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Mesenchymal stem cells and derived extracellular vesicles as next-generation biological drugs for tissue regeneration

Edited by
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Mesenchymal stem cells and derived extracellular vesicles as next-generation biological drugs for tissue regeneration

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Editorial: Mesenchymal stem cells and derived extracellular vesicles as next-generation biological drugs for tissue regeneration

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Editorial on the Research Topic
Mesenchymal stem cells and derived extracellular vesicles as next-generation biological drugs for tissue regeneration

Mesenchymal stem cells (MSCs) have emerged as a potent tool in regenerative medicine owing to their capacity for self-renewal, multipotency, and immunomodulation (Barbon et al., 2022; Barbon et al., 2021; Di Liddo et al., 2016; Di Liddo et al., 2014). The therapeutic efficacy of MSCs derived from different tissues (i.e., bone marrow, adipose tissue, peripheral blood, umbilical cord, placenta and amniotic membrane/fluid) has been rigorously investigated in preclinical models and clinical trials across an extensive range of human diseases, comprising autoimmune disorders, neurodegenerative diseases, inflammatory disorders, and musculoskeletal injuries (Han et al., 2025). Initially ascribed to direct cellular differentiation or cell-to-cell communication with immune cells, the therapeutic role of MSCs is now recognized to be largely mediated through paracrine modulation of the tissue microenvironment (Patel et al., 2025). The key effectors of this activity are extracellular vesicles (EVs) derived from MSCs (e.g., exosomes and microvesicles), which serve as natural nanocarriers of bioactive molecules with trophic, immunomodulatory, and pro-regenerative effects. This could indicate a conceptual shift from cell-based transplantation to cell-derived, cell-free treatments, leading to new therapeutic approaches focused on the precise regulation of inflammatory pathways, the enhancement of tissue repair, and the restoration of immune-regenerative equilibrium (Figure 1).

In this context, recent evidence also acknowledges the therapeutic use of MSC-EVs as integrative platforms for personalized medicine, especially for cancer diagnosis and treatment. Notably, EVs have recently been proposed as key mediators of tumor-microenvironment interplay, unveiling their value as non-invasive biomarkers in oncology. The review by Bernad et al. (2025) highlights how tumor-derived EVs carry disease-specific molecular signatures (i.e., proteins, lipids, and nucleic acids such as miRNAs) that can be leveraged for liquid biopsy, cancer diagnosis, prognosis, and monitoring of therapeutic response.

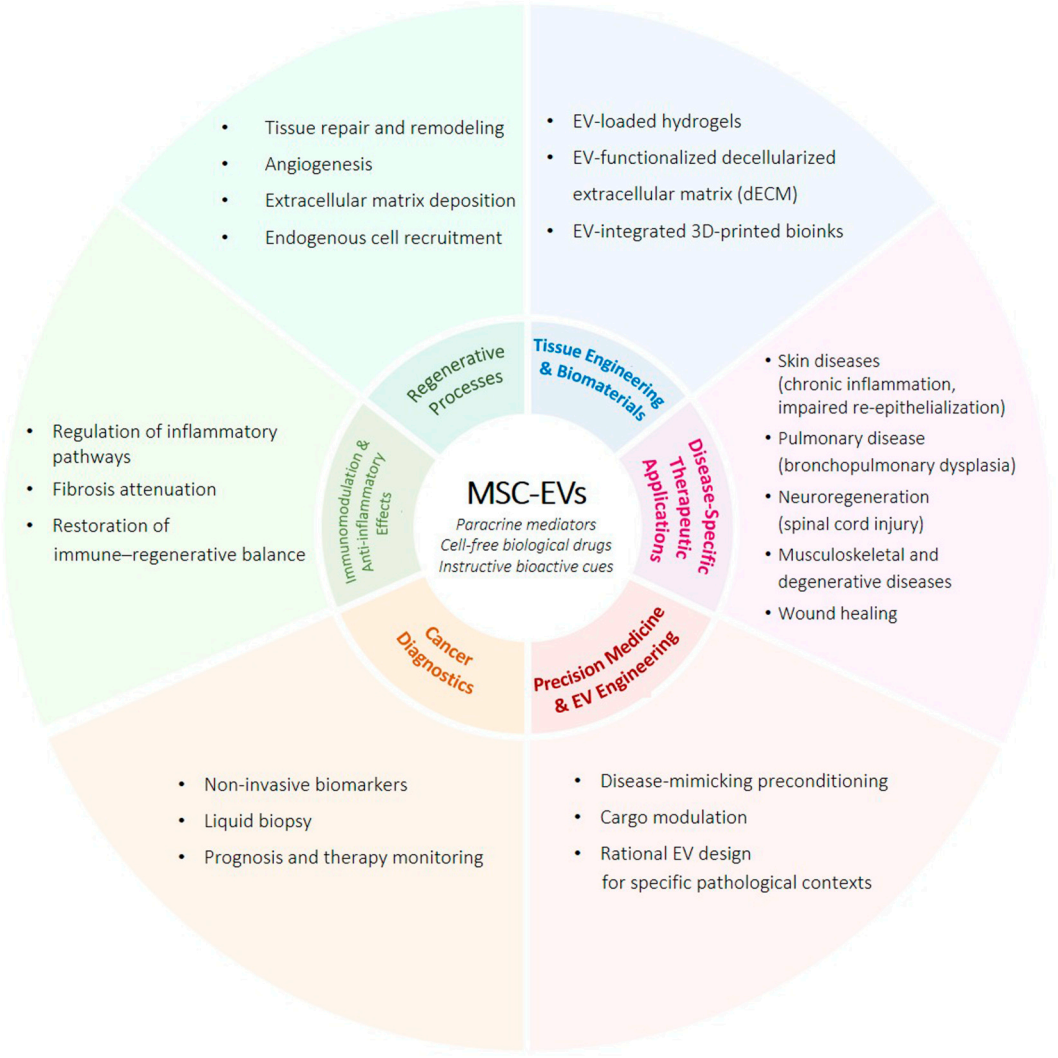


FIGURE 1
Overview of the biological functions and translational applications of mesenchymal stem cell–derived extracellular vesicles (MSC-EVs). (Created with BioRender.com).

In this Research Topic, some works examine how the therapeutic potential of MSCs can be refined and controlled through EV-based approaches. Gong et al. present a comprehensive analysis of adipose-derived MSC (ADMSC) exosomes, exploring their role in modulating inflammatory pathways and supporting tissue remodeling in degenerative and immune-mediated conditions (Gong et al.). Besides underlining exosome potential as programmable drug carriers which can be engineered for enhanced targeting and synergy, the authors point out that their clinical translation depends on overcoming technical and regulatory barriers. In line with this work, Gurney et al. demonstrate how the selective enhancement of EV cargo can promote wound healing, showing that vesicles are not only natural mediators of tissue regeneration but can be specifically engineered to activate key biological pathways (Gurney et al.). Both the studies highlight a major trend in this research field: EVs are undergoing a fundamental redefinition, from passive biomarkers of physiological states to actively engineered, customizable therapeutic platforms.

The integration of MSCs with biomaterials is another crucial aspect of this translational shift. Barbon et al. discuss hydrogels as delivery platforms that prolong MSC viability, locally concentrate their paracrine secretion, and improve functional outcomes in several tissues, bridging preclinical applications and early clinical use (Barbon et al.). Notably, the authors present hydrogels not as inert carriers, but as bioactive and cell-instructive scaffolds capable of influencing cellular behavior via mechanotransduction, a critical insight for engineering mechanically dynamic tissues. Interestingly, a growing body of evidence shows that hydrogels and scaffold-based systems are also effective in the targeted delivery of MSC-EVs, enhancing localized retention, sustaining cargo release and avoiding rapid clearance (Chen et al., 2021; Hu et al., 2020; Zhang et al., 2018; Liu et al., 2017). Starting form this concept, MSC-EVs are overcoming the boundaries of cell-based approaches, being increasingly investigated as bioactive tools that reshape structural biomaterials into instructive cell-free constructs. Indeed, MSC-EVs are now directly integrated into hydrogels, decellularized extracellular matrices, and 3D-printed bioinks

to provide sustained and spatially controlled delivery of vesicle cargo, regulate endogenous cell recruitment, guide matrix deposition, and promote neo-vascularization for functional tissue formation. Based on this approach, EVs are used not only as therapeutic agents but as bioactive cues that functionalize biomaterials, supporting the design of next-generation engineered scaffolds (Lu et al., 2022).

The therapeutic potential of MSC and EV treatments is increasingly being validated within the complex pathophysiology of organ-specific diseases. Matwiejuk et al. address dermatological applications considering skin diseases like atopic dermatitis, psoriasis, systemic sclerosis, contact dermatitis, graft-versus-host disease, alopecia areata, and systemic lupus erythematosus. In these conditions, chronic inflammation, fibrosis, and impaired re-epithelialization are crucial pathological features that require effective therapeutic intervention (Matwiejuk et al.). The authors' analysis supports a role for MSC-EVs as multi-target regulators capable of integrating immune modulation with structural repair. Ye et al., further expand the paradigm by demonstrating that preconditioning MSC-EVs under disease-mimicking environments enhances therapeutic precision, reinforcing the concept that regenerative interventions should reflect the complexity of pathological niches. Consequently, the field is evolving from indiscriminate administration toward the rational design of EVs preconditioned for specific disorders, such as systemic inflammation.

Large-scale analytical studies try to define emerging clinical priorities. The bibliometric analysis by Bai and Xin trace two decades of research on MSCs in neonatal bronchopulmonary dysplasia (BPD), documenting a clear thematic shift from whole-cell therapies towards MSC-derived exosomes as a dominant cell-free approach after 2018 (Bai and Xin). Although preclinical efficacy of MSC-EVs in BPD has been extensively reported in the scientific literature (Bisaccia et al., 2024; Porzionato et al., 2021; Porzionato et al., 2019), this work outlines a significant translational gap, as only 8.2% of studies report clinical outcomes. This suggests the need for future research advancement to bridge preclinical findings with standardized clinical trials and mechanistic studies. Yang et al. present a complementary bibliometric overview in degenerative joint and bone diseases, revealing increasing attention towards the therapeutic use of MSC-EVs to repair cartilage and bone, to regulate the immune system, and to act as targeted delivery vehicles for advanced regenerative strategies (Yang et al.).

Neuroregeneration is represented by Cipriano et al., who provide preclinical evidence that human umbilical cord MSC-EVs improve survival, motor function, and neuroinflammatory parameters in a rat model of spinal cord injury (Cipriano et al.). Beyond functional benefits, biodistribution analyses confirm selective EV retention at the injury site, reinforcing their suitability as targeted modulators of neuroinflammation and glial scarring. This work demonstrates how EVs can overcome limitations of direct MSC transplantation in the central nervous system, such as low engraftment, embolic risk, and safety concerns associated with proliferating cells.

Finally, the contribution by Shibili P. et al., broadens the scope beyond organ repair by examining the molecular interplay between endoplasmic reticulum (ER) stress and exosome-mediated communication in inflammatory bowel disease. Their review highlights a bidirectional regulatory axis in which ER stress alters vesicle biogenesis and cargo, while stress-modified vesicles propagate inflammatory signaling across epithelial and immune cells. This mechanistic insight positions EVs not only as therapeutic tools but also as pathogenic mediators, reinforcing the need for nuanced targeting strategies that distinguish beneficial from deleterious signaling (Shibili P. et al.).

Collectively, the articles in this Research Topic explore several themes that define the current trends of MSC-based regenerative medicine. First, therapeutic action is increasingly attributed to highly orchestrated paracrine networks rather than direct cell replacement. Second, EVs provide a scalable, cell-free platform with minimized safety risks and greater engineering potential compared to live cell therapies. Third, disease specificity achieved through preconditioning, cargo modulation, or biomaterial delivery appears essential to achieving clinically significant outcomes. Finally, bibliometric analyses are laying the groundwork for regulatory approval by clarifying therapeutic targets, methodological gaps, and translational priorities.

Future efforts should focus on standardizing EV isolation protocols, defining potency assays, improving in-tissue retention, and developing regulatory standards for the production and use of MSCs and derived EVs as effective biological drugs. As the field progresses, the integration of omics-based characterization, mechanobiology, and smart biomaterials will enable a new generation of precision therapeutic tools where MSCs represent natural bio-factory systems and derived EVs act as tunable agents that can modulate and heal the pathological microenvironments.

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References

Barbon, S., Rajendran, S., Bertalot, T., Piccione, M., Gasparella, M., Parnigotto, P. P., et al. (2021). Growth and differentiation of circulating stem cells after extensive *ex vivo* expansion. *Tissue Eng. Regen. Med.* 18, 411–427. doi:10.1007/s13770-021-00330-7

Barbon, S., Stocco, E., Rajendran, S., Zardo, L., Macchi, V., Grandi, C., et al. (2022). *In vitro* conditioning of adipose-derived mesenchymal stem cells by the endothelial microenvironment: modeling cell responsiveness towards non-genetic correction of haemophilia A. *Int. J. Mol. Sci.* 23, 7282. doi:10.3390/ijms23137282

Bernad, E. S., Vasilache, I.-A., Bernad, R. L., Hogea, L., Ene, D., Duica, F., et al. (2025). Extracellular vesicles and nanoparticles in regenerative and personalised medicine: diagnostic and therapeutic roles - a narrative review. *Pharmaceutics* 17, 1331. doi:10.3390/pharmaceutics17101331

Bisaccia, P., Magarotto, F., D'Agostino, S., Dedja, A., Barbon, S., Guidolin, D., et al. (2024). Extracellular vesicles from mesenchymal umbilical cord cells exert protection against oxidative stress and fibrosis in a rat model of bronchopulmonary dysplasia. *Stem Cells Transl. Med.* 13, 43–59. doi:10.1093/stcltm/szad070

Chen, B., Sun, Y., Zhang, J., Zhu, Q., Yang, Y., Niu, X., et al. (2021). Exosome-loaded injectable hydrogel for cartilage regeneration by modulating macrophage polarization. *Adv. Funct. Mater.* 31, 2100063. doi:10.1002/adfm.202100063

Di Liddo, R., Paganin, P., Lora, S., Dalzoppo, D., Giraudo, C., Miotto, D., et al. (2014). Poly-ε-caprolactone composite scaffolds for bone repair. *Int. J. Mol. Med.* 34, 1537–1546. doi:10.3892/ijmm.2014.1954

Di Liddo, R., Aguiari, P., Barbon, S., Bertalot, T., Mandoli, A., Tasso, A., et al. (2016). Nanopatterned acellular valve conduits drive the commitment of blood-derived multipotent cells. *Int. J. Nanomedicine* 11, 5041–5055. doi:10.2147/IJN.S115999

Han, X., Liao, R., Li, X., Zhang, C., Huo, S., Qin, L., et al. (2025). Mesenchymal stem cells in treating human diseases: molecular mechanisms and clinical studies. *Signal Transduct. Target. Ther.* 10, 262. doi:10.1038/s41392-025-02313-9

Hu, C., Li, L., Peng, H., Jiang, W., Liu, Q., Zhao, H., et al. (2020). Exosome-loaded thermosensitive hydrogel for wound healing and skin regeneration. *J. Nanobiotechnology* 18, 1–15. doi:10.1186/s12951-020-00657-0

Liu, X., Yang, Y., Li, Y., Niu, X., Zhao, B., Wang, Y., et al. (2017). Integration of stem cell-derived exosomes with *in situ* hydrogel glue as a promising tissue patch for articular cartilage regeneration. *Nanoscale* 9, 4430–4438. doi:10.1039/C7NR00353E

Lu, Y., Yang, Y., Liu, S., and Ge, S. (2022). Biomaterials constructed for MSC-derived extracellular vesicle loading and delivery - a promising method for tissue regeneration. *Front. Cell Dev. Biol.* 10, 898394. doi:10.3389/fcell.2022.898394

Patel, J. C., Shukla, M., and Shukla, M. (2025). From bench to bedside: translating mesenchymal stem cell therapies through preclinical and clinical evidence. *Front. Bioeng. Biotechnol.* 13, 1639439. doi:10.3389/fbioe.2025.1639439

Porzionato, A., Zaramella, P., Dedja, A., Guidolin, D., Van Wemmel, K., Macchi, V., et al. (2019). Intratracheal administration of clinical-grade mesenchymal stem cell-derived extracellular vesicles reduces lung injury in a rat model of bronchopulmonary dysplasia. *Am. J. Physiol. Lung Cell Mol. Physiol.* 316, L6–L19. doi:10.1152/ajplung.00109.2018

Porzionato, A., Zaramella, P., Dedja, A., Guidolin, D., Bonadies, L., Macchi, V., et al. (2021). Intratracheal administration of mesenchymal stem cell-derived extracellular vesicles reduces lung injuries in a chronic rat model of bronchopulmonary dysplasia. *Am. J. Physiol. Lung Cell Mol. Physiol.* 320, L688–L704. doi:10.1152/ajplung.00148.2020

Zhang, K., Zhao, X., Chen, X., Wei, Y., Du, W., Wang, Y., et al. (2018). Enhanced therapeutic effects of MSC-derived exosomes with an injectable hydrogel for hindlimb ischemia treatment. *ACS Appl. Mater. Interfaces* 10, 3008–3018. doi:10.1021/acsami.7b18153

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A bibliometric and visualized analysis of extracellular vesicles in degenerative musculoskeletal diseases (from 2006 to 2024)

Jun-Jie Yang[1,2], Sha-Qi He[1], Bei Huang[1], Peng-Xin Wang[1], Feng Xu[3], Xiao Lin[1]* and Jun Liu[1,4]*

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Background: With the rapid development of extracellular vesicles (EVs) in regenerative medicine research, they have become a promising new direction in the mechanistic, diagnosis and treatment studies of degenerative musculoskeletal diseases (DMDs), and has attracted increasing attention. However, there is currently a lack of comprehensive and objective summary analysis to help researchers quickly and conveniently understand the development trajectory and future trends of this field.

Method: This study collected articles and reviews published from 2006 to 2024 on EVs in DMDs from the Web of Science database. Bibliometric and visual analysis was conducted using several tools, including Microsoft Excel Office, VOSviewer, CiteSpace, Pajek, and R packages.

Results: 1,182 publications were included in the analysis from 2006 to 2024. Notably, there was a rapid increase in the number of publications starting in 2016, suggesting that this field remains in a developmental stage. Co-authorship analysis revealed that China ranked first in terms of publications, whereas the United States led in citations. The journal with the highest number of publications was International Journal of Molecular Sciences (INT J MOL SCI). The most prolific authors were Ragni, E with 23 publications, while the most cited author was Toh, WS. Additionally, nine of the top 10 institutions were from China, with Shanghai Jiao Tong University leading in the number of publications. The most cited article was "MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity", authored by Zhang, S, and published in BIOMATERIALS in 2018.

Conclusion: This study, through bibliometric and visual analysis, clearly illustrates the collaborative relationships among countries, authors, institutions, and journals, providing valuable insights for researchers seeking academic collaboration opportunities. Moreover, the analysis of keywords and citations allows researchers to better understand key research hotspots and frontiers in

this field, and points toward promising directions for future research. The growing interest in EV research in DMDs over recent years indicates increasing attention and a dynamic progression in this field.

KEYWORDS

degenerative musculoskeletal diseases, bibliometric analysis, extracellular vesicles, senescence, cell-free therapy

1 Introduction

Degenerative musculoskeletal diseases (DMDs) involve structural and functional impairments of the musculoskeletal system, leading to symptoms such as pain and restricted movement, which significantly impact patients' quality of life (Wen et al., 2023; Yin et al., 2023). Common types of DMDs include osteoarthritis (OA) (Hunter and Bierma-Zeinstra, 2019), osteoporosis (OP) (Compston et al., 2019), degenerative disc disease (DDD) (Xiang et al., 2024), and sarcopenia (Cruz-Jentoft and Sayer, 2019). As life expectancy rises and the global population ages, these DMDs have led to substantial economic and societal burdens (Scheuing et al., 2023; Clynes et al., 2020; Kirk et al., 2020). The primary treatment for OP focuses on pharmacological interventions, including calcium, vitamin D, estrogen receptor modulator, RANKL receptor agonists, and parathyroid hormone analogs (LeBoff et al., 2022; Reid and Billington, 2022). Nevertheless, several drugs cause serious adverse reactions or are not suitable for prolonged use (Song et al., 2022). For OA, there is currently no cure. Treatments mainly aim to relieve pain and maintain joint function. Nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen are typically first-line therapies, while total joint replacement surgery is considered the gold standard for patients with severe OA (not respond to conservative treatments or the quality of life severely affected by pain) (Abramoff and Caldera, 2020). Treatment for sarcopenia focuses on avoiding mobility impairment and significant health events like fractures, primarily through strength exercise and enhanced dietary energy intake (Chen et al., 2022; Coletta and Phillips, 2023; Dent et al., 2018). DDD can only alleviate pain through physical therapy, medications, and surgical intervention (Zhu et al., 2023). However, there is a high risk of recurrence and surgical complications. However, they do not address the underlying causes. This highlights the need for more direct and effective treatment strategies that can reverse the disease by eliminating pathogenic factors and promoting tissue repair.

Extracellular vesicles (EVs) are nanoscale lipid bilayer structures derived from cells that encapsulate proteins, lipids, RNA, metabolites, growth factors, and cytokines (Weng et al., 2021). EVs play multiple roles in intercellular communication by transporting their cargo to recipient cells, where they can modulate cellular functions or physiological activities, and even affect organs (Jeppesen et al., 2023). Additionally, EVs may contribute to the emergence of several diseases, such as cancer (Ciardiello et al., 2016) and neurological disorders (Izquierdo-Altarejos et al., 2024), by transporting abnormal cargo. The pivotal role of EVs in intercellular communication positions them as promising candidates for therapeutic applications, drug delivery systems, and disease biomarkers (Kumar M. A. et al., 2024). As a drug delivery system, EVs offer advantages over cell therapies, including low immunogenicity, high biocompatibility, low oncogenicity, the capacity to traverse biological barriers such as the blood-brain barrier, and a lipid membrane structure that protects enclosed bioactive molecules (Kou et al., 2022; Elsharkasy et al., 2020; Vader et al., 2016). Moreover, EVs can be engineered to modify their surfaces (Chen Z. et al., 2024; Zhang et al., 2023) and combined with biomaterials (Yang et al., 2024; Yin et al., 2022; Liao et al., 2022; Fang et al., 2023) to enhance their targeting and retention time at the target. Interestingly, the use of EVs as drug delivery vehicles for treating DMDs is gaining increasing research attention.

In addition, EVs are increasingly recognized for their potential as biomarkers in disease diagnosis, particularly for early cancer detection (Kumar M. A. et al., 2024; Kalluri, 2016; Wang et al., 2022). Although EVs as a diagnostic tool for DMDs are still in the preclinical stage, it is potential to replace conventional clinical diagnostics in the future, truly enabling early diagnosis and treatment.

Bibliometrics involves analyzing abstracts, keywords, and citations of published papers, using statistical data to describe or display relationships among published papers (Ninkov et al., 2022). While numerous high-quality reviews on EVs in the field of DMDs have been published, traditional literature review methods have become inadequate for researchers to track the state, development, and evolution, to identify gaps, and to categorize the knowledge system (Öztürk et al., 2024). Bibliometric analysis addresses this limitation by considering all literatures related to the research, revealing the scientific trends in different fields and providing insights into ongoing discussions about evaluating science and its researchers (Ellegaard, 2018). Therefore, this study primarily utilizes data visualization tools, including Citespace and VOSviewer, to perform bibliometric and visual analyses of EVs research in DMDs over the last 18 years. This study aims to provide new perspectives for future research, allowing researchers to easily access the latest trends and developments in the field, and offering useful references for possible collaborations.

2 Materials and methods

2.1 Data sources and search strategies

The bibliometric data for this study were sourced from the Web of Science Core Collection (WoSCC), a widely recognized and standardized academic database. We retrieved all online articles and reviews on EVs in DMDs published between 2006 and 2024 from the WoSCC SCI-expanded database. The search strategy employed was: "TS=("extracellular vesicle* "OR" exosom*") AND TS=("osteopor* "OR" osteoarthr*" OR "degenerative disc disease* "OR" degenerative musculoskeletal disease* "OR" sarcopenia* "OR" myopathy")". The search

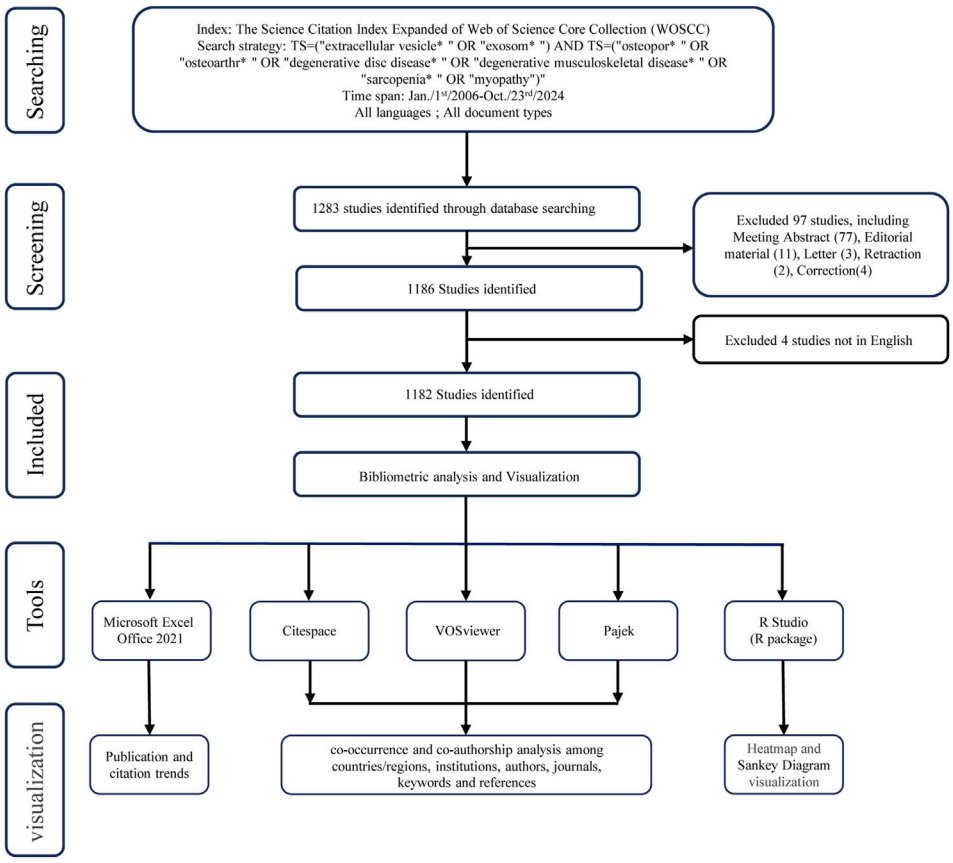


FIGURE 1
Flowchart of literature search and selection.

interval was from 1 January 2006 to 23 October 2024. The document types chosen were limited to "Article" and "Review," with English as the language. In total, 1,182 original articles and reviews met the criteria for inclusion in the analyses, and were further analyzed visually (Figure 1).

2.2 Data analysis and visualization

The comprehensive data was collected from WoSCC and loaded into Microsoft Excel Office 2021 (Microsoft Corporation, Redmond, United States), VOSviewer 1.6.20 (Leiden University, Netherlands), Citespace version 6.3. R1/6.2. R7 (Chaomei Chen, China), Pajek version 5.19 (University of Ljubljana, Slovenia), GraphPad Prism version 10.1.2 (GraphPad Software, San Diego, California United States, www.graphpad.com), and the chorddiag R package (R Studio, version 4.3.2). VOSviewer and Pajek were utilized to conduct co-occurrence and co-authorship analysis to identify patterns of co-occurrence and co-authorship among countries/regions, institutions, authors, journals, keywords. Citespace was utilized for visualization, allowing the creation of visual maps related to keywords, co-cited references, journals, and citation bursts (Chen, 2004; Chen et al., 2010; Chen, 2006). Bubble chart was plotted by https://www.bioinformatics.com.cn, an online platform for data analysis and visualization (Tang et al., 2023). Additionally, Bibliometrix package in R software (Aria and Cuccurullo, 2017) was used for visual analysis of counties/regions, authors and keywords.

3 Results

3.1 Analysis of annual publications

We collected 1,182 relevant documents, including reviews and articles, from 1 January 2006 to 23 October 2024 for the study of EVs in DMDs (Figure 2). The number of scholarly publications in this field has shown a steady increase over the past decade (Figure 2A). Prior to 2016, the volume of publications in this field was modest, suggesting that Scholarly exploration in this area had only just begun. However, the number of publications began to rise significantly after 2016, reaching a peak in 2023 with 240 annual publications, marking a 24-fold increase compared to 2016. However, the total number of publications on this topic remains relatively modest, with only 1,182 cumulative publications as of November 2024. Nevertheless, the high growth rate suggests promising prospects for the development of this field. To project future trends, we apply the exponential equation $y = 1.0704^{0.4088x}$ to model the anticipated trajectory of publication growth. The R^2 value of 0.9748 reflects the model's excellent predictive ability, allowing for

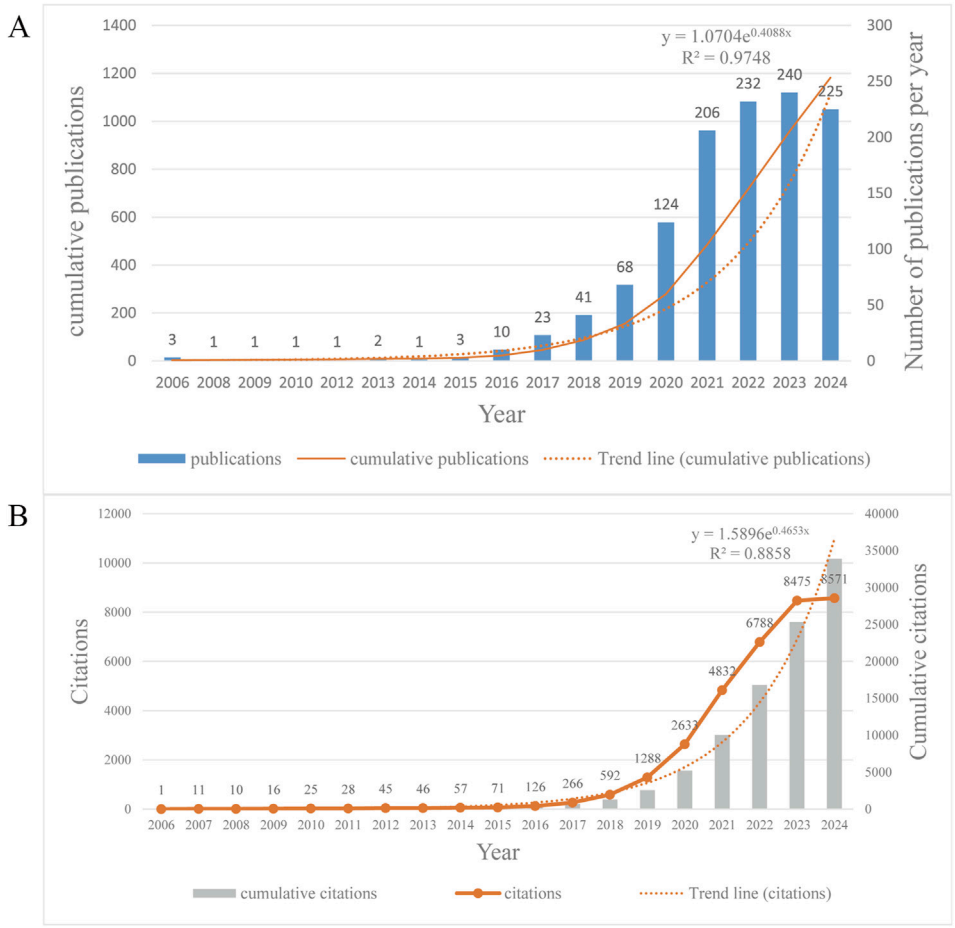


FIGURE 2
(A) Annual and cumulative publications from 2006 to 2024, and the trend line of annual publications. It should be noted that the articles we analyzed were published up to 23 October 2024, so the publications of this year are incomplete and lower compared to the previous year, but the prediction still shows an upward trend. **(B)** Annual and cumulative citations from 2006 to 2024, and the trend line of annual citations.

accurate forecasting of future publication trends. The prediction curve indicates a consistent rise in the annual number of articles, along with an increase in research activity in this field. Between 2006 and 2024, the total number of citations for all publications has reached 33,896, with the highest annual citation count occurring in 2024. On average, each publication has received 28.68 citations. The annual citation count exhibits an exponential growth trend, with particularly rapid increases observed since 2018, becoming even more pronounced after 2020. By 2023, the annual citations had reached 8,475. Based on the prediction model, the annual citations are expected to surpass 10,000 by the end of 2024. The exponential growth model ($y = 1.5896e^{0.4653x}$, $R^2 = 0.8858$) demonstrates a strong fit to the data, indicating that the upward trajectory of annual citations is likely to continue. This trend reflects the growing influence and increasing recognition of research in this field over time (Figure 2B).

3.2 Countries/regions analysis

EVs research in the field of DMDs involves 61 countries/regions. We screened 32 countries based on the requirement of at least five publications per country or region in DMDs, visualizing the number of publications in each country/region and the collaborations between countries/regions (Figure 3). As seen in Table 1, the top 10 countries/regions ranked by publications are China (n = 716), the United States (n = 132), and Italy (n = 92) respectively. Notably, although China significantly surpasses the United States in publications, its total link strength is lower than that of the United States, reflecting a lack of collaborative communication with other countries compared to the United States. This phenomenon can also be visually observed in Figure 3C. Statistical analysis revealed a significant correlation between the number of publications (NP) and total link strength, which represents the level of international collaboration among countries ($P < 0.0001$). Furthermore, countries were divided into high, medium, and low total link strength groups using tertile classification. A comparison of NP across these groups revealed that countries with high link strength published significantly more papers than the other two groups. Despite China achieving the highest publication output globally with relatively low total link strength, these findings underscore the critical role of international collaboration in accelerating research growth, as countries with stronger global partnerships tend to experience faster

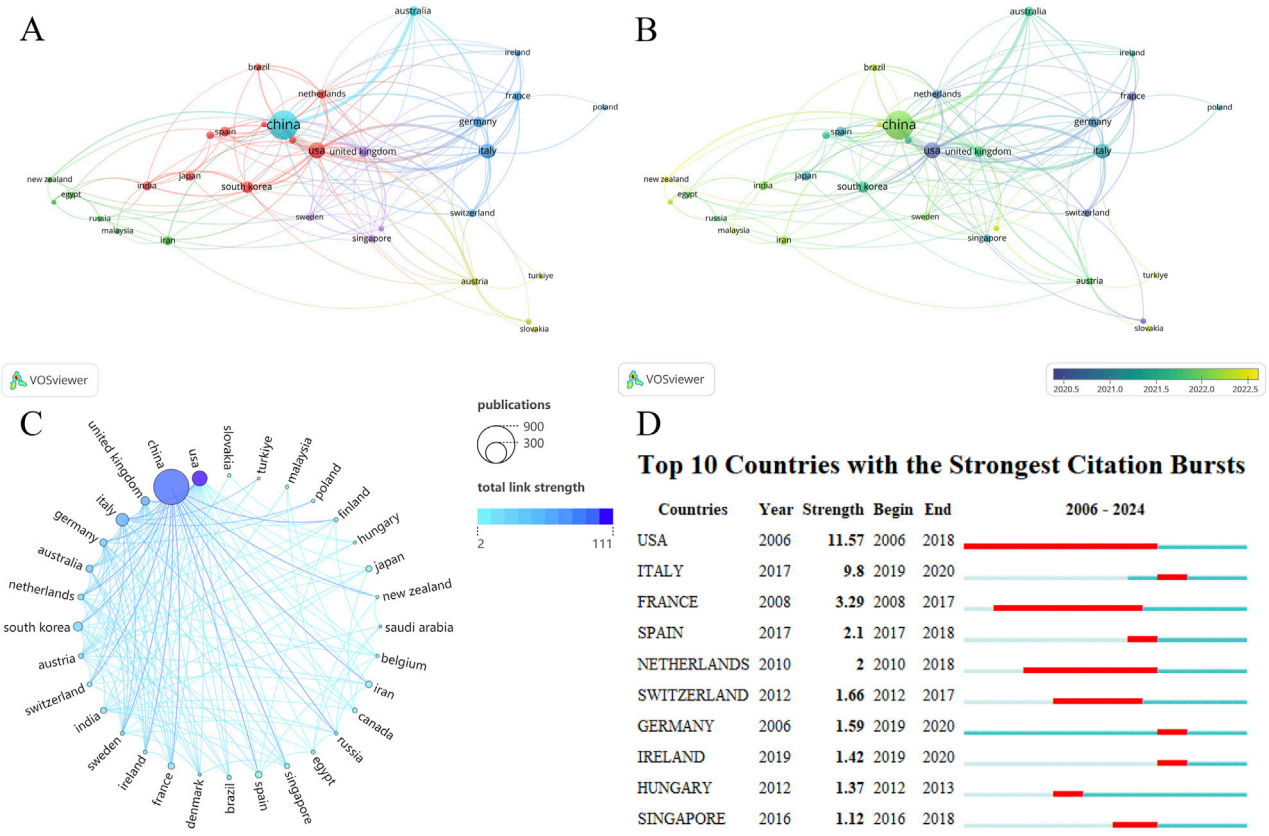


FIGURE 3
(A) Countries/regions network visualization. **(B)** Countries/regions overlay visualization. The size of the nodes also represents the publications of each country, and the color of the nodes reflects the average time of publication. **(C)** The chord diagram for countries/regions partnership. The United States has significantly fewer publications than China but has stronger cooperation with other countries and a higher Total link strength. **(D)** Top 10 countries with the strongest citation bursts.

TABLE 1 Top 10 Countries/regions ranked by publications with NC and total link strength.

Rank	Country/region	NP	NC	Total link strength
1	China	716	19739	94
2	United States	132	5211	111
3	Italy	92	2220	57
4	South Korea	48	1181	24
5	United Kingdom	37	573	61
6	Germany	31	935	42
7	Australia	30	846	36
8	Spain	26	721	11
9	France	25	1179	15
10	Iran	25	524	9

development in this domain (Supplementary Figures 1A, C). Most countries began research in this field after 2016, while a few, such as the United States, Germany, and France, started earlier and have established solid research foundations. However, the majority of publications have been concentrated in the past 3 years. Although China currently has a large number of publications, it is still in its early stages in this field, indicating a very rapid pace of development (Figures 3A, B; Supplementary Figure 2A). The national cooperation map offers a more intuitive visualization of collaborative relationships between countries (Supplementary Figure 2B). Figure 3D depicts the top 10 countries with the strongest citation bursts, with the United States showing both the highest intensity and the longest duration. This indicates that the U.S. has long been a leader in research hotspots and trends in this field. Overall, research in this field is still in its early stages globally, but growing interest suggests significant potential and promising prospects for future development.

3.3 Keywords analysis

Keywords often reflect the core content of an article. Through keyword co-occurrence analysis, research hotspots in a specific field can be identified. Using VOSviewer, a keyword co-occurrence network view was drawn for 1,182 publications, visualizing keywords with a frequency of eight or more (Figure 4). Table 2 shows the top 30 keywords by frequency. Figures 4A, B reveal the frequency of keywords and the results of cluster analysis, the top

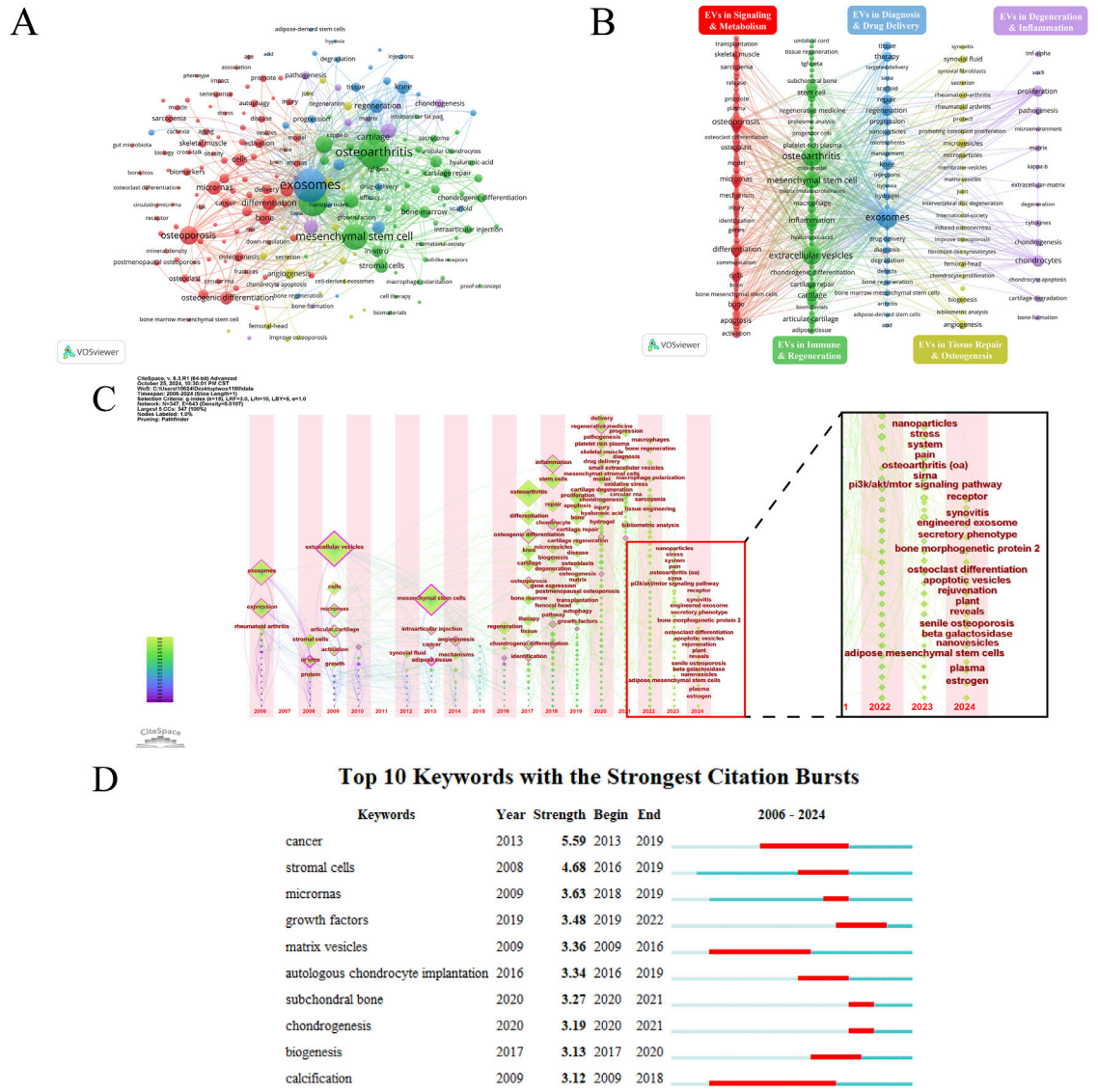


FIGURE 4
(A) Keyword network visualization. Circles and labels form the nodes, and the size of the node represents the frequency of the keyword, the higher the frequency, the larger the node. **(B)** Analysis of Keyword Clustering. The lines between the nodes represent the association strength between keywords. The color of the nodes represents their clusters, with different colors representing different clusters, divided into five clusters based on different research directions. **(C)** Temporal overlay visualization of the keyword co-occurrence network. The position of the nodes reflects the first appearance year of the corresponding keywords, the size of the nodes reflects the frequency of keyword occurrences, and the annual ring color reflects all years of keyword occurrence. The zoomed-in image shows the keywords that have emerged in the past 2 years. **(D)** Top 10 keywords with the strongest citation bursts.

three keywords are exosomes, osteoarthritis and extracellular vesicles. Five clusters are generated which can represent the five main research directions in this field. The average year of keyword occurrence can be used to understand the frontiers and hotspots in this field (e.g., drug delivery, hydrogel, oxidative stress, nanoparticles, bone regeneration, macrophage polarization, sarcopenia, intervertebral disc degeneration, etc.) (Supplementary Figure 3A). The current high research interest in exosomes combined with new biomaterials makes exosomes and hydrogels appear close to each other in the density visualization (Supplementary Figure 3B). Many new keywords began to appear after 2016, and most of them were heavily cited during this period (Supplementary Figures 3C, D). This indicates that the field is an emerging research area, with continuous advancements and numerous new research directions, aligning with the surge in publications. Figure 4C illustrates the evolving research themes over time by tracking changes in keywords from 2006 to 2024. Figure 4D is the top 10 keywords with strongest citation burst, which reveals the changing trends of research hotspots in this academic field over time. It also highlights which keywords gained more citations during specific periods, reflecting the academic community's focus on these topics.

TABLE 2 Top 30 keywords ranked by the frequency of occurrences, with total link strength.

Rank	Keyword	Occurrences	Total link strength
1	Exosomes	682	4,843
2	Osteoarthritis	607	4,428
3	Extracellular vesicles	522	3877
4	Mesenchymal stem cell	392	3110
5	Osteoporosis	211	1,309
6	Inflammation	182	1,465
7	Cartilage	151	1,205
8	Chondrocytes	135	1,030
9	Differentiation	134	962
10	Stem cell	129	1,033
11	Proliferation	128	989
12	Regeneration	118	933
13	Micrornas	118	866
14	Stromal cells	117	9,997
15	Apoptosis	109	798
16	Knee	106	809
17	Osteogenic differentiation	104	744
18	Cells	97	609
19	Bone	95	642
20	Repair	92	789
21	Articular-cartilage	85	695
22	*In-vitro*	81	667
23	Therapy	79	593
24	Bone-marrow	78	666
25	Microvesicles	67	583
26	Angiogenesis	63	459
27	Microrna	62	508
28	Osteoblast	62	495
29	Mechanism	59	454
30	Activation	58	392

3.4 Co-cited reference analysis

This section analyzes the field's development process and the evolution of research hotspots through co-cited references and citation bursts. The co-cited reference analysis was conducted using CiteSpace (Figure 5). Table 3 lists the top 10 most-cited articles. These highly cited articles often reflect major discoveries and research achievements in the field at the time. Analyzing them provides valuable insights into the field's developmental process. Figure 5A shows the cited articles and the co-citation relationships among them. In Figure 5B, each cluster represents a research topic or field, and the title of each cluster summarizes its main research topic. There are a total of 10 clusters: 0# (extracellular vesicles), 1# (orthopedic diseases), 2# (cell-based therapy), 3# (Mesenchymal stem Cells), 4# (regenerative medicine), 5# (clinical application), 6# (immune regulation), 7# (human adipose-derived stem cells), 8# (rheumatoid arthritis), 9# (articular cartilage vesicles). The two most active clusters are 0# (extracellular vesicles) and 1# (orthopedic disease), each containing over 100 keywords. As shown in Figure 5C, a sudden sharp increase in citations often indicates that the article has likely addressed a significant issue in the research field. Figure 5D shows the top 10 references in citation burst analysis. Most articles are heavily cited in 2016 ~ 2018 when this research field was rapidly growing.

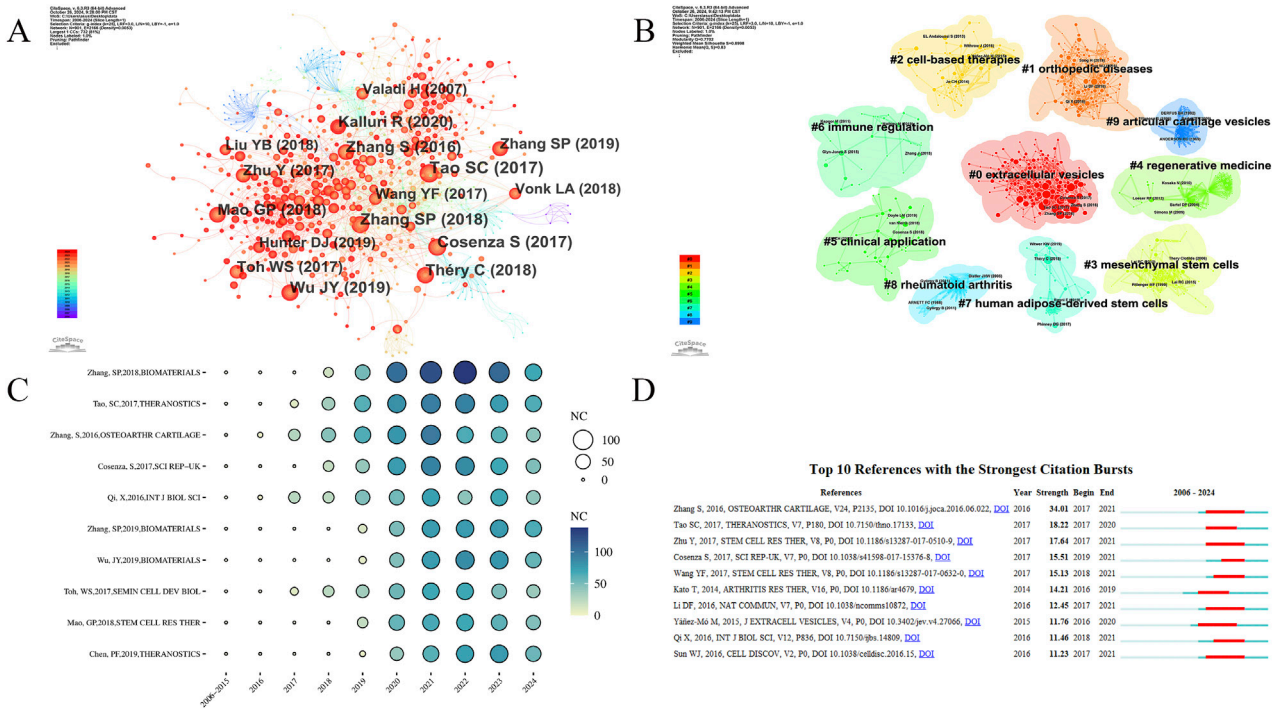


FIGURE 5
(A) Analysis of the network of references by citespace. Each node corresponds to each cited reference. The lines between nodes represent co-citation relationships. Various colors from purple to red represent different years, with the time range from 2006 to 2024. **(B)** The clustered network map of co-cited references. Different colors represent different clusters, which reflect the strength of association between articles. **(C)** Bubble chart of the top 10 cited references. The size and color of the bubbles intuitively show the citations of the articles and their trends over the years. **(D)** Top 10 references with the strongest citation bursts.

3.5 Journals analysis

A total of 384 journals have published research articles on EVs in the field of DMDs. We set a minimum threshold of five articles per journal and selected 60 journals for visualization analysis (Figure 6). Table 4 lists the top 10 journals by publications, along with their number of publications (NP), citation (NC), H-index, and impact factor (IF), and most of which have grown rapidly since 2016 (Supplementary Figure 4B). The journal with the most publications in this field is INT J MOL SCI (NP = 55), the most influential journal is STEM CELL RES THER (NC = 2,303, H-Index = 23), and the top three journals by impact factor are BIOACT MATER (IF = 18), J NANOBIOTECHNOL (IF = 10.6), and STEM CELL RES THER (IF = 7.1).

The journal network and density visualizations offer insights into the journals with the most publications and the collaborative relationships between them (Figure 6A; Supplementary Figure 4A). We used VOSviewer to analyze the 384 journals in this field across different categories. Most journals publish research articles concentrated in biomedicine and chemical physics, with a particular emphasis on biomedicine (Figure 6B). Table 5 lists the top 10 fields by publications, the top three fields in terms of publication output are Cell Biology (270), Biochemistry and Molecular Biology (160), and Experimental Medicine Research (160). The number of publications across various fields began to increase rapidly in 2019, with biology-related fields dominating this growth. However, since 2021, there has been a decline in publication within biology-related fields, while other interdisciplinary fields have shown more significant growth. This trend indicates that interdisciplinary studies are becoming the future focus, rather than being solely confined to biology (Supplementary Figure 5). Current research on EVs in the field of DMDs is primarily focused on basic research and clinical translation.

The overlay of dual-map journals effectively illustrates the interdisciplinary distribution of journals, citation patterns, and shifts in research center. We used VOSviewer and CiteSpace to visualize the overlay of dual-map journals (Figure 6C; Supplementary Figures 4C–E). The curves between the maps represent citation relationships between journals on both sides, highlighting the understanding of interdisciplinary connections in this field. Currently, interdisciplinary research on extracellular vesicles in DMDs is lacking. However, as interest grows in combining extracellular vesicles with biomaterials for treatment, interdisciplinary collaboration is likely to become a future trend in this field.

3.6 Authors analysis

6,720 authors have published research articles on EVS in the field of DMDs. We set a minimum threshold of 7 articles per author and selected 38 authors for visualization analysis (Figure 7). Table 6 shows the top 10 authors by publications, including their affiliations,

TABLE 3 Top 10 most-cited articles with journal, IF and total citation.

Rank	Year	Article	Journal	If (2023)	Total citation
1	2018	MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity	BIOMATERIALS	12.8	607
2	2017	Exosomes derived from miR-140-5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model	THERANOSTICS	12.4	514
3	2016	Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration	OSTEOARTHR CARTILAGE	7.2	482
4	2017	Mesenchymal stem cells derived exosomes and microparticles protect cartilage and bone from degradation in osteoarthritis	SCI REP-UK	3.8	429
5	2016	Exosomes Secreted by Human-Induced Pluripotent Stem Cell-Derived Mesenchymal Stem Cells Repair Critical-Sized Bone Defects through Enhanced Angiogenesis and Osteogenesis in Osteoporotic Rats	INT J BIOL SCI	8.2	378
6	2019	MSC exosomes alleviate temporomandibular joint osteoarthritis by attenuating inflammation and restoring matrix homeostasis	BIOMATERIALS	12.8	355
7	2019	miR-100-5p-abundant exosomes derived from infrapatellar fat pad MSCs protect articular cartilage and ameliorate gait abnormalities via inhibition of mTOR in osteoarthritis	BIOMATERIALS	12.8	352
8	2017	MSC exosome as a cell-free MSC therapy for cartilage regeneration: Implications for osteoarthritis treatment	SEMIN CELL DEV BIOL	6.2	346
9	2018	Exosomes derived from miR-92a-3p-overexpressing human mesenchymal stem cells enhance chondrogenesis and suppress cartilage degradation via targeting WNT5A	STEM CELL RES THER	7.1	319
10	2019	Desktop-stereolithography 3D printing of a radially oriented extracellular matrix/mesenchymal stem cell exosome bioink for osteochondral defect regeneration	THERANOSTICS	12.4	307

countries, publication counts, citation counts, and H-index. The top three authors are Ragni, E (23), De Girolamo, L (22), and Su, JC (16). The most cited author is Toh, WS (2066), reflecting the significance of these authors in the research field. Interestingly, six of the top 10 authors belong to the same research team at IRCCS Istituto Ortopedico Galeazzi in Italy. This team primarily focuses on the basic research and clinical translation of mesenchymal stem cells. Keeping track of their research progress provides valuable insights into the current hotspots and frontiers of this field. In Figures 7A, B, it is evident that close collaboration among authors in this field is lacking. Most collaborative relationships are confined to the same country or institution. Furthermore, there is almost no collaboration among authors from the top 10 countries, which hinders the field's progress. Encouraging international academic exchange and collaboration is essential for its development.

The total number of authors involved in the author co-citation analysis is 36,373. We set a minimum threshold of 20 citations per author and selected 577 authors for visualization analysis (Figure 7C). Tao SC, Zhang SP, and Zhang Y are the top three most cited authors, exerting significant academic influence in this field, potentially providing a foundation or inspiration for future studies. Among the top 10 authors with the strongest citation bursts (Figure 7D), Su Jiacan has shown the highest burst strength in the past 2 years (4.33). Notably, the burst periods for all top 10 authors have occurred in recent years, reflecting the research hotspots of their respective periods. By analyzing these authors and their research, other researchers can gain insights into academic trends, stay updated on the latest developments, and follow emerging dynamics in the field.

3.7 Institutions analysis

1,452 institutions have published research articles on EVs in the field of DMDs. We set a minimum threshold of 10 articles per institution and selected 41 institutions for visualization analysis (Figure 8). Table 7 displays the top 10 institutions ranked by publications, along with the number of their publication and citation. Shanghai Jiao Tong University in China ranks first with 71 publications. Notably, 9 of the top 10 institutions are from China, highlighting the country's high level of activity in this field. Figures 8A, B illustrate the publication output and institutional collaborations, emphasizing the lack of international cooperation, as most collaborations take place within domestic institutions. Statistical analysis revealed no significant correlation between the number of publications (NP) and total link strength among institutions ($p > 0.05$). Similarly, grouping institutions into high, medium, and low total link strength categories and comparing their NP revealed no statistically significant differences among the three groups (Supplementary Figures 1B, D). These findings suggest that, at the institutional level, total link strength may not have a direct influence on publication output in this field. However, this lack of statistical significance could be attributed to several factors. First, differences in institutional research capacity, resource allocation, and focus areas may moderate the relationship between collaboration intensity and NP. Second, total link strength reflects the quantity of collaborations but does not account for their quality, which may play a more critical role in driving publication output. Nevertheless, the importance of mutual collaboration in advancing research cannot be entirely dismissed.

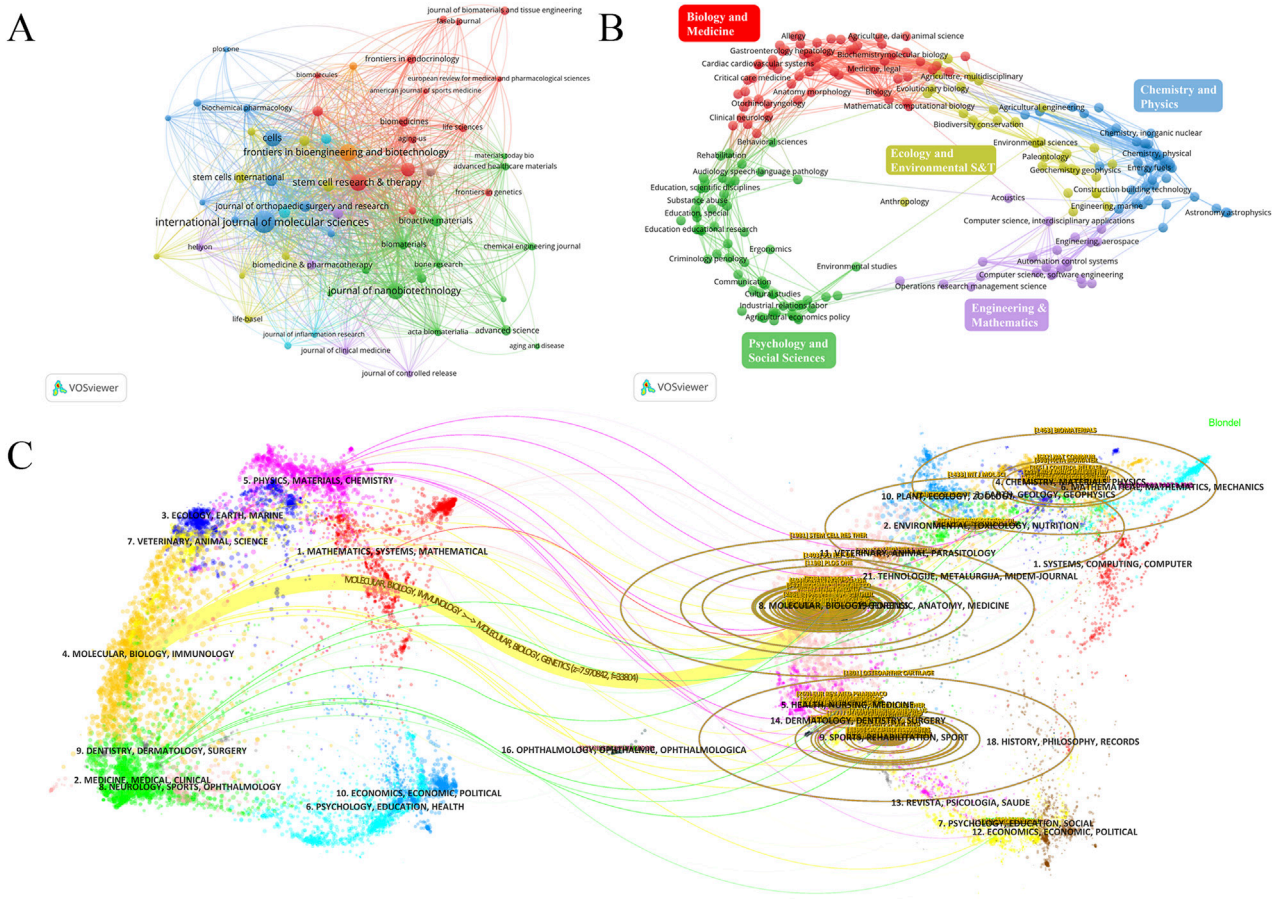


FIGURE 6
(A) journal network visualization. The size of the nodes represents the number of publications of the journals, and the colors represent different clusters. **(B)** Analyses of research subject areas. Different colors represent different fields. **(C)** The overlay of dual-map journals. The map is divided into two parts: the left side shows the distribution of the citing journals. The right side provides the distribution of the cited journals, which can be considered the research foundation for the former. The dots represent different journals, different colors represent the fields of interest shared by journals in the same cluster, and the ellipses represent the number of publications and the ratio of authors to publications for a journal. The length of the ellipses represents the number of authors, and the width represents the number of publications.

TABLE 4 The top 10 journals ranked by publications with NP, NC, H-index, and IF.

Rank	Journal	NP	NC	H-index	If (2023)
1	INT J MOL SCI	55	756	15	4.9
2	STEM CELL RES THER	32	2,303	23	7.1
3	FRONT BIOENG BIOTECH	32	681	16	4.3
4	Cells	32	565	15	5.1
5	J NANOBIOTECHNOL	32	516	11	10.6
6	Front Cell Dev Biol	23	690	15	4.6
7	J ORTHOP SURG RES	16	317	8	2.8
8	ARTHRITIS RES THER	15	672	10	4.4
9	BIOACT MATER	15	817	12	18
10	FRONT IMMUNOL	15	347	9	5.7

TABLE 5 The top 10 fields ranked by publications.

Rank	Field	Record count	% Of total
1	Cell Biology	270	22.843
2	Biochemistry Molecular Biology	160	13.536
3	Experimental Medicine Research	160	13.536
4	Pharmacology Pharmacy	136	11.506
5	Cell Tissue Engineering	132	11.168
6	Engineering Biomedical	116	9.814
7	Biotechnology Applied Microbiology	110	9.306
8	Chemistry Multidisciplinary	101	8.545
9	Nanoscience Nanotechnology	100	8.46
10	Orthopedics	69	5.838

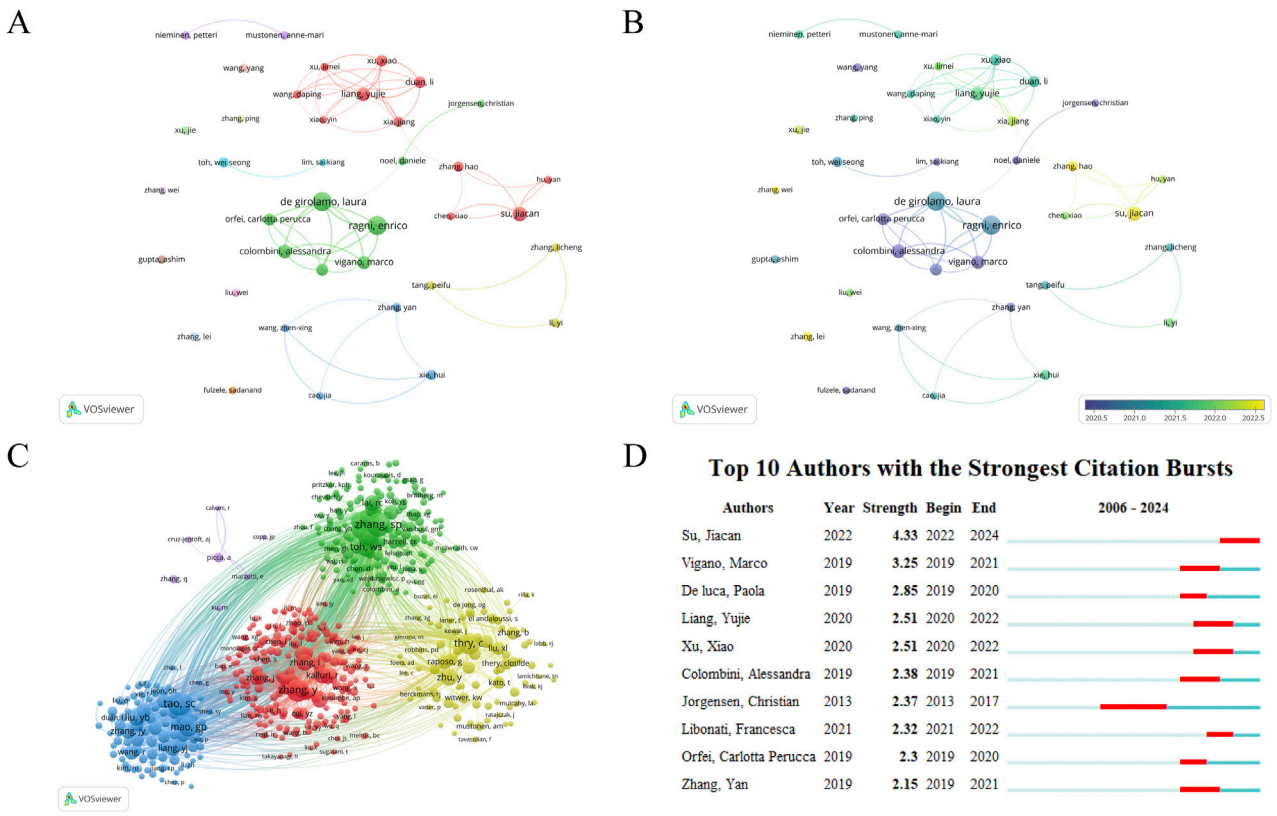


FIGURE 7
(A) Author network visualization. **(B)** Author overlay visualization. The size of circles in the network represents the publications of authors. The thickness of lines between circles represents the strength of collaboration among authors. The label view reflects the average publication year of authors based on the darkness of circle colors. **(C)** Author Co-citation Analysis by VOSviewer. Co-citation analysis refers to multiple authors' articles being cited in the same literature. The size of circles reflects the citation count of authors. Lines represent co-citation relationships, and the thickness of lines can indicate the frequency of co-citation. A higher frequency of co-citation between two authors may indicate that their research areas or topics are closely related. If the two authors have different academic backgrounds, it can reflect the interdisciplinary research potential of this field. **(D)** Top 10 authors with the strongest citation bursts.

In addition, Figure 8B also uses color gradients to clearly represent the publication volume of each institution. Supplementary Figure 6 illustrates the cross-linking between countries, institutions, and authors for the top 10. It is evident that the most prominent research power is concentrated in China, the United States of America, Singapore and Italy.

TABLE 6 The top 10 authors ranked by publications with their affiliations, countries, NP, NC, and H-index.

Rank	Author	Affiliation	Country	NP	NC	H-index
1	Ragni, E	IRCCS Istituto Ortopedico Galeazzi	Italy	23	509	14
2	De Girolamo, L	IRCCS Istituto Ortopedico Galeazzi	Italy	22	476	13
3	Su, JC	Shanghai Jiao Tong University	China	16	791	12
4	Colombini, A	IRCCS Istituto Ortopedico Galeazzi	Italy	15	438	12
5	Orfei, CP	IRCCS Istituto Ortopedico Galeazzi	Italy	14	290	8
6	Viganò, M	IRCCS Istituto Ortopedico Galeazzi	Italy	14	434	12
7	Liang, YJ	Jining Medical University	China	13	744	9
8	De Luca, P	IRCCS Istituto Ortopedico Galeazzi	Italy	13	350	9
9	Toh, WS	National University of Singapore	Singapore	12	2066	9
10	Xie, H	Xiangya Hospital	China	12	623	9

4 Discussion

4.1 General information

In this study, we conducted a bibliometric analysis of literatures on EVs research in DMDs from 2006 to 2024. The aim is to comprehensively understand the current research status and trends in this field, providing scholars with a systematic reference framework to understand important achievements and frontiers. Our results indicate that the period from 2006 to 2016 was the initial stage of research in this field, with annual publications fewer than 10 articles. However, since 2016, the field has experienced explosive growth, with annual publications exceeding 100 in 2020. Notably, in the past 2 years alone, the publication volume doubled in 2022. Up to now, the continuous rapid growth in annual publication volume indicates that EVs research in DMDs is gaining increasing attention and research enthusiasm.

The visualization analysis of countries, institutions, and authors shows that China, the United States, and Italy are the top three countries in terms of publication volume in this field. China leads significantly, with 716 publications (NP) and 19,739 citations (NC), far surpassing other countries. However, China's collaboration with other countries is relatively low (link strength = 94), with most research being independently conducted. In contrast, the United States has only 132 publications, but its link strength is as high as 111, indicating a strong emphasis on collaborative research with other countries. This model of international cooperation and extensive academic exchange is essential for the faster and better development of this field. The top three institutions by publication volume are Shanghai Jiao Tong University (71), Central South University (37), and Sichuan University (34). Understanding their publication volume, citation counts, and research directions provides valuable insights for choosing research collaborations and academic exchanges.

Ragni, E, De Girolamo, L, and Su, JC are currently the top three authors by NP and have conducted extensive research in this field. Ragni, E and De Girolamo, L are from the same laboratory, focusing mainly on the roles of secreted factors and extracellular vesicles derived from mesenchymal stem cells in regenerative medicine. This includes research related to DMDs, particularly the therapeutic mechanisms of mesenchymal stem cells from different sources for diseases such as OA (Ragni et al., 2019; Ragni et al., 2020). Su, JC mainly focuses on the basic research and clinical translation of engineered extracellular vesicles for treating degenerative diseases. This includes using bone-targeting extracellular vesicles to deliver therapeutic drugs for treating OP and other degenerative diseases, as well as research and applications related to biomaterials (Hu et al., 2021; Jiang et al., 2022; Liu et al., 2022a; Liu et al., 2022b).

We analyzed journals that published articles on EVs related to DMDs. The top three journals by NP are INT J MOL SCI (IF = 4.9, Q1), FRONT BIOENG BIOTECH (IF = 4.3, Q1), and STEM CELL RES THER (IF = 7.1, Q1). Among the top 10 journals by NP in this field, nine are Q1 journals, including BIOACT MATER, the top journal in the biomaterials field. This indicates that the field attracts many excellent scholars and the quality of published research is high. The overlay map reveals that the main fields of EVs research in DMDs are biomedicine and chemical physics. Combining this with the dual-map overlay results, it reveals a lack of multidisciplinary integration in this research direction. The focus is mainly on molecular biology, biology, and immunology, with a strong emphasis on basic research and a noticeable shortage of clinical studies.

The keyword analysis highlights the main research directions and hotspots in the field. Additionally, the time zone map provides insights into the progression of research over time. The red cluster relates to how EVs regulate signaling pathways, immune responses, and gene expression, impacting bone and muscle metabolism, regeneration, and degeneration. This cluster addresses pathological processes linked to aging, osteoporosis, and other DMDs. The green cluster focuses on how EVs regulate immune cells (such as T cells and macrophages) and their secretions to promote cartilage and bone repair, as well as their combination with stem cells, regenerative medicine, and biomaterials to advance treatment strategies. The blue cluster highlights the role of EVs in diagnosing and treating DMDs by promoting bone and cartilage regeneration, enabling targeted drug delivery, and integrating with biomaterials. The purple cluster explores the regulation of cartilage and bone degeneration, inflammatory responses, and the cellular microenvironment, influencing disease progression. Keywords in the yellow cluster mainly focus on the role of EVs in tissue repair,

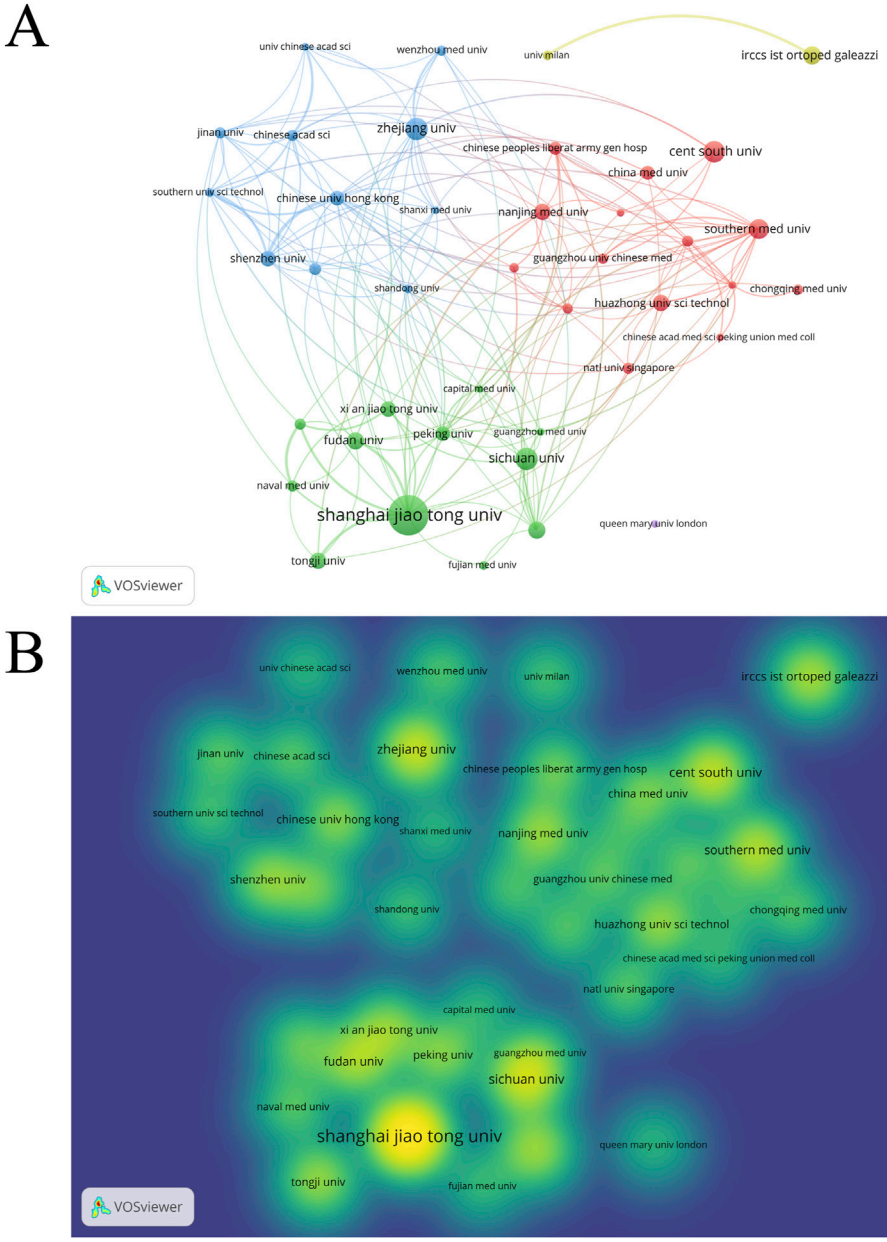


FIGURE 8
(A) institution network visualization. **(B)** Institution density visualization by VOSviewer.

osteogenesis and chondrogenesis, regulation of inflammatory responses, and relevant biomarkers. The connections between the red, green, and blue clusters indicate substantial overlap among these research directions (Figures 4A, B).

Moreover, the research history of extracellular vesicles shows a progression from initially studying exosomes, to extracellular vesicles, and more recently, to small extracellular vesicles. Exosomes are named based on their biogenesis, originating from the endosomal system. When the subcellular origin of vesicles cannot be determined, the term "exosomes" is not recommended for naming extracellular vesicles. Subsequently, methods such as differential ultracentrifugation (dUC) have been employed to differentiate extracellular vesicle subtypes by size, with those smaller than 200 nm classified as small EVs (sEVs) (Crescitelli et al., 2021). Additionally, study has identified non-vesicular extracellular particles, which can co-isolate with extracellular vesicles, as multimolecular assemblies lacking a lipid bilayer, and their influence on extracellular vesicle studies is increasingly recognized. In summary, the evolving terminology of extracellular vesicles reflects the advancements in this field (Welsh et al., 2024). Nodes with purple outer rings (e.g., exosomes, articular cartilage, extracellular vesicles, mesenchymal stem cells) indicate keywords with high betweenness centrality, playing crucial bridging roles in this field. Additionally, by examining the newly emerged keywords in the past 2 years, we can identify the research frontiers (Figure 4C). Based on keywords emerging in the past 2 years, the research hotspots of EVs in the field of DMDs primarily focus on the

TABLE 7 The top 10 institutions ranked by publications with their NP and NC.

Rank	Institutions	NP	NC
1	Shanghai Jiao Tong University	71	2657
2	Central South University	37	1391
3	Sichuan University	34	572
4	Zhejiang University	34	1275
5	Southern Medical University	30	359
6	IRCCS Istituto Ortopedico Galeazzi	28	600
7	Peking University	26	592
8	Fudan University	25	954
9	Sun Yat Sen University	25	1135
10	Nanjing Medical University	24	1017

following aspects: First, the application of EVs in bone metabolism and bone joint diseases, especially in osteoarthritis, senile osteoporosis, as well as bone reconstruction and repair, has become a key area of research. Additionally, engineered exosomes have gained widespread attention for enhancing therapeutic effects, particularly in bone regeneration and joint repair. Studies have also delved into the characteristics and potential applications of different types and sources of EVs, such as exosomes, apoptotic vesicles, nanovesicles, and plant-derived vesicles, in bone joint diseases. In terms of signaling pathways, the role of the PI3K/Akt/mTOR pathway in bone metabolism mediated by EVs has been highlighted, while the mechanisms by which EVs regulate cellular responses through receptor interactions are also becoming clearer. Stem cell-derived EVs, especially those derived from adipose mesenchymal stem cells and mesenchymal stem cells, are considered an important research direction for tissue repair and regeneration. Furthermore, the potential of EVs in alleviating symptoms such as chronic pain, synovitis, and stress responses has also attracted attention. As these fields continue to advance, EVs are showing increasing promise for application in the treatment of DMDs.

In the co-citation analysis, the top 10 cited articles each have over 200 citations, reflecting the foundation of this research field and the main research directions over various periods, indicating high influence. With the most-cited being "MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity" by Zhang S et al., published in BIOMATERIALS in 2018, with 607 citations. The second and third most-cited articles are "Exosomes derived from miR-140-5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent OA of the knee in a rat model" (514 citations) and "Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration" (482 citations). In 2014, Kato T et al. revealed the role of EVs in the pathogenesis of OA (Kato et al., 2014). And then, Zhang S (2016), and Tao SC (2017) et al. proposed the limitations of MSC-based cell therapies for cartilage repair and discovered the potential of cell-free therapies, opening new avenues for the treatment of DMDs (Zhang et al., 2016; Tao et al., 2017), which might be the reason for the rapid increase in the annual publications in this research field since 2016. Subsequently, Zhang S (2018) et al. addressed the unresolved issues in these two articles and revealed the mechanism by which extracellular vesicles derived from mesenchymal stem cells mediate cartilage repair, including promoting chondrocyte proliferation, migration, and matrix formation, inhibiting chondrocyte apoptosis, and modulating immune reactivity, laying the foundation for subsequent research (Zhang et al., 2018). Due to the limitations of cell therapy such as tumorigenicity and biocompatibility (Tan et al., 2024), safer and more effective cell-free therapies are emerging. This article provides an important theoretical basis for subsequent research on cell-free therapies for the treatment of diseases such as OA. This might also be the reason for its high citation.

4.2 Frontiers and hotspots

Keywords can reflect the research hotspots and development trends in this field. We analyze keyword co-occurrence, clustering, keyword bursts, and the timezone view to understand the research hotspots and frontiers in this field.

4.2.1 The role of EVs in the treatment of DMDs

Currently, the clinical treatment of DMDs mainly relies on drug interventions to alleviate symptoms (LeBoff et al., 2022; Reid and Billington, 2022), making it difficult to achieve complete recovery. For severe cases, such as patients with advanced osteoarthritis (OA) where conservative treatments are ineffective or pain significantly impacts their quality of life, total joint replacement surgery may be necessary. However, this procedure often brings substantial physical pain and financial burden to patients (Abramoff and Caldera, 2020). With the continuous progress of related research, stem cell therapy has become a focal point in the field of regenerative medicine. Because of their rapid proliferation, strong differentiation potential, and ability to migrate to injury sites (Margiana et al., 2022), mesenchymal stem cells (MSCs) from both autologous and allogeneic sources have been extensively researched for treating various DMDs, demonstrating promising therapeutic outcomes (Harrell et al., 2019). However, there are also limitations and risks, such as heterogeneity of MSCs from different sources, immune rejection, and tumorigenicity, which restrict their clinical application (Musiał-Wysocka et al., 2019). Recent research has revealed that the function of MSCs in tissue regeneration is primarily driven by their paracrine effects. EVs in the secretome have attracted significant attention due to their benefits, including low immunogenicity, targeted delivery, and biocompatibility (Tang et al., 2021). Growing evidence suggests that cell-free therapy using EVs has emerged as a highly potential treatment approach for DMDs.

EVs can act as mediators of crosstalk between multiple organs. Extracellular vesicles secreted by other organs (such as muscle and liver) influence bone metabolism through circulation, thereby contributing to the treatment of degenerative diseases. The study by Ma S et al. showed that mu-EVs promote glycolysis in bone marrow mesenchymal stem cells (BMSCs) by delivering lactate dehydrogenase A to BMSCs, which in turn promotes the osteogenic differentiation of BMSCs. This offers a new approach for the treatment of OP and also reveals a new mechanism by which

skeletal muscle affects bone metabolism (Ma et al., 2023). Additionally, research by Lin L and colleagues has found an interaction between liver and bone metabolism. Liver-specific SIRT2 deficiency can ameliorate bone loss caused by OP. Extracellular vesicles from SIRT2 $^{(-/-)}$ hepatocytes, carrying LRG1, are transferred to bone marrow-derived macrophages (BMDM) via circulation and inhibit osteoclastogenesis by suppressing the activation of NF-κB p65, thereby serving a therapeutic role in OP (Lin et al., 2023).

Engineered exosomes target disease-related receptor cells to deliver therapeutic cargo, achieving precise treatment. Natural EVs have low drug loading capacity and lack the ability to be effectively delivered to target sites for clinical applications. Some engineering methods can modify the surface of EVs to deliver them to specific target tissues and further explore methods for loading therapeutic drugs into EVs, thereby achieving efficient drug delivery (Komuro et al., 2022). Zhang H et al. engineered a cartilage affinity peptide (CAP) on the EVs surface through lipid insertion, obtaining chondrocyte-targeting exosomes—CAP-Exo. Then, they loaded siRNA targeting MMP13 (siMMP13) into these exosomes, resulting in CAP-Exo/siMMP13. These exosomes delivered siRNA to chondrocytes, specifically silencing MMP13 in chondrocytes, thereby alleviating cartilage degradation caused by OA (Zhang et al., 2024). Similarly, Yan W et al. used an engineered method where sortase A was used to link EVs and cartilage affinity peptide (CAP), followed by incubation with cholesterol-modified ASO-MMP13 to construct chondrocyte-targeting drug delivery exosomes (CAP-exoASO), which also reduced cartilage damage caused by OA (Yan et al., 2024). Besides single-surface engineering of EVs, more efficient drug loading can also be achieved through combined engineering approaches. Liu W et al. utilized dual-engineered EVs by loading exogenous miR-223 into hUC-EVs via electroporation and engineering a collagen II-targeting peptide (WYRGRL) onto the surface of hUC-EVs, thereby enabling more specific and effective RNA delivery to chondrocytes, which provides a more effective treatment for OA (Liu W. et al., 2023). Moreover, advanced exosome preparation methods can greatly increase the success rate of clinical translation by improving the yield of exosomes used for disease treatment. Yang Z et al. invented a revolutionary exosome preparation method called cell nanoporation, which combines the steps of purification and drug loading. By using nanoporation, cells secrete exosomes loaded with target mRNA, significantly improving their yield, targeting, and applicability (Yang et al., 2020).

The therapeutic efficacy of EVs relies on their internalization by target cells and/or their close interaction with receptors on the target cell surface, both of which necessitate the prolonged presence of EVs at the target site. Therefore, although EVs have therapeutic potential, their effects are short-lived when used alone. EVs administered systemically have a short half-life and are rapidly eliminated from the body, making it challenging to attain sustained therapeutic effects with EVs treatment (Leung et al., 2022). Studies have shown that biomaterial-based delivery systems can extend the retention time of EVs at target sites. Additionally, local delivery based on biomaterials can reduce the dose of EVs required to achieve therapeutic effects, thereby reducing associated costs and potential side effects (Murali and Holmes, 2021). Therefore, the combination of EVs and biomaterial-based delivery modes is gaining increasing attention. Chen M et al. used the CRISPR/Cas9 method to create cartilage-affinity peptide (CAP)-conjugated exosomes containing FGF18-targeted gene editing tools (CAP/FGF18-hyEXO), efficiently stimulating the FGF18 gene in OA chondrocytes at the genetic level *in vivo*. In addition, CAP/FGF18-hyEXO is encapsulated in methacrylic anhydride-modified hyaluronic acid (HAMA) hydrogel microspheres via microfluidics and photopolymerization, forming an injectable microgel system with a renewable hydration layer (CAP/FGF18-hyEXO@HMs). This system provides continuous lubrication to counter friction and wear, demonstrating the ability to work synergistically to promote cartilage regeneration, reduce inflammation, and prevent ECM degradation, both *in vitro* and *in vivo* (Chen M. et al., 2024). Pang L, Wan J, and Li X utilized methacryloyl gelatin (GelMA) hydrogels to load engineered exosomes for therapeutic purposes, allowing for sustained release at the treatment site and addressing the challenges of rapid elimination and limited retention of exosomes. In the study by Pang L et al., GelMA hydrogels loaded with MSC-NVs (GelMA-NVs) regulated chondrogenesis and macrophage polarization through MSC-NVs, providing a therapeutic effect on OA (Pang et al., 2023). Wan J et al. used GelMA hydrogels to load engineered exosomes modified with targeting peptides, enhancing the targeting and retention capabilities of exosomes, thereby maximizing their therapeutic effects. In addition to enhancing the retention of exosomes, biomaterials can also be used to achieve controlled release strategies for exosomes, enabling more precise treatments (Wan et al., 2023; Li X. et al., 2024). Li S et al. proposed a hydrogel composite with a logic gate function, the KM13E@PGE system. It uses matrix metalloproteinase 13 (MMP13)-sensitive self-assembling peptide hydrogel (KM13E) and polyethylene glycol diacrylate/gelatin methacryloyl (PGE/GelMA) hydrogel microspheres to encapsulate interleukin-10 (IL-10) EVs, which promote M2 macrophage polarization, and SRY-box transcription factor 9 (SOX9) EVs, which enhance cartilage matrix synthesis. The release times of the two types of EVs are controlled to achieve an initial anti-inflammatory effect followed by cartilage repair (Li S. et al., 2024). This is an effect that cannot be achieved by a single extracellular vesicle. Developing biomaterials that address specific therapeutic needs is crucial for the clinical translation of natural and engineered exosomes and aligns with the trend of interdisciplinary research, which has seen increasing interest in recent years.

Different pretreatments and culture conditions can influence the secretion of exosomes by altering the microenvironment. Studies have shown that controlling stem cell growth conditions through medium pretreatment with physical properties (such as culture systems and cell density), oxygen tension, and soluble factors can directly impact paracrine activity by modulating the cargo and biogenesis of EVs (Mas-Bargues and Borrás, 2021), thus improving therapeutic effectiveness. Hu H et al. discovered that hypoxic pretreatment enhances the biological functions of mesenchymal stem cells in challenging microenvironments and stimulates the production of small EVs with beneficial therapeutic properties. The produced sEVs (HP-sEVs) can reduce the inflammatory microenvironment of intervertebral disc degeneration, stimulate the proliferation of nucleus pulposus (NP) cells, and enhance proteoglycan synthesis and collagen

production, providing a new therapeutic strategy for treating IVD degeneration (Hu et al., 2023). Interestingly, It has been shown that EVs from aging mesenchymal stromal cells have a significantly impaired protective effect against OA. They also induce catabolic metabolism and pro-inflammatory gene expression, demonstrating that cellular aging is a factor that affects the secretion of EVs, thereby altering their original biological functions (Jérémy et al., 2024).

Besides human-derived EVs, studies have shown that plants can also secrete exosome-like nanovesicles containing miRNA, bioactive lipids, mRNA, and proteins, which can exert regenerative activity and have therapeutic effects on diseases such as OP. Hwang JH et al. showed that exosome-like nanovesicles from yam (YNV) can activate the BMP-2/p-p38-dependent Runx2 pathway to stimulate osteogenesis, providing a therapeutic effect on OP (Hwang et al., 2023). Zhan W et al. found that puerarin-derived exosome-like nanovesicles (PELNs) promote autophagy by degrading the gut microbiota metabolite trimethylamine-N-oxide (TMAO), which in turn promotes the differentiation and mineralization of hBMSCs, showing potential as a treatment for OP (Zhan et al., 2023).

4.2.2 EVs as biomarkers for the diagnosis of DMDs

Although new treatment methods for various DMDs have emerged in recent years, clinical translation still requires some time, and safety and efficacy need further verification through more clinical trials. Currently, conservative treatment remains the primary clinical approach, making timely early diagnosis and intervention particularly important. As early as 2004, EVs demonstrated great potential as disease detection tools. In the following years, the research interest in EVs as biomarkers for various diseases (Das et al., 2024) [such as cancer (Kumar M. A. et al., 2024; Yu et al., 2022; Lee et al., 2023), neurological disease (Chatterjee et al., 2024; Ricklefs et al., 2024; Kumar A. et al., 2024), metabolic diseases (Barreiro et al., 2020; Camino et al., 2022), etc.] has continuously increased. In 2016, ExoDx™ Lung (ALK) became the first biomarker to successfully complete clinical trials, marking a milestone for EVs as disease diagnostic biomarkers by diagnosing lung cancer through the detection of EMLA-ALK mutations (Sheridan, 2016). In the field of DMDs, there is substantial research on EVs as biomarkers for diagnosing OA. This research primarily involves early diagnosis of OA, identification of inflammation types, susceptibility assessment, and differential diagnosis of various types of joint diseases, enabling precise and personalized treatment of OA (Liu Z. et al., 2023).

Furthermore, existing studies have indicated that miRNAs from EVs can serve as potential biomarkers for early diagnosis of OP and prediction of fragility fracture risk (Huber et al., 2023; Pepe et al., 2022). Currently, there is a shortage of studies focusing on other degenerative diseases such as sarcopenia. While the prospect of EVs as disease diagnostic biomarkers is exciting, their limitations in specificity, reproducibility, and lack of single/unified molecules related to early disease processes, along with a lack of clinical studies in this field, require further research to be addressed.

4.2.3 The role of EVs in the mechanisms of DMDs development

As an essential medium for intercellular communication, EVs can transport therapeutic contents, and EVs derived from pathogenic cells can mediate disease occurrence by transporting pathogenic contents. Aging is a major cause of degenerative diseases, so EVs derived from various aging cells or those carrying aging-regulating factors may be involved in the disease development process. The reduced expression of miR-494-3p in exosomes from aging osteocytes suppresses osteogenic differentiation and promotes age-related bone loss via the PTEN/PI3K/AKT pathway (Yao et al., 2024). There have been previous reports that infrapatellar fat pad (IPFP) is closely related to the occurrence and progression of knee OA. Cao Y et al. found that small EVs (sEVs) secreted by the IPFP of OA patients can be delivered to articular chondrocytes. The high levels of let-7b-5p and let-7c-5p within these sEVs can aggravate the progression of OA by directly reducing the expression of the senescence-negative regulator Lamin B receptor (LBR) (Cao et al., 2024). M1 macrophages can impair joint homeostasis and lead to OA progression by producing nitric oxide and pro-inflammatory cytokines. Recently, studies have first demonstrated that EVs secreted by inflammatory macrophages induce atypical pyroptosis of chondrocytes, leading to cartilage catabolism and OA development (Ebata et al., 2023). Additionally, Liu B et al. found that the internalization of exosomes derived from inflammatory fibroblast-like synoviocytes (inf-exo) enhances M1 polarization of macrophages by triggering glycolysis activation, further inducing OA-like phenotypes in co-cultured chondrocytes. *In vivo* experiments also confirmed that inf-exo induces synovitis and exacerbates OA progression (Liu et al., 2024). In-depth research into the mechanisms by which EVs mediate disease occurrence can provide new targets and strategies for disease treatment. For example, limiting the uptake of EVs by receptor cells can inhibit their pathogenic effects. The latest research by Lin S et al. found that exosome-like nanovesicles derived from osteosarcoma, even after content removal, still possess targeting ability. By competitively inhibiting the uptake of normal osteosarcoma-derived exosomes by lung fibroblasts, they can inhibit lung fibroblast activation, pre-metastatic niche formation, and osteosarcoma lung metastasis (Lin et al., 2024). This research can also provide a reference for treating DMDs.

4.3 Limitations and future perspectives

EVs are gaining increasing attention in the field of DMDs research due to their roles in treatment, diagnosis, and elucidation of disease mechanisms. Currently, research in this field mainly focuses on the role of EVs in the treatment of DMDs and on using EVs to investigate new disease mechanisms, providing new therapeutic targets, essentially serving disease treatment. There are currently several limitations to using EVs for the treatment of DMDs: 1) The efficiency of EVs isolation and purification is not high enough, making it difficult to achieve satisfactory purity while maintaining high isolation efficiency. There is a lack of standardized strategies for isolation, purification, and storage, leading to poor reproducibility in experiments. 2) Current understanding of EVs biogenesis, internalization, and cargo transport processes is very limited. 3) Although engineering EVs can improve therapeutic effects and ability to target bone tissue, these strategies bring unpredictable immune responses and elevated production costs. Modifying the surface of EVs may cause the immune system to recognize them as foreign objects, triggering

host immune responses, leading to clearance or reduced efficacy. These modifications may also induce toxicity or adverse reactions, posing potential risks to the body. 4) A more specific understanding of the mechanisms by which EVs mediate disease occurrence is needed to enable precise intervention by inhibiting the formation, release, or uptake of relevant EVs. 5) There is limited understanding of the mechanisms behind the heterogeneity of EVs, the disruption caused by non-vesicular extracellular nanoparticles on EVs research, and the functional discrepancies among different EVs subgroups. Identifying which EVs subgroups are functionally active or contain specific therapeutic cargoes is essential for enhancing their effectiveness and increasing repeatability. Most of these problems are common to EVs in the field of regenerative medicine, and specific to DMDs, for example, most of the studies often neglect the effects of different pathological changes at different times of OA on the localization and biodistribution of EVs, the distribution of EVs used for treatment not in cartilage are unclear. The dosage level of EVs for OA treatment is unclear. The different methods of OA model construction in preclinical studies have different effects on EVs. And the therapeutic effects proposed in preclinical studies lack the clinical trials to test. These can affect the final therapeutic outcome and the efficiency of clinical translation. Moreover, most of the current attention to EVs for the treatment of OA is focused on the articular cartilage (Karoichan et al., 2024). But OA is a multifactorial disease, the other causative factors and Pain during disease progression should be considered at the same time. In future studies, simultaneous intervention of all aspects through various modification methods and biomaterials should be attempted, which may lead to better therapeutic outcomes. Other DMDs have the similar problems. The great potential of EVs for the early diagnosis of diseases has been widely recognized. Because of the absence of effective therapeutic tools currently, early diagnosis of degenerative musculoskeletal diseases is particularly important. However, there are relatively few studies in this field, which is an important research direction in the future.

In future research within this field, it is essential to avoid homogenization and focus on addressing the most pressing issues and challenges. To overcome the current limitations of EVs in DMDs treatment, future research should focus on several key areas: 1) Efforts should be made to develop standardized protocols for the isolation, purification, and storage of EVs. This will ensure high purity, reproducibility, and efficiency in experiments. Advanced techniques, such as optimized ultracentrifugation or immunoaffinity-based methods, should be explored for scalable and reliable EV isolation. 2) Future studies should prioritize investigating the biogenesis of EVs, their internalization by target cells, and the mechanisms of cargo loading and transport. This will help to unveil the fundamental processes that dictate EVs' function and potential therapeutic roles. 3) Engineering EVs holds great promise for enhancing therapeutic activity, but the associated immune responses and high production costs need to be addressed. Future research should focus on optimizing surface modifications to reduce immune recognition, while simultaneously developing more cost-effective production methods such as large-scale cell culture systems. 4) There is a need for more precise interventions targeting the specific mechanisms by which EVs mediate disease onset and progression. This could involve developing strategies to regulate EV formation, release, and uptake, enabling targeted therapies that are more effective in treating DMDs. 5) The functional discrepancies among different EV subgroups must be further explored to identify which subsets are functionally active or carry therapeutic cargo. Advanced techniques such as single-particle tracking and high-throughput sequencing could help identify the specific EV populations most relevant for therapeutic applications. 6) The previous points are common issues in EVs-related research. Specifically, in DMDs, there is a need for a more detailed understanding of how different pathological conditions (such as OA) influence the localization, biodistribution, and therapeutic effects of EVs. Research should address the effects of different OA stages on EV distribution and investigate the optimal dosage levels for EV-based treatments. 7) The transition from preclinical studies to clinical trials is crucial for the success of EV-based therapies. Future research should bridge this gap by incorporating more clinical trial data, and integrating disciplines such as biomaterials science, tissue engineering, and molecular medicine to enhance the clinical applicability of EVs in DMDs treatment. 8) Lastly, enhancing international collaboration and interdisciplinary research will accelerate the development of EV-based therapies. By leveraging expertise across various fields, including regenerative medicine, nanotechnology, and bioinformatics, the clinical translation of EV-based therapies can be achieved more efficiently, ultimately benefiting patients worldwide. These strategies aim to tackle the key challenges in EV research, improving their therapeutic potential and accelerating their clinical application in DMDs.

Although bibliometric analysis offers unique and valuable insights, it is not without limitations. First, the selection of databases, article types, and language restrictions can introduce biases, potentially affecting the comprehensiveness of the results. Second, visualization tools such as CiteSpace and VOSviewer may extract data differently from the original WoS database, resulting in potential discrepancies. Furthermore, differences in their underlying algorithms can lead to variations in analysis outcomes, causing inconsistencies in the visualization and interpretation of bibliometric data. Third, while bibliometric analysis is highly effective for quantitative evaluation, it is less adept at addressing qualitative aspects, such as identifying research hotspots and emerging frontiers, which often require complementary secondary analyses. To ensure clarity in the visualization results, the number of authors and institutions was reduced by raising the threshold in the analysis. However, this approach may have a potential drawback, as it could overlook some authors and institutions with innovative research emerging in the past 2 years. Fourth, to ensure the clarity of the visualization results, the number of authors and institutions was reduced by raising the threshold in the analysis. However, this approach has a potential drawback, as it may overlook some authors and institutions with innovative research emerging in the past 2 years. Finally, bibliometric analysis relies on static snapshots of data, limiting its ability to accurately predict future research trends.

5 Conclusion

EVs have shown great research prospects and popularity in the field of DMDs. Since 2016, the number of publications in this field has surged, with a trend expected to continue, indicating growing interest and ongoing increase in research activity. China has the

highest publication output, but less frequent collaboration with other countries compared to the United States, indicating high interest in this field within China and a need to strengthen cooperation with other countries. In addition, keyword and citation analysis reveals that biomaterial-based EVs and engineered EVs as drug delivery vehicles for treating DMDs are currently the main focus areas. However, many unresolved issues and challenges still hinder the clinical translation of basic research, which remains a major goal for future studies. Overall, this is a young but promising field of research.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

J-JY: Conceptualization, Data curation, Formal Analysis, Software, Validation, Visualization, Writing–original draft. S-QH: Conceptualization, Writing–original draft. BH: Conceptualization, Writing–original draft. P-XW: Conceptualization, Writing–original draft. FX: Conceptualization, Supervision, Writing–review and editing. XL: Conceptualization, Funding acquisition, Project administration, Writing–review and editing. JL: Conceptualization, Funding acquisition, Project administration, Writing–review and editing.

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Conflict of interest

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

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The author(s) declare that no Generative AI was used in the creation of this manuscript.

