta is an irradiation-induced stromal growth factor. *Cytokine*. 2013;**64**:131-137
- [137] Bigildeev AE, Zezina EA, Shipounova IN, Drize NJ. Interleukin-1 beta enhances human multipotent mesenchymal stromal cell proliferative potential and their ability to maintain hematopoietic precursor cells. *Cytokine*. 2015;**71**:246-254
- [138] Hackel A, Aksamit A, Bruderek K, Lang S, Brandau S. TNF- α and IL-1 β sensitize human MSC for IFN- γ signaling and enhance neutrophil recruitment. *European Journal of Immunology*. 2021;**51**:319-330
- [139] Burnham AJ, Foppiani EM, Goss KL, Jang-Milligan F, Kamalakar A, Bradley H, et al. Differential response of mesenchymal stromal cells (MSCs) to type 1 ex vivo cytokine priming: Implications for MSC therapy. *Cytotherapy*. 2023;**25**:1277-1284
- [140] Leitão L, Alves CJ, Sousa DM, Neto E, Conceição F, Lamghari M. The alliance between nerve fibers and stem cell populations in bone marrow: Life partners in sickness and health. *The FASEB Journal*. 2019;**33**:8697-8710

- [141] Doglio M, Crossland RE, Alho AC, Penack O, Dickinson AM, Stary G, et al. Cell-based therapy in prophylaxis and treatment of chronic graft-versus-host disease. *Frontiers in Immunology*. 17 Nov 2022;**13**:1045168
- [142] Zaripova LN, Midgley A, Christmas SE, Beresford MW, Pain C, Baildam EM, et al. Mesenchymal stem cells in the pathogenesis and therapy of autoimmune and autoinflammatory diseases. *International Journal of Molecular Sciences*. 7 Nov 2023;**24**(22):16040
- [143] Vianello F, Dazzi F. Mesenchymal stem cells for graft-versus-host disease: A double edged sword? *Leukemia*. 2008;**22**:463-465
- [144] Forte D, García-Fernández M, Sánchez-Aguilera A, Stavropoulou V, Fielding C, Martín-Pérez D, et al. Bone marrow mesenchymal stem cells support acute myeloid Leukemia bioenergetics and enhance antioxidant Defense and escape from chemotherapy. *Cell Metabolism*. 2020;**32**:829-843.e9
- [145] Ramuta TŽ, Kreft ME. Mesenchymal stem/stromal cells may decrease success of cancer treatment by inducing resistance to chemotherapy in cancer cells. *Cancers (Basel)*. 2 Aug 2022;**14**(15):3761
- [146] Antoon R, Overdevest N, Saleh AH, Keating A. Mesenchymal stromal cells as cancer promoters. *Oncogene*. Nov 2024;**43**(49):3545-3555
- [147] Lee MW, Ryu S, Kim DS, Lee JW, Sung KW, Koo HH, et al. Mesenchymal stem cells in suppression or progression of hematologic malignancy: Current status and challenges. *Leukemia*. 2019;**33**:597-611
- [148] Wong RSY. Mesenchymal stem cells: Angels or demons? *Journal of Biomedicine & Biotechnology*. 2011;**8**. Article ID: 459510. DOI: 10.1155/2011/459510
- [149] Qiao L, Xu ZL, Zhao TJ, Ye LH, Zhang XD. Dkk-1 secreted by mesenchymal stem cells inhibits growth of breast cancer cells via depression of Wnt signalling. *Cancer Letters*. 2008;**269**:67-77
- [150] Li L, Tian H, Yue W, Zhu F, Li S, Li W. Human mesenchymal stem cells play a dual role on tumor cell growth in vitro and in vivo. *Journal of Cellular Physiology*. 2011;**226**:1860-1867
- [151] Kidd S, Caldwell L, Dietrich M, Samudio I, Spaeth EL, Watson K, et al. Mesenchymal stromal cells alone or expressing interferon-beta suppress pancreatic tumors in vivo, an effect countered by anti-inflammatory treatment. *Cytotherapy*. 2010;**12**:615-625
- [152] Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature*. 2007;**449**:557-563
- [153] Suzuki K, Sun R, Origuchi M, Kanehira M, Takahata T, Itoh J, et al. Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. *Molecular Medicine*. 2011;**17**:579-587
- [154] Yu JM, Jun ES, Bae YC, Jung JS. Mesenchymal stem cells derived from human adipose tissues favor tumor cell growth in vivo. *Stem Cells and Development*. 2008;**17**:463-473
- [155] Lin G, Yang R, Banie L, Wang G, Ning H, Li LC, et al. Effects of transplantation of adipose tissue-derived stem cells on prostate tumor. *Prostate*. 2010;**70**:1066-1073

- [156] Klopp AH, Gupta A, Spaeth E, Andreeff M, Marini F. Concise review: Dissecting a discrepancy in the literature: Do mesenchymal stem cells support or suppress tumor growth? *Stem Cells*. 2011;**29**:11-19
- [157] Nobre AR, Risson E, Singh DK, Di Martino JS, Cheung JF, Wang J, et al. Bone marrow NG2+/nestin+ mesenchymal stem cells drive DTC dormancy via TGF- β 2. *Nature Cancer*. 2021;**23**(2):327-339
- [158] Ning H, Yang F, Jiang M, Hu L, Feng K, Zhang J, et al. The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: Outcome of a pilot clinical study. *Leukemia*. 2008;**22**:593-599
- [159] Zeng Z, Shi YX, Samudio IJ, Wang RY, Ling X, Frolova O, et al. Targeting the leukemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML. *Blood*. 2009;**113**:6215-6224
- [160] Konopleva M, Konoplev S, Hu W, Zaritskey AY, Afanasiev BV, Andreeff M. Stromal cells prevent apoptosis of AML cells by up-regulation of anti-apoptotic proteins. *Leukemia*. 2002;**16**:1713-1724
- [161] Jin L, Tabe Y, Konoplev S, Xu Y, Leysath CE, Lu H, et al. CXCR4 up-regulation by imatinib induces chronic myelogenous leukemia (CML) cell migration to bone marrow stroma and promotes survival of quiescent CML cells. *Molecular Cancer Therapeutics*. 2008;**7**:48-58
- [162] Vianello F, Villanova F, Tisato V, Lympieri S, Ho KK, Gomes AR, et al. Bone marrow mesenchymal stromal cells non-selectively protect chronic myeloid leukemia cells from imatinib-induced apoptosis via the CXCR4/CXCL12 axis. *Haematologica*. 2010;**95**:1081-1089
- [163] Zhang Y, Hu K, Hu Y, Liu L, Wang B, Huang H. Bone marrow mesenchymal stromal cells affect the cell cycle arrest effect of genotoxic agents on acute lymphocytic leukemia cells via p21 down-regulation. *Annals of Hematology*. 2014;**93**:1499-1508
- [164] Zhang X, Tu H, Yang Y, Wan Q, Fang L, Wu Q, et al. High IL-7 levels in the bone marrow microenvironment mediate imatinib resistance and predict disease progression in chronic myeloid leukemia. *International Journal of Hematology*. 2016;**104**:358-367
- [165] Cai J, Wang J, Huang Y, Wu H, Xia T, Xiao J, et al. ERK/Drp1-dependent mitochondrial fission is involved in the MSC-induced drug resistance of T-cell acute lymphoblastic leukemia cells. *Cell Death & Disease*. 2016;**7**(11):e2459, 11
- [166] Carter BZ, Mak PY, Chen Y, Mak DH, Mu H, Jacamo R, et al. Anti-apoptotic ARC protein confers chemoresistance by controlling leukemia-microenvironment interactions through a NF κ B/IL1 β signaling network. *Oncotarget*. 2016;**7**:20054-20067
- [167] Kamga PT, Bassi G, Cassaro A, Midolo M, Di Trapani M, Gatti A, et al. Notch signalling drives bone marrow stromal cell-mediated chemoresistance in acute myeloid leukemia. *Oncotarget*. 2016;**7**:21713-21727
- [168] Bernardo ME, Avanzini MA, Perotti C, Cometa AM, Moretta A, Lenta E, et al. Optimization of in vitro expansion of human multipotent mesenchymal stromal cells for cell-therapy approaches: Further insights in the search for a fetal calf serum substitute. *Journal of Cellular Physiology*. 2007;**211**:121-130

- [169] Kim J, Kang JW, Park JH, Choi Y, Choi KS, Park KD, et al. Biological characterization of long-term cultured human mesenchymal stem cells. *Archives of Pharmacal Research*. 2009;**32**:117-126
- [170] Choumerianou DM, Dimitriou H, Perdikogianni C, Martimianaki G, Riminucci M, Kalmanti M. Study of oncogenic transformation in ex vivo expanded mesenchymal cells, from paediatric bone marrow. *Cell Proliferation*. 2008;**41**:909-922
- [171] Wang Y, Huso DI, Harrington J, Kellner J, Jeong DK, Turney J, et al. Outgrowth of a transformed cell population derived from normal human BM mesenchymal stem cell culture. *Cytotherapy*. 2005;**7**:509-519
- [172] Røsland GV, Svendsen A, Torsvik A, Sobala E, McCormack E, Immervoll H, et al. Long-term cultures of bone marrow-derived human mesenchymal stem cells frequently undergo spontaneous malignant transformation. *Cancer Research*. 2009;**69**:5331-5339
- [173] Mutsaers AJ, Walkley CR. Cells of origin in osteosarcoma: Mesenchymal stem cells or osteoblast committed cells? *Bone*. 2014;**62**:56-63
- [174] Abarrategi A, Tornin J, Lucia MC, Hamilton A, Enrique MC, Rodrigo JP, et al. Osteosarcoma: Cells-of-origin, cancer stem cells, and targeted therapies. *Stem Cells International*. 2016;**2016**:3631764
- [175] Yamanaka R, Hayano A. Radiation-induced Schwannomas and neurofibromas: A systematic review. *World Neurosurgery*. 2017;**104**:713-722
- [176] Yamanaka R, Hayano A. Radiation-induced malignant peripheral nerve sheath Tumors: A systematic review. *World Neurosurgery*. 2017;**105**:961-970.e8
- [177] Inchaustegui ML, Kon-Liao K, Ruiz-Arellanos K, Silva GAE, GonzalezMR, Pretell-Mazzini J. Treatment and outcomes of radiation-induced soft tissue sarcomas of the extremities and trunk—A systematic review of the literature. *Cancers (Basel)*. 25 Nov 2023;**15**(23):5584
- [178] Kunisaki Y. Pericytes in bone marrow. *Advances in Experimental Medicine and Biology*. 2019;**1122**:101-114
- [179] Lebeau G, Ah-Pine F, Daniel M, Bedoui Y, Vagner D, Frumence E, et al. Perivascular mesenchymal stem/stromal cells, an immune privileged niche for viruses? *International Journal of Molecular Sciences*. 21 Jul 2022;**23**(14):8038

Section 4

Extracellular Vesicles,
Microenvironment,
and Cancer

Mesenchymal Stem Cell-Derived Extracellular Vesicles in Therapeutic Use

Yu Fu, Lufan Yang, Siyu Lan, Sha Zhang, Kai Zhang, Shengfeng Bai, Wen Qin, Chenxi Zheng and Bingdong Sui

Abstract

Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) have emerged as a promising cell-free therapeutic strategy in regenerative medicine and immunomodulation. These nanosized vesicles, carrying bioactive molecules like miRNAs, proteins, and lipids, exhibit dual capabilities in regulating immune responses and promoting tissue repair. MSC-EVs promote tissue regeneration, including revascularization, skeletal and dental tissue repair, wound healing, hepatic and renal regeneration, and neural repair. Simultaneously, their immunomodulatory properties-suppressing excessive inflammation and regulating immune cell activity, make them promising interventions for autoimmune, neurodegenerative, and inflammatory conditions. By integrating tissue repair with immune regulation, MSC-EVs represent a transformative paradigm in precision therapeutics, meriting accelerated clinical exploration.

Keywords: mesenchymal stem cell, extracellular vesicle, immunomodulation, tissue regeneration, translational medicine

1. Introduction

Mesenchymal stem cells (MSCs) are non-hematopoietic stromal cells that present in diverse tissues including bone marrow (BMSCs), umbilical cord blood (UCMSCs), umbilical cord tissue, placental tissue (hPMSCs), adipose tissue (ADSCs), and menstrual blood (MenSCs). These cells possess multi-directional differentiation capabilities [1] and feature a distinctive cytokine-secreting function. Cell models have been utilized in investigations on proliferation, transplantation, and differentiation, as well as in the *in vitro* identification of immune responses. Despite their unique advantages, MSC-based therapies still face several challenging limitations, including phenotypic changes, low homing efficiency, and reduced cell viability, which have prompted the shift toward cell-free therapeutic approaches. The finding that the majority of the therapeutic impacts of MSCs rely on their paracrine activity

and that extracellular vesicles (EVs) can substitute for their parent cells presents thrilling opportunities for researchers [2]. Concurrently, these vesicles exhibit profound regenerative and immunomodulatory capacities, capable of dampening excessive inflammation, inhibiting immune cell activation, and fostering a regenerative microenvironment [3].

The term EV is now accepted as a generic description of subcellular, membrane-delimited particles, ranging from tens of nanometers to several micrometers in size [4]. The origin of EVs can be traced back to the early 1940s when Chargaff and West conducted a series of experiments. These experiments hinted at the existence of tiny breakdown products of blood corpuscles in the blood that had clotting functions. It was not until the 1980s that Johnstone et al. identified “reticulocyte vesicles” as functional entities involved in antigen presentation, coining the term “exosomes” to describe their secretion by reticulocytes. Subsequent decades unraveled their complexity: EVs are now classified into three main categories including exosomes (Exos, size from 50 to 100 nm), microvesicles (MVs, 20–1000 nm), and apoptotic bodies (Abs, 1000–5000 nm)—each with unique biogenesis pathways and cargo profiles [5]. This chapter uses ‘MSC-EVs’ to refer to all MSC-derived vesicles unless specific subtypes are noted. Biochemically, EVs carry a repertoire of molecules reflective of their cellular origin, including membrane receptors, cytosolic proteins, lipid raft components, and nucleic acids, enabling them to shuttle information between cells over short or long distances. Proteomics analysis has showed that exclusive markers are expressed highly on their surfaces, such as ALG-2 interacting protein X (Alix), tumor suppressor genes (TSG101), CD9, and CD63 [6]. EVs exhibit biological characteristics encompassing stability, histocompatibility, and good material exchangeability. They are naturally secreted by cells and prevent immune rejection. The cell-free structure helps them prevent malignant differentiation *in vivo* [7].

The application of MSCs in cell therapy is rooted in their ability to regulate inflammatory responses and participate in tissue repair and regeneration [8]. Traditionally, the immunomodulatory capacity of MSCs has attracted significant attention, as they can sense and respond to the inflammatory milieu by secreting immunosuppressive cytokines, chemokines, and growth factors. These mediators act on various immune cell types to attenuate excessive inflammation and reestablish immune homeostasis [9]. Concurrently, MSCs play a pivotal role in tissue repair by secreting trophic factors that enhance angiogenesis, recruit resident progenitor cells, and stimulate regeneration in damaged tissues. This regenerative potential extends across multiple systems, including the musculoskeletal, cardiovascular, hepatic, renal, and nervous systems [2]. Emerging evidence suggests that EVs derived from MSCs retain these therapeutic effects, including both immunomodulatory and regenerative functions while avoiding safety concerns related to live cell transplantation. This review synthesizes recent advances in MSC-EVs therapeutic applications, with Section 2 examining their regenerative roles and Section 3 detailing their immunomodulatory mechanisms (**Figure 1**). This dual functionality-bridging tissue repair and immune balance-positions MSC-EVs as a cornerstone of modern regenerative medicine, with applications spanning degenerative diseases, autoimmune disorders, and inflammatory injuries. As such, a comprehensive understanding of the characteristics and mechanisms of MSC-EVs is crucial for unlocking their full potential in these therapeutic areas, which will be further explored in the following sections.

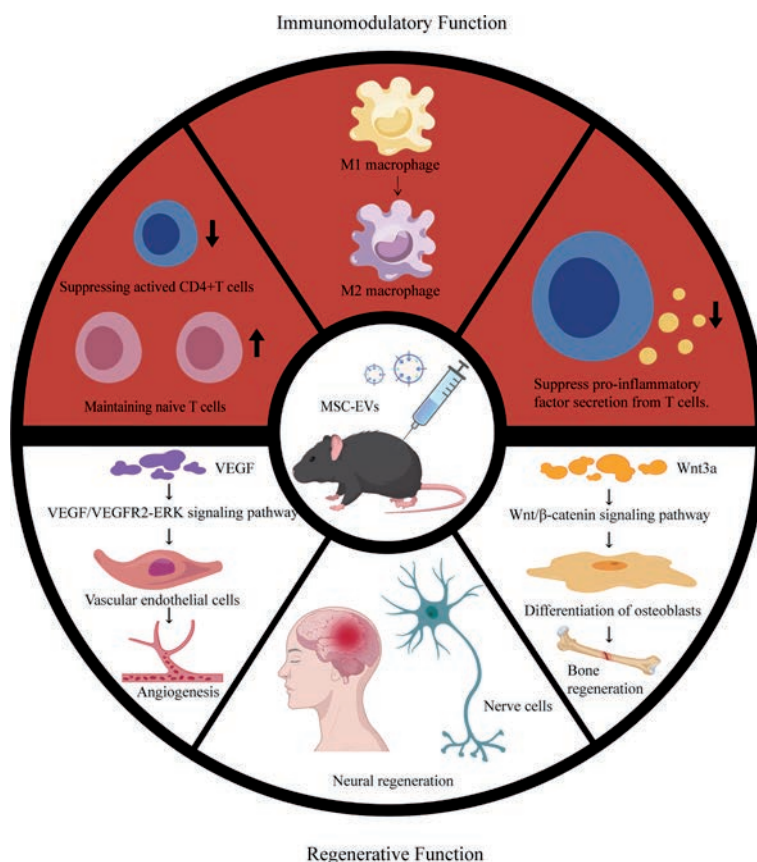


Figure 1.

The immunomodulatory and tissue regenerative capacities of MSC-EVs in various diseases. Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) combine immunomodulatory and tissue repair capabilities with advantages such as low immunogenicity and efficient delivery, emerging as a novel strategy in regenerative medicine. Studies have demonstrated that MSC-EVs not only retain the full immunomodulatory and tissue regenerative capacities of MSCs but also overcome the limitations of conventional cell therapies which exhibit remarkable advantages, including high biological barrier penetration efficiency, low immunogenicity, and stable bioactive components. From an immunomodulatory perspective, MSC-EVs exert their immunomodulatory effects through conserved mechanisms, including suppressing activated CD4⁺ T cells and maintaining naive T cells, polarization of immune cells, and suppression of pro-inflammatory cytokines. Their tissue regenerative potential is equally impressive, demonstrating the ability to target and repair damaged neural cells, vascular endothelial cells, and osteoblasts through sophisticated multi-mechanistic pathways, such as VEGF/VEGFR2-ERK signaling and Wnt/β-catenin signaling, that promote neural tissue regeneration, angiogenesis, and bone formation. Figure created with BioGDP.com.

2. Regenerative functions of MSC-EVs

The regenerative function of MSC-EVs is reflected in many aspects of the body. In a variety of pathological conditions such as cardiovascular disease, skeletal system disease, skin injury, dental and periodontal disease, liver disease, kidney disease, and nervous system disease (**Figure 1**). Generally, MSC-EVs promote tissue regeneration through diverse signaling pathways, each tailored to specific therapeutic targets. In osteogenesis, the Wnt/β-catenin pathway is activated by MSC-EV cargo to enhance bone formation, while the lnc-H19/miR-106a/Angpt1-Tie2-NO axis couples

angiogenesis with osteogenic differentiation. For anti-apoptosis and cell survival, the PI3K/AKT pathway and STAT3/CREB pathways are critical in cardiovascular and neuronal repair, respectively. In fibrotic diseases, MSC-EVs deliver miR-181-5p to suppress the STAT3/Bcl-2/Beclin1 pathway, mitigating collagen deposition. The IL-6/JAK2/STAT3 axis is targeted to resolve periodontal diseases. These mechanisms, detailed in subsequent sections, highlight the precision of MSC-EV-mediated therapy across tissues (**Table 1**).

2.1 MSC-EV therapy for revascularization and cardiac repair

Cardiovascular diseases (CVDs) are intimately linked with the processes of tissue regeneration and repair. While the cardiovascular system possesses a limited intrinsic capacity for self-renewal, this endogenous reparative potential is frequently inadequate following substantial injury. This limitation has driven the pursuit of exogenous regenerative strategies, particularly in the context of specific CVD subtypes. Among these, myocardial infarction (MI) represents a critical target for regenerative interventions due to its distinctive pathophysiological features [10]. Acute coronary artery occlusion precipitates sustained myocardial ischemia, during which the restricted proliferative capacity of terminally differentiated cardiomyocytes, coupled with progressive apoptotic cell death, culminates in irreversible myocardial damage. This damage is characterized not only by extensive cardiomyocyte loss but also by maladaptive ventricular remodeling and fibrotic scar formation, which collectively compromise cardiac contractile function and precipitate heart failure [11]. Conventional therapeutic modalities for myocardial infarction primarily aim to alleviate clinical symptoms and limit further damage, yet they fall short in promoting structural regeneration of the injured myocardium. This therapeutic gap has redirected attention toward stem cell-based regenerative strategies. Among these, MSC-EVs have emerged as a promising cell-free alternative, owing to their intrinsic regenerative and reparative capabilities. Accumulating evidence indicates that MSC-EV administration confers substantial cardioprotective and reparative benefits, mediated through a multifaceted array of mechanisms that synergistically promote myocardial regeneration and functional recovery [12]. In terms of promoting angiogenesis, MSC-EVs improve myocardial blood supply through a dual mechanism: On one hand, they carry angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor- β (FGF- β), and hepatocyte growth factor (HGF), which can directly activate endothelial cell function and promote neovascularization. On the other hand, MSC-EVs preconditioned by hypoxia significantly upregulate the expression of angiogenic genes, with characteristic miRNAs such as miR-125b-5p further enhancing vascular network formation through epigenetic regulation [13]. Notably, MSC-EVs also synergistically create a pro-angiogenic microenvironment by activating the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) signaling pathway and delivering matrix metalloproteinases (MMPs) [14]. In terms of inhibiting cardiomyocyte apoptosis, MSC-EVs exhibit multi-target regulatory properties: BMSC-EVs not only upregulate the expression of anti-apoptotic proteins such as B-cell lymphoma 2 (Bcl-2) by activating key survival signaling pathways like PI3K/protein kinase B (AKT)/mechanistic target of rapamycin (mTOR) but also silence pro-apoptotic factors through specific miRNAs such as miR-144. Meanwhile, they regulate protective miRNAs such as miR-21 to influence downstream apoptosis-related targets, forming a multi-layered anti-apoptotic protective network [15]. These research findings systematically elucidate the molecular mechanisms by which MSC-EVs promote myocardial

Disease	EV source	Biological molecule	Signaling	Target cells	Effect	Ref.
Cardiovascular diseases	BMSCs	miR-144	PTEN/AKT	H9C2 cardiomyocytes	anti-apoptosis	[15]
Bone defect	BMSCs	VEGF	VEGF/VEGFR2-ERK	Vascular endothelial cells	Promotion of vascularization	[17]
Bone defect	BMSCs	lnc-H19	lnc-H19/miR-106a/Angpt1-Tie2-NO	Endothelial cells	Promoting angiogenesis and osteogenic differentiation	[18]
Bone defect	Not stated	miR-27a	miR-27a/DKK2/Wnt/ β -catenin	Osteoblasts and osteoclasts	Promoting osteogenesis and inhibiting osteoclast	[20]
Bone defect	ADSCs	Osterix mRNA	Not stated	BMSCs	Contributing to bone differentiation	[21]
Skin defect	UCMSCs	Wnt4	Wnt/ β -catenin	Skin cells	Promoting skin regeneration and repairing	[25]
Periodontal injury	DPSCs	Not stated	IL-6/JAK2/STAT3	PDLSCs	Promoting periodontal healing	[28]
Chronic liver diseases	ADSCs	miR-181-5p	STAT3/Bcl-2/Bclm1	HST-T6	Anti-fibrosis	[31]
Acute kidney injury	TFAM-OE	TFAM mRNA, mtDNA	Not stated	Renal tubular cells	Reduction of kidney injury	[35]
Nerve injury	BMSCs	Cargo biomaterials	TLR2/IRAK1/NFkB	OPCs	Remyelination	[41]
Nerve injury	Not stated	miR-133b	ERK1/2, STAT3, and CREB	Neurons	Neuronal survival and axonal regeneration	[42]

Abbreviations: BMSCs, bone marrow mesenchymal stem cells; EVs, extracellular vesicles; ADSCs, adipose-derived mesenchymal stem cells; UCMSCs, umbilical cord blood mesenchymal stem cells; TFAM-OE, transcription factor an overexpression; PTEN, phosphatase; AKT, protein kinase b; VEGF, vascular endothelial growth factor; VEGFR2, vascular endothelial growth factor receptor 2; ERK, extracellular signal-regulated kinase; lnc-H19, long non-coding RNA h19; miR-106a, miRNA-106a; Angpt1, angiotensin-1-tyrosine kinase with immunoglobulin-like and EGF-like domains 2-nitric oxide; DKK2, dickkopf-related protein 2; Wnt, wingless-type integration site family; IL-6, interleukin-6; JAK2, janus kinase 2; STAT3, signal transducer and activator of transcription 3; Bcl-2, B-cell lymphoma 2; TLR2, toll-like receptor 2; IRAK1, interleukin-1 receptor-associated kinase 1; NFkB, nuclear factor kappa-light-chain-enhancer of activated b cells; CREB, cyclic amp response element-binding protein.

Table 1.
 MSC-EV therapy in tissue regeneration.

tissue repair through “multi-component and multi-pathway” synergistic effects, providing new strategic options for cardiovascular regenerative therapy. In particular, MSC-EVs can mimic the therapeutic effects of their parental stem cells while avoiding the risks associated with cell transplantation, making them a highly promising cell-free therapeutic strategy in the field of MI regenerative therapy.

2.2 MSC-EV therapy for skeletal regeneration and bone repair

Although the skeletal system possesses a robust intrinsic regenerative capacity, this ability is often insufficient in the context of large-scale bone defects, where complete structural and functional restoration cannot be achieved through endogenous repair alone. Bone regeneration is a highly orchestrated biological process involving sequential phases of inflammation resolution, neovascularization, and osteogenic differentiation. Disruption or dysregulation of any of these stages critically impairs the healing process and contributes to the failure of spontaneous bone regeneration. In clinical settings, critical-sized bone defects (CSBDs) are defined as the minimal defect dimensions at specific anatomical locations that do not heal without surgical or therapeutic intervention, thereby representing a significant challenge in orthopedic and reconstructive medicine [16]. Common etiologies include high-energy trauma, radical tumor resection, and chronic osteomyelitis. However, autografts face issues such as donor site morbidity, graft absorption, and limited availability, while allografts have drawbacks such as immune rejection, disease transmission risks, and poor bone integration. These limitations have prompted researchers to seek safer and more effective regenerative therapeutic strategies. MSC-EVs have emerged as a research hotspot due to their unique bone regeneration capabilities. MSC-EVs infusion therapy promotes bone defect repair through multi-dimensional mechanisms: In vascularized bone regeneration, they construct a sophisticated dual regulation system. On one hand, BMSC-EVs directly deliver angiogenic-osteogenic coupling factors such as VEGF and bone morphogenetic protein 2 (BMP2) [17]. On the other hand, they coordinate angiogenesis and osteogenesis through the long non-coding RNA H19 (lncRNA-H19)/ miR-23a-3p-angiopoietin 1 (ANGPT1)/phosphatase and tensin homolog (PTEN)/AKT signaling axis [18]. Notably, hypoxic preconditioning significantly enhances the pro-angiogenic activity of MSC-EVs by upregulating miR-126/miR-210-3p and activating the hypoxia-inducible factor (HIF) signaling pathway [19]. In terms of cell fate regulation, MSC-EVs exhibit precise bidirectional modulation capabilities: They promote the osteogenic differentiation of MSCs by activating the Wnt/ β -catenin pathway through osteogenic-related miRNAs such as miR-335 and miR-27 [20]. While ADSC-EVs simultaneously inhibiting osteoclast formation by secreting osteoprotegerin (OPG) and delivering bioactive components like miR-21-5p to suppress the receptor activator of nuclear factor kappa-B ligand (RANKL)/NF- κ B signaling pathway [21]. Existing studies have shown that strategies such as genetic engineering or biomolecular preconditioning can further optimize the bone repair efficacy of MSC-EVs. These important findings not only deepen our understanding of the mechanisms of bone regeneration but also provide a solid theoretical basis and translational pathway for the development of MSC-EVs-based targeted therapies.

2.3 MSC-EV therapy for skin regeneration and wound healing

As the largest organ of the human body, the skin possesses a significant capacity for self-repair. However, this regenerative process is often limited when facing

large-scale or chronic wounds. Wound healing in the skin is a highly coordinated physiological process involving precise regulation of three key stages: the inflammatory phase, the proliferative phase, and the remodeling phase. Disruption of any of these stages can lead to delayed healing or pathological scar formation. Among various types of skin injuries, chronic non-healing wounds and full-thickness skin defects pose significant challenges to regenerative medicine. These wounds are often accompanied by persistent inflammation, impaired angiogenesis, and abnormal remodeling of the extracellular matrix, making traditional therapeutic approaches less effective [22]. Recent studies have shown that MSC-EVs effectively promote skin tissue regeneration through multi-dimensional mechanisms. In terms of cellular function regulation, MSC-EVs from different tissue sources exhibit unique therapeutic advantages. BMSC-EVs enhance fibroblast proliferation and migration by activating the Akt/extracellular signal-regulated kinase (ERK)/signal transducer and activator of transcription 3 (STAT3) signaling axis and regulating the secretion of key growth factors [23]. In contrast, ADSC-EVs demonstrate precise control over extracellular matrix metabolism—promoting the synthesis and secretion of type I/III collagen to accelerate wound closure in the early stages of healing, while inhibiting excessive collagen deposition in the later stages to reduce pathological scar formation [24]. In terms of vascular reconstruction, UC-MSC-EVs exhibit remarkable angiogenic capabilities. Their carried miR-17-5p effectively improves angiogenic impairment in pathological conditions such as diabetes through the PTEN/AKT/hypoxia-inducible factor-1 (HIF-1)/VEGF signaling cascade [25]. Additionally, MSC-EVs can inhibit the differentiation of myofibroblasts by delivering characteristic miRNAs, thereby reducing the expression of transforming growth factor- β (TGF- β) and its downstream signaling. Notably, lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), which is enriched in adipose-derived MSC-EVs, regulates the epithelial-mesenchymal transition process and significantly accelerates re-epithelialization in ischemic wounds, providing a new molecular target for the treatment of chronic wounds [26]. These research findings not only elucidate the multiple mechanisms by which MSC-EVs promote skin regeneration but also lay an important foundation for the development of novel wound healing strategies based on MSC-EVs. Compared with traditional stem cell therapies, MSC-EVs offer better safety, stability, and tissue targeting, showing broad application prospects in the field of skin regenerative medicine.