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Supplementary material

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References

Abramoff, B., and Caldera, F. E. (2020). Osteoarthritis: pathology, diagnosis, and treatment options. *Med. Clin. North Am.* 104 (2), 293–311. doi:10.1016/j.mcna.2019.10.007

Aria, M., and Cuccurullo, C. (2017). *bibliometrix*: an R-tool for comprehensive science mapping analysis. *J. Inf.* 11 (4), 959–975. doi:10.1016/j.joi.2017.08.007

Barreiro, K., Dwivedi, O. P., Leparc, G., Rolser, M., Delic, D., Forsblom, C., et al. (2020). Comparison of urinary extracellular vesicle isolation methods for transcriptomic biomarker research in diabetic kidney disease. *J. Extracell. Vesicles* 10 (2), e12038. doi:10.1002/jev2.12038

Camino, T., Lago-Baameiro, N., Bravo, S. B., Molares-Vila, A., Sueiro, A., Couto, I., et al. (2022). Human obese white adipose tissue sheds depot-specific extracellular vesicles and reveals candidate biomarkers for monitoring obesity and its comorbidities. *Transl. Res.* 239, 85–102. doi:10.1016/j.trsl.2021.01.006

Cao, Y., Ruan, J., Kang, J., Nie, X., Lan, W., Ruan, G., et al. (2024). Extracellular vesicles in infrapatellar fat pad from osteoarthritis patients impair cartilage metabolism and induce senescence. *Adv. Sci. (Weinh)* 11 (3), e2303614. doi:10.1002/advs.202303614

Chatterjee, M., Özdemir, S., Fritz, C., Möbius, W., Kleineidam, L., Mandelkow, E., et al. (2024). Plasma extracellular vesicle tau and TDP-43 as diagnostic biomarkers in FTD and ALS. *Nat. Med.* 30 (6), 1771–1783. doi:10.1038/s41591-024-02937-4

Chen, C. M. (2004). Searching for intellectual turning points: progressive knowledge domain visualization. *Proc. Natl. Acad. Sci. U. S. A.* 101, 5303–5310. doi:10.1073/pnas.0307513100

Chen, C. M. (2006). CiteSpace II: detecting and visualizing emerging trends and transient patterns in scientific literature. *J. Am. Soc. Inf. Sci. Technol.* 57 (3), 359–377. doi:10.1002/asi.20317

Chen, C. M., Ibekwe-SanJuan, F., and Hou, J. H. (2010). The structure and dynamics of cocitation clusters: a multiple-perspective cocitation analysis. *J. Am. Soc. Inf. Sci. Technol.* 61 (7), 1386–1409. doi:10.1002/asi.21309

Chen, L.-K., Arai, H., Assantachai, P., Akishita, M., Chew, S. T. H., Dumlao, L. C., et al. (2022). Roles of nutrition in muscle health of community-dwelling older adults: evidence-based expert consensus from Asian Working Group for Sarcopenia. *J. Cachexia Sarcopenia Muscle* 13 (3), 1653–1672. doi:10.1002/jcsm.12981

Chen, M., Lu, Y., Liu, Y., Liu, Q., Deng, S., Liu, Y., et al. (2024b). Injectable microgels with hybrid exosomes of chondrocyte-targeted FGF18 gene-editing and self-renewable lubrication for osteoarthritis therapy. *Adv. Mater* 36 (16), e2312559. doi:10.1002/adma.202312559

Chen, Z., Xiong, M., Tian, J., Song, D., Duan, S., and Zhang, L. (2024a). Encapsulation and assessment of therapeutic cargo in engineered exosomes: a systematic review. *J. Nanobiotechnology* 22 (1), 18. doi:10.1186/s12951-023-02259-6

Ciardiello, C., Cavallini, L., Spinelli, C., Yang, J., Reis-Sobreiro, M., de Candia, P., et al. (2016). Focus on extracellular vesicles: new frontiers of cell-to-cell communication in cancer. *Int. J. Mol. Sci.* 17 (2), 175. doi:10.3390/ijms17020175

Clynes, M. A., Harvey, N. C., Curtis, E. M., Fuggle, N. R., Dennison, E. M., and Cooper, C. (2020). The epidemiology of osteoporosis. *Br. Med. Bull.* 133 (1), 105–117. doi:10.1093/bmb/ldaa005

Coletta, G., and Phillips, S. M. (2023). An elusive consensus definition of sarcopenia impedes research and clinical treatment: a narrative review. *Ageing Res. Rev.* 86, 101883. doi:10.1016/j.arr.2023.101883

Compston, J. E., McClung, M. R., and Leslie, W. D. (2019). Osteoporosis. *Lancet.* 393 (10169), 364–376. doi:10.1016/S0140-6736(18)32112-3

Crescitelli, R., Lässer, C., and Lötvall, J. (2021). Isolation and characterization of extracellular vesicle subpopulations from tissues. *Nat. Protoc.* 16 (3), 1548–1580. doi:10.1038/s41596-020-00466-1

Cruz-Jentoft, A. J., and Sayer, A. A. (2019). Sarcopenia. *Lancet.* 393 (10191), 2636–2646. doi:10.1016/S0140-6736(19)31138-9

Das, S., Lyon, C. J., and Hu, T. (2024). A panorama of extracellular vesicle applications: from biomarker detection to therapeutics. *ACS Nano* 18 (14), 9784–9797. doi:10.1021/acsnano.4c00666

Dent, E., Morley, J. E., Cruz-Jentoft, A. J., Arai, H., Kritchevsky, S. B., Guralnik, J., et al. (2018). International clinical practice guidelines for sarcopenia (ICFSR): screening, diagnosis and management. *J. Nutr. Health Aging* 22 (10), 1148–1161. doi:10.1007/s12603-018-1139-9

Ebata, T., Terkawi, M. A., Kitahara, K., Yokota, S., Shiota, J., Nishida, Y., et al. (2023). Noncanonical pyroptosis triggered by macrophage-derived extracellular vesicles in chondrocytes leading to cartilage catabolism in osteoarthritis. *Arthritis Rheumatol.* 75 (8), 1358–1369. doi:10.1002/art.42505

Ellegaard, O. (2018). The application of bibliometric analysis: disciplinary and user aspects. *SCIENTOMETRICS* 116 (1), 181–202. doi:10.1007/s11192-018-2765-z

Elsharkasy, O. M., Nordin, J. Z., Hagey, D. W., de Jong, O. G., Schiffelers, R. M., Andaloussi, S. E., et al. (2020). Extracellular vesicles as drug delivery systems: why and how? *Adv. Drug Deliv. Rev.* 159, 332–343. doi:10.1016/j.addr.2020.04.004

Fang, A., Wang, Y., Guan, N., Zuo, Y., Lin, L., Guo, B., et al. (2023). Porous microneedle patch with sustained delivery of extracellular vesicles mitigates severe spinal cord injury. *Nat. Commun.* 14 (1), 4011. doi:10.1038/s41467-023-39745-2

Harrell, C. R., Markovic, B. S., Fellabaum, C., Arsenijevic, A., and Volarevic, V. (2019). Mesenchymal stem cell-based therapy of osteoarthritis: current knowledge and future perspectives. *Biomed. and Pharmacother.* 109, 2318–2326. doi:10.1016/j.biopha.2018.11.099

Hu, H., Wang, Z., Yang, H., Bai, Y., Zhu, R., and Cheng, L. (2023). Hypoxic preconditional engineering small extracellular vesicles promoted intervertebral disc regeneration by activating mir-7-5p/NF-Kb/Cxcl2 Axis. *Adv. Sci. (Weinh)* 10 (35), e2304722. doi:10.1002/advs.202304722

Hu, Y., Li, X., Zhang, Q., Gu, Z., Luo, Y., Guo, J., et al. (2021). Exosome-guided bone targeted delivery of Antagomir-188 as an anabolic therapy for bone loss. *Bioact. Mater* 6 (9), 2905–2913. doi:10.1016/j.bioactmat.2021.02.014

Huber, J., Longaker, M. T., and Quarto, N. (2023). Circulating and extracellular vesicle-derived microRNAs as biomarkers in bone-related diseases. *Front. Endocrinol. (Lausanne)* 14, 1168898. doi:10.3389/fendo.2023.1168898

Hunter, D. J., and Bierma-Zeinstra, S. (2019). Osteoarthritis. *Lancet* 393 (10182), 1745–1759. doi:10.1016/S0140-6736(19)30417-9

Hwang, J.-H., Park, Y.-S., Kim, H.-S., Kim, D.-H., Lee, S.-H., Lee, C.-H., et al. (2023). Yam-derived exosome-like nanovesicles stimulate osteoblast formation and prevent osteoporosis in mice. *J. Control Release* 355, 184–198. doi:10.1016/j.jconrel.2023.01.071

Izquierdo-Altarejos, P., Moreno-Manzano, V., and Felipo, V. (2024). Pathological and therapeutic effects of extracellular vesicles in neurological and neurodegenerative diseases. *Neural Regen. Res.* 19 (1), 55–61. doi:10.4103/1673-5374.375301

Jeppesen, D. K., Zhang, Q., Franklin, J. L., and Coffey, R. J. (2023). Extracellular vesicles and nanoparticles: emerging complexities. *Trends Cell Biol.* 33 (8), 667–681. doi:10.1016/j.tcb.2023.01.002

Jérémy, B., Marie, M., Giuliana, B. M., Karine, T., Christian, J., and Daniéle, N. (2024). Extracellular vesicles from senescent mesenchymal stromal cells are defective and cannot prevent osteoarthritis. *J. Nanobiotechnology* 22 (1), 255. doi:10.1186/s12951-024-02509-1

Jiang, Y., Li, J., Xue, X., Yin, Z., Xu, K., and Su, J. (2022). Engineered extracellular vesicles for bone therapy. *Nano Today* 44, 101487. doi:10.1016/j.nantod.2022.101487

Kalluri, R. (2016). The biology and function of exosomes in cancer. *J. Clin. Invest.* 126 (4), 1208–1215. doi:10.1172/JCI81135

Karoichan, A., Boucenna, S., and Tabrizian, M. (2024). Therapeutics of the future: navigating the pitfalls of extracellular vesicles research from an osteoarthritis perspective. *J. Extracell. Vesicles* 13 (7), e12435. doi:10.1002/jev2.12435

Kato, T., Miyaki, S., Ishitobi, H., Nakamura, Y., Nakasa, T., Lotz, M. K., et al. (2014). Exosomes from IL-1β stimulated synovial fibroblasts induce osteoarthritic changes in articular chondrocytes. *Arthritis Res. Ther.* 16 (4), R163. doi:10.1186/ar4679

Kirk, B., Zanker, J., and Duque, G. (2020). Osteosarcopenia: epidemiology, diagnosis, and treatment-facts and numbers. *J. Cachexia Sarcopenia Muscle* 11 (3), 609–618. doi:10.1002/jcsm.12567

Komuro, H., Aminova, S., Lauro, K., and Harada, M. (2022). Advances of engineered extracellular vesicles-based therapeutics strategy. *Sci. Technol. Adv. Mater* 23 (1), 655–681. doi:10.1080/14686996.2022.2133342

Kou, M., Huang, L., Yang, J., Chiang, Z., Chen, S., Liu, J., et al. (2022). Mesenchymal stem cell-derived extracellular vesicles for immunomodulation and regeneration: a next generation therapeutic tool? *Cell Death Dis.* 13 (7), 580. doi:10.1038/s41419-022-05034-x

Kumar, A., Nader, M. A., and Deep, G. (2024b). Emergence of extracellular vesicles as "liquid biopsy" for neurological disorders: boom or bust. *Pharmacol. Rev.* 76 (2), 199–227. doi:10.1124/pharmrev.122.000788

Kumar, M. A., Baba, S. K., Sadida, H. Q., Marzooqi, S. A., Jerobin, J., Altemani, F. H., et al. (2024a). Extracellular vesicles as tools and targets in therapy for diseases. *Signal Transduct. Target Ther.* 9 (1), 27. doi:10.1038/s41392-024-01735-1

LeBoff, M. S., Greenspan, S. L., Insogna, K. L., Lewiecki, E. M., Saag, K. G., Singer, A. J., et al. (2022). The clinician's guide to prevention and treatment of osteoporosis. *Osteoporos. Int.* 33 (10), 2049–2102. doi:10.1007/s00198-021-05900-y

Lee, Y., Ni, J., Beretov, J., Wasinger, V. C., Graham, P., and Li, Y. (2023). Recent advances of small extracellular vesicle biomarkers in breast cancer diagnosis and prognosis. *Mol. Cancer* 22 (1), 33. doi:10.1186/s12943-023-01741-x

Leung, K. S., Shirazi, S., Cooper, L. F., and Ravindran, S. (2022). Biomaterials and extracellular vesicle delivery: current status, applications and challenges. *Cells* 11 (18), 2851. doi:10.3390/cells11182851

Li, S., Zheng, W., Deng, W., Li, Z., Yang, J., Zhang, H., et al. (2024b). Logic-based strategy for spatiotemporal release of dual extracellular vesicles in osteoarthritis treatment. *Adv. Sci. (Weinh)* 11 (26), e2403227. doi:10.1002/advs.202403227

Li, X., Fang, S., Wang, S., Xie, Y., Xia, Y., Wang, P., et al. (2024a). Hypoxia preconditioning of adipose stem cell-derived exosomes loaded in gelatin methacryloyl (GelMA) promote type H angiogenesis and osteoporotic fracture repair. *J. Nanobiotechnology* 22 (1), 112. doi:10.1186/s12951-024-02342-6

Liao, Z., Ke, W., Liu, H., Tong, B., Wang, K., Feng, X., et al. (2022). Vasorin-containing small extracellular vesicles retard intervertebral disc degeneration utilizing an injectable thermoresponsive delivery system. *J. Nanobiotechnology* 20 (1), 420. doi:10.1186/s12951-022-01624-1

Lin, L., Guo, Z., He, E., Long, X., Wang, D., Zhang, Y., et al. (2023). SIRT2 regulates extracellular vesicle-mediated liver-bone communication. *Nat. Metab.* 5 (5), 821–841. doi:10.1038/s42255-023-00803-0

Lin, S., Shu, L., Guo, Y., Yuan, J., Zhang, J., Wang, Y., et al. (2024). Cargo-eliminated osteosarcoma-derived small extracellular vesicles mediating competitive cellular uptake for inhibiting pulmonary metastasis of osteosarcoma. *J. Nanobiotechnology* 22 (1), 360. doi:10.1186/s12951-024-02636-9

Liu, B., Xian, Y., Chen, X., Shi, Y., Dong, J., Yang, L., et al. (2024). Inflammatory fibroblast-like synoviocyte-derived exosomes aggravate osteoarthritis via enhancing macrophage glycolysis. *Adv. Sci. (Weinh)* 11 (14), e2307338. doi:10.1002/advs.202307338

Liu, H., Li, M., Zhang, T., Liu, X., Zhang, H., Geng, Z., et al. (2022b). Engineered bacterial extracellular vesicles for osteoporosis therapy. *Chem. Eng. J.* 450, 138309. doi:10.1016/j.cej.2022.138309

Liu, H., Zhang, Q., Wang, S., Weng, W., Jing, Y., and Su, J. (2022a). Bacterial extracellular vesicles as bioactive nanocarriers for drug delivery: advances and perspectives. *Bioact. Mater* 14, 169–181. doi:10.1016/j.bioactmat.2021.12.006

Liu, W., Liu, A., Li, X., Sun, Z., Sun, Z., Liu, Y., et al. (2023a). Dual-engineered cartilage-targeting extracellular vesicles derived from mesenchymal stem cells enhance osteoarthritis treatment via miR-223/NLRP3/pyroptosis axis: toward a precision therapy. *Bioact. Mater* 30, 169–183. doi:10.1016/j.bioactmat.2023.06.012

Liu, Z., Zhuang, Y., Fang, L., Yuan, C., Wang, X., and Lin, K. (2023b). Breakthrough of extracellular vesicles in pathogenesis, diagnosis and treatment of osteoarthritis. *Bioact. Mater* 22, 423–452. doi:10.1016/j.bioactmat.2022.10.012

Ma, S., Xing, X., Huang, H., Gao, X., Xu, X., Yang, J., et al. (2023). Skeletal muscle-derived extracellular vesicles transport glycolytic enzymes to mediate muscle-to-bone crosstalk. *Cell Metab.* 35 (11), 2028–2043.e7. doi:10.1016/j.cmet.2023.10.013

Margiana, R., Markov, A., Zekiy, A. O., Hamza, M. U., Al-Dabbagh, K. A., Al-Zubaidi, S. H., et al. (2022). Clinical application of mesenchymal stem cell in regenerative medicine: a narrative review. *Stem Cell Res. Ther.* 13 (1), 366. doi:10.1186/s13287-022-03054-0

Mas-Bargues, C., and Borrás, C. (2021). Importance of stem cell culture conditions for their derived extracellular vesicles therapeutic effect. *Free Radic. Biol. Med.* 168, 16–24. doi:10.1016/j.freeradbiomed.2021.03.028

Murali, V. P., and Holmes, C. A. (2021). Biomaterial-based extracellular vesicle delivery for therapeutic applications. *Acta Biomater.* 124, 88–107. doi:10.1016/j.actbio.2021.01.010

Musiał-Wysocka, A., Kot, M., and Majka, M. (2019). The pros and cons of mesenchymal stem cell-based therapies. *Cell Transpl.* 28 (7), 801–812. doi:10.1177/0963689719837897

Ninkov, A., Frank, J. R., and Maggio, L. A. (2022). Bibliometrics: methods for studying academic publishing. *Perspect. Med. Educ.* 11 (3), 173–176. doi:10.1007/s40037-021-00695-4

Öztürk, O., Kocaman, R., and Kanbach, D. K. (2024). How to design bibliometric research: an overview and a framework proposal. *Rev. Manag. Sci.* 18, 3333–3361. doi:10.1007/s11846-024-00738-0

Pang, L., Jin, H., Lu, Z., Xie, F., Shen, H., Li, X., et al. (2023). Treatment with mesenchymal stem cell-derived nanovesicle-containing gelatin methacryloyl hydrogels alleviates osteoarthritis by modulating chondrogenesis and macrophage polarization. *Adv. Healthc. Mater* 12 (17), e2300315. doi:10.1002/adhm.202300315

Pepe, J., Rossi, M., Battafarano, G., Vernocchi, P., Conte, F., Marzano, V., et al. (2022). Characterization of extracellular vesicles in osteoporotic patients compared to osteopenic and healthy controls. *J. Bone Mineral Res.* 37 (11), 2186–2200. doi:10.1002/jbmr.4688

Ragni, E., Perucca Orfei, C., De Luca, P., Colombini, A., Viganò, M., and de Girolamo, L. (2020). Secreted factors and EV-miRNAs orchestrate the healing capacity of adipose mesenchymal stem cells for the treatment of knee osteoarthritis. *Int. J. Mol. Sci.* 21 (5), 1582. doi:10.3390/ijms21051582

Ragni, E., Perucca Orfei, C., De Luca, P., Lugano, G., Viganò, M., Colombini, A., et al. (2019). Interaction with hyaluronan matrix and miRNA cargo as contributors for *in vitro* potential of mesenchymal stem cell-derived extracellular vesicles in a model of human osteoarthritic synoviocytes. *Stem Cell Res. Ther.* 10 (1), 109. doi:10.1186/s13287-019-1215-z

Reid, I. R., and Billington, E. O. (2022). Drug therapy for osteoporosis in older adults. *Lancet.* 399 (10329), 1080–1092. doi:10.1016/S0140-6736(21)02646-5

Ricklefs, F. L., Wollmann, K., Salviano-Silva, A., Drexler, R., Maire, C. L., Kaul, M. G., et al. (2024). Circulating extracellular vesicles as biomarker for diagnosis, prognosis, and monitoring in glioblastoma patients. *Neuro Oncol.* 26 (7), 1280–1291. doi:10.1093/neuonc/noae068

Scheuing, W. J., Reginato, A. M., Deeb, M., and Acer Kasman, S. (2023). The burden of osteoarthritis: is it a rising problem? *Best. Pract. Res. Clin. Rheumatol.* 37 (2), 101836. doi:10.1016/j.berh.2023.101836

Sheridan, C. (2016). Exosome cancer diagnostic reaches market. *Nat. Biotechnol.* 34 (4), 359–360. doi:10.1038/nbt0416-359

Song, S., Guo, Y., Yang, Y., and Fu, D. (2022). Advances in pathogenesis and therapeutic strategies for osteoporosis. *Pharmacol. Ther.* 237, 108168. doi:10.1016/j.pharmthera.2022.108168

Tan, F., Li, X., Wang, Z., Li, J., Shahzad, K., and Zheng, J. (2024). Clinical applications of stem cell-derived exosomes. *Signal Transduct. Target Ther.* 9 (1), 17. doi:10.1038/s41392-023-01704-0

Tang, D. D., Chen, M. J., Huang, X. H., Zhang, G. C., Zeng, L., Zhang, G. S., et al. (2023). SRplot: a free online platform for data visualization and graphing. *Plos One* 18 (11), e0294236. doi:10.1371/journal.pone.0294236

Tang, Y., Zhou, Y., and Li, H.-J. (2021). Advances in mesenchymal stem cell exosomes: a review. *Stem Cell Res. Ther.* 12 (1), 71. doi:10.1186/s13287-021-02138-7

Tao, S.-C., Yuan, T., Zhang, Y.-L., Yin, W.-J., Guo, S.-C., and Zhang, C.-Q. (2017). Exosomes derived from miR-140-5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model. *Theranostics* 7 (1), 180–195. doi:10.7150/thno.17133

Vader, P., Mol, E. A., Pasterkamp, G., and Schiffelers, R. M. (2016). Extracellular vesicles for drug delivery. *Adv. Drug Deliv. Rev.* 106, 148–156. doi:10.1016/j.addr.2016.02.006

Wan, J., He, Z., Peng, R., Wu, X., Zhu, Z., Cui, J., et al. (2023). Injectable photocrosslinking spherical hydrogel-encapsulated targeting peptide-modified engineered exosomes for osteoarthritis therapy. *J. Nanobiotechnology* 21 (1), 284. doi:10.1186/s12951-023-02050-7

Wang, X., Tian, L., Lu, J., and Ng, I. O.-L. (2022). Exosomes and cancer - diagnostic and prognostic biomarkers and therapeutic vehicle. *Oncogenesis* 11 (1), 54. doi:10.1038/s41389-022-00431-5

Welsh, J. A., Goberdhan, D. C. I., O'Driscoll, L., Buzas, E. I., Blenkiron, C., Bussolati, B., et al. (2024). Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J. Extracell. Vesicles* 13 (2), e12404. doi:10.1002/jev2.12404

Wen, Z.-Q., Lin, J., Xie, W.-Q., Shan, Y.-H., Zhen, G.-H., and Li, Y.-S. (2023). Insights into the underlying pathogenesis and therapeutic potential of endoplasmic reticulum stress in degenerative musculoskeletal diseases. *Mil. Med. Res.* 10 (1), 54. doi:10.1186/s40779-023-00485-5

Weng, Z., Zhang, B., Wu, C., Yu, F., Han, B., Li, B., et al. (2021). Therapeutic roles of mesenchymal stem cell-derived extracellular vesicles in cancer. *J. Hematol. Oncol.* 14 (1), 136. doi:10.1186/s13045-021-01141-y

Xiang, H., Zhao, W., Jiang, K., He, J., Chen, L., Cui, W., et al. (2024). Progress in regulating inflammatory biomaterials for intervertebral disc regeneration. *Bioact. Mater* 33, 506–531. doi:10.1016/j.bioactmat.2023.11.021

Yan, W., Li, Y., Xie, S., Tao, W. A., Hu, J., Liu, H., et al. (2024). Chondrocyte-targeted delivery system of sortase A-engineered extracellular vesicles silencing MMP13 for osteoarthritis therapy. *Adv. Healthc. Mater* 13 (16), e2303510. doi:10.1002/adhm.202303510

Yang, C., Xue, Y., Duan, Y., Mao, C., and Wan, M. (2024). Extracellular vesicles and their engineering strategies, delivery systems, and biomedical applications. *J. Control Release* 365, 1089–1123. doi:10.1016/j.jconrel.2023.11.057

Yang, Z., Shi, J., Xie, J., Wang, Y., Sun, J., Liu, T., et al. (2020). Large-scale generation of functional mRNA-encapsulating exosomes via cellular nanoporation. *Nat. Biomed. Eng.* 4 (1), 69–83. doi:10.1038/s41551-019-0485-1

Yao, C., Sun, J., Luo, W., Chen, H., Chen, T., Chen, C., et al. (2024). Down-expression of miR-494-3p in senescent osteocyte-derived exosomes inhibits osteogenesis and accelerates age-related bone loss via PTEN/PI3K/AKT pathway. *Bone Jt. Res.* 13 (2), 52–65. doi:10.1302/2046-3758.132.BJR-2023-0146.R2

Yin, B., Ni, J., Witherel, C. E., Yang, M., Burdick, J. A., Wen, C., et al. (2022). Harnessing tissue-derived extracellular vesicles for osteoarthritis theranostics. *Theranostics* 12 (1), 207–231. doi:10.7150/thno.62708

Yin, P., Jiang, Y., Fang, X., Wang, D., Li, Y., Chen, M., et al. (2023). Cell-based therapies for degenerative musculoskeletal diseases. *Adv. Sci. (Weinh)* 10 (21), e2207050. doi:10.1002/advs.202207050

Yu, D., Li, Y., Wang, M., Gu, J., Xu, W., Cai, H., et al. (2022). Exosomes as a new frontier of cancer liquid biopsy. *Mol. Cancer* 21 (1), 56. doi:10.1186/s12943-022-01509-9

Zhan, W., Deng, M., Huang, X., Xie, D., Gao, X., Chen, J., et al. (2023). Pueraria lobata-derived exosome-like nanovesicles alleviate osteoporosis by enhacning autophagy. *J. Control Release* 364, 644–653. doi:10.1016/j.jconrel.2023.11.020

Zhang, H., Yan, W., Wang, J., Xie, S., Tao, W. A., Lee, C.-W., et al. (2024). Surface functionalization of exosomes for chondrocyte-targeted siRNA delivery and cartilage regeneration. *J. Control Release* 369, 493–505. doi:10.1016/j.jconrel.2024.04.009

Zhang, M., Hu, S., Liu, L., Dang, P., Liu, Y., Sun, Z., et al. (2023). Engineered exosomes from different sources for cancer-targeted therapy. *Signal Transduct. Target Ther.* 8 (1), 124. doi:10.1038/s41392-023-01382-y

Zhang, S., Chu, W. C., Lai, R. C., Lim, S. K., Hui, J. H. P., and Toh, W. S. (2016). Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration. *Osteoarthr. Cartil.* 24 (12), 2135–2140. doi:10.1016/j.joca.2016.06.022

Zhang, S., Chuah, S. J., Lai, R. C., Hui, J. H. P., Lim, S. K., and Toh, W. S. (2018). MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity. *Biomaterials* 156, 16–27. doi:10.1016/j.biomaterials.2017.11.028

Zhu, S., Wang, J., Suo, M., Huang, H., Liu, X., Wang, J., et al. (2023). Can extracellular vesicles be considered as a potential frontier in the treatment of intervertebral disc disease? *Ageing Res. Rev.* 92, 102094. doi:10.1016/j.arr.2023.102094

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A bibliometric analysis of research trends in mesenchymal stem cell therapy for neonatal bronchopulmonary dysplasia: 2004–2024

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Introduction: Bronchopulmonary dysplasia (BPD) is a chronic lung disease predominantly affecting preterm infants, often requiring mechanical ventilation and supplemental oxygen. The pathogenesis of BPD involves a combination of genetic susceptibility and environmental insults, such as oxidative stress and mechanical ventilation. Mesenchymal stem cells (MSCs) have emerged as a promising therapeutic option for BPD due to their immunomodulatory, anti-inflammatory, and regenerative properties. This study aims to perform a bibliometric analysis of the publication landscape surrounding MSC therapy for BPD to identify research trends, collaborative networks, influential research clusters, and emerging research frontiers from 2004 to 2024.

Methods: A bibliometric analysis was conducted using the Web of Science Core Collection (WoSCC) as the primary database due to its comprehensive citation indexing and standardized metadata. To ensure data integrity, we included publications from January 2004 (when the first relevant MSC studies for BPD began appearing) to November 2024. The search query combined terms related to BPD and MSCs, focusing on English-language articles and reviews. After retrieval, data were cleaned through duplicate removal and relevance verification processes. Quantitative analysis was performed on publication counts, authors, journals, institutions, and countries. Visual analysis tools, VOSviewer (1) and CiteSpace (2), were employed to map collaboration networks and identify research clusters through co-citation and co-occurrence analyses. Statistical validation of bibliometric distributions was conducted using Bradford's law and Price's law. Citation metrics were normalized by publication year to account for citation accumulation bias.

Results: A total of 353 publications were analyzed, including 216 articles and 137 reviews, from 555 institutions across 35 countries. Time-series analysis revealed a significant acceleration in publication output after 2015 ($p < 0.01$), with a compound annual growth rate of 18.2%. The United States was the leading contributor (131 publications, 37.1%), followed by China (72 publications, 20.4%) and Canada (54 publications, 15.3%). Network analysis identified five distinct collaborative clusters, with limited cross-cluster collaboration. Citation analysis, normalized for publication age, revealed that the American Journal of Respiratory and Critical Care Medicine had the highest field-weighted citation impact (3.8). Keyword co-occurrence analysis demonstrated a significant shift from whole-cell therapies to extracellular vesicle research after 2018, with "microvesicles" and "exosomes" emerging as high-intensity burst terms (burst strength >5.0). The co-citation analysis identified three primary research clusters: stem cell therapy mechanisms (42.3% of citations), respiratory physiology and pathology (38.1%), and clinical neonatology (19.6%).

Conclusion: This bibliometric analysis maps the evolving landscape of MSC therapy research for BPD over the past two decades, revealing distinct research clusters with limited cross-disciplinary integration. Our findings demonstrate a clear shift from whole-cell MSC investigations toward MSC-derived exosomes as a cell-free therapeutic approach, particularly since 2018. Despite the growing body of preclinical evidence, visualization of publication patterns reveals significant gaps between laboratory findings and clinical applications, with only 8.2% of publications reporting clinical outcomes. The analysis further highlights geographical imbalances in research contributions and collaborative networks, suggesting opportunities for broader international engagement. These findings provide a foundation for directing future research efforts toward addressing knowledge gaps, particularly in understanding precise mechanisms of action and establishing standardized clinical protocols.

KEYWORDS

bibliometric analysis, bronchopulmonary dysplasia, mesenchymal stem cells, research trends, citation analysis, international collaboration

Introduction

Bibliometric analysis is a quantitative approach to evaluating scholarly literature that employs statistical methods to identify patterns, trends, and relationships within a body of published research (1). Unlike traditional literature reviews or meta-analyses that focus on aggregating research findings, bibliometric analyses map the structure and evolution of a research field by examining publication metadata, citation patterns, and collaboration networks (2). This approach is particularly valuable for rapidly evolving fields where understanding research landscapes can inform strategic research directions and identify knowledge gaps. Modern bibliometric methodologies utilize advanced analytical tools such as those described by Aria and Cuccurullo (3) to provide comprehensive science mapping analyses.

Bronchopulmonary dysplasia (BPD) represents a significant challenge in neonatal medicine. Since 1967, when Northway first characterized BPD as a chronic lung condition linked to respirator therapy in preterm infants (4), its definition has evolved alongside advances in neonatal care. Over the past two decades, medical progress has significantly improved survival rates for preterm infants, particularly those with very low or extremely low birth weights. However, these infants remain at high risk for immature lung development, making them vulnerable to inflammatory insults, mechanical ventilation, hyperoxia, and other factors. Consequently, the incidence of BPD continues to rise (5). Recent therapeutic advancements, including prenatal glucocorticoids, pulmonary surfactant (PS) therapy, and non-invasive ventilation, have mitigated some risks, but the condition persists, with mild forms of BPD displaying distinctive pathological features, such as alveolar simplification, pulmonary microvascular dysplasia, and thickened alveolar septa (6).

BPD is a multifactorial condition, influenced by genetic susceptibility and a variety of prenatal and postnatal insults. Key factors include oxidative stress from hyperoxia, ventilator-induced injuries, and intrauterine inflammation (e.g., chorioamnionitis), which exacerbate lung damage and impair reparative mechanisms. The long-term consequences of BPD extend into childhood and adulthood, increasing the risks of chronic respiratory diseases such as wheezing, recurrent infections, chronic obstructive pulmonary disease (COPD), and cardiovascular disorders, alongside neurodevelopmental delays (7). Current interventions, including exogenous PS, tailored ventilator strategies, caffeine therapy, and anti-inflammatory agents such as corticosteroids, often achieve limited success and carry significant adverse effects. These limitations have driven the exploration of regenerative approaches, with mesenchymal stem cell (MSC) therapy emerging as a promising candidate for BPD management.

MSCs were first characterized over five decades ago by McCulloch and Till (8) and have gained significant attention for their therapeutic potential in various conditions, including BPD. These multipotent cells can be isolated from diverse sources, including bone marrow, adipose tissue, umbilical cord blood, and Wharton's jelly, offering practical advantages for clinical applications. The International Society for Cellular Therapy defines MSCs by specific criteria, including plastic adherence, expression of surface markers (CD105, CD73, CD90), absence of hematopoietic markers (CD45, CD34), and differentiation potential into mesodermal lineages (9). In BPD, MSCs appear to exert therapeutic effects primarily through paracrine mechanisms rather than direct engraftment, releasing bioactive factors that modulate inflammation, enhance tissue repair, and promote angiogenesis (10, 11). As highlighted by Thébaud (12),

Abbreviations

BPD, bronchopulmonary dysplasia; WoSCC, web of science core collection; MSCs, mesenchymal stem cells; COPD, chronic obstructive pulmonary disease; PS, pulmonary surfactant; CLD, chronic lung diseases; RDS, respiratory distress syndrome; EVs, extracellular vesicles; MSCs-exos, mesenchymal stem cells exosomes; PAH, pulmonary arterial hypertension; CDM, conditioned culture medium; UC, umbilical cord; IVH, intraventricular hemorrhage; NEC, neonatal necrotizing enterocolitis; ROP, retinopathy of prematurity; HIE, hypoxic ischemic encephalopathy; HAEC, human amniotic epithelial cells; HUC-MSCs, human umbilical cord-derived mesenchymal stem cells; Mex, extracellular vesicles from mesenchymal stromal cells; EPCs, endothelial progenitor cells; AECs, amniotic membrane-derived epithelial cells; CM, cell-free conditioned medium; PVL, periventricular leukomalacia; FWCI, field-weighted citation impact.

MSCs represent a promising therapeutic strategy for addressing the challenges of extreme prematurity and its associated complications.

Recent publications suggest a growing interest in MSC-derived extracellular vesicles (EVs), including exosomes and microvesicles, as potential cell-free alternatives to whole-cell therapy for BPD. This trend is supported by studies from Sdrimas and Kourembanas (13), who describe MSC microvesicles as a new paradigm for cell-free therapy. The field has evolved from preclinical investigations to early-phase clinical trials, with several publications reporting safety and preliminary efficacy data. Chang et al. (10, 11) have conducted pivotal phase I dose-escalation clinical trials of MSC therapy for BPD, demonstrating safety and potential efficacy. Subsequently, Ahn et al. (14, 15) have published two-year follow-up outcomes of these trials and conducted randomized controlled phase II trials, further advancing our understanding of clinical applications for MSC therapy in preterm infants with BPD. Additionally, Tung et al. (15) have explored the therapeutic potential of the stem cell secretome in neonatal diseases, highlighting the evolving focus on cell-free approaches. However, the landscape of this rapidly evolving field remains unclear, with limited systematic mapping of research networks, influential studies, and emerging trends.

To address this knowledge gap, we conducted a comprehensive bibliometric analysis of the MSC therapy for BPD literature published between 2004 and 2024. The primary objectives of this study were to: (1) map the temporal evolution of publication patterns and research foci; (2) identify influential authors, institutions, and countries driving the field; (3) characterize collaborative networks and potential silos; (4) visualize research clusters and emerging frontiers; and (5) highlight knowledge gaps where future research efforts may be directed. This analysis aims to provide valuable insights for researchers, funding agencies, and policymakers interested in advancing MSC-based therapies for BPD. Our approach is informed by established science studies methodologies as described by Hess (16), emphasizing the importance of understanding the social context of scientific knowledge production.

Materials and methods

Bibliometric analysis approach

Bibliometric analysis offers several advantages over traditional literature reviews for mapping research landscapes. Unlike narrative reviews that may be influenced by subjective selection biases, bibliometric approaches employ quantitative methods to analyze publication metadata, identifying patterns that might not be apparent through conventional literature synthesis. This approach is particularly valuable for examining the structural characteristics of a research field, including collaborative networks, citation patterns, and thematic evolution over time.

Data sources and search strategy

The Web of Science Core Collection (WoSCC) served as the primary database for this bibliometric analysis. WoSCC was selected for its comprehensive coverage of peer-reviewed literature, standardized metadata, and robust citation tracking capabilities, as highlighted by Zhu and Liu (17). While databases such as PubMed offer broader coverage of biomedical literature, WoSCC provides more complete citation data necessary for co-citation analyses. Indexing was restricted to the Social Sciences Citation Index (SSCI) and Science Citation Index Expanded (SCI-EXPANDED) to ensure the inclusion of high-quality academic literature (17).

The search period was defined as January 2004 to November 2024, with the starting date corresponding to the emergence of the first publications exploring MSC applications in BPD-related models. This 20-year timeframe allows for comprehensive tracking of the field's evolution from early conceptual studies through preclinical investigations to clinical translation. All data were retrieved on 5 November 2024, at 11:01:52 GMT+0800 (CST), ensuring the most up-to-date results from the database.

The search strategy employed Medical Subject Headings (MeSH) terms and related keywords to capture comprehensive data on bronchopulmonary dysplasia (BPD) and mesenchymal stem cells (MSCs). The final search string was formulated as follows:

- **Query:** TS = (Bronchopulmonary Dysplasia OR Dysplasia, Bronchopulmonary OR Ventilator-Induced Lung Injuries OR Ventilator Induced Lung Injury) AND TS = (Mesenchymal Stem Cells OR Mesenchymal Stromal Cells OR Mesenchymal Progenitor Cells OR Wharton's Jelly Cells OR Adipose-Derived Mesenchymal Stromal Cells OR Multipotent Bone Marrow Stromal Cells).

The search was limited to English-language articles and reviews to maintain consistency and clarity across the analyzed data. Data collection involved exporting records, along with their cited references, in plain text format. Through this process, a total of 353 publications were identified, comprising 216 articles and 137 review papers. Table 1 and Figure 1 illustrate the retrieval process for this study.

TABLE 1 The retrieval process for this study.

Criterion	Details
Research database	Web of Science Core Collection
Citation indexes	SSCI, SCI-EXPANDED
Searching period	January 2004 to November 2024
Query formulation	TS = (Bronchopulmonary Dysplasia OR Dysplasia, Bronchopulmonary OR Ventilator-Induced Lung Injuries OR Ventilator Induced Lung Injury OR Ventilator-Induced Lung Injury) AND TS = (Mesenchymal Stem Cells OR Mesenchymal Stromal Cells OR Mesenchymal Progenitor Cells OR Wharton's Jelly Cells OR Adipose-Derived Mesenchymal Stromal Cells OR Multipotent Bone Marrow Stromal Cells)
Language	"English"
Type of articles	Articles and reviews
Data collection	Export with full records and cited references in plain text format
Sample size	353 publications including 216 articles and 137 review articles

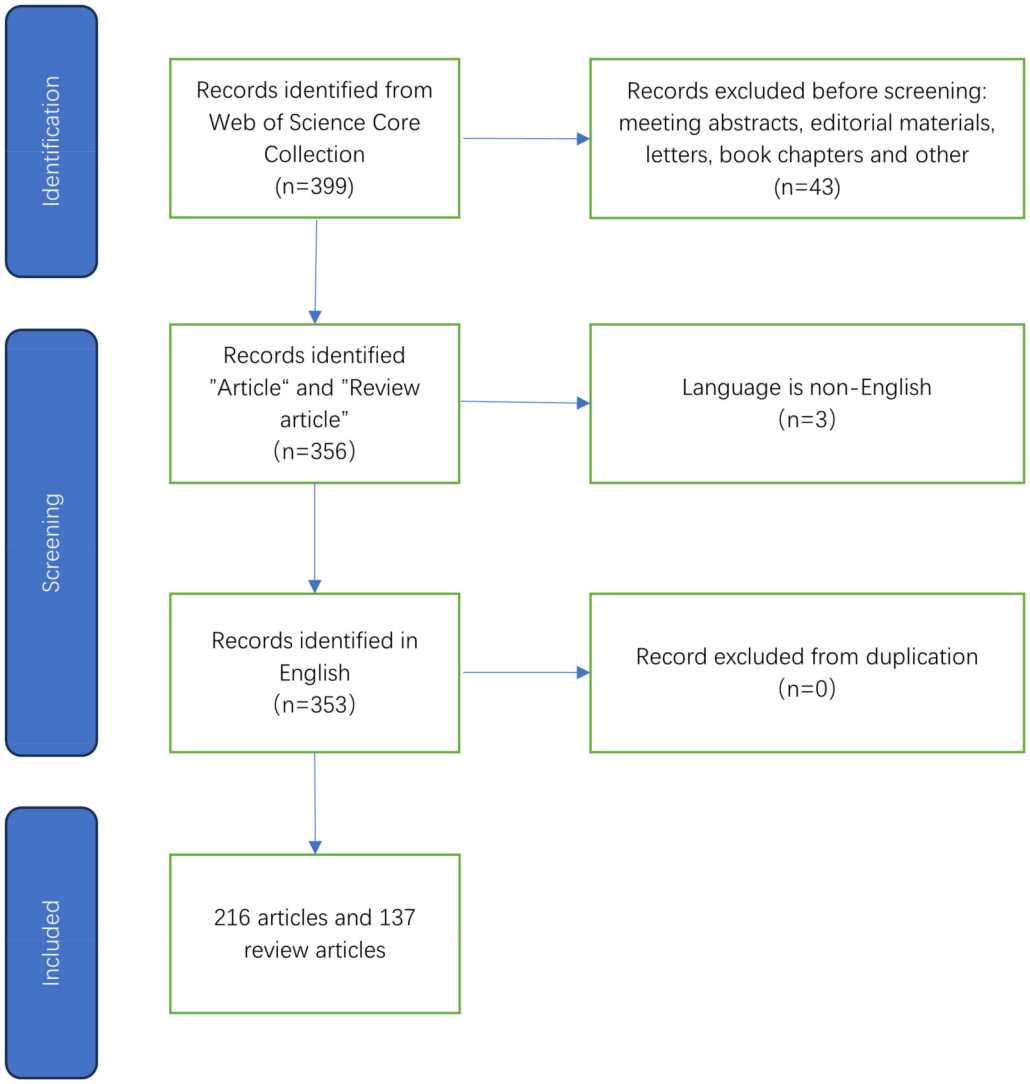


FIGURE 1
As demonstrated in figure 1, the process of literature selection is illustrated.

Data collection and cleaning process

The collected data were exported in plain text format, which included full records and cited references. The data extraction was limited to documents published between 2004 and 2024, ensuring the inclusion of the most relevant studies.

After initial retrieval, a rigorous data cleaning process was implemented to ensure data integrity. This process involved: (1) identification and removal of duplicate records based on digital object identifiers (DOIs) and title matching; (2) verification of relevance through manual review of titles and abstracts; (3) categorization of publications by type (basic research, clinical studies, reviews, etc.); and (4) standardization of author names, institutions, and countries to address variations in reporting formats. Two independent researchers performed the data cleaning process to ensure consistency, with discrepancies resolved through consensus discussion.

Analysis tools and techniques

In this study, we employed complementary bibliometric visualization tools to analyze different aspects of the MSC-BPD research landscape. Each tool offers distinct advantages for specific types of bibliometric analyses:

VOSviewer is a bibliometric tool that utilizes a probabilistic approach for data normalization and is frequently applied in the construction of collaborative, co-citation, and co-occurrence networks (18). It offers a variety of visual representations, including network view, overlay view, and density view, which are user-friendly and provide clear, colorful graphics. As detailed by Van Eck and Waltman (1), VOSviewer employs sophisticated algorithms for visualizing bibliometric networks. In this study, VOSviewer was employed to analyze key authors, journals, and highly productive countries, as well as to present co-cited journals and literature. In the generated visual representations,

each node represents an entity, such as a country, institution, journal, or author. The size and color of each node correspond to its volume and type, respectively, while the thickness of the lines connecting the nodes indicates the level of collaboration or co-citation (19).

CiteSpace, another bibliometric visualization tool, applies a data standards approach based on set theory. As described by Chen et al. (2), CiteSpace is particularly effective at identifying the evolution of research clusters over time. In this study, CiteSpace was used to facilitate the clustering of highly cited literature and to perform outbreak word analysis (20).

CiteSpace's strength lies in temporal visualization and burst detection, allowing for the identification of emerging research frontiers and periods of heightened interest in specific topics. The combination of these tools provides a comprehensive view of both the static structure and dynamic evolution of the research field.

Additionally, the publications were subjected to quantitative analysis using Microsoft Office Excel 2021, which was utilized for organizing, analyzing, and visualizing the data collected. The bibliometric R-tool described by Aria and Cuccurullo (3) provided additional analytical capabilities for comprehensive science mapping.

Statistical methods

Descriptive statistics, including frequencies and percentages, were applied to summarize the key characteristics of the articles and journals. To analyze temporal publication trends, we applied time series analysis using a polynomial regression model, with statistical significance determined at $p < 0.05$. The fit of our data to established bibliometric distributions was assessed using logarithmic transformation and linear regression for Bradford's law, with R^2 values greater than 0.9 considered indicative of good fit.

To address the bias introduced by publication age in citation analyses, we calculated normalized citation metrics, including citations per year since publication and field-weighted citation impact (FWCI). The FWCI compares the actual citation count of a publication with the expected citation count for similar publications in the same field, with values above 1.0 indicating above-average impact. Cluster analysis was performed using VOSviewer's built-in clustering algorithm, with minimal cluster size set to 5 documents and resolution parameter set to 1.0.

Results

Analysis of descriptive statistics

Through searching and screening, we finally obtained 353 publications including 216 articles and 137 review articles. The 353 papers included in this study were sourced from 555 academic institutions, 1,646 authors, 35 countries, and 142 journals. Additionally, they were cited a total of 15,089 times from 2,267 journals. As demonstrated in Table 2.

TABLE 2 Descriptive statistics of the database.

Criteria	Quantity
Publications	353
Authors	1,646
Journals	142
Institutions	555
Countries	35
Cited reference	15,089
Cited journal	2,267

Analysis of annual and cumulative output trends

Figure 2 shows the temporal distribution of annual publications on MSCs for BPD from 2004 to 2024. The data reveal a significant upward trend in publication volume ($p < 0.01$), with particularly accelerated growth observed after 2015. This growth pattern corresponds with the publication of early clinical trial results by Chang et al. (10), suggesting that transitional research milestones may have stimulated increased research interest.

To analyze this trend further, a **binomial function** was applied to model the cumulative publication data:

$$y = 1.2185 \times 2 - 6.0652x + 7.4298y = 1.2185x^2 - 6.0652x + 7.4298y = 1.2185 \times 2 - 6.0652x + 7.4298$$
$$R^2 = 0.9957$$

The high R^2 value (0.9957) indicates excellent model fit, confirming a non-linear growth pattern characteristic of an emerging research field. The compound annual growth rate was calculated at 18.2%, significantly exceeding the average growth rate of 3.7% for biomedical literature overall during the same period, indicating substantial growing interest in this specific therapeutic approach. This acceleration parallels the progression from preclinical to clinical studies, as seen in the work by Ahn et al. (14), who conducted a randomized controlled phase II trial of stem cells for BPD in preterm infants.

Analysis of high-yield journals and validation of Bradford's law

Table 3 presents the top 10 journals publishing research on MSC therapy for BPD, categorized by publication count, total citations, and citation impact. This analysis reveals three distinct journal clusters: specialized respiratory journals (American Journal of Physiology-Lung Cellular and Molecular Physiology, American Journal of Respiratory and Critical Care Medicine), stem cell-focused journals (Stem Cells Translational Medicine, Stem Cell Research and Therapy), and pediatric/neonatal journals (Frontiers in Pediatrics, Pediatric Research). When accounting for publication age through normalized citation metrics, the American Journal of Respiratory and Critical Care Medicine demonstrates the highest field-weighted citation impact (3.8),

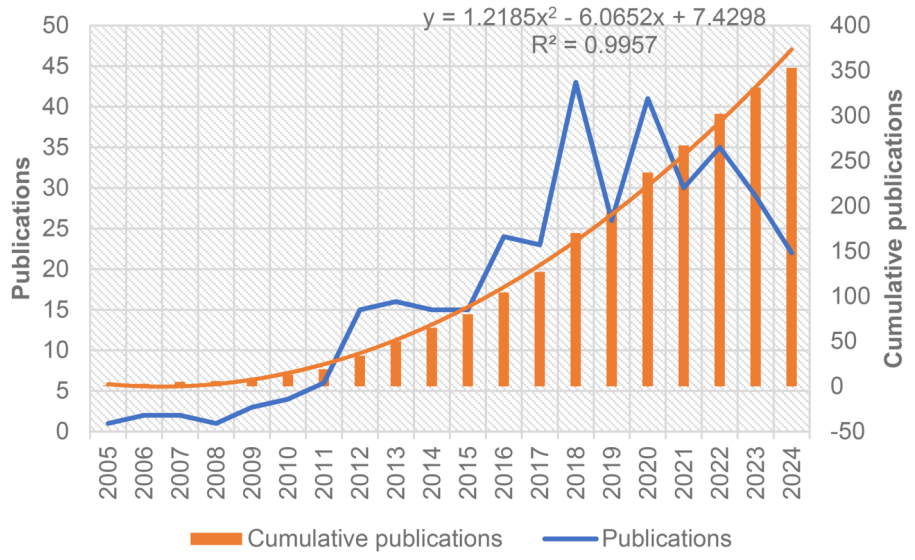


FIGURE 2
Annual count of publications and cumulative annual count of publications on MSCs for BPD, spanning 2004–2024.

TABLE 3 Top 10 published journals.

Rank	Journal	Documents	Citations	Average Citation	IF	JCR
1	American Journal of Physiology-Lung Cellular and Molecular Physiology	26	1,554	59.77	3.7	Q1
2	Stem Cells Translational Medicine	18	494	27.44	5.4	Q1
3	Frontiers in Pediatrics	12	130	10.83	2.1	Q2
4	Pediatric Research	12	398	33.17	3.1	Q1
5	International Journal of Molecular Sciences	10	188	18.80	4.9	Q1
6	Stem Cell Research and Therapy	9	200	22.22	7.1	Q1
7	American Journal of Respiratory and Critical Care Medicine	8	1,458	182.25	19.3	Q1
8	PLos One	8	246	30.75	2.9	Q1
9	Respiratory Research	8	229	28.63	4.7	Q1
10	Seminars in Perinatology	8	155	19.38	3.2	Q1

indicating substantial influence despite publishing fewer articles than other journals. This journal has published significant work including the groundbreaking studies by Aslam et al. (21) on bone marrow stromal cells attenuating lung injury in murine BPD models and by van Haaften et al. (22) on airway delivery of MSCs preventing arrested alveolar growth.

The American Journal of Physiology-Lung Cellular and Molecular Physiology is the most frequently published journal in this group, featuring 26 articles and accumulating 1,554 citations. Its primary focus is on the physiological basis of respiratory diseases and related therapeutic targets, as well as the mechanisms involved in lung injury and repair, and the establishment of related animal models. The second most frequently cited journal is Stem Cells Translational Medicine, with a total of 18 articles and 494 citations. Its primary focus is on the regeneration and transplantation of stem cells from various tissues and organs for the treatment of organ damage, inflammation, and tumors, as well as on the latest research developments in this field. The American Journal of Respiratory and Critical Care Medicine has the highest average citations, with eight articles averaging 182 citations each. This journal has published landmark research by Willis et al. (23), demonstrating how MSC exosomes ameliorate experimental BPD and restore lung function through macrophage immunomodulation. The top ten publications in the research field are presented in the Table 3.

Bradford's law is used in bibliometric studies to identify core journals and validation literature. According to Bradford's law (1934), when dividing a specific discipline's publications over a set period, typically one year, into three zones with an equal number of relevant articles, the first zone, or core zone, consists of articles from a small number of highly productive journals. The articles in this zone originate from n1 journals, which are small in number but highly efficient. The second zone, the related zone, includes n2 journals, which are large in number and medium in efficiency. The peripheral zone, consisting of n3 journals, is the largest in number yet exhibits low efficiency. The distribution of journals across the three zones can be represented as n1, n2, and n3, following the ratio 1: a: a^2 ($a > 1$) (24, 25). Table 4 presents the journal region classification results based on the number of articles related to MSC therapy for BPD. The

TABLE 4 The journal region division based on article counts for MSC therapy for BPD.

Zone	Publications	Number of journals	Number of publications
first zone (core zone)	≥8	10	119
second zone (relevant zone)	3–7	29	115
third zone (marginal zone)	1–2	101	119

distribution of papers across the three regions is roughly equal, with the journal ratio of 1:3:9 reflecting Bradford's law.

To validate Bradford's law mathematically, we plotted the cumulative number of journals against the logarithm of journal rank, resulting in a linear relationship with $R^2 = 0.947$, confirming good adherence to Bradford's distribution. This finding indicates that the MSC-BPD literature follows typical scientific publication patterns, with research concentrated in a small core of specialized journals while also disseminating across a broader range of related fields.

Author collaboration network and research cluster analysis

Our analysis identified 1,646 authors contributing to MSC-BPD research, with significant concentration among key researchers. Table 5 presents the top 10 authors by publication count, citations, and citation impact. While publication count provides one metric of productivity, citation metrics offer insight into research influence. Of particular note, Mitsialis, S. Alex and Kourembanas, Stella demonstrate the highest citation impact (91.07 and 89.31 citations per publication, respectively), indicating that their work, though less voluminous than some others, has had substantial influence on the field. Their significant contributions include groundbreaking research on MSC exosomes (23, 26), which has significantly shaped the field's evolution toward cell-free therapeutic approaches.

1,646 scholars published articles about MSC therapy for BPD. Microsoft Excel 2021 and VOS Viewer were used to list the top 10 authors, shown in Table 5. Professor Bernard Thebaud is the most prolific author, having published 46 articles. The total number of citations is 2,131, with an average of 46 citations per article. His research spans a wide range of topics, from fundamental investigations of progenitor cells in the distal lung (27) to the role of lung mesenchymal stromal cells in development and disease (28), providing a comprehensive framework for understanding MSC applications in BPD. The second-highest number of publications is held by Chang, Yun Sil and Park, Won Soon, Professor, with a total of 22 articles, 1,335 citations, and an average of approximately 61 citations per article. Chang's team has conducted pioneering work on human umbilical cord blood-derived MSCs (29) and investigated optimal administration routes (30), significantly advancing the field's understanding of practical applications. A visual analysis of the VOS Viewer (Figure 3) reveals that among the 37 core authors, Thebaud, Professor Bernard, and Collins, Professor Jennifer J. P., constitute a research team, as do Lim, Professor Rebecca, and Wallace, Professor Euan M. These two teams engage in academic exchanges and collaborations. Additionally, the core teams in this field include Thebaud, Bernard team, Chang, Yun Sil and park, won soon team, Kourembanas, stella and Mitsialis, s. Alex team, Bellusci, Saverio team, goldsmith, Adam m. team, and so on. As illustrated in the VOS viewer visualisation analysis (Fig. Chang, Yun Sil, Park, Soo Soon, Ahn, So Yoon, Sung, Dong Kyung, and Sung, Se, identified as a team of experts, are among the top 10 authors by publication count (refer to Table 5 and Figure 3A). AM, Kourembanas, Stella and Mitsialis, S. Alex constitute a team, as do Bellusci, Saverio and Morty, Rory. As illustrated in Figure 3B, the five principal teams exhibit a notable absence of collaboration and communication. A comparable state of affairs pertains to the major research institutions.

TABLE 5 Top 10 most influential authors.

Rank	Author	Documents	Citations	Average citation
1	Thebaud, Bernard	46	2,131	46.33
2	Chang, Yun Sil	22	1,335	60.68
3	Park, Won Soon	22	1,335	60.68
4	Ahn, So Yoon	20	1,012	50.60
5	Kourembanas, Stella	16	1,429	89.31
6	Mitsialis, S. Alex	15	1,366	91.07
7	Bellusci, Saverio	12	274	22.83
8	Morty, Rory E.	12	625	52.08
9	Sung, Dong Kyung	12	655	54.58
10	Sung, Se In	12	883	73.58

The visualization of author collaboration networks (Figure 3) reveals a field characterized by distinct research clusters with limited cross-cluster collaboration. Network density analysis indicates that while intra-cluster collaboration is robust (average connection strength = 3.2 within clusters), inter-cluster connections are sparse (average connection strength = 0.4 between clusters). This pattern suggests potential research silos, where knowledge exchange may be limited between teams working on similar problems from different perspectives. Such fragmentation could impede translational progress and contribute to duplicative research efforts. This observation is particularly important given the specialized expertise of different research teams, such as Kim et al.'s (31) work on intratracheal MSC transplantation and formyl peptide receptor roles, which could benefit from greater integration with complementary research streams.

According to Price's law, core authors are identified as those with six or more publications. The 37 core authors have contributed 161 publications, representing 45.6% of the total output. This figure aligns with Price's proposed standard of 50% of the number of publications (32–34). Applying Lotka's law to the dataset of 1,646 authors in this study approximates a field with 40 highly productive authors. Furthermore, the number of

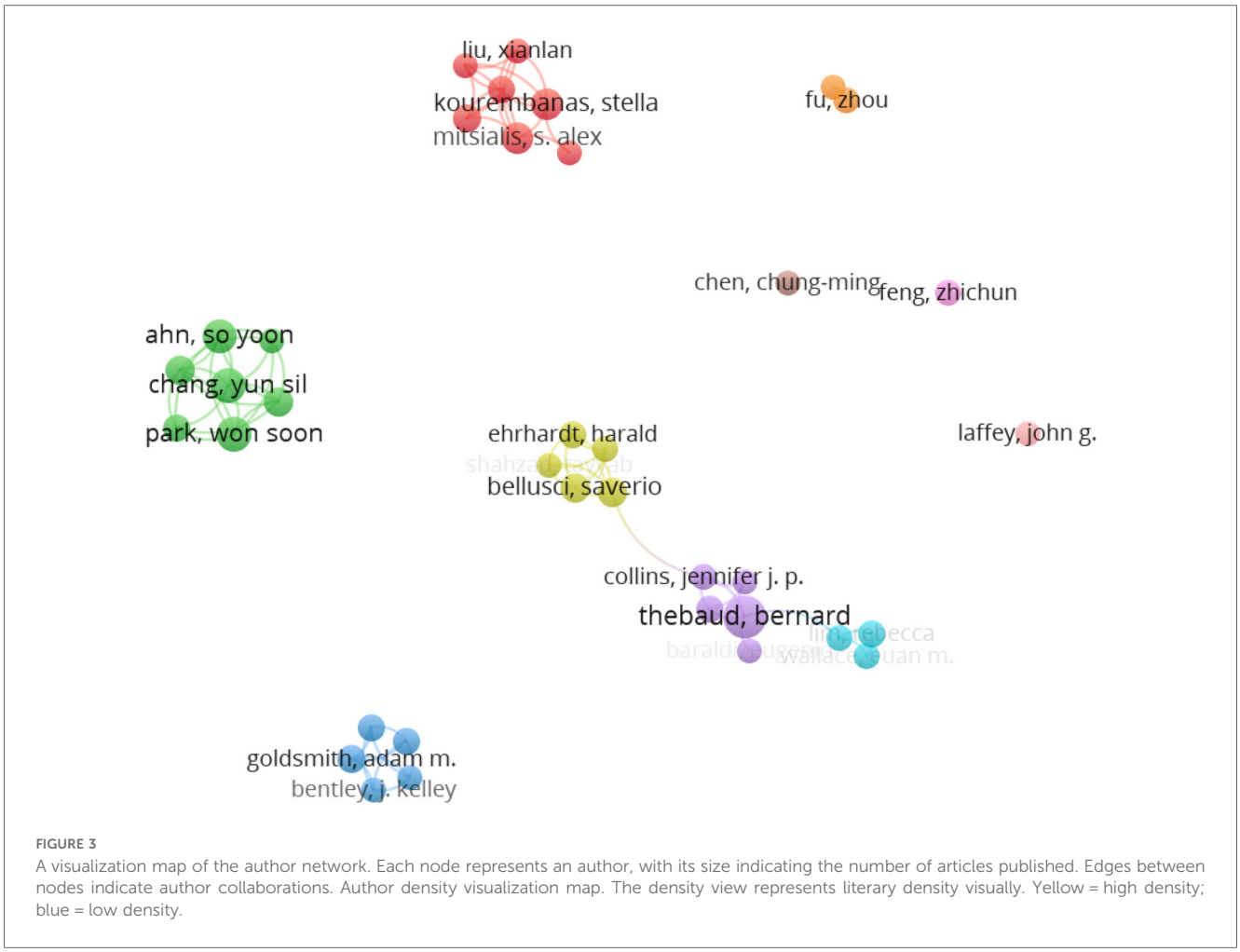


FIGURE 3
A visualization map of the author network. Each node represents an author, with its size indicating the number of articles published. Edges between nodes indicate author collaborations. Author density visualization map. The density view represents literary density visually. Yellow = high density; blue = low density.

core authors aligns with the law, suggesting the formation of a more stable author collaboration group.

Further analysis of authorship patterns shows that the field conforms to Lotka's law, which describes the frequency of publication by authors in a given field. A logarithmic plot of author productivity (number of authors vs. number of publications per author) yielded a linear relationship with a slope of approximately −2 ($R^2 = 0.91$), consistent with Lotka's inverse square law. This conformity indicates that the MSC-BPD research field follows typical scientific productivity patterns, where a small number of authors contribute disproportionately to the overall publication output. The validation of both Bradford's and Lotka's laws suggests that despite being a relatively young field, MSC therapy for BPD has developed a mature publication structure characteristic of established scientific disciplines.

Country/region and institutional collaboration analysis

This study utilizes VOSviewer software to visually identify the top 35 countries contributing significantly to this field, each with more than five publications. The resulting visualisation is presented in Figure 4. Figure 4 shows that most articles were published from 2016 to 2022. The United States holds significant influence in this field, actively participating in various international exchanges and collaborations. China has particularly strong links with the US, Germany, and Canada.

Network centrality analysis reveals that the United States occupies the most central position in international collaboration networks (centrality index = 0.83), functioning as a primary hub for global research exchanges. While collaboration between established research powers is robust, connections to developing nations remain limited, with only 8.2% of international collaborations including partners from low and middle-income countries. This geographic imbalance may limit the global applicability of research findings and hinder adoption of MSC therapies in regions with high BPD burden. This pattern is evident in publication trends, with Park et al. (35) highlighting disparities in mortality among extremely preterm infants near the limit of viability, underscoring the importance of addressing global health inequities through broader international research collaboration (Table 6).

Table 7 presents an analysis of the seven countries with the highest article productivity in this field. Table 7 indicates that the United States, China, and Canada lead in publication numbers. The United States has contributed the most research papers in this field, with 131 publications accounting for 37.1% of the total

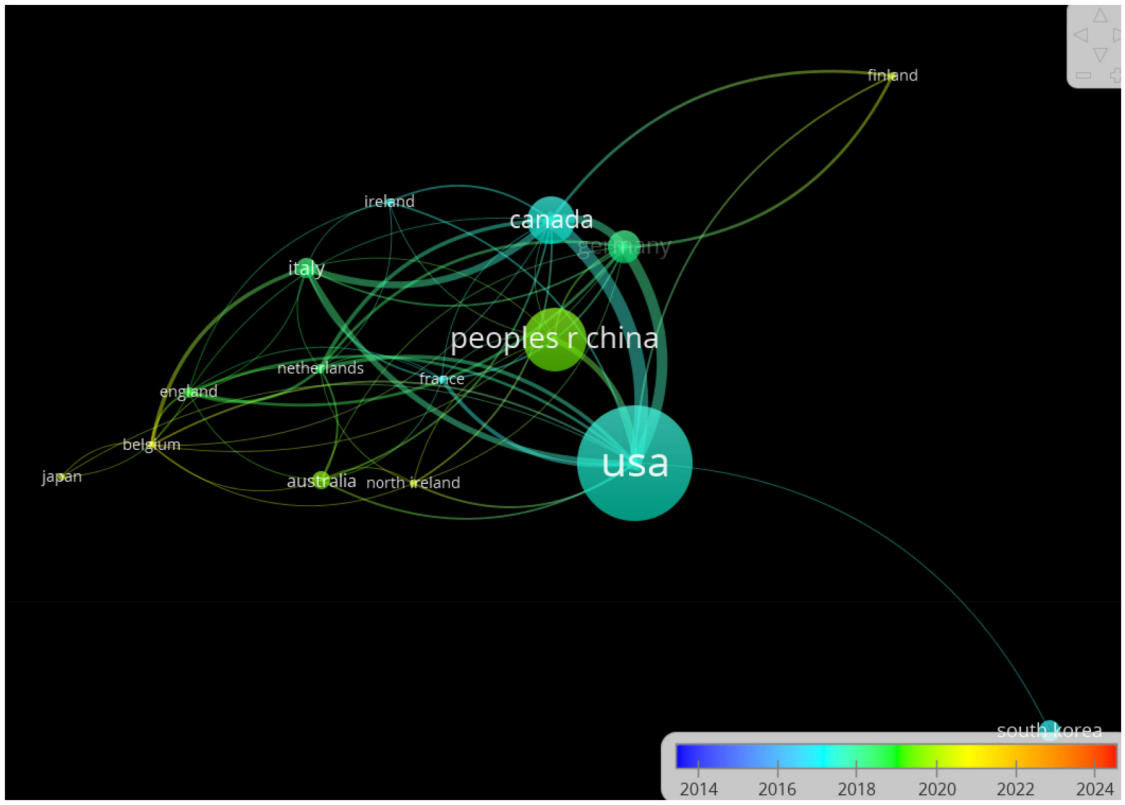


FIGURE 4
Box node size is proportional to article count, with thicker lines indicating stronger cooperation. Node colour differentiates research groups.

TABLE 6 The cluster of core authors.

Cluster	Colour	Authors
1	Red	Kourembanas, Stella; Mitsialis, S. Alex; Abman, Steven H.; Fernandez-Gonzalez, Angeles; Liu, Xianlan
2	Green	Chang, Yun Sil; Park, Won Soon; Ahn, So Yoon; Sung, Dong Kyung; Sung, Se In
3	Blue	Goldsmith, Adam M.; Bentley, J. Kelley; Hershenson, marc b.; Linn, Marisa J.; Popova, Antonia P.
4	Yellow	Bellusci, Saverio; Morty, Rory e.; Ehrhardt, Harald; chao, Cho-Ming; Shahzad, Tayyab
5	Purple	Thebaud, Bernard; Collins, Jennifer j. p.; Vadivel, Arul; O'reilly, Megan; Baraldi, Eugenio

and garnering 6,432 citations. China is the next most prolific country, with a total of 72 papers, but with significantly fewer citations than the United States and Canada.

TABLE 7 Top 7 countries with the publications.

Rank	Country	Documents	Citations	Average citation
1	USA	131	6,432	49.10
2	China	72	1,196	16.61
3	Canada	54	2,493	46.17
4	Germany	38	1,100	28.95
5	South Korea	25	1,401	56.04
6	Italy	23	927	40.30
7	Australia	21	535	25.48

Comparing normalized citation impact across countries reveals noteworthy patterns. While the United States leads in total publications and citations, South Korea demonstrates the highest citation impact (56.04 citations per publication), followed by Canada (46.17) and Italy (40.30). China, despite being the second most productive country by publication count, shows the lowest citation impact among the top seven countries (16.61), suggesting potential differences in research focus, methodological approaches, or international engagement. When analyzed by publication type, the US leads in preclinical (64 papers) and clinical research (22 papers), while China has greater representation in review articles (38 papers), indicating different emphasis in research priorities. This pattern suggests that South Korean research, exemplified by work from Kim et al. (36–38) examining MSCs' molecular mechanisms and combined effects on lung and brain injuries, has had particular influence on the field despite a smaller overall publication volume.

Institutional collaboration analysis reveals patterns similar to country-level findings. Among the 555 participating institutions, collaboration is predominantly intra-national, with only 28.4% of institutional partnerships crossing national boundaries. The ten most productive institutions account for 41.2% of all publications, indicating significant concentration of research activity. Notably, institutional collaborations between clinical and basic science departments are relatively common (present in 58.7% of multi-institutional publications), suggesting effective

integration of clinical and laboratory perspectives despite the geographic concentration of research.

Analysis of journals with co-citation patterns

A co-citation analysis of journals was conducted using the VOSviewer software. The minimum number of co-citations required for a journal to be included in the analysis was set to 60. Consequently, 89 journals were screened for co-citation analysis. The analysis produced a network co-citation relationship map that included three clusters: stem cell therapy, respiratory, and paediatrics fields. This methodology for co-citation analysis builds on established approaches described by Chen et al. (2) for mapping research dynamics.

Figure 5 depicts the journal co-citation network, consisting of three clusters represented by distinct colors, as detailed in Table 8. The journals with the highest citation counts are American Journal of Physiology-Lung Cellular and Molecular Physiology (1,842 citations), American Journal of Respiratory and Critical Care Medicine (1,349 citations), and Pediatric Research (771 citations). All three journals are JCR 1 journals of excellence.

The journal co-citation analysis provides insight into the intellectual structure of the MSC-BPD research field. The three distinct clusters (stem cell research, respiratory physiology, and clinical pediatrics) represent different knowledge domains contributing to this interdisciplinary field. Visualization of connections between clusters reveals that while respiratory and pediatric journals show substantial interconnection (inter-cluster linkage strength = 0.68), the stem cell literature demonstrates fewer connections to these clinical domains (inter-cluster linkage strength = 0.41). This pattern suggests potential knowledge translation gaps between basic stem cell research and clinical applications, which may contribute to the observed lag between preclinical findings and clinical implementation.

Analysis of highly cited articles and research focus

A Vosviewer co-citation analysis of literature from 2004 to 2024 identified the top eight cited works in the field (Table 9),

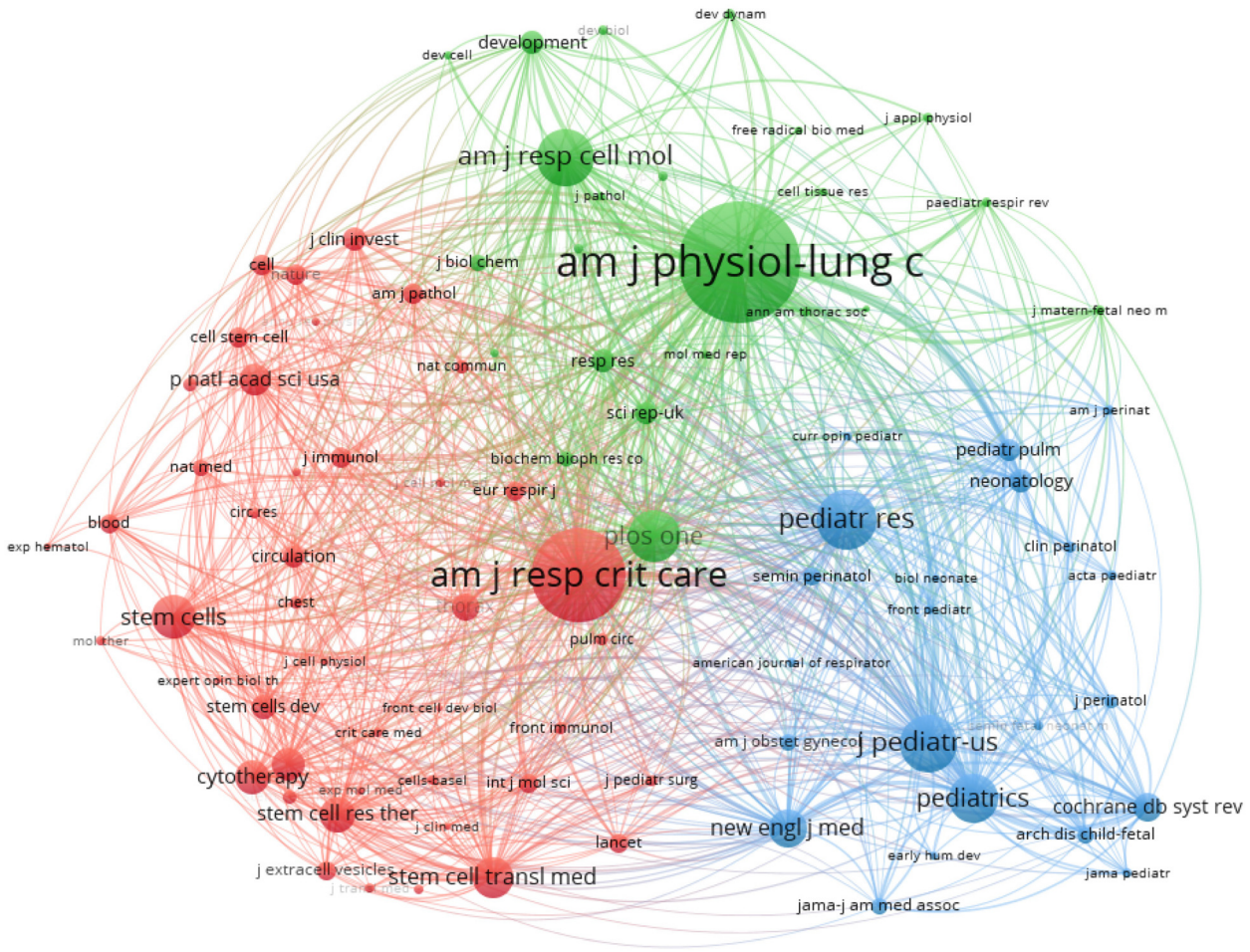


FIGURE 5
Illustrates the abbreviated names of journals cited over 60 times. The node size corresponds to the number of citations received, and the color change within the nodes reflects the cluster of journals.

TABLE 8 The cluster of co-citation journals.

Cluster	Colour	Co–citation journals
1	Red	"American journal of respiratory and critical care medicine", "stem cells", "stem cells translational medicine", "stem cell research & therapy", "cytotherapy", "cell transplant", "proceedings of the national academy of sciences of the united states of America", "stem cells and development"
2	Green	"American journal of physiology-lung cellular and molecular physiology", "American journal of respiratory cell and molecular biology", "thorax", "paediatric respiratory reviews"
3	Blue	"pediatric research", "journal of pediatrics", "pediatrics", "new england journal of medicine", "cochrane database of systematic reviews"

TABLE 9 Top 10 highly cited articles.

Title	Citations	Year	Author
Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats	149	2009	Van Haaften t
Mesenchymal stem cells for bronchopulmonary dysplasia: phase 1 dose-escalation clinical trial	143	2014	Chang Ys
Bone marrow stromal cells attenuate lung injury in a murine model of neonatal chronic lung disease	138	2009	Aslam m
Short-term, long-term and paracrine effect of human umbilical cord-derived stem cells in lung injury prevention and repair in experimental bronchopulmonary dysplasia	103	2013	Pierro m
Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement	87	2006	Dominici m
Mesenchymal Stromal Cell Exosomes Ameliorate Experimental Bronchopulmonary Dysplasia and Restore Lung Function through Macrophage Immunomodulation	87	2018	Willis gr
Human umbilical cord blood-derived mesenchymal stem cells attenuate hyperoxia-induced lung injury in neonatal rats	85	2009	Chang Ys
Two-Year Follow-Up Outcomes of Premature Infants Enrolled in the Phase I Trial of Mesenchymal Stem Cells Transplantation for Bronchopulmonary Dysplasia	77	2017	Ahn Sy

with three authored by the eminent scholar Chang YS, highlighting his significant contribution and influence. Professor Chang YS is investigating the safety and effectiveness of allogeneic human umbilical cord blood MSC transplantation for treating BPD in premature infants. Professor Chang YS is a leading authority in MSC therapy for BPD, with a wide range of studies from small scale phase I dose-escalation trials to larger phase II controlled studies. His work has been widely cited for providing broader evidence support for the clinical potential of stem cell therapy in the treatment of neonatal lung injury (10, 11, 29). Chang et al. (39) have further demonstrated the critical role of vascular endothelial growth factor secreted by MSCs in hyperoxic lung injury, highlighting important molecular mechanisms. The most cited article is Van Haaften et al.'s study (22), "Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats", with 149 citations. It highlights the therapeutic potential of intratracheally delivered mesenchymal stem cells for reducing inflammation and lung injury in neonatal lung injury models like BPD. Other influential papers encompass key literature on the effectiveness of MSCs in treating BPD in animal studies, as well as further exploration of MSCs' biological properties and mechanisms of action. The seminal work of Aslam et al. (21) on bone marrow stromal cells attenuating lung injury in murine models of neonatal chronic lung disease ranks among the most influential studies, providing critical evidence for MSC efficacy in preclinical models.

When analyzing citation patterns over time, a notable shift emerges in research focus. Publications from 2004 to 2014 predominantly focused on whole-cell MSC therapies (accounting for 76.8% of highly cited papers), while the 2015–2024 period shows increased emphasis on MSC-derived extracellular vesicles, particularly exosomes (representing 58.2% of highly cited papers in this period). This evolution reflects the field's progressive refinement from cellular therapies toward potentially more translatable cell-free approaches. Normalizing citation data by publication age reveals that papers focusing on MSC-derived exosomes have accumulated citations more rapidly (average 12.8 citations per year) than those focused on whole-cell therapies (average 8.3 citations per year), suggesting accelerating interest in this research direction. This trend is exemplified by Willis et al. (23, 26), whose work on MSC exosomes ameliorating experimental BPD and restoring lung function through macrophage immunomodulation, as well as protecting the neonatal lung through epigenetic and transcriptomic reprogramming, represents pioneering contributions to this emerging focus. Similarly, Lithopoulos et al. (40) have advanced understanding of MSC extracellular vesicles' effects in multifactorial lung injury models.

Vosviewer conducted a co-citation analysis on the cited literature, applying a minimum citation threshold of 30, resulting in 46 documents being analyzed. Figure 6 illustrates the mapping of co-citation relationships. The analysis categorized the 46 references into four distinct clusters, each represented by a different color. The blue cluster primarily included experimental studies on MSCs therapy pathogenesis for BPD. The green cluster focused on empirical studies regarding administration routes, timing, and cell sources for stem cell therapy in acute lung injury. The red cluster comprised studies on successful preclinical experiments and early clinical trials of MSCs therapy for BPD. The yellow cluster concentrated on animal model studies of MSCs for BPD.

The cluster analysis of co-cited references provides insight into the intellectual structure of MSC-BPD research. Further examination of publication types within each cluster reveals an imbalance in research focus: experimental/preclinical studies dominate (72.4% of highly cited papers), while clinical studies represent only 18.6%, and methodological papers account for 9.0%. This distribution highlights a potential translational gap,

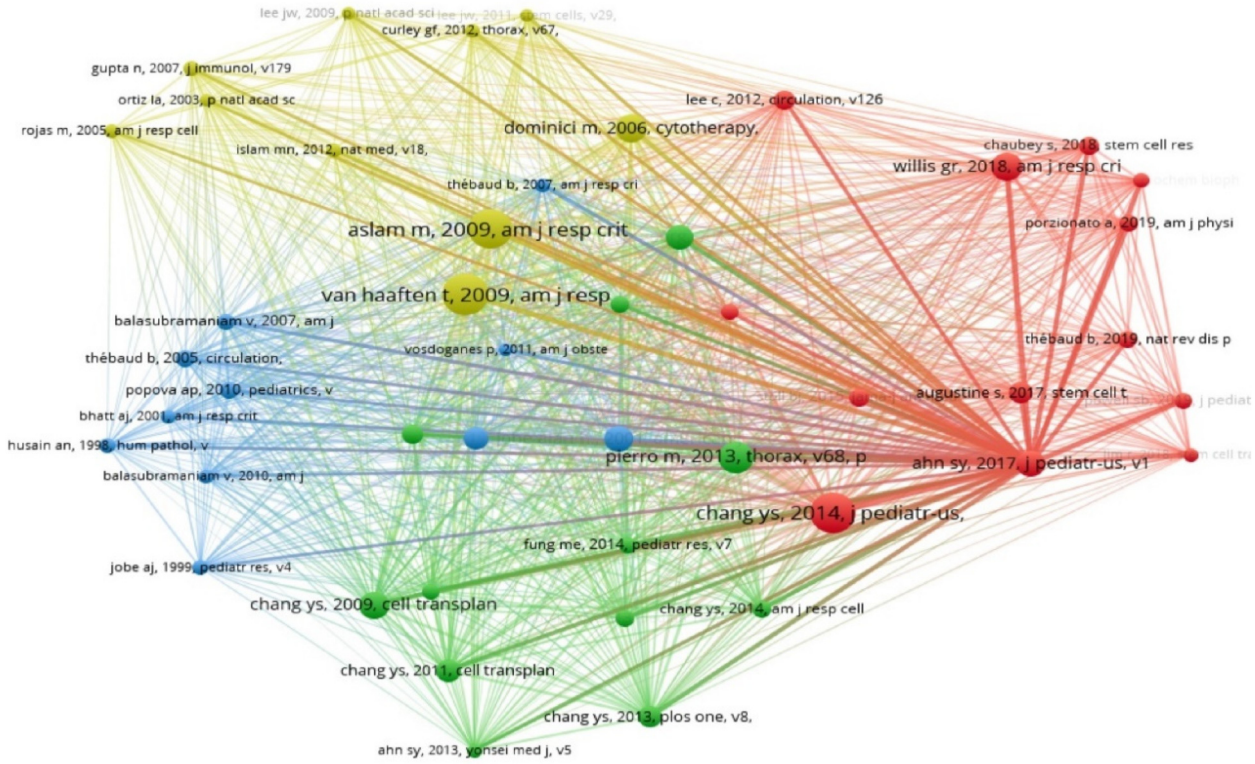


FIGURE 6
Illustrates the co-occurrence of co-cited references. This graph shows references cited over 30 times. Node size reflects occurrence frequency, while node color indicates the cluster of cited references. Clustering of co-cited references. Distinct colors denote separate clusters. Each node signifies a co-cited reference, with the number on the node denoting its cluster affiliation.

where abundant preclinical evidence has not yet led to proportional clinical investigation. When analyzed chronologically, the average year of publication for preclinical papers (2,013.4) precedes that of clinical papers (2,017.8) by over four years, suggesting a substantial lag in clinical translation. This translational challenge is reflected in the work of Baker et al. (41), who discuss the current status and future therapeutic potential of stem cell therapy for lung disease.

The cited literature was clustered into the above groups using Citespace software (Figure 6B). The clusters identified include "mesenchymal stem cell", "human mesenchymal stem cell", "pulmonary disease", "novel treatment approach", "bone marrow", "stem cell secretome", "resident lung stem cell", and "systematic review evidence map", highlighting the primary focus areas in MSC therapy for BPD over the last twenty years. The emergence of "stem cell secretome" as a cluster theme aligns with the work of Alphonse and Thébaud (42), who examined growth factors and stem cells in BPD pathogenesis and treatment.

Keyword analysis: identifying research hotspots and emerging trends

Figure 7 shows the keyword co-occurrence network view for the last five years, generated using Citespace. Node size reflects frequency of occurrence, with larger nodes indicating research hotspots. The most frequently occurring keywords are as follows: "bronchopulmonary dysplasia", "mesenchymal stem cells", "therapy", "brain injury", "preterm infants", "respiratory distress syndrome", "pulmonary hypertension", "animal models", "bone marrow", "extracellular vesicles" and so on. The emergence of "brain injury" as a significant keyword reflects the growing recognition of MSCs' potential for addressing multiple complications of prematurity simultaneously, as demonstrated by Kim et al. (29), who showed that intratracheal MSC transplantation can attenuate both lung and brain injuries in hyperoxic newborn rats.

Temporal analysis of keyword occurrence reveals distinct research phases in the MSC-BPD field. From 2004 to 2010, frequently occurring terms primarily focused on "bone marrow", "animal models", and "lung development", reflecting early foundational work. The 2011–2017 period saw increased emphasis on "inflammation", "hyperoxia", and "umbilical cord", corresponding with expanded preclinical investigations into different MSC sources and mechanisms. Since 2018, "extracellular vesicles", "exosomes", and "microvesicles" have rapidly increased in frequency, indicating a shift toward cell-free therapeutic approaches. This evolution suggests the field is progressing from proof-of-concept studies toward more refined and potentially more translatable therapeutic strategies. The

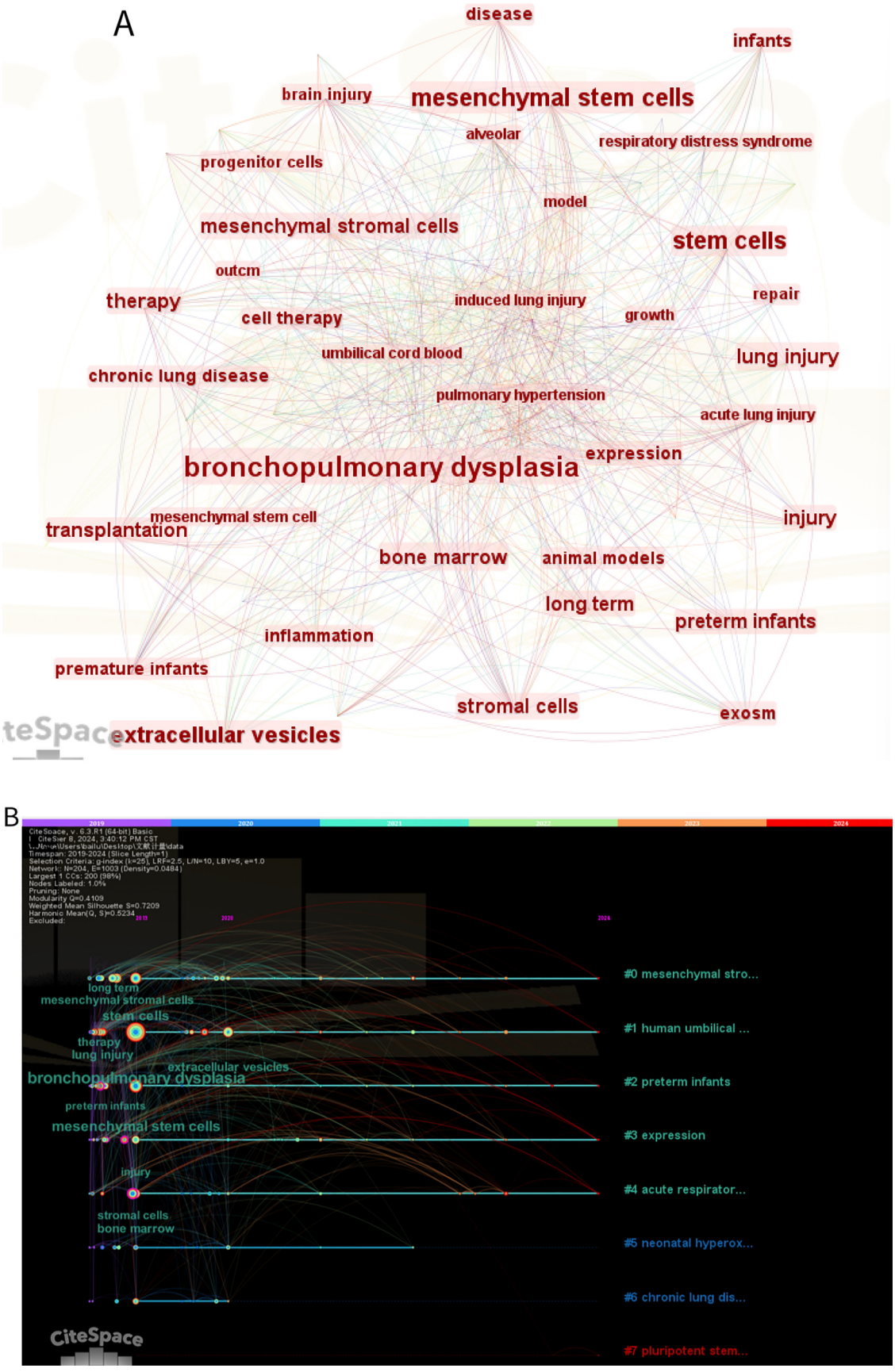


FIGURE 7
(Continued)

C

Keywords	Year	Strength	Begin	End	2019 - 2024
respiratory distress syndrome	2019	1.49	2019	2021	
clinical trial	2019	1.23	2019	2020	
prevention	2019	1.23	2019	2020	
repair	2019	1.22	2019	2020	
microvesicles	2020	1.73	2020	2021	
survival	2020	1.44	2020	2021	
mesenchymal stromal cell	2020	1.44	2020	2021	
risk	2021	1.32	2021	2022	
regenerative medicine	2020	1.26	2021	2022	
lung development	2019	1.26	2021	2022	
necrotizing enterocolitis	2020	1.38	2022	2024	
umbilical cord blood	2020	1.37	2022	2024	

FIGURE 7
(A) Keywords hotspot statistics (2019−-2024); (B) keywords clustering timeline. For each cluster, the location of each node represents when the literature was published, and the node size represents the frequency of occurrence. (C) Top 12 keywords with the highest citation bursts.

investigation of specific administration routes, as examined by Sung et al. (30), has contributed to understanding optimal delivery methods for MSC therapy.

In order to elucidate the research topics that have recently emerged as prominent in this field over the past five years (2019–2024), 12 key words were identified through the Bursts (Burst Words) analysis function of CiteSpace. Figure 7C illustrates that the distribution of these burst words in time is relatively even, with no instances of a sudden emergence of a large number of burst words in a specific year. Of the identified burst words, “microvesicles’ exhibits the highest intensity. The emergence of “umbilical cord blood” and “NEC” from 2022 onwards suggests that this area has become a prominent research frontier in recent years. This interest in umbilical cord blood as an MSC source is reflected in Chang et al.’s (29) work demonstrating that human umbilical cord blood-derived MSCs can attenuate hyperoxia-induced lung injury in neonatal rats.

The burst detection analysis identifies terms that have experienced rapid increases in usage frequency, indicating emerging research frontiers. The term “microvesicles” shows the highest burst strength (8.45), followed by “exosomes” (6.21) and “umbilical cord blood” (5.87). The timing of these bursts (all occurring after 2020) suggests recent acceleration in interest surrounding cell-free therapies and specific MSC sources. The emergence of “NEC” (necrotizing enterocolitis) in 2022 indicates expanding interest in MSC applications beyond lung pathology to other neonatal conditions, potentially reflecting recognition of MSCs’ broad immunomodulatory properties that could benefit multiple preterm complications simultaneously.

Discussion

This bibliometric analysis provides a comprehensive mapping of the MSC therapy for BPD research landscape over the past two decades, revealing significant insights into the field’s structure, evolution, and current frontiers. The findings demonstrate robust growth in research output, particularly since 2015, coinciding with the publication of early clinical trial results that appear to have catalyzed increased interest. The field displays characteristics of a maturing research domain, including conformity to established bibliometric laws and development of distinct research clusters with specialized focus areas.

The field of mesenchymal stem cell (MSC) therapy for bronchopulmonary dysplasia (BPD) has witnessed remarkable growth and development in recent years. The research interest in MSCs for BPD remains high and stable, reflecting the ongoing promise of this approach in treating a complex neonatal lung disorder. MSC-based therapies are increasingly recognized as a viable solution to address the multiple challenges associated with

BPD, including lung inflammation, fibrosis, and impaired lung growth. Numerous studies have demonstrated that MSCs can attenuate lung injury, promote tissue repair, and improve outcomes in preclinical models of BPD (43, 44). The development of novel therapeutic strategies, including MSC-derived extracellular vesicles (EVs), and a continued focus on MSC-based therapies have contributed significantly to advancements in this field, offering new hope for treating BPD (45, 46). Strueby and Thébaud (45, 46) have been instrumental in highlighting the potential of MSC-based therapies for chronic lung disease of prematurity and identifying novel therapeutic approaches for BPD.

Our analysis reveals distinct structural characteristics in the research landscape. The field is characterized by concentrated expertise, with 37 core authors (2.2% of all authors) contributing nearly half of all publications. This concentration is further reflected geographically, with three countries (USA, China, and Canada) accounting for over 70% of research output. The visualization of collaboration networks reveals distinct research clusters that largely operate as independent entities, with limited cross-cluster integration. This pattern of research silos may impede knowledge exchange and potentially slow clinical translation. The identification of these structural features through bibliometric analysis provides valuable context for understanding the field's development trajectory and highlights opportunities for enhanced collaboration across research groups. These observations align with those of Thébaud et al. (44), who identified both benefits and obstacles to cell therapy in neonates and emphasized the importance of accelerating translation of research through collaborative frameworks.

Despite the promising preclinical results, the precise molecular mechanisms by which MSCs exert their therapeutic effects remain unclear. While the therapeutic potential of MSCs is evident, their exact mechanisms of action, including how they influence cellular processes such as inflammation, oxidative stress, and angiogenesis, require further exploration. MSC-secreted factors, such as cytokines, growth factors, and EVs, play a key role in mediating these effects. The role of lung mesenchymal stromal cells in both development and disease has been extensively studied (28). Collins and Thébaud (28) have provided important insights into how lung mesenchymal stromal cells serve and protect in development and disease. In particular, researchers have focused on preventing BPD through various novel approaches, such as preconditioning MSCs or exosome-based therapies, to enhance their reparative capabilities and improve lung function (47). Álvarez-Fuente et al. (47) have highlighted the new tools emerging for the old challenge of preventing BPD, including stem cell-based approaches.

Our keyword analysis and burst detection findings highlight a significant shift in research focus over the past decade. The field has evolved from early studies predominantly investigating whole-cell MSC therapies to a growing emphasis on MSC-derived extracellular vesicles, particularly exosomes. This trend is evidenced by the high burst strength of terms like "microvesicles" and "exosomes" in recent years, as well as the increasing citation rates for publications focused on these topics. The transition toward cell-free approaches potentially addresses several limitations of whole-cell therapies, including concerns about cell survival, immunogenicity, and standardization. This evolution reflects the field's maturation and progressive refinement of therapeutic strategies based on accumulated evidence. The bibliometric identification of this shift provides valuable insight into the field's trajectory and suggests promising directions for future investigation.

MSC therapy for BPD primarily relies on their paracrine effects, where MSC-derived factors, particularly EVs, facilitate tissue repair and reduce inflammation. The mechanism of action of these EVs, including their immunomodulatory, anti-inflammatory, and regenerative effects, remains an area of active investigation. Studies suggest that MSC-exosomes can exert therapeutic effects by modulating immune responses, promoting cell survival, and stimulating tissue regeneration. The use of MSC-derived exosomes as a cell-free therapy has shown promise in preventing and repairing lung injuries in various models of neonatal lung diseases (26). Moreover, MSC microvesicles have emerged as a new paradigm for cell-free therapies, which offer an alternative to direct MSC transplantation, overcoming some of the challenges related to cell engraftment and differentiation (13). Recent studies have demonstrated that antenatal MSC-exosome therapy can prevent lung injury caused by preeclampsia, further highlighting the broad therapeutic potential of MSC-derived EVs in treating neonatal lung injuries (48).

The co-citation analysis reveals three distinct knowledge domains contributing to the MSC-BPD field: stem cell biology, respiratory physiology, and clinical neonatology. While these domains are interconnected, our analysis indicates weaker linkages between basic stem cell research and clinical literature, suggesting potential knowledge translation gaps. This finding is further supported by the observed temporal lag between preclinical and clinical publications, with an average delay of over four years. The relatively small proportion of clinical studies (18.6% of highly cited papers) despite abundant preclinical evidence highlights a translational challenge characteristic of many cell-based therapy fields. Bibliometric identification of these patterns provides objective evidence of translational bottlenecks that may inform strategies to accelerate clinical implementation of promising preclinical findings.

In clinical applications, MSC-based therapies face several challenges. For example, hyperoxia-induced lung injury, a key model of BPD, has shown impaired angiogenic supportive capacity and altered gene expression profiles of resident CD146+ mesenchymal stromal cells in neonatal rat lungs. These changes may hinder the natural reparative process in the lungs, making it difficult for them to recover from injury (49). However, MSC-based therapies may improve these conditions by promoting angiogenesis and vascular remodeling in the lung tissue. Collins et al. (49) have demonstrated that resident CD146+ mesenchymal stromal cells isolated from hyperoxia-injured neonatal rat lungs show impaired angiogenic supportive capacity and altered gene expression profiles. Studies have also shown that human-induced pluripotent stem cell-derived lung progenitor and alveolar epithelial cells can attenuate hyperoxia-induced lung injury in

neonatal rats, suggesting that induced pluripotent stem cell (iPSC)-derived cells can complement MSC-based therapies in treating lung injuries (50). Shafa et al. (50) have shown that human induced pluripotent stem cell-derived lung progenitor and alveolar epithelial cells can attenuate hyperoxia-induced lung injury. Furthermore, preconditioning MSCs has been found to enhance their paracrine effects, boosting their ability to prevent oxygen-induced neonatal lung injury (51). Waszak et al. (51) have demonstrated that preconditioning enhances the paracrine effect of MSCs in preventing oxygen-induced neonatal lung injury in rats.

Our geographical analysis reveals significant disparities in research contributions and international collaboration. While the United States, China, and Canada lead in publication volume, there is limited representation from regions with high BPD burden, particularly in developing countries. Only 8.2% of international collaborations include partners from low and middle-income countries, suggesting a potential disconnect between research activity and global clinical needs. This geographical imbalance may limit the generalizability of research findings and hinder the global implementation of MSC therapies. Increasing international collaboration, particularly with researchers in regions with high BPD prevalence, could enhance both the relevance and impact of future research. The bibliometric identification of these patterns provides an evidential basis for developing targeted strategies to promote more inclusive international research engagement.

While MSC-based therapies are showing considerable promise, their clinical translation requires a better understanding of their mechanisms of action and the optimal delivery methods. In particular, the biodistribution of MSCs and their long-term retention in target tissues remain crucial factors that impact their therapeutic efficacy. Future studies should explore various administration routes (e.g., intravenous, intraperitoneal, or endotracheal) and assess how these methods influence the efficacy and biodistribution of MSC-derived EVs. Intratracheal administration of MSC-derived EVs is particularly promising for lung diseases, as these vesicles tend to accumulate in the lung tissue and promote self-renewal of lung progenitor cells, potentially enhancing lung function and recovery after injury (40). Lithopoulos et al. (40) have examined the pulmonary and neurologic effects of MSC extracellular vesicles in a multifactorial lung injury model, providing important insights into their therapeutic potential.

MSC-derived small extracellular vesicles (sEVs) have also demonstrated potential in restoring lung architecture and improving exercise capacity in various animal models of lung injury (52). These findings suggest that MSC-derived vesicles could be a promising therapy not only for BPD but also for other neonatal lung conditions, such as acute lung injury, respiratory distress syndrome (RDS), and pulmonary arterial hypertension (PAH) (53). Given the immunomodulatory, regenerative, and anti-inflammatory properties of MSC-derived EVs, their use could extend beyond BPD to other organ injuries associated with preterm birth, such as intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), and retinopathy of prematurity (ROP). Thus, MSC therapy and MSC-derived exosomes represent a broad and promising treatment approach for a range of preterm-related diseases.

The identification of "necrotizing enterocolitis" as an emerging keyword after 2022 suggests broadening interest in MSC applications beyond pulmonary pathology to other neonatal conditions. This expansion reflects growing recognition of the multisystem benefits that might be achieved through MSC's immunomodulatory and regenerative effects. The temporal analysis of research topics further reveals increasing attention to standardization and manufacturing considerations in recent publications, indicating progression toward clinical implementation concerns. These trends, objectively identified through bibliometric analysis, provide valuable indicators of the field's future directions and highlight the increasing translational focus of MSC-BPD research.

Limitations of this study

This bibliometric analysis has several limitations that should be considered when interpreting our findings. First, our reliance on a single database (Web of Science Core Collection) may have excluded relevant publications indexed exclusively in other databases such as PubMed, Scopus, or Embase. A multi-database approach would provide more comprehensive coverage of the literature. Second, our analysis was restricted to English-language publications, potentially overlooking valuable contributions in other languages, particularly from non-Western research communities. Third, citation metrics have inherent limitations as proxies for research quality or clinical relevance, as they may be influenced by factors such as author reputation, journal visibility, and citation practices. Fourth, while we attempted to normalize citation metrics by publication age, other confounding factors such as journal impact factor and author self-citation were not fully accounted for. Finally, bibliometric analysis captures the structural characteristics of published research but cannot directly assess the quality, methodological rigor, or clinical relevance of individual studies. Future analyses could be strengthened by combining bibliometric approaches with systematic review methodologies to provide more comprehensive evaluation of the evidence base.

In summary, the use of mesenchymal stem cells and their secreted factors, especially extracellular vesicles, holds great promise for the treatment of bronchopulmonary dysplasia and other neonatal lung diseases. However, further research is required to understand their mechanisms of action, improve delivery methods, and evaluate long-term therapeutic effects in clinical settings. With continued advancements in MSC biology and cell-free therapies, MSC-based treatments could become a cornerstone of neonatal care, offering hope for the prevention and treatment of BPD and related disorders in preterm infants.

Conclusion

This bibliometric analysis provides a comprehensive mapping of the research landscape surrounding mesenchymal stem cell therapy for bronchopulmonary dysplasia over the past two

decades. Our findings reveal several key insights into the field's evolution, structure, and current frontiers:

First, the field has demonstrated substantial growth, particularly since 2015, with research output showing a compound annual growth rate of 18.2%. Publication patterns adhere to established bibliometric distributions, indicating development of a mature research domain despite its relatively recent emergence.

Second, the intellectual structure of the field comprises three distinct knowledge domains—stem cell biology, respiratory physiology, and clinical neonatology—with evidence of suboptimal integration between basic science and clinical research. This translational gap is further evidenced by the temporal lag between preclinical and clinical publications and the relatively small proportion (18.6%) of clinical studies among highly cited papers.