2.4 MSC-EV therapy for dental and periodontal tissue repair

Oral tissues possess unique regenerative characteristics, but their self-repair capacity is often limited when facing severe injury or inflammatory destruction. The regeneration of dental and periodontal tissues involves complex biological processes, including stem cell activation, mineralized tissue formation, and regulation of the immune microenvironment. Dysregulation of these processes is a key factor leading to the failure of dental tissue regeneration. In clinical oral treatment, pulp necrosis and periodontitis-induced tissue defects pose special challenges to regenerative medicine. These injuries not only cause defects in hard tissues (such as dentin and alveolar bone) but are also often accompanied by persistent inflammatory states and destruction of the stem cell microenvironment, making traditional repair methods unable to achieve true tissue regeneration [27]. Studies have shown that MSC-EVs exhibit unique therapeutic effects in the field of oral tissue regeneration. In the regeneration of the dental pulp-dentin complex, MSC-EVs infusion therapy operates through multiple coordinated mechanisms. They are efficiently internalized by dental

pulp stem cells (DPSCs) *via* caveolae-mediated endocytosis, subsequently activating the p38 mitogen-activated protein kinase (p38/MAPK) signaling pathway, which markedly upregulates the expression of key odontogenic genes such as dentin sialophosphoprotein (DSPP). Meanwhile, DPSC-EVs precisely recruit endogenous stem cells to the injury site through the stromal cell-derived factor-1 (SDF-1)/C-X-C chemokine receptor type 4 (CXCR4) chemotactic axis, thereby providing a sustained cellular source for tissue regeneration [28]. In the context of periodontal tissue regeneration, MSC-EVs exert a multifaceted regulatory influence through a tripartite mechanism. First, they finely modulate the OPG-RANKL-RANK signaling axis to maintain the critical balance between bone resorption and formation. Second, MSC-EVs contribute to the resolution of local inflammation by promoting macrophage polarization toward the anti-inflammatory M2 phenotype and enhancing TGF- β 1 secretion, thereby establishing a regenerative microenvironment conducive to tissue repair [29]. Breakthroughs have been achieved in animal experiments: hydrogel-based MSC-EVs delivery systems not only significantly reduce the RANKL/OPG ratio but also demonstrate alveolar bone regeneration efficacy comparable to clinically used guided tissue regeneration techniques. These important findings not only elucidate the molecular mechanisms by which MSC-EVs promote oral tissue regeneration but also provide solid translational evidence for the development of cell-free dental regenerative therapies based on MSC-EVs. Compared with traditional cell therapies, MSC-EVs offer better safety, stability, and tissue targeting, showing unique application value in the field of dental regenerative medicine [30].

2.5 MSC-EV therapy for hepatic regeneration and functional recovery

The liver, as the largest metabolic organ in the human body, has a significant capacity for regeneration. However, under the continuous influence of chronic injury factors, this regenerative capacity is often replaced by progressive fibrosis. Chronic liver diseases (CLDs) represent a spectrum of disorders characterized by liver fibrosis as the common terminal pathological change. Among them, nonalcoholic fatty liver disease (NAFLD) and its progressive form, nonalcoholic steatohepatitis (NASH), have become the main contributors to the global burden of liver diseases, with the proportion of patients with chronic liver diseases increasing year by year. The core pathological process of liver fibrosis involves the abnormal activation of hepatic stellate cells (HSCs), excessive deposition of the extracellular matrix, and a persistent inflammatory microenvironment. These changes ultimately lead to cirrhosis and liver failure [31]. In the field of liver disease treatment, MSC-EVs have garnered significant attention due to their unique multi-target intervention characteristics. Studies have shown that MSC-EVs infusion therapy exerts therapeutic effects through multi-level molecular mechanisms: In terms of signaling pathway regulation, they effectively inhibit the STAT3/Bcl-2/Beclin signaling axis by delivering regulatory RNAs such as miR-181-5p, significantly downregulating the expression of α -smooth muscle actin (α -SMA) and type I/III collagen, thereby blocking the activation of HSCs [32]. In terms of matrix remodeling, they reduce abnormal deposition of the extracellular matrix by modulating the Wnt/ β -catenin pathway, improving liver tissue structure [33]. MSC-EVs demonstrate dual regulatory effects on the hepatic microenvironment through two coordinated mechanisms: first, they suppress Kupffer cell overactivation and reduce the release of pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), thereby improving the inflammatory milieu; second, they activate regenerative pathways in hepatocytes

through proliferation-stimulating signals to promote tissue repair. This bifunctional activity enables MSC-EVs to simultaneously modulate inflammatory responses and enhance parenchymal regeneration in the liver microenvironment. Notably, MSC-EVs derived from different tissue sources exhibit significant differences in therapeutic efficacy. ADSC-EVs, enriched with regulatory molecules such as miR-181-5p, show stronger inhibition of the STAT3 signaling pathway and unique advantages in anti-fibrotic therapy [31]. These important findings not only deepen our understanding of the pathogenesis of liver fibrosis but also provide new ideas for the development of multi-target combined therapeutic strategies based on MSC-EVs. Compared with traditional drug therapies, MSC-EVs offer the advantages of multi-target synergy and good biocompatibility, showing broad application prospects in the field of chronic liver disease treatment. Future research should focus on optimizing the preparation process and delivery systems of MSC-EVs to further improve their targeting and therapeutic effects in liver disease treatment.

2.6 MSC-EV therapy for renal repair and regeneration

The kidneys, as important excretory and endocrine organs in the human body, possess a certain capacity for self-repair. However, in the face of severe injury or chronic lesions, this regenerative mechanism is often insufficient to maintain normal function. Acute kidney injury (AKI) and chronic kidney disease (CKD) constitute the main spectrum of causes leading to end-stage renal failure, with pathological processes involving renal tubular epithelial cell injury, inflammatory response, oxidative stress, and progressive fibrosis [34]. MSC-EVs have demonstrated significant therapeutic potential in AKI treatment by simultaneously targeting multiple pathological processes. Experimental studies have demonstrated that these nanovesicles facilitate renal recovery through three principal mechanisms. First, they directly promote the proliferation of renal tubular epithelial cells, thereby accelerating epithelial repair. Second, they modulate the Kelch-like ECH-associated protein 1 (Keap1)-nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway to enhance mitochondrial function and augment cellular antioxidant defenses. Finally, they markedly attenuate both local and systemic inflammatory responses, thereby reducing sustained inflammatory damage to renal tissue [35]. MSC-EVs demonstrate significant anti-fibrotic potential in CKD, particularly in diabetic nephropathy, by targeting multiple pathological processes simultaneously. MSC-EVs effectively suppress renal fibrosis through two primary mechanisms: first, they significantly downregulate the expression of TGF- β 1 and inhibit its downstream fibrotic signaling pathways; second, they activate mTOR-mediated autophagy to enhance the clearance of damaged cellular components and maintain intracellular homeostasis. These mechanisms collectively effectively delay the progression of renal interstitial fibrosis [36]. Existing preclinical research data indicate that MSC-EV therapy has a favorable safety profile, with no significant adverse reactions such as immune rejection or tumorigenicity observed [37]. Its therapeutic effects primarily stem from the targeted delivery of bioactive molecules it carries, including proteins, mRNA, and miRNAs, which precisely regulate key signaling pathways in renal repair. These important findings provide a new translational direction for the treatment of kidney diseases. Compared with traditional drug therapies or stem cell transplantation, MSC-EVs offer better targeting, stability, and safety advantages in the field of renal regenerative medicine, showing broad application prospects [35]. Future research should focus on solving key issues such

as large-scale production, quality control, and optimal dosing regimens of MSC-EVs to accelerate their clinical translation process. Meanwhile, exploring the therapeutic efficacy differences of MSC-EVs derived from different tissue sources and developing engineered MSC-EVs with enhanced effects will provide more effective solutions for kidney disease treatment.

2.7 MSC-EV therapy for neural regeneration and nerve repair

The nervous system presents unique regenerative challenges due to the limited self-repair capacity of mature neurons and the inhibitory microenvironment that develops after injury, making neurological repair—particularly for central nervous system injuries—one of the most significant challenges in regenerative medicine. Unlike most other tissues which possess robust regenerative abilities, the nervous system's combination of intrinsic neuronal growth limitations and extrinsic inhibitory factors creates substantial barriers to effective repair. These fundamental biological constraints explain why successful regeneration after neural injury remains an elusive goal, driving the need for innovative therapeutic approaches in this field [38]. In peripheral nerve repair, MSC-EVs have demonstrated significant promotional effects. Their mechanisms of action primarily involve activation of the JNK/c-Jun signaling pathway, which induces Schwann cells to adopt a reparative phenotype and enhances their secretion of neurotrophic factors [39]. Concurrently, MSC-EVs modulate the local inflammatory response by significantly downregulating key pro-inflammatory cytokines such as IL-6 and IL-1 β , thereby improving the microenvironment for nerve regeneration. These combined effects establish favorable conditions for axonal regrowth [40]. In contrast, the role of MSC-EVs in central nervous system regeneration is more intricate and multifaceted. They construct a sophisticated regulatory network: Inhibiting neuronal apoptosis through multiple signaling pathways such as Wnt/ β -catenin to protect surviving neurons; activating ERK/STAT3 signaling to promote synaptic plasticity and neural circuit reconstruction; promoting angiogenesis to improve blood supply in the injured area, while stimulating oligodendrocyte differentiation and myelin regeneration to restore nerve conduction function [41]. Notably, non-coding RNAs such as miR-133b carried by MSC-EVs play a central role in these regulatory processes, influencing the expression of multiple key target genes through epigenetic regulation [42]. These findings not only elucidate the molecular mechanisms by which MSC-EVs promote nerve regeneration but also provide important evidence for the development of new therapeutic strategies for nervous system injuries. Compared with traditional cell transplantation therapies, MSC-EVs offer better blood-brain barrier penetration and safety, showing unique advantages in the field of nerve regenerative medicine. Future research should focus on optimizing the delivery methods and dosages of MSC-EVs and exploring the optimal therapeutic time windows for different types of nerve injuries to further enhance their therapeutic effects.

2.8 Conclusion

In conclusion, MSC-EVs have emerged as a highly promising cell-free therapeutic strategy in regenerative medicine, demonstrating the remarkable potential for tissue repair and functional recovery across diverse pathologies including cardiovascular diseases, musculoskeletal disorders, and neurological conditions. These nanoscale vesicles exert their therapeutic effects through the targeted delivery of bioactive cargo

comprising proteins, nucleic acids, and lipids [43]. However, significant challenges persist in clinical translation, particularly regarding standardization of isolation protocols, scalability of production, and optimization of delivery efficiency. Notably, the therapeutic efficacy of MSC-EVs is intrinsically linked to the properties of their parent MSCs, which may vary due to donor heterogeneity, culture conditions, or passage-induced senescence. Critically, while MSCs themselves are considered low-risk for tumorigenesis, prolonged *in vitro* expansion can lead to genetic instability or malignant transformation—a concern that necessitates rigorous evaluation of whether derived EVs inherit any oncogenic potential, such as carrying pro-tumorigenic miRNAs or proteins. Future research must prioritize the development of robust characterization methods, innovative engineering approaches for enhanced targeting and cargo loading, and rigorous preclinical validation through standardized models. Crucially, establishing collaborative frameworks between researchers, clinicians, and regulatory bodies will be essential to define clear pathways for clinical implementation, with specific guidelines to address EV batch consistency, MSC sourcing ethics, and post-transplantation monitoring for unintended proliferation [44]. Through such multidisciplinary efforts, MSC-EVs hold tremendous potential to transition from experimental platforms to clinically viable next-generation therapeutics in regenerative medicine.

3. Immunoregulation of MSC-EVs

MSC-EVs exert their immunomodulatory effects through conserved mechanisms, including miRNA-mediated regulation of inflammatory pathways, polarization of immune cells, and suppression of pro-inflammatory cytokines (**Figure 1**). Their cell-free nature, low immunogenicity, and multi-targeted actions position MSC-EVs as a versatile therapeutic platform, bridging autoimmune, inflammatory, and degenerative diseases through shared immunoregulatory axes. This universality highlights their potential to redefine treatment strategies for complex, immune-driven pathologies. This section first consolidates core mechanisms, then examines system-specific applications.

3.1 Common immunomodulatory mechanisms

MSC-EVs exhibit multi-dimensional regulatory capabilities in immunomodulation through the delivery of bioactive molecules. Their core mechanisms can be summarized as miRNA-mediated signaling pathway reprogramming, immune cell phenotype polarization, and cytokine network regulation, which synergistically restore immune homeostasis (**Table 2**).