Third, our analysis demonstrates a clear shift in therapeutic focus from whole-cell MSC applications toward MSC-derived extracellular vesicles, particularly since 2018. This trend, identified through keyword burst analysis and citation patterns, reflects the field's refinement toward potentially more translatable cell-free therapeutic approaches.

Fourth, the research landscape shows significant geographical and institutional concentration, with limited representation from regions with high BPD burden. Enhanced international collaboration could improve both the relevance and implementation of research findings globally.

Fifth, emerging keywords indicate expansion of MSC applications beyond pulmonary pathology to other neonatal conditions, suggesting recognition of broader potential benefits of MSC therapies for preterm infants.

These findings provide an evidential basis for directing future research efforts toward: (1) strengthening translational pipelines between preclinical and clinical investigation; (2) standardizing isolation, characterization, and administration protocols for MSC-derived extracellular vesicles; (3) expanding international research collaborations, particularly with developing regions; (4) investigating mechanisms of action through integrated multi-omics approaches; and (5) designing pragmatic clinical trials that address implementation challenges in diverse healthcare settings.

By objectively mapping the structure and evolution of MSC-BPD research, this bibliometric analysis offers valuable insights for researchers, funding agencies, and policymakers seeking to advance this promising therapeutic approach for a devastating neonatal condition.

Author contributions

LB: Data curation, Methodology, Writing – original draft, Writing – review & editing. YX: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

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References

1. Van Eck NJ, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics*. (2010) 84(2):523–38. doi: 10.1007/s11192-009-0146-3

2. Chen C, Ibekwe-SanJuan F, Hou J. The structure and dynamics of cocitation clusters: a multiple-perspective cocitation analysis. *J Am Soc Inf Sci Technol*. (2010) 61(7):1386–409. doi: 10.1002/asi.21309

3. Aria M, Cuccurullo C. Bibliometrix: an R-tool for comprehensive science mapping analysis. *J Informetr*. (2017) 11(4):959–75. doi: 10.1016/j.joi.2017.08.007

4. Northway WH Jr, Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease: bronchopulmonary dysplasia. *N Engl J Med*. (1967) 276(7):357–68. doi: 10.1056/NEJM196702162760701

5. Thébaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, et al. Bronchopulmonary dysplasia. *Nat Rev Dis Primers*. (2019) 5(1):78. doi: 10.1038/s41572-019-0127-7

6. Shao XM, Ye HM, Qiu XS. *Practice of Neonatology*. Beijing: People's Medical Publishing House (2019).

7. Jensen EA, Schmidt B. Epidemiology of bronchopulmonary dysplasia. *Birth Defects Res A Clin Mol Teratol*. (2014) 100(3):145–57. doi: 10.1002/bdra.23235

8. McCulloch EA, Till JE. The radiation sensitivity of normal mouse bone marrow cells, determined by quantitative marrow transplantation into irradiated mice. *Radiat Res*. (1960) 13(1):115–25. doi: 10.2307/3570877

9. Thébaud B. Mesenchymal stromal cell therapy for respiratory complications of extreme prematurity. *Am J Perinatol*. (2018) 35(06):566–9. doi: 10.1055/s-0038-1639371

10. Chang YS, Ahn SY, Yoo HS, Sung SI, Choi SJ, Oh WI, et al. Mesenchymal stem cells for bronchopulmonary dysplasia: phase 1 dose-escalation clinical trial. *J Pediatr*. (2014) 164(5):966–72. doi: 10.1016/j.jpeds.2013.12.011

11. Ahn SY, Chang YS, Kim JH, Sung SI, Park WS. Two-year follow-up outcomes of premature infants enrolled in the phase I trial of mesenchymal stem cells transplantation for bronchopulmonary dysplasia. *J Pediatr*. (2017) 185:49–54. doi: 10.1016/j.jpeds.2017.02.061

12. Thébaud B. Stem cells for extreme prematurity. *Am J Perinatol*. (2019) 36(S02): S68–73. doi: 10.1055/s-0039-1691774

13. Sdrimas K, Kourembanas S. MSC microvesicles for the treatment of lung disease: a new paradigm for cell-free therapy. *Antioxid Redox Signal*. (2014) 21(13):1905–15. doi: 10.1089/ars.2013.5784

14. Ahn SY, Chang YS, Lee MH, Sung SI, Lee BS, Kim KS, et al. Stem cells for bronchopulmonary dysplasia in preterm infants: a randomized controlled phase II trial. *Stem Cells Transl Med*. (2021) 10(8):1129–37. doi: 10.1002/sctm.20-0330

15. Tung S, Delavogia E, Fernandez-Gonzalez A, Mitsialis SA, Kourembanas S. Harnessing the therapeutic potential of the stem cell secretome in neonatal diseases. *Semin Perinatol*. (2023) 47(3):151730. doi: 10.1016/j.semperi.2023.151730

16. Hess DJ. *Science Studies: An Advanced Introduction*. New York, NY: NYU Press (1997).

17. Zhu J, Liu W. A tale of two databases: the use of web of science and scopus in academic papers. *Scientometrics*. (2020) 123(1):321–35. doi: 10.1007/s11192-020-03387-8

18. Yeung AWK, Mozos I. The innovative and sustainable use of dental panoramic radiographs for the detection of osteoporosis. *Int J Environ Res Public Health*. (2020) 17(7):2449. doi: 10.3390/ijerph17072449

19. Zhang XL, Zheng Y, Xia ML, Wu YN, Liu XJ, Xie SK, et al. Knowledge domain and emerging trends in vinegar research: a bibliometric review of the literature from WoSCC. *Foods*. (2020) 9(2):166. doi: 10.3390/foods9020166

20. Chen C. Citespace II: detecting and visualizing emerging trends and transient patterns in scientific literature. *J Am Soc Inf Sci Technol*. (2006) 57(3):359–77. doi: 10.1002/asi.20317

21. Aslam M, Baveja R, Liang OD, Fernandez-Gonzalez A, Lee C, Mitsialis SA, et al. Bone marrow stromal cells attenuate lung injury in a murine model of neonatal chronic lung disease. *Am J Respir Crit Care Med*. (2009) 180(11):1122–30. doi: 10.1164/rccm.200902-0242OC

22. van Haaften T, Byrne R, Bonnet S, Rochefort GY, Akabutu J, Bouchentouf M, et al. Airway delivery of mesenchymal stem cells prevents arrested alveolar growth in neonatal lung injury in rats. *Am J Respir Crit Care Med*. (2009) 180(11):1131–42. doi: 10.1164/rccm.200902-0179OC

23. Willis GR, Fernandez-Gonzalez A, Anastas J, Vitali SH, Liu X, Ericsson M, et al. Mesenchymal stromal cell exosomes ameliorate experimental bronchopulmonary dysplasia and restore lung function through macrophage immunomodulation. *Am J Respir Crit Care Med*. (2018) 197(1):104–16. doi: 10.1164/rccm.201705-0925OC

24. Qiu JP. Informetrics (IV) lecture 4 law of discrete distribution of literature information—Bradford's law. *Inf Stud Theory Appl*. (2000) 23(04):315–4 + 316–320. doi: 10.16353/j.cnki.1000-7490.2000.04.028

25. Bradford SC. Sources of information on specific subjects. *Engineering*. (1934) 137:85–6.

26. Willis GR, Reis M, Gheinani AH, Fernandez-Gonzalez A, Taglauer ES, Yeung V, et al. Extracellular vesicles protect the neonatal lung from hyperoxic injury through the epigenetic and transcriptomic reprogramming of myeloid cells. *Am J Respir Crit Care Med*. (2021) 204(12):1418–32. doi: 10.1164/rccm.202102-0329OC

27. Collins JJ, Thébaud B. Progenitor cells of the distal lung and their potential role in neonatal lung disease. *Birth Defects Res A Clin Mol Teratol*. (2014) 100(3):217–26. doi: 10.1002/bdra.23227

28. Collins JJ, Thébaud B. Lung mesenchymal stromal cells in development and disease: to serve and protect? *Antioxid Redox Signal*. (2014) 21(13):1849–62. doi: 10.1089/ars.2013.5781

29. Chang YS, Oh W, Choi SJ, Sung DK, Kim SY, Choi EY, et al. Human umbilical cord blood-derived mesenchymal stem cells attenuate hyperoxia-induced lung injury in neonatal rats. *Cell Transplant*. (2009) 18(8):869–86. doi: 10.3727/096368909X471189

30. Sung DK, Chang YS, Ahn SY, Sung SI, Yoo HS, Choi SJ, et al. Optimal route for human umbilical cord blood-derived mesenchymal stem cell transplantation to protect against neonatal hyperoxic lung injury: gene expression profiles and histopathology. *PLoS One*. (2015) 10(8):e0135574. doi: 10.1371/journal.pone.0135574

31. Kim YE, Park WS, Ahn SY, Sung DK, Chang YS. Intratracheal transplantation of mesenchymal stem cells attenuates hyperoxia-induced lung injury by down-regulating, but not direct inhibiting formyl peptide receptor 1 in the newborn mice. *PLoS One*. (2018) 13(10):e0206311. doi: 10.1371/journal.pone.0206311

32. Qiu JP. Informetrics (VI) lecture 6 laws of distribution of authors of literature information—Lotka's law. *Inf Stud Theory Appl*. (2000) 23(06):475–8. doi: 10.16353/j.cnki.1000-7490.2000.06.026

33. Qiu JP. Informetrics (V) lecture 5 laws of word frequency distribution of information in literature—Zipf's law. *Inf Stud Theory Appl*. (2000) 23(05):396–400. doi: 10.16353/j.cnki.1000-7490.2000.05.026

34. Lotka AJ. The frequency distribution of scientific productivity. *J Wash Acad Sci*. (1926) 16(12):317–23.

35. Park JH, Chang YS, Sung S, Ahn SY, Park WS. Trends in overall mortality, and timing and cause of death among extremely preterm infants near the limit of viability. *PLoS One*. (2017) 12(1):e0170220. doi: 10.1371/journal.pone.0170220

36. Kim YE, Ahn SY, Sung DK, Chang YS, Park WS. Mesenchymal stem cells and formyl peptide receptor 2 activity in hyperoxia-induced lung injury in newborn mice. *Int J Mol Sci*. (2022) 23(18):10604. doi: 10.3390/ijms231810604

37. Kim YE, Park WS, Sung DK, Ahn SY, Chang YS. Antenatal betamethasone enhanced the detrimental effects of postnatal dexamethasone on hyperoxic lung and brain injuries in newborn rats. *PLoS One*. (2019) 14(8):e0221847. doi: 10.1371/journal.pone.0221847

38. Kim YE, Park WS, Sung DK, Ahn SY, Sung SI, Yoo HS, et al. Intratracheal transplantation of mesenchymal stem cells simultaneously attenuates both lung and brain injuries in hyperoxic newborn rats. *Pediatr Res*. (2016) 80(3):415–24. doi: 10.1038/pr.2016.88

39. Chang YS, Ahn SY, Jeon HB, Sung DK, Kim ES, Sung SI, et al. Critical role of vascular endothelial growth factor secreted by mesenchymal stem cells in hyperoxic lung injury. *Am J Respir Cell Mol Biol*. (2014) 51(3):391–9. doi: 10.1165/rcmb.2013-0385OC

40. Lithopoulos MA, Strueby L, O'Reilly M, Zhong S, Möbius MA, Eaton F, et al. Pulmonary and neurologic effects of mesenchymal stromal cell extracellular vesicles in a multifactorial lung injury model. *Am J Respir Crit Care Med*. (2022) 205(10):1186–201. doi: 10.1164/rccm.202012-4520OC

41. Baker HB, McQuilling JP, King NM. Stem cell therapy for lung disease: current status and future therapeutic potential. *Stem Cells Int*. (2016) 2016:5025901. doi: 10.1155/2016/5025901

42. Alphonse RS, Thébaud B. Growth factors, stem cells and bronchopulmonary dysplasia. *Neonatology*. (2011) 99(4):326–37. doi: 10.1159/000326621

43. Thébaud B. Stem cell-based therapies in neonatology: a new hope. *Arch Dis Child Fetal Neonatal Ed*. (2018) 103(6):F583–8. doi: 10.1136/archdischild-2017-314451

44. Thébaud B, Lalu M, Renesme L, Katwyk S, Presseau J, Thavorn K, et al. Benefits and obstacles to cell therapy in neonates: the INCuBAToR (innovative neonatal cellular therapy for bronchopulmonary dysplasia: accelerating translation of research). *Stem Cells Transl Med*. (2021) 10(7):968–75. doi: 10.1002/sctm.20-0508

45. Strueby L, Thebaud B. Novel therapeutics for bronchopulmonary dysplasia. *Curr Opin Pediatr*. (2018) 30(3):378–83. doi: 10.1097/MOP.0000000000000613

46. Strueby L, Thebaud B. Mesenchymal stromal cell-based therapies for chronic lung disease of prematurity. *Am J Perinatol*. (2016) 33(11):1043–9. doi: 10.1055/s-0036-1586115

47. Álvarez-Fuente M, Moreno L, Mitchell JA, Reiss IK, Lopez P, Elorza D, et al. Preventing bronchopulmonary dysplasia: new tools for an old challenge. *Pediatr Res*. (2019) 85(4):432–41. doi: 10.1038/s41390-018-0228-0

48. Taglauer ES, Fernandez-Gonzalez A, Willis GR, Reis M, Yeung V, Liu X, et al. Antenatal mesenchymal stromal cell extracellular vesicle therapy prevents preeclamptic lung injury in mice. *Am J Respir Cell Mol Biol*. (2022) 66(1):86–95. doi: 10.1165/rcmb.2021-0307OC

49. Collins JJ, Lithopoulos MA, Dos Santos CC, Issa N, Möbius MA, Ito C, et al. Impaired angiogenic supportive capacity and altered gene expression profile of resident CD146+ mesenchymal stromal cells isolated from hyperoxia-injured neonatal rat lungs. *Stem Cells Dev*. (2018) 27(16):1109–24. doi: 10.1089/scd.2017.0145

50. Shafa M, Ionescu LI, Vadivel A, Collins JJ, Xu L, Zhong S, et al. Human induced pluripotent stem cell–derived lung progenitor and alveolar epithelial cells attenuate hyperoxia-induced lung injury. *Cytotherapy*. (2018) 20(1):108–25. doi: 10.1016/j.jcyt.2017.09.003

51. Waszak P, Alphonse R, Vadivel A, Ionescu L, Eaton F, Thébaud B. Preconditioning enhances the paracrine effect of mesenchymal stem cells in preventing oxygen-induced neonatal lung injury in rats. *Stem Cells Dev*. (2012) 21(15):2789–97. doi: 10.1089/scd.2010.0566

52. Willis GR, Fernandez-Gonzalez A, Reis M, Yeung V, Liu X, Ericsson M, et al. Mesenchymal stromal cell-derived small extracellular vesicles restore lung architecture and improve exercise capacity in a model of neonatal hyperoxia-induced lung injury. *J Extracell Vesicles*. (2020) 9(1):1790874. doi: 10.1080/20013078.2020.1790874

53. Kim YE, Ahn SY, Sung SI, Yang M, Sung DK, Park WS, et al. Mesenchymal stem cells reduce the extracellular mitochondrial DNA-mediated TLR9 activation in neonatal hyperoxia-induced lung injury. *Biomedicines*. (2024) 12(3):686. doi: 10.3390/biomedicines12030686

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From laboratory to clinic: a precise treatment strategy of mesenchymal stem cells-derived exosomes pretreated by simulating disease microenvironment

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Mesenchymal Stem Cells (MSCs) and their secreted extracellular vesicles (EVs), particularly exosomes (Exos), have garnered significant attention for their potential in tissue repair, fibrosis, and tumor therapy. However, the therapeutic efficacy of mesenchymal stem cell-derived exosomes (MSC-Exos) is notably influenced by the disease-specific microenvironment. This review examines the mechanisms of action of MSCs and MSC-Exos in various diseases and analyzes the impact of inflammatory preconditioning on the functions and paracrine signaling of MSCs. We propose a personalized MSC preconditioning strategy based on the characteristics of the disease microenvironment to enhance the precision and efficacy of MSC-Exos therapy. Additionally, we discuss the limitations of traditional preconditioning strategies and introduce novel approaches for MSC preconditioning by simulating the disease microenvironment, such as using tissue homogenates and EVs derived from diseased tissues. These methods more accurately reflect the spatiotemporal features of the disease microenvironment, thereby improving the therapeutic potential of MSC-Exos. Finally, we explore the application of engineered exosomes loaded with key miRNAs targeting disease treatment, offering new insights for precision medicine.

KEYWORDS

mesenchymal stem cells, exosomes, disease microenvironment, inflammation, hypoxia, tissue repair, precision medicine, miRNA delivery

1 Introduction

In recent years, the rapid advancement of regenerative medicine and cell therapy technologies has propelled mesenchymal stem cells (MSCs) and their derivatives, particularly exosomes, to the forefront of novel therapeutic strategies (1–3). Studies have demonstrated that exosomes (MSCs-Exos) secreted by mesenchymal stem cells (MSCs) exhibit biological effects akin to those of MSCs (4–6). In the process of wound repair, MSCs-Exos can enhance the local microenvironment by modulating inflammatory responses, promoting angiogenesis, and facilitating cell proliferation and migration, ultimately contributing to improved wound healing (7–9). Compared with MSCs, MSC-Exos possess several advantages, including high efficiency, long-term stable storage, ease of transportation, and dose control, and almost no risk of tumor and thrombosis (10). These advantages make MSC-Exos regarded as an alternative treatment option to MSCs and have broad clinical application prospects (2, 11, 12). However, these biological agents exhibit significant heterogeneity in actual use. It is generally believed that cell source, culture environmental factors, and the local pathological microenvironment at the transplantation site affect cell growth, function, and the paracrine system (13–15).

Exosomes are a subtype of extracellular vesicles (EVs) with a lipid bilayer structure and sizes ranging from 30 to 150 nm. According to the 2014 guidelines on the " Minimum experimental requirements for extracellular vesicles and their functions" (MISEV2014) established by the International Society for Extracellular Vesicles (ISEV), the term "exosome" has emerged as the most frequently used descriptor for "extracellular vesicles" in the literature since 2004. In contrast, "EVs," introduced by the International Society for Extracellular Vesicles (ISEV) in 2011, refers to a heterogeneous group of particles released from cells through various pathways and involved in intercellular communication (16). The 2018 ISEV guidelines (MISEV2018) defined EVs as lipid bilayer-enclosed particles lacking a functional nucleus. EVs include exosomes, large vesicles, microvesicles, microparticles, and apoptotic bodies. However, specific markers for these subtypes remain elusive due to challenges in identifying subcellular origins. The guidelines recommend naming EV subtypes based on physical characteristics (e.g., size or density), specific surface markers (e.g., CD63+/CD81+), or conditions/cellular sources (e.g., hypoxic EVs) Despite ongoing nomenclature debates, the term "exosome" is still widely used (17). Despite the ongoing debate regarding nomenclature, the therapeutic potential of these subcellular components remains unchanged, and the term "exosome" continues to be widely adopted by researchers due to its familiarity. Notably, the 2023 guidelines on extracellular vesicles underscore the necessity for precise terminology and experimental rigor in EV research, emphasizing the importance of distinguishing between different EV subtypes based on their biophysical properties and biological functions. This precision is essential for advancing our understanding of EV biology and optimizing their therapeutic applications (18) (Figure 1).

MSC-Exos are capable of carrying a diverse array of biologically active molecules, including proteins, mRNA, and miRNA (19). They can not only transmit signal factors to regulate the growth, differentiation, and function of target cells but also serve as nano-carriers for targeted drug delivery, thus being widely applied in tissue repair, inflammation reduction, and fibrosis inhibition (20, 21). Although MSC-derived extracellular vesicles (MSCs-Exos) have demonstrated significant potential in basic research, their clinical application continues to encounter numerous challenges. Most current studies focus on culturing MSCs under traditional conditions to isolate extracellular vesicles (EVs) and subsequently investigate their therapeutic effects on various diseases (22). While notable efficacy has been observed, this approach may overlook the influence of the disease microenvironment on MSCs. A critical issue is that the biological effects of MSCs-Exos exhibit considerable variation across different disease microenvironments (13).

For instance, in chronic wounds, MSCs upregulate the expression of vascular endothelial growth factor (VEGF), thereby promoting angiogenesis and facilitating wound healing (23). Conversely, in malignant glioma, MSCs downregulate VEGF expression within tumor tissue, which serves to inhibit tumor growth (24). Even within the same disease context, adipose mesenchymal stem cells can enhance collagen I and III expression in fibroblasts when transplanted into open wounds, accelerating collagen synthesis and granulation tissue formation to promote wound healing (25). However, when these cells are transplanted post-epithelialization, their paracrine effects lead to a downregulation of collagen synthesis, counteracting fibrosis and scar formation (26). This discrepancy indicates that the therapeutic efficacy of MSCs-Exos is not solely reliant on their intrinsic biological properties but is also intricately linked to the 'temporal' and 'spatial' characteristics of the disease microenvironment (27). We have synthesized our thinking on this finding, current research on mesenchymal stem cell-derived exosomes (MSC-Exos) ignores an important scientific issue: the lack of targeted induction or preconditioning of MSCs for therapeutic use in disease pathological microenvironments. In particular, it is not fully considered that when mesenchymal stem cells are used as cell grafts, their paracrine composition and biological effects may change depending on the pathological microenvironment of the disease.

Abbreviations: MSCs, Mesenchymal stem cells; HUC-MSC, Human umbilical cord mesenchymal stem cell; Exos, Exosomes; MSC-Exos, Mesenchymal stem cell-derived exosomes; EVs, Extracellular vesicles; ISEV, International Society for Extracellular Vesicles; VEGF, Vascular endothelial growth factor; 3D, Three-dimensional; ADSCs, Adipose-derived mesenchymal stem cells; BMSCs, Bone marrow mesenchymal stem cells; ECM, Extracellular matrix; CAFs, Carcinoma-associated fibroblasts; IL-1β, Interleukin-1β; IFN-γ, Interferon-γ; IL-8, Interleukin-8; miRNAs, microRNAs; NF-κB, Nuclear factor kappa-B; LPS, Lipopolysaccharide; TNF-α, Tumor necrosis factor-α; HIF-1α, Hypoxia-inducible factor-1; GMSCs, Gingiva-derived mesenchymal stem cells; SCI, Spinal cord injury; HUVECs, Human umbilical vein endothelial cells; BDNF, Brain-derived neurotrophic factor; GMP, Good Manufacturing Practice; NKT, Natural Killer T; HCC, Hepatocellular Carcinoma.

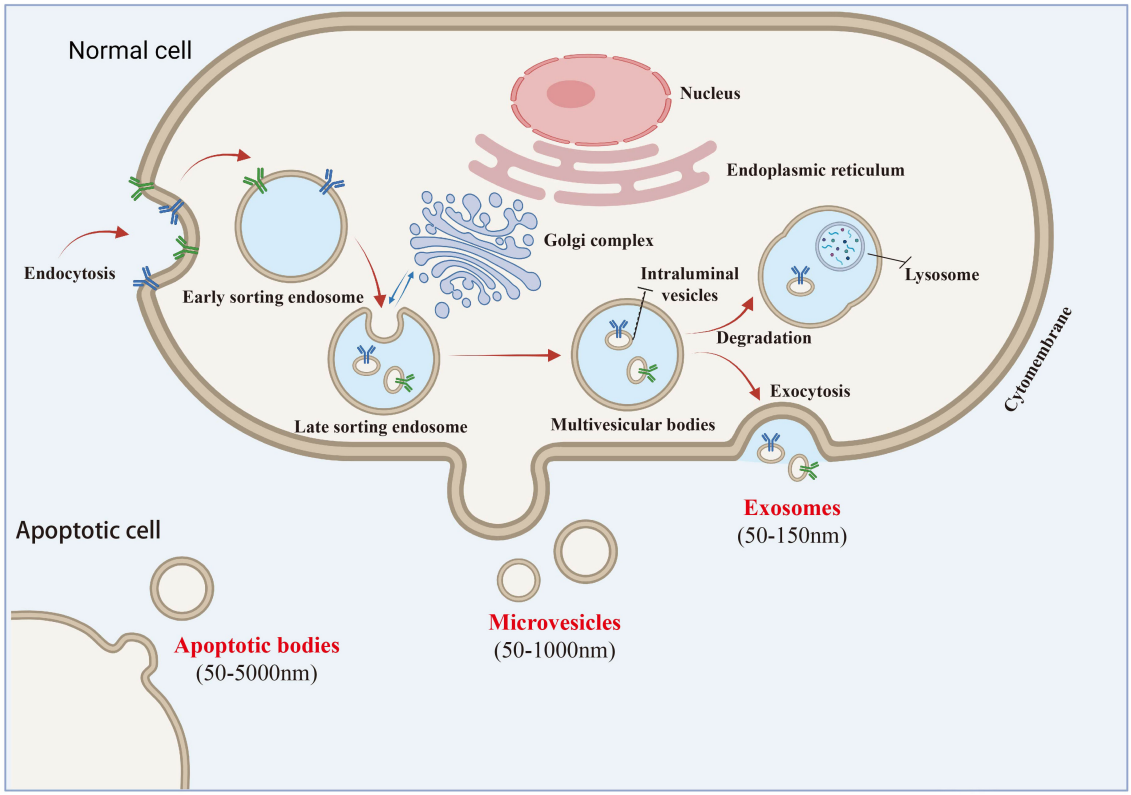


FIGURE 1
Extracellular vesicles and exosomes secretion pathway diagram.

Consequently, to enhance therapeutic outcomes, researchers have begun to pre-treat MSCs cultured *in vitro* in accordance with specific disease microenvironmental features (such as inflammation and hypoxia) to modify the biological functions of the EVs secreted by MSCs and improve their therapeutic efficacy in related diseases (28). To further enhance the therapeutic effect, researchers have started to pretreat MSCs cultured *in vitro* with certain characteristics of the disease microenvironment, such as inflammation and hypoxia, in order to alter the biological functions of EVs secreted by MSCs and improve their treatment effects on related diseases (28, 29). Additionally, three-dimensional (3D) cell culture technology has also achieved significant research results in MSCs pretreatment. Compared with two-dimensional culture, 3D culture can be closer to the *in vivo* environment, which helps maintain the pluripotency of MSCs and promotes the secretion of bioactive substances (30, 31).

Although traditional pretreatment strategies, such as inflammatory factor pretreatment, hypoxic culture and 3D culture, simulate certain characteristics of the disease microenvironment to some extent, they do not accurately replicate the 'personality' traits of this environment (32). Although the inflammation and hypoxia preconditioning to a certain extent simulates the pathological microenvironmental state of inflammation and hypoxia in some disease lesions, the temporal and spatial nature of the disease microenvironment is ignored. Consequently, the program exhibits a certain degree of lack of specificity, necessitating further refinement and optimization from the perspective of "precision" medicine.

In recent years, research has focused on optimizing the therapeutic effects of MSC-derived exosomes (MSCs-Exos) through the simulation of the disease microenvironment (33). For instance, the pretreatment of mesenchymal stem cells (MSCs) with the extract of tissue homogenate has been demonstrated to enhance the therapeutic effect by targeting the tissue (34). Furthermore, tissue-derived extracellular vesicles (EVs) are regarded as a more promising pretreatment solution, as they can more accurately reflect the characteristics of the disease microenvironment (35, 36). These studies provide a theoretical foundation for developing MSCs-Exos pretreatment strategies that are based on the disease microenvironment.

However, current research on MSC-derived exosome (MSCs-Exos) pretreatment strategies remains in its early stages, with numerous critical issues still to be addressed (37). For instance, how can we more accurately replicate the 'temporal' and 'spatial' characteristics of the disease microenvironment? Additionally, what engineering transformations can be implemented to further enhance the therapeutic efficacy of MSCs-Exos? Addressing these questions necessitates not only comprehensive foundational research but also interdisciplinary collaboration to facilitate the translation of findings from the laboratory to clinical settings. This review aims to systematically summarize the current advancements in MSCs-Exos pretreatment strategies in relation to the disease microenvironment, investigate the underlying molecular

mechanisms and regulatory factors, and evaluate the prospects and challenges associated with clinical translation. By organizing and analyzing existing research, we aspire to offer targeted recommendations for future investigations, advance exosome therapy from basic research to clinical application, and ultimately achieve the objectives of precision medicine.

Finally, taking complex wound repair as an example, we propose the viewpoint of optimizing the intervention scheme and therapeutic effect of MSCs-EVs treatment according to the pathological microenvironment characteristics of the disease to be treated. This hypothesis awaits verification by future experiments to further enhance the safety and effectiveness of mesenchymal stem cell treatment of diseases.

2 Mechanism of action of exosomes

2.1 Role of exosomes in intercellular communication

Exosomes play a crucial role in the transmission of information between cells. Molecules such as proteins, mRNA, and miRNA carried by exosomes can be internalized by recipient cells, thereby regulating their physiological functions (38). One of the key mechanisms by which extracellular vesicles (EVs) influence intercellular communication is through their ability to modulate the behavior of recipient cells. For instance, EVs derived from stem cells have been shown to promote microglial migration and modulate neuroinflammatory responses. This modulation occurs through specific receptor interactions, such as the activation of purinergic receptors (e.g., P2X4R) on microglial cells, which can enhance their motility and response to injury (39). By targeting these receptors or the signaling pathways they activate, researchers can potentially influence the efficacy of signal transmission via EVs. Furthermore, the composition of EVs can be manipulated to enhance their signaling capabilities. For example, the loading of specific proteins or RNAs into EVs can be optimized to ensure they convey the desired signals to recipient cells. This optimization can be achieved through various methods, including the genetic engineering of donor cells to overexpress certain molecules that are subsequently packaged into EVs (40). Additionally, the use of pharmacological agents to modulate the biogenesis and release of EVs can be explored to enhance their signaling potential. The microenvironment itself significantly impacts the efficacy of EV-mediated communication. Factors such as the presence of inflammatory cytokines, the composition of the extracellular matrix, and overall cellular density can influence the uptake of EVs by recipient cells and the effectiveness of their signal transmission. For instance, in the context of neuroinflammation, the presence of pro-inflammatory cytokines can enhance the uptake of EVs by microglia, thereby amplifying their signaling effects (41). Such mechanisms enable exosomes to selectively deliver specific bioactive molecules tailored to various pathological microenvironments, thereby modulating the physiological functions of recipient cells.

2.2 Application of MSC and MSC-Exos in different disease microenvironments

The mechanism of exosomes in various pathological microenvironments reflects their high adaptability and specificity (Figure 2). Sun et al. has shown that exosomes can selectively transport specific biological agents in accordance with different pathological contexts, thereby exerting distinct therapeutic effects (42).

2.2.1 Tissue repair

MSCs have been successfully applied in multiple tissue repair and disease scenarios. This process is associated with the ability of stem cells to differentiate into fibroblasts, chondrocytes, cardiomyocytes, and endothelial cells, thereby facilitating tissue repair (2, 43–45). For example, in chronic wound healing, MSCs function mainly by enhancing angiogenesis, promoting re-epithelialization, improving granulation formation, and accelerating wound healing (46). As of December 30, 2019, the Clinical Trials Database of the US National Institutes of Health (www.clinicaltrials.gov) has registered 38 clinical trials, mainly investigating the healing potential of MSCs for chronic wounds such as lower extremity ulcers and pressure sores (46). Additionally, in cardiovascular diseases such as myocardial infarction and heart failure, the therapeutic potential of MSCs has been confirmed by most studies. It promotes cardiomyocyte regeneration and angiogenesis through paracrine mechanisms, reverses dysfunction, and restores myocardial function (47). It is noteworthy that notably, adipose-derived mesenchymal stem cells (ADSCs) exhibit differences in their functions in wound treatment. During the process of tissue repair and injury, in the early stage of the same wound healing process, ADSCs can upregulate the expression of collagen I and III in fibroblasts, thereby accelerating collagen synthesis and granulation tissue formation and promoting wound healing (48, 49). However, after the epithelialization of the wound is completed, the paracrine effect of ADSCs in the later stage of healing manifests as downregulation of collagen synthesis and antagonism of fibrosis or scar formation (26, 50). These findings reveal the complex roles of mesenchymal stem cells under different disease and transplantation conditions.

Similarly, the use of MSC-Exos for tissue repair has also been verified. As an alternative cell-free therapeutic approach, MSC-Exos can transfer molecules such as mRNAs, miRNAs, proteins, and lipids to the local microenvironment and recipient cells in either a direct or indirect manner (51, 52). In so doing, they simulate the biological activity of mesenchymal stem cells, promote the activation of endogenous repair mechanisms, and accelerate tissue regeneration and functional restoration (53). For example, in wound repair, MSC-Exos enhance the biological characteristics of keratinocytes, fibroblasts, and endothelial cells (54). In myocardial repair, Exos have demonstrated therapeutic effects in preclinical models of ischemic heart disease (55).

Overall, MSCs and MSC-Exos function in tissue injury repair mainly by promoting angiogenesis, re-epithelialization, and granulation formation, as well as regulating immune and

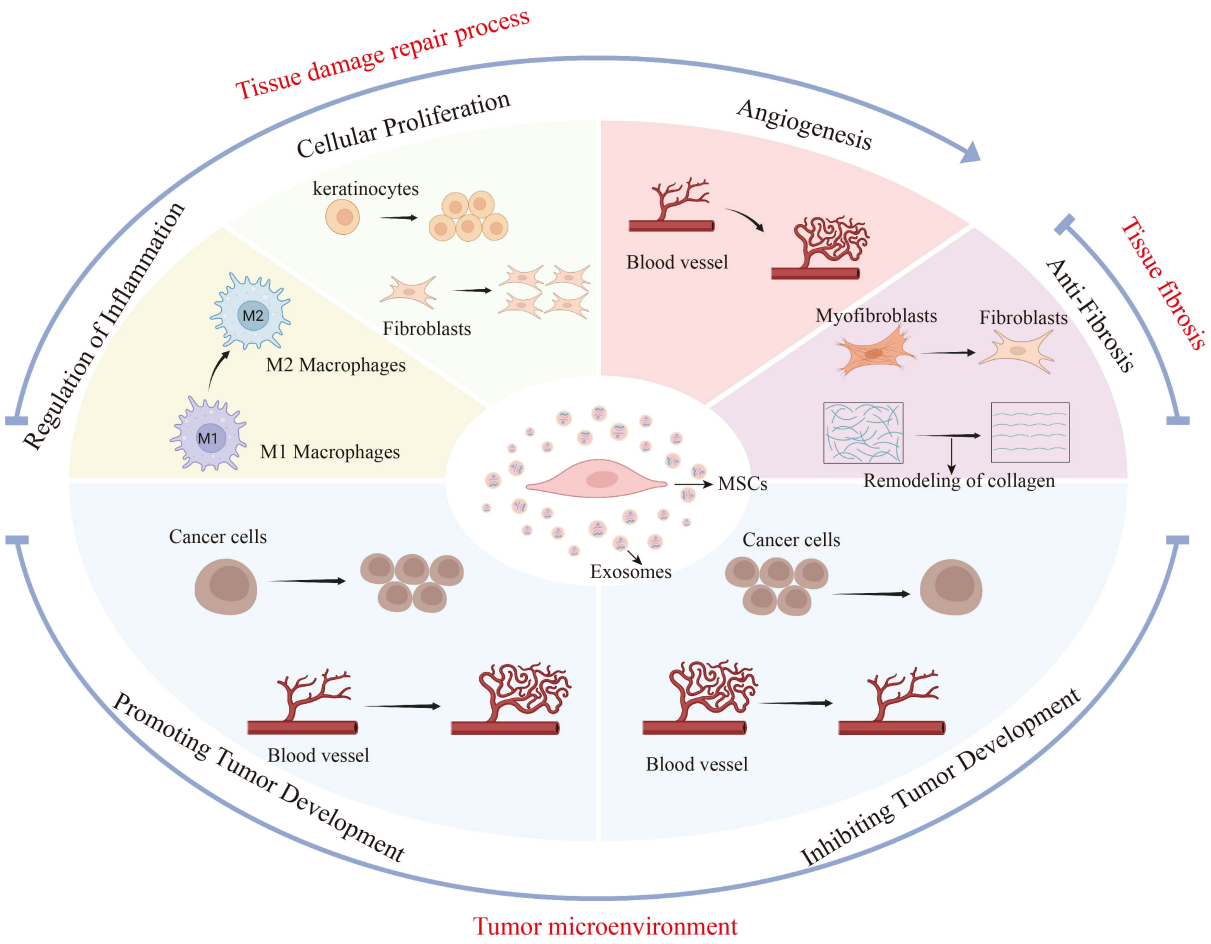


FIGURE 2
MSC-Exos have different biological effects in different disease microenvironments. This figure illustrates the diverse biological impacts of mesenchymal stem cell-derived exosomes (MSC-Exos) across various disease microenvironments, specifically in tissue repair, fibrosis, and tumor settings. During tissue repair, MSC-Exos regulate inflammation, fostering the shift from M1 to M2 macrophages, and enhance cellular proliferation and angiogenesis to expedite wound healing. In fibrosis, they counteract tissue stiffening by promoting myofibroblast-to-fibroblast transition and collagen remodeling. Within the tumor microenvironment, MSC-Exos may accelerate tumor progression by promoting tumor cell proliferation and angiogenesis. On the other hand, MSC-Exos also inhibited tumor progression by inhibiting tumor cell proliferation and angiogenesis. The figure highlights the influence of the disease microenvironment on the secretory effects of MSC-Exos and the possible promoting or inhibiting effects of MSC-Exos in different pathological states.

inflammatory responses and inhibiting apoptosis. The main repair mechanism of MSCs is the paracrine secretion of growth factors and cytokines. MSC-Exos, as bioactive vesicles secreted by MSCs, have potential as a new cell-free treatment.

2.2.2 Tissue fibrosis

MSCs have the potential to treat fibrotic diseases such as pulmonary fibrosis and liver fibrosis by regulating inflammatory responses, downregulating the expression of fibrosis-related genes, exerting anti-inflammatory and antioxidant effects, and promoting tissue repair (56–59). For example, ADSCs can reverse bleomycin-induced pulmonary fibrosis, as shown by decreased neutrophil infiltration, repair of damaged tissues, reduced collagen deposition, and inhibition of the NF-κB signaling pathway (60).

MSC-Exos exhibit similar therapeutic efficacy in fibrotic diseases (61). In a carbon tetrachloride (CCl4)-induced fibrotic liver model, HUC- MSC-Exos mitigated liver fibrosis by reducing the number of fibrous capsules, softening the texture, and decreasing inflammation and collagen deposition (62). This outcome is also thought to be associated with the TGF-β1/Smad2 signaling pathway (63, 64). Additionally, some studies have shown that MSC-Exos can alleviate fibrosis by reprogramming macrophage polarization by delivering miR-148a to macrophages and targeting the KLF6/STAT3 pathway (65). Moreover, MSC-Exos exert an antirenal fibrotic effect via miRNA-122a (66).

2.2.3 Tumor microenvironment

The use of MSCs and their Exos has led to certain advances in the treatment of tumors, yet there is also controversy (67–71). In terms of promoting tumors, bone marrow mesenchymal stem cells (BMSCs) can fuse with tumor cells through direct or indirect interactions and migrate to the tumor site, exhibiting a stronger proangiogenic effect *in vivo* and *in vitro* and promoting tumor growth (72). Another study revealed that the growth factors and

cytokines secreted by dental pulp MSCs increase the expression of Ki-67 in oral cancer cells, suggesting the possibility of promoting cancer development (73). However, MSCs also have antitumor effects. After entering the body of cancer patients, MSCs can effectively reduce the rate of cancer cell expansion by regulating immune components in the tumor environment (74). Research has also indicated that MSCs and HUC-MSCs can inhibit tumor growth by regulating the phenotype of inflammatory cells around tumors (75). Another study revealed that the secretome of HUC-MSCs exerts an anticancer effect by inducing the apoptosis of MCF-7 tumor cells (76). In addition, the antitumor activity of MSCs, which inhibits tumor angiogenesis, has also been proven (77).

Furthermore, MSC-Exos are capable of transmitting signal molecules to regulate tumor cell proliferation, angiogenesis, and metastasis (78), and they can also promote tumor resistance (79, 80). Additionally, MSC-Exos can also act as carriers for targeted tumor therapy (81–84). For example, BMSC-Exos loaded with doxorubicin target the treatment of osteosarcoma through the SDF1–CXCR4 axis (85).

As mentioned, MSCs and MSC-Exos can influence tumor progression through various pathways, exhibiting both pro-tumor and anti-tumor effects. On one hand, they promote tumor growth, angiogenesis, invasiveness, and drug resistance; on the other hand, they can inhibit tumors by modulating immune responses and intercellular signaling (86). This dual effect, while complicating tumor treatment, allows for personalized therapeutic strategies to be developed based on the patient's tumor type and pathological characteristics through the optimization of precise regulation of MSCs and MSC-Exos, providing additional support to existing treatment regimens (87). Although numerous studies have reported on the relevant effects of MSC-Exos, the biological transformations of MSC-Exos may vary significantly in different environments, and they contain various substances with complex biological activities, such as miRNAs, which show significant differences across different Exos Furthermore, Exos miRNAs participate in vast effect networks and may exert different or similar biological effects through various pathways. Therefore, our comprehensive understanding of their mechanisms remains very limited, and the controllability of their effects has yet to be fully validated. In the future, it is essential to explore more innovative research methods to further unveil their potential.

Further contradictions arise from the differences in experimental design across studies.Exos can be isolated from various tissues, including bone marrow, adipose tissue, umbilical cord, dental pulp, and placenta (88, 89). There are certain differences in the proteins, RNAs, and other components contained in MSC-Exos from different sources or with different pre-treatments (90). Any inconsistency at any stage may lead to varying effects on tumors. Moreover, different tumor types respond differently to the same MSC-Exos;For instance, exosomes derived from adipose-derived stem cells (ADSC-Exos) can enhance the anti-tumor effect of natural killer T (NKT) cells *in vivo*, which in turn contributes to the inhibition of hepatocellular carcinoma (HCC) (91) and they can also activate the Wnt signaling pathway to promote the migration of the MCF-7 human breast cancer cell line (92). The source, injection dosage, timing of administration, and tumor type of MSC-Exos collectively influence their biological effects in tumors (78). Additionally, the lack of standardized techniques for the extraction, purification, and storage of MSC-Exos further exacerbates the inconsistency of results (81). Therefore, there is a pressing need for a more comprehensive exploration of the mechanisms by which MSCs and MSC-Exos exert effects in tumors. It is essential to systematically elucidate the mechanisms of MSCs/MSC-Exos and establish standardized systems for preparation, storage, and functional assessment to enhance the reproducibility of research and accurately define their potential and risks in tumor therapy.

3 Temporal and spatial attributes of disease microenvironments

3.1 Complexity of the disease microenvironment

The complexity of the disease microenvironment is characterized by a diverse array of cell types that play crucial roles in disease progression (93, 94). We propose that, aside from laboratory conditions and systemic drug use, the varying effects exhibited by MSCs may stem from distinct pathological microenvironments associated with different organs and tissues, or varying stages of the same disease. These microenvironments possess unique features capable of inducing significant differences in the paracrine effects of MSCs. For instance, in the context of chronic wound healing, the micro-environment of wounds exhibits significant differences during the inflammatory, proliferation, and remodeling phases (95). During the inflammatory phase, the micro-loop is abundant in inflammatory cells and cytokines, such as TNF-α and IL-1β, which facilitate the removal of pathogens and necrotic tissue (96). In the proliferation phase, the microenvironment contains a wealth of growth factors and cells, alongside the extracellular matrix, which promotes cell proliferation and tissue repair (97). Finally, during the remodeling phase, the micro-ring is characterized by collagen deposition and tissue maturation (98).

Our review of the literature indicates that in chronic infections, inflammations, and cancers, the tissue microenvironment plays a crucial role in governing the behavior of local immune cells (99). Fibroblasts residing in diseased tissues are essential in regulating the activation or suppression of immune responses (100). These resident fibroblasts may originate from the circulation (including bone marrow-derived precursors and bone marrow-derived mesenchymal stem cells), local diseased tissues, or adjacent tissue cells such as adipocytes, adipose-derived mesenchymal stem cells, and pericytes (99).

3.2 Fibroblast heterogeneity and disease microenvironment

The fibroblast plays a crucial role in shaping the tissue microenvironment, and fibroblasts from different sources play distinct roles in disease progression (100–102). However, its

heterogeneity is closely linked not only to the tissue source but also to the specific characteristics of its anatomical location. A study identified 88 coding genes that were expressed at higher levels in lung fibroblasts relative to fibroblasts from other tissues, highlighting the unique transcriptional landscape associated with pulmonary fibroblast (103). Even fibroblasts from the same anatomical site can exhibit a range of phenotypes, influenced by local microenvironmental factor (104). For example, fibroblasts in the dermis can be categorized into distinct subpopulations, such as papillary and reticular fibroblasts, each contributing differently to the extracellular matrix (ECM) and tissue homeostasis (105). The papillary fibroblasts are known for their role in supporting epidermal maintenance, while reticular fibroblasts are more involved in the structural integrity of the dermis. Furthermore, the heterogeneity of fibroblast populations is closely associated with the overall health of the human body (106). In healthy tissues, fibroblasts maintain a balance that supports tissue homeostasis. However, in diseased states, the signals from damaged tissues, inflammatory cells, and the ECM can lead to the activation of fibroblasts and their subsequent differentiation into various subpopulations, each with distinct roles in the pathophysiology of the disease (107, 108).

Fibroblasts not only possess distinct tissue characteristics but also display significant temporal and spatial properties, enabling them to play rich and complex roles in the evolution and progression of diseases (106). Sole-Boldo et al. identified four populations of dermal fibroblasts through RNA-seq analysis, each with different functional transcriptome features and spatial distribution characteristics (109). Moreover, subbasal papillary fibroblasts have active Wnt signals, while reticular dermal fibroblasts highly express genes related to the ECM and immune signal transmission (106). Therefore, some researchers have summarized four key variables that determine the extensive heterogeneity of fibroblasts: tissue state, regional characteristics, microenvironment, and cell state (106). It is precisely the existence of this disparity that leads to the fact that even for the same disease, its pathological microenvironment will exhibit unique characteristics due to differences in disease location and disease stage.

For instance, in cases of tissue repair, organ fibrosis, and solid tumors, these diseases are closely associated with fibroblasts and each possesses a distinct pathological microenvironment (99). Particularly, carcinoma-associated fibroblasts (CAFs) have a strong connection between the heterogeneity of their subpopulations and the tumor microenvironment they reside in (110). This heterogeneity not only offers a new perspective for our understanding of tumor occurrence and development but also implies that there are tremendous opportunities and challenges on the path of future tumor precision medicine and individualized treatment.

Similarly, in skin wound repair, the external and internal environments of the skin wound are relatively independent yet interact with each other and are invariably closely associated with the dynamic pathophysiological changes throughout the repair process (111). Considering the alterations in the pathological microenvironment, it is believed that in the early stage of treatment after injury, greater emphasis might be placed on controlling inflammation and related cell proliferation. In contrast (112, 113), in the later stage, more attention may be required to focus on tissue repair and functional restoration. Generally speaking, the pathological microenvironment is a multifaceted entity that demands in-depth research to unveil its complexity and formulate effective treatment strategies.

In summary, fibroblasts, which are closely related to the disease microenvironment, exhibit extensive heterogeneity due to differences in tissue status, regional characteristics, cell status, and the microenvironment they inhabit. This heterogeneity can lead to variations in the pathological microenvironment of the same disease, depending on the organ or tissue involved and the stage of disease progression. Therefore, we speculate that the "unique" pathological microenvironment characteristics of diseases closely related to fibroblasts, such as wounds, cutaneous scars, organ fibrosis, and solid tumors, possess "tissue/site" attributes and "disease stage/progression" attributes. Transplanted MSCs can secrete intervention or therapeutic secretomes in response to the "unique" pathological microenvironments of the same disease at different sites (e.g., solid tumors at different locations) or even the same disease at the same site but at different stages of development (open wounds versus scars, or different stages of tumor progression). This may be one of the important reasons why MSCs have been proven to be effective in treating a variety of human diseases (2). Similarly, in addition to the above-mentioned laboratory factors, it may be that researchers have 'neglected' the 'temporal' and 'spatial' attributes of the disease microenvironment when studying the paracrine effects of MSCs, or that it is precisely because of the differences in 'time' and 'space' of the pathological microenvironment of the same disease or similar diseases that different researchers' research results are inconsistent (114).

4 The influence of pathological microenvironment on the secretion of MSC-Exos

4.1 Effect of pathological microenvironment on MSCs

Inflammation and hypoxia have been utilized as pretreatment approaches for MSCs cultured *in vitro* to boost the therapeutic effectiveness of corresponding MSCs-Exos for diverse diseases (28, 29). For example, the research of Yu et al. emphasizes that inflammatory cytokines such as interleukin-1β (IL-1β) and interferon-γ (IFN-γ) can impact the immune characteristics of human umbilical cord blood-derived MSCs (115). Antebi et al. reported that MSCs under hypoxic conditions possess enhanced therapeutic attributes (116, 117). Under short-term hypoxic circumstances, MSCs show accelerated cell proliferation, upregulated expression of VEGF, inhibited pro-inflammatory cytokine interleukin-8(IL-8), increased anti-inflammatory cytokine IL-1RA, and downregulated expression of apoptotic

genes BCL-2 and CASP3. On the contrary, under long-term hypoxic culture conditions, the proliferation rate of MSCs is significantly decelerated and the yield is lower. Continuous pathological stimulation may affect the long-term survival and biological functions of MSCs, leading to a reduction in therapeutic effects (118). These pretreatment strategies based on the characteristics of the pathological microenvironment have begun to emerge as an important means to enhance the biological effects of MSCs.

Furthermore, there are certain highly expressed factors in the pathological microenvironment, such as fibronectin and laminin, which might facilitate the adhesion and localization of MSCs (119). In tissue repair following injury, inflammation recruits MSCs to the damaged tissue. Simultaneously, the pro-inflammatory factors generated by MSCs play a crucial role in tissue repair (120). Yuan et al. also discussed that the changes in the local microenvironment of the liver are of great significance for the homing of MSCs and can enhance the efficacy of liver disease treatment (121). This indicates that inflammatory factors are involved in the recruitment of MSCs to the injured site and further supports the notion that the pathological microenvironment plays an important role in influencing the homing of MSCs.

4.2 Effect of pathological microenvironment on MSC-Exos secretion

The microenvironment in which MSCs reside has a profound influence on their paracrine signaling capacity and therapeutic efficacy (13). Research indicates that changes in the microenvironment, such as high levels of extracellular adenosine, can modify the secretome of MSCs and influence their interaction with other cell types (122). Recent studies have highlighted the significance of considering the microenvironment when investigating the potential therapeutic applications of MSC-Exos (123).

4.2.1 The pathological microenvironment alters the biological function of MSC-Exos

Specific factors within the pathological microenvironment can modify the composition of microRNAs (miRNAs) and proteins in extracellular vesicles of MSCs, thereby influencing the gene expression and function of recipient cells. For instance, Kim et al. investigated the anti-inflammatory effect and mechanism of extracellular vesicles secreted by MSCs pretreated with interleukin-1β (MSC-IL-Exos) in osteoarthritis SW982 cells. Compared to extracellular vesicles from MSCs without IL-1β treatment, MSC-IL-Exos not only inhibited the expression of pro-inflammatory cytokines but also enhanced the expression of anti-inflammatory factors. The study confirmed that MSC-IL-Exos mediate anti-inflammatory effects through miRNAs such as miR-147b, involving the inhibition of the nuclear factor kappa-B (NF-κB) pathway (124). The study by Cheng et al. also corroborated this. The EVs obtained by pretreating MSCs with IL-1β by their team enhanced the therapeutic effect of MSC-Exos in an *in vitro* lipopolysaccharide (LPS)-induced sepsis model. This indicates that pretreatment may enhance certain characteristics of Exos, thus playing a more effective role in the inflammatory response (125). In Han's review, it is also mentioned that MSCs pretreated with interferon-γ (IFNγ), tumor necrosis factor-α (TNFα), or IL-1 may exert their immune effects by altering the biological characteristics of the extracellular vesicles they secrete (126).

4.2.2 The pathological microenvironment regulates the secretion level of MSC-Exos

Certain factors within the pathological microenvironment can enhance the biological characteristics of Exos by elevating the secretion level of MSC-Exos. These pathological elements interact with the surface receptors of MSCs, activating signal transduction pathways related to the release of Exos and thereby promoting the secretion of Exos. Zhang et al. noted that under hypoxic conditions, hypoxia-inducible factor (HIF-1α) can mediate a significant upregulation in the expression of nSMase2 (127). And nSMase is a crucial regulator of Exos secretion, facilitating an increase in Exos secretion. Through increasing the secretion quantity of MSC-Exos, changes in the pathological microenvironment can not only raise the therapeutic concentration of Exos in the disease microenvironment but also endow Exos with more abundant biological characteristics, such as specificity. For instance, Nakao et al. discovered that pretreatment of human gingiva-derived mesenchymal stem cells (GMSCs) with TNF-α can not only increase the number of secreted Exos but also enhance the expression of CD73 in Exos, thereby inducing anti-inflammatory M2 macrophage polarization and strengthening the anti-inflammatory effect of Exos (128).

However, in the pathological microenvironment of tumors, the increased expression of MSC-Exos has a detrimental effect. The acidic condition in the tumor microenvironment may alter the secretion of Exos. Logozzi et al. verified and characterized the classic surface markers of Exos through Western Blotting and confirmed that the Exos released by tumor cells cultured in an acidic environment are significantly increased. Promoting the release of Exos means promoting and maintaining tumor progression (129). Wu et al. reported that the stiffness of the ECM is also one of the factors for changes in the pathological microenvironment. The rigid ECM can promote the release of Exos by tumor cells, which can activate the Notch signaling pathway and thereby promote tumor growth (130).

4.3 Summary

The complexity of the pathological microenvironment exerts multifaceted influences on MSCs and MSC-Exos. Thorough research is required to uncover its influence mechanism and offer new strategies for the precise treatment of diseases. The pathological microenvironment characteristic factors employed in existing studies as pretreatment methods for MSCs may not fully mimic

the actual pathological environment of diseases. Hence, it is essential to fully consider the heterogeneity of disease-related fibroblasts and study the interactions between fibroblasts and MSCs as well as MSC-Exos in different disease microenvironments. We are hopeful of providing new strategies and methods for the precise treatment of diseases. In the subsequent content, we will focus on three types of diseases with inflammatory pathological features, namely tissue injury repair, tissue fibrosis, and tumor diseases. We will analyze the treatment status of MSCs and their Exos and explore the therapeutic effects of MSC-Exos obtained after inflammatory pretreatment of MSCs in these three types of diseases.

5 Traditional preconditioning strategies and their limitations and challenges

5.1 Effect of inflammation pretreatment

Inflammatory factor pretreatment of MSCs will cause changes in the contents of their EVs, thereby exerting regulatory effects on cell communication and physiological processes. For example, TNF-α pretreatment can strengthen the osteogenic differentiation capacity of MSCs (131). Research has shown that exposing MSCs to inflammatory stimuli can lead to significant changes in their gene expression profiles, enhancing their ability to modulate immune responses and promote healing. Szűcs et al. have indicated that preconditioning MSCs with pro-inflammatory cytokines like TNF-α can elevate the expression of various interleukins, chemokines, and growth factors that are crucial for wound healing and tissue regeneration (132). This pre-activation can potentially lead to stronger wound healing responses and improved outcomes in conditions such as spinal cord injury (SCI) and other inflammatory diseases. Similarly, pretreatment with hypoxia and inflammatory factors (IL-1β, TNF-α, IFN-γ) can boost immunomodulatory effects without undermining biological properties (133).

However, owing to the complexity of the disease microenvironment, the therapeutic effects of MSCs following inflammatory pretreatment still vary in different diseases. One significant concern is the risk of inducing a pro-inflammatory phenotype in MSCs, which may lead to adverse effects rather than the desired therapeutic outcomes. The inflammatory environment can sometimes push MSCs towards a more inflammatory state, which could exacerbate tissue damage instead of promoting healing (132). Another limitation is the variability in response to inflammatory preconditioning among different MSC populations. Factors such as the source of MSCs (e.g., bone marrow, adipose tissue, umbilical cord), the specific inflammatory stimuli used, and the duration of exposure can all influence the outcome. This variability can complicate the standardization of preconditioning protocols, making it difficult to predict the behavior of MSCs in clinical settings (132, 134). From the perspective of precision medicine, we need to further refine and perfect pretreatment methods such as inflammation to increase the specificity of MSCs-Exos in treating different diseases and explore the mechanism of action of MSCs-Exos in diverse pathological microenvironments.

5.2 Effect of hypoxic pretreatment

Hypoxic preconditioning activates HIF-1α in MSCs by simulating the hypoxic microenvironment present *in vivo*, thereby enhancing the secretion of factors such as VEGF and HIF-1α. These factors play crucial roles in ischemic tissues, facilitating vascular neovascularization and tissue repair (135). Hypoxic preconditioning improves the therapeutic efficacy of MSCs-Exos. Research has shown that hypoxic preconditioning of human umbilical vein endothelial cells (HUVECs) enhances the angiogenic capacity of MSCs by stimulating the secretion of exosomes that promote MSC-mediated tube formation and effective nerve tissue repair in a rat model of spinal cord injury (136). Furthermore, Wang et al. have demonstrated that hypoxic preconditioning of BMSCs enhances the therapeutic efficacy of their derived exosomes (Hypo-Exos) in promoting facial nerve repair and regeneration (137).

Although hypoxic preconditioning has demonstrated significant efficacy in laboratory studies, it continues to face numerous challenges in clinical translation. First, variations in hypoxia concentrations and treatment durations significantly impact the biological properties of MSCs. The long-term effects of hypoxic preconditioning on stem cell behavior and function remain to be fully elucidated. While short-term exposure to hypoxia can enhance stem cell properties, prolonged hypoxic conditions may lead to unintended consequences, such as altered differentiation pathways or increased senescence, which could compromise the regenerative potential of the cells. Second, discrepancies in hypoxic conditions (e.g., oxygen concentration and treatment duration) employed across different laboratories have resulted in inconsistent findings, thereby affecting the feasibility of clinical translation (138–140).

5.3 Challenges

The existing pretreatment methods often overlook the unique characteristics of the disease microenvironment, which restricts their potential for clinical application (141). For instance, the addition of osteogenic factors can enhance the osteogenic differentiation of MSCs, while other factors may promote adipogenic or chondrogenic differentiation (142). the microenvironmental features of different diseases and individual patients can vary significantly, making it crucial to develop individualized pretreatment protocols tailored to specific circumstances in order to enhance therapeutic efficacy. Furthermore, pretreatment programs must achieve a higher level of precision to fulfill the requirements for clinical applications. For example, by simulating specific conditions present in the disease microenvironment (such as inflammation and hypoxia), preconditioning mesenchymal stem cells (MSCs) can

enhance the functionality of their secreted exosomes, thereby improving therapeutic outcomes (28, 29).

6 Preconditioning strategies by mimicking disease microenvironments

6.1 Pretreatment of homogenates based on disease tissue

The use of tissue homogenate supernatant to simulate a specific microenvironment is a common pretreatment method for MSCs. Xue et al. utilized mouse liver tissue homogenate supernatant to simulate the liver microenvironment and successfully induced HUC-MSCs to differentiate into liver-like cells with hepatocyte phenotype and function (143). Yang et al. also employed mouse brain tissue homogenate supernatant to simulate the brain tissue microenvironment, which can upregulate the secretion of brain-derived neurotrophic factor (BDNF) by progesterone-induced HUC-MSCs (144).

Additionally, Hao et al. used wound tissue homogenate supernatant in the inflammatory stage to simulate the wound microenvironment for pretreating MSCs. *In vitro* experiments demonstrated better effects on promoting fibroblast proliferation and migration than traditional culture conditions (145). At the same time, Wang et al. adopted wound tissue homogenate combined with inflammatory factors to pretreat MSCs (146). The results showed that MSC-Exs obtained by this approach are superior to those under traditional culture conditions in regulating wound inflammation and promoting repair. The above studies have confirmed our previous supposition that wound tissue homogenate simulates the pathological microenvironmental factors in the inflammatory stage of the wound. Moreover, the tissue homogenate combined with inflammation group is superior to the simple tissue homogenate group. Although using wound tissue homogenate supernatant to simulate the wound microenvironment can theoretically simulate the disease microenvironment to a certain extent, the disease microenvironment has "spatio-temporal" characteristics. Designing pretreatment schemes according to the spatio-temporal characteristics of wounds can become a future research direction.

6.2 Preprocessing based on disease tissue-derived EVs

Recent investigations have revealed that EVs originating from tissues hold extensive application prospects in disease research. For example, Brenna et al. characterized EVs within the brain tissue of mice under physiological states and following transient cerebral ischemia (147). Under steady-state conditions, microglia serve as the predominant source of EVs. In contrast, after cerebral ischemia, the major EVs are derived from astrocytes. Furthermore, the EVs released by astrocytes exhibit a further increase post-stroke. This research indicates that EVs sourced from brain ischemic tissue can reflect the pathological alterations in the brain tissue spatially following cerebral ischemia. These tissue-derived EVs are capable of reflecting tissue-specific pathological changes and participating in multiple physiological and pathological processes, such as angiogenesis and immune regulation. In comparison to EVs isolated from body fluids, EVs directly isolated from tissues possess advantages such as tissue specificity and accurately reflecting the tissue microenvironment (35, 36). In addition, currently, tumor tissue-derived EVs have been applied in multiple aspects, including tumor diagnosis, disease staging, progression assessment, and evaluation of treatment efficacy (148).

In conclusion, the relatively simple and controllable composition of tissue-derived EVs as key messengers for intercellular information exchange, the ability of their source cells to more accurately respond to the pathological state of disease by growing in the three-dimensional microenvironment of diseased tissues, and their property of analyzing the spatial and temporal heterogeneity of the tissue microenvironment, have collectively led to the establishment of preliminary guidelines for laboratory quality control and enhanced prospects for clinical translation (148). It is therefore anticipated that EVs derived from diseased tissue will become a more promising model for representing the pathological microenvironment of diseased tissues. To further substantiate our hypothesis, we propose that subsequent studies could employ disease tissue-derived EVs to mimic the *in vitro* microenvironment of the disease. Consequently, the EVs that can be obtained after intervening with the MSCs would be more specific for the treatment of the disease. This pre-treatment strategy of the MSCs is worthy of further investigation.

6.3 Challenges

Despite the significant potential demonstrated by the use of disease tissue-derived homogenates and Exos to simulate microenvironments for preconditioning MSCs, their reproducibility and standardization remain major bottlenecks for clinical implementation. Firstly, any research involving the procurement of human tissues must undergo rigorous ethical review; researchers are required to provide a detailed research plan outlining how tissue samples will be obtained and utilized, ensuring the privacy of donors is protected (149). This process often takes a considerable amount of time. In microenvironment simulations, tissue homogenates and EVs derived from healthy donors may serve as effective controls. However, it is sometimes challenging to collect healthy tissue or it may not meet ethical standards. Furthermore, when collecting pathological tissue, strict inclusion and exclusion criteria should be established in advance. In reality, the acquisition of high-quality disease tissue samples is very limited, and variations among different donors may affect the quality of tissue homogenates or EVs. Tissue homogenates themselves exhibit high complexity, as they contain a vast array of highly diverse information, such as DNA, RNA, proteins, lipids, and polar metabolites, with significant variations in their content and properties across different tissues and processing conditions (150). Although various preparation schemes have been

reported, the method for preparing tissue homogenates still requires continuous optimization. The variability in the methods for extracting EVs from tissue sources is influenced by multiple factors, including the type of tissue used, the effects of mechanical and enzymatic treatments on cells and EVs, the incubation time for tissue to release EVs, the number of separable Exos subpopulations, and the type of EVs isolation method employed (151, 152). Furthermore, different storage solvents, temperatures, and storage durations also impact the quality of EVs (153, 154). From 2014 to 2023, the evolution of the Minimal Information for Extracellular Vesicles (MISEV) guidelines reflects the ongoing efforts in this field to enhance research reproducibility and standardization (18).

In summary, advancing the preparation of tissue homogenates and tissue-derived EVs to clinical-grade Good Manufacturing Practice (GMP) production still faces multiple challenges. Donor heterogeneity and insufficient traceability of pathological tissue collection lead to significant inter-batch variability, affecting the consistency and reliability of the extracts. Furthermore, the lack of uniformity in ethical review processes and the absence of cross-center standards limit its widespread clinical application. Additionally, there is currently a lack of unified GMP-level production specifications, and critical quality attributes have not been clearly defined. To address these challenges, it is recommended to establish a rigorous donor screening and traceability system, while also formulating quality control standards and guidelines that comply with GMP requirements. These measures will help improve the quality, safety, and feasibility of its clinical translation.

7 Molecular mechanisms and regulatory factors

7.1 Role and regulation of miRNA

miRNAs in exosome form are pivotal molecules that regulate the function of receptor cells. miRNAs are a class of small non-coding RNAs that are able to regulate gene expression by binding to mRNAs, thereby either inhibiting their translation or promoting their degradation (155). Studies have demonstrated that miRNAs can be selectively absorbed by exosome and delivered to recipient cells to regulate their physiological activities (156). These non-coding molecules have been demonstrated to play pivotal roles in various aspects of cellular communication, including cell proliferation, differentiation, apoptosis, and inflammatory responses (156).

At present, EVs or exosome-derived miRNAs have become a focal point in research endeavors aimed at the diagnosis and treatment of a wide range of diseases, including tumors (157), trauma (158), and metabolic diseases (159). In the study of MSCs-Exos, researchers identified a variety of key microRNAs (miRNAs) that regulate therapeutic effects through high-throughput sequencing and bioinformatics analysis. These identified miRNAs play important roles in promoting tissue repair, suppressing inflammation, and regulating immune responses (160, 161). For instance, miR-126, which is highly expressed in MSC-Exos, has been shown to promote bone fracture healing by SPRED1/Ras/Erk signaling pathway (162).Additionally, the anti-inflammatory effects of miR-146a in MSCs-Exos were demonstrated by its capacity to attenuate inflammatory responses by targeting the NF-κB signaling pathway (163).Furthermore, miR-27b-3p, identified in MSC-Exos, has been shown to inhibit fibrosis by downregulating YAP/LOXL2 pathway. This indicates the potential of miR-27b-3p in treating fibrotic diseases such as liver fibrosis (164).

In conclusion, The loading of these key miRNAs into MSC-Exos to enhance efficacy has emerged as a novel research direction (159, 165). These studies lay the theoretical foundation for the engineering of modified exosome-based therapies. By loading specific microRNAs (miRNAs), the therapeutic efficacy of these vesicles can be enhanced, thus rendering them more widely applicable in a variety of diseases.

7.2 Application of engineered exosomes

By loading key miRNAs and other molecules to construct engineered exosome, specific therapeutic effects can be enhanced. Utilizing genetic engineering and nanotechnology, researchers have successfully augmented therapeutic outcomes by loading specific miRNAs or proteins into these vesicles, enabling the targeted release of these molecules upon reaching the intended cells, thereby improving treatment efficacy. For example, in the treatment of chronic wounds associated with diabetes, Yang et al. confirmed that engineered exosomes containing miR-31-5P significantly promoted angiogenesis, thereby enhancing blood vessel formation and facilitating the healing of diabetic wounds (166). In tumor therapy, engineered exosomes loaded with miR-317b-5p have been shown to effectively target and alter the bioactivities of tumor cells, leading to reduced tumor growth and improved survival rates in animal models (167).

In recent years, researchers have engineered exosomes using various methods, including electroporation, heat shock, saponin permeabilization, and cholesterol modification; however, these methods often result in low loading efficiency (168). Additionally, transfection reagents such as Exo-Fect have shown high transfection efficiency for miRNA, but Exo-Fect can interfere with the membrane structure of Exos, promoting Exos aggregation and altering their membrane barrier properties and surface charge, which may affect the biological functions of Exos and their subsequent applications. Surface modification of Exos enables targeted delivery. Researchers achieved targeted delivery to ischemic brain injury regions by coupling the cyclo(Arg-Gly-Asp-D-Tyr-Lys) peptide [c(RGDyK)] to the surface of Exos (169). Furthermore, by inserting cationic motifs on the surface of Exos, their transport rate and retention time were enhanced (170). Similarly, positively charged Exos have been utilized in gene therapy for osteoarthritis, improving their delivery efficiency in cartilage by reversing the charge (171). RNA nanotechnology-modified galactosamine (GalNAc)-decorated Exos have served as an effective means for targeted delivery to liver cancer, as GalNAc

binds to the overexpressed asialoglycoprotein receptor (ASGP-R) on liver cancer cells, endowing the Exos with targeting capability (172). These methods can effectively enhance the loading efficiency of miRNA into Exos and their functional delivery capabilities. However, optimizing these methods to balance efficacy, stability, and safety remains a significant challenge. Currently, the relevant findings are primarily at the preclinical stage, with further extensive clinical applications yet to be developed.

In summary, engineered exosomes loaded with key effector miRNAs hold significant promise for a range of therapeutic applications, including targeted drug delivery, cancer treatment, and the investigation of inflammatory processes. Their capacity to efficiently deliver miRNAs and selectively target specific pathways paves the way for novel therapeutic interventions. Consequently, engineered exosomes loaded with miRNAs are likely to play an increasingly vital role in the development of innovative therapies for various diseases.

8 Conclusion and future perspectives

This review reviews the biological effects of MSCs and MSC-Exos in the treatment of three different pathophysiological states: tissue repair, fibrosis, and tumor, and analyzes the impact of the disease microenvironment on MSCs function and paracrine signaling. This highlights the importance of individual differences in the disease microenvironment and dynamic changes in disease development for precision medicine. The in-depth understanding of the disease microenvironment makes us more demanding for precise treatment. Based on this, we have reviewed the research status of MSC-Exos treatment and proposed the "extracellular vesicle hypothesis of mesenchymal stem cell microenvironment" (173). MSC-Exos with enhanced therapeutic effects can be obtained by simulating the disease microenvironment of MSCs pretreatment. Finally, the engineered exosomes loaded with mirnas with key effects were prepared to improve the therapeutic effect. This strategy not only improves the targeting and effectiveness of MSC-Exos, but also provides a new idea for precision medicine.

Looking forward to the future, we look forward to more about MSC-Exos treatment research, to confirm or refute this review. Through continuous exploration and optimization, the safety and effectiveness of mesenchymal stem cells in the treatment of diseases can be improved, and the quality of life of patients can be improved.

Author contributions

HY: Conceptualization, Data curation, Resources, Visualization, Writing – original draft. QH: Conceptualization, Data curation, Resources, Visualization, Writing – original draft. FQ: Conceptualization, Writing – review & editing. GX: Conceptualization, Writing – review & editing. MQ: Conceptualization, Writing – review & editing. CD: Conceptualization, Project administration, Supervision, Writing – review & editing. ZW: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

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

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References

1. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. *Stem Cell Res Ther*. (2019) 10:68. doi: 10.1186/s13287-019-1165-5

2. Hoang DM, Pham PT, Bach TQ, Ngo ATL, Nguyen QT, Phan TTK, et al. Stem cell-based therapy for human diseases. *Signal Transduct Target Ther*. (2022) 7:272. doi: 10.1038/s41392-022-01134-4

3. 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 Rev Rep*. (2022) 18:1525–45. doi: 10.1007/s12015-022-10369-1

4. Liu Y, Zhao S, Luo L, Wang J, Zhu Z, Xiang Q, et al. Mesenchymal stem cell-derived exosomes ameliorate erection by reducing oxidative stress damage of corpus cavernosum in a rat model of artery injury. *J Cell Mol Med*. (2019) 23:7462–73. doi: 10.1111/jcmm.14615

5. Zhu Y, Wang Y, Zhao B, Niu X, Hu B, Li Q, et al. Comparison of exosomes secreted by induced pluripotent stem cell-derived mesenchymal stem cells and synovial membrane-derived mesenchymal stem cells for the treatment of osteoarthritis. *Stem Cell Res Ther*. (2017) 8:64. doi: 10.1186/s13287-017-0510-9

6. Li J, Huang Y, Sun H, Yang L. Mechanism of mesenchymal stem cells and exosomes in the treatment of age-related diseases. *Front Immunol*. (2023) 14:1181308. doi: 10.3389/fimmu.2023.1181308

7. Sun Y, Ju Y, Fang B. Exosomes from human adipose-derived mesenchymal stromal/stem cells accelerate angiogenesis in wound healing: implication of the EGR-1/lncRNA-SENCR/DKC1/VEGF-A axis. *Hum Cell*. (2022) 35:1375–90. doi: 10.1007/s13577-022-00732-2

8. Cheng B, Song X, Yin L, Lin J, Liu Z, Zhu Y, et al. HMOX1-overexpressing mesenchymal stem cell-derived exosomes facilitate diabetic wound healing by promoting angiogenesis and fibroblast function. *Biochem Biophys Res Commun*. (2024) 690:149271. doi: 10.1016/j.bbrc.2023.149271

9. Long C, Wang J, Gan W, Qin X, Yang R, Chen X. Therapeutic potential of exosomes from adipose-derived stem cells in chronic wound healing. *Front Surg*. (2022) 9:1030288. doi: 10.3389/fsurg.2022.1030288

10. Lelek J, Zuba-Surma EK. Perspectives for future use of extracellular vesicles from umbilical cord- and adipose tissue-derived mesenchymal stem/stromal cells in regenerative therapies-synthetic review. *Int J Mol Sci*. (2020) 21:799. doi: 10.3390/ijms21030799

11. Liu Y, Wang M, Yu Y, Li C, Zhang C. Advances in the study of exosomes derived from mesenchymal stem cells and cardiac cells for the treatment of myocardial infarction. *Cell Commun Signal: CCS*. (2023) 21:202. doi: 10.1186/s12964-023-01227-9

12. Petrenko Y, Vackova I, Kekulova K, Chudickova M, Koci Z, Turnovcova K, et al. A comparative analysis of multipotent mesenchymal stromal cells derived from different sources, with a focus on neuroregenerative potential. *Sci Rep*. (2020) 10:4290. doi: 10.1038/s41598-020-61167-z

13. Kusuma GD, Carthew J, Lim R, Frith JE. Effect of the microenvironment on mesenchymal stem cell paracrine signaling: opportunities to engineer the therapeutic effect. *Stem Cells Dev*. (2017) 26:617–31. doi: 10.1089/scd.2016.0349

14. Ghoneim MA, Gabr MM, El-Halawani SM, Refaie AF. Current status of stem cell therapy for type 1 diabetes: a critique and a prospective consideration. *Stem Cell Res Ther*. (2024) 15:23. doi: 10.1186/s13287-024-03636-0

15. Zhou T, Yuan Z, Weng J, Pei D, Du X, He C, et al. Challenges and advances in clinical applications of mesenchymal stromal cells. *J Hematol Oncol*. (2021) 14:24. doi: 10.1186/s13045-021-01037-x

16. Lötvall J, Hill AF, Hochberg F, Buzás EI, Di Vizio D, Gardiner C, et al. Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles. *J Extracell Vesicles*. (2014) 3:26913. doi: 10.3402/jev.v3.26913

17. Théry C, Witwer KW, Aikawa E, Alcaraz MJ, Anderson JD, Andriantsitohaina R, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles*. (2018) 7:1535750. doi: 10.1080/20013078.2018.1535750

18. Welsh JA, Goberdhan DCI, O'Driscoll L, Buzas EI, Blenkiron C, Bussolati B, et al. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. *J Extracell Vesicles*. (2024) 13:e12404. doi: 10.1002/jev2.12404

19. Marofi F, Alexandrovna KI, Margiana R, Bahramali M, Suksatan W, Abdelbasset WK, et al. MSCs and their exosomes: a rapidly evolving approach in the context of cutaneous wounds therapy. *Stem Cell Res Ther*. (2021) 12:597. doi: 10.1186/s13287-021-02662-6

20. Lai RC, Arslan F, Lee MM, Sze NSK, Choo A, Chen TS, et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res*. (2010) 4:214–22. doi: 10.1016/j.scr.2009.12.003

21. Gowen A, Shahjin F, Chand S, Odegaard KE, Yelamanchili SV. Mesenchymal stem cell-derived extracellular vesicles: challenges in clinical applications. *Front Cell Dev Biol*. (2020) 8:149. doi: 10.3389/fcell.2020.00149

22. Al-Masawa ME, Alshawsh MA, Ng CY, Ng AMH, Foo JB, Vijakumaran U, et al. Efficacy and safety of small extracellular vesicle interventions in wound healing and skin regeneration: A systematic review and meta-analysis of animal studies. *Theranostics*. (2022) 12:6455–508. doi: 10.7150/thno.73436

23. Shabbir A, Cox A, Rodriguez-Menocal L, Salgado M, Van Badiavas E. Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis *in vitro*. *Stem Cells Dev*. (2015) 24:1635–47. doi: 10.1089/scd.2014.0316

24. Zhang Q, Xiang W, Yi DY, Xue BZ, Wen WW, Abdelmaksoud A, et al. Current status and potential challenges of mesenchymal stem cell-based therapy for Malignant gliomas. *Stem Cell Res Ther*. (2018) 9:228. doi: 10.1186/s13287-018-0977-z

25. Hu L, Wang J, Zhou X, Xiong Z, Zhao J, Yu R, et al. Author Correction: Exosomes derived from human adipose mensenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. *Sci Rep*. (2020) 10:6693. doi: 10.1038/s41598-020-63068-7

26. Li Y, Zhang J, Shi J, Liu K, Wang X, Jia Y, et al. Exosomes derived from human adipose mesenchymal stem cells attenuate hypertrophic scar fibrosis by miR-192-5p/IL-17RA/Smad axis. *Stem Cell Res Ther*. (2021) 12:221. doi: 10.1186/s13287-021-02290-0

27. Liu J, Gao J, Liang Z, Gao C, Niu Q, Wu F, et al. Mesenchymal stem cells and their microenvironment. *Stem Cell Res Ther*. (2022) 13:429. doi: 10.1186/s13287-022-02985-y

28. Chen S, Sun F, Qian H, Xu W, Jiang J. Preconditioning and engineering strategies for improving the efficacy of mesenchymal stem cell-derived exosomes in cell-free therapy. *Stem Cells Int*. (2022) 2022:1779346. doi: 10.1155/2022/1779346

29. Miceli V, Bulati M, Iannolo G, Zito G, Gallo A, Conaldi PG. Therapeutic properties of mesenchymal stromal/stem cells: the need of cell priming for cell-free therapies in regenerative medicine. *Int J Mol Sci*. (2021) 22:763. doi: 10.3390/ijms22020763

30. Zhu S, Xuan J, Shentu Y, Kida K, Kobayashi M, Wang W, et al. Effect of chitin-architected spatiotemporal three-dimensional culture microenvironments on human umbilical cord-derived mesenchymal stem cells. *Bioact Mater*. (2024) 35:291–305. doi: 10.1016/j.bioactmat.2024.01.014

31. Devarasetty M, Mazzocchi AR, Skardal A. Applications of bioengineered 3D tissue and tumor organoids in drug development and precision medicine: current and future. *BioDrugs*. (2018) 32:53–68. doi: 10.1007/s40259-017-0258-x

32. Zheng L, Gong H, Zhang J, Guo L, Zhai Z, Xia S, et al. Strategies to improve the therapeutic efficacy of mesenchymal stem cell-derived extracellular vesicle (MSC-EV): a promising cell-free therapy for liver disease. *Front Bioeng Biotechnol*. (2023) 11:1322514. doi: 10.3389/fbioe.2023.1322514

33. Lv K, Wu T, Liu S, Lou P, Zhou P, Wang Y, et al. Disease-derived circulating extracellular vesicle preconditioning: A promising strategy for precision mesenchymal stem cell therapy. *Acta Pharm Sin B*. (2024) 14:4526–43. doi: 10.1016/j.apsb.2024.06.027

34. Xing XK, Feng HG, Yuan ZQ. Differentiation of bone mesenchymal stem cells into hepatocyte-like cells induced by liver tissue homogenate. *Genet Mol Res*. (2016) 15 (3). doi: 10.4238/gmr.15038550

35. Tao SC, Guo SC. Role of extracellular vesicles in tumour microenvironment. *Cell Commun Signal*. (2020) 18:163. doi: 10.1186/s12964-020-00643-5

36. Clancy JW, D'Souza-Schorey C. Tumor-derived extracellular vesicles: multifunctional entities in the tumor microenvironment. *Annu Rev Pathol*. (2023) 18:205–29. doi: 10.1146/annurev-pathmechdis-031521-022116

37. Adelipour M, Lubman DM, Kim J. Potential applications of mesenchymal stem cells and their derived exosomes in regenerative medicine. *Expert Opin Biol Ther*. (2023) 23:491–507. doi: 10.1080/14712598.2023.2211203

38. Wu Y, Li J, Zeng Y, Pu W, Mu X, Sun K, et al. Exosomes rewire the cartilage microenvironment in osteoarthritis: from intercellular communication to therapeutic strategies. *Int J Oral Sci*. (2022) 14:40. doi: 10.1038/s41368-022-00187-z

39. Jonavičė U, Romenskaja D, Kriaučiūnaitė K, Jarmalavičiūtė A, Pajarskienė J, Kašėta V, et al. Extracellular vesicles from human teeth stem cells trigger ATP release and promote migration of human microglia through P2X4 receptor/MFG-E8-dependent mechanisms. *Int J Mol Sci*. (2021) 22(20):10970. doi: 10.3390/ijms222010970

40. Vilcaes AA, Chanaday NL, Kavalali ET. Interneuronal exchange and functional integration of synaptobrevin via extracellular vesicles. *Neuron*. (2021) 109:971–83.e5. doi: 10.1016/j.neuron.2021.01.007

41. George S, Rey NL, Tyson T, Esquibel C, Meyerdirk L, Schulz E, et al. Microglia affect α-synuclein cell-to-cell transfer in a mouse model of Parkinson's disease. *Mol Neurodegener*. (2019) 14:34. doi: 10.1186/s13024-019-0335-3

42. Sun Z, Yang S, Zhou Q, Wang G, Song J, Li Z, et al. Emerging role of exosome-derived long non-coding RNAs in tumor microenvironment. *Mol Cancer*. (2018) 17:82. doi: 10.1186/s12943-018-0831-z

43. Saadh MJ, Ramírez-Coronel AA, Saini RS, Arias-Gonzáles JL, Amin AH, Gavilán JCO, et al. Advances in mesenchymal stem/stromal cell-based therapy and their extracellular vesicles for skin wound healing. *Hum Cell*. (2023) 36(4):1253–64. doi: 10.1007/s13577-023-00904-8

44. Zhu Y, Fu W. Peripheral blood-derived stem cells for the treatment of cartilage injuries: A systematic review. *Front Bioeng Biotechnol*. (2022) 10:956614. doi: 10.3389/fbioe.2022.956614

45. Kulandavelu S, Balkan W, Hare JM. Next-Generation Stem Cell Therapy: Genetically Modified Mesenchymal Stem Cells for Cardiac Repair: Editorial to: "Mesenchymal Stem Cells with eNOS Over-Expression Enhance Cardiac Repair in Rats with Myocardial Infarction" by Leilei Chen et al. *Cardiovasc Drugs Ther*. (2017) 31:5–7. doi: 10.1007/s10557-017-6714-5

46. Huang Y-Z, Gou M, Da L-C, Zhang W-Q, Xie H-Q. Mesenchymal stem cells for chronic wound healing: current status of preclinical and clinical studies. *Tissue Eng Part B: Rev*. (2020) 26:555–70. doi: 10.1089/ten.teb.2019.0351

47. Poomani MS, Mariappan I, Perumal R, Regurajan R, Muthan K, Subramanian V. Mesenchymal stem cell (MSCs) therapy for ischemic heart disease: A promising frontier. *Global Heart*. (2022) 17:19. doi: 10.5334/gh.1098

48. Bian D, Wu Y, Song G, Azizi R, Zamani A. The application of mesenchymal stromal cells (MSCs) and their derivative exosome in skin wound healing: a comprehensive review. *Stem Cell Res Ther*. (2022) 13:24. doi: 10.1186/s13287-021-02697-9

49. Hu L, Wang J, Zhou X, Xiong Z, Zhao J, Yu R, et al. Exosomes derived from human adipose mensenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. *Sci Rep*. (2016) 6:32993. doi: 10.1038/srep32993

50. Lu C, Dali W, Zairong W, Bo W, Qi J, Guangfeng S. Effects of local transplantation of autologous adipose-derived mesenchymalstem cells on the formation of hyperplastic scar on rabbit ear. *Chin J Burn*. (2016) 32:582–7. doi: 10.3760/cma.j.issn.1009-2587.2016.10.003

51. Liu Y-Y, Li Y, Wang L, Zhao Y, Yuan R, Yang M-M, et al. Mesenchymal stem cell-derived exosomes regulate microglia phenotypes: a promising treatment for acute central nervous system injury. *Neural Regenerat Res.* (2023) 18:1657–65. doi: 10.4103/1673-5374.363819

52. Asgarpour K, Shojaei Z, Amiri F, Ai J, Mahjoubin-Tehran M, Ghasemi F, et al. Exosomal microRNAs derived from mesenchymal stem cells: cell-to-cell messages. *Cell Commun Signal: CCS.* (2020) 18:149. doi: 10.1186/s12964-020-00650-6

53. Norouzi-Barough L, Shirian S, Gorji A, Sadeghi M. Therapeutic potential of mesenchymal stem cell-derived exosomes as a cell-free therapy approach for the treatment of skin, bone, and cartilage defects. *Connect Tissue Res.* (2022) 63(2):83–96. doi: 10.1080/03008207.2021.1887855

54. Tutuianu R, Rosca A-M, Iacomi DM, Simionescu M, Titorencu I. Human mesenchymal stromal cell-derived exosomes promote *in vitro* wound healing by modulating the biological properties of skin keratinocytes and fibroblasts and stimulating angiogenesis. *Int J Mol Sci.* (2021) 22:6239. doi: 10.3390/ijms22126239

55. Balbi C, Vassalli G. Exosomes: Beyond stem cells for cardiac protection and repair. *Stem Cells (Dayton Ohio).* (2020) 38:1387–99. doi: 10.1002/stem.3261

56. Ghufran H, Azam M, Mehmood A, Umair M, Baig MT, Tasneem S, et al. Adipose tissue and umbilical cord tissue: potential sources of mesenchymal stem cells for liver fibrosis treatment. *J Clin Exp Hepatol.* (2024) 14:101364. doi: 10.1016/j.jceh.2024.101364

57. Lee MJ, Jung J, Na KH, Moon JS, Lee HJ, Kim JH, et al. Anti-fibrotic effect of chorionic plate-derived mesenchymal stem cells isolated from human placenta in a rat model of CCl(4)-injured liver: potential application to the treatment of hepatic diseases. *J Cell Biochem.* (2010) 111:1453–63. doi: 10.1002/jcb.22873

58. 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 Res Ther.* (2021) 12:470. doi: 10.1186/s13287-021-02551-y

59. Zhao X, Wu J, Yuan R, Li Y, Yang Q, Wu B, et al. Adipose-derived mesenchymal stem cell therapy for reverse bleomycin-induced experimental pulmonary fibrosis. *Sci Rep.* (2023) 13:13183. doi: 10.1038/s41598-023-40531-9

60. Chen Z, Ruan B, Long G, Lin W. Adipose tissue-derived mesenchymal stem cells attenuate lung inflammation and fibrosis in the bleomycin-induced pulmonary fibrosis rat model via caveolin-1/NF-kB signaling axis. *Physiol Res.* (2022) 71:657–66. doi: 10.33549/physiolres

61. 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 Dis.* (2022) 13:580. doi: 10.1038/s41419-022-05034-x

62. Li T, Yan Y, Wang B, Qian H, Zhang X, Shen L, et al. Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis. *Stem Cells Dev.* (2013) 22:845–54. doi: 10.1089/scd.2012.0395

63. Chen W, Lin F, Feng X, Yao Q, Yu Y, Gao F, et al. MSC-derived exosomes attenuate hepatic fibrosis in primary sclerosing cholangitis through inhibition of Th17 differentiation. *Asian J Pharm Sci.* (2024) 19:100889. doi: 10.1016/j.ajps.2024.100889

64. Hu J, Chen Y, Huang Y, Su Y. Human umbilical cord mesenchymal stem cell-derived exosomes suppress dermal fibroblasts-myofibroblats transition via inhibiting the TGF-β1/Smad 2/3 signaling pathway. *Exp Mol Pathol.* (2020) 115:104468. doi: 10.1016/j.yexmp.2020.104468

65. Tian S, Zhou X, Zhang M, Cui L, Li B, Liu Y, et al. Mesenchymal stem cell-derived exosomes protect against liver fibrosis via delivering miR-148a to target KLF6/STAT3 pathway in macrophages. *Stem Cell Res Ther.* (2022) 13:330. doi: 10.1186/s13287-022-03010-y

66. Li D, Qu J, Yuan X, Zhuang S, Wu H, Chen R, et al. Mesenchymal Stem Cells Alleviate Renal Fibrosis and Inhibit Autophagy via Exosome Transfer of miRNA-122a. *Stem Cells Int.* (2022) 2022:1981798. doi: 10.1155/2022/1981798

67. Vakhshiteh F, Atyabi F, Ostad SN. Mesenchymal stem cell exosomes: a two-edged sword in cancer therapy. *Int J Nanomed.* (2019) 14:2847–59. doi: 10.2147/IJN.S200036

68. Zhang L. The role of mesenchymal stem cells in modulating the breast cancer microenvironment. *Cell Transplant.* (2023) 32:09636897231220073. doi: 10.1177/09636897231220073

69. Kidd S, Spaeth E, Klopp A, Andreeff M, Hall B, Marini FC. The (in) auspicious role of mesenchymal stromal cells in cancer: be it friend or foe. *Cytotherapy.* (2008) 10:657–67. doi: 10.1080/14653240802486517

70. Prakash MD, Miller S, Randall-Demllo S, Nurgali K. Mesenchymal stem cell treatment of inflammation-induced cancer. *Inflammation Bowel Dis.* (2016) 22:2694–703. doi: 10.1097/MIB.0000000000000900

71. Vaiasicca S, Melone G, James DW, Quintela M, Xiao J, Yao S, et al. Transcriptomic analysis reveals the anti-cancer effect of gestational mesenchymal stem cell secretome. *Stem Cells Trans Med.* (2024) 13(7):693–710. doi: 10.1093/stcltm/szae024

72. Sun C, Dai X, Zhao D, Wang H, Rong X, Huang Q, et al. Mesenchymal stem cells promote glioma neovascularization *in vivo* by fusing with cancer stem cells. *BMC Cancer.* (2019) 19:1240. doi: 10.1186/s12885-019-6460-0

73. Raj AT, Kheur S, Khurshid Z, Sayed ME, Mugri MH, Almasri MA, et al. The growth factors and cytokines of dental pulp mesenchymal stem cell secretome may potentially aid in oral cancer proliferation. *Mol (Basel Switzerland).* (2021) 26:5683. doi: 10.3390/molecules26185683

74. François S, Usunier B, Forgue-Lafitte M-E, L'Homme B, Benderitter M, Douay L, et al. Mesenchymal stem cell administration attenuates colon cancer progression by modulating the immune component within the colorectal tumor microenvironment. *Stem Cells Trans Med.* (2019) 8:285–300. doi: 10.1002/sctm.18-0117

75. Fu Y, Li J, Li M, Xu J, Rong Z, Ren F, et al. Umbilical cord mesenchymal stem cells ameliorate inflammation-related tumorigenesis via modulating macrophages. *Stem Cells Int.* (2022) 2022:1617229. doi: 10.1155/2022/1617229

76. Mirabdollahi M, Haghjooyjavanmard S, Sadeghi-aliabadi H. An anticancer effect of umbilical cord-derived mesenchymal stem cell secretome on the breast cancer cell line. *Cell Tissue Banking.* (2019) 20:423–34. doi: 10.1007/s10561-019-09781-8

77. Javan MR, Khosrojerdi A, Moazzeni SM. New insights into implementation of mesenchymal stem cells in cancer therapy: prospects for anti-angiogenesis treatment. *Front Oncol.* (2019) 9:840. doi: 10.3389/fonc.2019.00840

78. Shojaei S, Hashemi SM, Ghanbarian H, Salehi M, Mohammadi-Yeganeh S. Effect of mesenchymal stem cells-derived exosomes on tumor microenvironment: Tumor progression versus tumor suppression. *J Cell Physiol.* (2019) 234:3394–409. doi: 10.1002/jcp.27326

79. Xu H, Han H, Song S, Yi N, Qian C, Qiu Y, et al. Exosome-transmitted PSMA3 and PSMA3-AS1 promote proteasome inhibitor resistance in multiple myeloma. *Clin Cancer Res.* (2019) 25:1923–35. doi: 10.1158/1078-0432.CCR-18-2363

80. Lin SS, Zhu B, Guo ZK, Huang GZ, Wang Z, Chen J, et al. Bone marrow mesenchymal stem cell-derived microvesicles protect rat pheochromocytoma PC12 cells from glutamate-induced injury via a PI3K/Akt dependent pathway. *Neurochem Res.* (2014) 39:922–31. doi: 10.1007/s11064-014-1288-0

81. Jahangiri B, Khalaj-Kondori M, Asadollahi E, Kian Saei A, Sadeghizadeh M. Dual impacts of mesenchymal stem cell-derived exosomes on cancer cells: unravelling complex interactions. *J Cell Commun Signaling.* (2023) 17:1229–47. doi: 10.1007/s12079-023-00794-3

82. Li T, Zhou X, Wang J, Liu Z, Han S, Wan L, et al. Adipose-derived mesenchymal stem cells and extracellular vesicles confer antitumor activity in preclinical treatment of breast cancer. *Pharmacol Res.* (2020) 157:104843. doi: 10.1016/j.phrs.2020.104843

83. Zhang M, Hu S, Liu L, Dang P, Liu Y, Sun Z, et al. Engineered exosomes from different sources for cancer-targeted therapy. *Signal Transduct Target Ther.* (2023) 8:124. doi: 10.1038/s41392-023-01382-y

84. Ghasempour E, Hesami S, Movahed E, Keshel SH, Doroudian M. Mesenchymal stem cell-derived exosomes as a new therapeutic strategy in the brain tumors. *Stem Cell Res Ther.* (2022) 13:527. doi: 10.1186/s13287-022-03212-4

85. Wei H, Chen F, Chen J, Lin H, Wang S, Wang Y, et al. Mesenchymal stem cell derived exosomes as nanodrug carrier of doxorubicin for targeted osteosarcoma therapy via SDF1-CXCR4 axis. *Int J Nanomed.* (2022) 17:3483–95. doi: 10.2147/IJN.S372851

86. Zhang F, Guo J, Zhang Z, Qian Y, Wang G, Duan M, et al. Mesenchymal stem cell-derived exosome: A tumor regulator and carrier for targeted tumor therapy. *Cancer Lett.* (2022) 526:29–40. doi: 10.1016/j.canlet.2021.11.015

87. Cao Y, Yang H, Jin L, Du J, Fan Z. Genome-wide DNA methylation analysis during osteogenic differentiation of human bone marrow mesenchymal stem cells. *Stem Cells Int.* (2018) 2018:8238496. doi: 10.1155/2018/8238496

88. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell.* (2008) 2:313–9. doi: 10.1016/j.stem.2008.03.002

89. Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: an update. *Cell Transplant.* (2016) 25:829–48. doi: 10.3727/096368915X689622

90. Oveili E, Vafaei S, Bazavar H, Eslami Y, Mamaghanizadeh E, Yasamineh S, et al. The potential use of mesenchymal stem cells-erived exosomes as microRNAs delivery systems in different diseases. *Cell Commun Signal.* (2023) 21:20. doi: 10.1186/s12964-022-01017-9

91. Ko SF, Yip HK, Zhen YY, Lee CC, Lee CC, Huang CC, et al. Adipose-derived mesenchymal stem cell exosomes suppress hepatocellular carcinoma growth in a rat model: apparent diffusion coefficient, natural killer T-cell responses, and histopathological features. *Stem Cells Int.* (2015) 2015:853506. doi: 10.1155/2015/853506

92. Lin R, Wang S, Zhao RC. Exosomes from human adipose-derived mesenchymal stem cells promote migration through Wnt signaling pathway in a breast cancer cell model. *Mol Cell Biochem.* (2013) 383:13–20. doi: 10.1007/s11010-013-1746-z

93. Kumari S, Advani D, Sharma S, Ambasta RK, Kumar P. Combinatorial therapy in tumor microenvironment: Where do we stand? *Biochim Biophys Acta Rev Cancer.* (2021) 1876(2):188585. doi: 10.1016/j.bbcan.2021.188585

94. Xue C, Zhou Q, Xi H, Zhou J. Radiomics: A review of current applications and possibilities in the assessment of tumor microenvironment. *Diagn Interv Imaging.* (2023) 104:113–22. doi: 10.1016/j.diii.2022.10.008

95. Ning T, Yang F, Chen D, Jia Z, Yuan R, Du Z, et al. Synergistically detachable microneedle dressing for programmed treatment of chronic wounds. *Adv Healthc Mater.* (2022) 11:e2102180. doi: 10.1002/adhm.202102180

96. Liu W, Gao R, Yang C, Feng Z, Ou-Yang W, Pan X, et al. ECM-mimetic immunomodulatory hydrogel for methicillin-resistant Staphylococcus aureus-infected chronic skin wound healing. *Sci Adv.* (2022) 8:eabn7006. doi: 10.1126/sciadv.abn7006

97. Wyles SP, Dashti P, Pirtskhalava T, Tekin B, Inman C, Gomez LS, et al. A chronic wound model to investigate skin cellular senescence. *Aging (Albany NY)*. (2023) 15:2852–62. doi: 10.18632/aging.204667

98. Sarian MN, Zulkefli N, Che Zain MS, Maniam S, Fakurazi S. A review with updated perspectives on *in vitro* and *in vivo* wound healing models. *Turk J Biol*. (2023) 47:236–46. doi: 10.55730/1300-0152.2659

99. Davidson S, Coles M, Thomas T, Kollias G, Ludewig B, Turley S, et al. Fibroblasts as immune regulators in infection, inflammation and cancer. *Nat Rev Immunol*. (2021) 21:704–17. doi: 10.1038/s41577-021-00540-z

100. Wong ZY, Nee E, Coles M, Buckley CD. Why does understanding the biology of fibroblasts in immunity really matter? *PloS Biol*. (2023) 21(2):e3001954. doi: 10.1371/journal.pbio.3001954

101. Liu X, Dai K, Zhang X, Huang G, Lynn H, Rabata A, et al. Multiple fibroblast subtypes contribute to matrix deposition in pulmonary fibrosis. *Am J Respir Cell Mol Biol*. (2023) 69:45–56. doi: 10.1165/rcmb.2022-0292OC

102. Setten E, Castagna A, Nava-Sedeño JM, Weber J, Carriero R, Reppas A, et al. Understanding fibrosis pathogenesis via modeling macrophage-fibroblast interplay in immune-metabolic context. *Nat Commun*. (2022) 13:6499. doi: 10.1038/s41467-022-34241-5

103. Horie M, Miyashita N, Mikami Y, Noguchi S, Yamauchi Y, Suzukawa M, et al. TBX4 is involved in the super-enhancer-driven transcriptional programs underlying features specific to lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol*. (2018) 314: L177–l91. doi: 10.1152/ajplung.00193.2017

104. Hannan RT, Peirce SM, Barker TH. Fibroblasts: diverse cells critical to biomaterials integration. *ACS Biomater Sci Eng*. (2018) 4:1223–32. doi: 10.1021/acsbiomaterials.7b00244

105. Singhal PK, Sassi S, Lan L, Au P, Halvorsen SC, Fukumura D, et al. Mouse embryonic fibroblasts exhibit extensive developmental and phenotypic diversity. *Proc Natl Acad Sci U S A*. (2016) 113:122–7. doi: 10.1073/pnas.1522401112

106. Shaw TJ, Rognoni E. Dissecting fibroblast heterogeneity in health and fibrotic disease. *Curr Rheumatol Rep*. (2020) 22:33. doi: 10.1007/s11926-020-00903-w

107. Krenkel O, Hundertmark J, Ritz TP, Weiskirchen R, Tacke F. Single cell RNA sequencing identifies subsets of hepatic stellate cells and myofibroblasts in liver fibrosis. *Cells*. (2019) 8(5):503. doi: 10.3390/cells8050503

108. Novotný J, Strnadová K, Dvořánková B, Kocourková Š, Jakša R, Dundr P, et al. Single-cell RNA sequencing unravels heterogeneity of the stromal niche in cutaneous melanoma heterogeneous spheroids. *Cancers (Basel)*. (2020) 12(11):3324. doi: 10.3390/cancers12113324

109. Solé-Boldo L, Raddatz G, Schütz S, Mallm JP, Rippe K, Lonsdorf AS, et al. Single-cell transcriptomes of the human skin reveal age-related loss of fibroblast priming. *Commun Biol*. (2020) 3:188. doi: 10.1038/s42003-020-0922-4

110. Yamamoto Y, Kasashima H, Fukui Y, Tsujio G, Yashiro M, Maeda K. The heterogeneity of cancer-associated fibroblast subpopulations: Their origins, biomarkers, and roles in the tumor microenvironment. *Cancer Sci*. (2023) 114:16–24. doi: 10.1111/cas.15609