3.1.1 T cell function regulation

The phenotypic remodeling of immune cells by MSC-EVs is a key aspect of their immunomodulatory function: regulatory T cell (Treg) induction and effector T cell suppression are two interconnected immunomodulatory functions of MSC-EVs in managing immune-related disorders. Interferon- γ (IFN- γ)-preconditioned MSC-EVs enhance Treg expansion by upregulating PD-L1/PD-L2 molecules to engage the programmed cell death 1 (PD-1) signaling pathway, a mechanism that effectively mitigates immune rejection in graft-versus-host disease (GVHD) [45]. Concurrently,

Disease	EV source	Key mechanism	Effect	Ref.
Rheumatoid arthritis	BMSCs	Inhibits Th17 <i>via</i> miR-150-5p, promotes Tregs	Reduces joint inflammation and bone erosion	[46]
Systemic lupus erythematosus	ADSCs	Delivers miR-19b to restore Th17/Treg balance	Suppresses B-cell hyperactivity	[52]
Colitis	Hypoxia-primed MSCs	Enhances M2 polarization <i>via</i> HIF-1 α	Accelerates mucosal healing	[51]
GvHD	IDO-overexpressing MSCs	miR-540-3p/JAK3 inhibits DC maturation	Suppresses alloimmune T cell activation	[49]
Parkinson's disease	Various MSCs	miR-133b promotes microglial M2 polarization	Reduces α -synuclein aggregation	[60]
Acute lung injury	UCMSCs	Inhibits NF- κ B/NLRP3 inflammasome activation; reduces M1 macrophage polarization	Alleviates pulmonary inflammatory infiltration and tissue damage	[53]
Glomerulonephritis	Various MSCs	IL-10-enriched EVs inhibit M1 macrophage activation; suppress TGF- β 1-mediated fibrosis	Reduces renal inflammation and interstitial fibrosis	[54]
Silicosis	UCMSCs	miR-223-3p targets circPWWP2A/NLRP3 axis; reduces silica-induced M1 polarization and fibrosis	Mitigates lung inflammation and collagen deposition	[65]
Idiopathic pulmonary fibrosis	BMSCs	Delivers miR-186 to downregulate SOX4/DKK1; inhibits fibroblast activation	Suppresses lung fibrosis and EMT	[66]

Abbreviations: BMSCs, bone marrow mesenchymal stem cells; ADSCs, adipose-derived mesenchymal stem cells; UCMSCs, umbilical cord blood mesenchymal stem cells; Th17, T helper 17 cells; Tregs, regulatory T cells; HIF-1 α , hypoxia-inducible factor 1-alpha; GvHD, graft-versus-host disease; IDO, indoleamine 2,3-dioxygenase; JAK3, Janus kinase 3; DC, dendritic cells; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3, NLR family pyrin domain containing 3; IL-10, interleukin 10; TGF- β 1, transforming growth factor beta 1; SOX4, SRY-box transcription factor 4; DKK1, dickkopf-related protein 1; EMT, epithelial-mesenchymal transition; miR-150-5p, microRNA-150-5p; α -synuclein, alpha-synuclein; circPWWP2A, circular RNA PWWP2A.

Table 2.
MSC-EV therapy in immunoregulation.

in conditions like rheumatoid arthritis (RA) and multiple sclerosis, MSC-EVs directly inhibit the proliferation of activated CD4⁺ T cells and suppress T helper 17 cells (Th17) cells differentiation, thereby addressing the root cause of autoimmune attacks by disrupting pro-inflammatory T cell responses [46, 47]. For autoimmune disorders, MSC-EVs modulate T cell responses: BMSC-EVs increase Tregs and reduce Th17 cells *via* TGF- β [48], while IDO-overexpressing MSC-EVs inhibit dendritic cell (DC) maturation through miR-540-3p/JAK3, indirectly dampening T cell activation in organ transplantation [49]. Together, these mechanisms highlight MSC-EVs' dual role in promoting immune tolerance and suppressing excessive inflammation to restore immune balance across diverse pathological contexts.

3.1.2 Macrophage repolarization

The M1 to M2 phenotype switch and enhanced phagocytic function represent two critical mechanisms by which MSC-EVs regulate macrophage activity in inflammatory diseases. BMSC-EVs induce anti-inflammatory M2 macrophage polarization *via* the PI3K-Akt signaling pathway, thereby alleviating cartilage degradation in osteoarthritis [50], while hypoxic preconditioned MSC-EVs enhance M2 polarization efficiency through HIF-1 α pathway activation, accelerating mucosal repair in colitis models [51]. In systemic lupus erythematosus (SLE), MSC-EVs further improve macrophage phagocytic capacity for apoptotic cells, reducing self-antigen exposure and suppressing systemic immune hyperactivation [52]. Together, these mechanisms demonstrate MSC-EVs' multifaceted ability to reprogram macrophage function, balancing inflammation resolution and tissue repair across diverse pathological contexts.

3.1.3 Cytokine network regulation

MSC-EVs reshape the inflammatory microenvironment through bidirectional regulation of the cytokine network, simultaneously suppressing pro-inflammatory responses and enhancing anti-inflammatory mechanisms. In acute lung injury (ALI) and colitis, MSC-EVs significantly reduce the secretion of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α , thereby alleviating pulmonary inflammatory infiltration and intestinal mucosal damage [48, 53]. Concurrently, in glomerulonephritis models, IL-10-enriched MSC-EVs inhibit M1 macrophage activation by increasing local anti-inflammatory cytokine levels, effectively reducing the degree of renal tissue inflammation [54]. This dual regulation highlights MSC-EVs' capacity to rebalance cytokine networks and resolve pathological inflammation across multiple organ systems.

3.2 System-specific applications

MSC-EVs exhibit tailored effects in distinct organ systems, leveraging their common mechanisms to address disease-specific pathologies.

3.2.1 MSC-EV therapy for immune-mediated skin disorders and regeneration

As the body's largest organ, the skin serves as a vital protective barrier and maintains homeostasis, with its health intricately linked to immune system balance. Skin diseases are increasingly recognized as closely associated with dysregulated inflammatory responses, encompassing a spectrum of conditions such as vitiligo and wound healing disorders. Recent studies have emphasized that MSC-EV injection can boost angiogenesis, regulate immune responses, facilitate extracellular matrix remodeling, and promote skin cell proliferation and migration. The effects collectively enhance skin quality and health.

Numerous studies have demonstrated that MSC-EVs can significantly alleviate a range of skin pathologies. These effects encompass a reduction in the number of blood eosinophils, decreased mast cell infiltration within the skin, diminished inflammation, and enhanced wound healing. In skin aging and photoaging, MSC-EVs counteract ultraviolet (UV)-induced oxidative stress and collagen degradation by modulating MAPK/activator protein-1 (AP-1) and TGF- β /Smad pathways [55]. For wound healing, MSC-EVs enhance fibroblast migration and ECM remodeling—a mechanism

overlapping with their suppression of inflammatory damage in Alzheimer's disease (AD) and vitiligo [56].

In conclusion, with their unique biological functions and advantages, MSC-EVs have demonstrated remarkable potential in treating various skin diseases and promoting wound repair, emerging as a highly promising novel therapeutic approach in the field of skin health.

3.2.2 MSC-EV therapy for neuroinflammation and central nervous system repair

Neurodegenerative diseases are the most common manifestations of central nervous system (CNS) pathologies, usually including Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). Even though the molecular mechanisms of CNS-related disorders' pathogenesis are still mostly unknown, research has shown that inflammatory responses are crucial in their development [47]. Studies have demonstrated that MSC-EVs are capable of releasing various immunomodulatory factors, such as cytokines and growth factors, which in turn inhibit the release of excessive inflammatory mediators from over-activated microglial cells, thereby reducing neural damage and apoptosis [57].

For AD, MSC-EVs play crucial roles. They target A β pathology by delivering siRNA to inhibit the activity of β - and γ -secretases, thus reducing A β production [58]. Meanwhile, Cui found that hypoxia-preconditioned MSC-EVs significantly reduced the levels of pro-inflammatory factors IL-1 β and TNF- α and increased the levels of anti-inflammatory factors IL-4 and IL-10 in an AD model. They inhibited the inflammatory activation of astrocytes and microglia. Mechanistically, MSC-EVs achieved global regulation of central neuroinflammation by inhibiting the activities of the STAT3 and NF- κ B signaling pathways [59]. Regarding PD, MSC-EVs are involved in α -synuclein (α -syn) regulation. They deliver miR-133b to promote neuronal synapse growth and clear α -syn aggregates through autophagy [60]. Moreover, Peng et al. developed a self-guided nanocarrier, PR-EXO/PP@Cur, combining therapeutic MSC-EVs with curcumin. This integration enhances the functional restoration of neurons and lessens neuroinflammatory responses by diminishing the levels of α -synuclein aggregates [61]. For ALS, EVs significantly alleviated the pathological characteristics and neuroinflammation in SOD1G93A astrocytes. Lee and colleagues showed that after treatment with EVs from ADSCs, neural stem cells in superoxide dismutase 1 (SOD1) transgenic ALS mice could differentiate. The analysis indicated a decline in cytosolic SOD1 aggregates. Additionally, there was an enhancement in mitochondrial protein markers, like the phospho-CREB/CREB ratio and PGC-1 α [62]. In the case of HD, MSC-EVs carry out immunomodulation by releasing cytokines like IL-10 to inhibit the overactivation of microglia and reduce neuroinflammation [57].

In conclusion, MSC-EVs show great potential in treating CNS neurodegenerative diseases. However, further research is needed to optimize their use, overcome technical challenges, and translate these findings into effective clinical therapies for patients.

3.2.3 MSC-EV therapy for immune-related pulmonary injury and repair

The respiratory system relies on immune system coordination for homeostasis. Respiratory diseases are increasingly recognized as intricately linked to dysregulated inflammatory responses, with examples including pneumonia, allergic asthma, idiopathic pulmonary fibrosis (IPF), and silicosis. MSC-EVs have been shown to regulate

immune cells in respiratory disorders, effectively alleviating the inflammation linked to respiratory impairment and tissue injury [63].

MSC-EVs possess immunomodulatory properties, as demonstrated in the context of murine cytomegalovirus (MCMV)-induced pneumonia. In this disease, MCMV activates the NF- κ B/NLRP3 signaling pathway, leading to hyperinflammation, macrophage M1 polarization, and pulmonary fibrosis. MSC-EVs, however, alleviate these conditions. They inhibit the NF- κ B/NLRP3 pathway, reduce pro-inflammatory cytokine release, and promote a shift from M1 to M2 macrophage polarization. This modulation helps mitigate lung inflammation and fibrosis, highlighting the potential of MSC-EVs in treating viral pneumonia [64]. Similarly, in COVID-19 pneumonia, MSC-EVs suppress hyperinflammation by transferring miR-223-3p to inhibit NLRP3 inflammasome activation [65]. In IPF, MSC-EVs deliver miR-186 to downregulate SOX4/DKK1, inhibiting fibroblast activation [66], while in silicosis, miR-223-3p targets the circPWWP2A/NLRP3 axis to curb silica-induced fibrosis, demonstrating shared anti-fibrotic and anti-inflammatory pathways [65].

In conclusion, MSC-EVs and their associated miRNAs play a crucial role in treating various respiratory diseases, from pneumonia like COVID-19 to chronic conditions such as asthma, IPF, and silicosis. They offer promising therapeutic strategies by modulating inflammation and fibrosis.

3.3 Conclusion

MSC-EVs demonstrate remarkable versatility as immunomodulatory agents across diverse pathological contexts, bridging autoimmune, inflammatory, and degenerative diseases through conserved mechanisms. By regulating T cell function, polarizing macrophages toward anti-inflammatory M2 phenotypes, and suppressing pro-inflammatory cytokines, MSC-EVs restore immune homeostasis in conditions ranging from lupus nephritis to Alzheimer's disease. While preclinical studies highlight their efficacy in skin repair, neuroinflammation suppression, and respiratory injury mitigation, challenges remain in standardizing isolation protocols, optimizing cargo engineering, and validating clinical safety. In the realm of immunology, MSC-EV-based therapy confronts several formidable challenges. Firstly, the immunomodulatory effects of MSC-EVs are highly context-dependent. Their impact on immune cell subsets, such as T cells and macrophages, can vary widely based on the disease microenvironment, making it difficult to precisely control the therapeutic immune response [67]. Secondly, the potential for immune evasion or unexpected immune reactions remains a concern. As foreign entities, MSC-EVs might trigger unanticipated immune responses in some patients, undermining their safety and efficacy. Moreover, standardizing the immunomodulatory potency of MSC-EVs across different production batches is a significant hurdle, given the variability in MSC sources and isolation techniques. Future research should prioritize mechanistic elucidation of context-dependent effects and large-scale trials to translate MSC-EVs from bench to bedside, ultimately redefining treatment paradigms for complex immune-driven pathologies.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (82371020, 82301028, and 82474351), Projects of State Key Laboratory of

Oral & Maxillofacial Reconstruction and Regeneration (2024MS04 and 2024KA01), the China Postdoctoral Science Foundation (BX20230485), and the Qin Chuang Yuan Traditional Chinese Medicine Industry Innovation Agglomeration Zone Project of Xianyang (L2024-QCY-ZYYJJQ-X25).

Conflict of interest

The authors declare that they have no conflicts of interest.

Author details

Yu Fu^{1†}, Lufan Yang^{1†}, Siyu Lan^{1†}, Sha Zhang¹, Kai Zhang¹, Shengfeng Bai¹, Wen Qin², Chenxi Zheng^{1*} and Bingdong Sui^{1*}

1 State Key Laboratory of Military Stomatology and National Clinical Research Center for Oral Diseases and Shaanxi International Joint Research Center for Oral Diseases, Center for Tissue Engineering, School of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi, China

2 State Key Laboratory of Military Stomatology and National Clinical Research Center for Oral Diseases and Shaanxi International Joint Research Center for Oral Diseases, Department of Orthodontics, School of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi, China

*Address all correspondence to: chenxizheng@fmmu.edu.cn;
bingdong@fmmu.edu.cn

† These authors contributed equally to this work.

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Maqsood M et al. Adult mesenchymal stem cells and their exosomes: Sources, characteristics, and application in regenerative medicine. *Life Sciences*. 2020;**256**:118002
- [2] Jafarinia M et al. Mesenchymal stem cell-derived extracellular vesicles: A novel cell-free therapy. *Immunological Investigations*. 2020;**49**(7):758-780
- [3] Rani S et al. Mesenchymal stem cell-derived extracellular vesicles: Toward cell-free therapeutic applications. *Molecular Therapy*. 2015;**23**(5):812-823
- [4] Gregory CD, Rimmer MP. Extracellular vesicles arising from apoptosis: Forms, functions, and applications. *The Journal of Pathology*. 2023;**260**(5):592-608
- [5] Casado-Díaz A, Quesada-Gómez JM, Dorado G. Extracellular vesicles derived from mesenchymal stem cells (MSC) in regenerative medicine: Applications in skin wound healing. *Frontiers in Bioengineering and Biotechnology*. 2020;**8**:146
- [6] O'Brien K et al. RNA delivery by extracellular vesicles in mammalian cells and its applications. *Nature Reviews. Molecular Cell Biology*. 2020;**21**(10):585-606
- [7] Tang Y, Zhou Y, Li HJ. Advances in mesenchymal stem cell exosomes: A review. *Stem Cell Research & Therapy*. 2021;**12**(1):71
- [8] Hosseini S et al. The robust potential of mesenchymal stem cell-loaded constructs for hard tissue regeneration after cancer removal. *Advances in Experimental Medicine and Biology*. 2019;**1084**:17-43
- [9] Li N, Hua J. Interactions between mesenchymal stem cells and the immune system. *Cellular and Molecular Life Sciences*. 2017;**74**(13):2345-2360
- [10] Mensah GA et al. Decline in cardiovascular mortality: Possible causes and implications. *Circulation Research*. 2017;**120**(2):366-380
- [11] Wu X et al. Angiogenesis after acute myocardial infarction. *Cardiovascular Research*. 2021;**117**(5):1257-1273
- [12] Gunasekaran M et al. Comparative efficacy and mechanism of action of cardiac progenitor cells after cardiac injury. *iScience*. 2022;**25**(8):104656
- [13] Ke X et al. Endothelial colony-forming cell-derived exosomal miR-21-5p regulates autophagic flux to promote vascular endothelial repair by inhibiting SIPL1A2 in atherosclerosis. *Cell Communication and Signaling: CCS*. 2022;**20**(1):30
- [14] Vrijnsen KR et al. Exosomes from cardiomyocyte progenitor cells and mesenchymal stem cells stimulate angiogenesis via EMMPRIN. *Advanced Healthcare Materials*. 2016;**5**(19):2555-2565
- [15] Wen Z et al. Mesenchymal stem cell-derived exosomes ameliorate cardiomyocyte apoptosis in hypoxic conditions through microRNA144 by targeting the PTEN/AKT pathway. *Stem Cell Research & Therapy*. 2020;**11**(1):36
- [16] Calciolari E, Donos N, Mardas N. Osteoporotic animal models of bone healing: Advantages and pitfalls. *Journal of Investigative Surgery*. 2017;**30**(5):342-350

- [17] Takeuchi R et al. Exosomes from conditioned media of bone marrow-derived mesenchymal stem cells promote bone regeneration by enhancing angiogenesis. *PLoS One*. 2019;**14**(11):e0225472
- [18] Behera J et al. Exosomal lncRNA-H19 promotes osteogenesis and angiogenesis through mediating Angpt1/Tie2-NO signaling in CBS-heterozygous mice. *Theranostics*. 2021;**11**(16):7715-7734
- [19] Castañón-Cortés LG et al. Current advances in the development of microRNA-integrated tissue engineering strategies: A cornerstone of regenerative medicine. *Frontiers in Bioengineering and Biotechnology*. 2024;**12**:1484151
- [20] Wang Y, Zhou X, Wang D. Mesenchymal stem cell-derived extracellular vesicles inhibit osteoporosis via microRNA-27a-induced inhibition of DKK2-mediated Wnt/ β -catenin pathway. *Inflammation*. 2022;**45**(2):780-799
- [21] Liang Z et al. Osterix mRNA enrichment in small extracellular vesicles derived from osteogenically induced ADSCs: A promoter of osteogenic differentiation in BMSCs. *Journal of Cellular and Molecular Medicine*. 2025;**29**(1):e70353
- [22] Zhao R et al. Inflammation in chronic wounds. *International Journal of Molecular Sciences*. 2016;**17**(12):17122085
- [23] Tang S et al. Global trends in the clinical utilization of exosomes in dermatology: A bibliometric analysis. *Frontiers in Medicine (Lausanne)*. 2024;**11**:1462085
- [24] Hu L et al. Exosomes derived from human adipose mesenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. *Scientific Reports*. 2016;**6**:32993
- [25] Zhang B et al. HucMSC exosome-delivered 14-3-3 ζ orchestrates self-control of the Wnt response via modulation of YAP during cutaneous regeneration. *Stem Cells*. 2016;**34**(10):2485-2500
- [26] Huang F et al. Bioinspired collagen scaffold loaded with bFGF-overexpressing human mesenchymal stromal cells accelerating diabetic skin wound healing via HIF-1 signal pathway regulated neovascularization. *ACS Applied Materials & Interfaces*. 2024;**16**(35):45989-46004
- [27] Zhou L et al. Therapeutic effect of mesenchymal stem cell-derived exosome therapy for periodontal regeneration: A systematic review and meta-analysis of preclinical trials. *Journal of Orthopaedic Surgery and Research*. 2025;**20**(1):27
- [28] Qiao X et al. Dental pulp stem cell-derived exosomes regulate anti-inflammatory and osteogenesis in periodontal ligament stem cells and promote the repair of experimental periodontitis in rats. *International Journal of Nanomedicine*. 2023;**18**:4683-4703
- [29] Mohammed E, Khalil E, Sabry D. Effect of adipose-derived stem cells and their exo as adjunctive therapy to nonsurgical periodontal treatment: A histologic and histomorphometric study in rats. *Biomolecules*. 2018;**8**(4):8040167
- [30] Wang D et al. Schwann cell-derived EVs facilitate dental pulp regeneration through endogenous stem cell recruitment via SDF-1/CXCR4 axis. *Acta Biomaterialia*. 2022;**140**:610-624
- [31] Qu Y et al. Exosomes derived from miR-181-5p-modified adipose-derived

mesenchymal stem cells prevent liver fibrosis via autophagy activation. *Journal of Cellular and Molecular Medicine*. 2017;**21**(10):2491-2502

[32] Kaffash Farkhad N, 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

[33] Rong X et al. Human bone marrow mesenchymal stem cells-derived exosomes alleviate liver fibrosis through the Wnt/ β -catenin pathway. *Stem Cell Research & Therapy*. 2019;**10**(1):98

[34] Wang DR, Pan J. Extracellular vesicles: Emerged as a promising strategy for regenerative medicine. *World Journal of Stem Cells*. 2023;**15**(4):165-181

[35] Zhao M et al. Mesenchymal stem cell-derived extracellular vesicles attenuate mitochondrial damage and inflammation by stabilizing mitochondrial DNA. *ACS Nano*. 2021;**15**(1):1519-1538

[36] Wang W, Wang J, Liao D. Effects and mechanisms of extracellular vesicles in different models of acute kidney injury. *Stem Cells International*. 2025;**2025**:1075016

[37] Nassar W et al. Erratum to: Umbilical cord mesenchymal stem cells derived extracellular vesicles can safely ameliorate the progression of chronic kidney diseases. *Biomaterials Research*. 2017;**21**:3

[38] Qi T et al. Experimental study on repairing peripheral nerve defects with novel bionic tissue engineering. *Advanced Healthcare Materials*. 2023;**12**(17):e2203199

[39] Mao Q et al. Gingiva-derived mesenchymal stem cell-extracellular

vesicles activate schwann cell repair phenotype and promote nerve regeneration. *Tissue Engineering Part A*. 2019;**25**(11-12):887-900

[40] Moghassemi S et al. Extracellular vesicles in nanomedicine and regenerative medicine: A review over the last decade. *Bioactive Materials*. 2024;**36**:126-156

[41] Zhang J et al. Exosomes derived from bone marrow mesenchymal stromal cells promote remyelination and reduce neuroinflammation in the demyelinating central nervous system. *Experimental Neurology*. 2022;**347**:113895

[42] Li D et al. Exosomes derived from miR-133b-modified mesenchymal stem cells promote recovery after spinal cord injury. *Frontiers in Neuroscience*. 2018;**12**:845

[43] Kou M et al. Mesenchymal stem cell-derived extracellular vesicles for immunomodulation and regeneration: A next generation therapeutic tool? *Cell Death & Disease*. 2022;**13**(7):580

[44] Padinharayil H et al. Mesenchymal stem cell-derived exosomes: Characteristics and applications in disease pathology and management. *Life Sciences*. 2024;**342**:122542

[45] Hackel A et al. Immunological priming of mesenchymal stromal/stem cells and their extracellular vesicles augments their therapeutic benefits in experimental graft-versus-host disease via engagement of PD-1 ligands. *Frontiers in Immunology*. 2023;**14**:1078551

[46] Liu H et al. Immunomodulatory effects of mesenchymal stem cells and mesenchymal stem cell-derived extracellular vesicles in rheumatoid arthritis. *Frontiers in Immunology*. 2020;**11**:1912

- [47] Stephenson J et al. Inflammation in CNS neurodegenerative diseases. *Immunology*. 2018;**154**(2):204-219
- [48] An JH et al. TSG-6 in extracellular vesicles from canine mesenchymal stem/stromal is a major factor in relieving DSS-induced colitis. *PLoS One*. 2020;**15**(2):e0220756
- [49] He JG et al. Exosomes derived from IDO1-overexpressing rat bone marrow mesenchymal stem cells promote immunotolerance of cardiac allografts. *Cell Transplantation*. 2018;**27**(11):1657-1683
- [50] Li K et al. Anti-inflammatory and immunomodulatory effects of the extracellular vesicles derived from human umbilical cord mesenchymal stem cells on osteoarthritis via M2 macrophages. *Journal of Nanobiotechnology*. 2022;**20**(1):38
- [51] Gómez-Ferrer M et al. HIF-overexpression and pro-inflammatory priming in human mesenchymal stromal cells improves the healing properties of extracellular vesicles in experimental Crohn's disease. *International Journal of Molecular Sciences*. 2021;**22**(20):222011269
- [52] Zhang M et al. Mesenchymal stem cell-derived exosome-educated macrophages alleviate systemic lupus erythematosus by promoting efferocytosis and recruitment of IL-17(+) regulatory T cell. *Stem Cell Research & Therapy*. 2022;**13**(1):484
- [53] Liu JS et al. Exosomal miR-451 from human umbilical cord mesenchymal stem cells attenuates burn-induced acute lung injury. *Journal of the Chinese Medical Association*. 2019;**82**(12):895-901
- [54] Kitching AR et al. Endogenous interleukin-10 regulates Th1 responses that induce crescentic glomerulonephritis. *Kidney International*. 2000;**57**(2):518-525
- [55] Hajjaliasgari Najafabadi A, Soheilifar MH, Masoudi-Khoram N. Exosomes in skin photoaging: Biological functions and therapeutic opportunity. *Cell Communication and Signaling: CCS*. 2024;**22**(1):32
- [56] Kim H et al. Exosome-guided phenotypic switch of M1 to M2 macrophages for cutaneous wound healing. *Advanced Science (Weinheim)*. 2019;**6**(20):1900513
- [57] Jayaprakash B et al. The role of stem cell therapies in the treatment of neurodegenerative diseases. *Current Stem Cell Research & Therapy*. 2025;**20**(2):146-165
- [58] Shen H et al. Research progress of extracellular vesicles derived from mesenchymal stem cells in the treatment of neurodegenerative diseases. *Frontiers in Immunology*. 2025;**16**:1496304
- [59] Cui GH et al. Exosomes derived from hypoxia-preconditioned mesenchymal stromal cells ameliorate cognitive decline by rescuing synaptic dysfunction and regulating inflammatory responses in APP/PS1 mice. *The FASEB Journal*. 2018;**32**(2):654-668
- [60] Gassama Y, Favereaux A. Emerging roles of extracellular vesicles in the central nervous system: Physiology, pathology, and therapeutic perspectives. *Frontiers in Cellular Neuroscience*. 2021;**15**:626043
- [61] Peng H et al. Intranasal administration of self-oriented nanocarriers based on therapeutic exosomes for synergistic treatment of Parkinson's disease. *ACS Nano*. 2022;**16**(1):869-884

[62] Lee M et al. Adipose-derived stem cell exosomes alleviate pathology of amyotrophic lateral sclerosis in vitro. *Biochemical and Biophysical Research Communications*. 2016;**479**(3):434-439

[63] Holtzman J, Lee H. Emerging role of extracellular vesicles in the respiratory system. *Experimental & Molecular Medicine*. 2020;**52**(6):887-895

[64] Chen F et al. Mesenchymal stem cell-derived exosomes attenuate murine cytomegalovirus-infected pneumonia via NF- κ B/NLRP3 signaling pathway. *Viruses*. 2024;**16**(4):887-895

[65] Hou L et al. Human umbilical cord mesenchymal stem cell-derived extracellular vesicles alleviated silica induced lung inflammation and fibrosis in mice via circPWWP2A/miR-223-3p/NLRP3 axis. *Ecotoxicology and Environmental Safety*. 2023;**251**:114537

[66] Zhou J et al. microRNA-186 in extracellular vesicles from bone marrow mesenchymal stem cells alleviates idiopathic pulmonary fibrosis via interaction with SOX4 and DKK1. *Stem Cell Research & Therapy*. 2021;**12**(1):96

[67] Witwer KW et al. Defining mesenchymal stromal cell (MSC)-derived small extracellular vesicles for therapeutic applications. *Journal of Extracellular Vesicles*. 2019;**8**(1):1609206

The Dual Role of Mesenchymal Stem Cells in Cancer: From Immune Modulation to Therapy Resistance

Gulcin Sahingoz Erdal, Tamay Seda Tasci and Nilgun Isiksacan

Abstract

Mesenchymal stem cells (MSCs) have emerged as key modulators within the tumor microenvironment due to their ability to influence immune responses, tumor progression, and therapeutic resistance. While some evidence suggests anti-tumorigenic properties, numerous studies have revealed that MSCs can also promote immune evasion, enhance epithelial-mesenchymal transition, and support tumor growth. Furthermore, MSC-derived exosomes and secreted factors may contribute to resistance against chemotherapy, targeted therapies, and immunotherapies. This chapter will explore the dual role of MSCs in cancer biology, with a particular focus on their interactions with immune cells such as myeloid-derived suppressor cells (MDSCs), Tregs, macrophages, and natural killer (NK) cells, as well as their involvement in drug resistance mechanisms. Additionally, recent advances in engineering MSCs as therapeutic delivery systems will be discussed, highlighting their potential—and limitations—in future cancer treatments. Through a comprehensive review of current literature, this chapter aims to provide an updated and critical insight into the multifaceted role of MSCs in solid tumors.

Keywords: drug resistance, mesenchymal stem cells (MSCs), MSC-derived exosomes, immune modulation, tumor microenvironment

1. Introduction

1.1 Definition and historical background of mesenchymal stem cells (MSCs)

In this chapter, the concept of Mesenchymal stem cells (MSCs) will be used according to general use in oncology, although these cells are more accurately defined as mesenchymal stromal cells due to their heterogeneous nature. Friedenstein and colleagues first described MSCs in the 1960s as fibroblast-like cells derived from bone marrow [1]. These cells exhibited adherence to plastic surfaces, colony-forming capacity, and osteogenic differentiation potential. In 1991, Caplan coined the term “mesenchymal stem cells” and proposed that these cells could differentiate

into mesenchymal lineages such as osteoblasts, chondrocytes, and adipocytes [2]. However, subsequent studies revealed that these cells comprise a heterogeneous population with immunomodulatory and tissue-repairing properties that extend beyond the classical definition of stem cells, leading to the widespread use of the alternative term “mesenchymal stromal cells” [3].

1.2 Sources and phenotypic characteristics of MSCs

MSCs can be isolated not only from bone marrow but also from various tissues such as adipose tissue, umbilical cord, dental pulp, amniotic fluid, synovial fluid, perinatal tissues, and even tumor tissue [4, 5]. According to the International Society for Cellular Therapy (ISCT), the definition of MSCs is based on three key criteria: (i) adherence to plastic surfaces under standard *in vitro* conditions; (ii) expression of surface markers such as CD105, CD73, and CD90, and lack of expression of hematopoietic and immune markers such as CD45, CD34, CD14 or CD11b, CD79a, and HLA-DR; and (iii) the ability to differentiate into osteogenic, chondrogenic, and adipogenic lineages [6].

1.3 Physiological roles of MSCs

Under physiological conditions, MSCs play key roles in maintaining tissue homeostasis, facilitating wound healing, and modulating immune responses [7]. Their ability to home to injured tissues, recognize inflammatory signals, secrete anti-inflammatory cytokines such as IL-10 and TGF- β , and support regeneration has led to their widespread application in both experimental and clinical studies [8]. Their low immunogenicity also enables allogeneic transplantation between individuals, further enhancing their therapeutic potential [9].