111. Cheng B, Fu XB. Microenvironment control is the only way to achieve perfect wound repair. *Zhonghua Shao Shang Za Zhi*. (2020) 36(11):1003–8. doi: 10.3760/cma.j.cn501120-20201009-00429

112. Oishi Y, Manabe I. Macrophages in inflammation, repair and regeneration. *Int Immunol*. (2018) 30:511–28. doi: 10.1093/intimm/dxy054

113. Li L, Gan H, Jin H, Fang Y, Yang Y, Zhang J, et al. Astragaloside IV promotes microglia/macrophages M2 polarization and enhances neurogenesis and angiogenesis through PPARγ pathway after cerebral ischemia/reperfusion injury in rats. *Int Immunopharmacol*. (2021) 92:107335. doi: 10.1016/j.intimp.2020.107335

114. Lukomska B, Stanaszek L, Zuba-Surma E, Legosz P, Sarzynska S, Drela K. Challenges and controversies in human mesenchymal stem cell therapy. *Stem Cells Int*. (2019) 2019:9628536. doi: 10.1155/2019/9628536

115. Yu Y, Yoo SM, Park HH, Baek SY, Kim Y-J, Lee S, et al. Preconditioning with interleukin-1 beta and interferon-gamma enhances the efficacy of human umbilical cord blood-derived mesenchymal stem cells-based therapy via enhancing prostaglandin E2 secretion and indoleamine 2,3-dioxygenase activity in dextran sulfate sodium-induced colitis. *J Tissue Eng Regenerat Med*. (2019) 13:1792–804. doi: 10.1002/term.2930

116. Antebi B, Rodriguez LA 2nd, Walker KP 3rd, Asher AM, Kamucheka RM, Alvarado L, et al. Short-term physiological hypoxia potentiates the therapeutic function of mesenchymal stem cells. *Stem Cell Res Ther*. (2018) 9(1):265. doi: 10.1186/s13287-018-1007-x

117. Yang Y, Lee EH, Yang Z. Hypoxia-conditioned mesenchymal stem cells in tissue regeneration application. *Tissue Eng Part B Rev*. (2022) 28(5):966–77. doi: 10.1089/ten.TEB.2021.0145

118. Najar M, Krayem M, Merimi M, Burny A, Meuleman N, Bron D, et al. Insights into inflammatory priming of mesenchymal stromal cells: functional biological impacts. *Inflammation Res*. (2018) 67(6):467–77. doi: 10.1007/s00011-018-1131-1

119. Liesveld JL, Sharma N, Aljitawi OS. Stem cell homing: From physiology to therapeutics. *Stem Cells*. (2020) 38:1241–53. doi: 10.1002/stem.3242

120. Zhu M, Chu Y, Shang Q, Zheng Z, Li Y, Cao L, et al. Mesenchymal stromal cells pretreated with pro-inflammatory cytokines promote skin wound healing through VEGFC-mediated angiogenesis. *Stem Cells Transl Med*. (2020) 9:1218–32. doi: 10.1002/sctm.19-0241

121. Yuan M, Hu X, Yao L, Jiang Y, Li L. Mesenchymal stem cell homing to improve therapeutic efficacy in liver disease. *Stem Cell Res Ther*. (2022) 13:179. doi: 10.1186/s13287-022-02858-4

122. Pietrobono D, Giacomelli C, Marchetti L, Martini C, Trincavelli ML. High adenosine extracellular levels induce glioblastoma aggressive traits modulating the mesenchymal stromal cell secretome. *Int J Mol Sci*. (2020) 21(20):7706. doi: 10.3390/ijms21207706

123. Dali W. Some thoughts on the research of mesenchymal stem cell exosomes and wound microenvironment. *Zhonghua Shao Shang Yu Chuang Mian Xiu Fu Za Zhi*. (2023) 39(2):101–5. doi: 10.3760/cma.j.cn501225-20230112-00014

124. Kim M, Shin DI, Choi BH, Min B-H. Exosomes from IL-1β-primed mesenchymal stem cells inhibited IL-1β- and TNF-α-mediated inflammatory responses in osteoarthritic SW982 cells. *Tissue Eng Regenerat Med*. (2021) 18:525–36. doi: 10.1007/s13770-020-00324-x

125. Cheng X, Wang S, Li Z, He D, Wu J, Ding W. IL-1β-pretreated bone mesenchymal stem cell-derived exosomes alleviate septic endoplasmic reticulum stress via regulating SIRT1/ERK pathway. *Heliyon*. (2023) 9:e20124. doi: 10.1016/j.heliyon.2023.e20124

126. Han Y, Yang J, Fang J, Zhou Y, Candi E, Wang J, et al. The secretion profile of mesenchymal stem cells and potential applications in treating human diseases. *Signal Transduct Target Ther*. (2022) 7:1–19. doi: 10.1038/s41392-022-00932-0

127. Zhang W, Zhou X, Yao Q, Liu Y, Zhang H, Dong Z. HIF-1-mediated production of exosomes during hypoxia is protective in renal tubular cells. *Am J Physiol Renal Physiol*. (2017) 313:F906–F13. doi: 10.1152/ajprenal.00178.2017

128. Nakao Y, Fukuda T, Zhang Q, Sanui T, Shinjo T, Kou X, et al. Exosomes from TNF-α-treated human gingiva-derived MSCs enhance M2 macrophage polarization and inhibit periodontal bone loss. *Acta Biomater*. (2021) 122:306–24. doi: 10.1016/j.actbio.2020.12.046

129. Logozzi M, Mizzoni D, Angelini DF, Di Raimo R, Falchi M, Battistini L, et al. Microenvironmental pH and exosome levels interplay in human cancer cell lines of different histotypes. *Cancers (Basel)*. (2018) 10(10):370. doi: 10.3390/cancers10100370

130. Wu B, Liu D-A, Guan L, Myint PK, Chin L, Dang H, et al. Stiff matrix induces exosome secretion to promote tumour growth. *Nat Cell Biol*. (2023) 25:415–24. doi: 10.1038/s41556-023-01092-1

131. Mountziaris PM, Tzouanas SN, Mikos AG. Dose effect of tumor necrosis factor-alpha on *in vitro* osteogenic differentiation of mesenchymal stem cells on biodegradable polymeric microfiber scaffolds. *Biomaterials*. (2010) 31:1666–75. doi: 10.1016/j.biomaterials.2009.11.058

132. Szűcs D, Miklós V, Monostori T, Guba M, Kun-Varga A, Póliska S, et al. Effect of inflammatory microenvironment on the regenerative capacity of adipose-derived mesenchymal stem cells. *Cells*. (2023) 12(15):1966. doi: 10.3390/cells12151966

133. Li H, Ji X-Q, Zhang S-M, Bi R-H. Hypoxia and inflammatory factor preconditioning enhances the immunosuppressive properties of human umbilical cord mesenchymal stem cells. *World J Stem Cells*. (2023) 15:999. doi: 10.4252/wjsc.v15.i11.999

134. Peng X, Zhou X, Yin Y, Luo B, Liu Y, Yang C. Inflammatory microenvironment accelerates bone marrow mesenchymal stem cell aging. *Front Bioeng Biotechnol*. (2022) 10:870324. doi: 10.3389/fbioe.2022.870324

135. Wang Q, Zhang L, Sun Z, Chi B, Zou A, Mao L, et al. HIF-1α overexpression in mesenchymal stem cell-derived exosome-encapsulated arginine-glycine-aspartate (RGD) hydrogels boost therapeutic efficacy of cardiac repair after myocardial infarction. *Mater Today Bio*. (2021) 12:100171. doi: 10.1016/j.mtbio.2021.100171

136. Li L, Mu J, Zhang Y, Zhang C, Ma T, Chen L, et al. Stimulation by exosomes from hypoxia preconditioned human umbilical vein endothelial cells facilitates mesenchymal stem cells angiogenic function for spinal cord repair. *ACS Nano*. (2022) 16:10811–23. doi: 10.1021/acsnano.2c02898

137. Wang H, Zhao H, Chen Z, Cai X, Wang X, Zhou P, et al. Hypoxic Bone Mesenchymal Stem Cell-Derived Exosomes Direct Schwann Cells Proliferation, Migration, and Paracrine to Accelerate Facial Nerve Regeneration via circRNA_Nkd2/miR-214-3p/MED19 Axis. *Int J Nanomed*. (2024) 19:1409–29. doi: 10.2147/IJN.S443036

138. Rezabakhsh A, Sokullu E, Rahbarghazi R. Applications, challenges and prospects of mesenchymal stem cell exosomes in regenerative medicine. *Stem Cell Res Ther*. (2021) 12:521. doi: 10.1186/s13287-021-02596-z

139. Gao Y, Yuan Z, Yuan X, Wan Z, Yu Y, Zhan Q, et al. Bioinspired porous microspheres for sustained hypoxic exosomes release and vascularized bone regeneration. *Bioact Mater*. (2022) 14:377–88. doi: 10.1016/j.bioactmat.2022.01.041

140. Samal JRK, Rangasami VK, Samanta S, Varghese OP, Oommen OP. Discrepancies on the role of oxygen gradient and culture condition on mesenchymal stem cell fate. *Adv Healthc Mater*. (2021) 10:e2002058. doi: 10.1002/adhm.202002058

141. Çiçek G, Çiçek M, Utlu Özen E, Aktan TM, Özdengül F, Duman S. Brain-derived neurotrophic factor levels in mesenchymal stem cell culture fluid under different culture conditions. *Eurasian J Med*. (2023) 55:25–31. doi: 10.5152/eurasianjmed.2021.0138

142. Kozlowska U, Krawczenko A, Futoma K, Jurek T, Rorat M, Patrzalek D, et al. Similarities and differences between mesenchymal stem/progenitor cells derived from

various human tissues. *World J Stem Cells*. (2019) 11:347–74. doi: 10.4252/wjsc.v11.i6.347

143. Xue G, Han X, Ma X, Wu H, Qin Y, Liu J, et al. Effect of microenvironment on differentiation of human umbilical cord mesenchymal stem cells into hepatocytes *in vitro* and *in vivo*. *BioMed Res Int*. (2016) 2016:8916534. doi: 10.1155/2016/8916534

144. Yang J, Wang X, Liu S, Xue G. BDNF expression is up-regulated by progesterone in human umbilical cord mesenchymal stem cells. *Neurol Res*. (2016) 38:1088–93. doi: 10.1080/01616412.2016.1235248

145. Yi H, Qinxin Y, Qi W, Guangchao X, Fang Q, Chengliang D, et al. Research on feasibility of invitro inflammatory wound microenvironment simulated by using inflammatory wound tissue homogenate of mice. *Zhonghua Shao Shang Za Zhi*. (2020) 36(11):1024–34. doi: 10.3760/cma.j.cn501120-20200720-00351

146. Zhen W. Study on the effect of human umbilical cord mesenchymal stem cells exosomes induced by simulated wound microenvironment on wound healing. *Zunyi Medical College* (2022). doi: 10.27680/,dcnki.Gzyyc.2022.000125

147. Brenna S, Altmeppen HC, Mohammadi B, Rissiek B, Schlink F, Ludewig P, et al. Characterization of brain-derived extracellular vesicles reveals changes in cellular origin after stroke and enrichment of the prion protein with a potential role in cellular uptake. *J Extracell Vesicles*. (2020) 9:1809065. doi: 10.1080/20013078.2020.1809065

148. Li SR, Man QW, Gao X, Lin H, Wang J, Su FC, et al. Tissue-derived extracellular vesicles in cancers and non-cancer diseases: Present and future. *J Extracell Vesicles*. (2021) 10:e12175. doi: 10.1002/jev2.12175

149. Parker L. Using human tissue: when do we need consent? *J Med Ethics*. (2011) 37:759–61. doi: 10.1136/medethics-2011-100043

150. Hao Y, Horak J, Stijepic Z, Can SN, Tu L, Wolff JA, et al. Comprehensive tissue homogenization and metabolite extraction for application in clinical metabolomics. *Anal Chim Acta*. (2025) 1344:343728. doi: 10.1016/j.aca.2025.343728

151. Crescitelli R, Lässer C, Lötvall J. Isolation and characterization of extracellular vesicle subpopulations from tissues. *Nat Protoc*. (2021) 16:1548–80. doi: 10.1038/s41596-020-00466-1

152. Huang Y, Cheng L, Turchinovich A, Mahairaki V, Troncoso JC, Pletniková O, et al. Influence of species and processing parameters on recovery and content of brain tissue-derived extracellular vesicles. *J Extracell Vesicles*. (2020) 9:1785746. doi: 10.1080/20013078.2020.1785746

153. Görgens A, Corso G, Hagey DW, Jawad Wiklander R, Gustafsson MO, Felldin U, et al. Identification of storage conditions stabilizing extracellular vesicles preparations. *J Extracell Vesicles*. (2022) 11:e12238. doi: 10.1002/jev2.12238

154. Gelibter S, Marostica G, Mandelli A, Siciliani S, Podini P, Finardi A, et al. The impact of storage on extracellular vesicles: A systematic study. *J Extracell Vesicles*. (2022) 11:e12162. doi: 10.1002/jev2.12162

155. Rani V, Sengar RS. Biogenesis and mechanisms of microRNA-mediated gene regulation. *Biotechnol Bioeng*. (2022) 119:685–92. doi: 10.1002/bit.28029

156. Li Z, Wu Y, Tan G, Xu Z, Xue H. Exosomes and exosomal miRNAs: A new therapy for intervertebral disc degeneration. *Front Pharmacol*. (2022) 13:992476. doi: 10.3389/fphar.2022.992476

157. Li B, Cao Y, Sun M, Feng H. Expression, regulation, and function of exosome-derived miRNAs in cancer progression and therapy. *FASEB J*. (2021) 35:e21916. doi: 10.1096/fj.202100294RR

158. Wang J, Wang J, Li X, Shu K. Cell-derived exosomes as therapeutic strategies and exosome-derived microRNAs as biomarkers for traumatic brain injury. *J Clin Med*. (2022) 11(11):3223. doi: 10.3390/jcm11113223

159. Beuzelin D, Kaeffer B. Exosomes and miRNA-loaded biomimetic nanovehicles, a focus on their potentials preventing type-2 diabetes linked to metabolic syndrome. *Front Immunol*. (2018) 9:2711. doi: 10.3389/fimmu.2018.02711

160. Rozier P, Maumus M, Maria ATJ, Toupet K, Lai-Kee-Him J, Jorgensen C, et al. Mesenchymal stromal cells-derived extracellular vesicles alleviate systemic sclerosis via miR-29a-3p. *J Autoimmun*. (2021) 121:102660. doi: 10.1016/j.jaut.2021.102660

161. Baral H, Uchiyama A, Yokoyama Y, Sekiguchi A, Yamazaki S, Amalia SN, et al. Antifibrotic effects and mechanisms of mesenchymal stem cell-derived exosomes in a systemic sclerosis mouse model: Possible contribution of miR-196b-5p. *J Dermatol Sci*. (2021) 104:39–47. doi: 10.1016/j.jdermsci.2021.08.006

162. Liu W, Li L, Rong Y, Qian D, Chen J, Zhou Z, et al. Hypoxic mesenchymal stem cell-derived exosomes promote bone fracture healing by the transfer of miR-126. *Acta Biomater*. (2020) 103:196–212. doi: 10.1016/j.actbio.2019.12.020

163. Meng WT, Zhu J, Wang YC, Shao CL, Li XY, Lu PP, et al. Targeting delivery of miR-146a via IMTP modified milk exosomes exerted cardioprotective effects by inhibiting NF-κB signaling pathway after myocardial ischemia-reperfusion injury. *J Nanobiotechnol*. (2024) 22:382. doi: 10.1186/s12951-024-02631-0

164. Cheng F, Yang F, Wang Y, Zhou J, Qian H, Yan Y. Mesenchymal stem cell-derived exosomal miR-27b-3p alleviates liver fibrosis via downregulating YAP/LOXL2 pathway. *J Nanobiotechnol*. (2023) 21:195. doi: 10.1186/s12951-023-01942-y

165. Yao Z, Li J, Xiong H, Cui H, Ning J, Wang S, et al. MicroRNA engineered umbilical cord stem cell-derived exosomes direct tendon regeneration by mTOR signaling. *J Nanobiotechnol*. (2021) 19:169. doi: 10.1186/s12951-021-00906-4

166. Yan C, Chen J, Wang C, Yuan M, Kang Y, Wu Z, et al. Milk exosomes-mediated miR-31-5p delivery accelerates diabetic wound healing through promoting angiogenesis. *Drug Deliv*. (2022) 29:214–28. doi: 10.1080/10717544.2021.2023699

167. Xue Q, Yang Y, Yang L, Yan X, Shen Z, Liu J, et al. miR-371b-5p-engineered exosomes enhances tumor inhibitory effect. *Front Cell Dev Biol*. (2021) 9:750171. doi: 10.3389/fcell.2021.750171

168. de Abreu RC, Ramos CV, Becher C, Lino M, Jesus C, da Costa Martins PA, et al. Exogenous loading of miRNAs into small extracellular vesicles. *J Extracell Vesicles*. (2021) 10(10):e12111. doi: 10.1002/jev2.12111

169. Tian T, Zhang HX, He CP, Fan S, Zhu YL, Qi C, et al. Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. *Biomaterials*. (2018) 150:137–49. doi: 10.1016/j.biomaterials.2017.10.012

170. Millán Cotto HA, Pathrikar TV, Hakim B, Baby HM, Zhang H, Zhao P, et al. Cationic-motif-modified exosomes for mRNA delivery to retinal photoreceptors. *J Mater Chem B*. (2024) 12:7384–400. doi: 10.1039/D4TB00849A

171. Zhang C, Pathrikar TV, Baby HM, Li J, Zhang H, Selvadoss A, et al. Charge-reversed exosomes for targeted gene delivery to cartilage for osteoarthritis treatment. *Small Methods*. (2024) 8:e2301443. doi: 10.1002/smtd.202301443

172. Ellipilli S, Wang H, Binzel DW, Shu D, Guo P. Ligand-displaying-exosomes using RNA nanotechnology for targeted delivery of multi-specific drugs for liver cancer regression. *Nanomedicine*. (2023) 50:102667. doi: 10.1016/j.nano.2023.102667

173. Zairong W, Dali W. MSCs in wound repair——extracellular vesicles exosome microenvironment hypothesis. *Chin J Plast Surge*. (2019) 35:324–30. doi: 10.3760/cma.j.issn.1009-4598.2019.04.002

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The therapeutic efficacy of mesenchymal stem cells and their extracellular vesicles in the treatment of diverse skin diseases

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This review presents the current knowledge on the potential of mesenchymal stem cells (MSCs) and their extracellular vesicles (EVs) in the treatment of various skin diseases such as psoriasis, atopic dermatitis, contact dermatitis, systemic sclerosis, graft-versus-host disease, alopecia areata, and systemic lupus erythematosus. MSCs can modulate the immune response and release growth factors and cytokines that promote tissue regeneration and healing and reduce inflammation. In turn, EVs' ability to transport various biological molecules, including microRNAs (miRNAs), makes them potential therapeutic agents. Moreover, EVs have been shown to reduce scaling, thickness, and erythema in psoriasis. In atopic dermatitis, EVs can alleviate clinical symptoms, lower serum IgE levels, and reduce immune cell infiltration. Mesenchymal stem cells like bone marrow-derived mesenchymal stem cells (BM-MSCs) exert their immunomodulatory effects by directly targeting various immune cell populations, including T cells, B cells, dendritic cells, and natural killer (NK) cells. To fully realize the potential of EVs in clinical practice, further research is needed to conduct well-designed clinical trials to evaluate the safety and efficacy of EV-based therapies in different skin diseases. Overall, EVs have the potential to revolutionize the treatment of skin diseases by offering a targeted and effective approach to address various underlying mechanisms, although further large-scale studies are needed.

KEYWORDS

extracellular vesicles, exosomes, mesenchymal stem cells, skin diseases, psoriasis, atopic dermatitis, systemic sclerosis, graft-versus-host disease

1 Introduction

Psoriasis and atopic dermatitis (AD) are two autoimmune skin diseases with increasing prevalence.

Psoriasis is a long-lasting inflammatory skin disease that causes red, scaly patches on the skin. There is a significant genetic predisposition and autoimmune pathogenic traits.

While psoriasis vulgaris, also called plaque-type psoriasis, is the most prevalent type, there are different clinical subtypes of psoriasis, each with its own dermatological manifestation. These subtypes include guttate, inverse, pustular, and erythrodermic (1). The classical clinical manifestations of psoriasis vulgaris are sharply defined borders of the affected skin areas, erythema, itchiness (pruritus), and silvery scales covering the affected areas. The plaques can grow and merge, covering large portions of the skin. The most common locations include the trunk, extensor surfaces of the limbs (elbows, knees) and scalp (2, 3). Inverse psoriasis is also known as flexural psoriasis and affects skin folds, or intertriginous areas, such as the groin, armpits, under the breasts, perianal region. Moreover, an inverse psoriasis is characterized by slightly eroded, red patches on the skin and an absence of the typical silvery scales seen in plaque psoriasis (4). Guttate psoriasis typically develops suddenly and often is triggered by a streptococcal infection. It is characterized by small, red, scaly patches that resemble drops of water. This is the most common type of psoriasis in children and adolescents. About one-third of individuals with guttate psoriasis may develop plaque psoriasis later in life (5, 6). Furthermore, pustular psoriasis is a type of psoriasis characterized by the formation of pus-filled blisters (pustules) on the skin. It can be localized or generalized. Localized pustular psoriasis is divided into psoriasis pustulosa palmoplantaris (PPP), which affects the palms and soles of the feet and is characterized by multiple, small, sterile pustules, and acrodermatitis continua of Hallopeau (ACH), which affects the distal parts of the fingers and toes as well as the nails. In turn, generalized pustular psoriasis is a severe and potentially life-threatening condition with rapid onset of widespread redness (erythema) and numerous pustules. It is often accompanied by systemic symptoms like fever, chills, and joint pain. It is important to note that pustular psoriasis can be a debilitating condition, and prompt medical attention is crucial for effective management (7). However, inflammation in psoriasis is not limited to the skin areas. It can affect various organs and systems, contributing to a range of comorbidities. Psoriatic arthritis is a common complication of psoriasis, affecting the joints and causing pain, swelling, and stiffness. Psoriasis has been linked to several other health conditions, including cardiovascular disease, metabolic syndrome, depression, and kidney disease (1).

While the most visible manifestations of psoriasis occur in the epidermis (outermost layer), the underlying dermis also plays a crucial role. The interaction between keratinocytes and various immune cells (for instance T cells and dendritic cells) within the dermis drives the inflammatory process (8). Dendritic cells (DCs) are indeed key players in the early stages of psoriasis, acting as professional antigen-presenting cells (APCs). Their activation in psoriasis is a complex process involving various factors, including antimicrobial peptides (AMPs) released by keratinocytes. In response to injury or inflammation, keratinocytes release AMPs like LL37, β-defensins, and S100 proteins (9)—for instance, LL37 acts as an antimicrobial peptide as well as signal that activates the immune system (10). Activated pDCs release type I interferons (IFN-α and IFN-β) that act on myeloid dendritic cells (mDCs). This leads to the maturation and activation of mDCs. Mature mDCs present antigens to T cells, leading to their activation and differentiation. Moreover, type I interferons promote the differentiation of T cells into Th1 and Th17 cells. Afterwards, Th1 cells produce IFN-γ, while Th17 cells release IL-17. Finally, IFN-γ and IL-17 are potent pro-inflammatory cytokines, and they stimulate keratinocytes to proliferate and differentiate abnormally, leading to the formation of psoriatic plaques (11–13).

Atopic dermatitis is a recurrent, chronic, non-infectious inflammatory skin disease (14). It commonly develops within the first year of life and affects over 200 million individuals worldwide. It is estimated that the total global prevalence of AD is around 3% to 4% in adults with female predominance and about 15%–25% in children (15). The disease is characterized by eczema-like rashes, erythema, papules, and exudative lesions, usually worsening at night. AD is classified based on age of onset, and the distribution of skin lesions changes as a person ages. AD is often categorized into infantile, childhood, adolescent, and adult subtypes. In infants (usually aged 0–2 years), lesions often affect the face (cheeks, forehead), scalp, and extensor surfaces of the limbs (outer elbows and knees), but the diaper area is usually spared. In patients aged between 2 and 12 years, cutaneous manifestations often transfer to the flexural surfaces (antecubital fossa, popliteal fossa), wrists, ankles, and neck. In adult patients with AD (usually 12+ years), cutaneous lesions occur with local or widespread involvement (16). Atopic dermatitis is a member of a triad called the "atopic triad," along with allergic rhinitis and bronchial asthma. These conditions often occur together in individuals, either simultaneously or sequentially, and are linked by a shared predisposition and immune response. In patients with atopic dermatitis, the overall prevalence of rhinitis is 40.5%, asthma 25.7%, or both at 14.2%. On the other side, AD may be associated with non-atopic comorbidities such as autoimmune, cardiovascular, endocrine, infectious, ocular, and psychiatric diseases and some cancers (breast, brain, keratinocyte, lung, pancreatic cancer, lymphoma, and melanoma) (17). The pathogenesis of AD is complex and combines skin barrier dysfunction, dysregulation of innate and adaptive immune system, dysbiosis of the skin bacterial microbiome, genetic predisposition, and environmental factors. It is believed that the epidermal barrier defect is associated with mutations in the filaggrin gene, which, in combination with ceramide deficiency, lead to transepidermal water loss and increased penetration of irritants, allergens, and microorganisms into the skin. In turn, the disruption of the skin barrier leads to a cascade of events, including chronic inflammation, epidermal hyperplasia, and the infiltration of immune cells. These immune cells include dendritic cells, eosinophils, and T lymphocytes, all contributing to the inflammatory response. Dysregulation of a type-2 T-helper cell (Th2), Th17, and Th22 inflammatory response plays a crucial role in the pathogenesis of the disease. As a result, a broad range of pro-inflammatory cytokines like interleukins IL-2, IL-4, IL-5, IL-12, IL-13, IL-18, IL-21, and IL31, tumor necrosis factor α (TNF-α), and interferon γ (IFN-γ) are produced. These cytokines contribute to skin barrier dysfunction, inflammation, and disease progression (18).

One promising approach for treating inflammatory skin diseases involves the use of mesenchymal stem cells (MSCs).

MSCs have demonstrated the ability to modulate the immune system, repair damaged tissues, and stimulate angiogenesis (19). It has been provided a strong support for the therapeutic efficacy and safety of intravenously injected bone marrow-derived mesenchymal stem cells (BM-MSCs) in various inflammatory skin conditions—for example, in acute and chronic graft-versus-host disease (GvHD) with skin manifestations (20), systemic lupus erythematosus (SLE) (21), and systemic sclerosis (SSc) (22). The beneficial effects of MSCs' applications in tissue repair are attributed to their paracrine action via the secretion of vesicles such as extracellular vesicles (EVs) rather than to cell engraftment and response to the site of injury. Several studies indicate that MSC-derived EVs represent an alternative to MSC transplantation and can even replace stem cell-based therapy. Indeed MSC-derived EV therapy has been successfully used in various disease models, including diabetes, cancer, skin injuries, neurological, cardiovascular, immunological, renal disorders. While EV therapy holds promise, potential risks need careful consideration. Though generally less immunogenic than whole cells, EVs can still trigger immune responses or interact negatively with the host's immune system, especially at high doses or with prolonged use. This could affect hemostasis, potentially leading to thrombosis. Furthermore, the variability in EV production and isolation methods can impact consistency and therapeutic effectiveness, posing challenges for standardization and large-scale manufacturing.

Extracellular vesicles (EVs), naturally secreted by cells, have recently emerged as pathophysiological mediators of psoriasis. EVs come in various sizes, ranging from smaller exosomes (30–150 nm) to larger microvesicles (100–1,000 nm) and apoptotic bodies (1,000–5,000 nm) (12). Furthermore, EVs play a prominent role in the modulation of several biological processes. Firstly, EVs can attenuate inflammation by increasing the secretion of anti-inflammatory cytokines and promoting Treg polarization. They can also transport antigens and co-stimulatory molecules, contributing to immune cell memory and tolerance (23). Secondly, EVs can be involved in host defense against pathogens. They can contain antimicrobial factors like peptides and proteins, and they can also promote immune responses that help eliminate bacteria (24). Thirdly, EVs can influence cell growth and movement by delivering growth factors, signaling molecules, and extracellular matrix components. This is particularly important in processes like wound healing and development (25). Finally, EVs can stimulate the formation of new blood vessels, a process known as angiogenesis. This is essential for tissue growth and repair, but it can also contribute to pathological conditions like cancer (26, 27).

MSC-derived extracellular vesicles (EVs) universally suppress Th1/Th2/Th17 axis across different skin diseases such as psoriasis and atopic dermatitis. Psoriasis and AD are both inflammatory skin conditions, but their underlying immune mechanisms differ. Psoriasis is primarily driven by the Th17/IL-17 pathway, while AD involves multiple pathways, including Th2, Th22, and potentially Th17. This difference in immune involvement explains why targeting the Th17/IL-17 axis is highly effective for psoriasis, but a broader approach targeting multiple pathways is needed for AD. EVs can modulate immune responses, and their effects on different T helper cell subsets are distinct. For Th1 cells, EVs typically reduce the levels of pro-inflammatory cytokines like IFN-γ, TNF-α, and IL-2 (28). Conversely, for Th17 cells, the immunomodulatory effect of EVs is often characterized by a reduction in the levels of IL-17, IL-21, and IL-22. Both IL-17 and IL-22 drive keratinocyte proliferation and contribute to the inflammatory environment characteristic of psoriasis (29). Therefore, keratinocytes, the main cells of the epidermis, are a primary target of EVs in psoriasis. Mast cells are the target for EVs in AD. They contribute to inflammation not only through immediate hypersensitivity reactions but also by releasing a variety of cytokines and chemokines that further amplify the inflammatory response (29). This differential impact on cytokine profiles suggests that EVs can selectively influence the activity of these T helper cell subsets. MSC-derived EVs transfer miRNAs that suppress T-cell proliferation and cytokine production, inhibiting the differentiation of pro-inflammatory Th1 and Th17 cells and promoting the generation of regulatory T (Treg) cells. Th1 and Th17 cell differentiation is regulated by specific cytokines and transcription factors. Th1 cells are driven by IL-12 and T-bet via the STAT4 pathway, whereas Th17 cells are promoted by TGF-β, IL-6, and IL-23, engaging the RORγt and STAT3 pathways. EVs can modulate these pathways by delivering molecules like miRNA, proteins that inhibit the expression of key transcription factors such as T-bet and RORγt, thereby reducing the IFN-γ and IL-17 levels (30). In addition, EVs can also promote a shift in macrophage phenotype from pro-inflammatory (M1) to anti-inflammatory (M2) states, which are beneficial for tissue repair and inflammation resolution (30).

Extracellular vesicles are excellent candidates for the treatment of chronic inflammatory skin diseases due to their favorable biological properties such as biocompatibility, stability, and low toxicity. These small vesicles are involved in the intercellular transportation of biologically active molecules including nucleic acids, transcription factors, cytokines, carbohydrates, lipids, proteins, extracellular matrix proteins, receptors, and antigens. The great potential of the EVs is used for controlled drug delivery, gene delivery (due to their microRNA and mRNA content), and biomarker-driven therapies. Despite the innate benefits of MSC-derived EVs, the cell-free therapy has some drawbacks, including low targeting efficiency, low yield, limited tissue repair capabilities, and limited drug delivery capabilities. Therefore, some genetic modifications or pretreatment of EVs are proposed to increase the effectiveness of the cell-free strategy.

In recent years, researchers have mainly focused on different aspects of the use of MSC-derived EVs in the therapy of many diseases. While the exact implementation in dermatoses still remains not completely explained, the application of these EVs in skin conditions is an area of proceeding research. This narrative review aims to evaluate the potential implementation of EVs in widespread skin diseases such as psoriasis, atopic dermatitis, contact dermatitis, systemic sclerosis, graft-versus-host disease, alopecia areata, and systemic lupus erythematosus.

2 Discussion

2.1 Mesenchymal stem cell-derived extracellular vesicles

2.1.1 Psoriasis

Bakr et al. (31) investigated the therapeutic potential of BM-MSCs and their derived EVs (i.e., exosomes) in psoriasis-like inflammatory changes in animal models. The researchers used a psoriasis model induced by imiquimod (IMQ) in female albino rats. The rats were divided into four groups: control, IMQ-only, IMQ + BM-MSCs, and IMQ + BM-MSC-derived exosomes. The results showed that both BM-MSCs and exosomes reduced the inflammatory changes in the skin as observed through general observations and a microscopic examination of stained sections. Additionally, it was found that BM-MSCs secrete various cytokines that shift T lymphocyte populations from effector T cells to regulatory T cells. These cytokines include transforming growth factor-beta (TGF-β), hepatocyte growth factor (HGF), prostaglandin E2 (PGE2), nitric oxide (NO), and indoleamine 2,3-dioxygenase (IDO). Finally, BM-MSCs can directly interact with NK cells through cell–cell contact. This interaction leads to the downregulation of activating receptors on NK cells. Consequently, the cytotoxic activity of NK cells was reduced, leading to an immunosuppressive effect. Furthermore, BM-MSCs secrete various paracrine factors, such as IL-6, PGE2, and GRO-γ, which can suppress the maturation of dendritic cells (DCs). Additionally, BM-MSCs can interact with DCs through Jagged-2, further inhibiting their maturation. Overall, the study provided evidence that both BM-MSCs and their derived exosomes have potential as therapeutic agents for psoriatic skin lesions (31) (Table 1).

Zhang et al. (32) investigated how topically applied EVs (i.e., exosomes) alleviate psoriasis-associated inflammation. MSC-derived EVs were administered intraperitoneally or topically applied in a mouse model of imiquimod-induced psoriasis. Reduced levels of IL-17 and C5b-9 were demonstrated in psoriatic skin treated with exosomes. Most likely, MSC-derived exosomes act through CD59 to inhibit the formation of the terminal-activated C5b-9 complement complex, which subsequently inhibits NETosis (neutrophil extracellular trap formation) and the secretion of IL-17 by neutrophils. In conclusion, this type of treatment may lead to the mitigation of the spread and amplification of inflammatory signals within psoriatic skin lesions (32) (Table 2).

2.1.2 Contact dermatitis

Guo et al. (33) presented the potential therapeutic role of human umbilical cord mesenchymal stem cell-derived extracellular vesicles (hUC-MSC-derived EVs) in preventing contact hypersensitivity (CHS). hUC-MSC-derived EVs inhibited the proliferation of cytotoxic T cells (Tc1) and type 1 helper T cells (Th1), both of which play crucial roles in inflammation. Additionally, hUC-MSC-derived EVs promoted the generation of regulatory T cells (Tregs), which have immunosuppressive properties. hUC-MSC-derived EVs reduced TNF-α and IFN-γ, two key pro-inflammatory cytokines, and increased the production of IL-10, an anti-inflammatory cytokine (33). Collectively, these changes downregulate inflammatory responses such as macrophage activation and recruitment of additional immune cells to the site of inflammation and thereby ameliorate contact dermatitis (Table 1).

UC-MSC-EVs are often observed to outperform BM-MSC-EVs in contact dermatitis due to their enriched anti-inflammatory cargo (miRNAs, proteins, and lipids), stronger suppression of Th1/Th17 responses, enhanced skin repair capabilities, and biological advantages (younger cell source and easier to obtain and culture, leading to a higher yield of EVs and lower immunogenicity). These factors caused UC-MSC-EVs to be more effective at decreasing inflammation, restoring the skin barrier, and promoting resolution in CD models. Although UC-MSC-EVs appear to be more effective in treating contact dermatitis than BM-MSC-EVs, they are not effective in treating psoriasis, likely due to differences in the inflammatory environment and the specific mechanisms involved in each disease. In psoriasis, while MSC-EVs also play a role in immunomodulation, the disease's complex pathogenesis and the involvement of specific pathways like the NF-κB signaling pathway, which can be targeted by engineered MSC-EVs, might explain why certain engineered EVs show better results.

2.1.3 Systemic sclerosis

Jin et al. (34) demonstrated the potential of BM-MSC-EVs as a therapeutic approach for skin dysfunction in scleroderma (SSc). BM-MSC-EVs showed a similar efficacy to cell-based therapy in treating SSc but with fewer regulatory requirements, making them a more suitable therapeutic option. The therapeutic effects of BM-MSC-EVs were primarily attributed to the microRNAs (miRNAs) they carried, including miR-21a, miR-143, miR-27b, miR-29a, and let-7. These miRNAs are known to have immunomodulatory and anti-fibrotic effects—for instance, the let-7 family of microRNAs plays a role in regulating immune responses by modulating the production of pro-inflammatory cytokines and receptors. Specifically, let-7 inhibits the production of cytokines like IL-8 and receptors like IL1r1 and IL23r, which are involved in Th17 cell differentiation and regulate NKT cell function. Additionally, the miRNAs in BM-MSC-EVs were involved in regulating the proliferation and differentiation of multiple cell types and various EV-related biological processes. Precisely, BM-MSC-EV treatment led to a significant reduction in TGF-β1-positive cells, α-SMA-positive myofibroblasts, mast cells, infiltrating macrophages, and lymphocytes in the skin of SSc mice. Immunohistochemical analysis showed that BM-MSC-EV treatment significantly reduced the infiltration of inflammatory cells, such as F4/80+ macrophages and CD4+/CD8+ lymphocytes, in a mouse model of SSc. Moreover, BM-MSC-EVs decreased the mRNA levels of inflammatory cytokines: IL6 and TNF-α in SSc mice. To sum up, the fact that BM-MSC-derived EVs can achieve similar therapeutic outcomes to the cells themselves, while offering a more manageable, safe, and regulatory profile, strongly enhances their development as

TABLE 1 Summary of the studies on extravesicular vesicles.

Source of MSC-EVs	Disease	Doses	Model	Key observation	References
Bone marrow	Psoriasis	A dose of 1 million BM-MSCs in 1 mL phosphate buffer saline	50 albino rats	BM-MSCs and exosomes significantly ameliorated psoriasis-like skin inflammation in rats. BM-MSCs secrete various cytokines that shift T lymphocyte populations from effector T cells to regulatory T cells.	Bakr et al. (31)
Bone marrow	Psoriasis	n/a	Male Balb/C mice	MSC exosomes act through CD59 to inhibit the formation of the terminal-activated C5b-9 complement complex, which subsequently inhibits NETosis and the secretion of IL-17 by neutrophils.	Zhang et al. (32)
Human umbilical cord	Contact dermatitis	10 or 50 μg/mL hUC-MSC-derived EVs	6-week-old male BALB/c mice	hUC-*MSC*-derived EVs inhibited the proliferation of cytotoxic T cells (Tc1) and type 1 helper T cells (Th1) and promoted the generation of regulatory T cells (Tregs).	Guo et al. (33)
Bone marrow	Systemic sclerosis	A dose of BMSC-EV 15 μg/100 μL	Female C57BL/ 6 mice	BMSC-EV treatment led to a significant reduction in TGF-β1-positive cells, α-SMA-positive myofibroblasts, mast cells, infiltrating macrophages, and lymphocytes in the skin of SSc mice.	Jin et al. (34)
Bone marrow	Systemic sclerosis	n/a	BLM-treated and IL-33-knockout mice	BMSC-Exos inhibits the IL-33/ST2 axis leading to reduced proliferation, migration, and fibrotic gene expression in fibroblasts, ultimately mitigating skin fibrosis.	Xie et al. (35)
Bone marrow	Systemic sclerosis	UC-MSC exosomes (a dose of 1×10^9 particles/mL)	Human dermal fibroblasts	Treatment with UC-MSC exosomes led to an increase in the expression of collagen I and elastin, key proteins involved in skin rejuvenation.	Kim et al. (36)
Bone marrow	Systemic sclerosis	The dose of MSCs 0.1×10^6 cells per 10 g body weight	B6.Cg-Fbn1Tsk/J; *Tsk*/$^+$ mice	A downregulation of mTOR pathway activation to boost osteogenic differentiation and lower adipogenic differentiation	Chen et al. (37)
Bone marrow	Graft-versus-host disease	Dose of bone marrow cells, 8×10^6	10- to 12-week-old BALB/cJ$^{H\text{-}2d}$ female mice	MSCs-exo ameliorated fibrosis in the skin, lung, and liver and decreased the clinical and pathological scores of cGVHD. In pathomechanism, MSCs-exo exhibited potent immunomodulatory effects by inhibiting IL-17-expressing pathogenic T cells and inducing IL-10-expressing regulatory cells.	Lai et al. (38)
Mesenchymal	Graft-versus-host disease	The dose was 100 μg MSC-EVs	8- to 10-week-old female BALB/c (H-2d) mice	The study observed a decrease in macrophage percentage in the skin and spleen, suggesting that MSC-EVs reduced macrophage activity. Moreover, a reduction in TGF-β and SMAD2 production, key factors involved in fibrosis, was noted in the skin of MSC-EV-treated mice.	Guo et al. (39)
Mesenchymal	Alopecia areata	Th dose: 1 mL baricitinib-loaded EVs, (86.37 μg baricitinib and 2.48×10^{11} EV particles)	Twenty 6-week-old female C57BL/ 6 mice	All treatment groups (baricitinib alone, empty EVs, and EV-B) showed enhanced hair regrowth compared to the control group in mice with induced AA. EV-B decrease an inflammation, by downregulating the expression of IFN-γ, Jak-2, Stat-1, and IL-15. Additionally, to alleviate the inflammation, EV-B treatment led to a boost in the expression of β-catenin (it is responsible for hair follicle development and the anagen phase of the hair cycle).	Tang et al. (40)

BM-MSCs, bone marrow mesenchymal stem cells; IMQ, imiquimod; TGF-β, transforming growth factor-beta; HGF, hepatocyte growth factor; PGE2, prostaglandin E2; NO, nitric oxide; IDO, indoleamine 2,3-dioxygenase; DCs, dendritic cells; hUC-MSC-derived EVs, human umbilical cord mesenchymal stem cell-derived extracellular vesicles; Tc1, cytotoxic T cells; Th1, type 1 helper T cells; Tregs, regulatory T cells; SSc, scleroderma; miRNAs, microRNAs; BM-MSC-Exos, bone marrow mesenchymal stem cell-derived exosomes; BLM, bleomycin; TGF-β1, potent fibrosis-inducing growth factor; HDFs, human dermal fibroblasts; EM, effector memory; EMRA, effector memory RA; FASL, FAS ligand; TERT, telomerase reverse transcriptase; BRG1, bromodomain-containing protein 1; MSCT, mesenchymal stem cell transplantation; IF, immunofluorescence; MSCs-exo, mesenchymal stem cell-derived exosomes; cGVHD, chronic graft-versus-host disease; AA, alopecia areata.

a potential next-generation therapy for the debilitating skin manifestations of SSc (34) (Table 1).

Xie et al. (35) investigated the mechanism by which bone marrow mesenchymal stem cell-derived exosomes (BM-MSC-Exos) alleviate skin fibrosis. The researchers hypothesized that miR-214, carried within exosomes released by stem cells (BM-MSCs), plays a crucial role in the pathogenesis of SSc. A mouse model of skin fibrosis was induced using bleomycin (BLM), a drug known to cause lung fibrosis. Mice were treated with BM-MSC-Exos to investigate their therapeutic potential in reducing fibrosis.

TABLE 2 Summary of the studies on mesenchymal cells in different skin diseases.

Source of mesenchymal stem cells	Disease	Dose	Model	Key observation	References
Bone marrow	Atopic dermatitis	A dose of 2×10^5 cells	Balb/c mice	BM-MSCs inhibited the proliferation and cytokine production of T cells by downregulating transcription factors such as T-bet, GATA-3, and c-Maf. BM-MSCs into ovalbumin-induced AD mice led to a significant reduction in cell infiltration in skin lesions, decreased serum IgE levels, and suppressed IL-4 expression in both lymph nodes and skin.	Na et al. (41)
Bone marrow	Atopic dermatitis	A dose of 1.0×10^6 cells/kg	Five patients with atopic dermatitis	Changes were observed in certain cytokines, including a decrease in CCL-17, IL-13, and IL-22 and an increase in IL-17 in AD patients with sustained clinical response.	Shin et al. (42)
Bone marrow	Atopic dermatitis	The dose of BM-MSCs was 80 µL/day for 10 days.	BALB/c mice with AD lesions	Cell-based therapy reduced the RNA expression levels of inflammatory cytokines in the AD skin, suppressed the serum IgE levels, and decreased the proinflammatory cytokines like IL-1b, IL-4, IL-6, IL-10, and IL-13.	Ryu et al. (43)
Tonsil-derived-mesenchymal cells	Atopic dermatitis	The dose of TMSC was 2×10^4.	C57BL/6J mice with AD	TMSC treatment inhibited T-cell-mediated inflammatory responses by reducing the levels of IL-6, IL-1β, TNF-α, IL-4, and B-cell-mediated serum IgE in mice with AD.	Jung et al. (44)
Dental follicle mesenchymal stem cells	Atopic dermatitis	The dose of DF-MSCs was 5×10^4.	9 patients with AD and 6 patients with psoriasis	DF-MSCs alleviate inflammation by reducing T cell apoptosis, expanding Tregs, and stabilizing cytokines	Zibandeh et al. (45)
Human umbilical cord blood	Atopic dermatitis	The dose of hUCB-MSCs was 2×10^6 cells/200 µL	NC/Nga mice with AD	Activated hUCB-MSCs produce TGF-β1, which plays a crucial role in reducing mast cell degranulation by downregulating FcεRI expression.	Kim et al. (46)
Human umbilical cord blood	Atopic dermatitis	The dose of hUCB-MSCs was 1×10^6.	NC/Nga mice with AD	hUCB-MSCs more effectively suppress the activation of mast cells and B lymphocyte.	Lee et al. (4)
Human umbilical cord blood	Atopic dermatitis	Two doses of hUCB-MSCs: the higher dose of 5.0×10^7 cells and the lower dose of 2.5×10^7 cells.	Adult patients with moderate-to-severe AD	The serum IgE levels and blood eosinophils were downregulated by the treatment.	Kim et al. (47)
Human umbilical cord blood	Atopic dermatitis	The dose of hUCB-MSCs was 2×10^6.	NC/Nga mice with AD The dose of hUCB-MSCs was 2×10^6.	Pimecrolimus also impaired the immunomodulatory activity of hUCB-MSCs, including their ability to inhibit Th2 cell differentiation and mast cell activation.	Shin et al. (48)
Bone marrow	Systemic sclerosis	The dose of BM-MSCs was 6×10^5 cells/cm².	Patients with SSc and SSc murine	The human counterpart of murine IL-10-producing B cells was significantly increased after an injection of alloBM-MSCs in severe SSc patients, either in all recipients or in patients with clinical improvement. Moreover, it was described as the increase of $CD24^{hi}CD27^{pos}$ memory B-cell frequency and the increase of *IL10* expression by B cells.	Loisel et al. (49)

(Continued)

TABLE 2 Continued

Source of mesenchymal stem cells	Disease	Dose	Model	Key observation	References
Bone marrow	Systemic sclerosis	The dose of BMMSCs was 0.02 × 10^6 cells.	C57BL/6J, B6.129S-*Terttm1Yjc*/J (*TERT*$^{-/-}$), B6.Cg-*Fbn1Tsk*/J (Tsk/$^{+}$), and B6Smn – mice	Treatment with BM-MSCs expressing telomerase led to a significant reduction in skin hypodermal thickness in SSc mice. Reintroduction of telomerase activity through TERT transfection restored the immunomodulatory functions of TERT−/− BM-MSCs.	Chen et al. (50)
Human umbilical cord blood	Systemic lupus erythematosus	n/a	Patients suffering from SLE	UC-MSC infusion led to a significant decline in disease activity as measured by SLEDAI and BILAG scores. Anti-double-stranded DNA antibody and antinuclear antibody quantities were lowered after MSCT infusion.	Wang et al. (51)

ε-GLT, ε-globin gene-linked lymphocyte activator; ε-PST, ε-protein synthesis; EASI, Eczema Area and Severity Index; M-MSCs, mesenchymal stem cells derived from menstrual blood; MCMC, media concentrate; TMSCs, tonsil-derived mesenchymal stem cells; DNFB, 2,4-dinitrofluorobenzene; DF-MSCs, dental follicle mesenchymal stem cells; IDO, indoleamine 2,3 dioxygenase; hUCB-MSCs, human umbilical cord blood-derived mesenchymal stem cells; MDP, muramyl dipeptide; MC, mast cell; SCORAD, SCORing for Atopic Dermatitis; Th2, type 2 helper T; NFAT3, nuclear factor of activated T cells; anti-dsDNA, anti-double-stranded DNA antibody; ANA, antinuclear antibody; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; BILAG, British Isles Lupus Assessment Group.

Samples from SSc patients were used to measure the levels of miR-214, IL-33, and ST2 (a receptor for IL-33). miRs have been shown to post-transcriptionally regulate gene expression, and miR-214 can inhibit SSc-related fibrosis. The researchers found that BM-MSC-Exos deliver miR-214, which targets IL-33 and blocks the IL-33/ST2 axis. When BM-MSC-Exos deliver a miR-214 inhibitor, it leads to increased proliferation, migration, and expression of fibrotic genes in fibroblasts that have been stimulated with TGF-β1. This indicates that miR-214 normally suppresses these fibrotic processes. Subsequently, IL-33, which acts via its receptor ST2, also induces migration, proliferation, and fibrotic gene expression in fibroblasts, mirroring the effects observed after miR-214 inhibition. This further supports the profibrotic role of the IL-33/ST2 axis. In mice treated with bleomycin (BLM) to induce skin fibrosis, knocking out IL-33 inhibited the development of skin fibrosis. Importantly, BM-MSC-Exos delivering miR-214 have been shown to ameliorate skin fibrosis in BLM-treated mice. This highlights the therapeutic potential of BM-MSC-Exos carrying miR-214 to counter fibrosis by targeting this specific pathway. Summing up, this research emphasizes the rationale for developing BMSC-derived EVs as a cell-free therapy for SSc (35) (Table 1).

Kim et al. (36) reported that human umbilical cord blood-derived mesenchymal stem cells exosomes can be essential for skin rejuvenation. Exosomes may penetrate the outermost layer of the epidermis within 3 h and gradually reach deeper layers. It was shown that treatment with hUC-MSC-derived exosomes (at the dose of 1×10^9 particles/mL) led to an increase in the expression of collagen I and elastin, key proteins involved in skin rejuvenation. Moreover, *in vitro* experiments showed that exosomes can integrate into human dermal fibroblasts (HDFs) and promote their migration and collagen synthesis. The study found that UC-MSC exosomes were more potent than cell-based therapy in enhancing collagen and elastin expression. Overall, these results present a compelling case for the incorporation of UC-MSC exosomes into products aimed at skin rejuvenation and potentially for therapeutic applications in various skin conditions (36) (Table 1).

Chen et al. (37) observed in mice with SSc that mesenchymal stem cell transplantation (MSCT) led to a reduction in skin thickness. Immunofluorescence (IF) staining revealed that CD63, a marker of exosomes, co-localized with CD105, a marker of MSC, in both the femur and skin. This suggests that MSCs (at the dose of 0.1×10^6 cells per 10 g of body weight) may release exosomes that contribute to their therapeutic effects. Beyond improving skin thickness, MSCT also rescued osteoporosis and autoimmune phenotypes in Tsk/+ mice. An inhibition of IL4Rα expression downregulated mTOR pathway activation to boost osteogenic differentiation and lower adipogenic differentiation. In addition, administration of MSC together with Ad-miR-151, a microRNA known to regulate fibrosis, led to a reduction in skin hypodermal thickness. This study shows an optimistic way of treatment with the MSCT in a mouse model of SSc (37) (Table 1).

2.1.4 Graft-versus-host disease

Lai et al. (38) reported the potential of mesenchymal stem cell-derived exosomes (MSCs-exo) as a promising therapeutic approach for chronic graft-versus-host disease (cGVHD). It has been demonstrated that MSCs-exo effectively prolonged the survival of mice with cGVHD and reduced the severity of clinical and pathological manifestations. Furthermore, MSCs-exo ameliorated fibrosis in the skin, lung, and liver and decreased the clinical and pathological scores of cGVHD. It is postulated that MSCs-exo exhibited potent immunomodulatory effects by inhibiting IL-17-expressing pathogenic T cells and inducing IL-10-expressing regulatory cells. Precisely, MSCs-exo treatment significantly reduced the expression of key Th17-related pro-inflammatory cytokines, including IL-17A, IL-21, IL-22, and IL-2. Moreover, MSCs-exo treatment reduced the percentage of activated CD4+ T cells (CD4+, CD44+) in cGVHD mice compared to the control

groups. MSCs-exo treatment also significantly decreased the expression of CCR6, a chemokine receptor involved in the recruitment of Th17 cells, which is implicated in cGVHD pathogenesis. Summing up, based on the above-mentioned data, this treatment with MSCs-exo could offer a new therapeutic avenue for chronic graft-versus-host disease cGVHD (38) (Table 1).

Guo et al. (39) investigated the therapeutic potential of MSC-EVs in sclerodermatous cGVHD. They found a decrease in macrophage percentage in the skin and spleen, suggesting that the secretome-based therapy (at the dose of 100 μg MSC-EVs every 5 days) reduced the macrophage activity. Moreover, a reduction in TGF-β and SMAD2 production, key factors involved in fibrosis, was noted in the skin of MSC-EV-treated mice. MSC-EVs were found to decrease the infiltration of macrophages, particularly the pro-inflammatory CD11b+F4/80+ subset. In addition, MSC-EVs appeared to interfere with the interaction between TFH/GC B cells and also reduced the ratio of BAFF to B cells. By suppressing macrophage activation and B cell responses, MSC-EVs may help to alleviate the inflammatory processes associated with cGVHD. According to this study, MSC-EVs possess multifaceted immunomodulatory capabilities and could be used as a fibrosis alleviator (39) (Table 1).

2.1.5 Alopecia areata

Tang et al. (40) explored a new approach to treat alopecia areata (AA). In this study, EVs derived from mesenchymal stem cells were loaded with baricitinib (EV-B). Baricitinib is a known inhibitor of the JAK-STAT pathway, which is implicated in the inflammation associated with AA. The authors found out that all treatment groups (baricitinib alone, empty EVs, and EV-B) showed enhanced hair regrowth compared to the control group in mice with induced AA. Importantly, the EV-B group displayed the most significant improvement, with complete hair coverage in the treated area by day 20. The superior efficacy of EV-B was likely due to the improved delivery of baricitinib by the EVs and a potential synergistic effect between the drug and the EVs themselves. EV-B downregulated the expression of IFN-γ, Jak-2, Stat-1, and IL-15, all of which are essential components of the JAK-STAT signaling pathway. EV-B further demonstrated a more significant reduction in the expression of Jak-2, Stat-1, and IL-15 compared with baricitinib alone. Additionally, to alleviate inflammation, EV-B treatment increased the expression of β-catenin, a key signaling molecule in the Wnt/β-catenin pathway, which plays a crucial role in hair follicle development and the anagen phase of the hair cycle. In this case, β-catenin can be considered a marker of hair follicles in the anagen phase. In summary, EV-B promoted hair growth in a mouse model of AA by inhibiting inflammation through the downregulation of the JAK-STAT pathway and promoting hair follicle regeneration through the upregulation of the Wnt/β-catenin pathway. The use of EVs seems to be aimed at enhanced drug delivery and potentially contributes to the therapeutic effect. In summary, this type of modality is a more effective treatment strategy for AA by aiming at both the inflammatory and regenerative sides of this hair condition (40) (Table 1).

2.2 Mesenchymal stem cells

2.2.1 Atopic dermatitis

Na et al. (41) highlighted the therapeutic potential of BM-MSCs in treating AD. BM-MSCs effectively suppressed the activation of both T and B cells in the atopic skin of mice. Precisely, BM-MSCs inhibited the proliferation and cytokine production of T cells by downregulating transcription factors such as T-bet, GATA-3, and c-Maf. In addition, BM-MSCs were found to significantly decrease the levels of ε-globin gene-linked lymphocyte activator (ε-GLT) and ε-protein synthesis (ε-PST), two key genes involved in IgE production. In consequence, BM-MSCs suppressed the production of immunoglobulin E (IgE) by downregulating AID and BLIMP-1, key regulators of isotype class switching and B-cell differentiation. Moreover, an intravenous injection of BM-MSCs into ovalbumin-induced AD mice led to a significant reduction in cell infiltration in skin lesions, decreased serum IgE levels, and suppressed IL-4 expression in both lymph nodes and skin. BM-MSCs migrated to the skin lesions and draining lymph nodes, indicating their ability to target the affected areas. This study presents a possibility of developing a new therapeutic strategy for AD (41) (Table 2).

Shin et al. (42) demonstrated the efficacy and safety of multiple doses of allogeneic BM-MSC in adult patients with moderate to severe AD that was refractory to conventional treatments. The primary outcome measures, such as Eczema Area and Severity Index (EASI), showed significant improvement at 16 weeks, with 80% of patients achieving EASI-50 after one or two treatment cycles. Long-term follow-up revealed no serious side effects and maintained clinical response in some patients for over 84 weeks. Additionally, changes in certain cytokine levels were observed, including a decrease in CCL-17, IL-13, and IL-22 and an increase in IL-17 in patients with sustained clinical response. Both the first patient and the second one achieved a significant reduction in AD symptoms, as measured by the EASI-50 score, after the first cycle of treatment. The therapeutic effects of these compounds were sustained for a prolonged period, demonstrating long-term efficacy. Patients who responded well to the treatment had relatively higher levels of IL-17 in their blood compared to other patients (42) (Table 2). Despite promising results, it should be emphasized that the above-mentioned study was conducted on a small scale (n = 5), which significantly limits its reliability and the possibility of drawing conclusions. Large-scale studies are needed to confirm the effectiveness of the therapy.

Ryu et al. (43) compared the immunoregulatory functions of mesenchymal stem cells derived from menstrual blood (M-MSCs) and BM-MSCs. They found that M-MSCs, administered at a dose of 80 μL/day for 10 days, exhibited superior immunoregulatory properties compared to BM-MSCs. Further investigation focused on M-MSC conditioned media concentrate (MCMC) in mice with AD lesions. MCMC significantly reduced the RNA expression levels of inflammatory cytokines in the skin, suppressed the serum IgE levels, and decreased proinflammatory cytokines like IL-1b, IL-4, IL-6, and IL-13. The histopathological analysis also revealed a

significant improvement in skin lesions. These findings suggest that secretome from M-MSCs hold promise for effectively treating AD-related inflammatory lesions and highlight the potential of M-MSCs as a promising approach for next-generation AD therapy (43) (Table 2).

Jung et al. (44) investigated the therapeutic effects of tonsil-derived mesenchymal stem cells (TMSCs) in a mouse model of AD induced by 2,4-dinitrofluorobenzene (DNFB). A subcutaneous injection of TMSCs (2×10^4) significantly improved the inflammatory symptoms in the AD mice, demonstrating therapeutic rather than protective effects. TMSC treatment inhibited T-cell-mediated inflammatory responses by reducing the levels of IL-6, IL-1β, TNF-α, IL-4, and B-cell-mediated serum IgE. Additionally, TMSCs enhanced the anti-inflammatory cytokine TGF-β. Both *in vitro* and *in vivo* findings suggest that TMSC treatment effectively improved the inflammatory skin lesions in the DNFB-induced AD mice model through its immunomodulatory effects. TMSCs demonstrated the ability to inhibit T-cell- and B-cell-mediated responses while promoting anti-inflammatory responses. Based on this research, TMSCs' therapeutic benefits in the context of AD are primarily due to their immunomodulatory effects (44) (Table 2).

Zibandeh et al. (45) observed a reduction in the frequency of Fas, FasL, and TNFR II in T cells, suggesting that dental follicle mesenchymal stem cells (DF-MSCs) actively reduce the signaling pathways that promote T cell apoptosis. While reducing T cell apoptosis may initially seem counterintuitive to suppress a hyperactive immune response, in the context of a chronic inflammatory state such as AD, inappropriate T cell death or survival may contribute to the pathology. Tregs are critical for maintaining immune tolerance and suppressing excessive or erroneous immune responses. Their increased frequency signifies an enhanced ability of the immune system to self-regulate and dampen inflammatory processes, which is highly beneficial in autoimmune or allergic conditions such as AD. A more balanced T-cell turnover or specific modulation of apoptotic pathways may be beneficial. In addition, the authors observed a promotion of the frequency of regulatory T cells (Treg), which are critical for maintaining immune tolerance and suppressing excessive or erroneous immune responses (45).

Kim et al. (46) suggested that NOD2-activated human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) can be a promising therapeutic approach for AD. Precisely, stimulating the NOD2 receptor on hUCB-MSCs (2×10^6 cells/200 μL PBS) with a specific ligand, muramyl dipeptide (MDP), enhanced their therapeutic efficacy. In addition, activated hUCB-MSCs produce TGF-β1, which plays a crucial role in reducing mast cell degranulation by downregulating FcεRI expression. The authors suggested that PGE2 secreted by hUCB-MSCs plays a crucial role in suppressing mast cell degranulation. PGE2 exerts its inhibitory effect by binding to EP2 and EP4 receptors on mast cells. While cell-to-cell contact between hUCB-MSCs and mast cells can enhance the inhibitory effect, PGE2 alone is sufficient to inhibit degranulation. What is more, the subcutaneous administration of MDP-stimulated MSCs significantly reduces scratching behavior, lymphocyte infiltration, and eosinophil infiltration in AD mice, suggesting their ability to modulate the immune response. This data powerfully summarizes the therapeutic impact of hUCB-MSCs in the treatment course of AD (46) (Table 2).

Lee et al. (52) observed that pretreating hUCB-MSCs with mast cell (MC) granules can enhance their therapeutic efficacy for AD treatment. Pre-exposure of hUCB-MSCs to MC granules enhanced their ability to resolve allergic immune reactions and accelerate tissue regeneration. The priming hUCB-MSCs with mast cells enhanced their therapeutic potential for wound healing by 38% compared to CM from naive hUCB-MSCs. Treatment with MC granule-primed hUCB-MSCs (likely in an *in vivo* wound model) led to several beneficial histological alterations like suppression of granulation, suppression of immune cell infiltration, and accelerated re-epithelialization. Moreover, pretreated hUCB-MSCs more effectively suppress the activation of mast cells and B lymphocytes, both key players in AD pathogenesis. Histamine released from MC granules upregulates the COX-2 signaling pathway in hUCB-MSCs, contributing to the suppression of the allergic immune response. Interestingly, MC granule priming also enhances the wound healing ability of hUCB-MSCs. MC-primed UCB-MSCs were more effective in suppressing B cell proliferation and maturation compared to naïve UCB-MSCs. In conclusion, UCB-MSCs could serve as a huge enhancement for AD therapy (52) (Table 2).

Kim et al. (47) revealed that the hUCB-MSCs were well tolerated and effective in reducing the symptoms of AD, including eczema area and severity, itching, and inflammation. Specifically, the high-dose group (5.0×10^7 cells) showed a greater improvement in eczema index and severity index compared to the low-dose group (2.5×10^7 cells). The high-dose group had substantial reductions in AD symptoms, including a 51% decrease in lesion intensity, a 58% decrease in pruritus, and a 65% decrease in insomnia at week 12. The overall SCORing for Atopic Dermatitis (SCORAD) score, a measure of AD severity, decreased by 50%, and 45% of the patients achieved a SCORAD-50 response, indicating a significant clinical improvement. A significant decrease in blood eosinophil count was observed at weeks 8 and 12 in the high-dose hUCB-MSC-treated group compared to baseline levels, suggesting that hUCB-MSCs may have a beneficial effect on the immune system in AD (47) (Table 2).

Shin et al. (48) demonstrated that the coadministration of pimecrolimus with hUCB-MSCs affect the therapeutic efficacy of hUCB-MSCs in AD. hUCB-MSCs exert their immunomodulatory effects through the secretion of various soluble factors, including TGF-beta, HGF, IL-6, IL-10, NO, and PGE2. PGE2 is a particularly important factor in modulating the immune response in AD. It can regulate the activity of various immune cells, such as T cells, monocytes, B cells, and NK cells. The hUCB-MSCs were used at a dose of 2×10^6 in a murine AD model. Importantly, pimecrolimus (the dose was 100 ng/mL) disrupted the therapeutic effects of hUCB-MSCs. Pimecrolimus also impaired the immunomodulatory activity of hUCB-MSCs, including their ability to inhibit type 2 helper T (Th2) cell differentiation and mast cell activation. The drug decreased the production of PGE2, a crucial immunomodulatory factor in

hUCB-MSCs. Additionally, pimecrolimus downregulated the COX2–PGE2 axis by inhibiting the nuclear translocation of the nuclear factor of activated T cells (NFAT3). These findings suggest that the therapeutic efficacy of hUCB-MSCs in AD can be influenced by co-administration with certain drugs and emphasize the need for further research to optimize hUCB-MSC therapy for AD patients (48) (Table 2).

2.2.2 Systemic sclerosis

Loisel et al. (49) studied the impact of a single injection of BM-MSCs on B-cell phenotypic, transcriptomic, and functional profiles in SSc patients, with a shift from profibrotic to regulatory B cells in clinical responders. These data altogether confirm the activation of cytotoxic T cells, unlike $CD4^{pos}$ T cells, in SSc patients receiving alloBM-MSCs, independently from the observed clinical response. A transient increase in CD8+ T-cell frequency and count was observed at the first month post-treatment, and a decrease in naive CD8+ T cells was noted at the third month. The upregulation of HLA-DR on both effector memory (EM) and effector memory RA (EMRA) CD8+ T cells was observed early on. No significant variations in the frequency of CD4+ T-cell subsets or HLA-DR expression were observed, except for a transient decrease in EM CD4+ T cells at M1. The observed T-cell changes were independent of the clinical response to alloBM-MSC infusion. Overall, two Breg subsets, considered as the human counterpart of murine IL-10-producing B cells, were significantly increased after the injection of alloBM-MSCs in severe SSc patients, either in all recipients or in patients with clinical improvement. In addition, the increase of $CD24^{hi}CD27^{pos}$ memory B-cell frequency and the increase of *IL10* expression by B cells, as associated with the observed clinical response to alloBM-MSCs, highlight Breg as key targets of MSCs in SSc. This data serves as a significant contribution to the understanding of BM-MSC biology and their therapeutic potential in complex autoimmune conditions (49) (Table 2).

Chen et al. (50) provided insights into the role of telomerase in the immunomodulatory functions of BM-MSCs and their potential therapeutic application for SSc. BM-MSCs lacking telomerase activity (TERT−/−) lost their ability to inhibit T cells and ameliorate SSc disease phenotype in mice. Reintroduction of telomerase activity through TERT transfection restored the immunomodulatory functions of TERT−/− BM-MSCs. TERT, in combination with β-catenin and BRG1, formed a transcriptional complex that bound to the FAS ligand (FASL) promoter. The transcriptional complex upregulated FASL expression and enhanced the immunomodulatory function of BM-MSCs. As well as that, the study demonstrated a positive correlation between telomerase activity and FASL expression. The upregulation of FasL is mediated by the Wnt/β-catenin signaling pathway. A complex formed by telomerase reverse transcriptase (TERT), β-catenin, and bromodomain-containing protein 1 (BRG1) directly binds to the FASL promoter to enhance its transcriptional activity. By targeting the TERT/β-catenin/BRG1 complex, it may be possible to modulate FasL expression and immune responses. Interestingly, treatment with BM-MSCs expressing telomerase led to a significant reduction in skin hypodermal thickness in SSc mice, comparable to the control group. This study furthermore provides a promising new direction to develop more effective BM-MSC-based therapy for patients suffering from SSc (50) (Table 2).

2.2.3 Systemic lupus erythematosus

Wang et al. (51) found hUCB-MSCs as a safe and effective treatment option for severe and refractory SLE. hUCB-MSCs were intravenously administered to patients with SLE and a significant improvement in disease activity, as well as amelioration of cutaneous symptoms, was observed. Importantly, hUCB-MSCs were well tolerated by patients with SLE, with no serious adverse events reported. Moreover, it was shown that, in serum, an anti-double-stranded DNA antibody (anti-dsDNA) and antinuclear antibody (ANA) level were lowered after MSC transplantation (MSCT), with statistically significant differences spotted at the follow-up performed in 6 and 12 months. hUCB-MSC infusion led to a significant decline in disease activity, as measured by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and British Isles Lupus Assessment Group (BILAG) scores (51) (Table 2).

3 MSCs and MSC-derived EVs' efficacy across different skin diseases

BM-MSC-derived EVs hold significant promise for the treatment of cutaneous manifestations of scleroderma. They elicit therapeutic effects similar to those achieved by the cells themselves while offering improved safety and regulatory benefits. This makes them a compelling alternative to cell-based therapies for SSc, especially considering the challenges associated with cell-based approaches (34). Furthermore, preclinical studies have shown that MSC-EVs can alleviate GvHD symptoms, reduce mortality, and improve overall survival in animal models (38, 39). Similarly, the use of MSC-derived EVs in drug delivery (e.g., baricitinib) for alopecia areata (AA) shows promise to improve treatment outcomes. EVs can act as carriers for therapeutic agents, enhancing their delivery to targeted areas and potentially improving the efficacy. This targeted approach, combined with the ability of EVs to modulate inflammation and promote hair regeneration, suggests a more effective treatment strategy for AA than conventional methods (40).

Clinical studies have shown the effectiveness of BM-MSC transplantation in the reduction of AD symptoms, including Eczema Area and Severity Index (EASI) scores, Investigator's Global Assessment (IGA) scores, and pruritus (itching). Additionally, BM-MSCs can modulate the immune system by influencing the balance of T helper (Th) cell subsets (Th1/Th17) and reducing inflammatory factors (42). Furthermore, menstrual blood-derived mesenchymal stem cells (M-MSCs) have been shown to be more effective in treating AD than BM-MSCs due to their higher proliferation rate, easier and less controversial isolation, and potential for improved homing and engraftment at injury sites (43). The immunoregulatory potential of dental follicle-derived mesenchymal stem cells in AD has been confirmed. Specifically,

DF-MSCs have demonstrated the ability to reduce inflammatory cytokine levels and promote the frequency of regulatory T cells (Tregs), which play a crucial role in suppressing immune response (45). Finally, human umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs) are well tolerated and effective in reducing the symptoms of atopic dermatitis, including eczema area and severity, itching, and inflammation (47). While preclinical and small-scale clinical studies are promising, large clinical trials are needed to confirm the safety and efficacy of MSC therapy for treating skin diseases in humans.

4 Safety and adverse reactions and risks

Since the isolation of MSCs often requires the use of invasive procedures, approaches that only require an *in vitro* culture of MSCs and use of the released product (i.e., EVs) offer as alternatives to MSCs. MSC-EVs cannot self-replicate, which is a key advantage over native MSCs when considering cell-based therapies. This lack of self-replication addresses concerns about uncontrolled cell division and potential tumorigenicity associated with MSCs. However, in one study, researchers found that hUC-MSC-derived EVs exhibited the potential for uncontrolled differentiation and proliferation, which may lead to undesirable long-term side effects (33). This inherent feature of hUC-MSC-derived EVs has been reported as a factor limiting their clinical application (33). On the other hand, most of the available studies confirmed the safety and efficacy of MSCs and MSCs-derived EVs in the treatment of dermatoses—for example, Shin et al. (42), Kim et al. (47), Shin et al. (48), and Wang et al. (51) did not find any serious adverse events in their long-term follow-up or clinical studies. Kim et al. (47) reported that most of the adverse events were local reactions at the injection site (induration, bruising, erythema, pain), which were transient and mild. Interestingly, the investigators also noted a rare occurrence of transient and mild skin infections or gastrointestinal disorders. Furthermore, Kim et al. (47) found that hUCB-MSCs were well tolerated in their study. Similarly, Wang et al. (51) found that hUCB-MSC transplantation was safe, well tolerated, and not associated with any adverse transplant-related events. The available studies indicate that the proposed therapy has a good safety profile, with mild and transient local reactions, but no serious adverse events were reported.

5 Challenges and future perspectives

Although MSC-derived EV therapy has numerous advantages, there are several drawbacks that limit the broader translational use of EVs. First of all, the methods for the isolation and purification of EVs should be standardized to ensure reproducibility. Quality control and standardization of EVs are crucial for their safe and effective use as a therapeutic agent. Ensuring consistency in Evs' isolation, purification, characterization, and production is essential to minimize variability and maximize their therapeutic potential. Several techniques are employed to detect and isolate EVs based on their size, for example: ultrafiltration, sequential filtration, size exclusion chromatography, immunoaffinity chromatography, anion-exchange chromatography, electrophoresis, and dielectrophoresis (DEP) based techniques, commercial kits, field-flow fractionation, and hydrostatic filtration dialysis. There are three centrifugation-based protocols for isolating EVs from biological samples (e.g., cell culture media, urine, plasma, serum) based on their physical properties, i.e., differential ultracentrifugation, zonal gradient centrifugation, and isopycnic gradient centrifugation. Among them, differential ultracentrifugation is considered the "gold standard" for EVs' isolation due to its ability to effectively separate EVs based on size and density, yielding relatively pure samples. However, its scalability for clinical use is limited for several reasons. UC requires multiple centrifugation steps, often taking 4–24 h per sample, with manual handling that increases variability and labor costs. UCs are expensive and require specialized maintenance and trained personnel, which make them impractical for high-throughput clinical settings. Additionally, UC processes one sample at a time, which limits it for large-scale clinical applications where hundreds or thousands of samples may need to be evaluated. UC typically requires large starting volumes (e.g., 10–100 mL), which may not be feasible for clinical samples like blood or urine, where the volumes are often limited. While UC can achieve high purity, it often results in low EV yields (5%–20% recovery) and can co-isolate contaminants, for instance, protein aggregates, affecting the consistency for clinical diagnostics or therapeutics. Methods like size exclusion chromatography (SEC), tangential flow filtration (TFF), or microfluidics-based approaches are being explored for clinical use. These offer higher throughput, better scalability, and compatibility with smaller sample volumes, though they may compromise on purity or require optimization (53). Nevertheless, the choice of purification method depends on various factors, such as initial sample volume, purity, and yield, downstream application, and available resources (54). Another problem is the lack of unique markers for the heterogeneous subclasses of EVs. Although tetraspanins such as CD9, CD63, and CD81 are currently most commonly used to characterize extracellular vesicles, their expression can vary considerably depending on the cell type producing the EVs. The low presence of the above-mentioned tetraspanins directly limits the efficiency of immunoaffinity isolation of EVs from cell populations that naturally express low levels of CD9, CD63, or CD81. As a result, a significant amount of EVs may be missed or inefficiently isolated, leading to incomplete or biased exosome yields. Afterwards, clinical-grade EVs should be manufactured in accordance with good manufacturing practice (GMP) and quality control (QC) standards. The QC criteria include determining the quantity, size, identity, and purity of EVs. One method to monitor vesicle purity is to determine the particle-to-protein, protein-to-lipid, or RNA-to-particle ratios. Another approach is to determine the expression of intracellular proteins such as histones, lamin A/C, GRP94, or cytochrome C since extracellular vesicles do not contain these proteins. Moreover, contaminants from the cell culture process,

including antibiotics and serum, should also be monitored. It is also necessary to investigate which subpopulations of EVs have the highest therapeutic efficacy, as the composition of EVs may vary depending on the MSCs' origin and culture conditions. Finally, the appropriate therapeutic doses and optimal route(s) of Evs' administration should be established. EVs can be administered through various routes, including intravenous, topical, and intralesional—for example, Kim et al. showed that a dose of 5.0×10^7 cells (hUCB-MSCs) administered subcutaneously resulted in a significant reduction in AD symptoms, including the area and severity of eczema, itching, and inflammation (47). However, most of the available studies are animal studies or small-scale clinical trials, which have a significant impact on the inability to draw firm conclusions. Summing up, the ideal protocol should be characterized by a low level of sample contamination, preservation of vesicle integrity, high efficiency, reproducibility, versatility, low cost, high isolation rate from a large number of samples simultaneously (ideally for no more than 1 h), availability and simplicity of the equipment, and the possibility of process automation. In general, the final outcome (EVs purity and yield) highly depends on the chosen isolation protocol, and the best method is determined by the specific goal of the further study (55).

6 Conclusions

This review presents the potential of different types of mesenchymal stem cells (MSCs) and their EVs as a therapeutic strategy for immune-mediated inflammatory skin diseases. EVs possess immunosuppressive and immunomodulatory properties that can alleviate inflammation. Importantly, EVs are less immunogenic than cells, minimizing adverse immune responses. Available preclinical and clinical studies have shown the safety and good tolerability of MSCs and their EVs in the treatment of dermatoses. On the other hand, mild and transient local reactions have been demonstrated, but without serious adverse events. While the initial results are promising, larger-scale clinical trials are necessary to establish long-term safety and efficacy. Further research is also needed to elucidate the precise mechanisms of action of EVs in skin diseases. Exploring combination therapies with other modalities, such as drugs or other biological agents, may enhance therapeutic outcomes. Developing personalized EV-based therapies tailored to individual patient needs may improve the treatment efficacy and reduce side effects. Another issue is the difficulty in producing large quantities of clinically applicable extracellular EVs resulting from the technical challenges associated with long-term cell culture and obtaining homogenous MSCs for EV generation. These challenges include limited EV yield in standard cell cultures, the need for efficient isolation and purification methods, and ensuring consistency in EV cargo and properties. In addition, the biological properties of MSCs are not uniform and can vary significantly depending on several factors, including their origin (tissue source), the method of culture and expansion, the age of the donor, and the method used to isolate them. These variations can lead to inconsistencies in the cellular characteristics across different batches of MSCs, which can impact their therapeutic potential and the outcome of clinical trials. In conclusion, MSCs and EV-based therapies may hold great promise for treating immune-mediated inflammatory skin diseases, but robust clinical trials are needed to confirm their safety and efficacy in humans before routine clinical use.

Author contributions

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References

1. Rendon A, Schäkel K. Psoriasis pathogenesis and treatment. *Int J Mol Sci*. (2019) 20:1475. doi: 10.3390/ijms20061475

2. Ortonne J, Chimenti S, Luger T, Puig L, Reid F, Trüeb RM. Scalp psoriasis: European consensus on grading and treatment algorithm. *J Eur Acad Dermatol Venereol*. (2009) 23:1435–44. doi: 10.1111/j.1468-3083.2009.03372.x

3. Nestle FO, Kaplan DH, Barker J. Psoriasis. *N Engl J Med*. (2009) 361:496–509. doi: 10.1056/NEJMra0804595

4. Micali G, Verzì AE, Giuffrida G, Panebianco E, Musumeci ML, Lacarrubba F. Inverse psoriasis: from diagnosis to current treatment options. *Clin Cosmet Investig Dermatol*. (2019) 12:953–9. doi: 10.2147/CCID.S189000

5. Ko HC, Jwa SW, Song M, Kim MB, Kwon KS. Clinical course of guttate psoriasis: long-term follow-up study. *J Dermatol*. (2010) 37:894–9. doi: 10.1111/j.1346-8138.2010.00871.x

6. Martin BA, Chalmers RJ, Telfer NR. How great is the risk of further psoriasis following a single episode of acute guttate psoriasis? *Arch Dermatol*. (1996) 132:717–8. doi: 10.1001/archderm.1996.03890300147032

7. Navarini AA, Burden AD, Capon F, Mrowietz U, Puig L, Köks S, et al. European consensus statement on phenotypes of pustular psoriasis. *J Eur Acad Dermatol Venereol*. (2017) 31:1792–9. doi: 10.1111/jdv.14386

8. Di Meglio P, Villanova F, Nestle FO. Psoriasis. *Cold Spring Harb Perspect Med*. (2014) 4:a015354. doi: 10.1101/cshperspect.a015354

9. Morizane S, Gallo RL. Antimicrobial peptides in the pathogenesis of psoriasis. *J Dermatol*. (2012) 39:225–30. doi: 10.1111/j.1346-8138.2011.01483.x

10. Morizane S, Yamasaki K, Mühleisen B, Kotol PF, Murakami M, Aoyama Y, et al. Cathelicidin antimicrobial peptide LL-37 in psoriasis enables keratinocyte reactivity against TLR9 ligands. *J Invest Dermatol*. (2012) 132:135–43. doi: 10.1038/jid.2011.259

11. Santini SM, Lapenta C, Donati S, Spadaro F, Belardelli F, Ferrantini M. Interferon-α-conditioned human monocytes combine a Th1-orienting attitude with the induction of autologous Th17 responses: role of IL-23 and IL-12. *PloS One*. (2011) 6:e17364. doi: 10.1371/journal.pone.0017364

12. Andaloussi S EL, Mäger I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat Rev Drug Discov*. (2013) 12:347–57. doi: 10.1038/nrd3978

13. Buzas EI. The roles of extracellular vesicles in the immune system. *Nat Rev Immunol*. (2023) 23:236–50. doi: 10.1038/s41577-022-00763-8

14. Sroka-Tomaszewska J, Trzeciak M. Molecular mechanisms of atopic dermatitis pathogenesis. *Int J Mol Sci*. (2021) 22:4130. doi: 10.3390/ijms22084130

15. Tian J, Zhang D, Yang Y, Huang Y, Wang L, Yao X, et al. Global epidemiology of atopic dermatitis: a comprehensive systematic analysis and modelling study. *Br J Dermatol*. (2023) 190:55–61. doi: 10.1093/bjd/ljad339

16. Raimondo A, Lembo S. Atopic dermatitis: epidemiology and clinical phenotypes. *Dermatol Pract Concept*. (2021) 11:e2021146. doi: 10.5826/dpc.1104a146

17. Thyssen JP, Halling AS, Schmid-Grendelmeier P, Guttman-Yassky E, Silverberg JI. Comorbidities of atopic dermatitis-what does the evidence say? *J Allergy Clin Immunol*. (2023) 151:1155–62. doi: 10.1016/j.jaci.2022.12.002

18. Kanda N, Hoashi T, Saeki H. The roles of sex hormones in the course of atopic dermatitis. *Int J Mol Sci*. (2019) 20:4660. doi: 10.3390/ijms20194660

19. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol*. (2008) 8:726–36. doi: 10.1038/nri2395

20. Perez-Simon JA, Lopez-Villar O, Andreu EJ, Rifon J, Muntion S, Diez Campelo M, et al. Mesenchymal stem cells expanded *in vitro* with human serum for the treatment of acute and chronic graft-versus-host disease: Results of a phase I/II clinical trial. *Haematologica*. (2011) 96:1072–6. doi: 10.3324/haematol.2010.038356

21. Liang J, Zhang H, Hua B, Wang H, Lu L, Shi S, et al. Allogenic mesenchymal stem cells transplantation in refractory systemic lupus erythematosus: A pilot clinical study. *Ann Rheumatol Dis*. (2010) 69:1423–9. doi: 10.1136/ard.2009.123463

22. Christopeit M, Schendel M, Foll J, Muller LP, Keysser G, Behre G. Marked improvement of severe progressive systemic sclerosis after transplantation of mesenchymal stem cells from an allogeneic haploidentical-related donor mediated by ligation of CD137L. *Leukemia*. (2008) 22:1062–4. doi: 10.1038/sj.leu.2404996

23. Fernández-Messina L, Rodríguez-Galán A, de Yébenes VG, Gutiérrez-Vázquez C, Tenreiro S, Seabra MC, et al. Transfer of extracellular vesicle-microRNA controls germinal center reaction and antibody production. *EMBO Rep*. (2020) 21:e48925. doi: 10.15252/embr.201948925

24. Keller MD, Ching KL, Liang FX, Dhabaria A, Tam K, Ueberheide BM, et al. Decoy exosomes provide protection against bacterial toxins. *Nature*. (2020) 579:260–4. doi: 10.1038/s41586-020-2066-6

25. Sjoqvist S, Kasai Y, Shimura D, Ishikawa T, Ali N, Iwata T, et al. Oral keratinocyte-derived exosomes regulate proliferation of fibroblasts and epithelial cells. *Biochem Biophys Res Commun*. (2019) 514:706–12. doi: 10.1016/j.bbrc.2019.04.202

26. Li Q, Zhao H, Chen W, Huang P, Bi J. Human keratinocyte-derived microvesicle miRNA-21 promotes skin wound healing in diabetic rats through facilitating fibroblast function and angiogenesis. *Int J Biochem Cell Biol*. (2019) :114:105570. doi: 10.1016/j.biocel.2019.105570

27. Angioni R, Liboni C, Herkenne S, Sánchez-Rodríguez R, Borile G, Marcuzzi E, et al. $CD73^{+}$ extracellular vesicles inhibit angiogenesis through adenosine A_{2B} receptor signalling. *J Extracell Vesicles*. (2020) 9:1757900. doi: 10.1080/20013078.2020.1757900

28. Guttman-Yassky E, Krueger JG. Atopic dermatitis and psoriasis: two different immune diseases or one spectrum? *Curr Opin Immunol*. (2017) 48:68–73. doi: 10.1016/j.coi.2017.08.008

29. Lei X, Ring S, Jin S, Singh S, Mahnke K. Extracellular vesicles and their role in skin inflammatory diseases: from pathogenesis to therapy. *Int J Mol Sci*. (2025) 26:3827. doi: 10.3390/ijms26083827

30. Rodríguez-Morales P, Franklin RA. Macrophage phenotypes and functions: resolving inflammation and restoring homeostasis. *Trends Immunol*. (2023) 44:986–98. doi: 10.1016/j.it.2023.10.004

31. Bakr ASM, Raafat MH, Hammouda GA, Abdeltawab SM. The effect of bone marrow derived mesenchymal stem cells versus their exosomes on imiquimod-induced psoriasis-like skin inflammation in female albino rats: A histological study. *Egyptian Journal of Histology* 46:939–52. doi: 10.21608/ejh.2022.125141.1650

32. Zhang B, Lai RC, Sim WK, Choo ABH, Lane EB, Lim SK. Topical application of mesenchymal stem cell exosomes alleviates the imiquimod induced psoriasis-like inflammation. *Int J Mol Sci*. (2021) 22:720. doi: 10.3390/ijms22020720

33. Guo L, Lai P, Wang Y, Huang T, Chen X, Luo C, et al. Extracellular vesicles from mesenchymal stem cells prevent contact hypersensitivity through the suppression of Tc1 and Th1 cells and expansion of regulatory T cells. *Int Immunopharmacol*. (2019) 74:105663. doi: 10.1016/j.intimp.2019.05.048

34. Jin J, Ou Q, Wang Z, Tian H, Xu JY, Gao F, et al. BMSC-derived extracellular vesicles intervened the pathogenic changes of scleroderma in mice through miRNAs. *Stem Cell Res Ther*. (2021) 12:327. doi: 10.1186/s13287-021-02400-y

35. Xie L, Long X, Mo M, Jiang J, Zhang Q, Long M, et al. Bone marrow mesenchymal stem cell-derived exosomes alleviate skin fibrosis in systemic sclerosis by inhibiting the IL-33/ST2 axis via the delivery of microRNA-214. *Mol Immunol*. (2023) 157:146–57. doi: 10.1016/j.molimm.2023.03.017

36. Kim YJ, Yoo SM, Park HH, Lim HJ, Kim YL, Lee S, et al. Exosomes derived from human umbilical cord blood mesenchymal stem cells stimulates rejuvenation of human skin. *Biochem Biophys Res Commun*. (2017) 493:1102–8. doi: 10.1016/j.bbrc.2017.09.056

37. Chen C, Wang D, Moshaverinia A, Liu D, Kou X, Yu W, et al. Mesenchymal stem cell transplantation in tight-skin mice identifies miR-151-5p as a therapeutic target for systemic sclerosis. *Cell Res*. (2017) 27:559–77. doi: 10.1038/cr.2017.11

38. Lai P, Chen X, Guo L, Wang Y, Liu X, Liu Y, et al. A potent immunomodulatory role of exosomes derived from mesenchymal stromal cells in preventing cGVHD. *J Hematol Oncol*. (2018) 11:135. doi: 10.1186/s13045-018-0680-7

39. Guo L, Lai P, Wang Y, Huang T, Chen X, Geng S, et al. Extracellular vesicles derived from mesenchymal stem cells prevent skin fibrosis in the cGVHD mouse model by suppressing the activation of macrophages and B cells immune response. *Int Immunopharmacol*. (2020) 84:106541. doi: 10.1016/j.intimp.2020.106541

40. Tang H, Wang F, Yang R, Zhao Z, Zhang Y, Yang L, et al. Baricitinib-loaded EVs promote alopecia areata mouse hair regrowth by reducing JAK-STAT-mediated inflammation and promoting hair follicle regeneration. *Drug Discov Ther*. (2025) 18:368–74. doi: 10.5582/ddt.2024.01080

41. Na K, Yoo HS, Zhang YX, Choi MS, Lee K, Yi TG, et al. Bone marrow-derived clonal mesenchymal stem cells inhibit ovalbumin-induced atopic dermatitis. *Cell Death Dis*. (2014) 5:e1345. doi: 10.1038/cddis.2014.299

42. Shin HT, Lee SH, Yoon HS, Heo JH, Lee SB, Byun JW, et al. Long-term efficacy and safety of intravenous injection of clonal mesenchymal stem cells derived from bone marrow in five adults with moderate to severe atopic dermatitis. *J Dermatol*. (2021) 48:1236–42. doi: 10.1111/1346-8138.15928

43. Ryu B, Baek J, Kim H, Lee JH, Kim J, Jeong YH, et al. Anti-inflammatory effects of M-MSCs in DNCB-induced atopic dermatitis mice. *Biomedicines*. (2020) 8:439. doi: 10.3390/biomedicines8100439

44. Jung H, Son GM, Lee JJ, Park HS. Therapeutic effects of tonsil-derived mesenchymal stem cells in an atopic dermatitis mouse model. *In Vivo*. (2021) 35:845–57. doi: 10.21873/invivo.12325

45. Zibandeh N, Genc D, Ozgen Z, Duran Y, Goker K, Baris S, et al. Mesenchymal stem cells derived from human dental follicle modulate the aberrant immune response in atopic dermatitis. *Immunotherapy*. (2021) 13:825–40. doi: 10.2217/imt-2020-0257

46. Kim HS, Yun JW, Shin TH, Lee SH, Lee BC, Yu KR, et al. Human umbilical cord blood mesenchymal stem cell-derived PGE2 and TGF-β1 alleviate atopic dermatitis by reducing mast cell degranulation. *Stem Cells*. (2015) 33:1254–66. doi: 10.1002/stem.1913

47. Kim HS, Lee JH, Roh KH, Jun HJ, Kang KS, Kim TY. Clinical trial of human umbilical cord blood-derived stem cells for the treatment of moderate-to-severe atopic dermatitis: phase I/IIa studies. *Stem Cells*. (2017) 35:248–55. doi: 10.1002/stem.2401

48. Shin N, Jung N, Lee SE, Kong D, Kim NG, Kook MG, et al. Pimecrolimus interferes the therapeutic efficacy of human mesenchymal stem cells in atopic dermatitis by regulating NFAT-COX2 signaling. *Stem Cell Res Ther*. (2021) 12:482. doi: 10.1186/s13287-021-02547-8

49. Loisel S, Lansiaux P, Rossille D, Ménard C, Dulong J, Monvoisin C, et al. Regulatory B cells contribute to the clinical response after bone marrow-derived mesenchymal stromal cell infusion in patients with systemic sclerosis. *Stem Cells Transl Med*. (2023) 12:194–206. doi: 10.1093/stcltm/szad010

50. Chen C, Akiyama K, Yamaza T, You YO, Xu X, Li B, et al. Telomerase governs immunomodulatory properties of mesenchymal stem cells by regulating FAS ligand expression. *EMBO Mol Med*. (2014) 6:322–34. doi: 10.1002/emmm.201303000

51. Wang D, Li J, Zhang Y, Zhang M, Chen J, Li X, et al. Umbilical cord mesenchymal stem cell transplantation in active and refractory systemic lupus erythematosus: a multicenter clinical study. *Arthritis Res Ther*. (2014) 16:R79. doi: 10.1186/ar4520

52. Lee BC, Kim JJ, Lee JY, Kang I, Shin N, Lee SE, et al. Disease-specific primed human adult stem cells effectively ameliorate experimental atopic dermatitis in mice. *Theranostics*. (2019) 9:3608–21. doi: 10.7150/thno.32945

53. Monguió-Tortajada M, Gálvez-Montón C, Bayes-Genis A, Roura S, Borràs FE. Extracellular vesicle isolation methods: rising impact of size-exclusion chromatography. *Cell Mol Life Sci*. (2019) 76:2369–82. doi: 10.1007/s00018-019-03071-y

54. Doyle LM, Wang MZ. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cells*. (2019) 8:727. doi: 10.3390/cells8070727

55. Yakubovich EI, Polischouk AG, Evtushenko VI. Principles and problems of exosome isolation from biological fluids. *Biochem (Mosc) Suppl Ser A Membr Cell Biol*. (2022) 16:115–26. doi: 10.1134/S1990747822030096

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Mesenchymal stromal cell-laden hydrogels in tissue regeneration: insights from preclinical and clinical research

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Hydrogel-based delivery systems have emerged as a promising strategy to enhance the therapeutic efficacy of mesenchymal stromal cells (MSCs) in regenerative medicine. These biomimetic platforms provide a three-dimensional microenvironment that recapitulates key features of native extracellular matrix, supporting MSC viability, retention, and function upon transplantation. Beyond acting as passive carriers, hydrogels can be engineered with tunable biochemical and mechanical properties to modulate MSC behavior, including their differentiation potential, immunomodulatory activity, and paracrine signaling. Recent advances include the development of "smart" hydrogels responsive to physiological stimuli, enabling controlled release of encapsulated cells or bioactive molecules in response to local cues. Preclinical studies have demonstrated enhanced tissue repair in diverse pathological contexts, including musculoskeletal, cardiovascular, gastrointestinal, dermal, and neural injuries. Importantly, translation to clinical settings is being facilitated by the use of xeno-free, good manufacturing practices (GMP)-compliant components such as platelet derivatives and synthetic polymers. Selected early-phase clinical trials support the feasibility, safety, and therapeutic potential of MSC-laden hydrogels, although further studies are required to optimize delivery parameters and regulatory compliance. This review summarizes current progress in hydrogel-MSC systems across application areas, emphasizing design principles, preclinical outcomes, and translational challenges, with the aim of guiding future developments in stem cell-based tissue regeneration.

KEYWORDS

mesenchymal stromal cells, hydrogels, tissue regeneration, preclinical research, clinical trials

1 Introduction

Tissue regeneration remains a significant challenge in modern medicine, particularly for the restoration of structure and function in tissues damaged by trauma, chronic disease, or degenerative conditions related to aging. The intrinsic complexity of tissue architecture and the inflammatory and fibrotic responses following injury often hinder

spontaneous healing and limit the efficacy of conventional therapeutic strategies. In this context, regenerative medicine research aims to harness the therapeutic potential of stem cells to develop more effective, biologically integrated strategies for tissue repair and functional restoration (Hoang et al., 2022; Hussen et al., 2024).

Among the various cell types explored for regenerative purposes, mesenchymal stromal cells (MSCs) have emerged as one of the most promising candidates due to their ease of isolation, multipotent differentiation capacity, secretion of trophic factors, and immunomodulatory properties (Fan X. L. et al., 2020; Barbon et al., 2022; Barbon et al., 2021). These cells can contribute to tissue repair through both direct differentiation into target cell lineages and through paracrine mechanisms that modulate inflammation, promote angiogenesis, and recruit endogenous progenitor cells (Kaviarasan et al., 2024; Xue et al., 2024; Barbon et al., 2023).

However, the effective delivery and retention of MSCs at the injury site remain critical hurdles. Rapid cell death, washout due to mechanical forces, and lack of a supportive microenvironment compromise their regenerative potential (Bagno et al., 2022; Takayama et al., 2023). In particular, recent analyses have highlighted that cell-based interventions alone frequently fail to achieve long-term and uncomplicated wound healing across different tissue contexts. This limitation has been attributed to the transient survival of transplanted MSCs and the short-lived duration of their anti-inflammatory secretory activity, which may be insufficient to sustain the prolonged phases of tissue repair and remodeling (Zhao et al., 2021).

To overcome these barriers, biomaterial-based strategies have been developed to provide scaffolding for cell delivery, with hydrogels standing out as an optimal solution. Hydrogels are water-swollen, crosslinked polymer networks that closely mimic the physical and biochemical properties of the native extracellular matrix (ECM). Their biocompatibility, tunable mechanical strength, and ability to encapsulate and release cells or bioactive molecules make them ideal vehicles for supporting MSC survival and function (Seliktar, 2012).

The combination of MSCs and hydrogels has thus gained considerable attention in regenerative medicine, offering a synergistic approach to enhance tissue regeneration. Recent preclinical and clinical studies have explored the potential of MSC-laden hydrogels in various applications, including cartilage repair, wound healing, and myocardial regeneration (Gnecchi et al., 2016; Liang et al., 2019). Despite promising results, challenges such as optimal hydrogel composition, cell viability, and long-term therapeutic efficacy remain to be addressed.

This literature review examines the current preclinical and clinical research on MSC-laden hydrogels, evaluating their therapeutic outcomes, limitations, and future directions in tissue regeneration. By synthesizing findings from key studies, this review aims to provide a comprehensive understanding of the translational potential of MSC-hydrogel systems in regenerative medicine. Given the extensive body of research on hydrogel-assisted MSC delivery, this work does not aim to provide an exhaustive systematic review. Instead, a focused narrative approach was adopted, selecting representative preclinical and clinical studies that best illustrate the translational potential of MSC-laden hydrogels. Specifically, we prioritized *in vivo* investigations in clinically relevant animal models, along with published and ongoing clinical trials in humans. In vitro-only studies, early proof-of-principle experiments, and review articles were excluded from the scope. This strategy allows us to emphasize the therapeutic promise, current limitations, and future perspectives of MSC-hydrogel constructs within a clinically oriented framework.

2 Hydrogel properties enhancing MSC function

Hydrogels play a pivotal role in optimizing the therapeutic efficacy of MSCs for tissue regeneration by providing a biomimetic three-dimensional (3D) microenvironment that closely resembles the native ECM. This supportive niche not only facilitates MSC viability and engraftment but also modulates their paracrine and immunomodulatory functions. The mechanical and biochemical properties of hydrogels significantly influence MSC behavior, including cell survival, proliferation, migration, and lineage-specific differentiation, thereby directly impacting the regenerative process (Garcia-Aponte et al., 2025).

Hydrogels with tunable stiffness, porosity, and degradation kinetics can be engineered to mimic the mechanical properties of specific target tissues (Barbon et al., 2020; Todros et al., 2022a; Todros et al., 2022b). For example, softer hydrogels with elastic moduli in the range of 1–10 kPa have been shown to promote adipogenic or neurogenic differentiation, whereas stiffer matrices ranging from 25 to 40 kPa tend to favor osteogenic commitment (Engler et al., 2006). This mechanosensitivity underscores the importance of substrate stiffness in guiding stem cell fate decisions (Stocco et al., 2019a). Furthermore, pore architecture affects nutrient diffusion, waste elimination, and cell migration, all of which are essential for maintaining a viable and functionally active MSC population *in situ* (Zhou et al., 2023). Complementing these internal features, hydrogel surface geometry - including features such as roughness, curvature, and micro- or nano-topography - plays a critical role in modulating MSC adhesion, proliferation, and lineage commitment (Xiao et al., 2023; Stocco et al., 2021; Stocco et al., 2024; Di Liddo et al., 2016). These topographical cues can influence cytoskeletal organization and mechanotransduction pathways, thereby directing stem cell differentiation and enhancing tissue-specific integration.

The incorporation of bioactive molecules, such as ECM-derived peptides [e.g., arginine–glycine–aspartic acid (RGD), laminin], growth factors [e.g., vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), bone morphogenetic protein-2 (BMP-2)], and glycosaminoglycans (e.g., hyaluronic acid or chondroitin sulfate), further augments MSC functionality by facilitating cell adhesion, activating integrin-mediated signaling pathways, and enhancing the secretion of regenerative cytokines (Xiao et al., 2023; Porzionato et al., 2019; Di Liddo et al., 2014). These biochemical cues can be spatially and temporally regulated through hydrogel composition or surface modification, allowing more precise control of the cellular microenvironment.

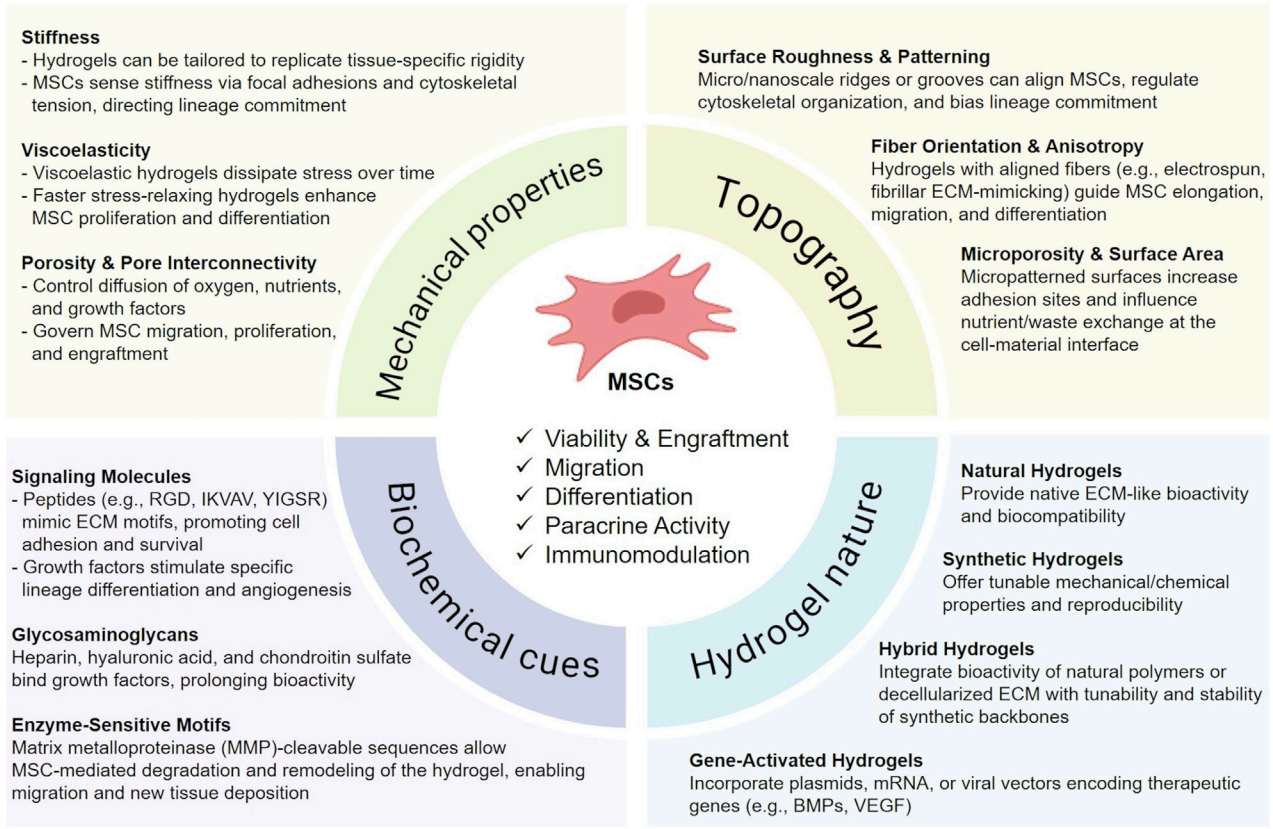


FIGURE 1
Key hydrogel design parameters influencing MSC therapeutic activity. Schematic overview of hydrogel features that regulate mesenchymal stromal cell (MSC) function and regenerative potential. Mechanical properties such as stiffness, viscoelasticity, and porosity govern MSC survival, migration, and lineage commitment. Topographical cues, including surface roughness, fiber alignment, and microporosity, modulate cell adhesion, elongation, and differentiation. Biochemical signals, provided by peptides, growth factors, glycosaminoglycans, and enzyme-sensitive motifs, enhance MSC survival, angiogenesis, and remodeling capacity. The intrinsic nature of the hydrogel further impacts therapeutic outcomes: natural hydrogels ensure ECM-like bioactivity, synthetic hydrogels provide reproducibility and tunability, hybrid systems combine both advantages, and gene-activated hydrogels enable controlled release of therapeutic genes. Collectively, these design parameters influence MSC viability, engraftment, paracrine activity, and immunomodulation in regenerative applications. This figure was created using BioRender.com.

Injectable hydrogels, including those based on natural polymers such as alginate, collagen, gelatin, or hyaluronic acid, enable minimally invasive administration, *in situ* gelation, and conformation to irregular defect geometries. This ensures precise MSC localization, retention, and protection within injured tissues (Burdick and Murphy, 2012). Synthetic variants such as polyethylene glycol (PEG) and polyvinyl alcohol (PVA) offer improved mechanical tunability and reproducibility, though often at the expense of bioactivity. Composite hydrogels combining natural and synthetic components aim to leverage the advantages of both material classes.

In addition to traditional natural and synthetic polymers, hydrogels derived from decellularized ECM have gained increasing attention as MSC carriers. These biomaterials closely mimic the native biochemical composition and architecture of tissues, thereby providing a bioactive microenvironment that promotes cell adhesion, survival, and lineage-specific differentiation. However, the intrinsic mechanical weakness and batch-to-batch variability of pure ECM hydrogels may limit their translational application. To address this, bio-hybrid systems combining ECM components with synthetic polymers have been developed, combining the bioactivity of ECM with the tunable mechanical and physicochemical properties of synthetic materials (Stocco et al., 2014; Stocco et al., 2016; Grandi et al., 2018; Stocco et al., 2022). Such composite scaffolds sowed to enhance MSC retention and regenerative capacity across different tissues, including cartilage, intestinal segments, and osteochondral units. *In vitro* and preclinical evidence further highlighted that ECM-derived hydrogels not only reproduce key integrin-binding and growth factor-retaining motifs, but also demonstrate promising preclinical outcomes in cartilage, bone, cardiac, and cutaneous repair. Based on that, hybrid ECM-synthetic systems emerge as promising next-generation carriers for MSC transport, capable of coupling structural stability with biochemical functionality to better meet the complex demands of tissue regeneration (Soltanmohammadi et al., 2025).

Parallel to these bio-hybrid strategies, advanced designs such as stimuli-responsive or dynamic hydrogels, incorporate environmental triggers (e.g., pH, temperature, enzymatic activity) to enable the controlled release of encapsulated cells or bioactive factors. These “smart” hydrogels can prolong therapeutic action, support tissue remodeling, and potentially provide on-demand modulation of MSC activity (Lu et al., 2024) (Figure 1).

A promising strategy to further refine hydrogel bioactivity involves engineering matrices with controlled surface or volumetric charge. (e.g., heparinization to confer anionic binding domains or incorporation of cationic moieties) to non-covalently sequester nucleic acids (microRNA, mRNA, plasmid DNA). Such gene-activated hydrogels can prolong local factor residence, protect labile cargos from degradation, and enable cell-responsive release, thereby extending and amplifying MSC paracrine activity *in vivo*. Preliminary studies further indicate that charge density and spatial distribution critically regulate payload loading/release kinetics and downstream MSC immunomodulation and differentiation (Krasilnikova et al., 2023; Liu et al., 2024).

Despite their potential, hydrogel-based strategies still have to face significant challenges. A key consideration is aligning hydrogel degradation rates with the timeline of native tissue healing to avoid premature scaffold loss or prolonged presence that could hinder integration. Batch-to-batch variability in natural polymers and potential immunogenicity also pose hurdles to clinical translation. Meanwhile, synthetic hydrogels often lack intrinsic bioactivity, requiring functionalization strategies to enhance their biological performance.

Future directions should prioritize the development of clinically scalable, off-the-shelf hydrogel-MSC formulations with optimized degradation kinetics, mechanical resilience, and biofunctionality. Strategies incorporating extracellular vesicles (EVs) or gene editing tools may further enhance therapeutic outcomes by augmenting MSC paracrine signaling or resistance to hostile injury microenvironments.

3 Paracrine signaling and immunomodulation

Mesenchymal stromal cell (MSC)-laden hydrogels exert their regenerative effects primarily through paracrine signaling and immunomodulation rather than direct differentiation. MSCs encapsulated in hydrogels secrete a diverse array of bioactive factors, including growth factors [e.g., VEGF, hepatocyte growth factor (HGF), transforming growth factor beta (TGF-β)], cytokines [e.g., interleukin-10 (IL-10), tumor necrosis factor-stimulated gene-6 (TSG-6)], and extracellular vesicles (exosomes), which collectively promote tissue repair by stimulating angiogenesis, reducing fibrosis, and modulating immune responses (Kuppa et al., 2022). The hydrogel matrix serves as a protective niche that prolongs MSC survival and sustains the release of these therapeutic factors, enhancing their local bioavailability compared to free MSC (Caplan and Correa, 2011) (Figure 2).

A key advantage of MSC-laden hydrogels is their ability to polarize macrophages from a pro-inflammatory (M1) to an anti-inflammatory (M2) phenotype, which is critical for resolving chronic inflammation in conditions like diabetic wounds or myocardial infarction (Saldanha-Araujo et al., 2020). Hydrogels

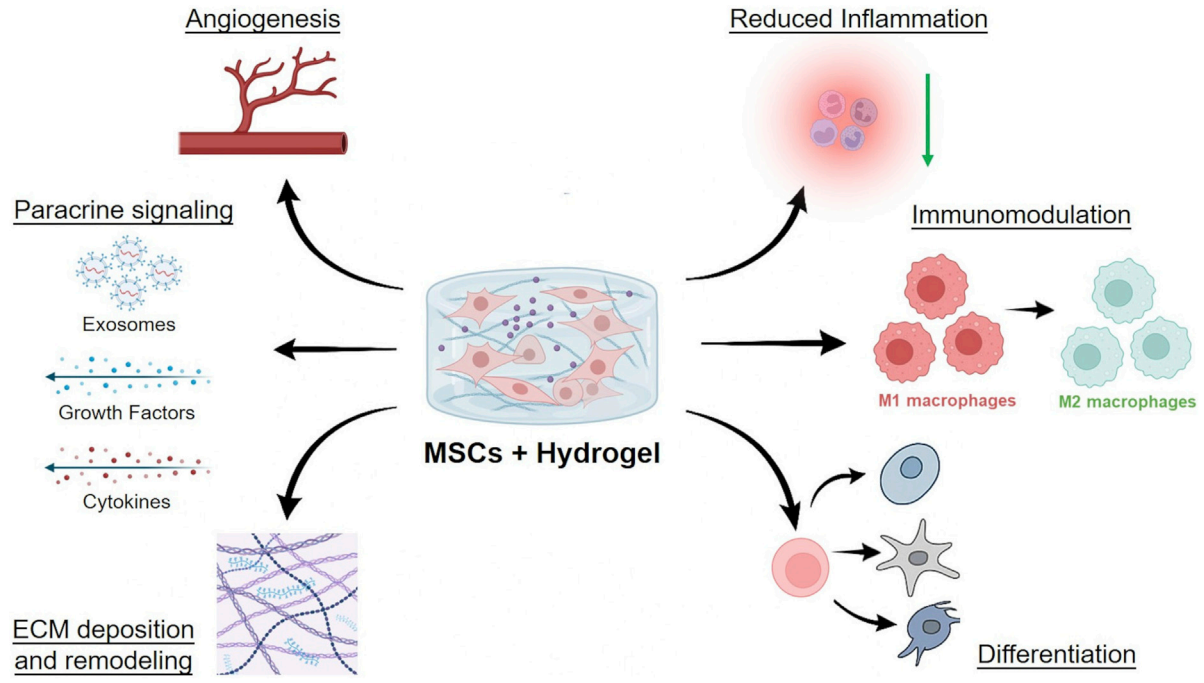


FIGURE 2
Mechanisms of MSC delivery and action within hydrogels. Schematic representation of how mesenchymal stromal cells (MSCs) act when encapsulated in hydrogel scaffolds. Hydrogels provide a protective scaffold that enhances MSC survival and retention, while enabling multiple therapeutic mechanisms. Paracrine signaling through cytokines, exosomes, and growth factors promotes angiogenesis and tissue repair. Immunomodulation drives macrophage polarization from pro-inflammatory M1 to anti-inflammatory M2 phenotypes, resulting in reduced inflammation. Differentiation into lineage-specific cells (e.g., chondrocytes, osteoblasts, neurons) and matrix deposition and remodeling contribute to structural regeneration. Collectively, these processes support vascularization, immune regulation, and tissue repair in regenerative medicine applications. This figure was created using BioRender.com.

can be further engineered to amplify these effects by incorporating immunomodulatory agents (e.g., IL-4, interferon gamma (IFN-γ)) or ECM components that synergize with MSC secretions (Xie et al., 2020).

Despite these benefits, challenges include variability in MSC secretomes due to donor differences and culture conditions, as well as the short-lived activity of some paracrine factors. Future strategies may involve genetic modification of MSCs to overexpress specific factors or the use of synthetic hydrogels with controlled release kinetics to optimize immunomodulatory outcomes. Importantly, in the context of wound healing stimulation, these paracrine effects may decrease before tissue repair is stabilized, resulting in incomplete, not functional regeneration. Hydrogel systems are being explored specifically to prolong factor release, modulate local immunity, and synchronize MSC activity with the protracted timeline required for durable and uncomplicated wound closure.

4 Hydrogel-based MSC therapies: preclinical studies

Preclinical studies represent a fundamental step in validating the therapeutic and translational potential of MSC-hydrogel systems across a wide range of disease models. Animal experiments provide essential proof-of-concept data and mechanistic insights into how biomaterial-assisted MSC therapy can overcome limitations such as poor cell survival, retention, and integration. Importantly, the outcomes of these studies depend strongly on the physiological niche in which the therapy is applied. In ischemic tissues, hydrogels protect MSCs from hypoxia and modulate macrophage-driven fibrosis; in neuroinhibitory niches, they attenuate glial scarring and reprogram microglia; in immune-active tissues such as skin and gut, they regulate inflammatory cascades; in mechanically demanding tissues like cartilage and bone, they provide physical support and engage osteo-immune cells; and in sensitive tissues, scaffold selection must minimize excessive immune activation. To reflect this, the following subsections review representative preclinical applications of hydrogel-assisted MSC delivery according to the major biological challenges that influence regenerative success. This integrated perspective highlights both the similarities and the context-specific differences across models, underscoring how hydrogel design can be strategically adapted to distinct tissue environments and host responses, and illustrating the versatility of MSC–hydrogel constructs across a spectrum of regenerative contexts (Table 1).

4.1 Ischemic and hypoxic environments

Tissues with limited oxygen supply, such as the heart and intervertebral discs, present severe survival barriers for transplanted MSCs. In the infarcted myocardium, hydrogels derived from Wharton's Jelly ECM combined with MSCs of the same origin promoted engraftment and enhanced secretion of paracrine factors, which improved left ventricular function, reduced fibrosis, and stimulated neovascularization in rats (Tavajjohi et al., 2025). Similarly, bone marrow-derived MSCs encapsulated in RGD-modified alginate hydrogel showed improved retention and viability in infarcted rat hearts, leading to increased ejection fraction, reduced infarct size, and greater capillary density (Levit et al., 2013; Guo et al., 2020). Beyond structural support, gene-modified MSC therapies have also been investigated. Wu and colleagues (2017) demonstrated that gene-modified MSCs delivered within a small-molecule hydrogel significantly improved angiogenesis and reduced infarct size in a rat model of myocardial infarction (MI), underscoring the synergistic potential of combining MSCs with gene-activated hydrogels in ischemic repair. Comparable challenges exist in the intervertebral disc, where low oxygen and nutrient supply limit regenerative capacity. In a goat model of disc degeneration, hyaluronic acid-based hydrogels encapsulating MSCs restored disc height and stiffness and decreased local inflammation (Zhang et al., 2021). In spinal fusion, gelatin methacryloyl (GelMA) carriers were developed to co-deliver MSCs and bone BMP-2, promoting osteogenesis *in vitro* and bone bridging *in vivo* (Li et al., 2024). These approaches show that in hypoxic and avascular tissues, hydrogels provide a metabolic buffer that sustains MSC survival and activity while modulating inflammatory macrophage infiltration.

4.2 Neuroinhibitory niches and scar formation

In the central nervous system, regeneration is hindered by glial scar formation and a highly specialized immune environment dominated by microglia.

To address these barriers, Sun et al. (2025) engineered a thermosensitive hydrogel incorporating BMP7-loaded nanoparticles, which promoted neuronal differentiation of bone marrow MSCs, enhanced axonal regeneration, reduced glial scarring, and improved locomotor recovery in rats with spinal cord injury (SCI). In a complementary design, Papa et al. (2018) encapsulated MSCs in a hyaluronic acid hydrogel with controlled release of CCL2, which preserved spinal cord cytoarchitecture, reduced lesion volume, and recruited host cells. Importantly, this system induced macrophage polarization toward a regenerative phenotype, highlighting the immune-modulatory role of hydrogels in the central nervous system.

These findings indicate that hydrogel scaffolds in neuroregeneration must not only sustain MSC viability but also regulate microglia and infiltrating macrophages to counteract inhibitory scar formation.

4.3 Highly immune-active tissues

Tissues such as the skin and gut are highly exposed to inflammation and microbial stress, requiring MSC-hydrogel systems that prolong paracrine signaling and shape immune responses. In a diabetic mouse model, Huang et al. (2025) demonstrated that perinatal MSCs delivered via a hydrogel scaffold accelerated wound closure through the activation of the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway.

TABLE 1 Overview of MSC laden-hydrogel preclinical research.

First author, year	Clinical target	Type of MSCs	Type of hydrogel	Animal model, tissue damage	Main *in vivo* outcomes
Wang et al. (2010)	Cartilage repair	BM-MSCs	PLGA sponge filled with fibrin gel + TMC/pDNA-TGF-β1 complexes	Rabbit, full-thickness femoral trochlear cartilage defect	- Hyaline-like cartilage formation with abundant type II collagen and GAGs - Improved surface smoothness and better integration - Evident subchondral bone remodeling
Chen et al. (2011)	Osteochondral regeneration	BM-MSCs	Bilayer gene-activated scaffold: a) plasmid TGF-β1-activated chitosan-gelatin as chondrogenic layer b) plasmid BMP-2-activated hydroxyapatite/chitosan-gelatin as osteogenic layer	Rabbit, knee osteochondral defect	- Simultaneous regeneration of cartilage and subchondral bone - Superior ICRS/Wakitani-type histology scores - Optimal cartilage–bone integration
Levit et al. (2013)	Myocardial infarction	BM-MSCs	Alginate-based encapsulating hydrogel	Rat LAD ligation	- Improved cell retention, cardiac function, and neovascularization
Li et al. (2013)	Cartilage repair	BM-MSCs	PLGA scaffold + fibrin gel + PEO-b-PLL/TGF-β1 plasmid DNA complexes	Rabbit, osteochondral defect	- Enhanced chondrogenesis - Higher histological repair scores - Improved defect fill and integration - More homogeneous hyaline-like tissue
Seol et al. (2013)	Cartilage repair/pain control	BM-MSCs	Reverse-thermal responsive Poloxamer hydrogel	Rabbit Articular cartilage lesion	- High hydrogel biocompatibility - Hyaline-like cartilage regeneration with smooth surface morphology - Good tissue integration, localized cell retention - No adverse immune reactions
Kopesky et al. (2014)	Cartilage repair	BM-MSCs	Self-assembling peptide hydrogel (TGF-β1 delivery)	Horse, articular cartilage defects	- Induced chondrogenesis - High glycosaminoglycan and collagen II deposition
Ha et al. (2015)	Cartilage repair	UCB-MSCs	Hyaluronic acid hydrogel	Minipig, full-thickness cartilage defect	- Hyaline-like cartilage regeneration - Good integration with native tissue
Bartlett et al. (2016)	Vocal fold repair	BM- MSCs	Hyaluronic acid hydrogel	Dog, vocal fold scarring model	- Decreased fibrosis - Partial restoration of vocal fold elasticity
Borg et al. (2016)	Pancreatic islet support	BM-MSCs	Macroporous PEG cryogel (heparin-functionalized)	Mouse, diabetic islet transplant model	- Improved islet viability - Immune modulation - Enhanced insulin secretion
Kolakshyapati et al. (2017)	Skin/sweat glands regeneration	BM-MSCs	Collagen-chitosan porous scaffold + Lipofectamine 2000/pDNA-EGF complexes	Mouse, full-thickness skin wound	- Formation of sweat-gland–like structures in regenerated skin - Upregulation of sweat-gland markers - Improved re-epithelialization and dermal remodeling
Lin et al. (2017)	Bone regeneration	BM-MSCs	Projection stereolithography-fabricated gene-activated matrix (BMP-2 pDNA embedded in photo-crosslinked hydrogel)	Mouse, implantation into the intramuscular compartment of upper long bone	- Robust ectopic bone formation - Mineralize bone and vascularization detected by histological studies
Wu et al. (2017)	Myocardial infarction	MSCs	Small-molecule self-assembling hydrogel	Rat LCA ligation	- Improved LVEF and fractional shortening - Reduced scar area and increased capillary density - Higher cell retention within infarct border zone

(Continued on following page)

TABLE 1 (*Continued*) Overview of MSC laden-hydrogel preclinical research.

First author, year	Clinical target	Type of MSCs	Type of hydrogel	Animal model, tissue damage	Main *in vivo* outcomes
Papa et al. (2018)	Spinal cord injury	BM-MSCs	PEG hydrogel releasing CCL2	Mouse, contusive SCI	- Preserved spinal cord architecture - Reduced inflammation - Improved locomotor recovery
Loozen et al. (2019)	Bone regeneration	MSCs	Alginate-ceramic scaffold with BMP-2 gene delivery	Rat, spinous process defect at the lumbosacral junction	- Robust bone regeneration - Substantial defect fill and restored bone volume at the micro-CT analysis - Histological evidence of well-organized, mineralized bone bridging the defect
Moussa et al. (2019)	Radiation-induced colonic damage	BM-MSCs	Heparan sulfate mimetic alginate-based hydrogel	Rat, localized colonic irradiation	- Reduced epithelial injury - Reduced colonic inflammation - Improved epithelial regeneration and restoration of mucosal architecture
Black et al. (2020)	Bone regeneration	Stro-4+ enriched MSCs	Bovine bone ECM hydrogel with PCL scaffold	Rat, femoral defect	- Enhanced mineralization, bone volume, and osteointegration
Sun et al. (2020)	Bone regeneration	BM-MSCs	Methacrylated gelatin-based hydrogel + rAAV encoding BMP2	Mouse, calvarial bone defect	- Accelerated bone bridging with higher bone volume and cortical continuity - Robust osteogenesis and vascularization - Mature trabecular bone tissue formation
Vivas et al. (2020)	Bone regeneration	BM-MSCs	Collagen-based GMP-grade hydrogel	Mouse, calvarial bone defect	- Early bone formation and vascularization - Clinically compliant MSC-hydrogel formulation
Zhang et al. (2021)	IVD degeneration	BM-MSCs	Fibrin-genipin hydrogel	Goat, moderate disc degeneration model	- Restored disc height and proteoglycan content - Reduced inflammatory cytokines
Lee et al. (2022)	Atopic dermatitis	MSCs	Thiolated hyaluronic acid hydrogel	Mouse DNCB-induced dermatitis	- Reduced epidermal thickness - Lowered cytokine expression - Improved skin barrier integrity
Canceill et al. (2023)	MSC delivery platform as ATMP	BM-MSCs	Platelet lysate-based fibrin hydrogel	Mouse, subcutaneous implantation	- No adverse immune reactions - Preserved MSC viability and differentiation potential
Hu et al. (2024)	Osteochondral regeneration	BM-MSCs	Nanozyme-functionalized bilayer hydrogel (oxidized dextran/gelatin + bioactive layer)	Rat, full-thickness osteochondral defect	- Improved cartilage repair - Subchondral bone formation - Reduced synovial inflammation
Li et al. (2024)	Spinal fusion	MSCs	Gelatin-methacryloyl composite hydrogel with BMP-2	Rat, posterolateral spinal fusion model	- Enhanced bone formation - Increased fusion rates - Osteoinduction
Huang et al. (2025)	Diabetic wound healing	Perinatal MSCs	Injectable PEG-based hydrogel	Mouse, streptozotocin-induced diabetic wounds	- Faster wound closure - Increased angiogenesis - Re-epithelialization via PI3K/AKT signaling
Sun et al. (2025)	Spinal cord injury	BM-MSCs	BMP7-loaded gelatin methacryloyl hydrogel	Rat T10 hemisection SCI	- Increased neuronal marker expression - Enhanced axon regeneration - Motor function recovery
Tavajjohi et al. (2025)	Myocardial infarction	Wharton's Jelly MSCs	Wharton's Jelly ECM-derived hydrogel	Rat LAD ligation	- Reduced infarct size - Enhanced angiogenesis - Improved ejection fraction - Decreased fibrosis

ATMP, advanced therapy medicinal product; BM-MSCs, Bone Marrow Mesenchymal Stromal Cells; BMP; Bone Morphogenetic Protein; DNCB, 2,4-dinitrochlorobenzene; ECM, Extracellular Matrix; EGF, Epidermal Growth Factor; GAGs, Glycosaminoglycans; ICRS, International Cartilage Repair Society; IVD, Intervertebral Disc; LAD, Left Anterior Descending Artery; LCA, Left Coronary Atery; LVEF, Left Ventricular Ejection Fraction; MSCs, Mesenchymal Stromal Cells; PCL, Polycaprolactone; pDNA, plasmid DNA; PEG, Polyethylene Glycol; PEO-b-PLL, poly (ethylene oxide)-b-poly (L-lysine); PI3K/AKT, phosphoinositide 3-kinase/protein kinase B; PLGA, Poly (Lactide-co-Glycolide); rAAV, recombinant adeno-associated viral vector; SCI, Spinal Cord Injury; TGF, Transforming Growth Factor; TMC, N,N,N-trimethyl chitosan chloride; UCB-MSCs, Umbilical Cord Blood Mesenchymal Stromal Cells.

This resulted in the promotion of keratinocyte migration, angiogenesis, and proliferation. In atopic dermatitis, thiolated hyaluronic acid hydrogels formed *in situ* after injection, improving MSC engraftment and retention. In treated mice, epidermal hyperplasia and dermal inflammation were significantly reduced, with decreased immune cell infiltration (Lee et al., 2022). Similarly, in gastrointestinal injury, MSCs embedded in hydrogels containing heparan sulfate mimetics (HS-m) named RGTA® showed to retain their immunoregulatory properties and reduced radiation-induced colonic inflammation, enhancing mucosal regeneration and restoring epithelial architecture (Moussa et al., 2019). Together, these studies show that in immune-rich tissues, hydrogels not only deliver MSCs but also provide a platform for reprogramming local macrophages and innate lymphoid cells, thereby facilitating resolution of inflammation.

4.4 Mechanically demanding and avascular tissues

Regeneration of cartilage, osteochondral interface, and bone is particularly difficult due to lack of vasculature, high mechanical stress, and poor intrinsic healing. In rabbits, Seol et al. (2013) reported that thermosensitive poloxamer 407 hydrogels carrying autologous MSCs filled full-thickness cartilage defects and promoted hyaline-like tissue formation with smooth surface morphology and improved histological scores. Hu and colleagues (2024) engineered a nanozyme-functionalized bilayer hydrogel conceptualized not only to promote osteochondral regeneration, but also to modulate the inflammatory microenvironment associate to tissue damage. The upper cartilage-inductive layer was composed of methacrylated gelatin (GelMA) incorporating kartogenin-loaded nanoparticles to stimulate chondrogenesis. The lower layer consisted of oxidized hyaluronic acid (OHA) and adipic acid dihydrazide (ADH) crosslinked hydrogel, embedded with manganese dioxide (MnO_2)-based nanozymes to scavenge reactive oxygen species (ROS) and reduce local inflammation. This construct significantly enhanced cartilage and subchondral bone repair in rabbits.

Several studies have specifically explored gene-activated MSC scaffolds for osteochondral regeneration. Wang et al. (2010) implanted poly (lactic-co-glycolic acid) (PLGA) sponges filled with fibrin gel, bone marrow-derived MSCs, and DNA complexes into rabbit cartilage defects, achieving near-complete filling and formation of hyaline-like tissue. Chen et al. (2011) designed bilayered scaffolds with spatially controlled gene delivery, enabling simultaneous regeneration of articular cartilage and subchondral bone *in vivo*. Building on this, Li et al. (2013) fabricated PLGA scaffolds containing MSCs and plasmid DNA encoding transforming growth factor beta-1 (TGF-β1), which markedly enhanced cartilage repair quality and ECM deposition. In larger animal models, umbilical cord-derived MSCs embedded in hyaluronic acid hydrogels promoted cartilage repair in minipigs (Ha et al., 2015), while self-assembling peptide hydrogels delivering TGF-β1 induced MSC chondrogenesis and ECM deposition in equine cartilage defects (Kopesky et al., 2014).

Bone regeneration also benefits from gene-activated approaches. Loozen and colleagues (2019) demonstrated that BMP-2 gene delivery in both MSC-laden and even cell-free constructs enhanced osteogenesis in rodent models, showing that sustained morphogen release can substitute for continuous cellular input. Building on this principle, Lin et al. (2017) used projection stereolithography to fabricate BMP-2 gene-activated scaffolds with high spatial precision, achieving robust bone tissue formation. Similarly, Sun et al. (2020) applied an injectable BMP-2 gene-activated scaffold to successfully repair cranial bone defects in mice, highlighting the translational relevance of minimally invasive delivery systems. Beyond gene activation, clinically oriented MSC–hydrogel formulations have been developed to meet regulatory and translational requirements. Vivas and colleagues (2020) designed a good manufacturing practices (GMP)-compliant osteogenic preparation consisting of bone marrow-derived MSCs embedded in a fibrin matrix enriched with micronized bone particles (0.25–1 μm). When implanted subcutaneously in immunodeficient mice, the construct showed no signs of toxicity or adverse immune response, while supporting early osteogenesis and vascularization. Importantly, this work emphasized safety, scalability, and compliance with good manufacturing practices, underlining the feasibility of advancing MSC-hydrogel therapies toward clinical application. Complementing these findings, Black et al. (2020) created a hybrid scaffold that combined a bovine ECM-derived hydrogel with a melt-electro-written polycaprolactone (PCL) framework. In a bovine critical-sized bone defect model, this construct promoted not only extensive new bone formation but also vascular ingrowth and stable integration with host tissue.

In these mechanically demanding tissues, hydrogel systems not only provide structural integration but also coordinate MSC crosstalk with osteoclasts, osteoblasts, and osteal macrophages, underscoring the role of osteo-immunity in skeletal regeneration.

4.5 Immune-sensitive and specialized tissues

Certain tissues present unique regenerative challenges, requiring repair strategies that minimize local immune activation or support complex graft integration. In a preclinical rabbit model of vocal fold scarring, Bartlett et al. (2016) evaluated the therapeutic efficacy and safety of bone marrow-derived MSCs delivered alone or in combination with a hyaluronic acid-based hydrogel (HyStem-VF). The study highlighted that the injection of MSCs alone improved viscoelastic recovery and ECM remodeling, while MSC-hydrogel combinations unexpectedly triggered inflammation and impaired repair. This underscores that in specialized tissues, biomaterial selection must be carefully tuned to avoid counterproductive immune responses.

In pancreatic transplantation, Borg et al. (2016) developed polyethylene glycol (PEG)–heparin cryogels to co-deliver pancreatic islets and MSCs. Implanted subcutaneously in mice, this system preserved insulin secretion and prolonged graft survival, with MSCs providing paracrine and immunomodulatory support.

Gene-activated constructs have also been applied to skin appendages. Kolakshyapati and collaborators (2017) reported that MSCs seeded in gene-activated matrices regenerated sweat gland-like structures *in vivo*, suggesting potential for adnexal tissue repair in burn injuries.

4.6 Safety and translational considerations

Clinical translation of hydrogel-MSC systems demands not only therapeutic efficacy but also rigorous demonstration of safety, biocompatibility, and compliance with regulatory standards. Accordingly, Canceill et al. (2023) reported that platelet lysate–based fibrin hydrogels encapsulating MSCs were biocompatible when implanted in immunocompetent rats, showing no acute inflammation, toxicity, or ectopic tissue formation. Platelet-rich formulations, already rich in growth factors and anti-inflammatory mediators, are well-known to enhance MSC activity, serving as valuable xeno-free components that sustain cell viability and function, as well as support tissue repair and immune modulation in a physiologically relevant manner (Barbon et al., 2018; Stocco et al., 2019b). The preclinical data collected by Canceill et al. (2023) exemplify how clinically oriented matrices can serve as advanced therapy medicinal products (ATMPs), bridging the gap from preclinical testing to human trials.

5 Hydrogel-based MSC therapies: clinical research

The clinical translation of MSC-based therapies has progressed significantly over the past decade, with numerous trials exploring their potential across various disease contexts. Nevertheless, when the integration of MSCs with hydrogel-based delivery systems is considered, clinical research appears to be in its early stages. Only a limited number of trials have investigated this combinatorial approach in human patients, with encouraging preliminary findings (Table 2). The following sections summarize representative clinical applications of hydrogel-assisted MSC delivery for cardiovascular, dermatological, articular, and chronic wound regeneration (Figure 3).

5.1 Cardiovascular regeneration

The clinical translation of regenerative therapies for ischemic heart disease has taken into consideration the integration of cell-based interventions with biomaterial scaffolds to enhance therapeutic efficacy. As already mentioned, this combinatorial approach aims to address the limitations of poor cell retention, survival, and functional integration frequently observed with cell delivery alone. Within this framework, He et al. (2020) conducted a multicenter, randomized, double-blind, placebo-controlled clinical trial involving 114 patients with chronic ischemic heart disease. The intervention group received intramyocardial implantation of MSCs combined with a bioengineered collagen scaffold during coronary artery bypass graft surgery, while the control group received the surgery alone. Over a 12-month follow-up, patients treated with MSCs and scaffold showed significantly improved myocardial perfusion, greater left ventricular ejection fraction (LVEF), and increased 6-min walk distance compared to controls. Importantly, the procedure was well tolerated, with no increase in adverse events.

This trial offers compelling evidence that combining cell therapy with biomaterial scaffolds enhances functional outcomes in ischemic heart disease.

5.2 Skin and scar regeneration

Post-surgical scarring, especially hypertrophic or keloid scars following cesarean sections, presents significant aesthetic and functional concerns. Traditional treatments have limited success in promoting satisfying dermal regeneration. MSCs, with their anti-inflammatory and remodeling capabilities, offer a novel approach to improving scar quality.

Recently, Fan et al. (2020b) conducted a randomized, double-blind, placebo-controlled clinical trial to assess the efficacy of umbilical cord-derived MSCs for improving cesarean section scar

TABLE 2 Overview of MSC laden-hydrogel clinical research.

First author, year	Clinical target	Type of MSCs	Type of hydrogel	No. of patients	Surgery	Main clinical outcomes
Park et al. (2017)	Knee osteoarthritis	UCB-MSCs	Hyaluronate hydrogel	18	Intra-articular injection	- Cartilage regeneration - Pain relief - Joint function maintained over 7 years
Zeng et al. (2017)	Diabetic foot ulcer	PDMSCs	Sodium alginate hydrogel	1	-	- Accelerated wound healing - Re-epithelialization - Reduced inflammation
Fan et al. (2020b)	Cesarean section skin scars	UCB-MSCs	Transdermal delivery hydrogel	58	C-section (follow-up for scar management)	- Reduced scar thickness and erythema - Improved pliability
He et al. (2020)	Chronic ischemic heart disease	BM-MSCs	Collagen scaffold	114	CABG with MSC scaffold injection	- Improved myocardial perfusion - Greater LVEF - Increased 6-min walk distance

BM-MSCs, Bone Marrow Mesenchymal Stromal Cells; CABG, Coronary Artery Bypass Grafting; LVEF, left ventricular ejection fraction; MSCs, Mesenchymal Stromal Cells; PDMSCs, placenta-derived Mesenchymal Stromal Cells; UCB-MSCs, Umbilical Cord Blood Mesenchymal Stromal Cells.

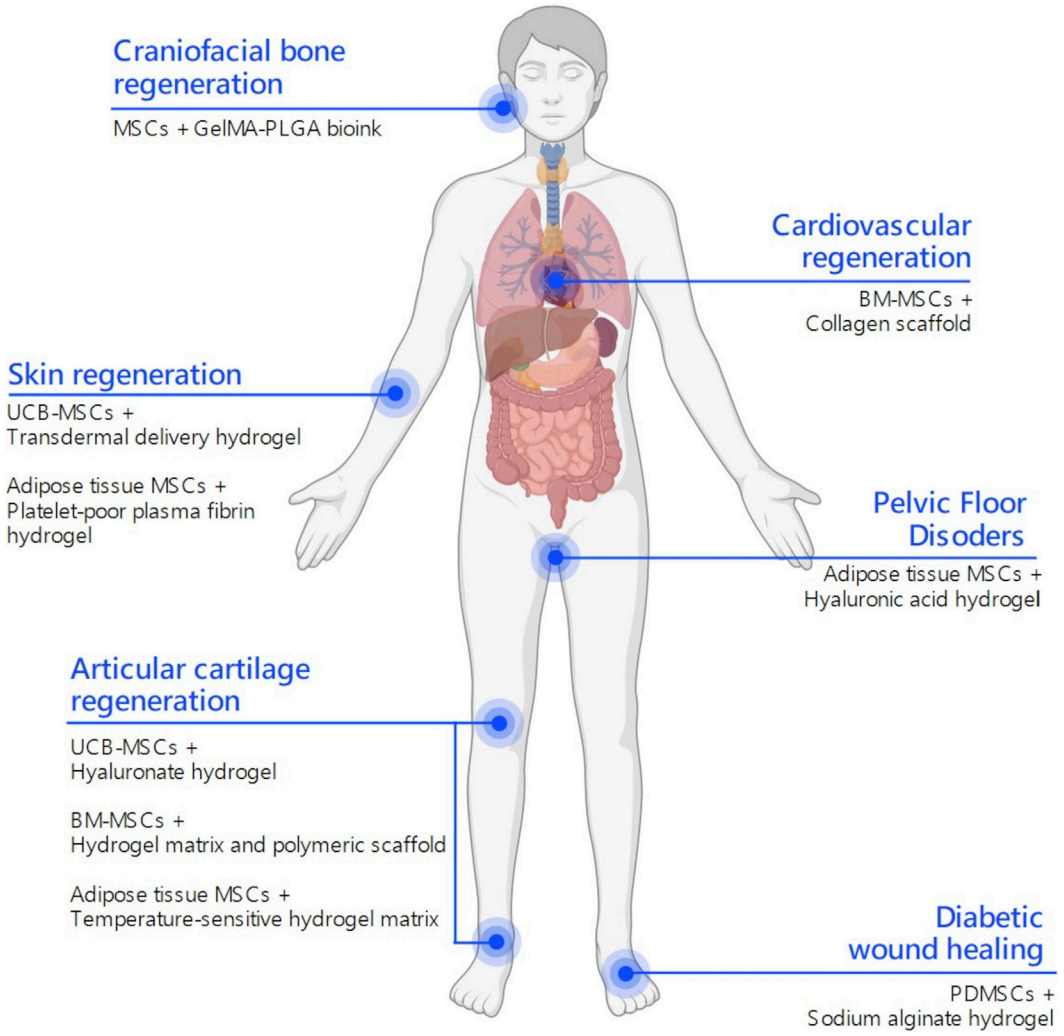


FIGURE 3
Clinical applications of MSC-laden hydrogels. Schematic representation of published and ongoing clinical trials on hydrogel-assisted mesenchymal stromal cell (MSCs) delivery to stimulate tissue regeneration. Depending on the anatomical site, distinct MSCs and hydrogel formulations are employed to address specific regenerative challenges. This integrative map highlights how hydrogel composition and MSC source can be strategically matched to different tissue microenvironments to improve regenerative outcomes. This figure was created using BioRender.com.

outcomes. Fifty-eight women were enrolled and randomly assigned to receive intradermal injections of MSCs or placebo along the surgical incision site. The stem cells were delivered in a transdermal hydrogel formulation designed to enhance local retention and therapeutic activity.

At 6 months post-treatment, scar assessments were performed using clinical examination, histological analysis, and the Vancouver Scar Scale (VSS). Results indicated modest improvements in scar appearance among MSC-treated patients, including reduced erythema, improved pliability, decreased scar thickness, and enhanced collagen remodeling. However, these differences did not reach statistical significance compared to the placebo group. Importantly, the treatment was well tolerated, with no adverse events or immune-related reactions reported.

These results suggest that UC-MSCs may be a safe and effective treatment to modulate fibroproliferative responses in human skin wounds. Beyond cosmetic benefits, this approach may reduce functional limitations and discomfort associated with scarring. However, larger trials are needed to standardize dosing and administration strategies.

5.3 Articular cartilage repair

Degenerative diseases affecting articular cartilage, such as osteoarthritis, are among the most common sources of chronic pain and functional impairment worldwide. Conventional treatments (pharmacologic management, physiotherapy, and surgical interventions) primarily aim to alleviate symptoms but fail in restoring cartilage integrity or halting disease progression. In this context, the integration of MSCs with biocompatible scaffolds, particularly hydrogels, represents a promising regenerative strategy. As part of this growing interest in cartilage regeneration therapies, Park and colleagues (2017) conducted an open-label, single-arm clinical trial in 18 patients with knee osteoarthritis. The treatment consisted of a composite product

containing allogeneic umbilical cord blood-derived MSCs mixed with hyaluronic acid hydrogel, injected intra-articularly. Clinical and arthroscopic assessments over 7 years showed sustained improvement in pain and mobility, with imaging revealing the development of hyaline-like cartilage and joint space preservation.

Together, these outcomes emphasize the long-term regenerative potential of MSC-hydrogel therapies in osteoarthritic conditions.

5.4 Diabetic wound healing

Chronic non-healing wounds such as diabetic foot ulcers are a major complication of diabetes, often leading to infection and amputation. Innovative therapies that promote re-epithelialization and vascularization are urgently needed. MSCs have appear to be promising therapeutic candidates due to their regenerative and immunomodulatory effects, particularly when delivered in a hydrogel vehicle.

In this scenario, Zeng et al. (2017) described a single-case application of placenta-derived MSCs embedded in a hydrogel matrix applied topically to a chronic diabetic foot ulcer. The wound was previously unresponsive to standard therapies. Over a 3-week period, the ulcer showed accelerated granulation tissue development, reduced local inflammation, and almost complete re-epithelialization. No complications were observed.

Despite being a case report, the study demonstrates the translational feasibility of topical MSC-hydrogel therapies. The favorable outcome supports further investigation through controlled trials. Numerous polymeric biomaterials are used recently for diabetic wound dressing including hydrogels, collagen-based scaffolds, whose efficacy can be further improved by loading therapeutic molecules, growth factors, and anti-microbial agents that could accelerate the process of wound closure by triggering collagen deposition and vascularization. However, advanced research in this field is essential to enhance the efficacy of polymeric wound dressings for efficient diabetic wound treatment (Sathyaraj et al., 2023). The selection of placenta-derived MSCs suggests a potential for enhanced immunomodulatory action and broader clinical applicability due to their allogeneic compatibility.

5.5 Ongoing clinical trials on MSC-laden hydrogels

Although the number of published clinical trials investigating hydrogel-assisted MSC therapies remains limited, other ongoing studies registered on the databases *ClinicalTrials.gov*, *EudraCT* (European Union Drug Regulating Authorities Clinical Trials) and *EU Clinical Trials Register* underscore the growing translational advancement in this field. These trials explore different hydrogel platforms, cellular sources, and delivery strategies, reflecting the adaptability of MSC-laden hydrogels across a range of regenerative approaches (Table 3).

In the context of autologous regenerative therapies, the multicenter, exploratory trial NCT03113747 entitled "Allogeneic ADSCs and Platelet-Poor Plasma Fibrin Hydrogel to Treat the Patients With Burn Wounds" investigates the safety and preliminary efficacy of a tissue-engineered construct combining allogeneic adipose tissue MSCs embedded in a platelet-poor plasma (PPP)-derived fibrin hydrogel. This study targets patients with deep second- or third-degree burns covering 10%–50% of their body surface. The composite graft is applied directly to wounds within 24 h post-injury to evaluate its potential to accelerate healing and reduce scarring. Specifically, the PPP-derived hydrogel aims to improve local MSC retention and bioactivity, thereby enhancing tissue healing responses. Currently, the study status is listed as "Unknown" due to the absence of updates since April 2017. This suggests possible interruption or prolonged inactivity, underscoring the criticisms often encountered in cell-based product development and clinical translation (Voronin, 2025).

Despite these challenges, further clinical research efforts are being pursued to validate and optimize regenerative therapies based on MSC-laden hydrogels. For example, the EudraCT 2021-002331-34 trial is a multicenter, randomized, double-blind Phase IIb study which evaluates the safety and efficacy of allogeneic adipose-derived MSCs embedded in a hyaluronic acid hydrogel for the treatment of structural fecal incontinence (FI). Patients with a confirmed anal sphincter defect and chronic FI were administered with the investigated MSC-hydrogel product via intralesional injection, comparing 2 cell doses (60 million vs. 120 million adipose tissue-derived MSCs) suspended in a hyaluronic acid matrix. This trial exemplifies the growing clinical interest in MSC-laden hydrogels as a novel treatment strategy for sphincter repair, combining regenerative potential of stem cells with their localized, minimally invasive delivery. Its outcomes may offer valuable insights into the clinical applicability of cell-based biomaterial therapies in pelvic floor disorders (FIMABIS, 2025).

The recent trial NCT06028763 titled "Development of Biomedical Technology for the Treatment of Ankle Cartilage Using Injectable Biocomposite Hydrogel" focuses on adipose tissue-derived MSCs embedded within a customized hydrogel matrix and administered locally for the treatment of ankle cartilage focal injuries. These lesions are particularly difficult to treat due to the avascular nature and limited intrinsic healing capacity of cartilage tissue. The trial addresses these challenges by delivering MSCs in a minimally invasive, injectable hydrogel that solidifies at body temperature, enabling mechanical support, targeted delivery and long-term cellular retention within the ankle joint (Center of National Scientific Center of Traumatology and Orthopedics, 2025).

The clinical challenge of articular cartilage regeneration is also being addressed by a recently registered Phase I clinical trial (EudraCT 2024-512977-28-01), designed to evaluate the safety and feasibility of a novel advanced therapy medicinal product (ATMP). This investigational approach combines autologous bone marrow-derived MSCs with a biocompatible hydrogel matrix and a polymeric scaffold for the treatment of focal femoral condyle cartilage lesions in the knee. The trial aims to assess not only the local tolerability of the composite implant, but also the operational feasibility of its GMP-compliant manufacturing and surgical application. Depending on its specific outcomes, this study may represent a key step toward the clinical translation of MSC-laden hydrogel systems in orthopedic regenerative medicine (Lamina Therapeutics, 2025).

TABLE 3 Ongoing clinical trials on MSC laden-hydrogels for tissue regeneration.

Identification number (study start year)	Clinical target	MSC type	Hydrogel type	Delivery route	Phase and status
NCT03113747 (2013)	2° or 3° degree burn wounds	Allogeneic MSCs from adipose tissue	Platelet-poor plasma fibrin hydrogel	Localized topical application to burn wound sites	Phase I/II Recruitment status "Unknown" *since last update in April 2017*
EudraCT 2021-002331-34 (2022)	Anal sphincter defect and chronic FI	Adipose tissue MSCs	Hyaluronic acid hydrogel	Intralesional injection	Phase IIb Status: Ongoing
NCT06028763 (2023)	Focal cartilage lesions in the ankle joint	Autologous MSCs from adipose tissue	Temperature-sensitive hydrogel matrix	Intra-articular injection of the cell-hydrogel composite into the knee joint	Phase I, Interventional Status: Recruiting
EudraCT 2024-512977-28-01 (2024)	Knee femoral cartilage lesions	Autologous BM-MSCs	Hydrogel matrix and polymeric scaffold	Local application within the knee joint	Phase I Status: Not yet recruiting
NCT06533150 (2024)	Craniofacial bone defects	Potential combination with MSCs	GelMA-PLGA bioink	Local implantation during craniofacial reconstructive surgery	Phase I, Interventional Status: Active, not yet recruiting

BM-MSCs, Bone Marrow MSCs; Mesenchymal Stromal Cells; FI, fecal incontinence; GelMA, Gelatin methacryloyl (GelMA); MSCs, Mesenchymal Stromal Cells; PLGA, poly (lactic-co-glycolic acid).

Finally, the trial NCT06533150 titled "Functionalized Bioink Delivering Biomolecules for the Treatment of Craniofacial Diseases (DART-CRAFT)" is developing an advanced Gelatin methacryloyl (GelMA) and poly (lactic-co-glycolic acid) (PLGA)-based bioink for potential MSC encapsulation and 3D bioprinting applications in craniofacial bone repair procedures. The bioink is applied intraoperatively during reconstructive surgery, where it serves as a moldable and *in situ* solidifying scaffold, tailored to support bone regeneration by enhancing local tissue integration and possibly incorporating MSCs. This innovative approach merges 3D bioprinting, controlled release technology, and biocompatible materials to address complex craniofacial defects with high precision. By enabling localized therapeutic delivery of growth factors or cells and structural support, the DART-CRAFT platform highlights the future of personalized regenerative strategies in maxillofacial surgery (Fondazione Policlinico Universitario Agostino Gemelli IRCCS, 2025).

Collectively, these ongoing trials demonstrate the continued evolution and clinical interest in hydrogel-based MSC delivery systems, highlighting their potential to enhance therapeutic efficacy, standardization, and applicability across multiple regenerative medicine applications.

6 Potential research gaps

Despite significant advancements in the preclinical and clinical application of MSC-laden hydrogels for tissue regeneration, several research gaps hinder their full clinical translation. One major challenge is the lack of standardized protocols for hydrogel composition, including optimal mechanical properties, degradation rates, and bioactive molecule incorporation, which vary depending on the target tissue (Burdick and Mauck, 2011). Additionally, while MSC-hydrogel systems have shown promise in preclinical studies, there is limited long-term data on cell survival, engraftment, and functional integration in human clinical trials (Galipeau and Sensébé, 2018). Another critical gap is the insufficient understanding of the paracrine mechanisms by which MSCs exert their therapeutic effects within hydrogels. While MSCs are known to secrete regenerative factors, the precise signaling pathways and their modulation by the hydrogel microenvironment remain unclear (Caplan and Correa, 2011). Furthermore, immune responses to implanted MSC-laden hydrogels, including potential foreign body reactions or unintended immunomodulatory effects, require further investigation (Andrzejewska et al., 2019).

Scalability and manufacturing consistency also pose challenges, as variations in MSC sources (e.g., autologous vs. allogeneic) and hydrogel fabrication techniques can impact therapeutic outcomes (Murphy et al., 2020). Finally, regulatory and ethical considerations surrounding MSC-based therapies necessitate clearer guidelines to facilitate clinical adoption (Sipp et al., 2018). Addressing these gaps through interdisciplinary research will be crucial for advancing MSC-laden hydrogels toward widespread clinical use in tissue regeneration.

7 Future clinical research focus

The clinical application of MSC-laden hydrogels holds immense potential for tissue regeneration, yet future research must address key challenges to optimize therapeutic efficacy and translation. A critical focus will be the development of smart hydrogels with tunable biomechanical and biochemical properties that can dynamically respond to the regenerative microenvironment (Daly et al., 2021). Such advanced biomaterials should enhance MSC retention, survival, and controlled differentiation while minimizing immune rejection (Zhu et al., 2022). Importantly, MSC-based therapies alone have shown to often fail to achieve long-term and uncomplicated wound

healing across different tissues, largely due to the transient survival of transplanted cells and the short-lived duration of their paracrine signaling. Embedding MSCs within hydrogels directly addresses this limitation, as these systems can provide a supportive matrix that prolongs MSC bioactivity, sustains the release of anti-inflammatory and trophic factors, and better synchronizes cellular functions with the extended phases of tissue repair and remodeling.

Building on this rationale, another priority is the standardization of MSC sources and hydrogel formulations for specific clinical indications, such as osteoarthritis, chronic wounds, and myocardial infarction. Large-scale, randomized controlled trials (RCTs) are needed to validate the safety and long-term functional outcomes of MSC-hydrogel therapies compared to conventional treatments (Wei et al., 2023). Additionally, integrating technologies like 3D bioprinting and gene editing could enable patient-specific constructs with spatially controlled MSC delivery (Gungor-Ozkerim et al., 2018).

Understanding the immune-modulatory mechanisms of MSCs within hydrogels will also be essential, particularly in allogeneic applications, to prevent adverse responses and harness their anti-inflammatory potential (Levy et al., 2020). Finally, regulatory frameworks must evolve to accommodate the unique challenges of combination products (cells + biomaterials), ensuring reproducibility and scalability for commercialization (Mendicino et al., 2022). By addressing these priorities, future clinical research can unlock the full regenerative potential of MSC-laden hydrogels.

Author contributions

SB: Conceptualization, Methodology, Writing – original draft. SR: Methodology, Writing – original draft. AB: Methodology, Writing – review and editing. PPP: Supervision, Writing – review and editing. RDC: Resources, Supervision, Writing – review and editing. VM: Resources, Supervision, Writing – review and editing. AP: Conceptualization, Supervision, Writing – review and editing.

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References

Andrzejewska, A., Lukomska, B., and Janowski, M. (2019). Concise review: mesenchymal stem cells: from roots to boost. *Stem Cells* 37, 855–864. doi:10.1002/stem.3016

Bagno, L. L., Salerno, A. G., Balkan, W., and Hare, J. M. (2022). Mechanism of action of mesenchymal stem cells (MSCs): impact of delivery method. *Expert Opin. Biol. Ther.* 22, 449–463. doi:10.1080/14712598.2022.2016695

Barbon, S., Stocco, E., Grandi, F., Rajendran, S., Borean, A., Pirola, I., et al. (2018). Biofabrication of a novel leukocyte-fibrin-platelet membrane as a cells and growth factors delivery platform for tissue engineering applications. *J. Tissue Eng. Regen. Med.* 12, 1891–1906. doi:10.1002/term.2713

Barbon, S., Stocco, E., Dalzoppo, D., Todros, S., Canale, A., Boscolo-Berto, R., et al. (2020). Halogen-mediated partial oxidation of polyvinyl alcohol for tissue engineering purposes. *Int. J. Mol. Sci.* 21, 801. doi:10.3390/ijms21030801

Barbon, S., Rajendran, S., Bertalot, T., Piccione, M., Gasparella, M., Parnigotto, P. P., et al. (2021). Growth and differentiation of circulating stem cells after extensive *ex vivo* expansion. *Tissue Eng. Regen. Med.* 18, 411–427. doi:10.1007/s13770-021-00330-7

Barbon, S., Stocco, E., Rajendran, S., Zardo, L., Macchi, V., Grandi, C., et al. (2022). *In vitro* conditioning of adipose-derived mesenchymal stem cells by the endothelial microenvironment: modeling cell responsiveness towards non-genetic correction of haemophilia A. *Int. J. Mol. Sci.* 23, 7282. doi:10.3390/ijms23137282

Barbon, S., Banerjee, A., Perin, L., De Caro, R., Parnigotto, P. P., and Porzionato, A. (2023). Editorial: therapeutic potential of mesenchymal stem cells in organ and tissue regeneration. *Front. Bioeng. Biotechnol.* 11, 1333281. doi:10.3389/fbioe.2023.1333281

Bartlett, R. S., Guille, J. T., Chen, X., Christensen, M. B., Wang, S. F., and Thibeault, S. L. (2016). Mesenchymal stromal cell injection promotes vocal fold scar repair without long-term engraftment. *Cytotherapy* 18, 1284–1296. doi:10.1016/j.jcyt.2016.07.005

Black, C., Kanczler, J. M., de Andrés, M. C., White, L. J., Savi, F. M., Bas, O., et al. (2020). Characterisation and evaluation of the regenerative capacity of Stro-4+ enriched bone marrow mesenchymal stromal cells using bovine extracellular matrix hydrogel and a novel biocompatible melt electro-written medical-grade polycaprolactone scaffold. *Biomaterials* 247, 119998. doi:10.1016/j.biomaterials.2020.119998

Borg, D. J., Welzel, P. B., Grimmer, M., Friedrichs, J., Weigelt, M., Wilhelm, C., et al. (2016). Macroporous biohybrid cryogels for co-housing pancreatic islets with mesenchymal stromal cells. *Acta Biomater.* 44, 178–187. doi:10.1016/j.actbio.2016.08.007

Burdick, J. A., and Mauck, R. L. (2011). *Hydrogels for tissue engineering: scaffold design variables and applications*. Berlin/Heidelberg, Germany: Springer.

Burdick, J. A., and Murphy, W. L. (2012). Moving from static to dynamic complexity in hydrogel design. *Nat. Commun.* 3, 1269. doi:10.1038/ncomms2271

Canceill, T., Jourdan, G., Kémoun, P., Guissard, C., Monsef, Y. A., Bourdens, M., et al. (2023). Characterization and safety profile of a new combined advanced therapeutic

medical product platelet lysate-based fibrin hydrogel for mesenchymal stromal cell local delivery in regenerative medicine. *Int. J. Mol. Sci.* 24, 2206. doi:10.3390/ijms24032206

Caplan, A. I., and Correa, D. (2011). The MSC: an injury drugstore. *Cell Stem Cell* 9, 11–15. doi:10.1016/j.stem.2011.06.008

Center of National Scientific Center of Traumatology and Orthopedics (2025). Development of biomedical technology for the treatment of ankle cartilage using injectable biocomposite hydrogel. *ClinicalTrials.gov*, identifier NCT06028763. Available online at: https://clinicaltrials.gov/study/NCT06028763 (Accessed on June 29, 2025).

Chen, J., Chen, H., Li, P., Diao, H., Zhu, S., Dong, L., et al. (2011). Simultaneous regeneration of articular cartilage and subchondral bone *in vivo* using MSCs induced by a spatially controlled gene delivery system in bilayered integrated scaffolds. *Biomaterials* 32 (21), 4793–4805. doi:10.1016/j.biomaterials.2011.03.041

Daly, A. C., Prendergast, M. E., Hughes, A. J., and Burdick, J. A. (2021). Bioprinting for the biologist. *Cell* 184, 18–32. doi:10.1016/j.cell.2020.12.002

Di Liddo, R., Paganin, P., Lora, S., Dalzoppo, D., Giraudo, C., Miotto, D., et al. (2014). Poly-ε-caprolactone composite scaffolds for bone repair. *Int. J. Mol. Med.* 34, 1537–1546. doi:10.3892/ijmm.2014.1954

Di Liddo, R., Aguiari, P., Barbon, S., Bertalot, T., Mandoli, A., Tasso, A., et al. (2016). Nanopatterned acellular valve conduits drive the commitment of blood-derived multipotent cells. *Int. J. Nanomed.* 11, 5041–5055. doi:10.2147/IJN.S115999

Engler, A. J., Sen, S., Sweeney, H. L., and Discher, D. E. (2006). Matrix elasticity directs stem cell lineage specification. *Cell* 126, 677–689. doi:10.1016/j.cell.2006.06.044

Fan, X. L., Zhang, Y., Li, X., and Fu, Q. L. (2020a). Mechanisms underlying the protective effects of mesenchymal stem cell-based therapy. *Cell. Mol. Life Sci.* 77, 2771–2794. doi:10.1007/s00018-020-03454-6

Fan, D., Zeng, M., Xia, Q., Chen, J., Liu, R., Wu, Z., et al. (2020b). Efficacy and safety of umbilical cord mesenchymal stem cells in treatment of cesarean section skin scars: a randomized clinical trial. *Stem Cell Res. Ther.* 11, 244. doi:10.1186/s13287-020-01695-7

FIMABIS (2025). A multicenter, randomized, double-blind phase IIb study to evaluate the safety and efficacy of the intralesional administration of two doses of expanded allogeneic adipose tissue adult mesenchymal stem cells (ADTCT), packed in hyaluronic acid hydrogel, as a treatment for patients with fecal incontinence. EU Clin. Trials Regist. *EudraCT Number 2021-002331-34*. Available online at: https://www.clinicaltrialsregister.eu/ctr-search/trial/2021-002331-34/ES/ (Accessed on June 29, 2025).

Fondazione Policlinico Universitario Agostino Gemelli IRCCS (2025). Functionalized bioink delivering biomolecules for the treatment of craniofacial diseases (DART-CRAFT). *ClinicalTrials.gov*, identifier NCT06533150. Available online at: https://clinicaltrials.gov/study/NCT06533150 (Accessed on June 29, 2025).

Galipeau, J., and Sensébé, L. (2018). Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. *Cell Stem Cell* 22, 824–833. doi:10.1016/j.stem.2018.05.004

Garcia-Aponte, O. F., Kahlenberg, S., Kouroupis, D., Egger, D., and Kasper, C. (2025). Effects of hydrogels on mesenchymal stem/stromal cells paracrine activity and extracellular vesicles production. *J. Extracell. Vesicles* 14, e70057. doi:10.1002/jev2.70057

Gnecchi, M., Danieli, P., Malpasso, G., and Ciuffreda, M. C. (2016). Paracrine mechanisms of mesenchymal stem cells in tissue repair. *Methods Mol. Biol.* 1416, 123–146. doi:10.1007/978-1-4939-3584-0_7

Grandi, F., Stocco, E., Barbon, S., Rambaldo, A., Contran, M., Fascetti Leon, F., et al. (2018). Composite scaffolds based on intestinal extracellular matrices and oxidized polyvinyl alcohol: a preliminary study for a new regenerative approach in short bowel syndrome. *Biomed. Res. Int.* 2018, 7824757. doi:10.1155/2018/7824757

Gungor-Ozkerim, P. S., Inci, I., Zhang, Y. S., Khademhosseini, A., and Dokmeci, M. R. (2018). Bioinks for 3D bioprinting: an overview. *Biomater. Sci.* 6, 915–946. doi:10.1039/C7BM00765E

Guo, Y., Yu, Y., Hu, S., Chen, Y., and Shen, Z. (2020). The therapeutic potential of mesenchymal stem cells for cardiovascular diseases. *Cell Death Dis.* 11, 349. doi:10.1038/s41419-020-2542-9

Ha, C. W., Park, Y. B., Chung, J. Y., and Park, Y. G. (2015). Cartilage repair using composites of human umbilical cord blood-derived mesenchymal stem cells and hyaluronic acid hydrogel in a minipig model. *Stem Cells Transl. Med.* 4, 1044–1051. doi:10.5966/sctm.2014-0264

He, X., Wang, Q., Zhao, Y., Li, X., Zhang, W., Chen, Y., et al. (2020). Effect of intramyocardial grafting collagen scaffold with mesenchymal stromal cells in patients with chronic ischemic heart disease: a randomized clinical trial. *JAMA Netw. Open* 3, e2016236. doi:10.1001/jamanetworkopen.2020.16236

Hoang, D. M., Pham, P. T., Bach, T. Q., Ngo, A. T. L., Nguyen, Q. T., Phan, T. T. K., et al. (2022). Stem cell-based therapy for human diseases. *Signal Transduct. Target. Ther.* 7, 272. doi:10.1038/s41392-022-01134-4

Hu, C., Huang, R., Xia, J., Hu, X., Xie, D., Jin, Y., et al. (2024). A nanozyme-functionalized bilayer hydrogel scaffold for modulating the inflammatory microenvironment to promote osteochondral regeneration. *J. Nanobiotechnol.* 22, 445. doi:10.1186/s12951-024-02723-x

Huang, J., Deng, Q., Tsang, L. L., Chang, G., Guo, J., Ruan, Y. C., et al. (2025). Mesenchymal stem cells from perinatal tissues promote diabetic wound healing *via* PI3K/AKT activation. *Stem Cell Res. Ther.* 16, 59. doi:10.1186/s13287-025-04141-8

Hussen, B. M., Taheri, M., Yashooa, R. K., Abdullah, G. H., Abdullah, S. R., Kheder, R. K., et al. (2024). Revolutionizing medicine: recent developments and future prospects in stem-cell therapy. *Int. J. Surg.* 110, 8002–8024. doi:10.1097/JS9.0000000000002109

Kaviarasan, V., Deka, D., Balaji, D., Pathak, S., and Banerjee, A. (2024). Signaling pathways in trans-differentiation of mesenchymal stem cells: recent advances. *Methods Mol. Biol.* 2736, 207–223. doi:10.1007/7651_2023_478

Kolakshyapati, P., Li, X., Chen, C., Zhang, M., Tan, W., Ma, L., et al. (2017). Gene-activated matrix/bone marrow-derived mesenchymal stem cells constructs regenerate sweat glands-like structure *in vivo*. *Sci. Rep.* 7, 17630. doi:10.1038/s41598-017-17967-x

Kopesky, P. W., Byun, S., Vanderploeg, E. J., Kisiday, J. D., Frisbie, D. D., and Grodzinsky, A. J. (2014). Sustained delivery of bioactive TGF-β1 from self-assembling peptide hydrogels induces chondrogenesis of encapsulated bone marrow stromal cells. *J. Biomed. Mater. Res. A* 102, 1275–1285. doi:10.1002/jbm.a.34789

Krasilnikova, O., Yakimova, A., Ivanov, S., Atiakshin, D., Kostin, A. A., Sosin, D., et al. (2023). Gene-activated materials in regenerative dentistry: narrative review of technology and study results. *Int. J. Mol. Sci.* 24, 16250. doi:10.3390/ijms242216250

Kuppa, S. S., Kim, H. K., Kang, J. Y., Lee, S. C., and Seon, J. K. (2022). Role of mesenchymal stem cells and their paracrine mediators in macrophage polarization: an approach to reduce inflammation in osteoarthritis. *Int. J. Mol. Sci.* 23, 13016. doi:10.3390/ijms232113016

Lamina Therapeutics (2025). *Feasibility and safety of a combined ATMP comprising a polymeric medical device and autologous bone marrow-derived MSCs in a hydrogel mixture for the treatment of isolated knee femoral cartilage lesions*. Strasbourg, France: EU Clinical Trials Register. Available online at: https://euclinicaltrials.eu/search-for-clinical-trials/?lang=en&EUCT=2024-512977-28-01 (Accessed on June 29, 2025).

Lee, H., Lee, T. W., Chandrasekharan, A., Sung, S. E., Yim, S. G., Kim, S., et al. (2022). Injectable self-crosslinkable thiolated hyaluronic acid for stem cell therapy of atopic dermatitis. *ACS Biomater. Sci. Eng.* 8, 1613–1622. doi:10.1021/acsbiomaterials.1c01374

Levit, R. D., Landázuri, N., Phelps, E. A., Brown, M. E., García, A. J., Davis, M. E., et al. (2013). Cellular encapsulation enhances cardiac repair. *J. Am. Heart Assoc.* 2, e000367. doi:10.1161/JAHA.113.000367

Levy, O., Kuai, R., Siren, E. M. J., Bhere, D., Milton, Y., Nissar, N., et al. (2020). Shattering barriers toward clinically meaningful MSC therapies. *Sci. Adv.* 6, eaba6884. doi:10.1126/sciadv.aba6884

Li, B., Yang, J., Ma, L., Li, F., Tu, Z., and Gao, C. (2013). Fabrication of poly(lactide-co-glycolide) scaffold filled with fibrin gel, mesenchymal stem cells, and poly(ethylene oxide)-b-poly(L-lysine)/TGF-β1 plasmid DNA complexes for cartilage restoration *in vivo*. *J. Biomed. Mater Res. A* 101 (11), 3097–3108. doi:10.1002/jbm.a.34618

Li, T., Zhang, X., Hu, Y., Gao, X., Yao, X., and Xu, Z. (2024). Development of gelatin-methacryloyl composite carriers for bone morphogenetic protein-2 delivery: a potential strategy for spinal fusion. *J. Biomater. Appl.* 39, 195–206. doi:10.1177/08853282241258302

Liang, Y., Zhao, X., Hu, T., Chen, B., Yin, Z., Ma, P. X., et al. (2019). Adhesive hemostatic conducting injectable composite hydrogels with sustained drug release and photothermal antibacterial activity to promote full-thickness skin regeneration during wound healing. *Small* 15, 1900046. doi:10.1002/smll.201900046

Lin, H., Tang, Y., Lozito, T. P., Oyster, N., Kang, R. B., Fritch, M. R., et al. (2017). Projection stereolithographic fabrication of BMP-2 gene-activated matrix for bone tissue engineering. *Sci. Rep.* 7, 11327. doi:10.1038/s41598-017-11051-0

Liu, J., Xi, Z., Fan, C., Mei, Y., Zhao, J., Jiang, Y., et al. (2024). Hydrogels for nucleic acid drugs delivery. *Adv. Healthc. Mater.* 13, e2401895. doi:10.1002/adhm.202401895

Loozen, L. D., Kruyt, M. C., Kragten, A. H. M., Schoenfeldt, T., Croes, M., Oner, C. F., et al. (2019). BMP-2 gene delivery in cell-loaded and cell-free constructs for bone regeneration. *PLoS One* 14 (7), e0220028. doi:10.1371/journal.pone.0220028

Lu, P., Ruan, D., Huang, M., Tian, M., Zhu, K., Gan, Z., et al. (2024). Harnessing the potential of hydrogels for advanced therapeutic applications: current achievements and future directions. *Signal Transduct. Target. Ther.* 9, 166. doi:10.1038/s41392-024-01852-x

Mendicino, M., Bailey, A. M., Wonnacott, K., Puri, R. K., and Bauer, S. R. (2022). MSC-Based product characterization for clinical trials: an FDA perspective. *Stem Cell Res. Ther.* 13, 1. doi:10.1186/s13287-021-02677-4

Moussa, L., Demarquay, C., Réthoré, G., Benadjaoud, M. A., Siñeriz, F., Pattapa, G., et al. (2019). Heparan sulfate mimetics: a new way to optimize therapeutic effects of hydrogel-embedded mesenchymal stromal cells in colonic radiation-induced damage. *Sci. Rep.* 9, 164. doi:10.1038/s41598-018-36631-6

Murphy, M. B., Moncivais, K., and Caplan, A. I. (2020). Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. *Exp. Mol. Med.* 52, 1–10. doi:10.1038/s12276-019-0350-4

Papa, S., Vismara, I., Mariani, A., Barilani, M., Rimondo, S., De Paola, M., et al. (2018). Mesenchymal stem cells encapsulated into biomimetic hydrogel scaffold gradually release CCL2 chemokine *in situ* preserving cytoarchitecture and promoting functional recovery in spinal cord injury. *J. Control Release* 278, 49–56. doi:10.1016/j.jconrel.2018.03.034

Park, Y. B., Ha, C. W., Lee, C. H., Park, K. S., Kang, M. C., Song, J. H., et al. (2017). Cartilage regeneration in osteoarthritic patients by a composite of allogeneic umbilical cord blood-derived mesenchymal stem cells and hyaluronate hydrogel: results from a clinical trial for safety and proof-of-concept with 7 years of extended follow-up. *Stem Cells Transl. Med.* 6, 613–621. doi:10.5966/sctm.2016-0157

Porzionato, A., Barbon, S., Stocco, E., Dalzoppo, D., Contran, M., De Rose, E., et al. (2019). Development of oxidized polyvinyl alcohol-based nerve conduits coupled with the ciliary neurotrophic factor. *Materials* 12, 1996. doi:10.3390/ma12121996

Saldanha-Araujo, F., Melgaço Garcez, E., Silva-Carvalho, A. E., Carvalho, J. L., Guilmeau-Becker, D., Meirelles, L. S., et al. (2020). Mesenchymal stem cells: a new piece in the puzzle of COVID-19 treatment. *Front. Immunol.* 11, 1563. doi:10.3389/fimmu.2020.01563

Sathyaraj, W. V., Prabakaran, L., Bhoopathy, J., Dharmalingam, S., Karthikeyan, R., and Atchudan, R. (2023). Therapeutic efficacy of polymeric biomaterials in treating diabetic wounds—An upcoming wound healing technology. *Polymers* 15, 1205. doi:10.3390/polym15051205

Seliktar, D. (2012). Designing cell-compatible hydrogels for biomedical applications. *Science* 336, 1124–1128. doi:10.1126/science.1214804

Seol, D., Magnetta, M. J., Ramakrishnan, P. S., Kurriger, G. L., Choe, H., Jang, K., et al. (2013). Biocompatibility and preclinical feasibility tests of a temperature-sensitive hydrogel for the purpose of surgical wound pain control and cartilage repair. *J. Biomed. Mater. Res. B Appl. Biomater.* 101, 1508–1515. doi:10.1002/jbm.b.32981

Sipp, D., Robey, P. G., and Turner, L. (2018). Clear up this stem-cell mess. *Nature* 561, 455–457. doi:10.1038/d41586-018-06756-9

Soltanmohammadi, F., Mahmoudi Gharehbaba, A., Alizadeh, E., and Javadzadeh, Y. (2025). Innovative approaches to tissue engineering: utilizing decellularized extracellular matrix hydrogels for mesenchymal stem cell transport. *Int. J. Biol. Macromol.* 290, 138893. doi:10.1016/j.ijbiomac.2024.138893

Stocco, E., Barbon, S., Dalzoppo, D., Lora, S., Sartore, L., Folin, M., et al. (2014). Tailored PVA/ECM scaffolds for cartilage regeneration. *Biomed. Res. Int.* 2014, 762189. doi:10.1155/2014/762189

Stocco, E., Barbon, S., Radossi, P., Rajendran, S., Dalzoppo, D., Bortolami, M., et al. (2016). Autologous chondrocytes as a novel source for neo-chondrogenesis in haemophiliacs. *Cell Tissue Res.* 366, 51–61. doi:10.1007/s00441-016-2408-8

Stocco, E., Barbon, S., Piccione, M., Belluzzi, E., Petrelli, L., Pozzuoli, A., et al. (2019a). Infrapatellar fat pad stem cells responsiveness to microenvironment in osteoarthritis: from morphology to function. *Front. Cell Dev. Biol.* 7, 323. doi:10.3389/fcell.2019.00323

Stocco, E., Barbon, S., Macchi, V., Porzionato, A., Dalzoppo, D., De Caro, R., et al. (2019b). New bioresorbable wraps based on oxidized polyvinyl alcohol and leukocyte-fibrin-platelet membrane to support peripheral nerve neurorrhaphy: preclinical comparison *versus* NeuraWrap. *Sci. Rep.* 9, 17193. doi:10.1038/s41598-019-53812-z

Stocco, E., Barbon, S., Lamanna, A., De Rose, E., Zamuner, A., Sandrin, D., et al. (2021). Bioactivated oxidized polyvinyl alcohol towards next-generation nerve conduits development. *Polymers* 13, 3372. doi:10.3390/polym13193372

Stocco, E., Barbon, S., Zeni, E., Cassari, L., Zamuner, A., Gloria, A., et al. (2022). Development of two-layer hybrid scaffolds based on oxidized polyvinyl alcohol and bioactivated chitosan sponges for tissue engineering purposes. *Int. J. Mol. Sci.* 23, 12059. doi:10.3390/ijms232012059

Stocco, E., Barbon, S., Ceroni, L., Confalonieri, M., Pulzato, G., Pressi, S., et al. (2024). Partially oxidized polyvinyl alcohol + functionalized water soluble multiwalled carbon nanotubes: a new conductive nanocomposite material with promising implications for neuroregeneration. *J. Sci. Adv. Mater. Devices* 9, 100762. doi:10.1016/j.jsamd.2024.100762

Sun, K., Lin, H., Tang, Y., Xiang, S., Xue, J., Yin, W., et al. (2020). Injectable BMP-2 gene-activated scaffold for the repair of cranial bone defect in mice. *Stem Cells Transl. Med.* 9 (12), 1631–1642. doi:10.1002/sctm.19-0315

Sun, X., Zhang, H., Huang, S., Li, K., and Wang, X. (2025). Dual sustained-release BMP7-nanoparticle hydrogel scaffolds for enhanced BMSC neuronal differentiation and spinal cord injury repair. *Spine* 50, 575–585. doi:10.1097/BRS.0000000000005307

Takayama, Y., Kusamori, K., Katsurada, Y., Obana, S., Itakura, S., and Nishikawa, M. (2023). Efficient delivery of mesenchymal stem/stromal cells to injured liver by surface PEGylation. *Stem Cell Res. Ther.* 14, 216. doi:10.1186/s13287-023-03446-w

Tavajjohi, Z., Sigaroodi, F., Rabbani, S., Barekat, M., Rouhani, M., Boroumand, S., et al. (2025). Therapeutic performance of hydrogel-derived extracellular Wharton's jelly matrix and Wharton's jelly mesenchymal stem cells in repairing infarcted myocardium of ischemic rats: a preclinical study. *Macromol. Biosci.* e70007, e70007. doi:10.1002/mabi.202400578

Todros, S., Barbon, S., Stocco, E., Favaron, M., Macchi, V., De Caro, R., et al. (2022a). Time-dependent mechanical behavior of partially oxidized polyvinyl alcohol hydrogels for tissue engineering. *J. Mech. Behav. Biomed. Mater.* 125, 104966. doi:10.1016/j.jmbbm.2021.104966

Todros, S., Spadoni, S., Barbon, S., Stocco, E., Confalonieri, M., Porzionato, A., et al. (2022b). Compressive mechanical behavior of partially oxidized polyvinyl alcohol hydrogels for cartilage tissue repair. *Bioengineering* 9, 789. doi:10.3390/bioengineering9120789

Vivas, D., Grau-Vorster, M., Oliver-Vila, I., García-López, J., and Vives, J. (2020). Evaluation of a cell-based osteogenic formulation compliant with good manufacturing practice for use in tissue engineering. *Mol. Biol. Rep.* 47, 5145–5154. doi:10.1007/s11033-020-05588-z

Voronin, A. V. (2025). Allogeneic ADSCs and platelet-poor plasma fibrin hydrogel to treat the patients with burn wounds. *ClinicalTrials.gov*, identifier NCT03113747. Available online at: https://clinicaltrials.gov/study/NCT03113747 (Accessed on June 29, 2025).

Wang, W., Li, B., Li, Y., Jiang, Y., Ouyang, H., and Gao, C. (2010). *In vivo* restoration of full-thickness cartilage defects by poly(lactide-co-glycolide) sponges filled with fibrin gel, bone marrow mesenchymal stem cells and DNA complexes. *Biomaterials* 31 (23), 5953–5965. doi:10.1016/j.biomaterials.2010.04.029

Wei, W., Dai, H., Zhang, Y., Li, Z., Guo, R., Huang, B., et al. (2023). Hydrogel-based delivery of MSCs for osteoarthritis treatment. *Nat. Rev. Rheumatol.* 19, 112–126. doi:10.1038/s41584-022-00831-8

Wu, Z., Chen, G., Zhang, J., Hua, Y., Li, J., Liu, B., et al. (2017). Treatment of myocardial infarction with gene-modified mesenchymal stem cells in a small molecular hydrogel. *Sci. Rep.* 7, 15826. doi:10.1038/s41598-017-15870-z

Xiao, L., Sun, Y., Liao, L., and Su, X. (2023). Response of mesenchymal stem cells to surface topography of scaffolds and the underlying mechanisms. *J. Mater. Chem. B* 11, 2550–2567. doi:10.1039/d2tb01875f

Xie, M., Tao, H., Zhu, H., Mei, Y., Chen, L., Wu, A., et al. (2020). Bionic detectors based on low-bandgap inorganic perovskite for selective NIR-I photon detection and imaging. *Adv. Mater.* 32, 1905362. doi:10.1002/adma.201905362

Xue, Z., Hu, D., Tang, H., Xue, M., Zhu, Y., Li, Y., et al. (2024). Mechanical force regulates the paracrine functions of ADSCs to assist skin expansion in rats. *Stem Cell Res. Ther.* 15, 250. doi:10.1186/s13287-024-03822-0

Yang, M., Zhang, Z. C., Yuan, F. Z., Deng, R. H., Yan, X., Mao, F. B., et al. (2022). An immunomodulatory polypeptide hydrogel for osteochondral defect repair. *Bioact. Mater.* 19, 678–689. doi:10.1016/j.bioactmat.2022.05.008

Zeng, X., Tang, Y., Hu, K., Wang, L., Li, S., Liu, J., et al. (2017). Three-week topical treatment with placenta-derived mesenchymal stem cells hydrogel in a patient with diabetic foot ulcer: a case report. *Med. Baltim.* 96, e9212. doi:10.1097/MD.0000000000009212

Zhang, C., Gullbrand, S. E., Schaer, T. P., Boorman, S., Elliott, D. M., Chen, W., et al. (2021). Combined hydrogel and mesenchymal stem cell therapy for moderate-severity disc degeneration in goats. *Tissue Eng. Part A* 27, 117–128. doi:10.1089/ten.TEA.2020.0103

Zhao, Y., Wang, M., Liang, F., and Li, J. (2021). Recent strategies for enhancing the therapeutic efficacy of stem cells in wound healing. *Stem Cell Res. Ther.* 12, 588. doi:10.1186/s13287-021-02657-3

Zhou, J., Xiong, S., Liu, M., Yang, H., Wei, P., Yi, F., et al. (2023). Study on the influence of scaffold morphology and structure on osteogenic performance. *Front. Bioeng. Biotechnol.* 11, 1127162. doi:10.3389/fbioe.2023.1127162

Zhu, Y., Wang, Y., Jia, Y., Yuan, H., Chen, H., Sun, L., et al. (2022). Selenium-containing nanoparticles synergistically enhance Pemetrexed&NK cell-based chemoimmunotherapy. *Biomaterials* 280, 121321. doi:10.1016/j.biomaterials.2021.121321

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Modulation of ASC-derived extracellular vesicles containing cargo that specifically enhances wound healing

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Introduction: We have developed a bioreactor-based production system for manufacturing Human Adipose Stromal Cell (ASC) extracellular vesicles (EVs), which includes exosomes, using a highly controlled and tunable environment that can modify the cargo of these nanovesicles. The patented innovation focuses on engineering novel pro-healing EVs with therapeutic activity and using a topical formulation to treat diabetic ulcers.

Methods: To evaluate biological activity of tuned ASC EVs, functional activity assays were performed using human primary dermal fibroblast and keratinocyte culture models. Molecular and biochemical assays were used to assess cytokine regulation, collagen production and cell migration. Rodent wound healing models were used to assess therapeutic potential of modified exosomes. A Human volunteer case study was carried out with a consenting individual suffering from chronic diabetic ulcers.

Results: Herein we demonstrate that our proprietary engineered ASC EVs, eXo^3 exosomes, contain a unique activity profile that reduces inflammatory cytokines, stimulates collagen production, as well as activates keratinocyte and fibroblast proliferation and migration. When formulated with an emollient and topically applied to an *in vivo* excisional wound model, tuned eXo^3 exosomes demonstrated enhanced wound closure, increased keratinization, collagen deposition, and overall improved recover rate. In a clinical case study addressing non-healing diabetic foot ulcers, conducted under informed consent, topical treatment with tuned eXo^3 exosomes formulated in a proprietary gel serum showed complete wound closure and dermal regeneration.

Conclusion: Our current research efforts have developed an EV manufacturing system that can be directed to improve the healing capacity of ASC-derived EVs. We show enhanced wound healing and repair activity *in vitro* and *in vivo*. Our data supports the regenerative properties of exosomes and reinforces their strong therapeutic potential.

KEYWORDS

exosomes, regenerative medicine, wound healing, adipose-derived stromal/stem cells, diabetic ulcer, extracellular vesicles, therapeutic vehicle, mesenchymal stem cells

1 Introduction

Intercellular and extracellular communication is essential for maintaining homeostasis in multicellular organisms, especially in response to tissue injury. One key mode of communication involves extracellular vesicles (EVs) that serve as vehicles to facilitate the exchange of bioactive cargo, including proteins, RNAs, and lipids, that influence target cell behavior (Zhang et al., 2019). Current research has shown that EVs are secreted by all cells and provide paracrine communication by employing different targeting mechanisms as well as carry different cargos to effector cells (Gurunathan et al., 2021; Zou et al., 2023). The underlying processes involved in regulating secretion and packaging of vesicular content are not well understood, but harnessing these bioactive systems provides an attractive modality for enhancing the regenerative capacity of innate biological material. Many studies have demonstrated that EVs are differentially produced and secreted according to the cellular environment (Kowal et al., 2014; Pote and Gacche, 2025; Skotland et al., 2019). Our studies have built upon this premise by developing a bioreactor-based production system for manufacturing Human Adipose Stromal Cell (ASC) exosomes using a highly controlled and tunable environment that can modify EV production and cargo that reduces inflammatory cytokines, stimulates collagen production, as well as activates keratinocyte and fibroblast proliferation and migration (Zhang et al., 2015; Zhang et al., 2020). When formulated with an emollient and topically applied to an *in vivo* excisional wound model, tuned eXo^3 exosomes demonstrated enhanced wound closure, increased keratinization, collagen deposition, and overall improved recover rate. In a clinical case study addressing non-healing diabetic foot ulcers, conducted under informed consent, topical treatment with engineered eXo^3 exosomes formulated in a proprietary gel serum showed complete wound closure and dermal regeneration.

2 Materials and methods

2.1 ASC culturing and exosome purification

Research has shown that both exosome cargos and stem cell paracrine activity are dramatically altered by the environment, particularly with regard to the inflammatory and healing state of tissue (Shabbir et al., 2015; Shi et al., 2021; Rani and Ritter, 2016). Critical *ex vivo* environmental cues can tune stem cell-derived EVs to be pro-healing. Leveraging our expertise in stem cell biology, we have developed a proprietary bioreactor-based production system for manufacturing "tuned" EVs from adult stem cells. Modified EVs are termed "eXo^3" throughout the manuscript and described in Section 2.9. By manipulating the cellular bioreactor environment, we have found that we can alter stem cell EV production and secretion as well as exosomal cargo packaging. Additionally, and most importantly, we have shown this manipulation of the bioreactor environment can improve the pro-healing capacity of the stem cell-derived EVs. Isolation and culturing of the Human Mesenchymal Stem Cells (MSC) subtype–adipose-derived stem cells (ASC), and exosome isolation and physical characterization, have been previously reported (Ludlow and Buehrer, 2024). The results within this report characterize enhanced features of eXo^3 EVs by comparing to "native" EVs which were prepared in tandom from the same lot of ASCs cultured in an identical bioreactor system under traditional culturing conditions with no modification steps.

It is important to maintain a controlled environment while manufacturing EVs that retain wound healing properties. An aging microenvironment will promote cellular senescence that can shift EV production containing pro-inflammatory characteristics that negatively affect tissue repair and exacerbate cellular pathology (Pirmoradi et al., 2018; Smirnova et al., 2023; Liu et al., 2017). The secretome of MSCs with senescence-associated secretory phenotype (SASP) generates a proinflammatory microenvironment affecting surrounding cells (Siraj et al., 2023). ADSCs are more resistance to senescence shifts and comparative studies show long-term cultures have higher proliferation rates and decreased oxidative stress (Wagner et al., 2005; Wagner et al., 2008; Gruber et al., 2012). Additionally, hollow-fiber bioreactor systems retain differentiation capacity, surface phenotype, genetic stability, and show no evidence of increased aging after large-scale culture (Vymetalova et al., 2020). Our commercial-scale ADSC bioreactor process monitors glucose consumption, cell proliferation, and cell cycle to ensure that the cells are not senescent, thus safeguarding against SASP vesicle formation which may compromise the efficacy of the product.

Briefly, EVs are isolated from adipose-derived stem cells (ASCs) using a hollow-fiber cartridge containing bioreactor (Ludlow and Bueher, 2022). The ASCs used are isolated from human adipose tissue and expanded in culture using established protocols (Buehrer and Cheatham, 2013). To prepare the bioreactor, ASCs from multiple donors with similar demographics were seeded onto the cartridge. After seeding, the separate cell cartridge was filled with serum-free medium for ASC maintenance and EV production, and growth medium was continually circulated through the bioreactor with medium exchanges every 3–5 days. Bioreactor cultures can be maintained in this manner for up to 3 months while the ASCs retain their ISCT-mandated MSC characteristics.

EVs were collected every 3–5 days after the bioreactor culture was established. The conditioned serum-free medium was removed from the cell cartridge and subjected to two centrifugation steps as follows: The supernatant was aspirated, and the pelleted EVs containing exosomes were suspended in DPBS and stored at −80 °C for further analysis.

2.2 EV characterization methods

Particle size and concentration was determined using ZetaView NTA (Particle Metrix) instrumentation. Concentrated EV preps were diluted in particle free water to a range of 5 × 107 particles per milliliter and analyzed using defined acquisition settings. Size distribution was determined and mean, median, and mode diameters were used to characterize EVs.

Transmission electron microscopy was performed as follows: Size 300 mesh copper EM grids were glow-discharge formvar/carbon film coated for 45 s. Once completed, 5 µL of EV suspension (previously fixed with 30 µL of 2% PFA) was added to the grid and incubate for 1 min. Following removal of the excess EV solution, one drop of filtered 1% uranyl acetate was added and the excess removed before rinsing the grid on a drop of water 3 times. The grids were allowed to dry for 10 min at room

temperature followed by imaging on Tecnai 12 electron microscope at 120 kV.

EV surface markers were analyzed using a Miltenyi MACSQuant Analyzer 10 for sample acquisition and MACSQuantify Software for data analysis. The MACSPlex Exosome Kit (Miltenyi Biotec) was used to evaluate each sample for the expression of 37 surface markers according to manufacturer's protocol. Protein concentration was determined for each sample using BCA protein quantification kit (Thermo). 20 μg of prepared exosomes was gently rotated at 4 °C overnight with capture beads. The beads were washed and then incubated for 1 h with detection bead mixture consisting of CD9, CD63 and CD81 exosome markers. The flow cytometer was calibrated and background settings were adjusted to unlabeled beads and gating strategies were used to identify bead populations for each analyte. Batch analysis quantified median intensities for each bead population and analyte surface expression was calculated for each sample.

2.3 *In vitro* inflammatory cytokine assay

To measure EV effects on inflammation, *in vitro* gene expression for the inflammatory cytokines IL-6, IL-8 and MCP-1 was performed via RT-qPCR. Primary human fibroblasts (PHFs) were seeded at a density of 390,000 cells per well in growth medium (DMEM-LG, 10% FBS and antibiotics) using 6-well plates. The cells were incubated overnight to allow the PHFs to attach. To induce inflammatory cytokine production, growth medium was removed and replaced with Test Medium (DMEM-HG, ascorbic acid and antibiotics) containing 1 × 107/mL heat killed P. gingivalis (HKPG, InvivoGen) with and without 6.7 μg/mL ASC EVs. After overnight incubation, the cells were washed and RNA extracted using RNeasy Mini Kit (Qiagen). cDNA was prepared using 1 μg of purified RNA in a 20 μL reaction and High-Capacity cDNA Reverse Transcription Kit (Life Technologies) and qPCR performed with 10 ng cDNA per reaction using iTaq Universal Probes Master Mix (BioRad) and a QuantStudio 12K Flex machine (Life Technologies).

2.4 *In vitro* migration assay

Radius™ Cell Migration Assay plates were used for this assay. Briefly, plates were prepared and human primary skin epithelial cells were seeded (100,000 cells/well) into a 24-well Radius plate in 10% Serum containing Media (1 mL) as per manufacturer's instructions. Following a 4h incubation at 37 °C in a 5% CO_2 –containing atmosphere to allow cells to attach, wells were washed and the hydrogel dissolved from the wells. The wells were washed again, and either Serum Free, 1% Serum containing media, 10% serum containing media, or 1% serum containing media supplemented with 50 μL of indicated number of EVs. Photographs of each well were taken at T = 48 following addition of EVs.

2.5 *In vitro* cell proliferation assay

EVs were also added to low density *in vitro* PHF and primary human keratinocytes (PHKs) cultures (3,000 cells/well) in 96-well culture plates using serum-free medium and incubated for 3 days. To compare the proliferative effects of EVs, cells were treated with other inducers, including 10% FBS, PDGF-BB, KGF, TGF-β1, or IGF-1. After 3 days, the cells were treated with Cell Titer Blue reagent (Promega) for 2 h to assess proliferation by fluorescence signal (excitation: 560 nm, emission: 590 nm).

2.6 *In vitro* collagen production assay

Dermal fibroblasts were seeded at 10,000 cells per well and allowed to attach overnight. The next day cells were treated with EV and controls in assay medium +/− ascorbic acid. Cells were treated for 48 h in replicates of 3. After 48 h, conditioned media was collected and frozen at −80 °C until ready to assay. Procollagen-1 levels were determined by ELISA (Takara, cat# MK101) following the manufacturer's instructions.

2.7 Excisional wound model

All animal husbandry is done in accordance to The Guide for the Care and Use of Laboratory Animals published by the Institute of Laboratory Animal Resources Commission on Life Science National Research Council, NATIONAL ACADEMY PRESS, Eighth Edition, and applicable USDA regulations without exception. Animals were housed on site (Synchrony Laboratories, Durham, NC, Study # 169-03-17) for a minimum of 5 days prior to study assignment. Animals were handled by staff daily to acclimate to human contact and reduce stress. Appropriate SOPs and protocols were followed for housing conditions, health status review, diet, pre-op instructions, and post-operative care. Marking and identification of animals was achieved by two non-invasive methods. 1. A black sharpie marker was used to inscribe a number on the fur. 2. Each animal was housed individually with identification number affixed to the cage. Both circular and linear skin injury models were developed and investigated. For circular wounds, 4x 2 cm diameter full thickness (entire skin layer) wounds were cut on the back of each animal. Sterile saline (phosphate buffered, PBS) was added to each wound and the injury covered with a transparent sterile, gas-permeable bandage or surgical tape. Each wound was measured and photographed daily for up to 30 days post-surgery. For linear wounds, 4x 2 cm linear full thickness cuts were made in the back skin. Sterile saline (phosphate buffered, PBS) was added to each wound and the injury covered with a transparent sterile, gas-permeable bandage or surgical tape. Each wound was measured and photographed daily for up to 30 days post-surgery. Baseline wound healing rates were established using measurements derived from these preliminary studies. Circular or linear wound healing models were generated on back skin of rodents as described above. Of the 4 wounds per rat, one was treated with PBS as a vehicle control. The other 3 were treated with an exosome preparation derived from adipocyte conditioned media. The treatments were randomized across subjects to avoid positional bias and assessment was blinded to avoid observer bias. Matched conditions on the same animal improved statistical power by allowing paired analysis. Up to 4 experimental groups (with different exosome preparations) were tested, 6 rodents per group. All rodents were evaluated for wound

healing response as described above. At the end of the study period, rodents were euthanized and the wound site dissected out for evaluation by molecular and histological techniques.

2.8 Topical formulation (test article)

eXo^3 exosomes are registered under International Nomenclature of Cosmetic Ingredients (INCI) name: Human Adipose Stromal Cell Exosome. eXo^3 exosomes personal care formulations are registered in compliance with FDA under the Modernization of Cosmetics Regulation Act of 2022. The product described is not a drug product and was initially used solely to improve the condition and appearance of the skin. For all cell-based assays and animal studies, eXo^3 exosomes were diluted in PBS and PBS alone was used as a vehicle control. For the patient case study, eXo^3 Exosomes were blended with a base formulation consisting of 1.0% hyaluronic acid-based gel formulation developed by Exotropin, LLC and used as an Over The Counter (OTC) personal care product.

2.9 Diabetic wound healing in patient volunteer- a single case study

The presented case involves a 55-year-old male patient with a 30-year diagnosis of diabetes mellitus type 2 with stable A1C and other investigations within normal limits, presenting with multiple Grade 1 non-healing ulcers over the foot that had been previously treated for over 6 months by a foot surgeon. The surgeon had not been successful in healing these wounds using conventional therapy including debridement, off-loading with a soft cast, infection management and weekly dressing changes. Over the prior 6-month treatment period to promote healing, various dressing types were tried including alginate, silver, hydrocolloid and honey impregnated dressings. Despite active treatment, 3 large ulcers remained on the foot and one on the toe. Due to the patient's history of chronic non-healing foot ulcers, an alternate solution was sought to accelerate healing.

Patient volunteer was treated once weekly with topical application of eXo^3 exosome test article. Ulcers were thoroughly cleaned but not debrided. Exosome gel was applied topically directly to the wound bed, sufficiently to cover ulcers followed by Tegaderm to cover the gel as well as a secondary dressing to cushion and protect the treatment areas. The wounds were monitored regularly for signs of infection or complications but treatment and dressing were only changed and reapplied weekly. Foot wounds were photographed by care provider and ImageJ was used to detect open wound and quantify pixel area.

2.10 Statistical analysis

Quantitative data were expressed as mean ± standard deviation (SD) based on at least three independent assays in duplicates or triplicates. Students unpaired t-tests were performed using GraphPad Prism (GraphPad Company) for all *in vitro* assays. ANOVA was used to determine significance in the described animal wound healing study as well as in the human case study. Graphs are presented mean ± SD or mean ± s.e.m. All p-values below 0.05 (*), 0.01 (**), and 0.001 (***) were considered statistically significant.

3 Results

3.1 Extracellular vesicles characterization

The manufacturing process using ASC-derived EVs was adapted from previously published protocols (Basu and Ludlow, 2016). To ensure lot consistency, MISEV guidelines for EV characterization were followed using multiple methods for identification (Zhang et al., 2024; Welsh et al., 2024). Each lot was screened using flow cytometry to identify the presence of exosomes surface markers (Figure 1A). Purified particles were stained with antibodies targeting tetraspanin proteins CD9, CD63, and CD81. Positive identification was confirmed while negative expression of lymphocytic markers CD3, CD4, and CD19 illustrated preparation purity (Figure 1B). Particle size was determined using ZetaView NTA analysis showing a distribution range from 65 to 200 nm, consistent with published exosome biology (Gurunathan et al., 2021; Zhang et al., 2020). TEM was used to validate vesicle size and morphology, confirming isolation and purification of EVs (Figure 1C).

3.2 eXo^3 exosomes stimulate collagen production and reduce inflammation

Chronic wound tissue is associated with degradation of the extracellular matrix and collagen fiber degeneration. The first stages of wound healing require collagen deposition to support extra cellular matrix formation which facilitates cell migration and tissue regeneration. We evaluated whether ASC-derived EVs could modulate collagen production using primary human fibroblasts (PHFs). EVs isolated from different controlled bioreactor microenvironments were applied to PHF cultures to stimulate collagen production. Serum-free conditioned medium was used for baseline comparison and TGFβ-1 was used as positive control. Native ASC EVs showed a dose dependent stimulation of collagen that did not reach levels of TGFβ-1 stimulation (Figure 2A). Interstingly, "tuned exosomes", eXo^3 exosome treatment was significantly higher than native EVs and matched a response similar to TGFβ-1. Figure 2B revealed reduced inflammatory cytokine production in response to exosome treatment.

3.3 eXo^3 exosomes stimulate cell migration

When cells are seeded in the well of the Radius plate, they will adhere everywhere except in the center of the well where there is a biocompatible hydrogel spot of a defined and constant area. Once cells form a monolayer, the assay is initiated by gently dissolving the gel with a removal solution, leaving a gap across which cell migration can occur. As shown in Figure 3, at T = 48 h, serum free media

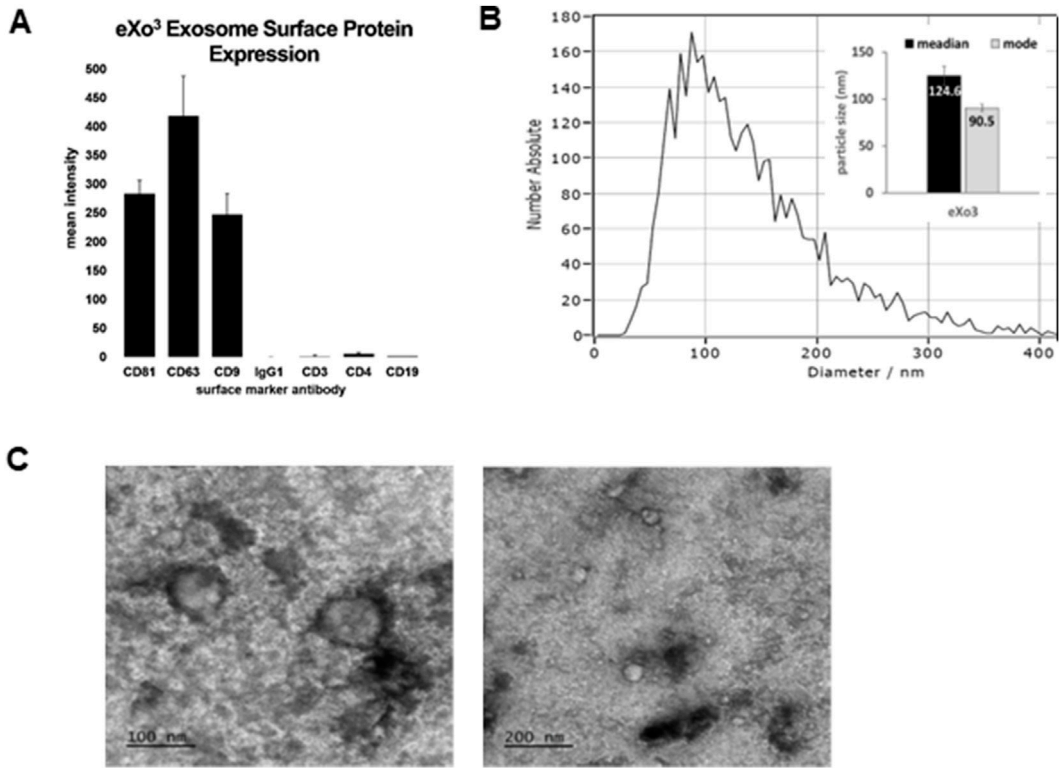


FIGURE 1
Manufactured EVs isolated from cultured ASCs were characterized using MISEV guidelines for EV classification. **(A)** Shows the expression of classical EV surface markers CD9, CD63, and CD81 detected in isolated eXo^3 EVs. **(B)** Shows EV size distribution using ZetaView NTA. **(C)** Shows TEM images depicting bi-membrane vesicles sized at 80–140 nm in diameter.

(negative control) shows minimal migration. As expected, 1% FBS media shows slightly more migration while10% FBS media shows full closure of the gap. Addition of 25 × 10E6 EVs to the 1% FBS media reveals slightly better gap closure compared to the 1% FBS media alone, while 10 × 10E7 EVs elicits complete gap closure when added to the 1% FBS media.