1.4 Tumor microenvironment and MSCs

Tumors are not composed solely of proliferating malignant cells. Today, the tumor is recognized as a heterogeneous structure encompassing a complex microenvironment (tumor microenvironment, TME), which includes immune cells, fibroblasts, endothelial cells, pericytes, and stromal components [10]. MSCs are considered key players in this microenvironment and exhibit the ability to migrate to tumor sites in response to inflammatory signals (chemotaxis). Upon arrival, MSCs can differentiate into stromal cells, fibroblasts, or even cancer-associated fibroblasts (CAFs), contributing to critical processes such as immune suppression, tumor cell proliferation, angiogenesis, metastasis, and therapy resistance [11–13].

1.5 Dual role of MSCs in cancer

The role of MSCs in cancer progression remains controversial. Some studies suggest that MSCs exert tumor-suppressive effects by secreting antitumor cytokines or stimulating the immune response [14]. However, many other studies indicate that MSCs may promote tumor growth, facilitate metastasis, and contribute to therapy resistance through immunosuppression [15, 16]. These contradictory outcomes are influenced by several factors, including the tissue origin of the MSCs (e.g., bone marrow, adipose tissue, and umbilical cord), the characteristics of the target tissue, the duration of exposure within the tumor microenvironment, and phenotypic changes acquired during this interaction [17].

Current literature suggests that MSCs may act as a “double-edged sword” within the cancer microenvironment, exhibiting both tumor-promoting and tumor-suppressing roles simultaneously [18]. Therefore, a detailed understanding of the impact of MSCs on tumor biology is essential to ensure the safety and efficacy of MSC-based therapeutic applications in oncology.

2. Pro-tumor effects

Among the dual roles of mesenchymal stem cells (MSCs) in cancer biology, their pro-tumor effects have been more frequently reported in the literature. These effects result from the multifaceted interactions that MSCs establish with the tumor microenvironment. The primary mechanisms contributing to these effects include extracellular vesicle (EV) release, immune suppression, direct cell-cell contact with tumor cells, extracellular matrix (ECM) modification, and metabolic reprogramming [4, 6, 8, 9].

2.1 Deepening of immunosuppressive mechanisms

The immunosuppressive features of MSCs affect not only the adaptive immune response but also the innate immune system, thus facilitating immune escape within the tumor microenvironment. For example, MSCs downregulate the expression of MHC-II on dendritic cells and inhibit the expression of co-stimulatory molecules such as CD80 and CD86, thereby weakening the antigen presentation capacity [16]. This significantly reduces the activation potential of T cells.

Furthermore, MSCs have been shown to inhibit the cytotoxic activity of natural killer (NK) cells by decreasing the production of perforin and granzyme B [8]. Several studies indicate that this suppression is mediated through immunomodulatory molecules such as IL-6, PGE2, and HLA-G. In addition, it has been reported that in the presence of pro-inflammatory cytokines such as interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α), MSCs markedly increase their IDO expression, thereby enhancing the level of immunosuppression.

Interestingly, some MSC subtypes have also been reported to induce polarization of macrophages toward the M2 phenotype, thereby supporting the maintenance of a persistently immunosuppressive environment within the tumor microenvironment (**Figure 1**) [12].

2.2 Novel contributions to angiogenesis

The release of pro-angiogenic factors is considered one of the fundamental mechanisms by which MSCs support tumor progression. However, recent literature suggests that this effect is not solely dependent on paracrine signaling but also involves the delivery of pro-angiogenic microRNAs *via* extracellular vesicles (EVs) secreted by MSCs. Notably, microRNAs such as miR-21, miR-210, and miR-126, carried within MSC-derived exosomes, have been shown to activate VEGF signaling in tumor endothelial cells and thereby accelerate neovascularization [4, 9].

In addition, MSCs significantly influence the extracellular matrix (ECM). It has been demonstrated that MSCs secrete matrix-degrading enzymes such as MMP-1 and MMP-9, which degrade the basement membrane and facilitate the invasion of new blood vessels into the tumor tissue [15].

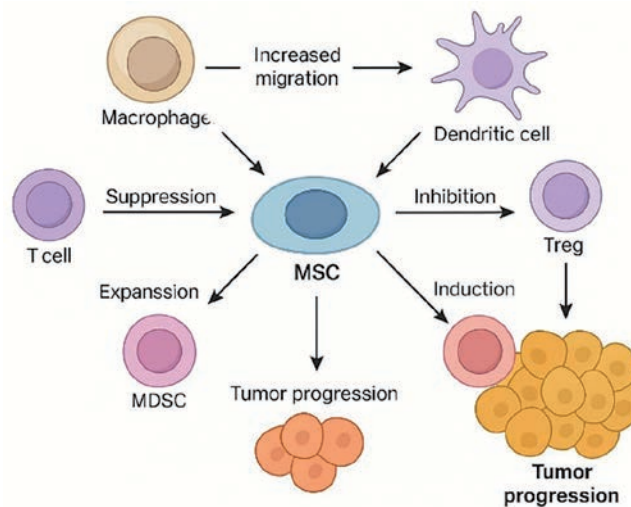


Figure 1.
Immunomodulatory effects of mesenchymal stem/stromal cells (MSCs) contributing to tumor progression.

2.3 Molecular dimensions of epithelial-mesenchymal transition (EMT) modulation

In recent years, there has been growing evidence indicating that MSC-induced epithelial-mesenchymal transition (EMT) occurs through more refined and diverse molecular mechanisms. For example: MSC secretes paracrine factors that activate the WNT/AN-Catenin signal road, resulting in the nucleus of the nucleus and upgrading the mesenchymal markers, including Daman, N-Cadherin, and vimentin [14], epithelium, and epithelium markers as e-cadherin. This infection increases the mobility and invasion of the tumor cell. In addition, IL-6/JAK/STAT3 signaling triggered by MSCs leads to increased expression of downstream effectors such as Cyclin D1, Survivin, and BCL-XL, contributing to both epithelial-mesenchymal transition (EMT) and therapy resistance [7]. MSC-derived exosomes containing MIR-221 and MIR-222 strengthen these effects and activate STAT3.

Additionally, MSCs have been reported to enhance the stabilization of hypoxia-inducible factor 1-alpha (HIF-1 α) in hypoxic tumors, further exacerbating the EMT process. Moreover, the heterogeneity of MSC populations plays a significant role in these effects. Certain MSC subpopulations have been found to exhibit high levels of TGF- β 1 production, while others lack this capacity, indicating that the phenotypic variability among MSCs can critically influence their impact on tumor biology (Table 1) [11].

2.4 Emerging observations in metastasis facilitation

The role of MSCs in metastasis extends beyond merely guiding or transporting tumor cells. They also actively contribute to the formation of pre-metastatic niches. The literature highlights several critical findings: MSCs increase the expression of ECM proteins such as fibronectin, tenascin-C, and periostin in pre-metastatic regions, thereby facilitating the adhesion of circulating tumor cells [9]. In target organs like the lung, liver, and bone marrow, MSCs utilize chemokine receptors and adhesion molecules to “prime” these sites for future metastasis.

Pathway/miRNA	Key targets/effects	Outcome
Wnt/ β -catenin	$\uparrow$ β -catenin, $\downarrow$ E-cadherin, $\uparrow$ N-cadherin, vimentin	EMT induction, invasion
STAT3 (via IL-6)	$\uparrow$ Cyclin D1, BCL-XL, Survivin	Proliferation, immune evasion, and drug resistance
miR-21 (MSC-exosomal)	Targets PTEN, $\uparrow$ AKT signaling	Anti-apoptosis, invasion
miR-221/222	Enhances STAT3 activation	EMT, therapy resistance
miR-145	$\downarrow$ OCT4, SOX2 (stemness markers)	Anti-stemness, anti-proliferative effects
miR-122	$\uparrow$ chemosensitivity in hepatocellular carcinoma (HCC)	Drug response modulation

Table 1.
Key signaling pathways and microRNA targets involved in MSC-mediated tumor modulation.

Additionally, MSCs activate the CCL2/CCR2 and CXCL12/CXCR4 chemokine axes, promoting the chemotactic migration of tumor cells.

In the murine models of breast and colorectal cancer, the metastatic burden decreased by ~40% as a result of the medicinal prohibition of CCL2/CCR2 and CXCR4 chemokine axes, as measured by the number of lung and liver metastases ($p < 0.01$) [15]. However, these effects were model-dependent and were mainly seen in early-phase metastatic proliferation, indicating potential boundaries in clinical extracts.

2.5 Novel mechanisms underlying therapy resistance

The impact of MSCs on therapy resistance is not limited to their role as exosomal carriers; it also involves the metabolic reprogramming of tumor cells. MSC-derived IL-6 and CXCL8 enhance glycolytic activity in tumor cells, thereby promoting the Warburg effect. This metabolic shift enables tumor cells to survive even under hypoxic or drug-induced stress conditions [6].

Moreover, MSCs activate the nuclear factor kappa B (NF- κ B) signaling pathway, which upregulates anti-apoptotic genes such as BCL-XL and Survivin, thus reducing the effectiveness of chemotherapeutic agents. In certain contexts, specific MSC subtypes have been proposed to bind and neutralize chemotherapeutic drugs directly, altering their pharmacodynamic profile and further contributing to resistance [12]. These characteristics suggest that MSCs should not be considered merely passive stromal components but rather as active participants in shaping therapy-resistant tumor phenotypes.

3. Antitumor effects

Although the role of mesenchymal stem cells (MSCs) in the tumor microenvironment is predominantly characterized as tumor-promoting, numerous studies have demonstrated that under specific conditions, MSCs may also exert tumor-suppressive effects. These effects are generally mediated through genetically modified MSCs, MSC-derived molecules that stimulate immune responses, or cell-based strategies that enable targeted delivery. Furthermore, some MSC subpopulations originating

from specific tissues have been shown to exhibit direct anti-proliferative or pro-apoptotic properties. This section discusses the antitumor effects of MSCs under five major subheadings.

3.1 Tumor-specific homing and targeted therapeutic delivery

MSCs possess the ability to migrate to sites of inflammation or tumor tissue following systemic administration, making them attractive candidates for use as biological drug delivery vehicles [8, 17]. Chemokine and adhesion pathways such as CXCR4/SDF-1 α , CCL2/CCR2, and VCAM-1/integrin regulate this homing capacity. Through genetic engineering, MSCs can be modified to carry antitumor proteins (e.g., TRAIL, IFN- β , IL-12) directly into the tumor, thereby minimizing systemic toxicity while enhancing local therapeutic efficacy. It has been shown that TRAIL-expressing MSCs can effectively induce apoptosis in both colorectal cancer and glioblastoma models [19]. Moreover, MSCs loaded with nanoparticles have demonstrated the ability to deliver drug-containing vesicles specifically to tumor tissues, thereby improving drug penetration and efficacy against resistant tumor cells [20].

3.2 Antitumor effects of MSC-derived exosomes

Exosomes secreted by MSCs can exhibit not only tumor-promoting but also antitumor effects. These exosomes carry specific microRNAs and proteins that can suppress proliferation or induce apoptosis in target tumor cells. For instance, miR-16 delivered *via* MSC-derived exosomes has been shown to downregulate BCL2 gene expression, promoting apoptosis [21]. Similarly, exosomes containing miR-145 have been reported to suppress stemness markers such as SOX2 and OCT4 in breast cancer cells, thereby reducing their cancer stem cell-like features [22]. Additionally, the PTEN/AKT/mTOR signaling axis may be regulated *via* MSC-exosomes, inhibiting tumor cell growth and metastatic potential [23]. Furthermore, proteins such as TIMP-2 and Dickkopf-1 carried by these exosomes have been shown to suppress Wnt signaling, thereby slowing tumor progression [24].

3.3 Activation of apoptosis and cell cycle arrest mechanisms

Certain MSC subpopulations, particularly those derived from umbilical cord (UC-MSCs) and adipose tissue, have demonstrated the potential to induce cell cycle arrest and apoptosis in various tumor cells [20, 25]. These effects typically involve arresting the cell cycle at the G0/G1 or G2/M phase *via* modulation of regulatory proteins such as p21, p27, and Cyclin D1, as well as altering the BAX/BCL2 ratio and activating caspase-3 pathways. Additionally, inhibition of HIF-1 α under hypoxic conditions disrupts tumor cell adaptation, further facilitating apoptosis. For example, UC-MSCs have been shown to activate mitochondrial-mediated apoptosis in hepatocellular carcinoma cells by increasing BAX and decreasing BCL2 expression levels [26].

3.4 Enhancement of immune response and antitumor immunity

Although most MSCs lack direct antigen-presenting capabilities, certain subtypes can promote antitumor immune responses when they adopt a pro-inflammatory phenotype. Activation of Toll-like receptor 4 (TLR4) has been shown to induce

such a phenotype, with increased secretion of cytokines like IL-12 and IL-6 [27]. This transition enhances CD8⁺ T cell infiltration into tumor tissue, strengthens M1 macrophage polarization, reduces T_{reg} cell levels, and restores NK cell cytotoxic activity. TLR4 stimulation, as opposed to TLR3, may thus redirect MSCs toward an immune-stimulatory phenotype, making them suitable candidates for use in preclinical immunotherapy models [17].

3.5 Preclinical evidence and therapeutic potential

Although the majority of MSC-related antitumor effects have been demonstrated at the preclinical level, several early-phase clinical studies have reported encouraging outcomes. For instance, IFN- β -expressing MSCs have been safely administered to patients with glioblastoma multiforme, achieving local tumor control [27]. Similarly, TRAIL-expressing MSCs administered intravenously have been shown to reduce pulmonary metastases in colorectal cancer models [19]. Furthermore, UC-MSCs combined with IL-24 have demonstrated anti-proliferative and pro-apoptotic effects in hepatocellular carcinoma [28]. These findings suggest that MSCs hold promise not only as supportive stromal components but also as active therapeutic agents in cancer treatment.

4. Effects of mesenchymal stem cells (MSCs) according to cancer types

The influence of mesenchymal stem cells (MSCs) within the tumor microenvironment varies significantly depending on the type of cancer, its anatomical location, and the structural characteristics of the microenvironment [7, 9]. In some malignancies, MSCs exhibit tumor-suppressive effects, while in others, they contribute to tumor progression [8, 12]. This dual behavior is influenced by factors such as the phenotypic heterogeneity of MSCs, their tissue of origin, the duration of exposure to the tumor microenvironment, and the degree of interaction with the immune system [10]. In this section, the potential roles of MSCs in various solid tumor types are comprehensively reviewed in light of the current scientific literature [13].

Among these cancer types, breast cancer stands out as one in which this dichotomy is most prominently observed. Therefore, the effects of MSCs in breast cancer will be discussed first.

4.1 Breast cancer

Breast cancer is one of the most extensively studied malignancies in terms of MSC-related interactions, with both pro-tumorigenic and anti-tumorigenic effects being well documented. In aggressive subtypes such as triple-negative breast cancer (TNBC), the role of MSCs becomes even more pronounced.

In terms of pro-tumor effects, it has been shown that MSCs promote epithelial-mesenchymal transition (EMT) and tumor progression through several interconnected mechanisms:

- Activation of EMT-related signaling pathways such as IL-6/STAT3 and TGF- β 1, enhancing the metastatic potential of tumor cells [14].
- Expansion of CD44⁺/CD24⁻ cancer stem-like cell populations, which contributes to the formation of treatment-resistant tumor subclones [20].