3.4 eXo^3 exosomes improve recovery in In vitro rodent wound healing study

We used an excisional wound model in which 2 cm diameter wounds were created in the dorsal skin of Sprague-Dawley rats (Figure 4A) and were treated topically with a single dose of vehicle (300 µL) or HS-tuned ASC EVs (25 × 106 particles). The wounds were measured every 2–3 days to monitor the healing process. As shown in Figure 4B, the wound closure rate was increased by HS-tuned EV treatment (green) after 14 days compared to the vehicle control (red). Figure 4B demonstrates that HS-tuned EVs subjected to lyophilization (green) and room temperature storage for 7 days prior to reconstitution maintain their capacity to improve wound healing *in vivo*. Figure 4C is a graphic representation of the closure rates over time. Note that lyophilized EVs, when reconstituted with sterile water, resulted in a wound closure rate comparable to that of freshly-isolated EVs. The wound areas were excised after 19 days and analyzed by immunohistochemistry. EV-treated wound tissue demonstrated darker staining for Ki-67 (Figure 4D) than vehicle, suggesting that there is more proliferation in EV-treated wounds.

3.5 Human volunteer single case study: topical application of eXo^3 exosomes promotes the appearance of ulcer healing

Given that eXo^3 significantly enhanced wound healing in the rodent model (Figure 4), we formulated the active ingredient for human skin application. The topical eXo^3 exosomes formulated in a hyaluronic acid gel was initially used solely to improve the condition and appearance of the skin. However, when applied to subjects suffering from diabetic ulcers which were previously refractive to currently accepted and practiced methods of treatment, we saw dramatic effects. Figure 5 documents a human volunteer single case study where eXo^3 exosome gel was applied topically directly to the wound bed. Figure 5A shows baseline wound state. The four ulcers were sufficiently covered with eXo^3 gel followed by Tegaderm to cover the gel as well as a secondary dressing to cushion and protect the treatment areas. The wounds were monitored regularly for signs of infection or complications but treatment and dressing were only changed and reapplied weekly. Wound healing was documented after each dressing change. After 4 weeks of treatment, three lesions were completely healed, while the largest lesion showed >75%

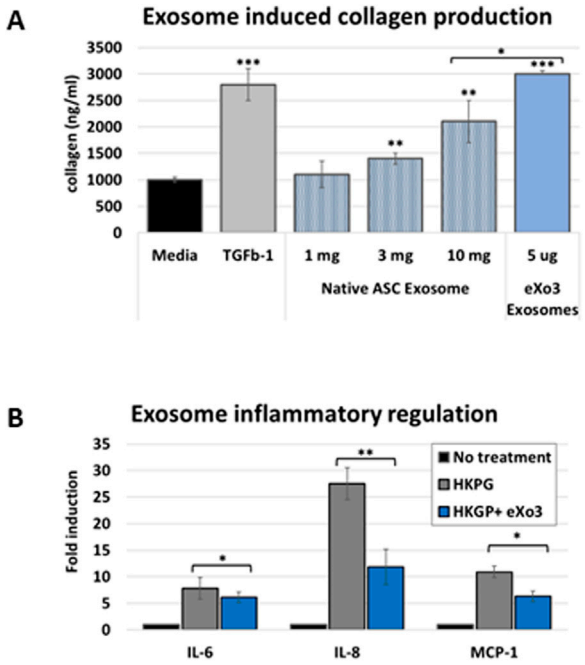


FIGURE 2
(A) Human dermal fibroblasts are treated with native or modified ASC-derived EVs. Media was harvested and analyzed for collagen accumulation. eXo^3 EVs show significant stimulation of collagen production that is comparable to TGFb-1 treatment. **(B)** Dermal fibroblasts are stimulated with HKGB to activate an immune response. Co-incubated eXo^3 EV samples show a restrains cytokine release detected by ELISA targeting IL-6, IL-8, and MCP-1. p-values below 0.05 (*), 0.01 (**), and 0.001 (***).

recovery (Figure 5B). Treatment was continued for 3 more weeks and resulted in nearly 100% recovery (Figures 5C,D). Quantitative image analysis of identified lesions in images identified open wound area and calculated the lesion size. Each wound site was normalized to baseline area at time 0 and the percent closure was calculated through out the term of collection These reported findings were unexpected; however, they support the ability to "tune" pro-healing EVs that provide therapeutic benefit beyond traditional pharmacological treatments. While encouraging in this single human volunteer case study, we recognize that further investigation is warranted to strengthen these anecdotal results.

4 Discussion

Stem cell therapies represent a compelling means of tissue repair and have demonstrated wound healing and soft tissue regeneration in animal models. Stem cell therapeutic approaches have the advantage of being capable of potentially regenerating an assortment of tissues by virtue of their pluripotency. Such versatility is a much-needed option for various medical conditions. The downsides of stem cell therapy include their complexity in isolation, characterization, and patient delivery. The greatest concern continues to be the potential for uncontrolled cell growth, proliferation, and tumorigenesis. The use of embryonic stem cells for therapeutic applications also raises ethical concerns and continues to be a passionately debated topic. Additionally, the intuitive concept that therapeutic stem cells engraft and differentiate at sites of tissue damage is not well supported given the low numbers of cells retained over time at application sites *in vivo* (Lotfy et al., 2023). This suggests that their mechanisms of action occur through paracrine modalities such as secretion of bioactive vesicles, including exosomes. Hence, exploiting stem cell-derived exosomes as a biologic-derived therapy, rather than delivering transient stem cells to treat chronic wounds, is an enticing approach.

Secreted extracellular vesicles (EVs), such as exosomes, are packed with potent pro-repair proteins and RNA cargos that are both cell type-specific, as well as, differentially produced and secreted according to the cellular environment (Zhang et al., 2019; Kim et al., 2017; Qiu et al., 2020). Exosomes offer a novel approach to tissue engineering and regenerative medicine therapies having several advantages over the use of cells. Since exosomes are cell-free, there is a much lower risk of immune rejection allowing for their allogenic use in the clinic. Exosome regenerative and wound healing capabilities are also heightened by having an elevated concentration of growth factors compared to cells and manufacturing large-scale, GMP-grade product is accomplished at sustainably lower costs. While encouraging, it needs to be recognized that exosome therapy is still early in development,

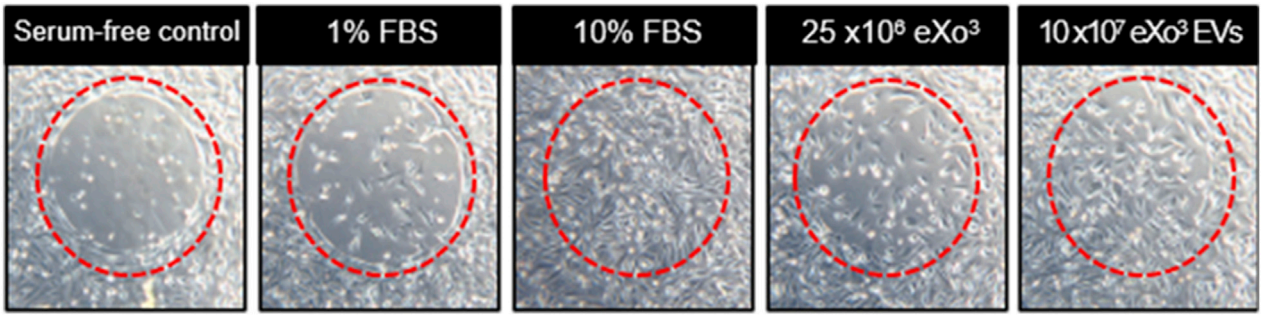


FIGURE 3
From left to right, serum free media serves as the negative control. 1% FBS media is the vehicle control. 10% FBS media is the positive control. Vehicle supplemented with EVs are shown in the last 2 panels.

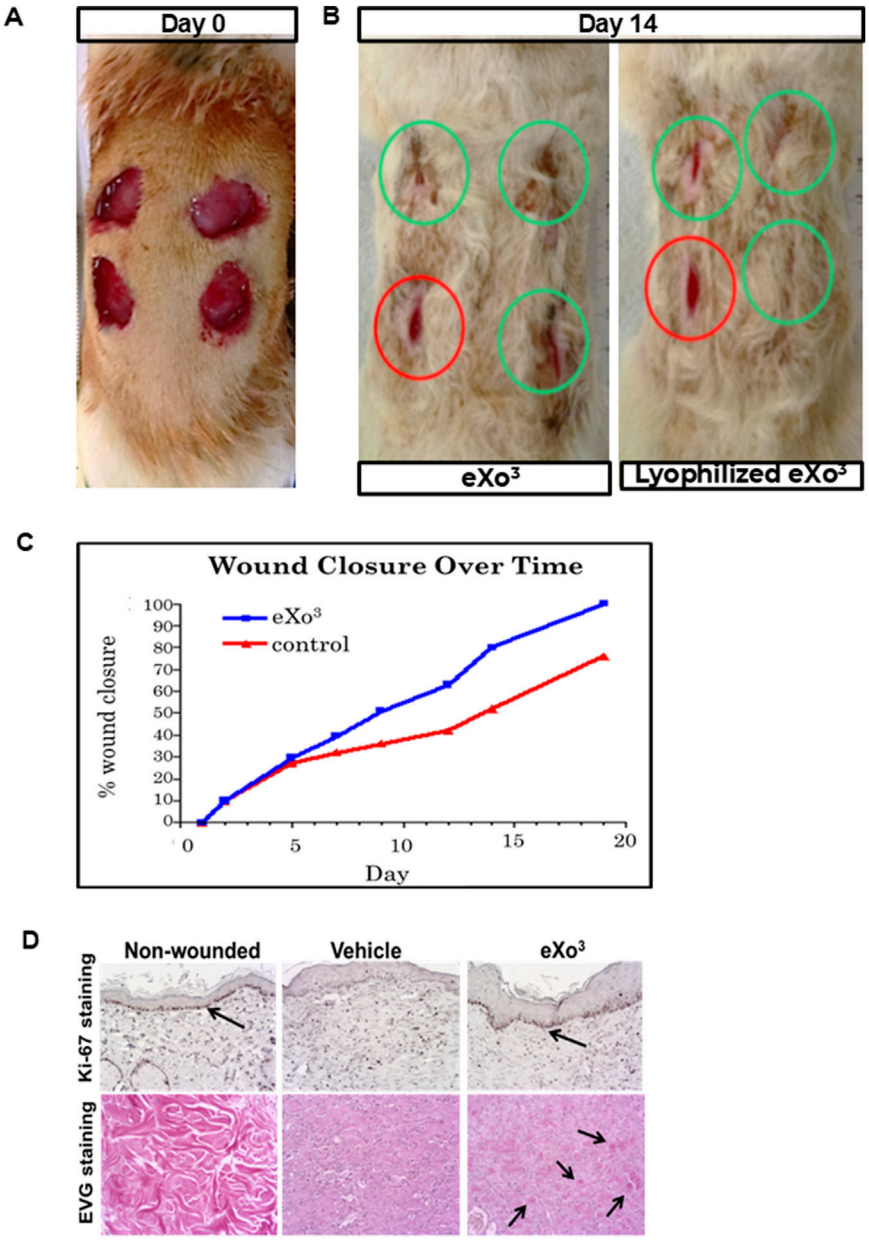


FIGURE 4
Rat wound healing model. **(A)** Four excisional wounds shown on animal at Day 0; **(B)** Same animal at Day 14 of treatement with either vehicle, eXo^3 exosomes, or lyophilized eXo^3 exosomes. Red circles-Vehicle; Green circles-eXo^3 treatment. **(C)** Wound diameter was measured daily and percent closure data is graphed over 20 days. eXo^3 treatment shows enhanced wound closure. ANOVA $p < 0.05$. **(D)** Histological sections of non-wounded, vehicle-treated, and eXo^3-treated tissue, stained with Ki-67 and EVG show increased Ki-67 and collagen staining in eXo^3-treated wounds. Differences in cellular activity and tissue structure are shown with arrows.

and regulatory considerations such as purity, batch-to-batch consistency, and potency need to be addressed before widespread adoption for clinical use. The data presented here supports the idea that manipulation of the cellular bioreactor environment improves the repair activity of stem cell-derived EVs *in vitro* and *in vivo*. Current investigation into the cell-signaling elicited by these EVs will facilitate further understanding of the mechanisms involved in wound healing and tissue repair. Towards this goal, we have partially analysed miRNA cargo contained in eXo^3 and found several sequences implicated in wound healing. Additionally, current proteomic research has identified cell cycle regulatory proteins and stress response proteins such as HSP70 that may contribute to the observed pro-healing function of eXo^3. Once our analysis is complete, we will report on the miRNA and protein cargos we believe to be important for this healing effect and potentially lead to additional tissue engineering and regenerative medicine applications.

The singe human volunteer case study reported herein shows promising therapeutic benefit and wound healing potential of eXo^3 application. Diabetic foot ulcers remain a therapeutic area of high

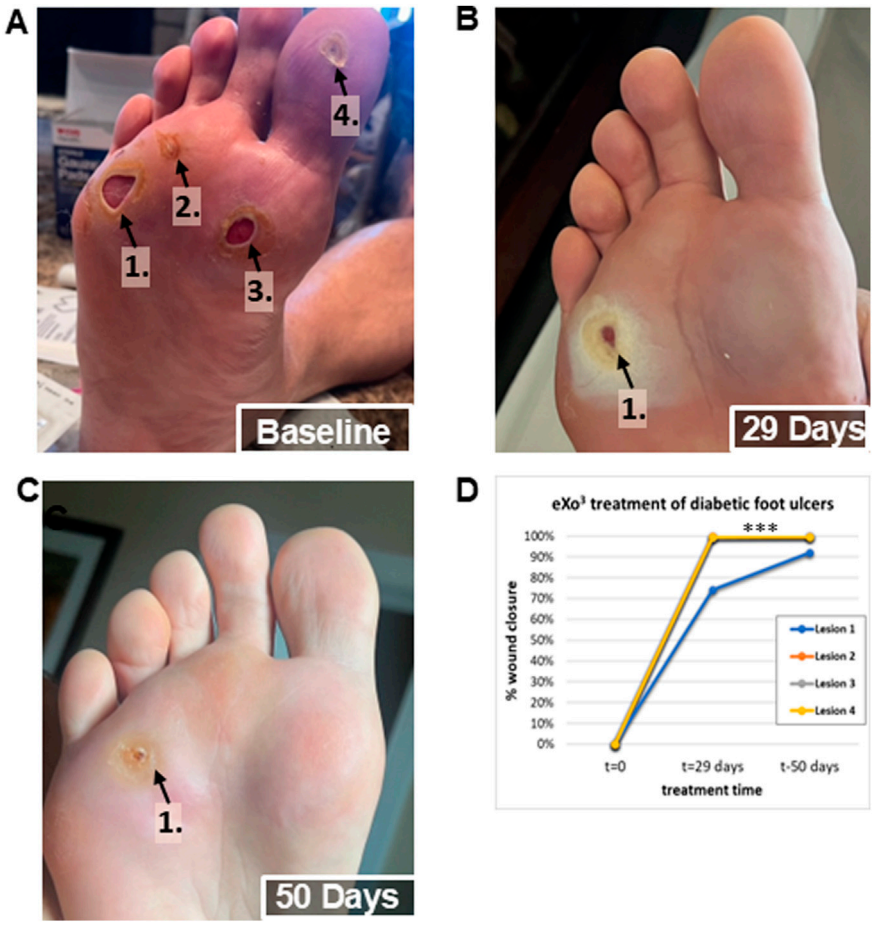


FIGURE 5
Human volunteer single case study observations after topical application of eXo^3 exosomes to chronic diabetic ulcers. **(A)** Baseline: Non-healing diabetic ulcers after 6 months of treatment attempts with other therapeutic agents. **(B)** 29 Days: After 4 once weekly treatments with topical eXo^3 exosomes formulated in a hyaluronic acid gel. **(C)** 50 Days: After 7 once weekly treatments with topical eXo^3 exosomes formulated in a hyaluronic acid gel. **(D)** Digital image analysis of wound closure area over time of treatment.

medical unmet need. The preclinical data presented, including the animal studies, combined with the morbidity and mortality associated with diabetic foot ulcers served to highlight the critical need to treat this patient. Given that 70% of diabetic foot ulcers remain unhealed at 20 weeks, the rapid closure of these wounds is significant. The rationale for why this patient was treated as well as the successful healing presented will hopefully serve as justification for larger clinical trials in this area of unmet need. Follow-up studies over time are required to adequately assess the effectiveness of eXo^3 in its current product formulation. If eXo^3 were to be administered in a manner other than topically, such as oral ingestion, subcutaneous or systemic injection, the product must be manufactured in a cGMP compliant manner following the guidelines put forth by the FDA for clinical trial products. While we are not planning to submit a clinical trial application at this time, there are currently 134 exosome-based studies investigating EV therapeutic use according to ClinicalTrials.gov (National Library of Medicine, 2025). With a growing number of studies focused on wound healing, our data presented and research efforts provide valuable translational evidence supporting the therapeutic benefits of exosomes (Hu et al., 2016; Qiu et al., 2020; Shabbir et al., 2015; Shi et al., 2021).

5 Conclusion

The innovation demonstrated here focuses on co-opting the cellular context-dependence of ASC loading and secretion of EVs for engineering a potentially novel pro-healing therapeutic. Here we also show that "tuned" EVs from adipose derived stem cells have the ability to facilitate accelerated and improved skin wound healing upon application in a topical formulation. The bioreactor-based production approach enables large quantities of exosomes to be isolated from relatively small volumes of conditioned culture media, when compared to culturing in traditional culture plastic ware generating multiple liters of media. The bioreactor is also considered a "closed system", thus addressing at least one recommendation by regulatory agencies for products derived from cell culture. This approach reduces the cost of goods for

large scale applications, such as OTC personal care products and potential FDA-allowed medicinal products.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The participant provided their written informed consent to participate in this study. The requirement of ethical approval was waived by participant through written informed consent for studies involving humans. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. The studies were conducted in accordance with the local legislation and institutional requirements. The animal study was approved by Institute of Laboratory Animal Resources Commission on Life Science National Research Council. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

JG: Data curation, Project administration, Formal Analysis, Methodology, Visualization, Funding acquisition, Conceptualization, Writing – review and editing, Investigation, Resources, Writing – original draft, Supervision. JL: Writing – original draft, Data curation, Resources, Investigation, Validation, Funding acquisition, Supervision, Conceptualization, Formal Analysis, Project administration, Writing – review and editing, Methodology. MV: Data curation, Formal Analysis, Validation, Methodology, Conceptualization, Writing – original draft, Writing – review and editing, Visualization, Investigation. RS: Formal Analysis, Project administration, Data curation, Conceptualization, Funding acquisition, Supervision, Resources, Writing – review and editing.

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Conflict of interest

Author MV is a consultant to Exotropin, LLC. Author JL is employed by ZenBio, a BioIVT Company. Authors JG and RS are shareholders of Exotropin, LLC.

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References

Basu, J., and Ludlow, J. W. (2016). Exosomes for repair, regeneration and rejuvenation. *Expert Opin. Biol. Ther.* 16 (4), 489–506. doi:10.1517/14712598.2016.1131976

Buehrer, B. M., and Cheatham, B. (2013). Isolation and characterization of human adipose-derived stem cells for use in tissue engineering. *Methods Mol. Biol. Clift. N. J.* 1001, 1–11. doi:10.1007/978-1-62703-363-3_1

Gruber, H., Somayaji, S., Riley, F., Hoelscher, G. L., Norton, H. J., Ingram, J., et al. (2012). Human adipose-derived mesenchymal stem cells: serial passaging, doubling time and cell senescence. *Biotech. Histochem.* 87 (4), 303–311. doi:10.3109/10520295.2011.649785

Gurunathan, S., Kang, M.-H., and Kim, J.-H. (2021). A comprehensive review on factors influences biogenesis, functions, therapeutic and clinical implications of exosomes. *Int. J. Nanomedicine* 16, 1281–1312. doi:10.2147/IJN.S291956

Hu, L., Wang, J., Zhou, X., Xiong, Z., Zhao, J., Yu, R., et al. (2016). Exosomes derived from human adipose mensenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. *Sci. Rep.* 6, 32993. doi:10.1038/srep32993

Kim, K. M., Abdelmohsen, K., Mustapic, M., Kapogiannis, D., and Gorospe, M. (2017). RNA in extracellular vesicles. *Wiley Interdiscip. Rev. RNA* 8 (4), e1413. doi:10.1002/wrna.1413

Kowal, J., Tkach, M., and Théry, C. (2014). Biogenesis and secretion of exosomes. *Curr. Opin. Cell Biol.* 29, 116–125. doi:10.1016/j.ceb.2014.05.004

Liu, M., Lei, H., Dong, P., Fu, X., Yang, Z., Yang, Y., et al. (2017). Adipose-derived mesenchymal stem cells from the elderly exhibit decreased migration and differentiation abilities with senescent properties. *Cell Transplant.* 26 (9), 1505–1519. doi:10.1177/0963689717721221

Lotfy, A., AboQuella, N. M., and Wang, H. (2023). Mesenchymal stromal/stem cell (MSC)-derived exosomes in clinical trials. *Stem Cell Res. Ther.* 14 (1), 66. doi:10.1186/s13287-023-03287-7

Ludlow, J. W., and Buehrer, B. M. (2022). "Manipulating the cellular environment generates differntial extracellular vesicle packaging and biological activity," in *Advances in exosome research*. Available online at: https://www.biocompare.com/3262-eBooks/583797-Advances-in-Exosome-Research/?arev=true (Accessed May 25, 2025).

Ludlow, J. W., and Buehrer, B. M. (2024). Analyses and utilization of selectively tuned human adipose-derived stromal/stem cell exosomes. *Methods Mol. Biol. Clift. N. J.* 2783, 309–322. doi:10.1007/978-1-0716-3762-3_22

National Library of Medicine (2025). *ClinicalTrials.gov: Search results for "exosome therapy"*. Available online at: https://clinicaltrials.gov/search?intr=exosome%20therapy.

Pirmoradi, S., Fathi, E., Farahzadi, R., Pilehvar-Soltanahmadi, Y., and Zarghami, N. (2018). Curcumin affects adipose tissue-derived mesenchymal stem cell aging through TERT gene expression. *Drug Res.* 68 (4), 213–221. doi:10.1055/s-0043-119635

Pote, M. S., and Gacche, R. N. (2025). Exosomal signaling in cancer metastasis: molecular insights and therapeutic opportunities. *Archives Biochem. Biophysics* 764, 110277. doi:10.1016/j.abb.2024.110277

Qiu, H., Liu, S., Wu, K., Zhao, R., Cao, L., and Wang, H. (2020). Prospective application of exosomes derived from adipose-derived stem cells in skin wound healing: a review. *J. Cosmet. Dermatology* 19 (3), 574–581. doi:10.1111/jocd.13215

Rani, S., and Ritter, T. (2016). The exosome - a naturally secreted nanoparticle and its application to wound healing. *Adv. Mater. Deerf. Beach, Fla.* 28 (27), 5542–5552. doi:10.1002/adma.201504009

Shabbir, A., Cox, A., Rodriguez-Menocal, L., Salgado, M., and Van Badiavas, E. (2015). Mesenchymal stem cell exosomes induce proliferation and migration of normal and chronic wound fibroblasts, and enhance angiogenesis *in vitro*. *Stem Cells Dev.* 24 (14), 1635–1647. doi:10.1089/scd.2014.0316

Shi, H., Wang, M., Sun, Y., Yang, D., Xu, W., and Qian, H. (2021). Exosomes: emerging cell-free based therapeutics in dermatologic diseases. *Front. Cell Dev. Biol.* 9, 736022. doi:10.3389/fcell.2021.736022

Siraj, Y., Galderisi, U., and Alessio, N. (2023). Senescence induces fundamental changes in the secretome of mesenchymal stromal cells (MSCs): implications for the therapeutic use of MSCs and their derivates. *Front. Bioeng. Biotechnol.* 11, 1148761. doi:10.3389/fbioe.2023.1148761

Skotland, T., Hessvik, N. P., Sandvig, K., and Llorente, A. (2019). Exosomal lipid composition and the role of ether lipids and phosphoinositides in exosome biology. *J. Lipid Res.* 60 (1), 9–18. doi:10.1194/jlr.R084343

Smirnova, A., Yatsenko, E., Baranovskii, D., and Klabukov, I. (2023). Mesenchymal stem cell-derived extracellular vesicles in skin wound healing: the risk of senescent drift induction in secretome-based therapeutics. *Mil. Med. Res.* 10 (1), 60. doi:10.1186/s40779-023-00498-0

Vymetalova, L., Kucirkova, T., Knopfova, L., Pospisilova, V., Kasko, T., Lejdarova, H., et al. (2020). Large-scale automated hollow-fiber bioreactor expansion of umbilical cord-derived human mesenchymal stromal cells for neurological disorders. *Neurochem. Res.* 45 (1), 204–214. doi:10.1007/s11064-019-02925-y

Wagner, W., Wein, F., Seckinger, A., Frankhauser, M., Wirkner, U., Krause, U., et al. (2005). Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. *Exp. Hematol.* 33 (11), 1402–1416. doi:10.1016/j.exphem.2005.07.003

Wagner, W., Horn, P., Castoldi, M., Diehlmann, A., Bork, S., Saffrich, R., et al. (2008). Replicative senescence of mesenchymal stem cells: a continuous and organized process. *PloS One* 3 (5), e2213. doi:10.1371/journal.pone.0002213

Welsh, J. A., Goberdhan, D. C. I., O'Driscoll, L., Buzas, E. I., Blenkiron, C., Bussolati, B., et al. (2024). Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J. Extracell. Vesicles* 13 (2), e12404. doi:10.1002/jev2.12404

Zhang, J., Guan, J., Niu, X., Hu, G., Guo, S., Li, Q., et al. (2015). Exosomes released from human induced pluripotent stem cells-derived MSCs facilitate cutaneous wound healing by promoting collagen synthesis and angiogenesis. *J. Transl. Med.* 13, 49. doi:10.1186/s12967-015-0417-0

Zhang, Y., Liu, Y., Liu, H., and Tang, W. H. (2019). Exosomes: biogenesis, biologic function and clinical potential. *Cell Biosci.* 9, 19. doi:10.1186/s13578-019-0282-2

Zhang, Y., Bi, J., Huang, J., Tang, Y., and Du, S. (2020). Exosome: a review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications. *Int. J. Nanomedicine* 15, 6917–6934. doi:10.2147/IJN.S264498

Zhang, Y., Lan, M., and Chen, Y. (2024). Minimal information for studies of extracellular vesicles (MISEV): ten-year evolution (2014-2023). *Pharmaceutics* 16 (11), 1394. doi:10.3390/pharmaceutics16111394

Zou, Z., Li, H., Xu, G., Hu, Y., Zhang, W., and Tian, K. (2023). Current knowledge and future perspectives of exosomes as nanocarriers in diagnosis and treatment of diseases. *Int. J. Nanomedicine* 18, 4751–4778. doi:10.2147/IJN.S417422

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Adipose-derived stem cell exosomes: emerging roles and therapeutic application

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Stem cell-derived exosomes have broad application prospects in different medical fields, and are increasingly being considered a replacement for Mesenchymal stromal cells (MSCs) therapy. Adipose-derived stem cells (ADSCs) are an efficient and high-quality source of stem cell exosomes because ADSCs can be easily obtained from autologous adipose tissue and there are only minor ethical concerns, also ADSCs shown multipotent differentiation potential, self-renewal potential, low immunogenicity, and high proliferation rate. Exosomes derived from ADSCs have the function of promoting tissue regeneration through activation or inhibition of multiple signaling pathways (such as Wnt/βcatenin, PI3K/Akt), and immunomodulation, angiogenesis, cell migration, proliferation and differentiation, and tissue remodeling. This review presents the current state of knowledge on ADSCs exosomes and summarizes the use of ADSCs exosomes in stem cell-free therapies for the treatment of diabetes mellitus, cardiovascular, wound healing, neurodegenerative, skeletal, respiratory diseases, and skin aging and other conditions, thus providing novel insights into the clinical applications of MSC-derived exosomes in disease management.

KEYWORDS

adipose-derived stem cells, exosomes, tissue regeneration, cell-free therapy, regulatory pathway

1 Introduction

1.1 MSCs sources and characteristics

MSCs are a group of multipotent progenitor cells found in a number of tissues such as bone marrow and adipose, which can be isolated from adult and foetal tissues. MSCs show promising applications in clinical tissue regeneration, as they have the ability to proliferate and differentiate into a variety of cell lineages, such as into adipocytes, chondrocytes, and osteoblasts, as well as hepatocytes and neuronal cells (Kariminekoo et al., 2016; Crapnell et al., 2013).

1.2 Limitations of MSCs therapy

Initial studies suggested that MSCs may play a key role in tissue repair, but with continued research it has been found that the low survival rate and low engraftment potential of MSCs in damaged tissue regions limit the effectiveness of MSCs in tissue repair (Hade et al., 2021). It was later shown after several studies that the reparative effect of MSCs in injured tissues does not depend on their implantation and differentiation at the site of injury, but on their paracrine activity (Barreca et al., 2020). MSCs can produce and release a variety of growth factors, chemokines and cytokines. These factors can reduce apoptosis and tissue fibrosis, stimulate extracellular matrix remodelling, inhibit local inflammation, regulate immune responses, induce regeneration, salvage damaged cells, reduce tissue damage, and ultimately accelerate organ repair through paracrine effects (Keshtkar et al., 2018). However, safety concerns regarding MSCs persist. These include a potential risk of malignant transformation due to the *in vitro* expansion required for therapy, as well as the ability of allogeneic MSCs to partially evade immune recognition. In addition, MSCs have the potential to trigger adaptive immunity and compromise efficacy; MSCs can modulate the immune system by being phagocytosed by antigen-presenting cells (APCs), and subsequent MSC antigens presented by APCs result in decreased anti-inflammatory activity and therapeutic efficacy. On the other hand, recognition and clearance of MSCs by the host immune system may also limit the duration and possible efficacy of treatment for many MSCs (Caplan et al., 2019). Through further research, it was found that MSCs are able to release a large number of extracellular vesicles (EVs), which can carry the active factors produced by MSCs to deliver information to damaged cells or tissues to participate in tissue regeneration, exerting biological activities similar to those of MSCs (Rani et al., 2015).

1.3 Extracellular vesicles biology and classification

Extracellular vesicles (EVs) are membrane-enclosed nanoscale particles released from cells. Almost all cells, both prokaryotic and eukaryotic, release EVs as part of their normal physiology and in response to pathological conditions (O'Brien et al., 2020). EVs are a general concept that can be further broken down into different categories based on the size of EVs, including apoptotic vesicles (1,000–5,000 nm), microvesicles (100–1,000 nm), and exosomes (30–200 nm).

1.4 Role of paracrine factors and exosomes

Exosomes carry various biomolecules, including proteins, lipids, and nucleic acids, from their parent cells. Exosomes transmit information in the extracellular space, altering the biological response of the recipient cell by delivering proteins, nucleic acids, and other substances that can inhibit disease but may also promote it (Kalluri and LeBleu, 2020). Based on this property of regulating intracellular pathways, exosomes play a potential utility in the treatment and control of many diseases, including neurodegenerative diseases and tumour therapy (Younas et al., 2022; Zhang and Yu, 2019). In regulating the tumour microenvironment and promoting tumourigenesis, exosomes have potential functions related to immunomodulation and have been implicated as carriers of anti-tumour immune responses (Urabe et al., 2020; van Niel et al., 2018).

1.5 Exosomes derived from adipose-derived stem cells

Adipose-derived stem cells(ADSCs) have received widespread attention due to their multifaceted, abundant, and easily isolated characteristics. A large number of studies have demonstrated that ADSCs are promising for a wide range of applications in regenerative medicine. ADSCs are an important stem cell type isolated from adipose tissue with properties of multidirectional differentiation and self-renewal (Deng and Chen, 2022). Increasingly, research is focusing on exosomes derived from adipose-derived stem cells (ADSCs-exos). ADSCs-exos promote tissue regeneration, immunomodulation, angiogenesis, cell migration, proliferation, differentiation, and tissue remodeling by modulating multiple signaling pathways (Li Y. et al., 2023). This paper reviews the current state of research on ADSCs-exos and summarises the application of ADSCs-exos in stem cell-free treatment of diabetes, cardiovascular, wound healing, neurodegenerative, skeletal, respiratory disorders, and skin ageing, thus providing new insights into the clinical application of ADSCs-exos in disease management.

2 Adipose tissue as a source of adult stem cells: functions and advantages

Stem cells are undifferentiated cells characterized by their extensive proliferative and self-renewal capacities, as well as their potential to differentiate into various cell types and tissues (Kolios and Moodley, 2013). Stem cells are capable of differentiating into different cell populations including adipocytes, osteoblasts, myoblasts, and chondrocytes (Figure 1), which can act as a repository of cells to maintain, repair, and regenerate different tissues (Rodriguez et al., 2005; Laplane and Solary, 2019). Stem cells are divided into three main categories, namely embryonic stem cells, adult stem cells and induced pluripotent stem cells. Although embryonic stem cells exhibit unlimited differentiation potential both *in vitro* and *in vivo*, their clinical application is constrained by ethical, legal, safety, and efficacy concerns. Stem cells from adult tissues can circumvent these issues, and although adult stem cells can only differentiate into a limited number of cell types, these cells can be used in an autologous form, overcoming ethical and legal issues (Gimble and Guilak, 2003; Bacakova et al., 2018). Adult stem cells are undifferentiated cells residing in various tissues throughout the body. Typically maintained in a quiescent, non-dividing state, they can be activated to proliferate and differentiate following tissue damage or cell death to replace lost cells. Due to this proliferative property and the ability to regenerate tissues, adult stem cells have the potential to treat various degenerative diseases as well as aging (Cable et al., 2020).

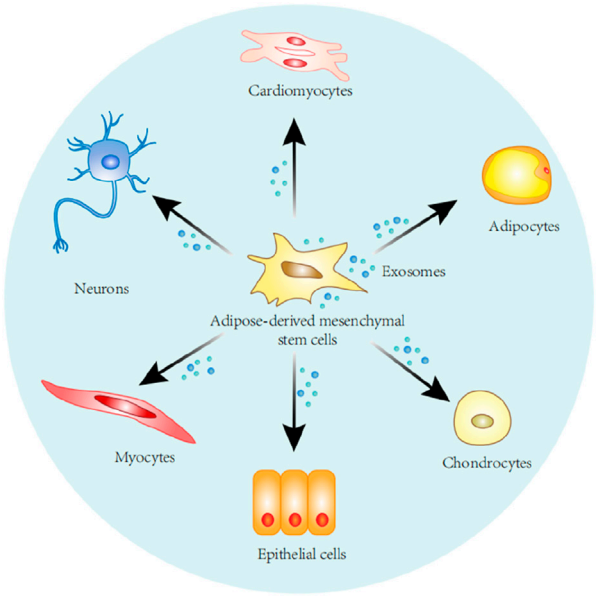


FIGURE 1
Stem cells derived from adipose tissue can self-renew and differentiate along various cell lines, including adipocytes, chondrocytes, epithelial cells, myocytes, nerve cells, cardiomyocytes.

Adipose tissue is usually considered to be inert in terms of biological activity, but with research it has been found to be rich in stem cells. Adipose tissue-derived adult stem cells are Mesenchymal stromal cells found in the subcutaneous tissue at the base of the hair follicle (dermal papilla cells), the dermis (dermal layer cells), the interfollicular dermis, and subcutaneous dermis (Bunnell, 2021; Mazini et al., 2020). The main advantages over other adult stem cells include abundant source of adipose tissue, easy tissue collection and cell isolation for extraction (Banas et al., 2007). Based on these advantages make ADSCs one of the most favourable stem cells for clinical cell therapy (Mazini et al., 2020). After a number of studies, it has been shown that the differentiation of ADSCs is not limited to the adipocyte lineage, but is capable of differentiating into a variety of cell lineages or a variety of cells of mesodermal origin including chondrocytes, osteoblasts, and adipocytes under appropriate stimulation *in vitro* (Gimble and Guilak, 2003; Mazini et al., 2020; Veronesi et al., 2014). ADSCs cultured under horse serum conditions can express myosin light chain kinase (a protein marker for skeletal muscle cell lineage), which provides a research idea for ADSCs to differentiate skeletal muscle cells for repairing damaged skeletal muscle (Gimble and Guilak, 2003).

Adult stem cells can be isolated from various tissues and their regenerative potential has been widely noted, so researchers need to conduct comparative studies on adult stem cells from different tissues (Heo et al., 2016). The first application for cell therapy was bone marrow stem cells, but bone marrow is relatively difficult to obtain, and it can be traumatic for patients during bone marrow harvesting, and the number of stem cells extracted from bone marrow is small, and it is often necessary to undergo *in vitro* expansion to increase the number of cells. Numerous studies have demonstrated that ADSCs can be an alternative to bone marrow stem cells. The ideal source of adult stem cells needs to be easily accessible and capable of extracting a certain number of stem cells, and ADSCs meet these requirements (Banas et al., 2007; Veronesi et al., 2014; Zuk et al., 2001). Treatment by osteogenic differentiation medium induced ADSCs to differentiate towards osteogenesis, exhibiting upregulation of osteogenesis-specific genes and mineralisation, suggesting that ADSCs cells have the potential to promote skeletal recovery (Im, 2013). ADSCs were demonstrated to reverse wall thinning in areas of myocardial scarring in studies performed from rats. Regeneration of cardiomyocytes and blood vessels was found after stem cells were cultured into monolayers and transplanted into rats with infarcted myocardium (Miyahara et al., 2006). It has also been found that these stem cells can improve ventricular function through growth factor-mediated paracrine effects (Madonna et al., 2009). Chandra et al. found that islet-like cell aggregates expressing pancreatic hormones were generated from stem cells cultured from mouse epididymal fat pads and transplanted intraperitoneally into STZ-induced diabetic mice, and blood glucose levels of the diabetic mice returned to normal within 2 weeks (Chandra et al., 2009). Yu et al. found that ADSCs can improve insulin sensitivity by reducing chronic inflammation in diabetic patients, which in turn delays or treats diabetic complications (Yu et al., 2019). Sen et al. found that therapeutic diabetes medications can alter the differentiation pathways of ADSCs, which can respond to the individual's efficacy of diabetes medications. This ability to predict the efficacy of diabetes treatments suggests that ADSCs can be used as a cell-based biomarker. However, there are fewer studies of ADSCs as biomarkers responding to drug efficacy, and additional experiments are needed to confirm this (Sen, 2019). Adenovirus, herpes simplex virus, lentivirus and retroviral vectors are all capable of infecting ADSCs cells *in vitro*, and the question of whether ADSCs can serve as cellular vectors for the delivery of genes has gradually attracted the attention of researchers. The basic fibroblast growth factor (bFGF) gene was introduced into ADSCs cells and peak secretion of bFGF protein was observed 3 days after viral transduction. This study validated the conjecture of ADSCs as gene carriers, but further studies are needed for the clinical application of ADSCs as gene carriers (Gimble and Guilak, 2003; Katz et al., 1999). The above studies have shown that ADSCs have the ability to differentiate into a variety of cell lines, which are differentiated into specific tissue cells through *in vitro* culture under certain conditions, and that injection of these treated ADSCs into organisms can promote cell regeneration and tissue repair. In addition, ADSCs can be taken from autologous bodies and applied to themselves, avoiding legal and ethical issues, and these features and advantages make ADSCs have an extremely wide range of applications in various clinical disciplines.

3 Overview of adipose-derived stem cell exosomes: exosome biogenesis, secretion and uptake isolation of exosomes

Recent studies have increasingly focused on the role of exosomes in health and disease, which play an important role in promoting human health and treating diseases, including the promotion of organismal development, immune response, and tissue homeostasis,

as well as the treatment of cardiovascular diseases, neurodegenerative diseases, and cancers (Pegtel and Gould, 2019). ADSCs-exos have been considered a versatile therapeutic tool for the treatment of tissue damage (Xing et al., 2020a). A large number of studies have confirmed that ADSCs-exos promotes damaged tissue repair and that ADSCs-exos can be used as a stem cell-free therapeutic tool to regenerate different tissues such as bone, cartilage, nerves and skin tissues (An et al., 2021; Zhang S. et al., 2018; Feng et al., 2019; Lu et al., 2019).

Exosomes are nanoscale membrane-bound vesicles secreted by donor cells that are able to participate in intercellular transport of substances (Liang et al., 2021). Exosomes contain biomolecules such as proteins, lipids, nucleic acids, and metabolites. By passing these biomolecules between cells the recipient cell undergoes relevant biological reactions (Kalluri and LeBleu, 2020). To further understand how exosomes are involved in intercellular communication mechanisms, a large number of experiments have been performed to study the processes of exosome biogenesis, secretion and uptake (Pegtel and Gould, 2019). Currently, the prevailing view is that the biogenesis of exosomes is as follows: the cell membrane invaginates to form endosomes, endosomes form intracellular multivesicular bodies (MVBs) through secondary invagination, and finally the MVBs fuse with the cytoplasmic membrane and secrete the intraluminal vesicles (ILVs) within the MVBs outside the cell by cytotoxic secretion into exosomes (Kalluri and LeBleu, 2020). The process of exosome biogenesis is divided into four main steps, including the sorting of content uptake, the formation and maturation of MVBs, the transport of MVBs, and the fusion of MVBs with the plasma membrane (Han et al., 2022). It follows that the process of exosome biogenesis is realised mainly around MVBs, which are special intracellular body compartments enriched with ILVs, and MVBs are able to segregate specific proteins, lipids and other cytoplasmic components (Gurung et al., 2021). The general process of MVBs formation is the internalization of extracellular proteins, lipids, small molecule free substances and other components into the cell through plasma membrane invagination to form cytoplasmic membrane bud vesicles, which are called early sorting endosomes (ESEs), which in turn fuse with secreted vesicles such as the endoplasmic reticulum (ER), the trans Golgi body (TGN) and the mitochondria to form late sorting endosomes (LSEs). Subsequently, the LSE plasma membrane invaginates twice to form many ILVs, and the LSEs carrying ILVs are then called MVBs (Krylova and Feng, 2023). Rab5 and its effector VPS34/p150 have been found to be key regulators of ESE to LSE conversion in the plasma membrane. It acts by recycling extracellular material to the cell membrane within minutes after the ESE begins to form ILVs through inward membrane outgrowth, which ultimately results in the segregation and distribution of the contents into vesicles (Mashouri et al., 2019). When the LSE membrane is invaginated, LSE inclusions are mixed and encapsulated to form multiple intraluminal vesicles. Depending on the amount of invagination, this process produces ILVs with different inclusions and different sizes. The remaining membrane of the invaginated LSE membrane acts as an outer membrane, concentrating the formed ILVs within the LSE lumen, a process that leads to further formation of MVBs in the LSE (Gurunathan et al., 2021). Formation of MVBs is at the centre of exosome biogenesis, where ILVs production and membrane outgrowth are key to exosome production (Hurley et al., 2010). It was found that the formation of MVBs and ILVs can be driven by the endosomal sorting complex required for transport (ESCRT), which consists of the ESCRT-0, -I, -II, -III subcomplexes and the atpase VPS4. Among them, the ESCRT subcomplexes jointly promote the formation of MVBs and ILVs through different functions. This ESCRT-driven formation of MVBs and ILVs is an ESCRT-dependent pathway, and ESCRT-dependent pathways mainly include: the Alix-dependent pathway, HD-PTP-dependent pathway and other pathways (Han et al., 2022; Colombo et al., 2014). In addition, MVBs and ILVs can also be formed independently of ESCRT-driven formation. For example, ILVs can be formed in the absence of ESCRT by sorting through the four-spanning protein CD63, which is an ESCRT-independent mechanism. ESCRT-independent pathways mainly include:The nSMase2-ceramide-dependent pathway, Caveolin-1, Flotillins, and Cholesterol (Han et al., 2022; Stuffers et al., 2009; Edgar et al., 2014). Therefore, it is currently believed that MVBs and ILVs can be formed through the ESCRT-dependent pathway and the ESCRT-independent pathway, which may be related to the classification of cellular contents (Carayon et al., 2011).

MVBs can be fused with autophagosomes, and eventually their contents can be degraded in lysosomes, MVBs can also be directly fused with lysosomes for degradation, and the degradation products can be recycled by cells (Kalluri and LeBleu, 2020). In addition, MVBs can also be transported to the plasma membrane through the cytoskeleton and microtubule network and fused to the plasma membrane with the help of MVBs docking proteins, and through cytosolisation, ILVs in MVBs can be secreted outside the cell and become exocytosed (Colombo et al., 2014). MVBs can be classified into two categories based on their different orientations; MVBs that are degraded by fusion with lysosomes are referred to as degradative MVBs (dMVBs), and those that are fused to the plasma membrane are referred to as secretory MVBs (secretory MVBs) (Han et al., 2022). During the fusion of MVBs with the plasma membrane, the RAB family of small GTPase proteins controls the transport of intracellular vesicles, such as vesicle outgrowth, movement of vesicles through cytoskeletal roles, and the docking of vesicles to target membranes, which leads to plasma membrane fusion. Some researchers extracted different RABs involved in docking endosomes at different stages of maturation along the endosomal pathway and found that RAB11 and RAB35 can be associated with recirculation and early sorting of endosomes, whereas RAB27A and RAB27B are associated with late sorting of endosomes and secretory compartments (Stenmark, 2009; Kowal et al., 2014). In addition to the RAB family of proteins, soluble nsf attachment protein receptor (SNARE) complexes contribute to the fusion of lipid bilayers (Zylbersztejn and Galli, 2011). Specific pairing of vesicular SNAREs (v-SNAREs) with homologous target membrane SNAREs (tSNAREs) forms the SNARE complex, which drives the fusion of two opposing membranes in a zipper-like manner (Yue et al., 2020). These RAB proteins, as well as SNAREs, are thought to be required for the final fusion of MVBs with the cytoplasmic membrane (PM) (Kowal et al., 2014). Eventually MVBs fuse with the plasma membrane and release exosomes into the extracellular space (Yue et al., 2020). Exosomes can be transferred to receptor cells after release by at least three mechanisms: endocytosis, direct membrane fusion, and receptor-ligand interactions (Figure 2) (Abels and Breakefield, 2016). Ligand-receptor interactions may explain exosome-targeted biological effects, including those caused by exosome-borne growth factors, and extracellular matrix (ECM) proteins

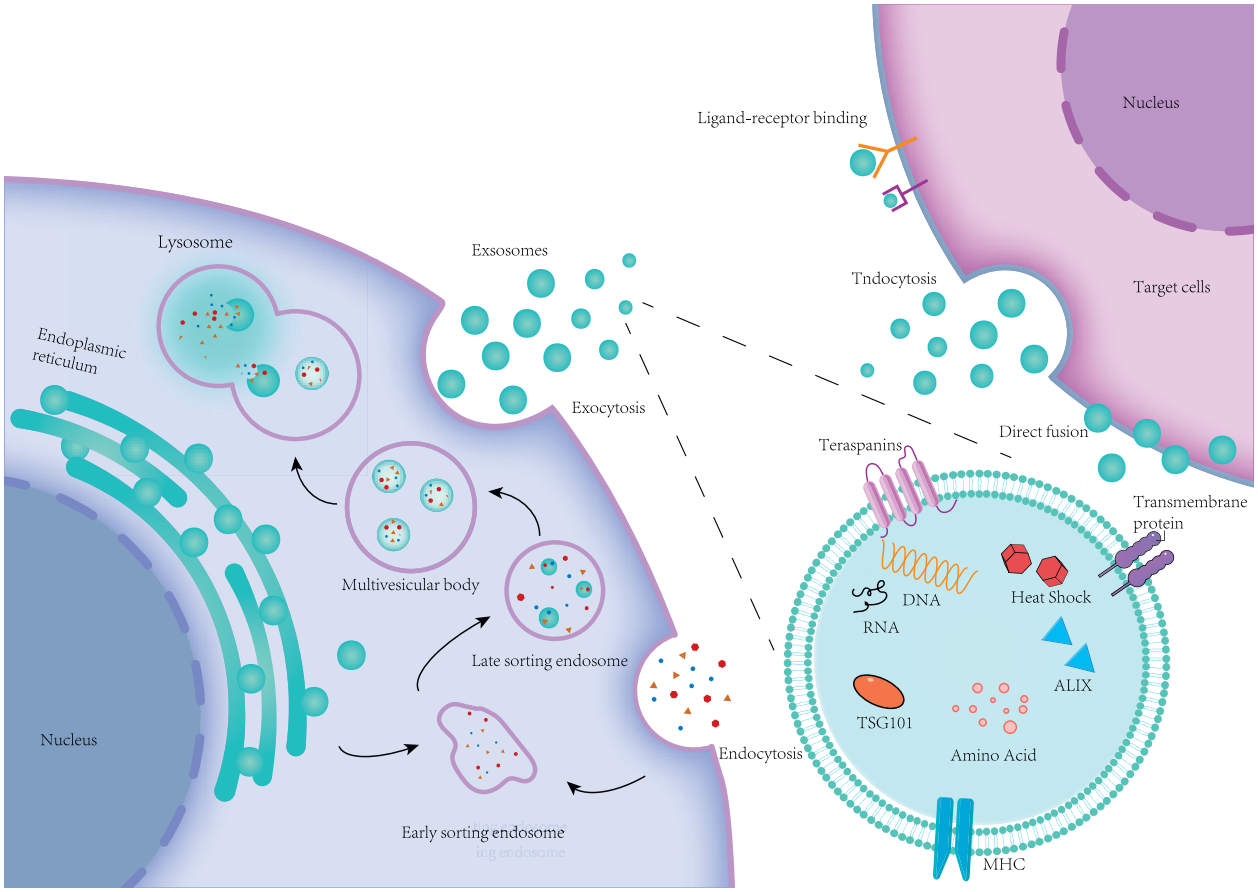


FIGURE 2
Cell membrane invagination endocytoses some extracellular proteins, lipids, small molecules and cell surface proteins to form vesicles, namely early sorting endosomes (ESE). ESE fuses with vesicles secreted from the Golgi and endoplasmic reticulum to form the late sorting endosome (LSE). The LSE reorganizes its contents through membrane invagination to form a collection of luminal vesicles (ILVs) of different sizes, which are multivesicular bodies (MVBs). MVBs can be fused to lysosomes for degradation, or they can release ILVs outside the cell to form exosomes through exocytosis. Exosomes that are ultimately released between cells deliver the vesicular contents to target cells through endocytosis, direct membrane fusion, and receptor-ligand interactions.

(Maas et al., 2017). However, an increasing number of studies have shown that endocytosis is the main pathway for the uptake of exosomes, which can be internalised by endocytosis mediated by lattice proteins, vesicle proteins and lipid rafts (Jadli et al., 2020). The mode of exosome uptake may also be related to the cell type and its physiological state, as well as whether or not the ligand on the exosome surface recognises the receptor on the cell surface. Ultimately, exosomes internalise the above pathways to deliver their contents to the receptor cell, thus enabling exosome messaging between cells (Abels and Breakefield, 2016; Mathieu et al., 2019).

4 Adipose-tissue derived exosome for wound healing

ADSCs-exos, as a new cell-free therapeutic strategy, has a wide range of applications in the field of regenerative medicine. A large number of studies have shown that ADSCs-exos can promote cell proliferation and tissue regeneration by activating signalling pathways or regulating gene expression to enhance or inhibit the activity of specific proteins, which provides a new therapeutic idea to promote wound healing in the clinic. Li et al. found that ADSCs-exos can promote the protein SRY-related (SRY), high-mobility-group box 9 (SOX9), Collagen I, and Collagen II (Collagen II), and the protein SRY-related (SRY), by inhibiting the expression of miR-19b. High-mobility-group box 9 (SOX9), Collagen I and Collagen III expression by inhibiting miR-19b expression, thereby promoting skin fibroblast (HSF) cell proliferation (Qian et al., 2021). The Wnt/βcatenin pathway may promote stem cell differentiation to regulate biological activity (Liu et al., 2019). Ma et al. used Western blot to detect the expression levels of Wnt and β-catenin in HaCaT cells, and β-catenin expression was elevated in the exosomes group as compared to the control group (Ma et al., 2019). In addition, SOX9 upregulated by ADSCs-exos can activate the Wnt/β-catenin pathway and promote HSF cell proliferation, migration, and invasion (Qian et al., 2021). MALAT1 serves as a transcriptional regulator of a variety of genes involved in tumour metastasis and cell migration and is involved in cell cycle regulation. He et al. found that MALAT1-containing ADSCs-exos targeting miR-124 via the Wnt/

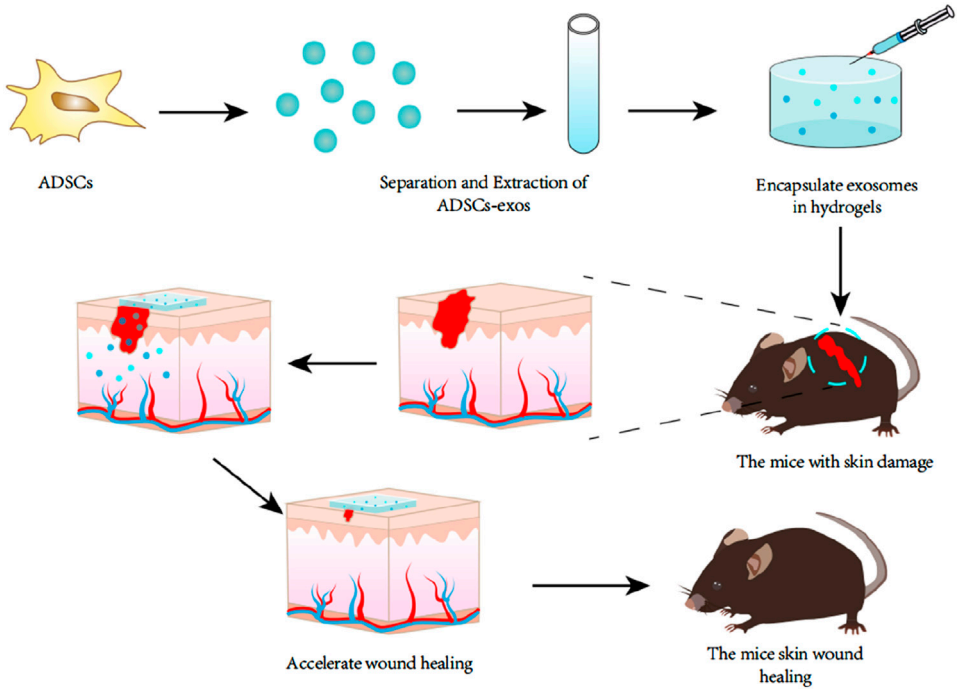


FIGURE 3
Exosomes were extracted from adipose tissue stem cells and encapsulated in a specific hydrogel, which was then locally applied to a mouse model of refractory skin injury. It was found that this hydrogel encapsulated with exosomes could promote skin wound healing better than the application of exosomes or hydrogel alone.

β-catenin pathway could promote cell proliferation and skin healing (He et al., 2020). Zhang et al. found that ADSCs-exos promoted elevated levels of phosphorylated Akt, Col 1, and Col 3 proteins, and that treatment of fibroblasts with Ly294002 (phosphatidylinositol kinase inhibitor) treatment of fibroblasts, Ly294002 was able to significantly inhibit exosome-induced protein level elevation (Zhang W. et al., 2018). Yang et al. found that miR-21, which enhances matrix metalloproteinase 9 (MMP-9) expression by the PI3K/AKT pathway, promotes the migration and proliferation of HaCaT cells and thus promotes wound healing (Yang et al., 2020). Ren et al. found that ADSCs-exos stimulated the AKT and ERK signalling pathways in fibroblasts resulting in significant upregulation of gene expression of proliferation markers (cyclin D1, cyclin D2, cyclin A1, cyclin A2) and growth factors (VEGFA, PDGFA, EGF, FGF2) (Ren S. et al., 2019). Furthermore, miR-19b carried by ADSCs-exos exerts its therapeutic effects on skin wounds by regulating the CCL1/TGF-β signalling axis targeting the chemokine C-C motif ligand 1 (CCL1) (L et al., 2019), and by releasing Neat1 to inhibit the expression of miR-17-5p thereby activating Ulk1-regulated autophagy (An et al., 2023). These studies suggest that ADSCs-exos can activate multiple signalling pathways to promote cell proliferation and wound healing. Zhou et al. encapsulated ADSCs-exos in Pluronic F-127 hydrogel and applied the complex topically to whole skin wounds in mice (Figure 3), and found that increasing the expression of Ki67, α-SMA, and CD31 proteins in the epithelial cells, and up-regulating the expression of the skin barrier proteins KRT1 and AQP3, the complex was more likely to promote the healing of skin wounds compared with either ADSCs-exos or PF-127 hydrogel alone. 127 hydrogel, the PF-127/ADSCs-exos complex was more able to promote skin wound healing compared with ADSCs-exos or PF-127 hydrogel alone, and this study provides a new idea for the combined application of ADSCs-exos and tissue engineering materials (Zhou et al., 2022).

Skin wounds in diabetic patients tend to be more difficult to heal than in the general population and are characterised by a prolonged skin wound healing process, leading to the development of chronic ischaemic ulcers. In contrast, ADSCs-exos are able to induce endothelial cell migration and angiogenesis by regulating several mirna, including miR-31-5p, miR-125a-5p, miR-126-3p miR-221-3p, miR-130a and miR-132 (Pomatto et al., 2021). Hypoxia, infection, reduced vascularisation and elevated oxidative stress may be key factors contributing to non-healing of chronic diabetic wounds. Wang et al. found that under hypoxic conditions, ADSCs-exos could upregulate miR-21-3p, miR-126-5p, miR-31-5p, and downregulate miR-99b and miR-146-a, genes related to wound healing. Genes, as well as activating the PI3K/Akt signalling pathway to inhibit inflammation to promote diabetic wound healing (Wang et al., 2021). Li et al. found that the transcription factor Nrf2 has an anti-oxidative stress protective effect, and the researchers applied ADSCs-exos to a diabetic rat foot ulcer model and found that ADSCs-exos could overexpress Nrf2 in a high-glucose environment thereby promoting the value-added of endothelial progenitor cells as well as angiogenesis (Li X. et al., 2018). Shiekh et al. supplemented a loaded oxygen-releasing antioxidant wound dressing OxOBand with ADSCs-exos for the treatment of diabetic wounds and found that this ADSCs-exos-added adjuvant was able to enhance collagen deposition to accelerate

re-epithelialisation, as well as increase neovessel formation and decrease oxidative stress, thereby accelerating wound healing (Shiekh et al., 2020). Despite the unfavourable factors such as high glucose, high oxidative stress, and hypoxia in wounds of diabetic patients, ADSCs-exos are able to promote wound healing in this environment by modulating a variety of small molecule nucleic acids, and this study provides a new therapeutic idea for treating diabetic patients' wound healing in the clinic. The current study only focuses on skin wounds caused by simple diabetes. Future studies should also consider the effect of diabetes type (type 1 and type 2), chronic wound and patient variability on the treatment of ADSCs-derived exosomes, which will be critical for clinical translation.

ADSCs-exos also has a wide range of applications in the field of aesthetic medicine. Kwon et al. treated acne scars with a combination of fractional carbon dioxide laser (FCL) and ADSCs-exos, and found that ADSCs-exos could promote skin recovery and reduce the appearance of post-procedural erythema after ablation of FCL by increasing a variety of anti-inflammatory factors and regenerative growth factors. Skin recovery and reduced the appearance of post-procedural erythema by increasing multiple anti-inflammatory factors and regenerative growth factors (Kwon et al., 2020). Trzyna et al. found that ADSCs-exos not only reduced inflammation and apoptosis to repair flaps, but also prolonged the survival of vascularised composite allografts after transplantation by down-regulating $CD4^+$ T and Th1 cells, and up-regulating Tr1 and Treg cells, by which the success rate of allograft implantation could be further improved (Trzyna and Banaś-Ząbczyk, 2021). Reducing healing time and scar formation in soft tissue trauma recovery is a pressing clinical need today. Current conventional methods to accelerate healing and reduce scar formation include skin grafting, laser therapy and topical application of some growth factors or gene therapy. However, these methods may lead to adverse consequences such as atrophic scars, pigmentation abnormalities, and skin necrosis. In the early stage of wound healing, ADSCs-exos increased the production of collagen types I and III to promote wound healing, whereas in the later stage, ADSCs-exos inhibited collagen expression and reduced scar formation (Hu et al., 2016). This conclusion is also supported by a study that ADSCs-exos play different roles at different stages of wound healing and scarring phase. Li et al. found that ADSCs-exos could regulate the ratio of fibroblast Col-III to Col-I, TGF-3 to TGF-1, and MMP3 to TIMP-1 during the late healing phase, shifting fibroblasts to an endogenous state and inhibiting fibroblast differentiation thereby reducing scarring (Li C. et al., 2022). This mechanism may be that ADSCs-exos inhibit the expression of Smad3 and Notch-1 proteins as well as transforming growth factor β2 (TGF-β2) in KFs, promote the expression of TGF-β3 as well as inhibit the expression of collagen type I (COL-1), collagen type III (COL-3), fibronectin (FN) and α-smooth muscle actin (α-SMA) gene and protein expression (Li J. et al., 2022). In addition, miR-192-5p is also a regulatory mode of ADSCs-exos to reduce the level of proliferative scar fibrosis (Li Y. et al., 2021). These studies demonstrated that ADSCs-exos also plays a great potential in inhibiting scar formation in the medical aesthetic field, whether it is skin reimplantation, allograft or scar repair, ADCSs-exos can promote the repair of damaged skin by regulating or activating specific signalling pathways.

5 Musculoskeletal regeneration

There is still a high clinical demand for effective bone regeneration therapy. Autologous and allogeneic bone grafts may lead to various complications, and the safety of cellular therapy needs to be further demonstrated. Exosomes have been found to have a possible role in the regeneration of the musculoskeletal system (Qin et al., 2016). ADSCs-exos can be used as a biomimetic tool to regulate and induce the differentiation of stem cells to osteoblasts. The osteogenesis-promoting effects of ADSCs-exos have been attributed to four main mechanisms; reduction of apoptosis in ischemic and necrotic microenvironments, recruitment of MSCs and promotion of their proliferation, promotion of angiogenesis, and direct promotion of osteogenic differentiation of MSCs (Liu et al., 2017; Zhang et al., 2016; Qi et al., 2016). Chen et al. found that miR-375 could be overexpressed in adipose stem cells and enriched in exosomes, and these exosomes carrying miR-375 stimulated mRNA expression of relevant osteogenic genes (Chen S. et al., 2019). Insulin-like growth factor binding protein 3 (IGFBP3), an inhibitor of osteoblast differentiation, inhibits bone morphogenetic protein 2 (BMP - 2) signalling, and miR-375 can downregulate IGFBP3 expression (Chen S. et al., 2019; Eguchi et al., 2018). Lu et al. treated ADSCs-exos with tumour necrosis factor-α (TNF-α) and found that ADSCs-exos were able to promote the proliferation and osteogenic differentiation of primary osteoblasts (HOBs), and this osteogenic capacity was further enhanced after tumour necrosis factor-α (TNF-α) treatment. Pretreated exosomes showed elevated Wnt-3a content, and it was found that inhibition of the Wnt signalling pathway led to a significant reduction in the level of osteogenic gene expression, so this mechanism may provide a mechanism for the enhancement of primary progenitor generation by TNF-α pretreatment through increasing Wnt-3a content in exosomes proliferation of osteoblasts (Lu et al., 2017). An experiment comparing the effects of exosomes from different tissue stem cell sources on cartilage and bone regeneration analysed the role of ADSCs-exos, BMSCs- exos and SMSCs - exos in promoting cartilage differentiation *in vitro*, and found that the gene for the hypertrophy-associated marker collagen type X (Col X) was markedly downregulated in all three types of exos-treated BMSCs, but was most markedly downregulated in the ADSCs-exos group. In turn, hypertrophy adversely affects the chondrocyte differentiation process, suggesting that ADSCs-exos is more advantageous for chondrogenic differentiation in obese patients. This study also demonstrated that ADSCs-exos induced BMSCs into chondrogenesis more significantly. BMSCs treated with ADSCs-exos could more significantly express proteins COL I, COL II, and SOX9, which can promote cartilage matrix synthesis, and ADSCs-exos showed the best bone-related matrix production performance among all groups, demonstrating better bone regeneration ability of ADSCs-exos (Li Q. et al., 2021).

Parental cell pretreatment of exosomes can enhance the regenerative potential of exosomes for bone defects. With the development of various biomaterials, biomaterial-assisted exosomes have become a promising strategy for bone regeneration (Lu et al., 2023). ADSCs-exos treated with osteogenesis-directed culture solution could upregulate the mRNA expression of relevant osteogenic genes, such as RUNX2,

ALP, COL1A1, in addition to significantly promoting the osteogenic differentiation of BMSCs by enhancing ALP activity, and extracellular mineralisation nodules. Under certain conditions, BMSCs were immobilised on polydopamine-coated PLGA/pDA scaffolds, and this composite exosome PLGA/pDA scaffolds could consistently and effectively promote bone regeneration (Li W. et al., 2018). Chen et al. integrated miR-375-ADSCs-exos into a hydrogel and applied it to a rat model, and found that bone regeneration was enhanced in rats with cranial bone defects (Chen S. et al., 2019). Li et al. used tissue-specific decellularised extracellular matrix (dECM) and ADSCs-exos as materials for a novel biomimetic dual-network hydrogel scaffold prepared by 3D printing, and by monitoring cartilage-specific genes, it was found that the expression levels of genes including aggregated proteoglycan (ACAN), collagen type II (COL II) and sy -box transcription factor 9 (SOX9) were significantly upregulated in both the Hydrogel-DCM and Hydrog-cultured rBMSCs were significantly upregulated. It was also found that 3d-printed microenvironment-specific heterogeneous bilayer scaffolds effectively accelerated the simultaneous regeneration of cartilage and subchondral bone tissue in a rat OC defect model (Li Q. et al., 2023).

ADSCs-exos not only have pro-regenerative, anti-inflammatory and anti-apoptotic properties in orthopaedic diseases, but have also been found to play a key therapeutic role in muscle tissue regeneration. Figliolini et al. found that ADSCs-exos had a role in driving myocyte proliferation and differentiation in an *in vitro* ischaemia model. In addition researchers found that ADSCs-exos showed anti-apoptotic effects both *in vivo* and *ex vivo*, and that ADSCs-exos carried a variety of pro-angiogenic mrna and NRG1. In contrast, NRG1 is associated with muscle protection, vascular growth and inflammatory cell recruitment. The involvement of ADSCs-exos in vascular growth and muscle regeneration was confirmed by bioinformatics analysis of 18 molecules detected in ADSCs-exos (Figliolini et al., 2020). In a rat model of severe rotator cuff tear (MRCT), ADSCs-exos were injected into the lesion, and when compared with the control group group, it was found that not only muscle atrophy, fatty infiltration, inflammation, and vascularisation could be prevented in the ADSCs-exos group, but also muscle fibre regeneration and biomechanical properties of tendon sheaths of the rats treated with ADSCs-exos were significantly improved (Wang et al., 2019). Fu et al. found that ADSCs-exos could promote the proliferation and differentiation of tendon-derived stem cells (tdsc) through the paracrine pathway (Fu et al., 2021). In a controlled trial, Doan et al. found that the expression of genes Flk1, Vwf, Ang1, Tgfb1, Myod, and Myf5, which promote angiogenesis and muscle remodelling, was increased 4-8-fold in the ADSCs-exos group compared to the control group, by intravenously injecting ADSCs-exos into a mouse model of hindlimb ischemia compared to the placebo group (Doan et al., 2023). Mitchell et al. tested the regenerative effects of extracellular vesicles using a model of skeletal muscle injury and found that soluble factors secreted by ADSCs-exos promoted muscle regeneration *in vitro* and *in vivo* in a highly synergistic manner (Mitchell et al., 2019). Zhu et al. summarised the possible mechanisms of ADSCs-exos in muscle regeneration as follows: ADSCs-exos promotes the classical Wnt pathway to dominate the myogenic function of satellite cells, activates the ERK1/2 and JNK MAPK pathways to stimulate myoblast proliferation at an early stage, assists the PI3K/Akt pathway to promote protein synthesis and myofibre hypertrophy, and promotes the JAK2/STAT2/STAT3 pathway to advance the myoblast differentiation process (Zhu et al., 2022). Although there are fewer studies on the regeneration of the musculoskeletal system by ADSCs-exos, it can be shown from the above studies that ADSCs-exos play an important role in bone and muscle regeneration, and that these exosomes can stimulate the expression of relevant genes or activate specific signalling pathways to promote bone and muscle regeneration. In addition, these effects and advantages of exosomes can also be used to combine with biological tissue engineering to continue to play its role. These studies provide a strong theoretical basis and good innovative ideas for the subsequent application of exosomes in clinical treatment.

6 Cardiovascular regeneration

Existing studies suggest that ADSCs-exos may have a role in promoting cardiovascular regeneration, improving cardiovascular fibrosis, and preventing and reducing the extent of atherosclerosis. The mechanism of action may be through the induction of signalling pathways by mirna or small molecule proteins carried by ADSCs-exos. Insulin resistance is a known risk factor for cardiovascular disease, and it has been found that ADSCs-exos can reduce systemic insulin resistance. Injection of ADSCs-exos into the peritoneal cavity of a high-fat mouse model revealed that these mice showed improved glucose tolerance and insulin sensitivity, as well as reduced levels of serum triglycerides and total cholesterol *in vivo*, which reveals a possible role of ADSCs-exos in the prevention and treatment of cardiovascular-related metabolic diseases (Constantin et al., 2020; Ormazabal et al., 2018). Fan et al. measured increased markers of vascular endothelial cell activation VEGF, ET-1, VCAM-1, and ICAM-1 as well as decreased markers of inflammation CRP, IL-6, and TNFα, and also found reductions of atherosclerotic plaques in mice after an intravenous infusion of ADSCs-exos in atherosclerotic rats (Fan et al., 2019). Deng et al. found that ADSCs-exos were able to prevent oxidative stress-induced apoptosis in cardiomyocytes in in vitro experiments. In addition researchers injected ADSCs-exos intravenously into an animal model of myocardial infarction and found that ADSCs-exos were able to induce M2-type macrophage polarisation and immune cell transport, culminating in improved haemodynamic outcomes observed with echocardiography in these animals (Deng et al., 2019). Xing et al. investigated the effect of ADSCs-exos on the differentiation of ADSCs to endothelial cells and found that ADSCs-exos may reduce endothelial cell apoptosis by decreasing the expression of miR-324-5p, a potential marker and effector of atherosclerosis (Xing et al., 2020b). Cui et al. found that ADSCs-exos could protect cardiomyocytes from ischaemia-reperfusion injury by activating the Wnt/β-catenin signalling pathway (Cui et al., 2017). Lou et al. found that ADSCs-exos overexpressing miR −126 reduced cardiac fibrosis and pro-inflammatory cytokine levels while promoting angiogenesis in a rat model of myocardial infarction and a model of hypoxia-induced H9c2 cardiomyocyte injury (Luo et al., 2017). In addition, Liu et al. found that mir -93-5p-modified ADSCs-exos exhibited a stronger protective effect against acute myocardial infarction-induced

myocardial injury compared to unmodified ADSCs-exos, which was mediated by the reduction of atg7-mediated autophagy and inhibition of TLR4 (Liu et al., 2018). These studies can demonstrate that ADSCs-exos can regulate lipid levels *in vivo* and improve atherosclerosis as well as protect the myocardium to reduce myocardial fibrosis.

The very first studies found that ADSCs have the ability to differentiate towards endothelial and smooth muscle cells, which can be used in the preparation of artificial blood vessels. The use of uniaxial mechanical strain at a frequency of 1 Hz and TGF-β1 stimulation allowed the differentiation of ADSCs towards smooth muscle cells (Lee et al., 2007). Vascular endothelial growth factor (VEGF) has the ability to induce the differentiation of stem cells in the direction of vascular cells, and Rehman et al. showed that ADSCs cultured under hypoxic conditions were found to secrete a wide range of biologically active angiogenic and anti-apoptotic growth factors, and the proliferation of endothelial cells and the inhibition of their apoptosis are essential for the growth of new blood vessels, so that the secretion of these growth factors by ADSCs may promote angiogenesis (Kranz et al., 2000; Rehman et al., 2004). Yu et al. found that mirna-engineered modified ADSCs were able to promote cell proliferation and vascular regeneration (Yu et al., 2020). These studies imply that ADSCs play a role in cardiovascular regeneration. Zhu et al. demonstrated *in vitro* and *in vivo* that ADSCs-exos promoted angiogenesis by promoting VEGF secretion from vascular endothelial cells through the let-7/argonaute 1 (AGO1)/VEGF signalling pathway (Zhu et al., 2020). Xu et al. observed that some pro-angiogenic mirna was enriched in ADSCs-exos, which could be exocytosed and internalised into human umbilical vein vascular endothelial cells and could act as paracrine signals to promote angiogenesis (Xu et al., 2019). Lou et al. found that the area of myocardial infarction and fibrosis after acute infarction was reduced with miR-126-enriched ADSCs-exos. In an *in vivo* animal model, recovery of perfusion in ischaemic muscle was significantly increased by intramuscular injection of miR-126-enriched ADSCs-exos, suggesting that ADSCs-exos may reduce the area of myocardial infarction and fibrosis after acute infarction by restoring vascular perfusion (Luo et al., 2017). ADSCs-exos from a porcine model of hyperlipidaemia was found to alter gene expression in cardiac fibroblasts (CF) in a simulated ischaemic environment, resulting in the upregulation of cardiomyocyte-specific transcription factors and healing markers α- sma, such as GATA4, Nkx2.5, IRX4, and TBX5, in CF, and the finding that the biomarkers in fibroblasts vimentin, FSP1, podoplanin and cardiac biomarkers troponin- i and connexin-43 were downregulated. With these markers we can learn that ADSCs-exos can modulate specific genes to improve healing after myocardial ischaemic injury (Thankam et al., 2023). Song et al. found that ADSCs-exos transfected with miR-210 regulated protein tyrosine phosphatase 1B (PTP1B) and death-associated protein kinase 1 (DAPK1) to inhibit apoptosis and promote cardiovascular regeneration through the inhibition of Ephrin-A3 (EFNA3) under hypoxic conditions (Song et al., 2021). Lelek et al. found that ADSCs-exos had anti-inflammatory, anti-apoptotic reduction and pro-angiogenic effects by injecting them into ischaemic tissues (Lelek and Zuba-Surma, 2020). The above studies provide a rationale for the role of ADSCs-exos in promoting cardiovascular regeneration, preclinical evidence suggests that the bioactive factors carried by exosomes can promote the proliferation of vascular endothelial cells and neovascularisation. The application of ADSCs-exos in cardiovascular disease is promising, and future studies need to further explore the mechanisms of these effects and how their therapeutic efficacy can be applied in the clinic, but a number of challenges such as safety and efficacy will need to be addressed before they can be applied in the clinic.

7 Nervous system regeneration

Neurological injury caused by trauma or disease has always been a clinical challenge, and whether neuronal cells can be regenerated after injury is currently a focus of academic debate. Peripheral nerve injury (PNIs) is a common neurological disease, and it has been shown that ADSCs can play a regenerative therapeutic role in PNIs by secreting growth factors or promoting myelination through differentiated Schwann cells (SCs). ADSCs-exos may be the key to treating PNIs, and cell-free therapeutic studies of ADSCs-exos have provided new breakthroughs in neuronal cell regeneration after neurological injury (Ju et al., 2023; Jiang et al., 2022). Bucan et al. found that ADSCs-exos promoted Schwann cell proliferation, increased the expression of the cell cycle protein Ki67, and enhanced the axon length of dorsal root ganglion (DRG) neurons. In addition, researchers have confirmed the presence of nerve growth factors such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), nerve growth factor (NGF), fibroblast growth factor-1 (FGF-1), and glial cell-derived neurotrophic factor (GDNF) in ADSCs-exos (Bucan et al., 2019). Ching et al. found that ADSCs-exos transfer the same RNAs as Schwann cell-derived exosomes, and that these RNAs play an important role in promoting neurite growth (Ching et al., 2018). In PNIs model rats, ADSCs-exos reduced apoptosis in SCs after PNIs by up-regulating the expression of anti-apoptotic Bcl-2 mRNA and down-regulating the expression of pro-apoptotic Bax mRNA (Liu et al., 2020). Yang et al. found that ADSCs-exos may also inhibit the expression of chromosome 10 deletion phosphatase and tensin homologue (PTEN) through miRNA-22-3p, activating the phosphorylation of the AKT/mTOR axis to promote the proliferation and migration of SCs cells, and additionally it was found that ADSCs-exos could promote the growth of dorsal root ganglion (DRG) axons (Yang et al., 2022). Ren et al. found that ADSCs-exos modified with mir −133b significantly promoted neurological recovery in spinal cord injured animals by affecting the axonal regeneration-related signalling pathway and the expression of related proteins NF, GAP-43, GFAP and MBP (Ren Z. W. et al., 2019). ADSCs can differentiate towards the Schwann cell phenotype and exosomes derived from such differentiated cells have been found to secrete more neurotrophic and other growth factors (Liu et al., 2022). In a rat sciatic nerve severance model, ADSCs-exos internalised by Schwann cells significantly promoted SCs proliferation, migration, myelination and secretion of neurotrophic factors by up-regulating the corresponding genes, thereby promoting peripheral nerve regeneration (Chen J. et al., 2019). These findings suggest that exosomes promote Schwann cell proliferation for neuronal regeneration through the regulation of growth factors and miRNAs. Pigment epithelium-derived factor (PEDF), a 50-kDa secreted glycoprotein, has been shown to have protective effects on

cultured cortical neurons by inhibiting oxidative stress and apoptosis. ADSCs-exos modified by PEDF strongly inhibit neuronal apoptosis through caspase-dependent (caspase-9 and caspase-3) pathways (Huang et al., 2018; Sanchez et al., 2012). After neural injury, macrophage-induced autophagy occurs in the peripheral environment of innervated neurons, affecting the regulation of inflammatory responses. Whereas the immunosuppressant FK506 promotes nerve regeneration after nerve crush injury and allogeneic nerve transplantation, the combination of FK506 and ADSCs in a mouse sciatic nerve crush injury model revealed that ADSCs-FK506-exos significantly reduced autophagy in segmental macrophages after spinal cord injury (Kuo et al., 2021). However, another study in which ADSCs were pretreated with FK506 found that the exosomes secreted by both ADSCs pretreated with FK506 and untreated ADSCs did not show significant differences in their nerve regeneration-promoting effects, but histone deacetylases (HDACs), β-amyloid A4 (APP), and integrin β1 (ITGB1) detected in the exosomes of both were involved in the promotion of nerve regeneration (Rau et al., 2021). This suggests that FK506, although not able to enhance the effects of ADSCs-exos on nerve regeneration, can act synergistically with ADSCs-exos and together act as a neuroprotecting and nerve regenerating agent. Yin et al. found that the autophagy level and the expression of nuclear transporter protein subunit α2 (Kpna2) in Schwann cells were significantly increased after sciatic nerve injury through peripheral nerve injury rat model experiments. Researchers found that ADSCs-exos could inhibit SCs autophagy by down-regulating Kpna2 via miRNA-26b through *ex vivo* experiments (Yin et al., 2021). In addition, Farinazzo et al. found *in vitro* that low doses of ADSCs-exos were able to exert a protective effect on neuronal cells exposed to oxidative damage by H2O2, and that their mechanism of action may exert an anti-apoptotic effect by inhibiting the H2O2-induced apoptotic cascade response in neuronal cells and by increasing neuronal cell viability. Moreover, this study identified ADSCs-exos as a source of relevant regenerative factors that may modulate the microenvironment in neuroinflammatory and neurodegenerative diseases, thereby promoting neuronal regeneration (Farinazzo et al., 2015). These studies suggest that ADSCs-exos can promote neuronal regeneration by carrying associated factors and can also be neuroprotective by reducing phagocytosis by macrophages or inhibiting oxidative damage.

ADSCs-exos can play a role in neuroprotection and neuroregeneration in neurodegenerative diseases. Alzheimer's disease (AD) is a neurological disorder characterised by cognitive deficits and pathologically characterised by the accumulation of β-amyloid plaques and progressive neuronal death in the hippocampus and cerebral cortex (Molinuevo et al., 2018). Lee et al. found that ADSCs-exos reduced β-amyloid levels in AD neuronal cells as well as apoptosis in AD neuronal cells, and furthermore, the researchers found that ADSCs-exos were able to promote axonal growth in neural stem cells (Lee et al., 2018). By applying ADSCs-exos transfected with miRNA-22 to a mouse model of Alzheimer's disease (AD), Zhai et al. found that it could inhibit cellular pyroptosis to reduce the release of inflammatory factors to play a therapeutic role in AD (Zhai et al., 2021). Neurotrophic factor-3 (NT-3) is an important neurotrophic factor in the process of peripheral nerve regeneration. ADSCs-exos were used as a carrier of NT-3 mRNA, loaded in nerve-guided catheters and bridged to a rat model of deficient sciatic nerve, which ultimately revealed that NT-3 mRNA promotes nerve regeneration mediated by ADSCs-exos (Yang et al., 2021). These studies suggest that ADSCs-exos can also be used to treat neurodegenerative diseases or as a vehicle to promote nerve regeneration and treat nerve injuries. Based on the above studies ADSCs-exos is expected to provide a new therapeutic strategy for the clinical treatment of nerve injuries and the promotion of nerve regeneration. Furthermore, when using ADSCs-exos to promote neural regeneration, the following issues should also be taken into consideration, such as penetration of the blood-brain barrier, targeted delivery to the central nervous system, dosing/bioavailability, and standardization of exosome purity. These issues will be a series of challenges that ADSCs-exos therapy for nerve injury faces in its transition to clinical application.

8 The clinical application of adipose-derived stem cell exosomes

The therapeutic potential of ADSCs-exos in a variety of diseases has been unearthed in recent years through the study of ADSCs-exos. ADSCs-exos carry a variety of genetic material and proteins that enable them to participate in a wide range of biological processes, including cell proliferation, migration, and apoptosis, modulation of the immune and inflammatory response, regulation of osteoblast metabolism, promotion of angiogenesis, and influence on tumour growth [(Hong et al., 2019)]. Moreover, the therapeutic effects of ADSCs-exos have been demonstrated in different disease models, such as promoting diabetic skin wound healing, reducing scars after wound repair, improving fat grafting, regeneration of bone and muscular system, treating myocardial infarction and ischemia/reperfusion injuries, and delaying neurodegeneration (Table 1). This augurs well for the possible clinical application of ADSCs-exos as a cell-free therapy.

One of the most challenging complications of diabetes is delayed wound healing, and hypoxia, reduced vascularisation, elevated oxidative stress and infection are key factors in the non-healing of chronic diabetic wounds (Shiekh et al., 2020; Greenhalgh, 2003). Wang et al. demonstrated that ADSCs-exos could promote wound healing in diabetic mice by promoting angiogenesis, fibroblast proliferation and migration, and collagen synthesis (Wang et al., 2020). ADSCs-exos express antioxidant receptors (Nrf2) that promote angiogenesis to accelerate diabetic wound healing (Li X. et al., 2018). It has also been found that ADSCs-exos play different roles at different stages of wound healing, with ADSCs-exos regulating the ratio of fibroblast Col-III to Col-I in the late stages of healing to inhibit fibroblast differentiation as well as decreasing the level of proliferative keloid fibrosis through specific genes (Li C. et al., 2022; Li Y. et al., 2021). In terms of improving fat grafting, ADSCs-exos can increase the retention of fat graft volume by stimulating angiogenesis and modulating inflammatory responses. In addition, it was found that hypoxia-treated ADSCs-exos had a higher ability to promote angiogenesis in fat grafting. This ability to retain grafted fat volume greatly improves the success of fat grafting in medical aesthetics (Mou et al., 2019; Han et al., 2019). Based on the biocompatibility and cellular targeting of exosomes can be loaded into biological tissue materials, perpetuating the release of exosomes to enhance wound healing (An et al., 2021). Shilan et al. were able to significantly promote wound healing and local

TABLE 1 Adipose-derived stem cell exosomes promote the regeneration of various tissues by regulating related genes and signaling pathways.

Gene	Signal pathway	Reproduction organization
miR-19b, SOX9	Wnt, β- catenin, PI3K, AKT, ERK	epithelial tissue
miR-375, RUNX2, ALP, COL1A1	Wnt	bone tissue
Flk1, Vwf, Ang1, Tgfb1, Myod, Myf5	Wnt, ERK1/2, JNK MAPK, PI3K/Akt, JAK2/STAT2/STAT3	muscular tissue
miR-324-5p, miR -93-5p	Wnt/β-catenin	Cardiovascular
Bcl-2 mRNA, miR-22-3p, miR −133b, miR-26b	AKT/mTOR	Nervous tissue

angiogenesis in an *in vivo* study using exosomes loaded in sodium alginate gel as a bioactive scaffold (Shafei et al., 2020). These studies have demonstrated the therapeutic ability of ADSCs-exos in skin wound healing.

ADSCs-exos in the regeneration of the musculoskeletal system, treatment with ADSCs-exos prevented muscle atrophy, fatty infiltration, inflammation, and vascularisation in a rat massive rotator cuff tear (MRCT) model, resulting in significant improvements in tendon sheath muscle fibre regeneration and biomechanical properties (Wang et al., 2019). Li et al. could accelerate the repair of critical skull defects in mice by combining ADSCs-exos with polylactic acid-hydroxyacetic acid copolymer (PLGA) scaffolds and showed that PLGA scaffolds combined with exosomes recruited more MSCs *in vivo* by immunofluorescence staining (Li W. et al., 2018).

Neurodegenerative diseases such as Alzheimer's disease (AD), Huntington's chorea (HD), and Parkinson's disease (PD) are commonly characterised by progressive deterioration and death of nerve cells. ADSCs-exos were found to reduce β-amyloid levels in AD neuronal cells as well as reduce apoptosis in AD neuronal cells (Lee et al., 2018). Enkephalinase (NEP) is a therapeutic target for AD, and Katsuda et al. demonstrated that ADSCs-exos were able to secrete enzymatically active NEP, co-cultured ADSCs with neuroblastoma N2a cells, and ultimately found that ADSCs-exos led to a reduction in intracellular and extracellular Aβ levels (Katsuda et al., 2013). In a mouse model of amyotrophic lateral sclerosis (ALS) with increased superoxide dismutase (SOD-1) aggregation, ADSCs-exos were able to reduce SOD-1 aggregation and normalise aberrant mitochondrial protein levels as well as normalise PGC-1α and p-CREB/CREB ratios in ALS mutants (Lee et al., 2016). These studies were able to demonstrate the therapeutic potential of ADSCs-exos in neurodegenerative diseases.

The occurrence of ischaemia-reperfusion in any tissue or organ may lead to the generation of reactive oxidative stress and even lead to organ failure causing severe irreversible damage to the body. Some studies have shown that ADSCs-exos could be a new tool as a novel cell-free therapy to treat ischaemia-reperfusion injury (Yapca et al., 2013). Cui et al. found that ADSCs-exos could exert anti-apoptotic and pro-survival effects on cardiomyocytes by activating the Wnt/β-catenin signalling pathway, thereby protecting cardiomyocytes from ischemia-reperfusion injury (Cui et al., 2017). Lin et al. found that ADSCs-exos protected the kidneys from acute ischaemia-reperfusion injury and attenuated inflammatory response, oxidative stress and deterioration of renal function (Lin et al., 2016). Huang et al. found that ADSCs-exos modified with pedf were able to activate autophagy and inhibit neuronal apoptosis to attenuate cerebral ischaemia or reperfusion injury (Huang et al., 2018). In flaps after ischaemia-reperfusion injury, ADSCs-exos promote neovascularisation and attenuate inflammatory response and apoptosis (Bai et al., 2018). These studies demonstrated the therapeutic effects of ADSCs-exos on ischaemia-reperfusion injury in different organs, and ADSCs-exos cell-free therapy may become a valuable tool for the clinical treatment of ischaemia-reperfusion injury.

9 Conclusion

ADSCs-exos has an extremely outstanding performance in various tissue regeneration fields. It can inherit abundant bioactive molecules, such as proteins, nucleic acids and small molecule signalling molecules, from parent cell ADSCs, and carry these molecules to participate in cell-to-cell communication and a variety of biological processes in order to promote cell proliferation and tissue regeneration. ADSCs-exos, as a cell-free treatment, is also able to overcome the drawbacks of cellular therapies such as cellular immune rejection, which opens up the possibility of alternative cellular therapies. In addition, ADSCs-exos are also capable of synergising therapeutic effects by pre-treating the culture so that it carries specific small molecule nucleic acids, or acting as drug carriers for targeted drug transport to target tissues. However, there are still many difficulties in applying ADSCs-exos in the clinic. Although adipose tissue is widely available and easy to obtain, there are still technical bottlenecks in isolating, extracting and purifying exosomes and producing them on a large scale, and the current production methods are complicated, which still can't realise large-scale production. Moreover, the heterogeneity of the donors, variations in culture conditions (such as differences in serum batches), and the lack of standardization in the induction protocols all contribute to fluctuations in the production and function of exosomes across different batches. ADSCs-exos are also relatively unstable and prone to lose biological activity during storage and transport, and further research and development of effective preservation and transport methods are needed. It has been shown that artificial micro- and nano vesicles are able to possess a higher proliferation capacity than natural ADSCs-exos (Kim et al., 2017), which provides a good idea for the preparation of exosomes, although a large number of studies are needed to prove whether artificial nano vesicles are superior to natural exosomes in all aspects or in the treatment of a variety of diseases. An additional challenge that prevents ADSCs-exos from being used in the clinic is that it is still not possible to determine a

specific safe dose for use in humans, and further research is needed to determine the safe range of therapeutic measures. We also need to expand our knowledge of ADSCs-exos over a long period of time, with large samples, and gradually moving from animal to clinical trials. At the regulatory and production levels, the ambiguity of exosome classification, the absence of regulatory frameworks, and the challenges in selecting clinical indications (such as the design of endpoint indicators) collectively constitute barriers to industrialization. To break through these bottlenecks, interdisciplinary collaboration is required, encompassing the elucidation of basic mechanisms, standardization of processes, the development of innovative analytical methods, and the establishment of regulatory science cooperation, in order to facilitate a substantive leap from laboratory to clinical application of ADSC-Exos. Although there are some challenges in the application of ADSCs-exos to the clinic, ADSCs-exos has a broad prospect as a cell-free therapy in clinical application. With the advancement of technology, the preparation method and scale-up production technology of exosome will be gradually improved to make it more suitable for clinical application. Meanwhile, the biological activity and stability of exosomes will be further improved to enhance their effectiveness and safety in therapy. In the future, ADSCs-exos are expected to be applied to disease regeneration therapy, opening up new avenues for cell-free treatment.

Author contributions

YG: Writing – original draft. HM: Writing – review and editing, Writing – original draft, Supervision. ZZ: Writing – original draft. XW: Writing – review and editing. JZ: Writing – original draft. XZ: Funding acquisition, Supervision, Methodology, Writing – review and editing.

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Conflict of interest

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

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References

Abels, E. R., and Breakefield, X. O. (2016). Introduction to extracellular vesicles: biogenesis, RNA cargo selection, content, release, and uptake. *Cell Mol. Neurobiol.* 36 (3), 301–312. doi:10.1007/s10571-016-0366-z

An, Y., Lin, S., Tan, X., Zhu, S., Nie, F., Zhen, Y., et al. (2021). Exosomes from adipose-derived stem cells and application to skin wound healing. *Cell Prolif.* 54 (3), e12993. doi:10.1111/cpr.12993

An, Y., Huang, F., Tan, X., Zhu, S., Zhen, Y., Shang, Y., et al. (2023). Exosomes of adipose tissue-derived stem cells promote wound healing by sponging miR-17-5p and inducing autophagy protein Ulk1. *Plast. Reconstr. Surg.* 151 (5), 1016–1028. doi:10.1097/PRS.0000000000010083

Bacakova, L., Zarubova, J., Travnickova, M., Musilkova, J., Pajorova, J., Slepicka, P., et al. (2018). Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells - a review. *Biotechnol. Adv.* 36 (4), 1111–1126. doi:10.1016/j.biotechadv.2018.03.011

Bai, Y., Han, Y. d., Yan, X. L., Ren, J., Zeng, Q., Li, X. D., et al. (2018). Adipose mesenchymal stem cell-derived exosomes stimulated by hydrogen peroxide enhanced skin flap recovery in ischemia-reperfusion injury. *Biochem. Biophys. Res. Commun.* 500 (2), 310–317. doi:10.1016/j.bbrc.2018.04.065

Banas, A., Teratani, T., Yamamoto, Y., Tokuhara, M., Takeshita, F., Quinn, G., et al. (2007). Adipose tissue-derived mesenchymal stem cells as a source of human hepatocytes. *Hepatology* 46 (1), 219–228. doi:10.1002/hep.21704

Barreca, M. M., Cancemi, P., and Geraci, F. (2020). Mesenchymal and induced pluripotent stem cells-derived extracellular vesicles: the new frontier for regenerative medicine? *Cells* 9 (5), 1163. doi:10.3390/cells9051163

Bucan, V., Vaslaitis, D., Peck, C. T., Strauß, S., Vogt, P. M., and Radtke, C. (2019). Effect of exosomes from rat adipose-derived mesenchymal stem cells on neurite outgrowth and sciatic nerve regeneration after crush injury. *Mol. Neurobiol.* 56 (3), 1812–1824. doi:10.1007/s12035-018-1172-z

Bunnell, B. A. (2021). Adipose tissue-derived mesenchymal stem cells. *Cells* 10 (12), 3433. doi:10.3390/cells10123433

Cable, J., Fuchs, E., Weissman, I., Jasper, H., Glass, D., Rando, T. A., et al. (2020). Adult stem cells and regenerative medicine-a symposium report. *Ann. N. Y. Acad. Sci.* 1462 (1), 27–36. doi:10.1111/nyas.14243

Caplan, H., Olson, S. D., Kumar, A., George, M., Prabhakara, K. S., Wenzel, P., et al. (2019). Mesenchymal stromal cell therapeutic delivery: translational challenges to clinical application. *Front. Immunol.* 10, 1645. doi:10.3389/fimmu.2019.01645

Carayon, K., Chaoui, K., Ronzier, E., Lazar, I., Bertrand-Michel, J., Roques, V., et al. (2011). Proteolipidic composition of exosomes changes during reticulocyte maturation. *J. Biol. Chem.* 286 (39), 34426–34439. doi:10.1074/jbc.M111.257444

Chandra, V., Phadnis, S., Nair, P. D., and Bhonde, R. R. (2009). Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells. *Stem Cells* 27 (8), 1941–1953. doi:10.1002/stem.117

Chen, S., Tang, Y., Liu, Y., Zhang, P., Lv, L., Zhang, X., et al. (2019a). Exosomes derived from miR-375-overexpressing human adipose mesenchymal stem cells promote bone regeneration. *Cell Prolif.* 52 (5), e12669. doi:10.1111/cpr.12669

Chen, J., Ren, S., Duscher, D., Kang, Y., Liu, Y., Wang, C., et al. (2019b). Exosomes from human adipose-derived stem cells promote sciatic nerve regeneration *via* optimizing schwann cell function. *J. Cell Physiol.* 234 (12), 23097–23110. doi:10.1002/jcp.28873

Ching, R. C., Wiberg, M., and Kingham, P. J. (2018). Schwann cell-like differentiated adipose stem cells promote neurite outgrowth *via* secreted exosomes and RNA transfer. *Stem Cell Res. Ther.* 9 (1), 266. doi:10.1186/s13287-018-1017-8

Colombo, M., Raposo, G., and Théry, C. (2014). Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu. Rev. Cell Dev. Biol.* 30, 255–289. doi:10.1146/annurev-cellbio-101512-122326

Constantin, A., Filippi, A., Alexandru, N., Nemecz, M., and Georgescu, A. (2020). Extracellular vesicles from adipose tissue stem cells in diabetes and associated cardiovascular disease; pathobiological impact and therapeutic potential. *Int. J. Mol. Sci.* 21 (24), 9598. doi:10.3390/ijms21249598

Crapnell, K., Blaesius, R., Hastings, A., Lennon, D. P., Caplan, A. I., and Bruder, S. P. (2013). Growth, differentiation capacity, and function of mesenchymal stem cells expanded in serum-free medium developed *via* combinatorial screening. *Exp. Cell Res.* 319 (10), 1409–1418. doi:10.1016/j.yexcr.2013.04.004

Cui, X., He, Z., Liang, Z., Chen, Z., Wang, H., and Zhang, J. (2017). Exosomes from adipose-derived mesenchymal stem cells protect the myocardium against ischemia/reperfusion injury through Wnt/β-Catenin signaling pathway. *J. Cardiovasc Pharmacol.* 70 (4), 225–231. doi:10.1097/FJC.0000000000000507

Deng, H., and Chen, Y. (2022). The role of adipose-derived stem cells-derived extracellular vesicles in the treatment of diabetic foot ulcer: trends and prospects. *Front. Endocrinol. (Lausanne)* 13, 902130. doi:10.3389/fendo.2022.902130

Deng, S., Zhou, X., Ge, Z., Song, Y., Wang, H., Liu, X., et al. (2019). Exosomes from adipose-derived mesenchymal stem cells ameliorate cardiac damage after myocardial infarction by activating S1P/SK1/S1PR1 signaling and promoting macrophage M2 polarization. *Int. J. Biochem. Cell Biol.* 114, 105564. doi:10.1016/j.biocel.2019.105564

Doan, H. T., Van Pham, P., and Vu, N. B. (2023). Intravenous infusion of exosomes derived from human adipose tissue-derived stem cells promotes angiogenesis and muscle regeneration: an observational study in a murine acute limb ischemia model. *Adv. Exp. Med. Biol.*, 101–116. doi:10.1007/5584_2023_769

Edgar, J. R., Eden, E. R., and Futter, C. E. (2014). Hrs- and CD63-dependent competing mechanisms make different sized endosomal intraluminal vesicles. *Traffic* 15 (2), 197–211. doi:10.1111/tra.12139

Eguchi, K., Akiba, Y., Akiba, N., Nagasawa, M., Cooper, L. F., and Uoshima, K. (2018). Insulin-like growth factor binding Protein-3 suppresses osteoblast differentiation *via* bone morphogenetic protein-2. *Biochem. Biophys. Res. Commun.* 507 (1-4), 465–470. doi:10.1016/j.bbrc.2018.11.065

Fan, M., Bai, J., Ding, T., Yang, X., Si, Q., and Nie, D. (2019). Adipose-derived stem cell transplantation inhibits vascular inflammatory responses and endothelial dysfunction in rats with atherosclerosis. *Yonsei Med. J.* 60 (11), 1036–1044. doi:10.3349/ymj.2019.60.11.1036

Farinazzo, A., Turano, E., Marconi, S., Bistaffa, E., Bazzoli, E., and Bonetti, B. (2015). Murine adipose-derived mesenchymal stromal cell vesicles: *in vitro* clues for neuroprotective and neuroregenerative approaches. *Cytotherapy* 17 (5), 571–578. doi:10.1016/j.jcyt.2015.01.005

Feng, N., Jia, Y., and Huang, X. (2019). Exosomes from adipose-derived stem cells alleviate neural injury caused by microglia activation *via* suppressing NF-kB and MAPK pathway. *J. Neuroimmunol.* 334, 576996. doi:10.1016/j.jneuroim.2019.576996

Figliolini, F., Ranghino, A., Grange, C., Cedrino, M., Tapparo, M., Cavallari, C., et al. (2020). Extracellular vesicles from adipose stem cells prevent muscle damage and inflammation in a mouse model of hind limb ischemia: role of Neuregulin-1. *Arterioscler. Thromb. Vasc. Biol.* 40 (1), 239–254. doi:10.1161/ATVBAHA.119.313506

Fu, G., Lu, L., Pan, Z., Fan, A., and Yin, F. (2021). Adipose-derived stem cell exosomes facilitate rotator cuff repair by mediating tendon-derived stem cells. *Regen. Med.* 16 (4), 359–372. doi:10.2217/rme-2021-0004

Gimble, J., and Guilak, F. (2003). Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. *Cytotherapy* 5 (5), 362–369. doi:10.1080/14653240310003026

Greenhalgh, D. G. (2003). Wound healing and diabetes mellitus. *Clin. Plast. Surg.* 30 (1), 37–45. doi:10.1016/s0094-1298(02)00066-4

Gurunathan, S., Kang, M. H., and Kim, J. H. (2021). A comprehensive review on factors influences biogenesis, functions, therapeutic and clinical implications of exosomes. *Int. J. Nanomedicine* 16, 1281–1312. doi:10.2147/IJN.S291956

Gurung, S., Perocheau, D., Touramanidou, L., and Baruteau, J. (2021). The exosome journey: from biogenesis to uptake and intracellular signalling. *Cell Commun. Signal* 19 (1), 47. doi:10.1186/s12964-021-00730-1

Hade, M. D., Suire, C. N., and Suo, Z. (2021). Mesenchymal stem cell-derived exosomes: applications in regenerative medicine. *Cells* 10 (8), 1959. doi:10.3390/cells10081959

Han, Y., Ren, J., Bai, Y., and Pei, X. (2019). Exosomes from hypoxia-treated human adipose-derived mesenchymal stem cells enhance angiogenesis through VEGF/VEGF-R. *Int. J. Biochem. Cell Biol.* 109, 59–68. doi:10.1016/j.biocel.2019.01.017

Han, Q. F., Li, W. J., Hu, K. S., Gao, J., Zhai, W. L., Yang, J. H., et al. (2022). Exosome biogenesis: machinery, regulation, and therapeutic implications in cancer. *Mol. Cancer* 21 (1), 207. doi:10.1186/s12943-022-01671-0

He, L., Zhu, C., Jia, J., Hao, X. Y., Yu, X. Y., Liu, X. Y., et al. (2020). ADSC-exos containing MALAT1 promotes wound healing by targeting miR-124 through activating Wnt/β-catenin pathway. *Biosci. Rep.* 40 (5). doi:10.1042/BSR20192549

Heo, J. S., Choi, Y., Kim, H. S., and Kim, H. O. (2016). Comparison of molecular profiles of human mesenchymal stem cells derived from bone marrow, umbilical cord blood, placenta and adipose tissue. *Int. J. Mol. Med.* 37 (1), 115–125. doi:10.3892/ijmm.2015.2413

Hong, P., Yang, H., Wu, Y., Li, K., and Tang, Z. (2019). The functions and clinical application potential of exosomes derived from adipose mesenchymal stem cells: a comprehensive review. *Stem Cell Res. Ther.* 10 (1), 242. doi:10.1186/s13287-019-1358-y

Hu, L., Wang, J., Zhou, X., Xiong, Z., Zhao, J., Yu, R., et al. (2016). Exosomes derived from human adipose mensenchymal stem cells accelerates cutaneous wound healing *via* optimizing the characteristics of fibroblasts. *Sci. Rep.* 6, 32993. doi:10.1038/srep32993

Huang, X., Ding, J., Li, Y., Liu, W., Ji, J., Wang, H., et al. (2018). Exosomes derived from PEDF modified adipose-derived mesenchymal stem cells ameliorate cerebral ischemia-reperfusion injury by regulation of autophagy and apoptosis. *Exp. Cell Res.* 371 (1), 269–277. doi:10.1016/j.yexcr.2018.08.021

Hurley, J. H., Boura, E., Carlson, L. A., and Różycki, B. (2010). Membrane budding. *Cell* 143 (6), 875–887. doi:10.1016/j.cell.2010.11.030

Im, G. I. (2013). Adipose stem cells and skeletal repair. *Histol. Histopathol.* 28 (5), 557–564. doi:10.14670/HH-28.557

Jadli, A. S., Ballasy, N., Edalat, P., and Patel, V. B. (2020). Inside(sight) of tiny communicator: exosome biogenesis, secretion, and uptake. *Mol. Cell Biochem.* 467 (1-2), 77–94. doi:10.1007/s11010-020-03703-z

Jiang, L., Mee, T., Zhou, X., and Jia, X. (2022). Augmenting peripheral nerve regeneration with adipose-derived stem cells. *Stem Cell Rev. Rep.* 18 (2), 544–558. doi:10.1007/s12015-021-10236-5

Ju, Y., Hu, Y., Yang, P., Xie, X., and Fang, B. (2023). Extracellular vesicle-loaded hydrogels for tissue repair and regeneration. *Mater Today Bio* 18, 100522. doi:10.1016/j.mtbio.2022.100522

Kalluri, R., and LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. *Science* 367 (6478), eaau6977. doi:10.1126/science.aau6977

Kariminekoo, S., Movassaghpour, A., Rahimzadeh, A., Talebi, M., Shamsasenjan, K., and Akbarzadeh, A. (2016). Implications of mesenchymal stem cells in regenerative medicine. *Artif. Cells Nanomed Biotechnol.* 44 (3), 749–757. doi:10.3109/21691401.2015.1129620

Katsuda, T., Tsuchiya, R., Kosaka, N., Yoshioka, Y., Takagaki, K., Oki, K., et al. (2013). Human adipose tissue-derived mesenchymal stem cells secrete functional neprilysin-bound exosomes. *Sci. Rep.* 3, 1197. doi:10.1038/srep01197

Katz, A. J., Llull, R., Hedrick, M. H., and Futrell, J. W. (1999). Emerging approaches to the tissue engineering of fat. *Clin. Plast. Surg.* 26 (4), 587–603. doi:10.1016/s0094-1298(20)32659-6

Keshtkar, S., Azarpira, N., and Ghahremani, M. H. (2018). Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. *Stem Cell Res. Ther.* 9 (1), 63. doi:10.1186/s13287-018-0791-7

Kim, Y. S., Kim, J. Y., Cho, R., Shin, D. M., Lee, S. W., and Oh, Y. M. (2017). Adipose stem cell-derived nanovesicles inhibit emphysema primarily *via* an FGF2-dependent pathway. *Exp. Mol. Med.* 49 (1), e284. doi:10.1038/emm.2016.127

Kolios, G., and Moodley, Y. (2013). Introduction to stem cells and regenerative medicine. *Respiration* 85 (1), 3–10. doi:10.1159/000345615

Kowal, J., Tkach, M., and Théry, C. (2014). Biogenesis and secretion of exosomes. *Curr. Opin. Cell Biol.* 29, 116–125. doi:10.1016/j.ceb.2014.05.004

Kranz, A., Rau, C., Kochs, M., and Waltenberger, J. (2000). Elevation of vascular endothelial growth factor-A serum levels following acute myocardial infarction. Evidence for its origin and functional significance. *J. Mol. Cell Cardiol.* 32 (1), 65–72. doi:10.1006/jmcc.1999.1062

Krylova, S. V., and Feng, D. (2023). The machinery of exosomes: biogenesis, release, and uptake. *Int. J. Mol. Sci.* 24 (2), 1337. doi:10.3390/ijms24021337

Kuo, P. J., Rau, C. S., Wu, S. C., Lin, C. W., Huang, L. H., Lu, T. H., et al. (2021). Exosomes secreted by adipose-derived stem cells following FK506 stimulation reduce autophagy of macrophages in spine after nerve crush injury. *Int. J. Mol. Sci.* 22 (17), 9628. doi:10.3390/ijms22179628

Kwon, H. H., Yang, S. H., Lee, J., Park, B. C., Park, K. Y., Jung, J. Y., et al. (2020). Combination treatment with human adipose tissue stem cell-derived exosomes and fractional CO2 laser for acne scars: a 12-week prospective, double-blind, randomized, split-face study. *Acta Derm. Venereol.* 100 (18), adv00310. doi:10.2340/00015555-3666

L, P. K., Kandoi, S., Misra, R., S, V., K, R., and Verma, R. S. (2019). The mesenchymal stem cell secretome: a new paradigm towards cell-free therapeutic mode in regenerative medicine. *Cytokine Growth Factor Rev.* 46, 1–9. doi:10.1016/j.cytogfr.2019.04.002

Laplane, L., and Solary, E. (2019). Towards a classification of stem cells. *Elife* 8, e46563. doi:10.7554/eLife.46563

Lee, W. C., Maul, T. M., Vorp, D. A., Rubin, J. P., and Marra, K. G. (2007). Effects of uniaxial cyclic strain on adipose-derived stem cell morphology, proliferation, and differentiation. *Biomech. Model Mechanobiol.* 6 (4), 265–273. doi:10.1007/s10237-006-0053-y

Lee, M., Ban, J. J., Kim, K. Y., Jeon, G. S., Im, W., Sung, J. J., et al. (2016). Adipose-derived stem cell exosomes alleviate pathology of amyotrophic lateral sclerosis

in vitro. *Biochem. Biophys. Res. Commun.* 479 (3), 434–439. doi:10.1016/j.bbrc.2016.09.069

Lee, M., Ban, J. J., Yang, S., Im, W., and Kim, M. (2018). The exosome of adipose-derived stem cells reduces β-amyloid pathology and apoptosis of neuronal cells derived from the transgenic mouse model of alzheimer's disease. *Brain Res.* 1691, 87–93. doi:10.1016/j.brainres.2018.03.034

Lelek, J., and Zuba-Surma, E. K. (2020). Perspectives for future use of extracellular vesicles from umbilical Cord- and adipose tissue-derived mesenchymal stem/stromal cells in regenerative therapies-synthetic review. *Int. J. Mol. Sci.* 21 (3), 799. doi:10.3390/ijms21030799

Li, X., Xie, X., Lian, W., Shi, R., Han, S., Zhang, H., et al. (2018a). Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model. *Exp. Mol. Med.* 50 (4), 1–14. doi:10.1038/s12276-018-0058-5

Li, W., Liu, Y., Zhang, P., Tang, Y., Zhou, M., Jiang, W., et al. (2018b). Tissue-engineered bone immobilized with human adipose stem cells-derived exosomes promotes bone regeneration. *ACS Appl. Mater Interfaces* 10 (6), 5240–5254. doi:10.1021/acsami.7b17620

Li, Y., Zhang, J., Shi, J., Liu, K., Wang, X., Jia, Y., et al. (2021a). Exosomes derived from human adipose mesenchymal stem cells attenuate hypertrophic scar fibrosis by miR-192-5p/IL-17RA/Smad axis. *Stem Cell Res. Ther.* 12 (1), 221. doi:10.1186/s13287-021-02290-0

Li, Q., Yu, H., Sun, M., Yang, P., Hu, X., Ao, Y., et al. (2021b). The tissue origin effect of extracellular vesicles on cartilage and bone regeneration. *Acta Biomater.* 125, 253–266. doi:10.1016/j.actbio.2021.02.039

Li, C., Wei, S., Xu, Q., Sun, Y., Ning, X., and Wang, Z. (2022a). Application of ADSCs and their exosomes in scar prevention. *Stem Cell Rev. Rep.* 18 (3), 952–967. doi:10.1007/s12015-021-10252-5

Li, J., Li, Z., Wang, S., Bi, J., and Huo, R. (2022b). Exosomes from human adipose-derived mesenchymal stem cells inhibit production of extracellular matrix in keloid fibroblasts *via* downregulating transforming growth factor-β2 and Notch-1 expression. *Bioengineered* 13 (4), 8515–8525. doi:10.1080/21655979.2022.2051838

Li, Y., Tang, X., Gu, Y., and Zhou, G. (2023a). Adipocyte-derived extracellular vesicles: small vesicles with big impact. *Front. Biosci.* 28 (7), 149. doi:10.31083/j.fbl2807149

Li, Q., Yu, H., Zhao, F., Cao, C., Wu, T., Fan, Y., et al. (2023b). 3D printing of microenvironment-specific bioinspired and exosome-reinforced hydrogel scaffolds for efficient cartilage and subchondral bone regeneration. *Adv. Sci. (Weinh)* 10 (26), e2303650. doi:10.1002/advs.202303650

Liang, Y., Duan, L., Lu, J., and Xia, J. (2021). Engineering exosomes for targeted drug delivery. *Theranostics* 11 (7), 3183–3195. doi:10.7150/thno.52570

Lin, K. C., Yip, H. K., Shao, P. L., Wu, S. C., Chen, K. H., Chen, Y. T., et al. (2016). Combination of adipose-derived mesenchymal stem cells (ADMSC) and ADMSC-Derived exosomes for protecting kidney from acute ischemia-reperfusion injury. *Int. J. Cardiol.* 216, 173–185. doi:10.1016/j.ijcard.2016.04.061

Liu, X., Li, Q., Niu, X., Hu, B., Chen, S., Song, W., et al. (2017). Exosomes secreted from human-induced pluripotent stem cell-derived mesenchymal stem cells prevent osteonecrosis of the femoral head by promoting angiogenesis. *Int. J. Biol. Sci.* 13 (2), 232–244. doi:10.7150/ijbs.16951

Liu, J., Jiang, M., Deng, S., Lu, J., Huang, H., Zhang, Y., et al. (2018). miR-93-5p-Containing exosomes treatment attenuates acute myocardial infarction-induced myocardial damage. *Mol. Ther. Nucleic Acids* 11, 103–115. doi:10.1016/j.omtn.2018.01.010

Liu, D., Chen, L., Zhao, H., Vaziri, N. D., and Zhao, Y. Y. (2019). Small molecules from natural products targeting the Wnt/β-catenin pathway as a therapeutic strategy. *Biomed. Pharmacother.* 117, 108990. doi:10.1016/j.biopha.2019.108990

Liu, C. Y., Yin, G., Sun, Y. D., Lin, Y. F., Xie, Z., English, A. W., et al. (2020). Effect of exosomes from adipose-derived stem cells on the apoptosis of schwann cells in peripheral nerve injury. *CNS Neurosci. Ther.* 26 (2), 189–196. doi:10.1111/cns.13187

Liu, B., Kong, Y., Shi, W., Kuss, M., Liao, K., Hu, G., et al. (2022). Exosomes derived from differentiated human ADMSC with the schwann cell phenotype modulate peripheral nerve-related cellular functions. *Bioact. Mater* 14, 61–75. doi:10.1016/j.bioactmat.2021.11.022

Lu, Z., Chen, Y., Dunstan, C., Roohani-Esfahani, S., and Zreiqat, H. (2017). Priming adipose stem cells with tumor necrosis factor-alpha preconditioning potentiates their exosome efficacy for bone regeneration. *Tissue Eng. Part A* 23 (21-22), 1212–1220. doi:10.1089/ten.tea.2016.0548

Lu, J., Wang, Q. Y., and Sheng, J. G. (2019). Exosomes in the repair of bone defects: next-generation therapeutic tools for the treatment of nonunion. *Biomed. Res. Int.* 2019, 1983131. doi:10.1155/2019/1983131

Lu, Y., Mai, Z., Cui, L., and Zhao, X. (2023). Engineering exosomes and biomaterial-assisted exosomes as therapeutic carriers for bone regeneration. *Stem Cell Res. Ther.* 14 (1), 55. doi:10.1186/s13287-023-03275-x

Luo, Q., Guo, D., Liu, G., Chen, G., Hang, M., and Jin, M. (2017). Exosomes from MiR-126-Overexpressing adscs are therapeutic in relieving acute myocardial ischaemic injury. *Cell Physiol. Biochem.* 44 (6), 2105–2116. doi:10.1159/000485949

Ma, T., Fu, B., Yang, X., Xiao, Y., and Pan, M. (2019). Adipose mesenchymal stem cell-derived exosomes promote cell proliferation, migration, and inhibit cell apoptosis *via* Wnt/β-catenin signaling in cutaneous wound healing. *J. Cell Biochem.* 120 (6), 10847–10854. doi:10.1002/jcb.28376

Maas, S. L. N., Breakefield, X. O., and Weaver, A. M. (2017). Extracellular vesicles: unique intercellular delivery vehicles. *Trends Cell Biol.* 27 (3), 172–188. doi:10.1016/j.tcb.2016.11.003

Madonna, R., Geng, Y. J., and De Caterina, R. (2009). Adipose tissue-derived stem cells: characterization and potential for cardiovascular repair. *Arterioscler. Thromb. Vasc. Biol.* 29 (11), 1723–1729. doi:10.1161/ATVBAHA.109.187179

Mashouri, L., Yousefi, H., Aref, A. R., Ahadi, A. M., Molaei, F., and Alahari, S. K. (2019). Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. *Mol. Cancer* 18 (1), 75. doi:10.1186/s12943-019-0991-5

Mathieu, M., Martin-Jaular, L., Lavieu, G., and Théry, C. (2019). Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication. *Nat. Cell Biol.* 21 (1), 9–17. doi:10.1038/s41556-018-0250-9

Mazini, L., Rochette, L., Admou, B., Amal, S., and Malka, G. (2020). Hopes and limits of adipose-derived stem cells (ADSCs) and mesenchymal stem cells (MSCs) in wound healing. *Int. J. Mol. Sci.* 21 (4), 1306. doi:10.3390/ijms21041306

Mitchell, R., Mellows, B., Sheard, J., Antonioli, M., Kretz, O., Chambers, D., et al. (2019). Secretome of adipose-derived mesenchymal stem cells promotes skeletal muscle regeneration through synergistic action of extracellular vesicle cargo and soluble proteins. *Stem Cell Res. Ther.* 10 (1), 116. doi:10.1186/s13287-019-1213-1

Miyahara, Y., Nagaya, N., Kataoka, M., Yanagawa, B., Tanaka, K., Hao, H., et al. (2006). Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. *Nat. Med.* 12 (4), 459–465. doi:10.1038/nm1391

Molinuevo, J. L., Ayton, S., Batrla, R., Bednar, M. M., Bittner, T., Cummings, J., et al. (2018). Current state of Alzheimer's fluid biomarkers. *Acta Neuropathol.* 136 (6), 821–853. doi:10.1007/s00401-018-1932-x

Mou, S., Zhou, M., Li, Y., Wang, J., Yuan, Q., Xiao, P., et al. (2019). Extracellular vesicles from human adipose-derived stem cells for the improvement of angiogenesis and fat-grafting application. *Plast. Reconstr. Surg.* 144 (4), 869–880. doi:10.1097/PRS.0000000000006046

O'Brien, K., Breyne, K., Ughetto, S., Laurent, L. C., and Breakefield, X. O. (2020). RNA delivery by extracellular vesicles in Mammalian cells and its applications. *Nat. Rev. Mol. Cell Biol.* 21 (10), 585–606. doi:10.1038/s41580-020-0251-y

Ormazabal, V., Nair, S., Elfeky, O., Aguayo, C., Salomon, C., and Zuñiga, F. A. (2018). Association between insulin resistance and the development of cardiovascular disease. *Cardiovasc Diabetol.* 17 (1), 122. doi:10.1186/s12933-018-0762-4

Pegtel, D. M., and Gould, S. J. (2019). Exosomes. *Annu. Rev. Biochem.* 88, 487–514. doi:10.1146/annurev-biochem-013118-111902

Pomatto, M., Gai, C., Negro, F., Cedrino, M., Grange, C., Ceccotti, E., et al. (2021). Differential therapeutic effect of extracellular vesicles derived by bone marrow and adipose mesenchymal stem cells on wound healing of diabetic ulcers and correlation to their cargoes. *Int. J. Mol. Sci.* 22 (8), 3851. doi:10.3390/ijms22083851

Qi, X., Zhang, J., Yuan, H., Xu, Z., Li, Q., Niu, X., et al. (2016). Exosomes secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells repair critical-sized bone defects through enhanced angiogenesis and osteogenesis in osteoporotic rats. *Int. J. Biol. Sci.* 12 (7), 836–849. doi:10.7150/ijbs.14809

Qian, L., Pi, L., Fang, B. R., and Meng, X. X. (2021). Adipose mesenchymal stem cell-derived exosomes accelerate skin wound healing *via* the lncRNA H19/miR-19b/SOX9 axis. *Lab. Invest* 101 (9), 1254–1266. doi:10.1038/s41374-021-00611-8

Qin, Y., Sun, R., Wu, C., Wang, L., and Zhang, C. (2016). Exosome: a novel approach to stimulate bone regeneration through regulation of osteogenesis and angiogenesis. *Int. J. Mol. Sci.* 17 (5), 712. doi:10.3390/ijms17050712

Rani, S., Ryan, A. E., Griffin, M. D., and Ritter, T. (2015). Mesenchymal stem cell-derived extracellular vesicles: toward cell-free therapeutic applications. *Mol. Ther.* 23 (5), 812–823. doi:10.1038/mt.2015.44

Rau, C. S., Kuo, P. J., Wu, S. C., Huang, L. H., Lu, T. H., Wu, Y. C., et al. (2021). Enhanced nerve regeneration by exosomes secreted by adipose-derived stem cells with or without FK506 stimulation. *Int. J. Mol. Sci.* 22 (16), 8545. doi:10.3390/ijms22168545

Rehman, J., Traktuev, D., Li, J., Merfeld-Clauss, S., Temm-Grove, C. J., Bovenkerk, J. E., et al. (2004). Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 109 (10), 1292–1298. doi:10.1161/01.CIR.0000121425.42966.F1

Ren, S., Chen, J., Duscher, D., Liu, Y., Guo, G., Kang, Y., et al. (2019a). Microvesicles from human adipose stem cells promote wound healing by optimizing cellular functions *via* AKT and ERK signaling pathways. *Stem Cell Res. Ther.* 10 (1), 47. doi:10.1186/s13287-019-1152-x

Ren, Z. W., Zhou, J. G., Xiong, Z. K., Zhu, F. Z., and Guo, X. D. (2019b). Effect of exosomes derived from MiR-133b-modified ADSCs on the recovery of neurological function after SCI. *Eur Rev Med Pharmacol Sci.* 23 (1), 52–60. doi:10.26355/eurrev_201901_16747

Rodriguez, A. M., Elabd, C., Amri, E. Z., Ailhaud, G., and Dani, C. (2005). The human adipose tissue is a source of multipotent stem cells. *Biochimie* 87 (1), 125–128. doi:10.1016/j.biochi.2004.11.007

Sanchez, A., Tripathy, D., Yin, X., Luo, J., Martinez, J., and Grammas, P. (2012). Pigment epithelium-derived factor (PEDF) protects cortical neurons *in vitro* from oxidant injury by activation of extracellular signal-regulated kinase (ERK) 1/2 and induction of Bcl-2. *Neurosci. Res.* 72 (1), 1–8. doi:10.1016/j.neures.2011.09.003

Sen, S. (2019). Adult stem cells: beyond regenerative tool, more as a bio-marker in obesity and diabetes. *Diabetes Metab. J.* 43 (6), 744–751. doi:10.4093/dmj.2019.0175

Shafei, S., Khanmohammadi, M., Heidari, R., Ghanbari, H., Taghdiri Nooshabadi, V., Farzamfar, S., et al. (2020). Exosome loaded alginate hydrogel promotes tissue regeneration in full-thickness skin wounds: an *in vivo* study. *J. Biomed. Mater Res. A* 108 (3), 545–556. doi:10.1002/jbm.a.36835

Shiekh, P. A., Singh, A., and Kumar, A. (2020). Exosome laden oxygen releasing antioxidant and antibacterial cryogel wound dressing OxOBand alleviate diabetic and infectious wound healing. *Biomaterials* 249, 120020. doi:10.1016/j.biomaterials.2020.120020

Song, B. W., Lee, C. Y., Kim, R., Kim, W. J., Lee, H. W., Lee, M. Y., et al. (2021). Multiplexed targeting of miRNA-210 in stem cell-derived extracellular vesicles promotes selective regeneration in ischemic hearts. *Exp. Mol. Med.* 53 (4), 695–708. doi:10.1038/s12276-021-00584-0

Stenmark, H. (2009). Rab GTPases as coordinators of vesicle traffic. *Nat. Rev. Mol. Cell Biol.* 10 (8), 513–525. doi:10.1038/nrm2728

Stuffers, S., Sem Wegner, C., Stenmark, H., and Brech, A. (2009). Multivesicular endosome biogenesis in the absence of ESCRTs. *Traffic* 10 (7), 925–937. doi:10.1111/j.1600-0854.2009.00920.x

Thankam, F. G., Sedighim, S., Kuan, R., and Agrawal, D. K. (2023). Ischemia challenged epicardial adipose tissue stem cells-derived extracellular vesicles alter the gene expression of cardiac fibroblasts to cardiomyocyte like phenotype. *Transl. Res.* 254, 54–67. doi:10.1016/j.trsl.2022.10.004

Trzyna, A., and Banaś-Ząbczyk, A. (2021). Adipose-derived stem cells secretome and its potential application in stem cell-free therapy. *Biomolecules* 11 (6), 878. doi:10.3390/biom11060878

Urabe, F., Kosaka, N., Ito, K., Kimura, T., Egawa, S., and Ochiya, T. (2020). Extracellular vesicles as biomarkers and therapeutic targets for cancer. *Am. J. Physiol. Cell Physiol.* 318 (1), C29–c39. doi:10.1152/ajpcell.00280.2019

van Niel, G., D'Angelo, G., and Raposo, G. (2018). Shedding light on the cell biology of extracellular vesicles. *Nat. Rev. Mol. Cell Biol.* 19 (4), 213–228. doi:10.1038/nrm.2017.125

Veronesi, F., Maglio, M., Tschon, M., Aldini, N. N., and Fini, M. (2014). Adipose-derived mesenchymal stem cells for cartilage tissue engineering: State-Of-the-art in *in vivo* studies. *J. Biomed. Mater Res. A* 102 (7), 2448–2466. doi:10.1002/jbm.a.34896

Wang, C., Song, W., Chen, B., Liu, X., and He, Y. (2019). Exosomes isolated from adipose-derived stem cells: a new cell-free approach to prevent the muscle degeneration associated with torn rotator cuffs. *Am. J. Sports Med.* 47 (13), 3247–3255. doi:10.1177/0363546519876323

Wang, J., Yi, Y., Zhu, Y., Wang, Z., Wu, S., Zhang, J., et al. (2020). Effects of adipose-derived stem cell released exosomes on wound healing in diabetic mice. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi* 34 (1), 124–131. doi:10.7507/1002-1892.201903058

Wang, J., Wu, H., Peng, Y., Zhao, Y., Qin, Y., Zhang, Y., et al. (2021). Hypoxia adipose stem cell-derived exosomes promote high-quality healing of diabetic wound involves activation of PI3K/Akt pathways. *J. Nanobiotechnology* 19 (1), 202. doi:10.1186/s12951-021-00942-0

Xing, X., Han, S., Cheng, G., Ni, Y., Li, Z., and Li, Z. (2020a). Proteomic analysis of exosomes from adipose-derived mesenchymal stem cells: a novel therapeutic strategy for tissue injury. *Biomed. Res. Int.* 2020, 6094562. doi:10.1155/2020/6094562

Xing, X., Li, Z., Yang, X., Li, M., Liu, C., Pang, Y., et al. (2020b). Adipose-derived mesenchymal stem cells-derived exosome-mediated microRNA-342-5p protects endothelial cells against atherosclerosis. *Aging (Albany NY)* 12 (4), 3880–3898. doi:10.18632/aging.102857

Xu, F., Xiang, Q., Huang, J., Chen, Q., Yu, N., Long, X., et al. (2019). Exosomal miR-423-5p mediates the proangiogenic activity of human adipose-derived stem cells by targeting Sufu. *Stem Cell Res. Ther.* 10 (1), 106. doi:10.1186/s13287-019-1196-y

Yang, C., Luo, L., Bai, X., Shen, K., Liu, K., Wang, J., et al. (2020). Highly-expressed micoRNA-21 in adipose derived stem cell exosomes can enhance the migration and proliferation of the HaCaT cells by increasing the MMP-9 expression through the PI3K/AKT pathway. *Arch. Biochem. Biophys.* 681, 108259. doi:10.1016/j.abb.2020.108259

Yang, Z., Yang, Y., Xu, Y., Jiang, W., Shao, Y., Xing, J., et al. (2021). Biomimetic nerve guidance conduit containing engineered exosomes of adipose-derived stem cells promotes peripheral nerve regeneration. *Stem Cell Res. Ther.* 12 (1), 442. doi:10.1186/s13287-021-02528-x

Yang, J., Wang, B., Wang, Y., Feng, C., Chen, L., Liu, Y., et al. (2022). Exosomes derived from adipose mesenchymal stem cells carrying miRNA-22-3p promote schwann cells proliferation and migration through downregulation of PTEN. *Dis. Markers* 2022, 7071877. doi:10.1155/2022/7071877

Yapca, O. E., Borekci, B., and Suleyman, H. (2013). Ischemia-reperfusion damage. *Eurasian J. Med.* 45 (2), 126–127. doi:10.5152/eajm.2013.24

Yin, G., Yu, B., Liu, C., Xie, Z., and Hu, Y. (2021). Exosomes produced by adipose-derived stem cells inhibit schwann cells autophagy and promote the regeneration of the myelin sheath. *Int. J. Biochem. Cell Biol.* 132, 105921. doi:10.1016/j.biocel.2021.105921

Younas, N., Fernandez Flores, L. C., Hopfner, F., Höglinger, G. U., and Zerr, I. (2022). A new paradigm for diagnosis of neurodegenerative diseases: peripheral exosomes of brain origin. *Transl. Neurodegener.* 11 (1), 28. doi:10.1186/s40035-022-00301-5

Yu, S., Cheng, Y., Zhang, L., Yin, Y., Xue, J., Li, B., et al. (2019). Treatment with adipose tissue-derived mesenchymal stem cells exerts anti-diabetic effects, improves long-term complications, and attenuates inflammation in type 2 diabetic rats. *Stem Cell Res. Ther.* 10 (1), 333. doi:10.1186/s13287-019-1474-8

Yu, F., Witman, N., Yan, D., Zhang, S., Zhou, M., Yan, Y., et al. (2020). Human adipose-derived stem cells enriched with VEGF-modified mRNA promote angiogenesis and long-term graft survival in a fat graft transplantation model. *Stem Cell Res. Ther.* 11 (1), 490. doi:10.1186/s13287-020-02008-8

Yue, B., Yang, H., Wang, J., Ru, W., Wu, J., Huang, Y., et al. (2020). Exosome biogenesis, secretion and function of exosomal miRNAs in skeletal muscle myogenesis. *Cell Prolif.* 53 (7), e12857. doi:10.1111/cpr.12857

Zhai, L., Shen, H., Sheng, Y., and Guan, Q. (2021). ADMSC Exo-MicroRNA-22 improve neurological function and neuroinflammation in mice with alzheimer's disease. *J. Cell Mol. Med.* 25 (15), 7513–7523. doi:10.1111/jcmm.16787

Zhang, L., and Yu, D. (2019). Exosomes in cancer development, metastasis, and immunity. *Biochim. Biophys. Acta Rev. Cancer* 1871 (2), 455–468. doi:10.1016/j.bbcan.2019.04.004

Zhang, J., Liu, X., Li, H., Chen, C., Hu, B., Niu, X., et al. (2016). Exosomes/Tricalcium phosphate combination scaffolds can enhance bone regeneration by activating the PI3K/Akt signaling pathway. *Stem Cell Res. Ther.* 7 (1), 136. doi:10.1186/s13287-016-0391-3

Zhang, S., Chuah, S. J., Lai, R. C., Hui, J. H. P., Lim, S. K., and Toh, W. S. (2018a). MSC exosomes mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity. *Biomaterials* 156, 16–27. doi:10.1016/j.biomaterials.2017.11.028

Zhang, W., Bai, X., Zhao, B., Li, Y., Zhang, Y., Li, Z., et al. (2018b). Cell-free therapy based on adipose tissue stem cell-derived exosomes promotes wound healing *via* the PI3K/Akt signaling pathway. *Exp. Cell Res.* 370 (2), 333–342. doi:10.1016/j.yexcr.2018.06.035

Zhou, Y., Zhang, X. L., Lu, S. T., Zhang, N. Y., Zhang, H. J., Zhang, J., et al. (2022). Human adipose-derived mesenchymal stem cells-derived exosomes encapsulated in pluronic F127 hydrogel promote wound healing and regeneration. *Stem Cell Res. Ther.* 13 (1), 407. doi:10.1186/s13287-022-02980-3

Zhu, Y., Zhang, J., Hu, X., Wang, Z., Wu, S., and Yi, Y. (2020). Extracellular vesicles derived from human adipose-derived stem cells promote the exogenous angiogenesis of fat grafts *via* the let-7/AGO1/VEGF signalling pathway. *Sci. Rep.* 10 (1), 5313. doi:10.1038/s41598-020-62140-6

Zhu, A., Liu, N., Shang, Y., Zhen, Y., and An, Y. (2022). Signaling pathways of adipose stem cell-derived exosomes promoting muscle regeneration. *Chin. Med. J. Engl.* 135 (21), 2525–2534. doi:10.1097/CM9.0000000000002404

Zuk, P. A., Zhu, M., Mizuno, H., Huang, J., Futrell, J. W., Katz, A. J., et al. (2001). Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng.* 7 (2), 211–228. doi:10.1089/107632701300062859

Zylbersztejn, K., and Galli, T. (2011). Vesicular traffic in cell navigation. *Febs J.* 278 (23), 4497–4505. doi:10.1111/j.1742-4658.2011.08168.x

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Early intervention with extracellular vesicles derived from human umbilical cord mesenchymal stem cells enhances survival and functional recovery after spinal cord injury in rats

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Introduction: Spinal cord injury (SCI) is a devastating condition with high mortality and limited treatment options. Mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) have emerged as promising cell-free therapies due to their immunomodulatory and neuroprotective properties. Here, we evaluated the therapeutic potential of EVs derived from human umbilical cord MSCs in a rat model of SCI.

Methods: Adult male Wistar rats were randomized into three groups: control, SCI, and SCI treated with a single intralesional dose of EVs. Locomotor recovery was assessed by the Basso, Beattie & Bresnahan (BBB) score, while survival, neuroinflammation, histological alterations, and biodistribution were systematically analyzed.

Results: EV administration improved 30-day survival, and locomotor performance from day 7, and was associated with sustained reductions in pro-inflammatory cytokines (IL-1β, TNF-α) alongside increased levels of anti-inflammatory cytokines (IL-10) and neurotrophic factors (BDNF). Histological and immunofluorescence analyses showed attenuated microglial activation and astrocytic reactivity, accompanied by reduced lesion size and glial scar formation. *In vivo* imaging demonstrated accumulation of labeled EVs at the injury site, with peak retention at day 7 post-injection.

Discussion: Together, these findings demonstrate that early intralesional delivery of MSC-EVs enhances survival, modulates the inflammatory response, and promotes functional recovery after SCI, supporting further translational development of EV-based interventions for SCI.

KEYWORDS

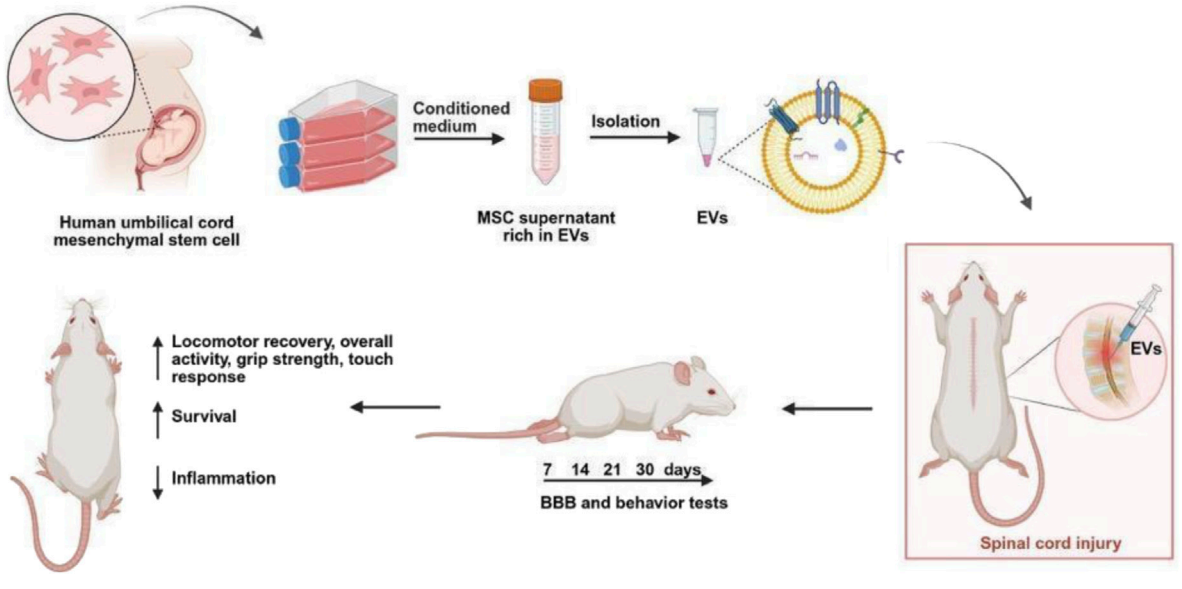
spinal cord injury, mesenchymal stem cells, extracellular vesicles, survival, neuroinflammation

1 Introduction

Spinal cord injury (SCI) remains a major public health challenge, both in terms of mortality and morbidity, due to its high incidence and the scarcity of effective therapeutic options (Courtine and Sofroniew, 2019). Most cases arise from traumatic events leading to spinal fractures and/or dislocations, often resulting in permanent paralysis due to the limited regenerative capacity of the central nervous system (CNS) (Holmes, 2017). Mortality is particularly elevated in the first year after injury - largely due to respiratory complications, infections, and thromboembolic events - and survival rates of SCI patients remain reduced compared with the general population despite advances in acute care and rehabilitation, reinforcing the urgent need for interventions capable of mitigating early mortality while promoting functional recovery (Zadra et al., 2024).

Following the primary insult, SCI initiates a cascade of secondary injury mechanisms that amplify tissue damage and neurological deficits (Eli et al., 2021). Disruption of the blood-spinal cord barrier permits the infiltration of peripheral immune cells and plasma proteins, which synergize with the rapid activation of resident microglia (Sterner et al., 2023). Activated microglia, together with infiltrating macrophages, amplify tissue damage by releasing pro-inflammatory cytokines, ROS, and proteases. In parallel, astrocytes undergo reactive transformation, initially attempting to restore homeostasis but later contributing to glial scar formation and deposition of inhibitory extracellular matrix molecules (Tran et al., 2018). Ultimately, these events converge to neuronal death and glial cell activation, particularly of astrocytes, microglia, and endothelial cells, which in turn release pro-inflammatory cytokines and chemokines, amplifying a widespread inflammatory response (Zhang M., et al., 2021). Excitotoxicity, oxidative stress, ischemia, and edema further drive neuronal and oligodendrocyte death. Ultimately, SCI promotes a hostile inflammatory microenvironment that hampers regeneration, promotes gliosis, and creates long-lasting barriers to functional recovery (Zhang M. et al., 2021; Li et al., 2017).

Intercellular communication plays a central role in orchestrating these injury responses, with extracellular vesicles (EVs) emerging as key mediators of crosstalk between neurons, glia, endothelial cells, and infiltrating immune cells (Cao et al., 2025). According to the International Society for Extracellular Vesicles (ISEV), EVs comprise a heterogeneous population of membrane-bound vesicles (30–1000 nm in diameter) secreted by virtually all cell types. EVs carry proteins, lipids, and nucleic acids, including mRNAs and microRNAs, that can either propagate inflammation or promote repair, depending on their cellular origin (Welsh et al., 2024; Ragni et al., 2025; Sohrabi et al., 2022). Harnessing this natural signaling system, therapeutic strategies have increasingly focused on mesenchymal stem cell (MSC)-derived EVs, which combine the neuroprotective and immunomodulatory effects of MSCs with the practical advantages of a cell-free product (Volarevic et al., 2017; Wang et al., 2023).



GRAPHICAL ABSTRACT

Therapeutic efficacy has been previously demonstrated in MSC-based therapy studies using cells obtained from different sources, including bone marrow-derived MSCs (BM-MSCs) (Huang et al., 2023), adipose tissue-derived MSCs (ASCs) (Vialle et al., 2023), and human umbilical cord-derived MSCs (Sun et al., 2023). In SCI, most preclinical studies with MSC-EVs have primarily focused on motor recovery and histological outcomes. However, a translational gap remains: while SCI in patients is strongly associated with high early mortality due to systemic complications, most preclinical studies have limited their analyses to motor and histological endpoints. Whether EV-based interventions can also extend survival has not been addressed. Here, we evaluated the therapeutic efficacy of MSC-derived EVs in a rat model of acute SCI, focusing on survival, locomotor recovery, neuroinflammation, and neurotrophic signaling.

2 Materials and methods

2.1 MSC isolation and culture

Human Wharton's jelly-derived MSCs were obtained and banked under approval of the Research Ethics Committee of São Rafael Hospital (CAAE: 09803819.30000.0048), with written informed consent provided by all participants. MSCs were sourced from the study biorepository at the Center for Biotechnology and Cell Therapy, Hospital São Rafael (D'OR Institute for Research and Education) at passage 3 (P3). Approximately 6×10^3 cells/cm^2 were seeded into T175 culture flasks and expanded in low-glucose DMEM (Gibco), supplemented with 2 mM L-glutamine (Gibco), Pen-Strep (Gibco), and 3% platelet lysate. Cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2, and the medium was replaced every 3–4 days. At 70%–80% confluence, cultures were washed and maintained for 48 h in complete low-glucose DMEM without platelet lysate. The conditioned medium was then collected for EV isolation.

2.2 MSC characterization

2.2.1 Immunophenotyping

MSCs were characterized by flow cytometry using a Navios cytometer (Beckman Coulter) and the BD Stemflow™ hMSC Analysis Kit. Cells were stained with antibodies for positive markers (CD73, CD90, CD105) and negative markers (CD34, CD45, CD11b, CD19, HLA-DR). After incubation and washing, samples (≥10,000 events) were analyzed with Kaluza software. MSCs were defined as CD73$^+$CD90$^+$CD105$^+$ and negative for hematopoietic/endothelial markers. Appropriate controls were included for gating and compensation.

2.2.2 Multi-lineage differentiation

MSCs were induced to differentiate into osteogenic, chondrogenic, and adipogenic lineages using commercial kits (StemPro, Thermo Fisher). Differentiation was confirmed by Alizarin Red staining (osteogenesis), Alcian Blue (chondrogenesis), and Oil Red O (adipogenesis), after 14 days (osteogenic and adipogenic) or 21 days (chondrogenic). Representative images were acquired with a Nikon Eclipse Ti1 inverted microscope (Nikon, Tokyo, Japan).

2.2.3 Cytogenetic analysis

Cytogenetic evaluation was performed by G-band karyotyping to detect structural or numerical chromosomal alterations. MSCs were cultured at 4000 cells/cm^2 and, at 80% confluence, treated with 0.1 μg/mL colcemid (Gibco). Cells were exposed to hypotonic 0.075 M KCl, fixed with Carnoy's solution (3:1 methanol:acetic acid), and slides were aged at 60 °C for 16 h before GTG banding. Twenty metaphases were analyzed under a BX61 microscope (Olympus) with a digital imaging system (Applied Spectral Imaging, Carlsbad, CA), and images were processed with Lucia Karyo software (Lucia Cytogenetics, Prague, Czech Republic).

2.2.4 Functional assays

The immunomodulatory potential of MSCs was evaluated by co-culture with activated lymphocytes. PBMCs (2×10^5/well) were stimulated with CD3/CD28 beads (Thermo Fisher Scientific, 1:5 bead-to-cell ratio) and co-cultured with mitomycin C-treated MSCs at a 1:10 MSC:PBMC ratio, as previously described (Silva, et al., 2025). After 5 days at 37 °C/5% CO_2, supernatants were harvested for cytokine quantification. TNF-α and IL-10 levels were measured by ELISA (R&D Systems, Minneapolis, MN, United States), with absorbance read at 450 nm (540 nm correction) on a GloMAX Explorer. Senescence was assessed with the CellEvent™ Senescence Green Detection Kit (Thermo Fisher) following manufacturer's instructions. Nuclei were counterstained with Hoechst (1:1000). Hydrogen peroxide–treated MSCs (100–200 μmol/L for 2 h at 37 °C) served as positive controls. Quantification was performed in quadruplicate, analyzing 20 quadrants per well using an Operetta high-performance microscope (PerkinElmer, Waltham, MA, United States). Images were analyzed with Harmony 3.8 software (PerkinElmer), and data were expressed as mean ± SD of SA-βGal-positive cells.

2.3 EV isolation

The conditioned medium was centrifuged at 3000 *g* for 30 min at 4 °C to remove debris and filtered through a 0.22 μm membrane. Supernatants were concentrated using Amicon® Ultra-15 Centrifugal Filter Units (Merck Millipore, Cork, Ireland), with 100 kDa pore, followed by overnight incubation with the Total Exosome Isolation reagent (Thermo Fisher Scientific) according to manufacturer's instructions. The mixture was centrifuged at 10,000 × g for 1 h, and the resulting EV pellet was resuspended in PBS. EVs were stored at −80 °C until use.

2.3.1 EV characterization

Nanoparticle tracking analysis (NTA) was performed with a ZetaView PMX-220 system (Particle Metrix). Measurements were taken in triplicate across 11 positions. Calibration was performed with 100 nm polystyrene beads, and data were processed with ZetaView software. Protein concentration was determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). To evaluate morphology, transmission electron microscopy (TEM) was

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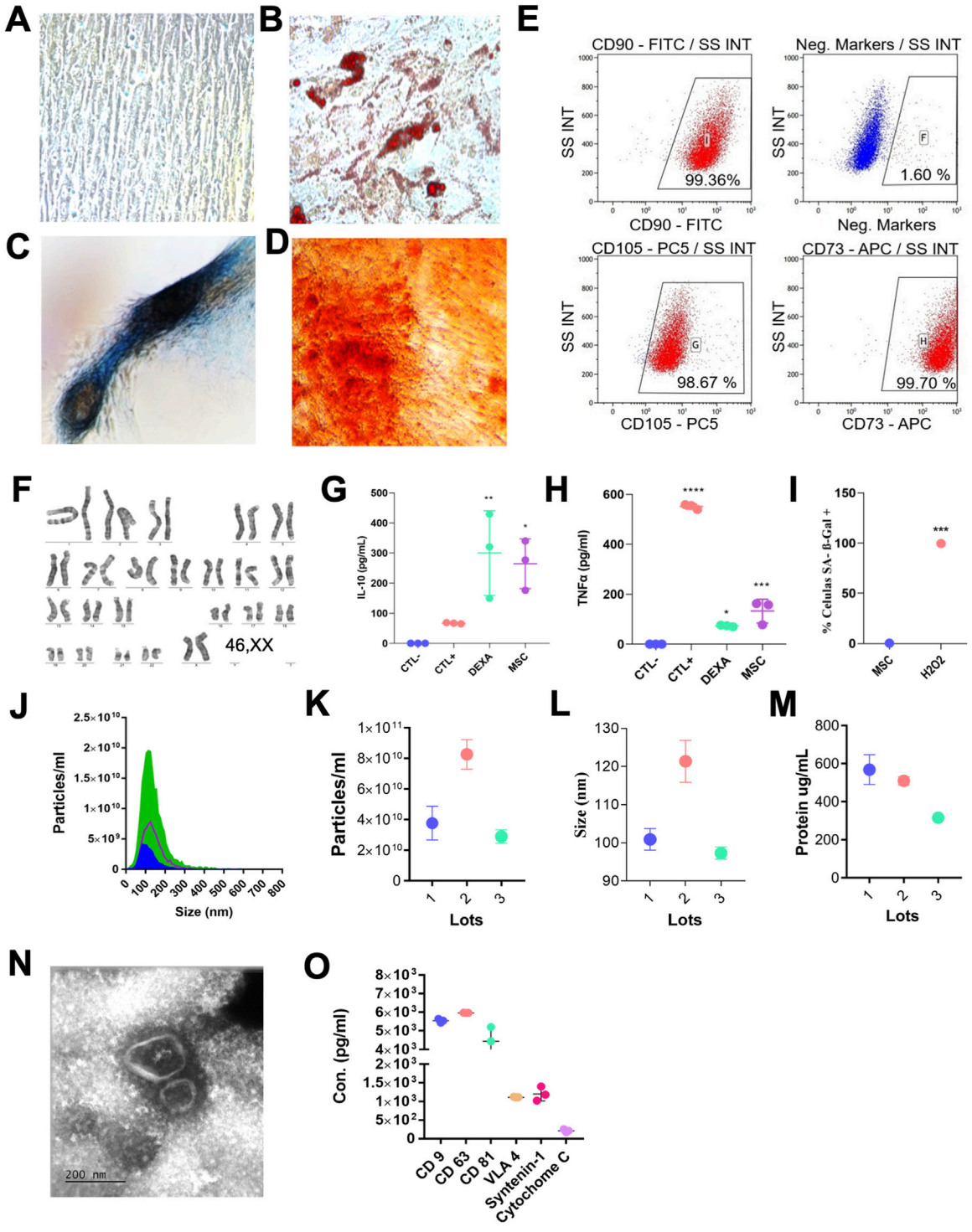


FIGURE 1
Characterization of MSCs and EVs. **(A)** Representative image of undifferentiated MSCs and differentiated MSCs toward adipogenic **(B)**, chondrogenic **(C)**, and osteogenic **(D)** lineages *in vitro*. **(E)** Representative flow-cytometry dot plots showing positive expression of MSC surface markers (CD73, CD90, CD105) and lack of hematopoietic markers (CD34, CD45, CD11b, CD19, HLA-DR). Data representative of three independent analyses. **(F)** Representative G-banded karyotype confirming genomic stability (46,XX). **(G,H)** Immunomodulatory potency of MSCs assessed in co-culture with activated PBMCs, showing IL-10 upregulation and TNF-α suppression. Experimental conditions: Ctl−, unstimulated PBMCs; Ctl+, PBMCs stimulated with anti-CD3/CD28; Dexa, PBMCs stimulated with anti-CD3/CD28 and treated with dexamethasone; MSC, PBMCs stimulated with anti-CD3/CD28 and co-cultured with MSCs for 5 days. Cytokines were quantified by ELISA. **(I)** Quantification of SA-β-gal-positive cells showing low senescence levels in MSCs compared with H_2O_2-treated controls. **(J–L)** Nanoparticle tracking analysis (NTA) showing EV particle size distribution and concentration obtained from

(*Continued*)

FIGURE 1 (Continued)
three independent EV batches. **(M)** Total protein content of EV samples (μg/mL); values represent mean ± SD of three EV lots. **(N)** Representative transmission electron microscopy image showing typical cup-shaped EV morphology (50–150 nm; scale bar, 200 nm). **(O)** EV protein marker profiling showing strong expression of canonical exosomal markers (CD9, CD63, CD81) together with VLA-4 and syntenin-1, and minimal levels of cytochrome C (assessed by Luminex; n = 3 EV lots). Statistical analysis: **(G,H)** one-way ANOVA with Tukey multiple-comparison test versus Ctl+; **(I)** unpaired t-test (two-tailed). Data are presented as mean ± SD; n = 3 biological replicates per condition. *p < 0.05; **p < 0.01; ***p < 0.001.

conducted using a JEOL 1230 microscope at 80 kV. Samples (10 μL) were adsorbed onto formvar carbon-coated copper grids for 5 min, stained with 2% uranyl citrate for 1 min, and air-dried for 24 h, as previously described (Costa-Ferro et al., 2024). The presence of EV markers (CD9, CD63, CD81) was confirmed with the ProcartaPlex™ Human Exosome 6-plex Panel on a Luminex MAGPIX system (Luminex Corporation, Austin, TX, United States). Data were analyzed using Luminex software.

2.4 SCI model

All animal protocols were approved by the Ethics Committee of Fiocruz-BA (#020/21, issued on March 15, 2021). Adult male Wistar rats (45–50 days, 212.6 ± 5.8 g) were maintained under standard conditions (22 °C ± 1 °C, 60% humidity, 12 h light/dark cycle) with free access to food and water. After 1 week of acclimatization, the rats were randomly assigned to experimental groups: Control (no injury), SCI (vehicle only), and SCI + EV (treated with a single intralesional dose of EVs, 1×10^9 particles in 10 μL PBS).

Surgical procedure: SCI was induced as described previously (Vanický et al., 2001). Briefly, rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg, i. p.), and a midline incision was made to expose T9. After laminectomy, a groove was drilled in T11 to guide insertion of a 2-French Fogarty catheter (Baxter Healthcare Corporation, Irvine, CA) into the epidural space. The balloon was inflated with 10 μL saline for 90 s, then deflated and removed. EVs were administered at the lesion site. Then, the catheter was deflated and removed and the soft tissues and skin were sutured in anatomical layers. The SCI model was established and EVs were injected into the lesion site. Post-injury, the muscle and skin layers were sutured with 5–0 silk sutures, and the rats were placed on a heating pad to maintain body temperature during recovery.

Postoperative care: Animals received postoperative care including a single dose of dipyrone (125 mg/kg, i. p.) for analgesia, hydration, daily manual bladder expression until spontaneous voiding, and monitoring for signs of distress or complications. Animals were euthanized at 24 h, 7 days, and 30 days post-injury for tissue collection.

2.4.1 *In vivo* biodistribution of DiR-labeled EVs

The biodistribution of MSC-derived EVs was assessed using the near-infrared lipophilic dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR; Invitrogen, United States), following labeling procedures previously described by our group (Costa-Ferro et al., 2024; Costa-Ferro et al., 2025). DiR-labeled EVs (1×10^9 particles in 10 μL PBS) were administered intralesionally immediately after SCI. *In vivo* fluorescence imaging was performed at 1, 7, and 14 days post-injection using the AMI HTX System (Spectral Instruments Imaging, Tucson, AZ, United States) with excitation/emission filters of 710/770 nm, and the fluorescence signal was quantified as total radiant efficiency using AMIView software. Identical regions of interest (ROIs) were applied for all animals, and background signal from control animals was subtracted.

2.4.2 Basso-beattie-bresnahan (BBB) scoring

The recovery of hind limb motor function on the 1st, 7th, and 14th days after injury was evaluated using the BBB scoring system (n = 7/group). During the test, each rat was placed in an open field and allowed to walk freely for a 5-min observation period. Hind limb movement was assessed on a 0–21 point scale. A score of 0 indicates complete paralysis with no observable leg movement, and a score of 21 represents normal locomotion. The BBB assesses various aspects of motor function, including hindlimb movement, joint movements, stepping ability, trunk stability, and coordination during open-field locomotion (Basso et al., 1995).

2.4.3 Survival and behavioral analyses

Clinical and general health parameters were assessed daily according to a 0–3 clinical scoring system (n = 7/group), assessed by two observers. Neuromuscular and autonomic parameters, including touch response, tail grip, tremors, muscle tone, piloerection, hypothermia, diarrhea, cyanosis, changes in urine color, and general activity were assessed daily for 1, 7 and 14 days following the administration of EVs using analog scales (n = 7/group) (Gale et al., 1985). Animal survival and behavioral recovery were monitored for up to 30 days after SCI, by two observers. Survival data were analyzed using the Kaplan–Meier estimator, and differences between groups were determined using the log-rank (Mantel–Cox) test (n = 20/group).

2.5 Quantitative real-time polymerase chain reaction (qRT-PCR)

Initially, spinal cord tissues were collected and snap-frozen. Total RNA was extracted using TRIzol™ (Thermo Fisher Scientific, Waltham, MA, United States) following the manufacturer's instructions. After extraction, the RNA purity was measured photometrically using NanoDrop™ 1000 (Thermo Fisher Scientific, Waltham, MA, United States). Then, RNA samples (1.5 μg per sample) were converted to cDNA using a high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific, Waltham, MA, United States). Aiming to quantify mRNA expression, TaqMan Master Mix and TaqMan™ probes were used at a final volume of 10 μL, following the manufacturer's

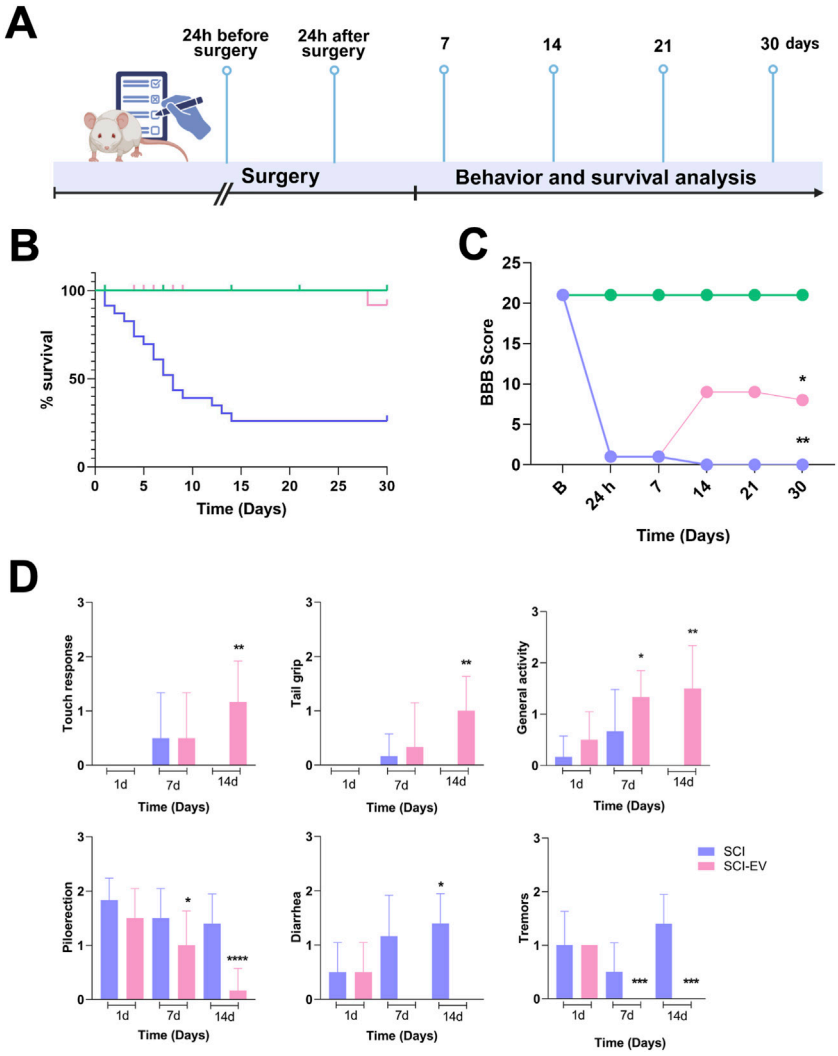


FIGURE 2
EVs improve survival and locomotor recovery after SCI. **(A)** Experimental timeline showing EV administration (1×10^9 particles/rat, administered intralesionally, immediately post-injury) and follow-up up to 30 days. **(B)** Survival curves of control (green), SCI (blue), and SCI-EVs (pink) groups showing markedly reduced survival after SCI, which was partially improved by EV treatment. Statistical analysis was performed using the log-rank (Mantel–Cox) test (n = 20 per group). *p < 0.05, **p < 0.01 vs. SCI. **(C)** Locomotor function assessed using the BBB scale by two independent blinded evaluators. SCI-EVs animals (pink) showed progressive functional improvement from day 14 onward compared to the SCI group (blue), while controls (green) maintained normal scores (n = 7 per group). **(D)** Clinical and behavioral recovery scoring according to a modified 0–3 scale (n = 7 per group). Upper panels: neuromuscular parameters (touch response, tail grip, general activity) were scored as 0 = severe impairment, 1 = moderate, 2 = mild, 3 = normal. Lower panels: autonomic/toxicity parameters (piloerection, diarrhea, tremors) were scored as 0 = normal, 1 = mild, 2 = moderate, 3 = severe. **(C,D)** Data are presented as mean ± SD. Statistical analysis was performed using two-way ANOVA followed by Dunnett's *post hoc* test. *p < 0.05, **p < 0.01, ***p < 0.001 compared with the SCI group.

instructions (all from Thermo Fisher Scientific, Waltham, MA, United States), for detection of interleukin 1 beta (IL-1β, Rn00580432_m1), tumor necrosis factor (TNF-α, Rn99999017_m1), interleukin 10 (IL-10, Rn01483988_g1) and brain-derived neurotrophic factor (BDNF, Rn02531967_s1) genes. Finally, the PCR amplification was performed in a ABI7500 real-time PCR system (Applied Biosystems, Foster City, CA, United States) under standard thermal cycling conditions. The thermal cycling was performed according to the manufacturer's recommendations. Relative mRNA expression levels were calculated using the comparative ΔΔCt method. Briefly, the Ct values of target genes were normalized to GAPDH (ΔCt), and fold changes in expression were determined relative to the control group (ΔΔCt).

2.6 Histological analysis

Spinal segments around the lesion center were removed and fixed 48 h in 10% formaldehyde. After stepwise dehydration with different concentrations of ethanol solution, the samples were paraffin-embedded, continuous transverse sections were prepared, hematoxylin and eosin (H&E) staining was performed according to the manufacturer's instructions and cut into 5 μm sections. Images were collected using a digital pathological section scanning system (Zeiss image.Z.2, Oberkochen, Germany). The cellular infiltrates and necrosis were classified as mild (0), moderate (1), and extensive (2). The prepared slides were then examined under a light microscope. Cellular infiltration and tissue necrosis were

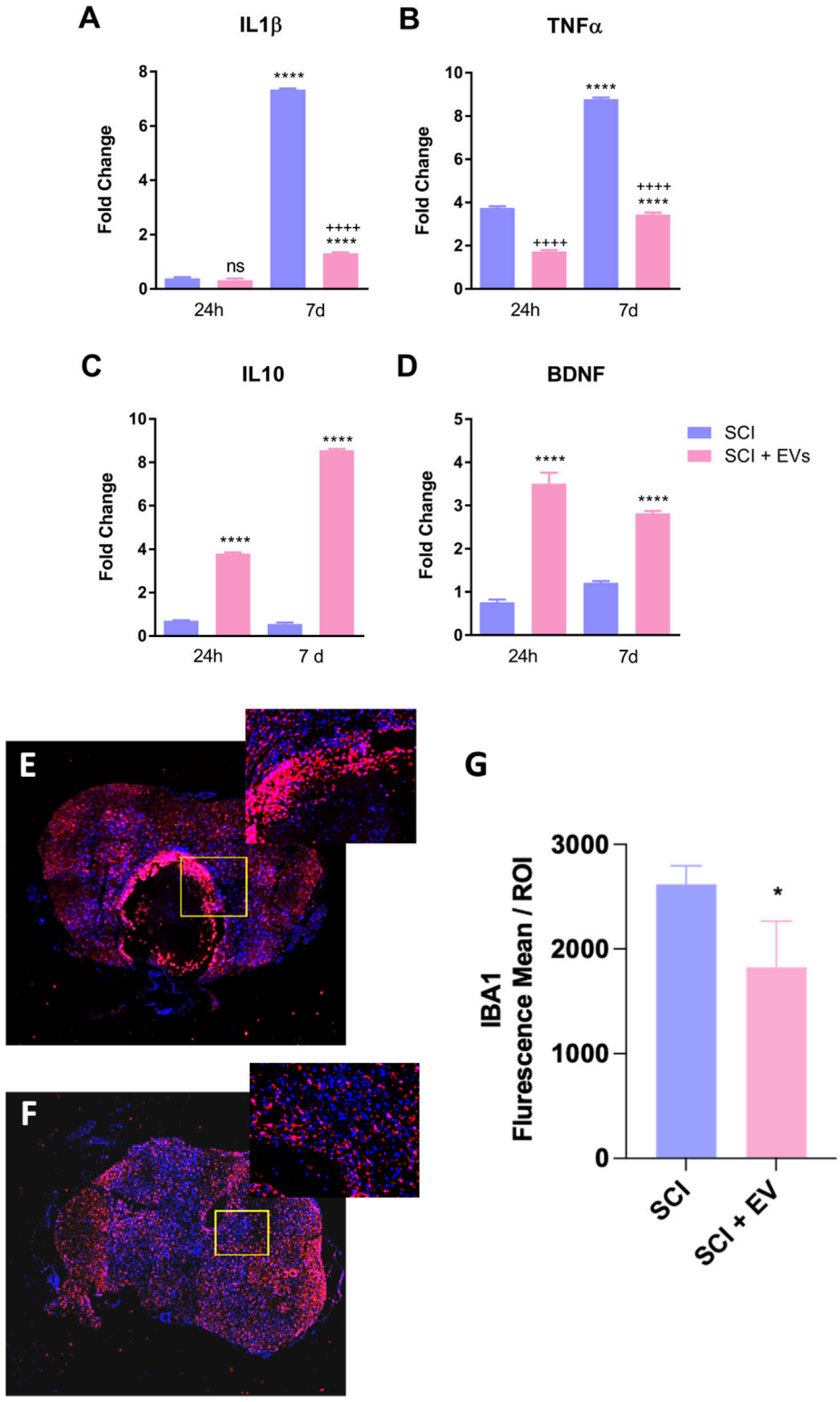


FIGURE 3
EV treatment modulates inflammatory cytokines and microglial activation after spinal cord injury. Relative gene expression of **(A)** IL-1β, **(B)** TNF-α, **(C)** IL-10, and **(D)** BDNF in SCI and SCI-EV groups was determined by RT-qPCR. Data were normalized to GAPDH and expressed as fold change relative to non-injured controls. **(E,F)**. Values represent mean ± SD (n = 7 per group/time point, one-way ANOVA with Tukey multiple-comparison test). Representative confocal images of IBA1 (red) and DAPI (blue) immunofluorescence in spinal cord sections from SCI **(E)** and SCI + EVs **(F)** groups at 7 days post-injury (200x magnification). Insets show higher magnification of the lesion border. **(G)** Quantification of IBA1 immunoreactivity (mean fluorescence intensity per ROI) showing decreased microglial activation in EV-treated rats (n = 5-7 per group, unpaired t-test, $p < 0.05$). ns = not significant; $p < 0.05$; $p < 0.001$ vs. SCI; "+" indicates downregulation relative to SCI; data presented as mean ± SD.

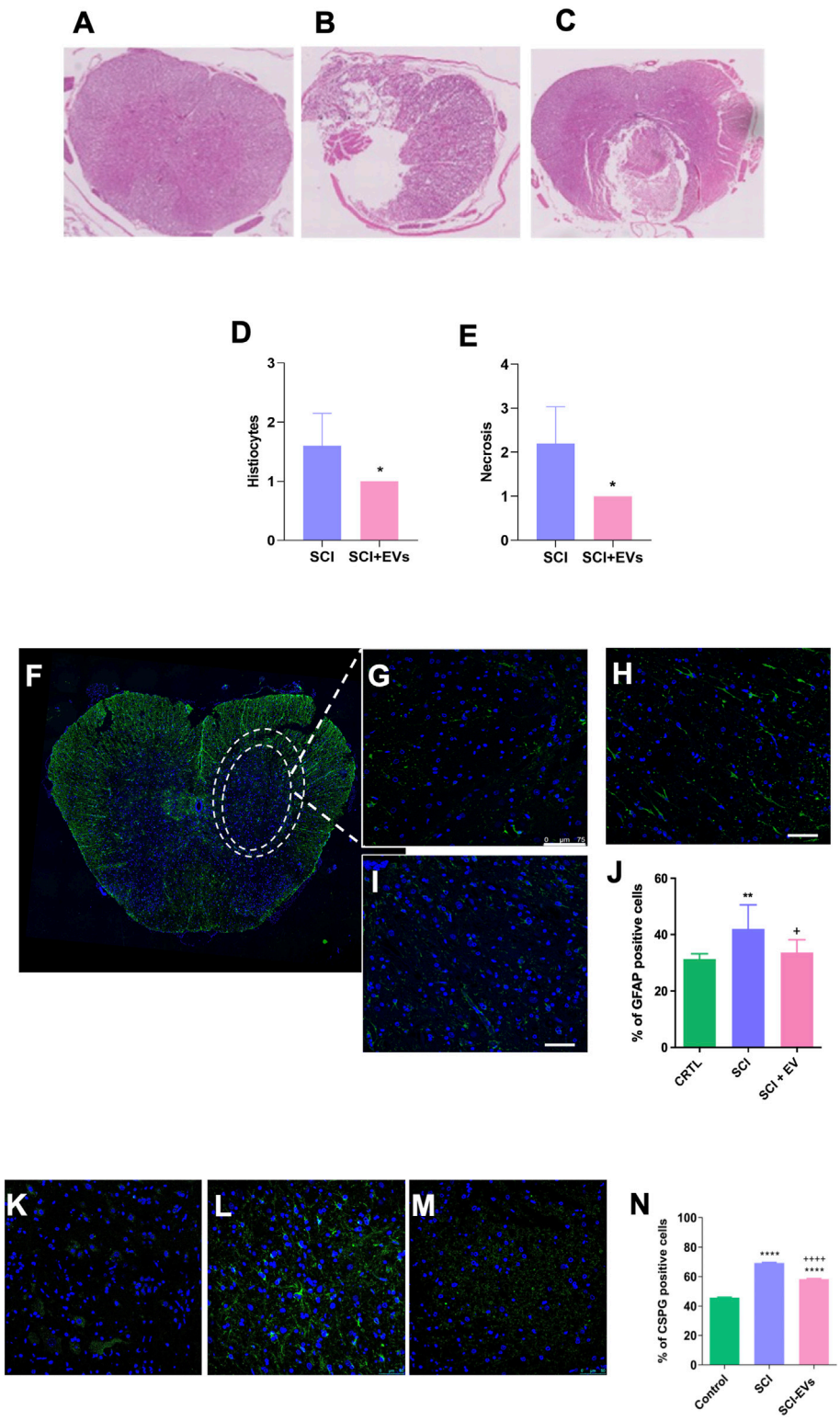


FIGURE 4
EV treatment reduces lesion severity, glial reactivity, and CSPG deposition after SCI. **(A–C)** Representative hematoxylin and eosin (H&E)-stained spinal cord sections from intact control, SCI, and SCI-EV groups at 7 days post-injury (200x magnification). Quantitative histological analysis reveals a significant reduction in histiocytic infiltration **(D)** and necrotic area **(E)** in the SCI-EVs group compared with SCI ($p < 0.05$). Representative immunofluorescence images of GFAP-positive astrocytes (green) with nuclear counterstaining (DAPI, blue) **(F,G)** intact control, **(H)** SCI, and **(I)** SCI-EVs (400x magnification). **(J)** Quantification of GFAP + astrocytes in the spinal cord. **(K–M)** CSPG representative immunofluorescence staining, with
(*Continued*)

FIGURE 4 (Continued)
CSPG (green), with a nuclear counterstaining with DAPI (blue): **(K)** intact control, **(H)** SCI, and **(I)** SCI-EVs (400x magnification). **(N)** Quantification of CSPG-positive cells demonstrates a significant increase after SCI and partial attenuation following EV administration. Data are presented as mean ± SD (n = 5-7 per group). Statistical significance was defined as *p < 0.05; ***p < 0.001 and +++p < 0.001."+" indicates downregulation relative to SCI.

semiquantitatively classified as mild (0), moderate (1), or extensive (2). All histological evaluations were performed in a blinded manner by an experienced pathologist.

2.7 Immunofluorescent staining

For the immunofluorescence staining, spinal cord segments, sectioned into paraffin sections measuring 5 μm in thickness, were deparaffinized using xylene (Exodo Cientifica) and rehydrated after successive washes using different concentrations of alcohol (100%, 90%, 70% and 30%) for later heat-induced antigen retrieval using citric acid for 25 min. After this time, spinal cord samples were incubated overnight with the primary antibody anti-GFAP (dilution of 1:400, Z0334, Invitrogen), anti-IBA1 (dilution of 1:200, ab178680, Abcam), and anti-CSPG (dilution of 1:100, SAB5700198, Merk). Following a thorough washing process, the sections were incubated with a secondary antibody (dilution of 1:800, A11011, Invitrogen) for 1 h at room temperature. Nuclei were stained with DAPI (Fluoroshield™ with DAPI, Sigma). Then, the presence of fluorescent cells was observed using a Confocal fluorescence microscope (Zeiss, Oberkochen, Germany), while the quantification of the number of fluorescent cells was performed using the CellInsight CX7 LED Pro HCS Platform (Thermo Fisher Scientific).

2.8 Statistical analyses

All statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, United States). Data are expressed as mean ± SD from independent biological replicates, and statistical significance was set at $p \leq 0.05$. Survival was analyzed by the Kaplan-Meier method and compared using the log-rank (Mantel–Cox) test. Behavioral and locomotor data, including BBB and clinical scoring, were analyzed by two-way repeated-measures ANOVA (treatment × time) followed by Dunnett's *post hoc* tests. Gene expression (RT-qPCR) and EV quantifications were analyzed by one- or two-way ANOVA with Tukey or Dunnett *post hoc* tests. Comparisons between two groups were performed using unpaired t-tests. When data did not meet normality assumptions (Shapiro–Wilk test), non-parametric alternatives (Mann–Whitney or Kruskal–Wallis with Dunn's multiple comparison) were applied, as indicated in the figure legends.

3 Results

3.1 Manufacturing and characterization of MSCs and EVs

MSCs demonstrated plastic adherence and exhibited the typical spindle-shaped morphology (Figure 1A). Their multipotency was confirmed by successful differentiation into osteogenic, adipogenic, and chondrogenic lineages (Figures 1B–D). As analyzed by flow cytometry, MSC surface negative markers (CD34, CD45, CD11b, CD19, HLA-DR) were minimally expressed and positive for MSC markers (CD73, CD90, CD105) were prominently expressed, confirming a characteristic MSC immunophenotype (Figure 1E). Cytogenetic analysis by G-banding revealed genomic stability, with a normal female karyotype (46,XX) and no structural or numerical abnormalities detected (Figure 1F).

To assess the biological activity of the MSCs, their immunomodulatory function was evaluated through co-culture assays with activated PBMCs. The cells showed the ability to inhibit TNF-α and induce IL-10 expression (Figures 1G,H), supporting their anti-inflammatory potential. Furthermore, senescence-associated β-galactosidase staining revealed low levels of cellular senescence (Figure 1I), indicating that the MSC population maintained proliferative capacity, genomic stability, and overall cellular fitness, consistent with a therapeutically competent phenotype suitable for EV production.

EVs were isolated from the supernatants of MSC cultures across three independent batches. The EV samples displayed consistent size profiles, with nanoparticle tracking analysis (NTA) indicating a size range between 35 and 200 nm (Figures 1J–L). Protein concentration of the EV preparations ranged from 316 ± 6 to 568.5 ± 55.5 μg/mL (Figure 1M). Transmission electron microscopy confirmed the typical cup-shaped morphology of EVs, revealing bilayer membrane structures with heterogeneous sizes (Figure 1N). Multiplex immunoassays demonstrated that EVs were enriched in canonical exosomal markers (CD9, CD63, and CD81, syntenin-1, and VLA-4, while the non-EV contaminant cytochrome C was either minimally detected or undetectable (Figure 1O).

3.2 EV therapy promotes functional recovery post-SCI model

In vivo, we evaluated the neuroprotective efficacy of EVs in an SCI model, following the experimental timeline demonstrated in Figure 2A. Following SCI, untreated animals exhibited severe urogenital complications, including marked urinary retention and, in some cases, hematuria post-injury, often accompanied by lethargic behavior. Despite antibiotic administration, the health status of untreated animals progressively deteriorated. EV treatment was associated with improved survival rates over the 30-day follow-up compared with untreated SCI animals (Figure 2B). To further assess the functional impact of EV therapy, locomotor recovery was evaluated using the BBB scoring system at predefined time points after injury. EV-treated animals showed a significant and progressive improvement in BBB scores, with differences from the SCI

group already evident at day 7 and sustained throughout the 30-day follow-up (Figure 2C). Moreover, a time-dependent improvement in clinical parameters was observed, compared with untreated SCI animals. EV-treated rats showed faster recovery of behavior parameter analyzed, including touch response, tail grip strength, general activity, reduced tremors, and improved autonomic regulation (Figure 2D).

3.3 EVs sustainably reduce inflammation and enhance neurotrophic support after the SCI model

Figure 3 shows the effects of SCI-EVs treatment on inflammatory cytokines and the neurotrophic factor BDNF at 24 h and 7 days after SCI, expressed as fold change relative to the control group. At 24 h, IL-1β remained near baseline (ns), whereas TNF-α ($p < 0.001$) was already elevated in SCI. EV treatment markedly attenuated TNF-α ($p < 0.001$) expression while strongly increasing both IL-10 ($p < 0.001$) and BDNF ($p < 0.001$). At 7 days post-injury, these modulatory effects were maintained, with a pronounced reduction in IL-1β ($p < 0.001$) and TNF-α ($p < 0.001$) and a robust increase in IL-10 ($p < 0.001$) and BDNF ($p < 0.001$) compared to untreated SCI animals. Immunofluorescence analysis of IBA-1-positive microglia revealed a marked reduction in activated IBA-1 cells in the lesion area of animals treated with EVs (Figures 3E,F). Quantification at 7 days post-injury (Figure 3G) confirmed a significant decrease in IBA-1 immunoreactivity in the SCI-EVs group compared with SCI controls ($p < 0.05$), supporting the anti-inflammatory action of EVs in the injured spinal cord.

3.4 EVs suppress SCI-induced glial scar formation around the lesion site after SCI

Histological analysis at 7 days post-injury revealed extensive tissue damage and cavity formation in the SCI group, whereas animals treated with EVs showed a reduced lesion area and tissue preservation (Figures 4A–C). Semiquantitative assessment of histiocytic infiltration and necrosis in H&E-stained sections demonstrated a significant reduction in lesion severity in the SCI-EV group compared with untreated SCI animals ($p < 0.05$; Figures 4D,E). Immunofluorescence staining further demonstrated a marked accumulation of GFAP-positive astrocytes in the SCI group, consistent with glial scar formation (Figures 4F–I). In contrast, EV-treated animals (Figure 4I) exhibited attenuated astrocytic reactivity as reflected by the reduced density of GFAP-positive cells (***$p < 0.001$; Figure 4J). Additionally, immunolabeling for chondroitin sulfate proteoglycans (CSPGs, Figures 4K–M) revealed pronounced matrix deposition in the SCI group (Figure 4L), whereas EV administration substantially reduced CSPG accumulation (+++$p < 0.001$; Figure 4N). Quantitative analysis confirmed that CSPG expression was significantly higher in the SCI group compared with controls (****$p < 0.0001$), and attenuated following EV treatment, indicating mitigation of glial scar formation.

3.5 *In vivo* biodistribution of EVs after SCI

In vivo tracking of DIR-labeled EVs demonstrated a selective accumulation at the spinal cord injury site in the SCI-EVs group compared with SCI controls (Figure 5). Representative fluorescence images (B) revealed a strong and localized signal in SCI-EVs-treated animals at days 1, 7, and 14 post-injection, with maximal intensity at day 7. Quantitative analysis (C) showed that, at day 1, photon emission was significantly higher in the SCI-EVs group ($1.2 \pm 0.6 \times 10^9$ photons/s) compared with the SCI group ($0.4 \pm 0.1 \times 10^9$ photons/s; $p < 0.05$). At day 7, the signal in the SCI-EVs group remained elevated ($0.5 \pm 0.2 \times 10^9$ photons/s; $p < 0.01$). By day 14, the signal in the SCI-EVs group decreased ($1.0 \pm 0.5 \times 10^9$ photons/s) but remained above baseline, whereas SCI controls presented minimal or undetectable fluorescence across all time points. These findings indicate a sustained retention of EVs at the lesion site, particularly during the first week after administration.

4 Discussion

SCI is a severe condition associated with high mortality, disability, and a substantial socioeconomic burden on patients' families (Eli et al., 2021). The sudden onset and complex pathophysiology of SCI highlight the urgent need for improved therapeutic strategies. EVs from bone marrow, adipose, and umbilical cord MSCs have shown significant therapeutic potential (Mou et al., 2025). In this study, we demonstrate that early administration of MSC-EVs confers significant neuroprotection and functional benefits in a rat model of SCI. EV administration not only promoted partial motor recovery, as evidenced by improved BBB scores, but also mitigated the severe systemic complications observed in untreated animals, significantly promoting survival.

We first confirmed the multilineage differentiation potential of MSCs and verified the expression of characteristic surface markers by flow cytometry, together with normal genomic stability by karyotype analysis. Subsequently, MSC-EVs were successfully isolated and exhibited the typical cup-shaped morphology under transmission electron microscopy, as well as the expected expression profile of canonical exosomal markers, consistent with previous studies from our group (Costa-Ferro et al., 2024; Costa-Ferro et al., 2025). Importantly, we demonstrated that MSC-EVs promote functional improvements when administered in the acute phase of SCI in rats.

A key mechanism underlying these functional improvements appears to be the modulation of the post-injury inflammatory environment. We observed that EV treatment robustly reduced microglia reactivity and production of pro-inflammatory cytokines such as IL-1β and TNF-α while enhancing anti-inflammatory (IL-10) and neurotrophic (BDNF) factors both in the acute and subacute phases after SCI model. In SCI, IL-10 can suppress the release of pro-inflammatory factors and reduce neuronal cell apoptosis, thereby promoting the functional recovery (Yang et al., 2016) and the reduced levels of pro-inflammatory cytokines (IL-1β and TNF-α) observed in our study agree with previous research has demonstrated that these

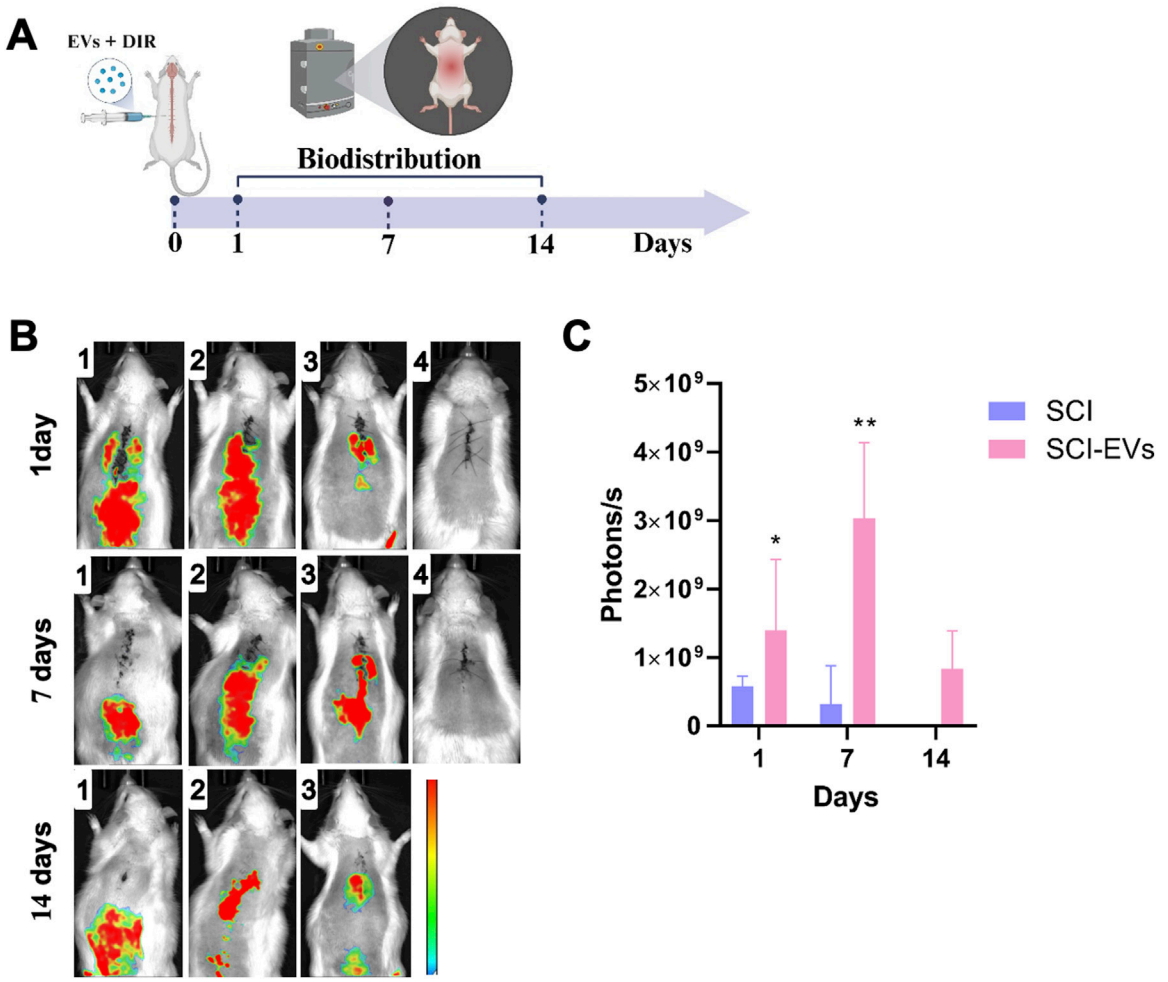


FIGURE 5
In vivo biodistribution of DiR-labeled EVs after local injection in SCI. **(A)** Schematic representation of the experimental timeline and marked imaging time points. EVs were labeled with the near-infrared dye DiR and locally injected at the lesion site in SCI rats. **(B)** Representative fluorescence images acquired at days 1, 7, and 14 post-injection. Each row represents the SCI-EVs group, and each column corresponds to a different animal (1–3) and control (4), showing fluorescence signals predominantly localized in the thoracic spinal cord region. **(C)** Quantification of photon emission revealed significantly higher fluorescence intensity in the SCI-EVs group compared to SCI. Data are presented as mean ± SD (SCI, n = 3; SCI-EVs, n = 6) and were analyzed using two-ANOVA followed by Tukey's *post hoc* test. *p < 0.05; **p < 0.01.

vesicles can mitigate neuroinflammation via various mechanisms (Huang et al., 2017; Sun et al., 2018; Fan et al., 2021).

This dual effect suggests that EVs not only suppress inflammation but also promote a regenerative microenvironment that may facilitate neuronal survival and plasticity. These results are consistent with previous reports highlighting the immunomodulatory and trophic cargo of MSC-EVs, including miRNAs (Wang et al., 2023; Lai et al., 2022) and growth factors known to attenuate neuroinflammation and support axonal repair. Zhang Y. et al. (2021) found that bone marrow-derived MSC-EVs inhibited the level of inflammatory factors in SCI rats, thereby protecting the damaged neurons and promoting the recovery of motor function in rats (Zhang M. et al., 2021).

Histological analyses further revealed that EV therapy reduced lesion cavity formation, immune cell infiltration, tissue necrosis, and astrocytic reactivity. This attenuation of glial scar formation is particularly important, excessive astrocyte reactivity leads to the formation of the glial scar, characterized by dense fibrous tissue and high CSPG content (Park et al., 2022), which restricts regenerative axonal sprouting (Schiera et al., 2024) and reactive astrocytes are major contributors to secondary damage and inhibitors of axonal regeneration after SCI (Karimi-Abdolrezaee et al., 2012; Romanelli et al., 2019) demonstrated that intravenous administration of hUC-MSCs in female rats after traumatic spinal cord injury (tSCI), more effective than their parental cells, markedly attenuates astrogliosis and fibroglial scar formation after tSCI, suggesting that the therapeutic effect is primarily mediated by secreted factors rather than cellular integration.

Another important aspect of our study is the biodistribution profile of EVs. *In vivo* imaging revealed a selective accumulation of EVs at the lesion site, with peak retention observed at day 7 post-injection. Their sustained retention at the lesion could enhance therapeutic efficacy by prolonging the availability of bioactive cargo during the critical window of secondary injury progression. However, previous studies have shown that systemically administered EVs can also modulate cytokine release and

promote neuroprotection and functional recovery following SCI (Huang et al., 2017).

Taken together, our results support the concept that EV therapy acts through a multifaceted mechanism: (i) modulating systemic and local inflammation, (ii) enhancing neurotrophic support, (iii) preserving tissue integrity, and (iv) sustaining the delivery of bioactive molecules at the injury site. These combined effects contribute to improved functional outcomes after SCI. Importantly, the survival benefit observed in EV-treated animals highlights their translational relevance, as systemic complications are a major determinant of mortality in SCI patients; traumatic spinal cord injuries have reduced overall life spans and higher mortality, especially in the first year after an injury, compared with the general population. Patients with traumatic spinal cord injuries have reduced overall life spans and higher mortality, especially in the first year after an injury, compared with the general population (Ahuja et al., 2017).

Although survival in rodent models cannot be directly extrapolated to human clinical outcomes due to species-specific differences in physiology, systemic responses, and post-injury care, its inclusion as an endpoint provides complementary translational value. Most preclinical studies of SCI report only functional or histological improvements, whereas survival integrates systemic factors such as neurogenic shock, systemic inflammation, and acute respiratory dysfunction (Zhang Y. et al., 2021). These processes are also associated with early mortality in patients (Shao et al., 2011). Therefore, the improved survival observed in EV-treated animals likely reflects a broader modulation of the acute systemic response to injury rather than a mere local effect at the lesion site, reinforcing the overall robustness of the therapeutic benefit.

This study has some limitations that should be acknowledged. First, we evaluated the overall therapeutic effects of hUCMSC-derived EVs in SCI but did not dissect the contribution of specific EV cargo (e.g., miRNAs, proteins, lipids) to the observed outcomes. Such mechanistic studies will be essential to better understand and refine their therapeutic potential. Second, we used a single dose and route of administration, which precludes conclusions about optimal dosing strategies or alternative delivery methods. Third, EVs were administered only in the acute phase after SCI, and their potential efficacy in subacute or chronic stages of injury remains to be determined.

5 Conclusion

In conclusion, this study demonstrates that hUCMSC-derived EV therapy exerts neuroprotective effects after SCI, characterized by reduced inflammation, attenuated glial scar formation, and improved functional recovery. Importantly, EV administration was also associated with enhanced survival, a clinically relevant outcome that has been rarely addressed in preclinical SCI studies. Together, these findings provide a compelling rationale for further investigation of EV-based approaches, including mechanistic studies, optimized dosing strategies, and evaluation across different stages of injury, to advance their development as a promising therapeutic strategy for spinal cord injury.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

Ethics statement

The studies involving humans were approved by Comitê de Ética em Pesquisa Hospital São Rafael. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which ethical approval was obtained. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements. The animal study was approved by Instituto Gonçalo Moniz (FIOCRUZ). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

BC: Formal Analysis, Investigation, Methodology, Writing – original draft. RC: Investigation, Methodology, Writing – original draft. TS: Formal Analysis, Investigation, Methodology, Writing – original draft. KS: Formal Analysis, Investigation, Methodology, Writing – original draft. EL: Investigation, Methodology, Writing – original draft. JF: Investigation, Methodology, Writing – original draft. ES: Investigation, Methodology, Writing – original draft. AL: Investigation, Methodology, Writing – original draft. ER: Formal Analysis, Investigation, Methodology, Writing – original draft. IS: Formal Analysis, Writing – original draft. RN: Investigation, Methodology, Writing – original draft. SC: Conceptualization, Supervision, Writing – review and editing. ZC-F: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – original draft. BS: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review and editing.

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Conflict of interest

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

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References

Ahuja, C. S., Wilson, J. R., Nori, S., Kotter, M. R. N., Druschel, C., Curt, A., et al. (2017). Traumatic spinal cord injury. *Nat. Rev. Dis. Prim.* 3, 17018. doi:10.1038/NRDP.2017.18

Basso, D. M., Beattie, M. S., and Bresnahan, J. C. (1995). A sensitive and reliable locomotor rating scale for open field testing in rats. *J. Neurotrauma* 12 (1), 1–21. doi:10.1089/NEU.1995.12.1

Cao, J., Zhang, X., Guo, J., Wu, J., Lin, L., Lin, X., et al. (2025). An engineering-reinforced extracellular vesicle–integrated hydrogel with an ROS-Responsive release pattern mitigates spinal cord injury. *Sci. Adv.* 11 (14), eads3398. doi:10.1126/sciadv.ads3398

Costa-Ferro, Z. S. M., Rocha, G. V., da Silva, K. N., Paredes, B. D., Loiola, E. C., Silva, J. D., et al. (2024). GMP-compliant extracellular vesicles derived from umbilical cord mesenchymal stromal cells: manufacturing and pre-clinical evaluation in ARDS treatment. *Cytotherapy* 26 (9), 1013–1025. doi:10.1016/j.jcyt.2024.04.074

Costa-Ferro, Z. S. M., Cunha, R. S., Rossi, E. A., Loiola, E. C., Cipriano, B. P., Figueiredo, J. C. Q., et al. (2025). Extracellular vesicles derived from mesenchymal stem cells alleviate depressive-like behavior in a rat model of chronic stress. *Life Sci.* 366-367, 123479–367. doi:10.1016/j.lfs.2025.123479

Courtine, G., and Sofroniew, M. v. (2019). Spinal cord repair: advances in biology and technology. *Nat. Med.* 25 (6), 898–908. doi:10.1038/S41591-019-0475-6

Eli, I., Lerner, D. P., and Ghogawala, Z. (2021). Acute traumatic spinal cord injury. *Neurol. Clin.* 39 (2), 471–488. doi:10.1016/J.NCL.2021.02.004

Fan, L., Dong, J., He, X., Zhang, C., and Zhang, T. (2021). Bone marrow mesenchymal stem cells-derived exosomes reduce apoptosis and inflammatory response during spinal cord injury by inhibiting the TLR4/MyD88/NF-κB signaling pathway. *Hum. Exp. Toxicol.* 40 (10), 1612–1623. doi:10.1177/09603271211003311

Gale, K., Kerasidis, H., and Wrathall, J. R. (1985). Spinal cord contusion in the rat: behavioral analysis of functional neurologic impairment. *Exp. Neurol.* 88 (1), 123–134. doi:10.1016/0014-4886(85)90118-9

Holmes, D. (2017). Repairing the neural highway. *Nature* 552 (7684), S50–S51. doi:10.1038/D41586-017-07551-8

Huang, J. H., Yin, X. M., Xu, Y., Xu, C. C., Lin, X., Ye, F. B., et al. (2017). Systemic administration of exosomes released from mesenchymal stromal cells attenuates apoptosis, inflammation, and promotes angiogenesis after spinal cord injury in rats. *J. Neurotrauma* 34 (24), 3388–3396. doi:10.1089/NEU.2017.5063

Huang, L. Y., Sun, X., Pan, H. X., Wang, L., He, C. Q., and Wei, Q. (2023). Cell transplantation therapies for spinal cord injury focusing on bone marrow mesenchymal stem cells: advances and challenges. *World J. Stem Cells* 15 (5), 385–399. doi:10.4252/WJSC.V15.I5.385

Karimi-Abdolrezaee, S., and Billakanti., R. (2012). Reactive astrogliosis after spinal cord injury-beneficial and detrimental effects. *Mol. Neurobiol.* 46 (2), 251–264. doi:10.1007/S12035-012-8287-4

Lai, X., Wang, Y., wang, X., Liu, B., and Rong, L. (2022). miR-146a-5p-modified hUCMSC-derived exosomes facilitate spinal cord function recovery by targeting neurotoxic astrocytes. *Stem Cell Res. Ther.* 13 (1), 487. doi:10.1186/S13287-022-03116-3

Li, X., Zhao, Y., Cheng, S., Han, S., Shu, M., Chen, B., et al. (2017). Cetuximab modified collagen scaffold directs neurogenesis of injury-activated endogenous neural stem cells for acute spinal cord injury repair. *Biomaterials* 137, 73–86. doi:10.1016/J.BIOMATERIALS.2017.05.027

Mou, C., Xia, Z., Wang, X., Dai, X., Wang, J., Zhang, C., et al. (2025). Stem cell-derived exosome treatment for acute spinal cord injury: a systematic review and meta-analysis based on preclinical evidence. *Front. Neurology* 16, 1447414. doi:10.3389/fneur.2025.1447414

Park, H. H., Kim, Y. M., Anh Hong, L. T., Kim, H. S., Kim, S. H., Jin, X., et al. (2022). Dual-functional hydrogel system for spinal cord regeneration with sustained release of arylsulfatase B alleviates fibrotic microenvironment and promotes axonal regeneration. *Biomaterials* 284, 121526. doi:10.1016/j.biomaterials.2022.121526

Ragni, E. (2025). Extracellular vesicles: recent advances and perspectives. *Front. Biosci. Landmark Ed.* 30 (6), 36405. doi:10.31083/FBL36405

Romanelli, P., Bieler, L., Scharler, C., Pachler, K., Kreutzer, C., Zaunmair, P., et al. (2019). Extracellular vesicles can deliver anti-inflammatory and anti-scarring activities of mesenchymal stromal cells after spinal cord injury. *Front. Neurology* 10, 1225. doi:10.3389/FNEUR.2019.01225

Schiera, G., di Liegro, C. M., Schirò, G., Sorbello, G., and di Liegro, I. (2024). Involvement of astrocytes in the formation, maintenance, and function of the blood–brain barrier. *Cells* 13 (2), 150. doi:10.3390/CELLS13020150

Shao, J., Zhu, W., Chen, X., Jia, L., Song, D., Zhou, X., et al. (2011). Factors associated with early mortality after cervical spinal cord injury. *J. Spinal Cord. Med.* 34, 555–562. doi:10.1179/2045772311Y.0000000024

Silva, K. N., Marim, F. M., Rocha, G. V., Costa-Ferro, Z. S. M., França, L. S. de A., Nonaka, C. K. V., et al. (2025). Functional heterogeneity of mesenchymal stem cells and their therapeutic potential in the K18-hACE2 mouse model of SARS-CoV-2 infection. *Stem Cell Res. Ther.* 16, 1–17. doi:10.1186/S13287-024-04086-4/FIGURES/6

Sohrabi, B., Dayeri, B., Zahedi, E., Khoshbakht, S., Nezamabadi Pour, N., Ranjbar, H., et al. (2022). Mesenchymal stem cell (MSC)-derived exosomes as novel vehicles for delivery of miRNAs in cancer therapy. *Cancer Gene Ther.* 29 (8–9), 1105–1116. doi:10.1038/S41417-022-00427-8

Sterner, R. C., and Sterner, R. M. (2023). Immune response following traumatic spinal cord injury: pathophysiology and therapies. *Front. Immunol.* 13, 1084101. doi:10.3389/FIMMU.2022.1084101

Sun, G., Li, G., Li, D., Huang, W., Zhang, R., Zhang, H., et al. (2018). hucMSC derived exosomes promote functional recovery in spinal cord injury mice *via* attenuating inflammation. *Mater. Sci. Eng. C* 89, 194–204. doi:10.1016/j.msec.2018.04.006

Sun, X. C., Wang, H., Ma, X., and Xia, H. F. (2023). Application of human umbilical cord mesenchymal stem cells in rat spinal cord injury model. *ASAIO J.* 69 (6), E256–E264. doi:10.1097/MAT.0000000000001938

Tran, A. P., Warren, P. M., and Silver, J. (2018). The biology of regeneration failure and success after spinal cord injury. *Physiol. Rev.* 98 (2), 881–917. doi:10.1152/PHYSREV.00017.2017

Vanický, I., Urdzíková, L., Saganová, K., Čízková, D., and Gálik, J. (2001). A simple and reproducible model of spinal cord injury induced by epidural balloon inflation in the rat. *J. Neurotrauma* 18 (12), 1399–1407. doi:10.1089/08977150152725687

Vialle, E. N., Fracaro, L., Barchiki, F., Dominguez, A. C., Arruda, A. de O., Olandoski, M., et al. (2023). Human adipose-derived stem cells reduce cellular damage after experimental spinal cord injury in rats. *Biomedicines* 11 (5), 1394. doi:10.3390/BIOMEDICINES11051394

Volarevic, V., Gazdic, M., Markovic, B., Jovicic, N., Djonov, V., and Arsenijevic, N. (2017). Mesenchymal stem cell-derived factors: immuno-modulatory effects and therapeutic potential. *BioFactors* 43 (5), 633–644. doi:10.1002/BIOF.1374

Wang, X., Yang, Y., Li, W. H. , M. Y., and Xu, Y. S. (2023). Umbilical mesenchymal stem cell-derived exosomes promote spinal cord functional recovery through the miR-146b/TLR4 -mediated NF-κB p65 signaling pathway in rats. *Biochem. Biophysics Rep.* 35, 101497. doi:10.1016/j.bbrep.2023.101497

Welsh, J. A., Goberdhan, D. C. I., O'Driscoll, L., Buzas, E. I., Blenkiron, C., Bussolati, B., et al. (2024). Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J. Extracell. Vesicles* 13 (2), e12404. doi:10.1002/JEV2.12404

Yang, R., He, J., and Wang, Y. (2016). Activation of the niacin receptor HCA2reduces demyelination and neurofilament loss, and promotes functional recovery after spinal cord injury in mice. *Eur. J. Pharmacol.* 791, 124–136. doi:10.1016/j.ejphar.2016.08.020

Zadra, A., Bruni, S., de Tanti, A., Saviola, D., Ciavarella, M., Cannavò, G., et al. (2024). Life expectancy and long-term survival after traumatic spinal cord injury: a systematic review. *Eur. J. Phys. Rehabilitation Med.* 60 (5), 822–831. doi:10.23736/S1973-9087.24.08462-4

Zhang, M., Wang, L., Huang, S., and He, X. (2021). Exosomes with high level of miR-181c from bone marrow-derived mesenchymal stem cells inhibit inflammation and apoptosis to alleviate spinal cord injury. *J. Mol. Histology* 52 (2), 301–311. doi:10.1007/S10735-020-09950-0

Zhang, Y., Mamun, A. A. L., Yuan, Y., Lu, Q., Xiong, J., Yang, S., et al. (2021). Acute spinal cord injury: pathophysiology and pharmacological intervention. *Mol. Med. Rep.* 23, 417. doi:10.3892/MMR.2021.12056

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Endoplasmic reticulum stress and exosomes secretion in the pathogenesis of inflammatory bowel disease: a concise summary of research findings

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Cellular stress responses and intercellular communication play a crucial role in the pathogenesis of Inflammatory bowel disease (IBD). Among these, endoplasmic reticulum (ER) stress and exosome-mediated signaling have emerged as interconnected drivers of chronic intestinal inflammation. Persistent ER stress, primarily through unfolded protein response pathways involving PERK, IRE1, and ATF6, disrupts epithelial barrier integrity, alters immune cell function, and promotes pro-inflammatory gene expression. ER stress not only affects intracellular homeostasis but also modulates intercellular communication through the secretion of exosomes, which carry proteins, lipids, and nucleic acids. This bidirectional relationship ensures that stress-altered exosomes can amplify ER stress and inflammatory signals in neighboring cells, sustaining intestinal inflammation. For this review, relevant research and review articles were retrieved from established search engines and databases, including PubMed, Google Scholar, and ScienceDirect, using key terms such as "endoplasmic reticulum stress," "exosome secretion," "exosome cargo," "inflammatory bowel disease," "intestinal inflammation," and "intercellular communication." The literature search primarily focused on studies published in the last 5 years, prioritizing clinical and preclinical studies (*in vivo* and *in vitro* models). Published literature addressing ER stress, exosome biology, and their interconnection in IBD were included, whereas studies lacking relevance or study quality were excluded. Recent findings highlight a dynamic interconnection between ER stress and exosomes, where ER stress modulates exosome biogenesis, secretion, and cargo composition. In contrast, stress-altered exosomes amplify ER stress signals and inflammatory mediators in neighboring cells. This review aims to summarize the current evidences on the interconnection of ER stress and exosomes in modulating the intestinal microenvironment, driving inflammation, and contributing to epithelial and

immune dysregulation in IBD. This review also highlights experimental insights, existing challenges, and therapeutic prospects for targeting the ER stress–exosome axis to restore mucosal homeostasis in IBD management.