- Delivery of oncogenic microRNAs *via* exosomes, particularly miR-21 and miR-222, which activate anti-apoptotic genes and support sustained tumor growth [21].

Conversely, genetically engineered MSCs that deliver molecules such as miR-145 or TRAIL have been shown to induce apoptosis in breast cancer cells [19, 22]. miR-145, in particular, reduces cancer stem cell properties by suppressing key stemness markers like OCT4 and SOX2 [22]. Additionally, MSCs expressing IFN- β have demonstrated antitumor activity in various preclinical studies [27].

From a clinical standpoint, although MSC-based targeted therapy strategies are still at the preclinical stage, their immunosuppressive properties necessitate careful evaluation before clinical translation.

4.2 Colorectal cancer

Colorectal cancer (CRC) serves as a balanced model in which mesenchymal stem cells (MSCs) exhibit both tumor-supportive and tumor-suppressive functions. The heterogeneous nature of CRC and the complexity of its tumor microenvironment contribute to the diverse effects observed with MSCs.

From a pro-tumorigenic perspective, MSCs secrete chemokines such as CXCL12 and CCL2, which enhance the invasive potential of CRC cells [9]. Furthermore, MSCs have been reported to promote chemoresistance by upregulating anti-apoptotic genes, including BCL-2 and Survivin [15]. Additionally, microRNAs delivered *via* MSC-derived exosomes have been shown to support tumor growth and enhance cancer cell viability.

On the other hand, MSCs engineered to express TRAIL have demonstrated the ability to selectively induce apoptosis in colorectal cancer cells [19]. Moreover, certain subtypes of umbilical cord-derived MSCs (UC-MSCs) have been found to arrest the cell cycle in the G0/G1 phase, thereby inhibiting tumor cell proliferation [25]. Some studies also suggest that MSCs exert anti-inflammatory effects by suppressing pro-tumorigenic cytokines, indirectly contributing to tumor suppression.

These findings have heightened interest in the potential use of MSCs as therapeutic delivery vehicles or targeted treatment agents in CRC. Nevertheless, due to their immunomodulatory properties and possible long-term contributions to tumor progression, preclinical outcomes must be carefully interpreted, and the safety profile of MSC-based therapies should be thoroughly assessed before clinical translation.

4.3 Gynecological cancers (ovarian and cervical)

Among gynecologic malignancies, mesenchymal stem cells (MSCs) exhibit particularly prominent tumor-promoting effects in ovarian cancer. In this setting, MSCs contribute to immune suppression and create a tumor-supportive microenvironment. Through the secretion of immunomodulatory mediators such as CCL5, IL-6, and PGE2, MSCs suppress T cell function and facilitate immune evasion. In parallel, activation of the STAT3 signaling pathway promotes epithelial-mesenchymal transition (EMT), leading to enhanced cellular invasiveness [15].

Conversely, MSCs genetically modified to express TRAIL have demonstrated selective apoptotic effects in cervical cancer cells [19]. Furthermore, miR-124, delivered *via* MSC-derived exosomes, has been shown to target MCT1 in regulatory T cells within the ovarian tumor microenvironment, thereby reducing lactate uptake. This results in decreased immunosuppressive activity, suppression of tumor growth, and

enhanced response to PD-1 checkpoint blockade therapy [29]. Additionally, preclinical models have indicated that IFN- β -expressing MSCs may elicit antitumor responses *via* immunostimulatory mechanisms [27].

Although these findings are promising at the preclinical level, MSC-based therapies in gynecologic oncology have yet to be translated into clinical application. Further research is warranted to assess the safety and efficacy profiles and determine the potential of MSCs in gynecologic cancer treatment strategies.

4.4 Gastrointestinal system cancers

Gastrointestinal system (GIS) cancers represent a group of malignancies in which mesenchymal stem cells (MSCs) exert pronounced effects within the tumor microenvironment. In both gastric and hepatic tumors, MSCs have demonstrated dual roles with both tumor-promoting and tumor-suppressive potentials.

In gastric cancer, gastric cancer-associated MSCs (GC-MSCs) have been shown to secrete key cytokines such as IL-8, PDGF-DD, and CXCL16, which promote tumor cell proliferation and migration. Furthermore, these MSCs suppress natural killer (NK) cell cytotoxicity, thereby facilitating immune evasion by the tumor [30].

In hepatocellular carcinoma (HCC) models, MSCs exhibit bidirectional effects. On one hand, they enhance metastatic potential by promoting epithelial-mesenchymal transition (EMT) *via* TGF- β signaling; on the other hand, certain UC-MSC subsets can induce apoptosis by altering the BAX/BCL-2 ratio. Moreover, exosomes derived from MSCs that carry miR-122 have been found to sensitize HCC cells to chemotherapy and reduce drug resistance [31].

Combinatorial approaches involving MSCs and therapeutic agents have also shown promise in preclinical research. For example, the MSC + IL-24 combination has demonstrated tumor-suppressive activity in experimental models; however, these strategies remain unverified in clinical settings and require further safety evaluations [32].

4.5 Glioblastoma

Glioblastoma (GBM) is one of the most promising tumors for the clinical application of mesenchymal stem cell (MSC)-based therapeutic strategies. The invasive nature of GBM, the presence of stem cell-like tumor cells, and the pronounced immunosuppressive microenvironment significantly limit the effectiveness of conventional treatments. In this context, MSCs capable of delivering targeted molecules directly to the tumor site have emerged as a potential alternative.

It has been demonstrated that MSCs can specifically migrate toward glioma lesions *via* chemokine pathways such as SDF-1/CXCR4, HGF/c-Met, VEGF, and PDGF-BB, making them suitable carriers for direct therapeutic delivery [33]. However, some studies have suggested that MSCs may fuse with glioma cells and contribute to tumor progression by inducing genetic instability [34, 35]. Therefore, careful consideration of the source, culture conditions, and mode of application is essential to avoid potential protumoral effects of MSCs. Therapeutically engineered MSCs have shown promising results in glioma treatment. Particularly, MSCs expressing IFN- β or TRAIL have been found to induce selective apoptosis in glioma cells and suppress tumor mass without causing toxicity to surrounding tissues [27, 36]. Phase I/II clinical studies by Chiocca et al. (2008) have demonstrated the safety of these cells, although more data are needed to establish long-term efficacy [34].

Furthermore, the bidirectional interaction between MSCs and glioma stem cells is critical; while some MSC subtypes can suppress tumor stem cell activity, others may support their viability and invasiveness [33, 36]. This indicates that the source of MSCs—such as bone marrow, umbilical cord, or induced pluripotent stem cell origin—can directly influence treatment outcomes. Recent studies suggest that MSC-based delivery systems, when combined with genetic engineering to enhance targeting capability, may offer a valuable therapeutic approach in treatment-resistant brain tumors like GBM.

4.6 Lung cancer

Lung cancer is one of the most prominent tumor types where mesenchymal stem cells (MSCs) exhibit dual functional effects. Particularly in the context of non-small cell lung cancer (NSCLC), MSCs have been shown to interact with the tumor microenvironment and activate signaling pathways such as TGF- β /Smad and PI3K/AKT, which are closely associated with proliferation and metastasis. Following their migration into the tumor site, MSCs contribute to epithelial-to-mesenchymal transition (EMT), angiogenesis, and tumor invasion through the secretion of cytokines and the delivery of exosomal content. It has been reported that MSC-derived exosomes carry oncogenic microRNAs such as miR-21 and miR-210, which can activate anti-apoptotic genes and promote tumor progression [37, 38]. Conversely, preclinical studies have demonstrated that genetically engineered MSCs expressing TRAIL induce apoptosis in NSCLC cells and significantly reduce tumor volume. Similarly, MSC-derived exosomes loaded with tumor-suppressive microRNAs, such as miR-34a and miR-126, have been shown to inhibit cell proliferation, induce cell cycle arrest, and enhance chemosensitivity [39, 40]. These findings highlight the bidirectional nature of MSCs in lung cancer, where they may act both as facilitators of tumor progression and as potential therapeutic delivery vehicles. Therefore, before translating MSC-based applications into clinical use, comprehensive biosafety analyses and targeted engineering strategies must be thoroughly implemented.

4.7 Urogenital system cancers

Urogenital system tumors, particularly prostate, bladder, and testicular cancers, represent a critical group of malignancies in which mesenchymal stem cells (MSCs) may exert dual functional roles. It has been reported that MSCs can produce either pro-tumorigenic or anti-tumorigenic effects through cytokine signaling and cell-cell interactions within the tumor microenvironment [4, 6, 41]. In prostate cancer, MSCs have been shown to activate TGF- β and Wnt/ β -catenin signaling pathways, thereby promoting tumor cell proliferation and invasion and contributing to stromal remodeling [41]. In addition, certain microRNAs carried by MSC-derived exosomes may promote an immunosuppressive environment in the tumor microenvironment, facilitating immune evasion [16, 42].

On the other hand, MSCs engineered to express tumor-suppressive agents such as TRAIL or miR-145 have been reported to exert pro-apoptotic and tumor-inhibitory effects in prostate cancer models [19, 22]. In bladder cancer, miR-145 carried by MSC-derived exosomes has been shown to suppress tumor cell proliferation and migration, partly through targeting IL-6 signaling and modulating inflammatory responses [6, 22]. Nevertheless, some studies indicate that long-term exposure of certain MSC subtypes to tumor tissue may induce their conversion into immunosuppressive phenotypes, thereby contributing to tumor progression [6, 41].

In the context of testicular cancer, direct data on MSC interactions remain limited. However, some preclinical studies suggest that MSCs recruited into testicular tumor microenvironments may differentiate into M2-like macrophages or other immunosuppressive cell types, resulting in attenuated antitumor immune responses [5, 6].

In conclusion, the effects of MSCs in urogenital system tumors vary depending on cell origin, tumor subtype, exposure duration, and local micro-environmental factors. As such, therapeutic applications of MSCs in this area remain experimental, and extensive safety profiling is required before clinical translation can be considered.

4.8 Hematologic malignancies

Although hematologic malignancies exhibit biological characteristics distinct from solid tumors, they present a compelling area of investigation with respect to their interactions with mesenchymal stem cells (MSCs). In diseases such as acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and multiple myeloma, MSCs within the bone marrow microenvironment have been shown to establish close relationships with malignant cells, thereby facilitating disease progression [6, 16].

In AML patients, bone marrow-derived MSCs have been found to possess altered transcriptomic profiles compared to those from healthy individuals. These cells secrete high levels of immunosuppressive cytokines, such as IL-6 and TGF- β , which support the survival of leukemic cells [41]. In addition, MSCs contribute to chemotherapy resistance by activating anti-apoptotic signaling mechanisms in leukemic cells [6].

In multiple myeloma, MSCs interact directly with plasma cells through cell-to-cell contact, promoting proliferation, bone degradation, and drug resistance. Moreover, MSCs have been reported to support myeloma cell growth *via* secretion of factors such as CXCL12/SDF-1, VEGF, and IL-6, while also actively contributing to the remodeling of the bone marrow stroma [5, 16].

Conversely, preclinical evidence suggests that genetically modified MSCs carrying antitumor cytokines (e.g., IFN- β) or apoptosis-inducing agents may exert cytotoxic effects against leukemic or myeloma cells [19, 36]. However, such strategies remain at the preclinical stage and require thorough evaluation of safety and efficacy before clinical application can be considered.

4.9 Skin cancers and melanoma

Among skin cancers, melanoma represents one of the best-studied models in which mesenchymal stem cells (MSCs) exhibit dual effects on the tumor microenvironment. Despite its highly immunogenic nature, melanoma effectively develops immune escape mechanisms, making the immunosuppressive properties of MSCs particularly relevant [4].

MSCs have been shown to suppress local immune responses by secreting factors such as TGF- β , IL-10, and PGE2, thereby facilitating the immune evasion of tumor cells [20, 36]. Additionally, microRNAs such as miR-21 and miR-155 delivered *via* MSC-derived exosomes have been reported to promote tumor cell proliferation, migration, and resistance to apoptosis [20].

Conversely, genetically engineered MSCs—modified to express molecules like IFN- β or TRAIL—have demonstrated antitumor activity against melanoma cells. In such applications, MSCs serve as targeted carriers for therapeutic molecules, potentially reducing systemic toxicity while enhancing treatment specificity [19, 36].

Cancer type	Pro-tumor effects	Antitumor effects
Breast cancer	EMT <i>via</i> IL-6/STAT3 and TGF- β , CSC support, therapy resistance, exosomal miR-21/222	Apoptosis <i>via</i> TRAIL or miR-145, downregulation of OCT4/SOX2, IFN- β -modified MSCs
Colorectal cancer	CXCL12/CCL2-mediated invasion, upregulation of BCL-2, survivin, chemoresistance	TRAIL-induced apoptosis, G0/G1 arrest by UC-MSCs
Gynecologic cancers	Immunosuppression <i>via</i> IL-6, CCL5, and PGE2; EMT <i>via</i> STAT3	TRAIL-triggered apoptosis in cervical cancer; exosomal miR-124 suppresses proliferation
Gastric and HCC	EMT <i>via</i> IL-8, PDGF-DD, TGF- β ; NK suppression; miR-21-mediated proliferation and invasion	Apoptosis and chemosensitization <i>via</i> IL-24 or miR-122-modified MSCs
Glioblastoma	Possible cell fusion, inflammatory enhancement, and tumor-promoting interactions	Apoptosis <i>via</i> IFN- β and TRAIL-loaded MSCs; Phase I/II trials ongoing
Lung cancer	EMT, angiogenesis <i>via</i> exosomal miR-21/210; activation of TGF- β /Smad and PI3K/AKT pathways	TRAIL-induced apoptosis; exosomal miR-126/34a enhances chemosensitivity
Urogenital cancers	TME immunosuppression, Wnt/ β -catenin, and IL-6 signaling; support of tumor invasion	TRAIL-induced apoptosis, suppression of proliferation, and migration <i>via</i> miR-145
Hematologic malignancies	IL-6 and TGF- β secretion support leukemic survival; chemoresistance <i>via</i> anti-apoptotic signals	Apoptosis <i>via</i> IFN- β and genetically engineered MSCs in preclinical studies
Melanoma	Immune evasion <i>via</i> IL-10, TGF- β , and PGE2; support of CSCs <i>via</i> miR-155	TRAIL- or IFN- β -expressing MSCs induce apoptosis in melanoma cells

Table 2.
Overview of MSC effects across cancer types.

Some studies have also suggested that the interaction between MSCs and melanoma-derived cancer stem-like cells may enhance the viability and therapeutic resistance of these subpopulations [12]. These findings indicate that the tumor-modulating effects of MSCs are highly context-dependent, influenced by factors such as cell source, genetic modification, and application strategy. Therefore, MSC-based approaches must be rigorously defined and validated through preclinical evaluations prior to clinical translation.

The dual effects of MSCs across different cancer types (**Table 2**). Pro-tumor mechanisms include induction of EMT, support of cancer stem cells (CSCs), immunosuppression, and promotion of metastasis. Antitumor effects are predominantly observed when MSCs are genetically modified to express therapeutic agents such as TRAIL, IFN- β , or miRNAs.

5. Clinical applications and future perspectives

The use of mesenchymal stem cells (MSCs) in cancer therapy has become a notable area of research, particularly due to their carrier functions. Their ability to home toward the tumor microenvironment and their modifiability through genetic

engineering have positioned MSCs as promising vectors for delivering drugs, genes, and proteins. However, translating this potential into clinical practice has progressed more slowly than expected.

Most MSC-based approaches remain at the preclinical or early clinical trial (Phase I–II) stages. Studies in glioblastoma, colorectal cancer, and hepatocellular carcinoma have investigated MSCs engineered to deliver therapeutic agents such as IFN- β , TRAIL, IL-24, or miR-122, assessing their safety and biological effects. Nevertheless, these findings are constrained by limited sample sizes and short follow-up periods, and MSC-based therapies have yet to reach routine clinical application in oncology [25, 34, 35].

One of the major obstacles to clinical use is heterogeneity. MSCs derived from different tissues display variable phenotypic and functional properties, complicating standardization in production and application [4]. Furthermore, certain MSC subtypes are known to exert tumor-promoting effects, raising safety concerns for therapeutic use [20]. Long-term *in vitro* expansion can result in genetic instability, potentially increasing oncogenic risk. Additionally, the uncontrolled differentiation potential of MSCs may lead to loss of therapeutic specificity or undesired stromal effects.

To overcome these challenges, several innovative bioengineering strategies have been developed. These include inducible gene expression systems, CRISPR/Cas9-mediated gene editing, and artificial modification of MSC surfaces with ligands or antibodies to enhance tumor specificity. Moreover, MSC-derived exosomes are gaining attention as cell-free alternatives that retain MSC-like delivery properties while minimizing cellular risks [31].

Experimental studies are also exploring the combination of MSCs with chemotherapy, immunotherapy, or immune checkpoint inhibitors. Such combinatory approaches aim to simultaneously target tumor cells and modulate the immune response [18].

6. Conclusions

Mesenchymal stem cells represent a paradoxical but promising tool in oncology. Their dual nature—supporting or suppressing tumors depending on context—places them at the center of ongoing debate. While MSCs may contribute to immune suppression, enhanced invasion, metastasis formation, and treatment resistance, they also offer potential as genetically modifiable carriers for precise therapeutic delivery.

Antitumor effects are mostly observed in engineered MSCs programmed to express agents such as TRAIL, IFN- β , or IL-24. These outcomes are not inherent to MSCs themselves but rather result from external manipulation. This distinction is important, as it underscores the need for controlled programming to unlock their therapeutic utility.

Despite encouraging laboratory findings, clinical results remain limited, and MSCs have not yet become part of standard oncologic practice. Key barriers include variability in cell source, genetic instability during culture, lack of functional characterization markers, and insufficient control over *in vivo* behavior. Importantly, conventional surface markers often fail to predict MSC function, necessitating more advanced omics-based characterization approaches.

Future directions include the use of MSC-derived exosomes, synthetic biology-based sensor systems, and combination therapies that integrate MSCs with existing

treatments. These approaches may help position MSCs not only as passive drug carriers but also as programmable and intelligent therapeutic platforms. However, occasional reports of MSC-induced disease progression—particularly in immunocompromised animal models—underscore the importance of rigorous biosafety assessment, careful patient selection, and long-term monitoring in future clinical trials.

In conclusion, MSCs hold unique potential in future cancer therapy—not only due to their targeting capacity but also because of their dynamic interaction with the tumor microenvironment. However, realizing this potential will require strict control over their behavior and transformation into programmable, safe, and tumor-specific bioagents. If achieved, MSCs could become a cornerstone of next-generation, biologically engineered cancer therapeutics.

Funding

This research received no external funding.

Conflict of interest

The authors declare no conflict of interest.

List of abbreviations

CXCR4/SDF-1	chemokine receptor/ligand axis
ECM	extracellular matrix
EMT	epithelial-mesenchymal transition
IDO	indoleamine 2,3-dioxygenase
IFN- β	interferon beta
IL	interleukin
miR	microRNA
MMP	matrix metalloproteinase
MSC	mesenchymal stem/stromal cell
NF- κ B	nuclear factor kappa-light-chain-enhancer of activated B cells
NK	natural killer cell
TLR	toll-like receptor
TME	tumor microenvironment
TRAIL	TNF-related apoptosis-inducing ligand
Treg	regulatory T cell
VEGF	vascular endothelial growth factor

Author details

Gulcin Sahingoz Erdal¹, Tamay Seda Tasci² and Nilgun Isiksacan^{2*}

1 Department of Oncology, University of Health Sciences, Bakırkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkiye

2 Department of Biochemistry and Immunology, University of Health Sciences, Bakırkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkiye

*Address all correspondence to: nisiksacan@gmail.com

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *Development*. 1966;**16**(3):381-390
- [2] Caplan AI. Mesenchymal stem cells. *Journal of Orthopaedic Research*. 1991;**9**(5):641-650
- [3] Dominici ML, Le Blanc K, Mueller I, Slaper-Cortenbach 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
- [4] Slama Y, Ah-Pine F, Khettab M, et al. The dual role of mesenchymal stem cells in cancer pathophysiology: Pro-tumorigenic effects versus therapeutic potential. *International Journal of Molecular Sciences*. 2023;**24**(17):13511
- [5] Antoon R, Overdevest N, Saleh AH, Keating A. Mesenchymal stromal cells as cancer promoters. *Oncogene*. Nov 2024;**43**(49):3545-3555. DOI: 10.1038/s41388-024-03183-1. Epub 2024 Oct 16
- [6] Minev T, Balbuena S, Gill JM, et al. Mesenchymal stem cells-the secret agents of cancer immunotherapy: Promises, challenges, and surprising twists. *Oncotarget*. 2024;**15**:793
- [7] Tang J, Chen Y, Wang C, et al. The role of mesenchymal stem cells in cancer and prospects for their use in cancer therapeutics. *MedComm*. 2024;**5**(8):e663
- [8] Xiang Z, Hua M, Hao Z, et al. The roles of mesenchymal stem cells in gastrointestinal cancers. *Frontiers in Immunology*. 2022;**13**:844001
- [9] Wang M, Li J, Wang D, et al. The effects of mesenchymal stem cells on the chemotherapy of colorectal cancer. *Biomedicine & Pharmacotherapy*. 2023;**160**:114373
- [10] Patsalias A, Kozovska Z. Personalized medicine: Stem cells in colorectal cancer treatment. *Biomedicine & Pharmacotherapy*. 2021;**141**:111821
- [11] Starska-Kowarska K. Role of mesenchymal stem/stromal cells in head and neck cancer—Regulatory mechanisms of tumorigenic and immune activity, chemotherapy resistance, and therapeutic benefits of stromal cell-based pharmacological strategies. *Cells*. 2024;**13**(15):1270
- [12] Timaner M, Letko-Khait N, Kotsofruk R, et al. Therapy-educated mesenchymal stem cells enrich for tumor-initiating cells. *Cancer Research*. 2018;**78**(5):1253-1265
- [13] Miyazaki Y, Oda T, Mori N, et al. Adipose-derived mesenchymal stem cells differentiate into pancreatic cancer-associated fibroblasts *in vitro*. *FEBS Open Bio*. 2020;**10**(11):2268-2281
- [14] Ridge SM, Sullivan FJ, Glynn SA. Mesenchymal stem cells: Key players in cancer progression. *Molecular Cancer*. 2017;**16**(1):31
- [15] Xuan X, Tian C, Zhao M, et al. Mesenchymal stem cells in cancer progression and anticancer therapeutic resistance. *Cancer Cell International*. 2021;**21**(1):595
- [16] Whiteside TL. Exosome and mesenchymal stem cell cross-talk in the tumor microenvironment. In: *Seminars in Immunology*. Vol. 35. Amsterdam, Netherlands: Academic Press; 2018. pp. 69-79

- [17] Nakamizo A, Marini F, Amano T, et al. Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas. *Cancer Research*. 2005;**65**(8):3307-3318
- [18] Balyasnikova IV, Ferguson SD, Sengupta S, et al. Mesenchymal stem cells modified with a single-chain antibody against EGFRvIII successfully inhibit the growth of human xenograft malignant glioma. *PLoS One*. 2010;**5**(3):e9750
- [19] Loebinger MR, Eddaoudi A, Davies D, et al. Mesenchymal stem cell delivery of TRAIL can eliminate metastatic cancer. *Cancer Research*. 2009;**69**(10):4134-4142
- [20] Li Z, Fan D, Xiong D. Mesenchymal stem cells as delivery vectors for anti-tumor therapy. *Stem Cell Investigation*. 2015;**2**:6
- [21] Jothimani G, Pathak S, Dutta S, et al. A comprehensive cancer-associated microRNA expression profiling and proteomic analysis of human umbilical cord mesenchymal stem cell-derived exosomes. *Tissue Engineering and Regenerative Medicine*. 2022;**19**(5):1013-1031
- [22] Sheykhhasan M, Kalhor N, Sheikholeslami A, et al. Exosomes of mesenchymal stem cells as a proper vehicle for transfecting miR-145 into the breast cancer cell line and its effect on metastasis. *BioMed Research International*. 2021;**2021**(1):5516078
- [23] Pakravan K, Babashah S, Sadeghizadeh M, et al. MicroRNA-100 shuttled by mesenchymal stem cell-derived exosomes suppresses in vitro angiogenesis through modulating the mTOR/HIF-1 α /VEGF signaling axis in breast cancer cells. *Cellular Oncology*. 2017;**40**:457-470
- [24] Xu Y, Hu J, Lv Q, et al. Endometrium-derived mesenchymal stem cells suppress progression of endometrial cancer via the DKK1-Wnt/ β -catenin signaling pathway. *Stem Cell Research & Therapy*. 2023;**14**(1):159
- [25] Tang YM, Bao WM, Yang JH, et al. Umbilical cord-derived mesenchymal stem cells inhibit growth and promote apoptosis of HepG2 cells. *Molecular Medicine Reports*. 2016;**14**(3):2717-2724
- [26] Waterman RS, Tomchuck SL, Henkle SL, et al. A new mesenchymal stem cell (MSC) paradigm: Polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype. *PLoS One*. 2010;**5**(4):e10088
- [27] Mao J, Cao M, Zhang F, et al. Peritumoral administration of IFN β upregulated mesenchymal stem cells inhibits tumor growth in an orthotopic, immunocompetent rat glioma model. *Journal for Immunotherapy of Cancer*. 2020;**8**(1):e000164
- [28] Zhao D, Hou L, Pan M, et al. Inhibitory effect and mechanism of mesenchymal stem cells cultured in 3D system on hepatoma cells HepG2. *Applied Biochemistry and Biotechnology*. 2018;**184**:212-227
- [29] Tian GA, Yong-Qing LI, Hai-Yan YE, et al. miR-124 delivered by BM-MSCs-derived exosomes targets MCT1 of tumor-infiltrating Treg cells and improves ovarian cancer immunotherapy. *Neoplasma*. 2023;**70**(6):713-721
- [30] Yang Y, Meng WJ, Wang ZQ. Cancer stem cells and the tumor microenvironment in gastric cancer. *Frontiers in Oncology*. 2022;**11**:803974
- [31] Lou G, Chen Z, Zheng M, et al. Mesenchymal stem cell-derived exosomes as a new therapeutic strategy

for liver diseases. *Experimental & Molecular Medicine*. 2017;**49**(6):e346

[32] Gu H, Yan C, Wan H, et al. Mesenchymal stem cell-derived exosomes block malignant behaviors of hepatocellular carcinoma stem cells through a lncRNA C5orf66-AS1/microRNA-127-3p/DUSP1/ERK axis. *Human Cell*. 2021;**34**(6):1812-1829

[33] Bajetto A, Thellung S, Dellacasagrande I, et al. Cross talk between mesenchymal and glioblastoma stem cells: Communication beyond controversies. *Stem Cells Translational Medicine*. 2020;**9**(11):1310-1330

[34] Chiocca EA, Smith KM, McKinney B, et al. A phase I trial of ad. hIFN- β gene therapy for glioma. *Molecular Therapy*. 2008;**16**(3):618-626

[35] Motaln H, Schichor C, Lah TT. Human mesenchymal stem cells and their use in cell-based therapies. *Cancer: Interdisciplinary International Journal of the American Cancer Society*. 2010;**116**(11):2519-2530

[36] Han XX, Jin S, Yu LM, et al. Interferon-beta inhibits human glioma stem cell growth by modulating immune response and cell cycle related signaling pathways. *Cell Regeneration*. 2022;**11**(1):23

[37] Wang L, He J, Hu H, et al. Lung CSC-derived exosomal miR-210-3p contributes to a pro-metastatic phenotype in lung cancer by targeting FGFR1. *Journal of Cellular and Molecular Medicine*. 2020;**24**(11):6324-6339

[38] Rahman MA, Barger JF, Lovat F, et al. Lung cancer exosomes as drivers of epithelial mesenchymal transition. *Oncotarget*. 2016;**7**(34):54852

[39] Wu J, Li S, Zhang P. Tumor-derived exosomes: Immune properties and

clinical application in lung cancer. *Cancer Drug Resistance*. 2022;**5**(1):102

[40] Grimalizzi F, Monaco F, Leoni F, et al. Exosomal miR-126 as a circulating biomarker in non-small-cell lung cancer regulating cancer progression. *Scientific Reports*. 2017;**7**(1):15277

[41] Nwabo Kamdje AH, Seke Etet PF, Simo Tagne R, et al. Tumor microenvironment uses a reversible reprogramming of mesenchymal stromal cells to mediate pro-tumorigenic effects. *Frontiers in Cell and Developmental Biology*. 2020;**8**:545126

[42] Valadi H, Ekström K, Bossios A, et al. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biology*. 2007;**9**(6):654-659

*Edited by Rui Damásio Alvites
and Ana Colette Maurício*

Mesenchymal stem cells (MSCs) are among the most promising foundations of modern regenerative medicine. Endowed with a remarkable capacity for self-renewal, differentiation, and immune system modulation, these cells play an essential role in tissue repair processes, cell-based therapies, and the development of advanced biotechnological products. The growing scientific and clinical interest in MSCs reflects not only their therapeutic potential but also the diversity of fields in which they are applied, from cell and molecular biology to tissue engineering, gene therapy, oncology, and neurosciences. A deeper understanding of their mechanisms of action and interactions with the cellular microenvironment has driven the development of new translational medicine strategies aimed at regeneration and the treatment of complex diseases. The book *Mesenchymal Stem Cells – Biology, Therapeutics, and Beyond* brings together contributions from researchers across different specialties, exploring topics ranging from genetic engineering and genome editing to clinical and preclinical applications in regenerative medicine, neuroprotection, immunomodulation, and the use of extracellular vesicles. This diversity of perspectives highlights the multidisciplinary and constantly evolving nature of MSC research, as well as its increasing impact on contemporary biomedicine. With a comprehensive approach, this book provides an integrated view of the biology and therapeutic potential of MSCs, serving as an essential theoretical and practical resource for researchers, clinicians, and students interested in this rapidly expanding field.

Andrei Surguchov, Biochemistry Series Editor

Published in London, UK

© 2025 IntechOpen
© monsiti / iStock

IntechOpen

ISSN 2632-0983

ISBN 978-1-83635-445-1



9 781836 354451