KEYWORDS

endoplasmic reticulum stress, exosomes, immune dysregulation, inflammatory bowel disease, intercellular communication, unfolded protein response

1 Introduction

Inflammatory Bowel Disease (IBD) is a chronic and relapsing inflammatory condition of the gastrointestinal tract, primarily encompassing two major clinical entities: Crohn's disease (CD) and Ulcerative colitis (UC) (Hirten et al., 2024). While UC is limited to the colon and rectum, CD may affect any region of the gastrointestinal tract, often resulting in complications such as bowel strictures, fistulas, malnutrition, anemia, and hepatic disorders (Ber et al., 2021). Globally, IBD has emerged as a significant public health concern, with incidence rates that were once confined to high-income Western countries now increasing rapidly in newly industrialized regions such as East Asia and Latin America (Lin et al., 2024; Park et al., 2023). This epidemiological shift is largely attributed to industrialization, urbanization, dietary transitions, and other environmental factors interacting with genetic susceptibility (Shan et al., 2022). According to Global Burden of Disease (GBD) analyses, the age-standardized incidence of IBD showed a modest increase, rising from 4.22 per 100,000 individuals in 1990 to 4.45 per 100,000 in 2021 (Lin et al., 2024). By 2019, an estimated 4.9–5.0 million people worldwide were living with IBD, with crude prevalence rising from about 3.3 million in 1990 to 4.9 million in 2019 (Park et al., 2023; Wang et al., 2023). The pathogenesis of IBD involves multiple interlinked components, including immune dysregulation, genetic susceptibility, epithelial barrier dysfunction, and environmental triggers (Calvez et al., 2025). Over the past decade, there has been increasing recognition that chronic intestinal inflammation in IBD has become increasingly evident, and it arises not only from persistent immune cell activation but also from profound disruptions in epithelial cell homeostasis, barrier integrity, and cellular stress response pathways (Calvez et al., 2025; Guan, 2019). Among these, endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) have emerged as central regulatory mechanisms linking the pathological onset of intestinal inflammation and mucosal damage (Zheng et al., 2025; Ma et al., 2017). ER stress refers to a cellular condition in which the folding capacity of the ER is disrupted, leading to the accumulation of misfolded or unfolded proteins within the ER lumen (Chen et al., 2023). The ER is essential for protein folding, calcium homeostasis, and lipid metabolism; however, under inflammatory, oxidative, or metabolic disregulation, ER stress is triggered, leading to activation of the UPR (English and Voeltz, 2013; Singh et al., 2024). UPR is an adaptive cellular mechanism that helps to restore ER homeostasis by suppressing protein synthesis, optimizing protein folding, and eliminating misfolded proteins (Hetz et al., 2020). However, persistent ER stress can trigger apoptosis, autophagy, and modulation of inflammatory pathways, leading to epithelial barrier disruption, immune system activation, and sustained intestinal inflammation (Chen et al., 2023). Moreover, genetic polymorphisms in ER stress-related genes such as X-box-binding protein 1 (*XBP1*) and Orosomucoid-like protein 3 (*ORMDL3*) have been associated with increased IBD susceptibility (Vanhove et al., 2018; Kaser et al., 2008). In intestinal epithelial cells (IECs) and immune cells, persistent ER stress promotes inflammation by enhancing pro-inflammatory cytokine release, impairing mucosal healing, and altering microbiota composition (Cao, 2018). In addition to intracellular stress mechanisms, extracellular signaling pathways have gained attention for their role in regulating immune and epithelial responses. Among these, extracellular vesicles (EVs), particularly exosomes, are increasingly recognized as key mediators of intercellular communication in intestinal homeostasis and inflammation (Wu et al., 2024). Exosomes are small membrane-bound vesicles (30–150 nm) that carry proteins, lipids, and nucleic acids, and play major roles in immune regulation, maintaining epithelial barrier integrity, modulating gut microbial communities, and facilitating intercellular communication in the intestinal microenvironment (Chavda et al., 2023; Kalluri and LeBleu, 2020; Ayyar and Moss, 2021). It has been reported that ER stress is mechanistically linked to exosome biogenesis, influencing secretory dynamics and altering the molecular content of exosomes, thereby modulating their biological activity (Ye and Liu, 2022; Jahangiri et al., 2022). ER stress-induced exosomes may carry specific microRNAs or proteins that promote inflammatory responses or enhance tumor cell survival and dissemination. For instance, immune cell-derived exosomal miR-155 and miR-21 contribute to inflammation by targeting regulatory pathways essential for maintaining immune balance (Nail et al., 2023). Furthermore, exosomes derived from ER-stressed cells may influence immune responses by modulating the activity of macrophages, dendritic cells (DCs), and T cells, thereby worsening the inflammatory landscape within the intestinal microenvironment (Zhang et al., 2019). The dynamic relationship between ER stress and exosomes may play a significant role in the development of IBD. Elucidating how ER stress influences the composition and function of exosomes, and how these vesicles, in turn, affect intestinal homeostasis and immune responses, could provide novel insights into the underlying mechanisms of IBD. In this review, we aim to discuss the current research exploring how ER stress influences exosome biogenesis, alters their molecular composition, and modulates their biological functions within the intestinal microenvironment. This review further examines how ER stress–induced exosomes contribute to intestinal inflammation, epithelial barrier dysfunction, and immune

TABLE 1 Cellular factors contributing to ER stress and their associated pathological outcomes.

Factor	Mechanism	Pathological impact	References
Oxidative stress	Disrupts redox balance, inhibits disulfide bonds.	Protein misfolding, inflammation, cancer, IBD.	Cao and Kaufman (2014)
Calcium disruption	Impairs chaperone activation (calnexin, calreticulin).	ER dysfunction, IBD, and neurodegeneration.	Stutzmann and Mattson (2011); Mekahli et al. (2011)
Nutrient deprivation	Affects the folding and glycosylation of the proteins.	UPR activation, tumor stress response.	Chen and Shoulders (2024)
Hypoxia	Reduces ATP and the folding capacity of protein.	Tumor survival, metastasis.	Akman et al. (2021)
Infections	Overload of the ER by viral/bacterial proteins.	Inflammation, IBD	Da Fonseca et al. (2024)
Genetic mutations	Causes persistent misfolded proteins.	Cystic fibrosis, IBD, ER overload.	Chen et al. (2023)
Inflammatory cytokines	Disrupt signalling pathways; induce ER stress.	Chronic inflammation, IBD, autoimmunity.	Salminen et al. (2020)

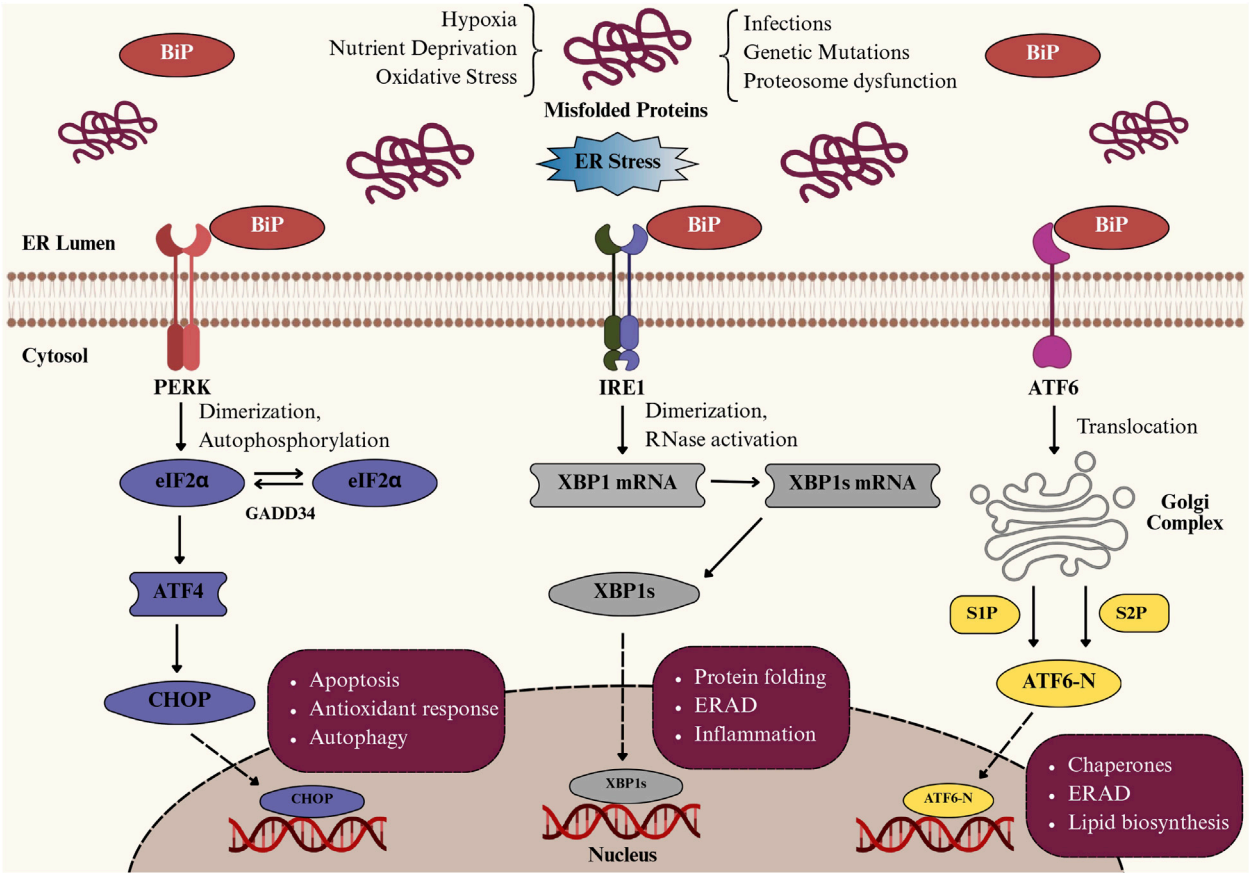


FIGURE 1
Pathways of the unfolded protein response (UPR) activated during endoplasmic reticulum (ER) stress. Schematic representation of unfolded protein response (UPR) activation in intestinal epithelial cells under ER stress. Accumulation of misfolded proteins due to hypoxia, oxidative stress, or nutrient deprivation activates three key sensors: PERK, IRE1, and ATF6. PERK phosphorylates eIF2α, inducing ATF4/CHOP-mediated apoptosis and autophagy; IRE1 splices XBP1 mRNA, generating XBP1s that regulate protein folding, ER-associated degradation (ERAD), and inflammation; and ATF6 translocates to the Golgi, where cleavage produces ATF6-N, which upregulates chaperones and lipid biosynthesis to restore ER homeostasis.

dysregulation, thereby promoting IBD progression. In addition, the review also highlights the potential of targeting ER stress–exosome interactions as a future therapeutic approach for the management of IBD.

2 Endoplasmic reticulum (ER) stress

The ER is a multifunctional organelle composed of interconnected tubules and cisternae that extend throughout the

cytoplasm, playing a central role in protein synthesis, protein folding, post-translational modifications, lipid metabolism, and calcium homeostasis (Singh et al., 2024; Deka et al., 2022; Girigoswami et al., 2023). Under physiological conditions, chaperone proteins such as Glucose-Regulated Protein 78 (GRP78)/BiP and protein folding enzymes including calnexin and calreticulin maintain protein quality control; however, factors like oxidative stress, hypoxia, nutrient deprivation, calcium imbalance, genetic mutations, and inflammation can disrupt ER function, causing the accumulation of misfolded or unfolded proteins and thereby inducing ER stress (Table 1) (Chen et al., 2023; Singh et al., 2024). This activates the UPR, an adaptive mechanism regulated by three primary ER membrane sensors, including protein kinase RNA-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor-6 (ATF6) (Chen et al., 2023), as illustrated in Figure 1. Upon ER stress, GRP78 dissociates, enabling PERK to oligomerize and phosphorylate eIF2α, which transiently inhibits overall protein translation while selectively enhancing Activating Transcription Factor 4 (ATF4) translation. ATF4 promotes expression of genes involved in amino acid metabolism, antioxidant defense, and ER-associated degradation (ERAD), but also triggers C/EBP homologous protein (CHOP), a transcription factor that suppresses Bcl-2, upregulates pro-apoptotic mediators (BIM, PUMA), and promotes reactive oxygen species (ROS) generation and calcium release, thereby initiating mitochondrial stress (Verjan Garcia et al., 2023; Hu et al., 2019; Smolková et al., 2020). IRE1, once activated by oligomerization and autophosphorylation, splices XBP1 mRNA to generate the transcriptionally active XBP1s, which enhance the transcription of genes related to ERAD, protein folding, and lipid synthesis (Park et al., 2021; Jiang et al., 2015). Prolonged IRE1 activation also leads to Regulated IRE1-Dependent Decay (RIDD), c-Jun N-terminal Kinase (JNK) activation *via* TRAF2–ASK1, and degradation of specific microRNAs, thereby linking ER stress to inflammation and apoptosis (Siwecka et al., 2021; Hotamisligil, 2010). Meanwhile, upon release from GRP78, ATF6 translocates to the Golgi, where it is sequentially cleaved by site-1 and site-2 proteases (S1P and S2P). This cleavage generates the cytosolic fragment ATF6p50, which migrates to the nucleus and activates genes encoding ER chaperones (GRP78, GRP94), protein-folding enzymes (PDIs), and components of ERAD (Hetz et al., 2020; Yamamoto et al., 2007). Persistent ER stress overcomes these pathways, leading to apoptosis, inflammation, and cellular dysfunction, particularly in highly secretory cells like IECs (Salminen et al., 2020; Song et al., 2008; Lin et al., 2008).

2.1 Intracellular and extracellular matrix–mediated stress in IBD

IBD arises from a complex interaction between intracellular stress responses and extracellular matrix (ECM)-mediated stress, both of which play pivotal roles in sustaining chronic intestinal inflammation, epithelial barrier disruption, and fibrosis (Zhang et al., 2025; Lin M. et al., 2025). At the cellular level, ER stress disrupts epithelial cell function by activating the UPR (He et al., 2025). When this adaptive mechanism fails, it triggers apoptosis, compromises barrier integrity, and promotes pro-inflammatory signaling cascades that sustain mucosal inflammation (Kong et al., 2024; Otte et al., 2023). These intracellular changes disrupt cellular communication and tissue regeneration, fostering a microenvironment that promotes chronic inflammation (Saez et al., 2023). Concurrently, extracellular stress arises from ECM remodeling, where the ECM undergoes significant compositional and mechanical alterations (Prakash and Shaked, 2024). Normally, the ECM provides a structural and regulatory framework for the cells, but during IBD progression, excessive deposition of collagen and fibronectin, fragmentation of hyaluronan, and upregulation of matrix metalloproteinases (MMPs) disrupt ECM homeostasis (Zhao et al., 2025; Petrey and de la Motte, 2017). These changes increase tissue stiffness and alter cell–matrix interactions, promoting fibroblast activation, angiogenesis, and immune cell infiltration (Petrey and de la Motte, 2017; Lin S. N. et al., 2025). The remodeled ECM imposes additional mechanical and biochemical stress on epithelial and mesenchymal cells *via* altered mechanotransduction pathways, thereby sustaining inflammation and promoting fibrogenesis (Mierke, 2024; Horta et al., 2023). Mechanical cues at the cellular level further influence ER stress development. Cells constantly experience forces from tissue tension, fluid shear, and ECM stiffness, which are transmitted through integrins and cytoskeletal networks to intracellular organelles (Mierke, 2024). Elevated mechanical load, such as that found in regions of stiffened ECM or fibrotic tissue, can deform the ER membrane, disrupt protein folding, and enhance ER stress signaling (Lenna and Trojanowska, 2012). Likewise, changes in osmotic pressure or cytoskeletal tension can disrupt ER-calcium homeostasis, which further amplifies the UPR (Carreras-Sureda et al., 2018). The cytoskeleton also acts as a critical mechanotransductive bridge between ECM remodeling and intracellular ER stress (Huang et al., 2023). Comprised of actin filaments, microtubules, and intermediate filaments, the cytoskeleton transmits mechanical and biochemical signals from the ECM to intracellular organelles, including the ER (Momotyuk et al., 2025; Gurel et al., 2014). Alterations in ECM stiffness, composition, or ligand availability are detected by integrins and focal adhesion complexes, which transmit these mechanical cues through the cytoskeleton to intracellular organelles, particularly the ER, thereby modulating its structure, calcium homeostasis, and protein-folding capacity (Di et al., 2023; Derricks et al., 2015; Khoonkari et al., 2024). Conversely, ER stress can modify cytoskeletal composition through UPR-mediated signaling, specifically affecting actin filament dynamics and microtubule stability, which, in turn, influence cell shape, motility, and mechanotransduction (van Vliet and Agostinis, 2017; Khoonkari et al., 2024). This coordinated interplay may establish a dynamic network in which ECM remodeling, cytoskeletal dynamics, and ER stress mutually amplify each other, driving epithelial barrier disruption, immune activation, and fibrotic remodeling in IBD.

2.2 ER stress–mediated immune dysregulation and molecular outcomes in intestinal epithelium

Gut homeostasis depends on a balanced immune–epithelial interface that regulates tolerance and inflammation (Bretto et al.,

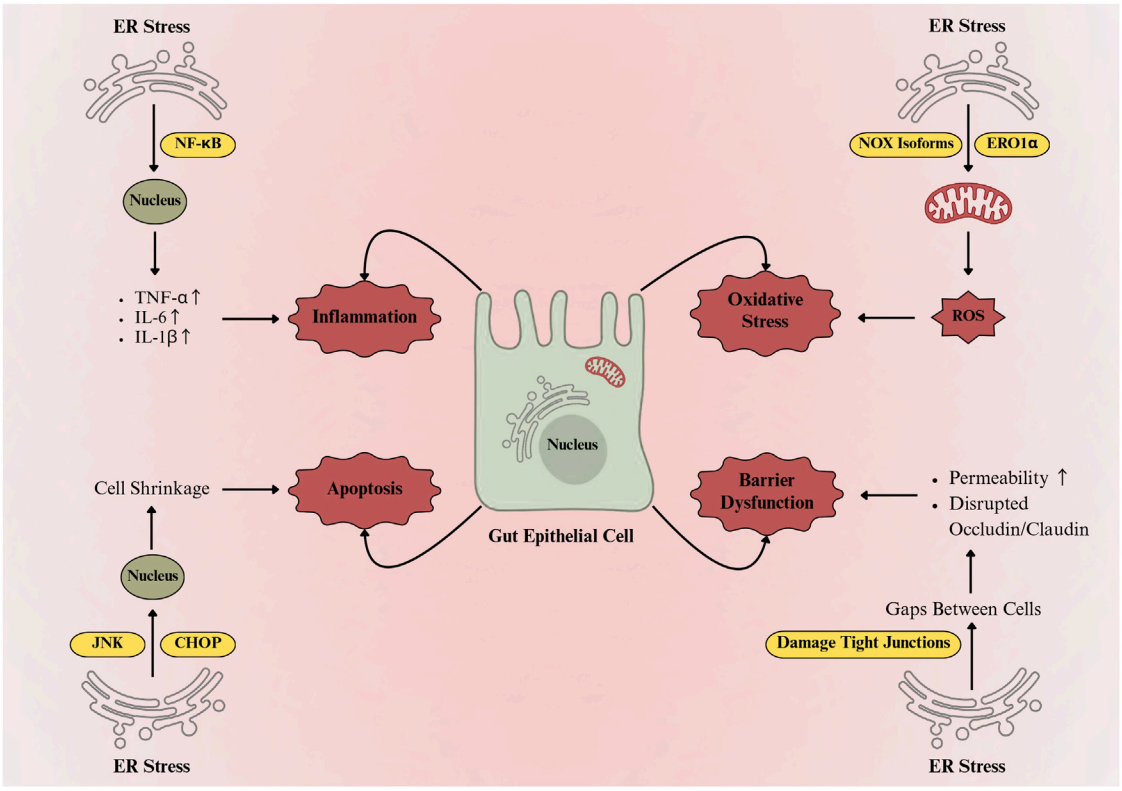


FIGURE 2
ER stress-mediated damage and functional impairment in gut epithelial cells. ER stress activates NF-κB and JNK/CHOP pathways, inducing proinflammatory cytokines (TNF-α, IL-6, IL-1β) and apoptosis. Concurrently, ROS generation *via* NADPH oxidase isoforms and ER oxidoreductin-1α disrupts tight junctions, increases epithelial permeability, and impairs intestinal barrier integrity.

2025). Paneth and goblet cells, derived from $Lgr5^{+}$ intestinal stem cells, are central to mucosal defence (Leushacke and Barker, 2014). Paneth cells sense microbes *via* MyD88-dependent Toll-like receptors and secrete antimicrobial peptides RegIII-β, RegIII-γ, and α-defensins (Yang et al., 2025), while goblet cells produce Mucin 2 (MUC2) to form a protective mucus barrier. Defective MUC2 folding or glycosylation compromises barrier function and predisposes to colitis and colorectal cancer (Kang et al., 2022). Different physiological and pathological conditions can produce ER stress, triggering the UPR. However, if ER stress persists, UPR imbalance can drive programmed cell death and inflammatory responses, which are particularly critical in intestinal epithelial cells that rely on UPR for their normal function (Di Mattia et al., 2025). Patients with active IBD often exhibit increased ER stress markers in both ileal and colonic epithelial tissues (Rodrigues et al., 2025; Cao, 2018). IBD patients also show signs of impaired integrated stress response, including decreased phosphorylation of eukaryotic initiation factor 2 alpha (eIF2α), which normally protects against oxidative and ER stress, infection, and inflammation. The IRE1α-XBP1 signaling pathway plays a critical role in intestinal immune modulation. Knockdown of XBP1 in IECs promotes ER stress, triggers inflammatory responses, and induces Paneth cell apoptosis (Luo and Cao, 2015). Prolonged ER stress activates all three UPR branches (IRE1α, PERK, and ATF6), leading to inflammation and apoptosis (Martinotti and Ranzato, 2025). IRE1α–TRAF2 activates JNK (Riaz et al., 2020), while PERK–ATF4–CHOP signalling induces apoptosis and NF-κB–driven cytokine transcription (Rozpedek et al., 2016). ATF6 supports chaperone synthesis but also amplifies CHOP and NF-κB expression (Chen et al., 2023). Sustained UPR activation disrupts tight junction proteins and epithelial permeability (Di Mattia et al., 2025). Prolonged ER stress also induces oxidative stress, mitochondrial dysfunction, and lipid peroxidation, resulting in excessive reactive oxygen species (ROS) production that damages lipids, proteins, and DNA, impairs mitochondrial function, and disrupts tight junction proteins such as occludin, claudins, and ZO-1, thereby compromising epithelial integrity and increasing intestinal epithelial permeability (Loncke et al., 2021; Bhandary et al., 2012). In gut epithelium, prolonged ER stress also triggers a highly regulated adaptive response known as autophagy, which acts as a protective mechanism to maintain cellular homeostasis (Shi et al., 2024). However, mutations in the autophagy-related genes *ATG16L1*, *IRGM*, and *NOD2* linked to Crohn's disease disrupt autophagy and thereby intensify ER stress (Alula and Theiss, 2023). These effects are closely linked to the major molecular and cellular consequences of prolonged ER stress in IECs, as illustrated in Figure 2. Recent studies (Ye and Liu, 2022; Jahangiri et al., 2022) indicate that ER stress not only drives inflammation, apoptosis, autophagy, and oxidative stress but also affects cell-to-cell communication *via* exosomes. ER stress influences exosome biogenesis and cargo composition, which in turn contributes to intestinal inflammation (Zhou et al., 2025). The

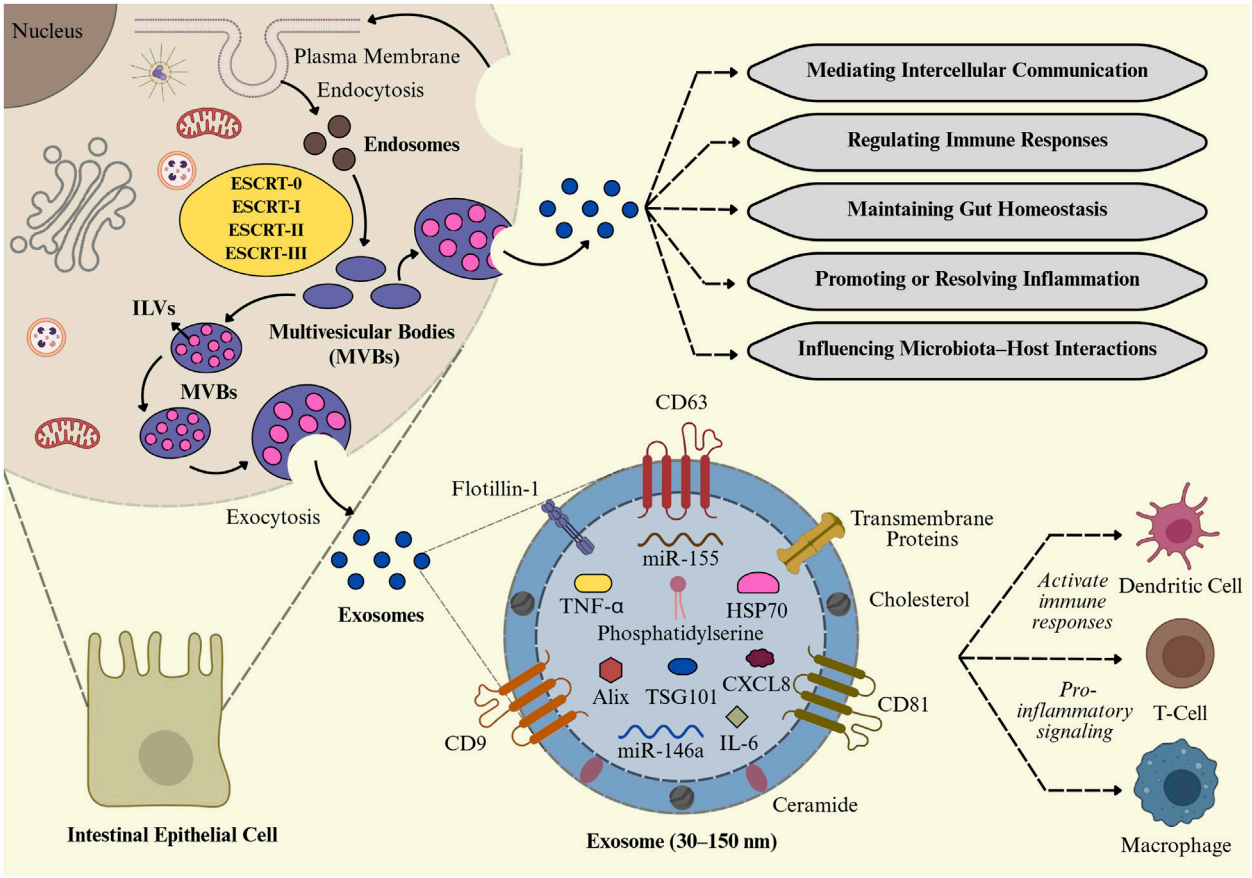


FIGURE 3
Biogenesis and functional roles of intestinal exosomes. Exosome formation in intestinal epithelial cells (IECs) *via* the ESCRT-mediated multivesicular body (MVB) pathway. These exosomes, enriched with cytokines (TNF-α, IL-6), miRNAs (miR-155, miR-146a), and proteins (CD63, TSG101, HSP70), regulate intercellular communication, immune responses, gut homeostasis, and microbiota–host interactions, while also contributing to proinflammatory signaling and immune cell activation.

role of ER stress in modulating exosome-mediated communication in IBD will be discussed in detail below.

3 Exosomes in intestinal inflammation

3.1 Overview of exosomes and their molecular cargo

Exosomes are nano-sized (30–150 nm) EVs of endosomal origin, secreted by almost all cell types, and play key roles in intercellular communication. They carry a broad spectrum of bioactive molecules, including proteins, lipids, metabolites, mRNA, DNA, and non-coding RNAs such as miRNAs, lncRNAs, and circRNAs (Kumar et al., 2024; Ruivo et al., 2017; Banerjee et al., 2020). In IBD, exosomes contribute to chronic inflammation by promoting immune cell activation, epithelial barrier disruption, and pro-inflammatory signaling (Ocansey et al., 2020). Exosomal proteins such as tetraspanins (CD9, CD63), ALIX, TSG101, and heat shock proteins (Hsp70, Hsp90) are associated with the amplification of intestinal inflammation and stress-induced epithelial damage (Schopf et al., 2017; Mazurov et al., 2013). miRNAs like miR-21, miR-126, and miR-23a, frequently enriched in exosomes, amplify inflammatory responses, promote angiogenesis, and compromise barrier integrity in the gut mucosa (Grimolizzi et al., 2017; Hu et al., 2020). Similarly, exosomal lncRNAs, including H19 and UCA1, are associated with promoting epithelial-mesenchymal transition and influencing immune regulatory pathways that intensify mucosal inflammation and contribute to therapeutic resistance (Xia et al., 2023; Fan et al., 2014). Also, exosomal circRNAs, such as circ-IARS and circ-DLEU2, affect immune signaling and epithelial function by binding to specific miRNAs and blocking their normal gene regulatory activities (Li et al., 2018; Wu et al., 2018).

3.2 Exosome biogenesis in IBD

The endosomal system generates tiny EVs, as part of the intricate intracellular process known as exosome biogenesis, as illustrated in Figure 3 (Gurung et al., 2021). It begins with the inward budding of the plasma membrane to form early-sorting endosomes, which can integrate cargo from both the extracellular environment and intracellular compartments such as the Golgi

apparatus and ER (Xie et al., 2022). As early-sorting endosomes mature into late-sorting endosomes, they undergo a second inward budding process, forming multivesicular bodies (MVBs) that include numerous intraluminal vesicles (ILVs), which are exosome precursors (Kalluri and LeBleu, 2020). Exosome biogenesis involves not only membrane budding and vesicle formation but also highly regulated molecular machinery that decides which proteins, lipids, and nucleic acids are packaged into ILVs (Ha et al., 2016). This sorting process is mainly carried out by a set of protein complexes collectively known as the endosomal sorting complex required for transport ESCRT), as well as by ESCRT-independent mechanisms. The ESCRT pathway comprises four main protein complexes, including ESCRT-0, I, II, and III, that work together to regulate vesicle fission, membrane budding, and the recognition of ubiquitinated cargo (Ha et al., 2016; Zhang et al., 2019). ESCRT-0 first recognizes, and confines ubiquitinated cargo proteins on the endosomal membrane, followed by the recruitment of ESCRT-I and ESCRT-II to assist membrane deformation. ESCRT-III completes vesicle fragmentation with ATPase VPS4 (Dai et al., 2020). Proteins like TSG101 and ALIX facilitate cargo selection and membrane budding (Taha et al., 2019). In the ESCRT-independent mechanism, ceramides derived from sphingomyelin promote vesicle budding through membrane curvature and phase separation (Horbay et al., 2022). Tetraspanins such as CD9, CD63, and CD81 organize membrane microdomains and assist in cargo sorting *via* tetraspanin-enriched microdomains (Toribio and Yáñez-Mó, 2022). MVBs may either fuse with lysosomes for cargo degradation or with the plasma membrane for extracellular exosome release. This secretion is mediated by Rab GTPases (Rab27a/b, Rab11), SNARE proteins, and syntenin-1, which regulate membrane trafficking and fusion (Xu et al., 2022; Kalluri and LeBleu, 2020). Released exosomes are 30–150 nm in diameter and appear cup-shaped with the transmission electron microscope or spheroidal under electron microscopy (Jung and Mun, 2018; Fakhredini et al., 2022). It has been reported that exosomes released by immune cells such as DCs, macrophages, T cells, and B cells actively contribute to the pathogenesis of IBD by persistent inflammation, modulating immune responses, and altering intestinal homeostasis (Hazrati et al., 2022). The role of these exosomes derived from the immune cells is discussed below.

3.3 Role of immune cell-derived exosomes in IBD

The immune microenvironment of the intestinal mucosa is significantly influenced by the immune cell-derived exosomes, which provide effective communication *via* both autocrine and paracrine pathways. By carrying proteins, lipids, and nucleic acids, immune cell-derived exosomes can alter the function of other immune cells, either promoting or inhibiting immunological responses (Hazrati et al., 2022). Depending on the cytokine environment, macrophages can polarize into pro-inflammatory M1 or anti-inflammatory M2 phenotypes (Shapouri-Moghaddam et al., 2018). Pro-inflammatory mediators such as TNF-α, IL-6, IL-23, and miR-155 are prevalent in exosomes released by M1 macrophages. These exosomes may increase inflammation by stimulating NLRP3 inflammasomes, Toll-like receptors, and TNF-related pathways in recipient cells (Wang et al., 2019; Tian et al., 2022). Additionally, these exosomes promote the migration of leukocytes and sustained inflammatory signaling in the intestinal mucosa, which are characteristics of IBD, by increasing the expression of endothelial cell adhesion molecules like ICAM-1 (Osada-Oka et al., 2017). Similarly, exosomes secreted by T cells, including $CD8^+$ cytotoxic T cells, $CD4^+$ helper T cells, and regulatory T cells (Tregs), serve as carriers of bioactive molecules, including proteins, lipids, and microRNAs, which influence target cell behavior (Table 2). Exosomes derived from activated T cells activate the RAS/MAPK signaling cascade, inducing ERK phosphorylation and amplifying inflammatory responses in recipient immune cells, thereby contributing to IBD pathophysiology (Zhang et al., 2024). Exosomes produced by $CD8^+$ T cells, which include cytotoxic substances, such as granzyme and perforin, could contribute to chronic inflammation by causing tissue damage. Exosomes generated from $CD4^+$ T cells interact with immune cells, including macrophages and NK cells, and may alter immunological activities by stimulating TCRs to release major histocompatibility complex (MHC) class II-like peptides (Wang and Shi, 2022). Upon activation of B cells *via* B cell receptor or Toll-like receptor (TLR) signaling, B cells release exosomes rich in immune molecules, including MHC I/II, costimulatory markers (CD40, CD80, CD86), and B cell marker CD19. These exosomes can stimulate T cell responses by presenting antigens through MHC-II-peptide complexes (Azoulay-Alfaguter and Mor, 2018). However, this activation frequently results in a proliferation of T cells with limited capacity to proliferate and a decreased production of cytokines (Hazrati et al., 2022), which may be a contributing factor to the persistent inflammation observed in IBD. Likewise, DCs produce exosomes that vary in function depending on their maturation status and the local cytokine environment. DC progenitors develop into mature DCs under pro-inflammatory conditions, which are characterized by high levels of cytokines like IL-6 and TNFα (Hazrati et al., 2022). These cells then produce exosomes that are enriched with immunostimulatory molecules such as MHC class II, CD40, CD80, and CD86 (Tkach et al., 2017). Naïve T cells are extensively activated by these mature DC-derived exosomes, leading them to differentiate into effector T cells and increasing the adaptive immune response. Moreover, these exosomes promote the proliferation and activation of natural killer cells by expressing IL-15 receptor alpha (IL-15Rα) and NKG2D ligands such as MIC-A, MIC-B, and ULBP-1, resulting in increased IFN-γ secretion that impairs mucosal inflammation (Bassani et al., 2019; Viaud et al., 2009). DC-derived exosomes also increase cytokine production and immune activation by releasing inflammatory microRNAs, such as miR-155, to recipient immune cells. Their integrin and adhesion molecule content facilitates the influx of immune cells to inflamed tissues, contributing to the longevity of inflammation (Lindenbergh et al., 2019). Thus, mature DC-derived exosomes in IBD function as potent pro-inflammatory agents that drive immune cell activation, promote the production of cytokines, and maintain mucosal dysregulation, thereby worsening the severity and progression of IBD.

TABLE 2 Key immune cell-derived exosomes and their functional roles in IBD.

Immune cell type	Key exosomal cargo	Effect in IBD	Inflammatory role	References
Macrophages (M1/M2)	M1: TNF-α, IL-6, IL-23, miR-155 M2: TGF-β, IL-10, miR-21-5p, miR-155-5p	M1 promotes leukocyte recruitment and inflammation; M2 reduces inflammation but may promote tumorigenesis	Pro-inflammatory (M1)/Anti-inflammatory, pro-tumorigenic (M2)	Wang et al. (2019); Tian et al. (2022); Ma et al. (2021)
T Cells (CD4+/CD8+)	Granzyme, perforin, MHC II-like peptides	Cytotoxicity, cytokine release, and immune activation	Pro-inflammatory	Wang and Shi, (2022); Zhang et al. (2024)
Dendritic cells	MHC II, CD40, CD80, CD 86, IL-15Rα, MIC-A/B, ULBP-1, miR-155	Stimulate T/NK cells; mucosal inflammation	Strongly pro-inflammatory	Tkach et al. (2017); Bassani et al. (2019)
Neutrophils	Myeloperoxidase, miR-23a, miR-155	Barrier damage, impaired wound healing	Strongly pro-inflammatory	Ayyar and Moss (2021)
Mast cells	Histamine, IL-4, TNF-α, tryptase, chymase, miR-223	Increase permeability, promote fibrosis	Pro-inflammatory	Li et al. (2020)
Natural killer cells	Perforin, granzyme B, IFN-γ, miR-186	Epithelial injury and inflammation	Pro-inflammatory	Hazrati et al. (2022)

4 Exosome-mediated miRNA dysregulation and barrier dysfunction in IBD

In IBD, exosomes carrying dysregulated miRNAs and inflammatory cargo contribute to gut barrier breakdown, microbial imbalance, and inflammation. Exosomal miR-223 derived from immune cells downregulates Claudin-8 and CLDN8 expression, leading to the disruption of tight junctions and increased epithelial permeability through activation of the IL-23 pathway. This, in turn, promotes microbial translocation and enhances inflammatory cell infiltration (Li et al., 2020; Zhou et al., 2021). Similarly, elevated miR-21 and miR-301a in epithelial exosomes, induced by pro-inflammatory stress, promote *AKT* activation and suppress cadherin-1 or *PTEN* expression, disrupting junctional integrity and promoting inflammation-driven permeability (Zhou et al., 2021). Moreover, aberrant exosomal miR-29a expression in IECs has been linked to reduced occludin levels and enhanced epithelial permeability, particularly in aging and inflammatory conditions marked by elevated levels of IFN-γ, IL-6, and IL-1β (Park et al., 2022). These exosomes can also promote macrophage polarization toward the pro-inflammatory M1 phenotype through delivery of miR-155, which enhances NF-κB activity and the secretion of TNFα and IL-6, amplifying epithelial injury and mucosal immune activation (Shen et al., 2021). Moreover, exosomes derived from the serum of IBD patients have been found to upregulate Interleukin-8 (IL-8) in epithelial cells, triggering macrophage chemotaxis and sustaining immune cell infiltration, which further disrupts the mucosal barrier and disturbs microbial homeostasis (Shen et al., 2021). Furthermore, exosomal cargo altered by infection with *Escherichia coli* strains exhibiting adherent-invasive characteristics, such as the downregulation of let-7b, can alter macrophage activation, hinder autophagy, and activate fibrogenic and ER stress pathways, thereby intensifying CD progression (Carrière et al., 2016). Notably, host-derived exosomes can also directly influence microbial gene expression. Fecal exosomes containing miR-515-5p or miR-1226-5p can enter gut bacteria like *Fusobacterium nucleatum* or *E. coli*, altering their transcriptional activity, favoring the expansion of pro-inflammatory species, and contributing to dysbiosis (Liu et al., 2016). Additionally, disruptions in Rab27a-mediated exosomal secretion from myeloid cells can limit the release of anti-inflammatory miRNAs such as miR-146a, leading to excessive macrophage activation and worsening colitis severity (Bauer et al., 2022). In addition, *F. nucleatum*-derived EVs have been shown to intensify the experimental colitis by disrupting epithelial barrier function and promoting inflammatory autophagy (Wei S. et al., 2023). In Dextran Sulfate Sodium (DSS)-treated mice, oral administration of *F. nucleatum*-derived EVs significantly worsened colitis severity by downregulating miR-574-5p and activating the CARD3-dependent autophagy pathway in IECs, which led to reduced expression of tight junction proteins ZO-1 and occludin, elevated IL-1β, IL-6, and TNFα secretion, and pronounced epithelial damage (Wei Z. et al., 2023). Wei S. et al. (2023) employed a DSS-induced colitis mouse model along with an experiment on cultured IECs to investigate the effects of *F. nucleatum*-derived EVs. The study utilized targeted knockdown and overexpression approaches to dissect the role of miR-574-5p and the CARD3-dependent autophagy pathway and quantified tight junction proteins and pro-inflammatory cytokines using standard molecular and immunological techniques. Inhibition of autophagy or targeting the miR-574-5p/CARD3 axis alleviated both barrier disruption and colitis symptoms, highlighting a direct pathogenic mechanism by which bacterial EVs can impair gut integrity in IBD (Wei Z. et al., 2023).

5 Interconnection of ER stress and exosome biogenesis in IBD

The interconnection between ER stress and exosome release may significantly contribute to the pathogenesis and progression of IBD, with significant implications for intestinal inflammation and immune modulation. Inherited genetic variations in major ER stress regulators such as XBP1, ARG2, and ORMDL3, along with external factors like microbial-derived molecules and pro-inflammatory

cytokines, can significantly impair ER homeostasis (Kaser and Blumberg, 2010). This dysregulation of ER stress can activate pro-inflammatory signaling pathways in the gut, reduce the integrity of the mucosal barrier, and induce epithelial cell apoptosis (Qiao et al., 2021). ER stress, arising from the accumulation of misfolded proteins, activates the UPR, leading to alterations in cellular homeostasis, and this stress response not only influences intracellular functions but also modulates EV dynamics, particularly exosome biogenesis (Ye and Leu, 2022). ER stress influences the formation of MVBs and the release of exosomes, thereby establishing a crucial link between intracellular stress responses and intercellular communication (Jahangiri et al., 2022). As exosomes serve as crucial mediators of intercellular communication, in the context of IBD, their interplay with ER stress may play an important role in disease onset, progression, and recovery. Under ER stress conditions, cells initiate adaptive mechanisms to restore homeostasis, which not only regulate protein folding and degradation but also influence cellular processes such as autophagy and exosome formation (Senft and Ronai, 2015; Jahangiri et al., 2022). It has been reported that ER stress enhances the formation of MVBs, the precursors to exosomes, by promoting the inward budding of late endosomes (Wu et al., 2021). This process is facilitated by the upregulation of proteins involved in vesicle trafficking and membrane remodeling, such as CD63 (Gurunathan et al., 2021). Consequently, ER stress may lead to an increase in exosome secretion, thereby altering the extracellular matrix and influencing neighboring cells. Exosomes derived from ER-stressed cells often carry stress-related biomolecules, including proteins such as GRP78 and CHOP, lipids such as ceramides and lysophosphatidylcholine, and RNAs, including miR-23a, which can transmit ER stress and activate inflammatory signals in recipient cells (Ye and Leu, 2022). In IBD, where the intestinal epithelium is subjected to chronic inflammatory stimuli, this mechanism amplifies persistent ER stress, disrupting epithelial cell function and integrity and contributing to barrier dysfunction and increased intestinal permeability (Shi et al., 2024). Simultaneously, enhanced exosome production under ER stress conditions may facilitate the transfer of pro-inflammatory mediators, including cytokines, microRNAs, and damaged proteins, to adjacent cells (Ye and Leu, 2022). In the mucosal microenvironment of IBD, enriched with inflammatory mediators and damage-associated molecular patterns (DAMPs), this ER stress–exosome interplay creates a feedback loop that continues and amplifies inflammation (Ocansey et al., 2020). Exosomes derived from stressed epithelial cells can deliver inflammatory signals to immune cells, continuing the inflammatory characteristic of IBD, while the altered protein and RNA composition of exosomes under ER stress may influence the differentiation, activation, and polarization of immune cells such as macrophages, dendritic cells, and T cells, thereby worsening the inflammatory response (Ocansey et al., 2020). The mutual interaction between ER stress and exosome biogenesis may further intensify the pathophysiology of IBD, as exosomes not only serve as vehicles for transmitting stress-induced signals (Liao et al., 2019) but also participate in the modulation of ER stress pathways in recipient cells (Kanemoto et al., 2016). This intercellular communication loop may underscore the complexity of cellular adaptations to stress and highlights the potential for exosomes to propagate pathological conditions beyond their cell of origin. A more detailed analysis of how ER stress interfaces with exosome biogenesis in IBD will be discussed in the following sections.

5.1 ER stress-induced exosome release and cargo modifications

Under ER stress, cells upregulate pathways that significantly enhance exosome biogenesis and secretion. Kanemoto et al. (2016) demonstrated that ER stress significantly enhances multivesicular body formation and exosome secretion, establishing a direct link between ER stress signaling and extracellular vesicle biogenesis using HeLa cells as well as IRE1α/β and PERK knockout mouse embryonic fibroblasts (MEFs), treated with tunicamycin. By inhibiting the key UPR sensors, the stress-related increase in exosome release was prevented. This is because certain key molecules involved in the generation of exosomes, such as ESCRT proteins and ceramide, are activated by ER stress pathways, particularly those involving IRE1α and PERK (Ye and Leu, 2022). This highlights a strong connection between ER stress and increased exosome production. Likewise, triggering intense ER stress in choriocarcinoma cells with tunicamycin results in a significant increase in the release of EVs (Collet et al., 2018). These findings suggest that increased exosome production is a common cellular response to stress, indicating that cells deliberately adjust exosome release to cope with stressful conditions (Collet et al., 2018). ER stress also changes the composition of exosomal cargo. Under stress, cells are more likely to load vesicles with DAMPs and proinflammatory molecules. In a study by Collet et al. (2018), it was revealed that inducing severe ER stress in placental cells led to the release of EVs that were enriched with high levels of DAMPs, HMGB1, and HSP70. These EVs containing DAMPs naturally promote inflammation. In the metabolic context, obesity-induced metabolic stress, which is characterized by elevated palmitate levels, activates NF-κB and ER stress pathways in adipocytes, which in turn significantly enhance the enrichment of certain microRNAs into exosomes (Li et al., 2024). Similarly, inflammatory signals in the gut, such as cytokines and hypoxia, are likely to alter the RNA composition of exosomes. In fact, exosomal miRNA profiles are disrupted in IBD; for instance, miR-21, a pro-inflammatory miRNA elevated in inflamed colonic tissue, is selectively incorporated into epithelial cell-derived exosomes in response to stress-related stimuli like substance P/NK-1R activation (Bakirtzi et al., 2019). Similarly, neutrophil-derived exosomes carry miRNAs like miR-23a and miR-155, which negatively affect colonic epithelial wound healing (Ayyar and Moss, 2021). Additionally, exosomal miR-223 from macrophages has been shown to intensify intestinal barrier damage in a DSS-induced colitis model (Chang et al., 2023). Also, IBD patient-derived exosomes contain a highly pro-inflammatory molecular profile. Mitsuhashi et al. (2016) collected intestinal luminal aspirates (~10 mL of fluid) from the colon of IBD patients and healthy controls during colonoscopy to isolate luminal EVs and compare their molecular contents. It has been reported that EVs from IBD patients contained significantly elevated levels of proinflammatory cytokines IL-6, IL-8, TNFα, and the anti-inflammatory cytokine IL-10, along with specific proteins and miRNAs associated with inflammation. It also

TABLE 3 Exosome-mediated propagation of ER stress and inflammatory signals in IBD.

Source of exosomes	Key cargo	Effect on intestinal microenvironment	References
ER-stressed IECs derived exosomes	dsDNA-rich exosomes	Activate cGAS–STING in macrophages → excessive cytokine release	Zhou et al. (2021)
IBD patient-derived exosomes	Pro-inflammatory cytokines, miRNAs, proteins	Increased IL-8 production, disrupted barrier integrity	Mitsuhashi et al. (2016); Bakirtzi et al. (2019)
Neutrophil-derived exosomes	miR-23a, miR-155, myeloperoxidase	Impaired wound healing, barrier damage	Ayyar and Moss (2021)
Adipose tissue exosomes	miR-155	Promote M1 polarization, worsen colitis	Wei Z. et al. (2023)
Salivary exosomes	Multiple inflammatory mediators	Disrupt tight junctions, worsen colitis	Yang et al. (2024)

demonstrated that these EVs could induce proinflammatory responses in cultured IECs and macrophages, suggesting that they originate from ER-stressed intestinal epithelial and immune cells within inflamed mucosal tissue and actively contribute to disease-associated inflammation.

5.2 Exosome-mediated transfer of ER stress signals in intestinal inflammation

Exosomes released from ER-stressed IECs can transmit stress signals to neighboring cells within the gut microenvironment. Gut-resident secretory cells under proteostatic stress, such as Paneth or goblet cells with XBP1 dysfunction, are particularly prone to releasing exosomes enriched with UPR components or misfolded proteins (Bhattacharya and Chatterji, 2024). Mahadevan et al. (2011) revealed that macrophages exposed to conditioned medium from ER-stressed tumor cells were used to investigate intercellular communication of stress signals. They observed that macrophages not only upregulate UPR genes but also produce elevated levels of proinflammatory cytokines, demonstrating that ER stress can be transmitted from tumor cells to recipient myeloid cells, thereby activating the UPR and promoting an inflammatory response Moreover, ER stress-associated exosomes have the potential to modulate immune responses. He et al. (2020) found that macrophages exposed to exosomes derived from ER-stressed HepG2 cells significantly increase the expression of IL-6, MCP-1, and IL-10. These exosomes might contain phospholipids or vesicular miRNAs that activate NFκB or JNK signaling pathways, or they may carry UPR-related transcription factors or chaperones like GRP78 either within their cargo or on their surface, thereby amplifying stress responses in recipient cells. Functionally, recipient cells react by triggering UPR sensors, elevating cytokine production, and frequently exhibiting impaired barrier integrity. For instance, exosomal miR-21 can target tight junction regulators and promote epithelial migration, while other miRNAs or proteins in IBD EVs may disrupt mucosal integrity (Bakirtzi et al., 2019). Furthermore, neutrophil-derived exosomes, which are abundant in inflamed tissues, secrete myeloperoxidase and specific miRNAs (e.g., miR-23a and miR-155). These miRNAs are taken up by IECs and have been shown to impair wound healing (Ayyar and Moss, 2021). Collectively, these mechanisms suggest that ER stress in a single cell can be transmitted through exosomes, triggering ER stress markers along with inflammatory responses in nearby gut cells. A summary of the exosome-mediated interactions and their functional consequences in IBD is represented in Table 3.

5.3 Exosome-ER stress loop in gut inflammation

Exosomes carrying inflammatory and ER stress signals amplify cytokine activity and cellular dysfunction, which subsequently enhances ER stress and triggers further exosome release (Figure 4). For instance, Mitsuhashi et al. (2016) showed that exosomes from IBD patients promote macrophage migration and cytokine release, while IL-8-containing exosomes attract neutrophils, collectively exacerbating ER stress in epithelial cells. Notably, this ER stress significantly influences the molecular composition of the exosomes, leading to their enrichment with specific proteins such as Pregnancy zone protein (Shao et al., 2021) as well as lipids like ceramides associated with IRE1α activation (Dasgupta et al., 2020), and potentially distinct miRNAs (miR-223, miR-23a, and miR-155) that reflect the inflammatory and stressed state of the originating cell (Wang et al., 2020; Ayyar and Moss, 2021). At the molecular level, exosomes carrying miRNAs or DAMPs can strongly stimulate the NF-κB and JAK/STAT signaling pathways in immune cells. Mahadevan et al. (2011) demonstrated that macrophages exposed to tumor-derived ER stress signals not only activate their own stress response pathways but also acquire a pronounced proinflammatory phenotype. These macrophages showed increased levels of stress-related markers such as Grp78, Gadd34, CHOP, and spliced Xbp1, indicating that they were deeply affected by the tumor environment. Also, Chen et al. (2022) showed that a high-fat diet worsens TNBS-induced colitis in mice *via* mesenteric adipose tissue-derived exosomes enriched with *MALAT1*. These exosomes are taken up by colonic epithelial cells, where *MALAT1* suppresses miR-15a-5p, leading to activation of the ATF6-mediated ER stress pathway. This cascade results in amplified inflammatory signaling, increased epithelial cell damage, and worsening of colitis pathology, highlighting the interplay between diet, exosome signaling, and ER stress in intestinal inflammation. Similarly, Jin et al. (2025) demonstrated that IEC-specific Prdx3 deficiency leads to severe DSS-induced colitis through increased oxidative stress and exosomal release of miR-1260b. These exosomes impair epithelial barrier integrity and activate p38 MAPK/NF-κB signaling, promoting inflammation. This further supports that the stress-induced

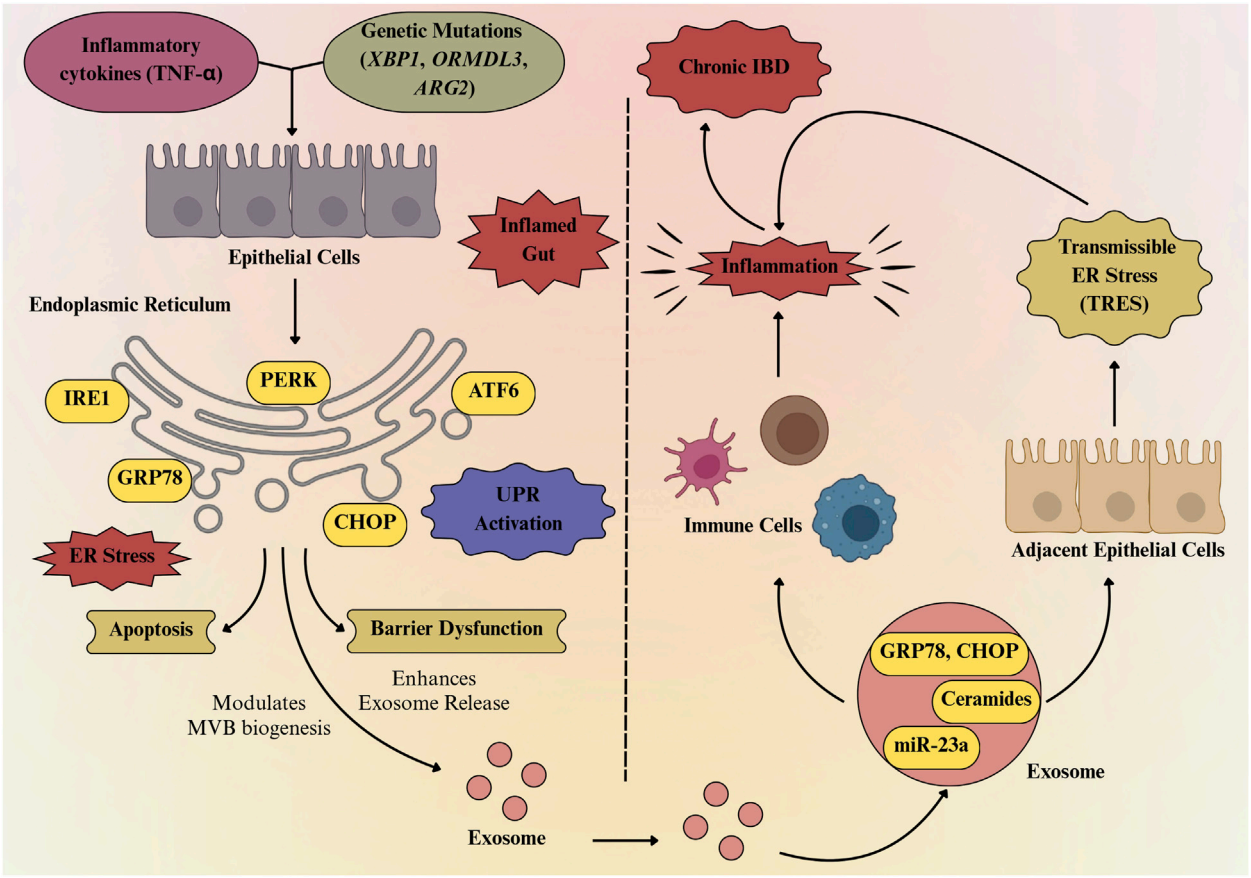


FIGURE 4
Exosome-mediated bidirectional loop linking ER stress to chronic gut inflammation. Inflammatory cytokines and genetic mutations (XBP1, ORMDL3, ARG2) trigger ER stress in intestinal epithelial cells, activating the unfolded protein response (UPR) through PERK, IRE1, and ATF6 pathways. Persistent ER stress induces CHOP-mediated apoptosis and barrier dysfunction, while also promoting exosome release enriched with GRP78, CHOP, ceramides, and miR-23a. These exosomes propagate inflammation, activate immune cells, and transmit ER stress to neighboring cells, thereby sustaining chronic IBD.

exosomal cargo amplifies inflammatory signaling and epithelial dysfunction, thereby sustaining ER stress and contributing to disease severity in IBD.

6 Preclinical evidence linking ER stress–exosome to IBD pathogenesis

Growing experimental evidence reveals that ER stress in IECs promotes exosome-mediated immune activation, thereby amplifying intestinal inflammation. Zhou et al. (2021) demonstrated that ER-stressed IECs, in both DSS-induced colitis models and in colonic biopsies from CD patients, secrete exosomes enriched with mitochondrial and nuclear double-stranded DNA (dsDNA), suggesting a mechanism by which ER stress contributes to intestinal inflammation. When intestinal macrophages absorb these exosomal dsDNAs, they function as strong DAMPs that trigger the cytosolic cGAS–STING DNA-sensing pathway. This activation leads to the induction of proinflammatory cytokines, including TNF-α, IL-6, and IFN-β (Zhou et al., 2021). Additionally, in DSS-induced colitis mice, therapeutic suppression of exosome secretion with GW4869 not only decreased STING activation in immune cells but also reduced the severity of the disease, suggesting a transmissible loop between immunological activation and epithelial stress. Furthermore, Kaser et al. (2008) showed that deletion of XBP1 in IECs leads to spontaneous enteritis and increased vulnerability to colitis as a result of Paneth cell malfunction and epithelial hyperactivity to inflammatory and microbial stimuli such as TNFα and flagellin. The study revealed and validated the link between XBP1 genetic variants and both forms of human IBD (UC and CD), with novel hypomorphic variants emerging as risk factors. By activating macrophages and disrupting the intestinal barrier, Gong et al. (2022) showed that serum-derived exosomes from individuals with CD increase intestinal inflammation. These exosomes increased macrophage count and proinflammatory cytokine expression *in vitro*, while also enhancing epithelial permeability. The study also demonstrated how CD altered the exosomal miRNA profile, specifically lowering the levels of let-7b-5p (Gong et al., 2022). The let-7b-5p/TLR4 pathway was found to be a major mechanistic regulator of inflammation mediated by macrophages. These findings collectively highlight a pathogenic feedback loop in which ER stress in the gut epithelium induces the release of pro-inflammatory exosomes, which in turn activate immune responses

TABLE 4 Preclinical insights into ER stress–exosome–mediated intestinal inflammation.

Source/Model	Key findings	Type of study	References
DSS-induced colitis mice	ER-stressed IECs release dsDNA-rich exosomes → activate cGAS–STING in macrophages → excessive cytokine release.	*In vivo / ex vivo*	Zhou et al. (2021)
XBP1-deficient mice	XBP1 deletion → spontaneous enteritis, Paneth cell dysfunction, ↑ colitis susceptibility.	*In vivo*	Kaser et al. (2008)
Serum exosomes (from CD patients)	↑ macrophage cytokines, ↑ epithelial permeability; ↓ let-7b-5p/TLR4 drives inflammation.	*In vitro / ex vivo*	Gong et al. (2022)
Salivary exosomes (from IBD patients)	Disrupted tight junctions in Caco-2 cells induced macrophage inflammation, worsened colitis.	*In vitro / in vivo*	Yang et al. (2024)
Adipose exosomes (from high-fat diet mice)	miR-155-enriched exosomes → M1 macrophage polarization → worsened DSS colitis.	*In vivo*	Wei S. et al. (2023)

that further intensify ER stress and epithelial injury (Gong et al., 2022). This emerging axis provides novel insights into the chronicity of intestinal inflammation in IBD and identifies potential therapeutic targets such as UPR modulators and exosome release inhibitors to disrupt the pathological interplay between epithelial stress responses and immune activation. Also, Yang et al. (2024) demonstrated that salivary exosomes from active IBD patients aggravated colitis in mice. These exosomes disrupted intestinal epithelial integrity by reducing tight junction proteins in Caco-2 cells and induced inflammatory responses in THP-1 macrophages, potentially intensifying ER stress in IECs. Moreover, the distinct miRNA profiles found in these salivary exosomes indicate that their cargo may influence cellular stress pathways, immune responses, and gut microbiota balance (Yang et al., 2024). Another study by Wei S. et al. (2023) showed that visceral adipose tissue-derived exosomes from high-fat diet-fed mice intensified DSS-induced colitis by shifting their miRNA cargo toward a pro-inflammatory profile, particularly enriching miR-155. These exosomes infiltrated the intestinal lamina propria and promoted M1 macrophage polarization, thereby enhancing intestinal inflammation. Key preclinical evidences connecting ER stress and exosomes in IBD pathogenesis is summarized in (Table 4).

7 Therapeutic and diagnostic implications

Exosomes play a dual role in the pathogenesis and treatment of IBD, acting both as mediators of inflammation and as potential therapeutic agents. Exosomes display a context-dependent balance between pro-inflammatory and anti-inflammatory functions. For instance, serum-derived exosomes enriched with proinflammatory mediators, such as let-7b-5p, can activate macrophages *via* the p38/ERK pathway, thereby intensifying colitis and promoting tissue damage (Huang et al., 2025). In contrast, mesenchymal stem cell–derived exosomes (e.g., from adipose or umbilical cord sources) are enriched with anti-inflammatory cargos, particularly those carrying IL-10, TGF-β, and miR-146a. These exosomes suppress NF-κB signaling, reduce cytokine production, and promote epithelial repair, highlighting their therapeutic promise (Lee et al., 2023; Kheradmand et al., 2025). A study by Banerjee et al. (2015) has demonstrated that UCMSCs effectively prevented DSS-induced colitis in immunodeficient mice by reducing inflammation, tissue damage, and ER stress, highlighting the therapeutic potential of targeting ER stress in IBD. The study also showed that UCMSC-derived factors can likely directly modulate intestinal injury, suggesting a novel diagnostic and treatment approach for IBD. Recent studies (Wei Z. et al., 2023; Saleem et al., 2024) highlight the ability of exosomes to carry bioactive molecules that influence immune responses and epithelial integrity, making them desirable targets for therapeutic intervention. Various sources of exosomes have been explored as a therapeutic target, including those derived from mesenchymal stem cells, immune cells, intestinal epithelial cells, and even plant and food-derived nanovesicles (Ocansey et al., 2020). Several emerging therapeutic strategies targeting exosomes from different sources and ER stress modulation have shown promise in alleviating IBD. Exosomes derived from mesenchymal stem cells, particularly those isolated from adipose tissue or bone marrow, have demonstrated anti-inflammatory and immunomodulatory properties that reduce intestinal inflammation and promote mucosal healing in colitis models (Wei S. et al., 2023; Zubair et al., 2025). These exosomes can carry beneficial microRNAs like miR-146a, miR-223, which inhibit NF-κB activation and suppress pro-inflammatory cytokine expression in macrophages and IECs, thereby restoring immune balance (Zubair et al., 2025). Additionally, plant-derived exosome-like nanoparticles from ginger, grape, or broccoli are gaining attention due to their natural origin and ability to deliver bioactive molecules directly to the gut (Li et al., 2023). It has been reported that, in DSS-induced colitis models, ginger-derived nanoparticles were found to be predominantly absorbed by intestinal epithelial and immune cells, decreasing pro-inflammatory cytokines (TNF-α, IL-6), and improving barrier function (Zhang et al., 2016). Another study by Deng et al. (2017) has revealed that broccoli-derived nanoparticles (BDNs) play a protective role in mouse models of colitis by modulating DC function through activation of AMP-activated protein kinase (AMPK). BDNs induced the formation of tolerogenic DCs and suppressed their activation, thereby maintaining intestinal immune homeostasis (Deng et al., 2017). The anti-inflammatory activity was primarily associated with the sulforaphane component of BDNs, since defense against DSS-induced colitis was exclusively provided by $AMPK^{+/+}$ DCs and BDN lipids, including sulforaphane (Deng et al., 2017). Moreover, in mouse models of IBD, it has been demonstrated that chemical chaperones like tauroursodeoxycholic acid and 4-phenylbutyric acid reduce ER stress by stabilizing protein

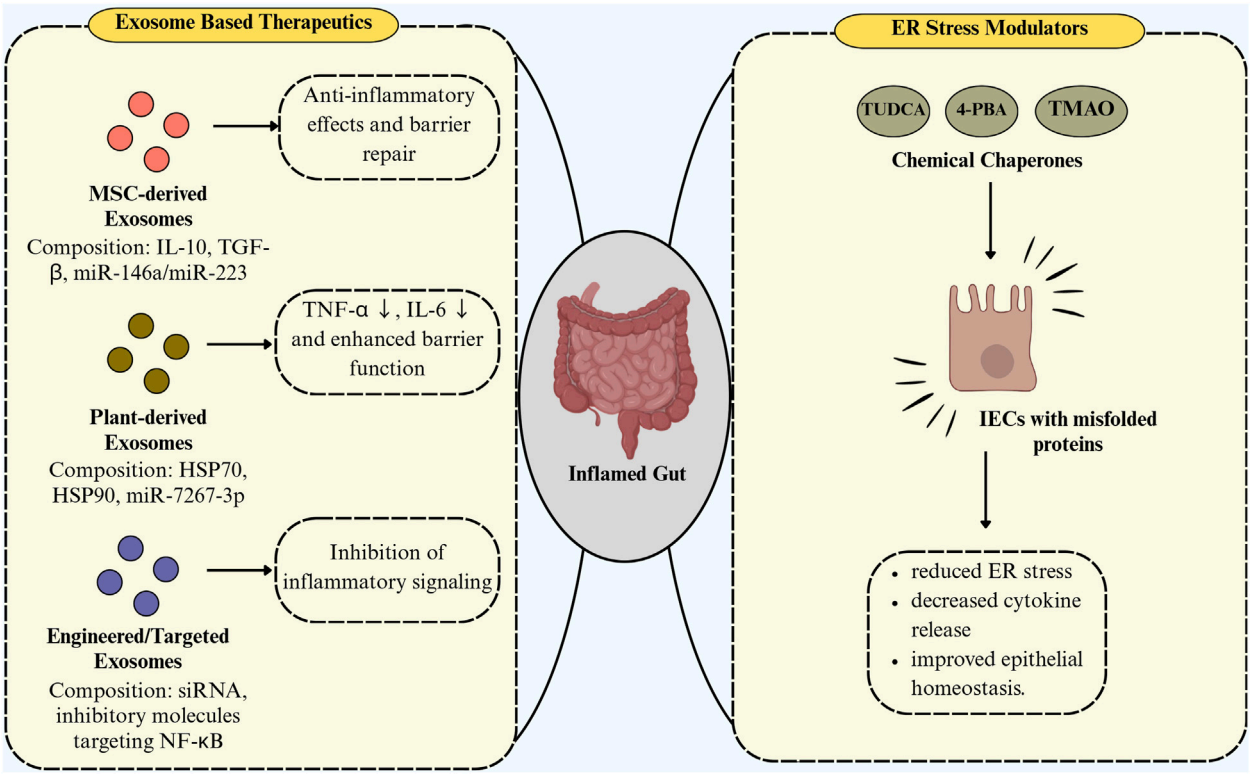


FIGURE 5
Exosome-based therapeutics and ER stress modulators in intestinal inflammation. Therapeutic strategies for intestinal inflammation include exosome-based approaches—MSC-derived exosomes promoting anti-inflammatory effects and barrier repair, plant-derived exosomes reducing TNF-α/IL-6 and enhancing barrier function, and engineered exosomes inhibiting NF-κB signaling—and ER stress modulators (TUDCA, 4-PBA, TMAO) acting as chemical chaperones to alleviate ER stress, decrease cytokine release, and restore epithelial homeostasis.

conformation and reducing the accumulation of unfolded proteins, which restores epithelial homeostasis and decreases inflammation (Cao et al., 2013). Thus, a dual approach including modulating the origin and cargo of exosomes, and thereby reducing ER stress, may offer a combinational potential in decreasing IBD severity and promoting gut homeostasis. A schematic representation of these therapeutic opportunities is illustrated in Figure 5.

7.1 Human clinical trials

Exosome research is gradually advancing toward clinical application, with several early-phase human trials underway (https://clinicaltrials.gov/). While most of the exosome-based studies focus on cancer, regenerative medicine, and drug delivery, an increasing number of studies are now exploring the potential roles of exosomes in IBD. A Phase I clinical trial (NCT06853522) is currently investigating the safety and therapeutic potential of human umbilical cord mesenchymal stem cell–derived exosomes (hUC-MSC-Exos) in patients with active UC. The trial seeks to determine whether hUC-MSC-Exos can be safely administered to patients while also providing early indications of their ability to alleviate intestinal inflammation and promote mucosal healing. In addition, a randomized pilot study (NCT04879810) is evaluating the safety and therapeutic potential of ginger-derived exosomes, with or without curcumin, in patients with IBD. This trial assesses whether exosomes alone or in combination with curcumin can reduce symptoms, improve disease scores, and modulate inflammatory biomarkers. The study plans to enroll up to 90 patients across three groups, with the combination therapy expected to provide greater symptom improvement, supporting the development of novel nutraceutical-based approaches for patients with persistent IBD. Similarly, a Phase I clinical trial (NCT06742203) is evaluating WPMDE1, a whey protein milk-derived exosome nutritional supplement, in healthy adults. The trial aims to assess tolerability, usability, and optimal dosing of WPMDE1, with participants receiving ascending doses over 7–30 days. Blood sampling, vital sign monitoring, and gastrointestinal symptom tracking will be performed to guide the design of future studies targeting patients with irritable bowel syndrome and IBD. Likewise, phase I/II clinical studies (NCT05499156, NCT05402748) are evaluating human placenta–derived MSC exosomes for the treatment of complex perianal or anal fistulas in CD patients who have not responded to prior therapies. In both studies, exosomes were injected directly into the fistula tract weekly for three consecutive weeks, and patients were monitored for fistula healing by MRI, changes in inflammatory biomarkers (CRP, IL-6, TNF-α, calprotectin), and quality of life over 12 weeks. These studies provide preliminary evidence that MSC-derived exosomes may promote fistula closure, reduce local inflammation, and improve patient-reported outcomes, supporting their potential as a novel therapeutic option in Crohn's-related perianal disease. A summary

TABLE 5 Human clinical trials of exosome-based therapies in IBD.

Trial ID	Sources of exosomes	Phase of study	Intervention/Design	Key outcomes
NCT06853522	hUC-MSC	Phase I	Administration of hUC-MSC-Exosomes in UC patients	Evaluation of safety, inflammation reduction, and mucosal healing
NCT04879810	Ginger with/without curcumin	Pilot study	Exosomes with/without curcumin supplementation	Assessment of symptoms, disease activity scores, and biomarkers
NCT06742203	Whey protein milk-derived	Phase I	Ascending dose administration over 7–30 days	Determination of tolerability, optimal dosing, and gastrointestinal symptom response
NCT05499156	Placenta-derived MSC	Phase I/II	Weekly injections of exosomes into the fistula tract	Evaluation of fistula healing, biomarker modulation, and quality of life improvement
NCT05402748	Placenta-derived MSC	Phase I/II	Weekly injections of exosomes into the fistula tract	Evaluation of fistula closure and inflammation.

of these human clinical trials, highlighting their design, interventions, and key findings in IBD, is provided in Table 5.

8 Challenges and future directions

Despite significant advances in understanding the ER stress–exosome axis in IBD, several key challenges and substantial knowledge gaps remain, particularly concerning how these processes operate in human disease and how current insights can be effectively translated into clinical applications. Most of the recent research is based on animal models or *in vitro* cell cultures, which do not accurately represent the complexity of the human gut, leading to variability and inconsistencies in results across studies. This complicates the understanding of the interaction between exosomes and ER stress in IBD patients. Moreover, few clinical studies directly validated the role of ER stress–exosome interactions in IBD, leaving a critical gap between preclinical findings and patient outcomes. Another major challenge is the lack of advanced models that can accurately mimic the complex environment of the human intestine. Conflicting results regarding exosome cargo composition, release mechanisms, and functional effects further underscore the need for more robust, standardized investigations. Current experimental models are limited in replicating the intricate microenvironment of the human gut. Although emerging tools such as organoids (mini-gut models) (Yin et al., 2019), gut-on-a-chip systems (Ashammakhi et al., 2020), and single-cell technologies (Zheng, 2023) offer promising platforms, yet they remain in the early stages of development and are not fully explored. Technical limitations in isolating, characterizing, and analyzing exosomes, due to their heterogeneity and overlap with other EVs, (Tzng et al., 2023), hinder reproducibility, and limit the discovery of reliable biomarkers or therapeutic targets. From the clinical application point of view, ensuring the safety, specificity, and scalability of exosome-based therapies, as well as addressing complex regulatory requirements, are additional challenges. (Rezaie et al., 2022). Future research should focus on elucidating the precise molecular mechanisms by which ER stress pathways, such as PERK, IRE1α, and ATF6, regulate exosomal cargo loading, including proteins, lipids, and miRNAs that influence intestinal inflammation. To better understand the dynamic connection between ER stress and exosomes during disease progression and treatment, and to identify novel diagnostic and therapeutic approaches for IBD, future studies could employ a range of experimental strategies. CRISPR/Cas9-mediated gene editing could be utilized to selectively modulate ER stress components (PERK, IRE1α, or ATF6) or miRNA cargo regulators to determine their specific roles in exosomal cargo loading and intestinal inflammation (Xu et al., 2025). Engineered exosomes carrying therapeutic miRNAs or silencing RNAs could be developed and tested in organoid models to assess their capacity to ameliorate ER stress-induced inflammation. Integration of spatial transcriptomics like GeoMx or 10x Xenium platforms on intestinal biopsies from IBD patients will enable precise mapping of the cellular microenvironments where ER stress and exosome interactions occur within inflamed tissue, revealing the spatial relationship between stressed epithelial cells and immune infiltrates (Liu et al., 2024; Mennillo et al., 2024). Single-cell and spatial transcriptomic profiling of patient-derived intestinal organoids will bridge the gap between *in vitro* mechanistic studies and clinical pathophysiology (Tsalikis et al., 2016). These combined approaches will provide unprecedented resolution of how exosomal cargo heterogeneity impacts localized inflammatory microenvironments. Co-immunoprecipitation coupled to mass spectrometry (Co-IP/MS) can be used to identify protein-protein interactions within exosome cargo complexes and to monitor changes in these interactions in response to ER stress induction (Fu et al., 2024). RNA immunoprecipitation (RNA-IP) followed by high-throughput sequencing should be employed to identify ER stress-responsive miRNAs and their interaction with RNA-binding proteins within exosomes, elucidating selective cargo loading mechanisms (Wozniak et al., 2020). Machine learning algorithms could also be applied to multi-omics datasets like proteomics, lipidomics, and transcriptomics to identify predictive biomarker profiles within exosomes that distinguish disease states and predict treatment response (Lin M. et al., 2025). Deep learning models trained on exosomal cargo profiles could facilitate the development of advanced diagnostic tools for early IBD detection and patient stratification. Additionally, live-cell imaging to track exosome biogenesis and trafficking under stress conditions, and longitudinal human studies to validate findings and monitor disease progression. Collectively, these complementary methodologies could provide a comprehensive framework for understanding ER stress-exosome interactions in IBD.

9 Conclusion

ER stress and exosomes are recognized as two critical contributors to IBD pathogenesis. Persistent ER stress not only disrupts epithelial integrity and immune homeostasis but also alters exosome biogenesis and cargo composition, enabling the transmission of stress and inflammatory signals within the intestinal microenvironment. Previous experimental studies have demonstrated that ER stress can modulate exosome composition, including miRNAs, proteins, and lipids, which in turn affect immune responses and barrier function in gut epithelium, highlighting a mechanistic link between ER stress and exosome-mediated signaling. ER stress in IECs has shown altered exosomal miRNA profiles, such as increased miR-21 and miR-155, which target tight junction proteins and inflammatory regulators, thereby intensifying epithelial permeability and cytokine production. Conversely, stress-induced exosomes have been shown in *in vitro* and *in vivo* models to amplify immune dysregulation and epithelial dysfunction. ER stress–induced exosomes derived from IECs, or macrophages have been shown to activate NF-κB and the NLRP3 inflammasome in murine models of colitis, supporting their role in amplifying mucosal inflammation. Additionally, inhibition of the IRE1α–XBP1 pathway or blocking exosome release using GW4869 has been shown to mitigate colitis severity, providing functional validation of this mechanism. Despite these advances, the molecular profiles of ER stress–derived exosomes and their precise contributions to intercellular communication remain incompletely defined, and further experimental validation is needed to establish their clinical relevance as biomarkers or therapeutic targets. Overall, the current evidences suggest that the dynamic connection between ER stress and exosome-mediated signaling is a key mediator of chronic inflammation and epithelial dysfunction in IBD, providing novel insights into disease mechanisms and potential therapeutic strategies aimed at restoring gut homeostasis. Further mechanistic studies, together with advances in exosome engineering and ER stress–targeted interventions, could offer innovative approaches for improving clinical outcomes in IBD.

Author contributions

AS: Writing – original draft. AB: Conceptualization, Writing – original draft, Writing – review and editing. SC: Writing – original draft. SK: Writing – review and editing. AP: Writing – review and editing. SB: Writing – review and editing. SP: Writing – original draft, Writing – review and editing.

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References

Akman, M., Belisario, D. C., Salaroglio, I. C., Kopecka, J., Donadelli, M., De Smaele, E., et al. (2021). Hypoxia, endoplasmic reticulum stress, and chemoresistance: dangerous liaisons. *J. Exp. and Clin. Cancer Res.* 40 (1), 28. doi:10.1186/s13046-020-01824-3

Alula, K. M., and Theiss, A. L. (2023). Autophagy in Crohn's disease: converging on dysfunctional innate immunity. *Cells* 12 (13), 1779. doi:10.3390/cells12131779

Ashammakhi, N., Nasiri, R., Barros, N. R., Tebon, P., Thakor, J., Goudie, M., et al. (2020). Gut-on-a-chip: current progress and future opportunities. *Biomaterials* 255, 120196. doi:10.1016/j.biomaterials.2020.120196

Ayyar, K. K., and Moss, A. C. (2021). Exosomes in intestinal inflammation. *Front. Pharmacol.* 12, 658505. doi:10.3389/fphar.2021.658505

Azoulay-Alfaguter, I., and Mor, A. (2018). Proteomic analysis of human T cell-derived exosomes reveals differential RAS/MAPK signaling. *Eur. J. Immunol.* 48 (11), 1915–1917. doi:10.1002/eji.201847655

Bakirtzi, K., Man Law, I. K., Fang, K., Iliopoulos, D., and Pothoulakis, C. (2019). MiR-21 in substance P-induced exosomes promotes cell proliferation and migration in human colonic epithelial cells. *Am. J. Physiol.-Gastrointestinal Liver Physiol.* 317 (6), G802–G810. doi:10.1152/ajpgi.00043.2019

Banerjee, A., Bizzaro, D., Burra, P., Di Liddo, R., Pathak, S., Arcidiacono, D., et al. (2015). Umbilical cord mesenchymal stem cells modulate dextran sulfate sodium-induced acute colitis in immunodeficient mice. *Stem Cell Res. Ther.* 6 (1), 79. doi:10.1186/s13287-015-0073-6

Banerjee, A., Jain, S. M., S Abrar, S., Kumar, M. M., Mathew, C., and Pathak, S. (2020). Sources, isolation strategies, and therapeutic outcome of exosomes at a glance. *Regen. Med.* 15 (12), 2361–2378. doi:10.2217/rme-2020-0077

Bassani, B., Baci, D., Gallazzi, M., Poggi, A., Bruno, A., and Mortara, L. (2019). Natural killer cells as key players of tumor progression and angiogenesis: old and novel tools to divert their pro-tumor activities into potent anti-tumor effects. *Cancers* 11 (4), 461. doi:10.3390/cancers11040461

Bauer, K. M., Nelson, M. C., Tang, W. W., Chiaro, T. R., Brown, D. G., Ghazaryan, A., et al. (2022). CD11c+ myeloid cell exosomes reduce intestinal inflammation during colitis. *JCI Insight* 7 (19), e159469. doi:10.1172/jci.insight.159469

Ber, Y., García-Lopez, S., Gargallo-Puyuelo, C. J., and Gomollón, F. (2021). Small and large intestine (II): inflammatory bowel disease, short bowel syndrome, and malignant tumors of the digestive tract. *Nutrients* 13 (7), 2325. doi:10.3390/nu13072325

Bhandary, B., Marahatta, A., Kim, H. R., and Chae, H. J. (2012). An involvement of oxidative stress in endoplasmic reticulum stress and its associated diseases. *Int. J. Mol. Sci.* 14 (1), 434–456. doi:10.3390/ijms14010434

Bhattacharya, A., and Chatterji, U. (2024). Exosomal misfolded proteins released by cancer stem cells: dual functions in balancing protein homeostasis and orchestrating tumor progression. *Discov. Oncol.* 15 (1), 392. doi:10.1007/s12672-024-01262-z

Bretto, E., Urpì-Ferreruela, M., Casanova, G. R., and González-Suárez, B. (2025). The role of gut Microbiota in gastrointestinal immune homeostasis and inflammation: implications for inflammatory bowel disease. *Biomedicines* 13 (8), 1807. doi:10.3390/biomedicines13081807

Calvez, V., Puca, P., Di Vincenzo, F., Del Gaudio, A., Bartocci, B., Murgiano, M., et al. (2025). Novel insights into the pathogenesis of inflammatory bowel diseases. *Biomedicines* 13 (2), 305. doi:10.3390/biomedicines13020305

Cao, S. S. (2018). Cellular stress responses and gut microbiota in inflammatory bowel disease. *Gastroenterol. Res. Pract.* 2018, 7192646. doi:10.1155/2018/7192646

Cao, S. S., and Kaufman, R. J. (2014). Endoplasmic reticulum stress and oxidative stress in cell fate decision and human disease. *Antioxidants Redox Signal.* 21 (3), 396–413. doi:10.1089/ars.2014.5851

Cao, S. S., Zimmermann, E. M., Chuang, B. M., Song, B., Nwokoye, A., Wilkinson, J. E., et al. (2013). The unfolded protein response and chemical chaperones reduce protein misfolding and colitis in mice. *Gastroenterology* 144 (5), 989–1000.e6. doi:10.1053/j.gastro.2013.01.023

Carreras-Sureda, A., Pihán, P., and Hetz, C. (2018). Calcium signaling at the endoplasmic reticulum: fine-tuning stress responses. *Cell Calcium* 70, 24–31. doi:10.1016/j.ceca.2017.08.004

Carrière, J., Bretin, A., Darfeuille-Michaud, A., Barnich, N., and Nguyen, H. T. (2016). Exosomes released from cells infected with crohn's disease-associated adherent-invasive *Escherichia coli* activate host innate immune responses and enhance bacterial intracellular replication. *Inflamm. Bowel Dis.* 22 (3), 516–528. doi:10.1097/MIB.0000000000000635

Chang, X., Song, Y. H., Xia, T., He, Z. X., Zhao, S. B., Wang, Z. J., et al. (2023). Macrophage-derived exosomes promote intestinal mucosal barrier dysfunction in inflammatory bowel disease by regulating TMIGD1 *via* mircroRNA-223. *Int. Immunopharmacol.* 121, 110447. doi:10.1016/j.intimp.2023.110447

Chavda, V. P., Pandya, A., Kumar, L., Raval, N., Vora, L. K., Pulakkat, S., et al. (2023). Exosome nanovesicles: a potential carrier for therapeutic delivery. *Nano Today* 49, 101771. doi:10.1016/j.nantod.2023.101771

Chen, K., and Shoulders, M. D. (2024). Protein glycosylation patterns shaped by the IRE1-XBP1s arm of the unfolded protein response. *Israel J. Chem.* 64 (12), e202300162. doi:10.1002/ijch.202300162

Chen, D., Lu, M. M., Wang, J. H., Ren, Y., Xu, L. L., Cheng, W. X., et al. (2022). High-fat diet aggravates colitis *via* mesenteric adipose tissue-derived exosome metastasis-associated lung adenocarcinoma transcript 1. *World J. Gastroenterol.* 28 (29), 3838–3853. doi:10.3748/wjg.v28.i29.3838

Chen, X., Shi, C., He, M., Xiong, S., and Xia, X. (2023). Endoplasmic reticulum stress: molecular mechanism and therapeutic targets. *Signal Transduct. Target. Ther.* 8 (1), 352. doi:10.1038/s41392-023-01570-w

Collett, G. P., Redman, C. W., Sargent, I. L., and Vatish, M. (2018). Endoplasmic reticulum stress stimulates the release of extracellular vesicles carrying danger-associated molecular pattern (DAMP) molecules. *Oncotarget* 9 (6), 6707–6717. doi:10.18632/oncotarget.24158

da Fonseca, F. G., Serufo, Â. V., Leão, T. L., and Lourenço, K. L. (2024). Viral infections and their ability to modulate endoplasmic reticulum stress response pathways. *Viruses* 16 (10), 1555. doi:10.3390/v16101555

Dai, J., Su, Y., Zhong, S., Cong, L., Liu, B., Yang, J., et al. (2020). Exosomes: key players in cancer and potential therapeutic strategy. *Signal Transduct. Target. Ther.* 5 (1), 145. doi:10.1038/s41392-020-00261-0

Dasgupta, D., Nakao, Y., Mauer, A. S., Thompson, J. M., Sehrawat, T. S., Liao, C. Y., et al. (2020). IRE1A stimulates hepatocyte-derived extracellular vesicles that promote inflammation in mice with steatohepatitis. *Gastroenterology* 159 (4), 1487–1503.e17. doi:10.1053/j.gastro.2020.06.031

Deka, D., D'Incà, R., Sturniolo, G. C., Das, A., Pathak, S., and Banerjee, A. (2022). Role of ER stress-mediated unfolded protein responses and ER stress inhibitors in the pathogenesis of inflammatory bowel disease. *Dig. Dis. Sci.* 67 (12), 5392–5406. doi:10.1007/s10620-022-07467-y

Deng, Z., Rong, Y., Teng, Y., Mu, J., Zhuang, X., Tseng, M., et al. (2017). Broccoli-derived nanoparticle inhibits mouse colitis by activating dendritic cell AMP-activated protein kinase. *Mol. Ther.* 25 (7), 1641–1654. doi:10.1016/j.ymthe.2017.01.025

Derricks, K. E., Trinkaus-Randall, V., and Nugent, M. A. (2015). Extracellular matrix stiffness modulates VEGF calcium signaling in endothelial cells: individual cell and population analysis. *Integr. Biol.* 7 (9), 1011–1025. doi:10.1039/c5ib00140d

Di, X., Gao, X., Peng, L., Ai, J., Jin, X., Qi, S., et al. (2023). Cellular mechanotransduction in health and diseases: from molecular mechanism to therapeutic targets. *Signal Transduct. Target. Ther.* 8 (1), 282. doi:10.1038/s41392-023-01501-9

Di Mattia, M., Sallese, M., and Lopetuso, L. R. (2025). Unfolded protein response: an essential element of intestinal homeostasis and a potential therapeutic target for inflammatory bowel disease. *Acta Physiol.* 241 (2), e14284. doi:10.1111/apha.14284

English, A. R., and Voeltz, G. K. (2013). Endoplasmic reticulum structure and interconnections with other organelles. *Cold Spring Harb. Perspect. Biol.* 5 (04), a013227. doi:10.1101/cshperspect.a013227

Fakhredini, F., Mansouri, E., and Orazizadeh, M. (2022). Isolation and characterization of exosomes derived from supernatant of rabbit kidney tubular cells culture. *J. Prev. Epidemiol.* 7 (2), e26161. doi:10.34172/jpe.2022.26161

Fan, Y., Shen, B., Tan, M., Mu, X., Qin, Y., Zhang, F., et al. (2014). Long non-coding RNA UCA1 increases chemoresistance of bladder cancer cells by regulating Wnt signaling. *FEBS J.* 281 (7), 1750–1758. doi:10.1111/febs.12737

Fu, X., Zhang, S., and Liu, P. (2024). Co-immunoprecipitation for identifying protein–protein interaction on lipid droplets. *Biophys. Rep.* 10 (2), 102–110. doi:10.52601/bpr.2024.240007

Girigoswami, K., Pallavi, P., and Girigoswami, A. (2023). Intricate subcellular journey of nanoparticles to the enigmatic domains of endoplasmic reticulum. *Drug Deliv.* 30 (1), 2284684. doi:10.1080/10717544.2023.2284684

Gong, L., Xiao, J., Yi, J., Xiao, J., Lu, F., and Liu, X. (2022). Immunomodulatory effect of serum exosomes from crohn disease on macrophages *via* Let-7b-5p/TLR4 signaling. *Inflamm. Dowel Dis.* 28 (1), 96–108. doi:10.1093/ibd/izab132

Grimolizzi, F., Monaco, F., Leoni, F., Bracci, M., Staffolani, S., Bersaglieri, C., et al. (2017). Exosomal miR-126 as a circulating biomarker in non-small-cell lung cancer regulating cancer progression. *Sci. Rep.* 7 (1), 15277. doi:10.1038/s41598-017-15475-6

Guan, Q. (2019). A comprehensive review and update on the pathogenesis of inflammatory bowel disease. *J. Immunol. Res.* 2019 (1), 7247238. doi:10.1155/2019/7247238

Gurel, P. S., Hatch, A. L., and Higgs, H. N. (2014). Connecting the cytoskeleton to the endoplasmic reticulum and Golgi. *Curr. Biol.* 24 (14), R660–R672. doi:10.1016/j.cub.2014.05.033

Gurunathan, S., Kang, M. H., Qasim, M., Khan, K., and Kim, J. H. (2021). Biogenesis, membrane trafficking, functions, and next generation nanotherapeutics medicine of extracellular vesicles. *Int. J. Nanomedicine* 16, 3357–3383. doi:10.2147/IJN.S310357

Gurung, S., Perocheau, D., Touramanidou, L., and Baruteau, J. (2021). The exosome journey: from biogenesis to uptake and intracellular signalling. *Cell Commun. Signal.* 19 (1), 47. doi:10.1186/s12964-021-00730-1

Ha, D., Yang, N., and Nadithe, V. (2016). Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. *Acta Pharm. Sin. B* 6 (4), 287–296. doi:10.1016/j.apsb.2016.02.001

Hazrati, A., Soudi, S., Malekpour, K., Mahmoudi, M., Rahimi, A., Hashemi, S. M., et al. (2022). Immune cells-derived exosomes function as a double-edged sword: role in disease progression and their therapeutic applications. *Biomark. Res.* 10 (1), 30. doi:10.1186/s40364-022-00374-4

He, C., Hua, W., Liu, J., Fan, L., Wang, H., and Sun, G. (2020). Exosomes derived from endoplasmic reticulum-stressed liver cancer cells enhance the expression of cytokines in macrophages *via* the STAT3 signaling pathway. *Oncol. Lett.* 20 (1), 589–600. doi:10.3892/ol.2020.11609

He, F., Zheng, Y., Elsabagh, M., Fan, K., Zha, X., Zhang, B., et al. (2025). Gut microbiota modulate intestinal inflammation by endoplasmic reticulum stress-autophagy-cell death signaling axis. *J. Animal Sci. Biotechnol.* 16 (1), 63. doi:10.1186/s40104-025-01196-8

Hetz, C., Zhang, K., and Kaufman, R. J. (2020). Mechanisms, regulation, and functions of the unfolded protein response. *Nat. Rev. Mol. cell Biol.* 21 (8), 421–438. doi:10.1038/s41580-020-0250-z

Hirten, R. P., Lin, K. C., Whang, J., Shahub, S., Churcher, N. K. M., Helmus, D., et al. (2024). Longitudinal monitoring of IL-6 and CRP in inflammatory bowel disease using IBD-AWARE. *Biosens. Bioelectron. X* 16, 100435. doi:10.1016/j.biosx.2023.100435

Horbay, R., Hamraghani, A., Ermini, L., Holcik, S., Beug, S. T., and Yeganeh, B. (2022). Role of ceramides and lysosomes in extracellular vesicle biogenesis, cargo sorting and release. *Int. J. Mol. Sci.* 23 (23), 15317. doi:10.3390/ijms232315317

Horta, C. A., Doan, K., and Yang, J. (2023). Mechanotransduction pathways in regulating epithelial-mesenchymal plasticity. *Curr. Opin. Cell Biol.* 85, 102245. doi:10.1016/j.ceb.2023.102245

Hotamisligil, G. S. (2010). Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell* 140 (6), 900–917. doi:10.1016/j.cell.2010.02.034

Hu, H., Tian, M., Ding, C., and Yu, S. (2019). The C/EBP homologous protein (CHOP) transcription factor functions in endoplasmic reticulum stress-induced apoptosis and microbial infection. *Front. Immunol.* 9, 3083. doi:10.3389/fimmu.2018.03083

Hu, C., Meiners, S., Lukas, C., Stathopoulos, G. T., and Chen, J. (2020). Role of exosomal microRNAs in lung cancer biology and clinical applications. *Cell Prolif.* 53 (6), e12828. doi:10.1111/cpr.12828

Huang, D., Chen, S., Xiong, D., Wang, H., Zhu, L., Wei, Y., et al. (2023). Mitochondrial dynamics: working with the cytoskeleton and intracellular organelles to mediate mechanotransduction. *Aging Dis.* 14 (5), 1511–1532. doi:10.14336/AD.2023.0201

Huang, S., Yan, F., Qiu, Y., Liu, T., Zhang, W., Yang, Y., et al. (2025). Exosomes in inflammation and cancer: from bench to bedside applications. *Mol. Biomed.* 6 (1), 41. doi:10.1186/s43556-025-00280-9

Jahangiri, B., Saei, A. K., Obi, P. O., Asghari, N., Lorzadeh, S., Hekmatirad, S., et al. (2022). Exosomes, autophagy and ER stress pathways in human diseases: cross-regulation and therapeutic approaches. *Biochimica Biophysica Acta (BBA)-Molecular Basis Dis.* 1868 (10), 166484. doi:10.1016/j.bbadis.2022.166484

Jiang, D., Niwa, M., and Koong, A. C. (2015). Targeting the IRE1α-XBP1 branch of the unfolded protein response in human diseases. *Seminars Cancer Biol.* 33, 48–56. doi:10.1016/j.semcancer.2015.04.010

Jin, J., Jung, M., Sonn, S. K., Seo, S., Suh, J., Kweon, H. Y., et al. (2025). Peroxiredoxin 3 deficiency exacerbates DSS-induced acute colitis *via* exosomal miR-1260b-Mediated barrier disruption and proinflammatory signaling. *Antioxidants Redox Signal.* 42 (1-3), 133–149. doi:10.1089/ars.2023.0482

Jung, M. K., and Mun, J. Y. (2018). Sample preparation and imaging of exosomes by transmission electron microscopy. *J. Vis. Exp. JoVE* (131), 56482. doi:10.3791/56482

Kalluri, R., and LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. *Science* 367 (6478), eaau6977. doi:10.1126/science.aau6977

Kanemoto, S., Nitani, R., Murakami, T., Kaneko, M., Asada, R., Matsuhisa, K., et al. (2016). Multivesicular body formation enhancement and exosome release during endoplasmic reticulum stress. *Biochem. Biophysical Res. Commun.* 480 (2), 166–172. doi:10.1016/j.bbrc.2016.10.019

Kang, Y., Park, H., Choe, B. H., and Kang, B. (2022). The role and function of mucins and its relationship to inflammatory bowel disease. *Front. Med.* 9, 848344. doi:10.3389/fmed.2022.848344

Kaser, A., and Blumberg, R. S. (2010). Endoplasmic reticulum stress and intestinal inflammation. *Mucosal Immunol.* 3 (1), 11–16. doi:10.1038/mi.2009.122

Kaser, A., Lee, A. H., Franke, A., Glickman, J. N., Zeissig, S., Tilg, H., et al. (2008). XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. *Cell* 134 (5), 743–756. doi:10.1016/j.cell.2008.07.021

Kheradmand, F., Yasaman Rahimzadeh, S. F., Esmaeili, S. A., Negah, S. S., Farkhad, N. K., Nazari, S. E., et al. (2025). Efficacy of umbilical cord-derived mesenchymal stem cells and exosomes in conjunction with standard IBD drug on immune responses in an IBD mouse model. *Stem Cell Res. Ther.* 16 (1), 5. doi:10.1186/s13287-024-04062-y

Khoonkari, M., Liang, D., Kamperman, M., van Rijn, P., and Kruyt, F. A. E. (2024). The unfolded protein response sensor PERK mediates mechanical stress-induced maturation of focal adhesion complexes in glioblastoma cells. *FEBS Lett.* 598 (24), 3021–3035. doi:10.1002/1873-3468.14996

Kong, C., Yang, M., Yue, N., Zhang, Y., Tian, C., Wei, D., et al. (2024). Restore intestinal barrier integrity: an approach for inflammatory bowel disease therapy. *J. Inflamm. Res.* 17, 5389–5413. doi:10.2147/JIR.S470520

Kumar, M. A., Baba, S. K., Sadida, H. Q., Marzooqi, S. A., Jerobin, J., Altemani, F. H., et al. (2024). Extracellular vesicles as tools and targets in therapy for diseases. *Signal Transduct. Target. Ther.* 9 (1), 27. doi:10.1038/s41392-024-01735-1

Lee, J. H., Won, Y. J., Kim, H., Choi, M., Lee, E., Ryoou, B., et al. (2023). Adipose tissue-derived mesenchymal stem cell-derived exosomes promote wound healing and tissue regeneration. *Int. J. Mol. Sci.* 24 (13), 10434. doi:10.3390/ijms241310434

Lenna, S., and Trojanowska, M. (2012). The role of endoplasmic reticulum stress and the unfolded protein response in fibrosis. *Curr. Opin. Rheumatol.* 24 (6), 663–668. doi:10.1097/BOR.0b013e3283588dbb

Leushacke, M., and Barker, N. (2014). *Ex vivo* culture of the intestinal epithelium: strategies and applications. *Gut* 63 (8), 1345–1354. doi:10.1136/gutjnl-2014-307204

Li, J., Li, Z., Jiang, P., Peng, M., Zhang, X., Chen, K., et al. (2018). Circular RNA IARS (circ-IARS) secreted by pancreatic cancer cells and located within exosomes regulates endothelial monolayer permeability to promote tumor metastasis. *J. Exp. Clin. Cancer Res.* 37 (1), 177. doi:10.1186/s13046-018-0822-3

Li, M., Zhao, J., Cao, M., Liu, R., Chen, G., Li, S., et al. (2020). Mast cells-derived MiR-223 destroys intestinal barrier function by inhibition of CLDN8 expression in intestinal epithelial cells. *Biol. Res.* 53 (1), 12. doi:10.1186/s40659-020-00279-2

Li, D. F., Tang, Q., Yang, M. F., Xu, H. M., Zhu, M. Z., Zhang, Y., et al. (2023). Plant-derived exosomal nanoparticles: potential therapeutic for inflammatory bowel disease. *Nanoscale Adv.* 5 (14), 3575–3588. doi:10.1039/d3na00093a

Li, M., Hou, Y., Chen, Y., Sun, C., Liang, M., Chu, X., et al. (2024). Palmitic acid promotes miRNA release from adipocyte exosomes by activating NF-κB/ER stress. *Nutr. Diabetes* 14 (1), 75. doi:10.1038/s41387-024-00334-x

Liao, W., Du, Y., Zhang, C., Pan, F., Yao, Y., Zhang, T., et al. (2019). Exosomes: the next generation of endogenous nanomaterials for advanced drug delivery and therapy. *Acta Biomater.* 86, 1–14. doi:10.1016/j.actbio.2018.12.045

Lin, J. H., Walter, P., and Yen, T. S. (2008). Endoplasmic reticulum stress in disease pathogenesis. *Annu. Rev. Pathology* 3, 399–425. doi:10.1146/annurev.pathmechdis.3.121806.151434

Lin, D., Jin, Y., Shao, X., Xu, Y., Ma, G., Jiang, Y., et al. (2024). Global, regional, and national burden of inflammatory bowel disease, 1990-2021: insights from the global burden of disease 2021. *Int. J. Colorectal Dis.* 39 (1), 139. doi:10.1007/s00384-024-04711-x

Lin, M., Guo, J., Gu, Z., Tang, W., Tao, H., You, S., et al. (2025). Machine learning and multi-omics integration: advancing cardiovascular translational research and clinical practice. *J. Transl. Med.* 23 (1), 388. doi:10.1186/s12967-025-06425-2

Lin, S. N., Wang, J., Mukherjee, P. K., Veisman, I., Mao, R., Chandra, J., et al. (2025). The functional role of the extracellular matrix in inflammatory bowel disease-associated gut fibrosis. *Matrix Biol.* 139, 29–48. doi:10.1016/j.matbio.2025.05.001

Lindenbergh, M. F. S., Koerhuis, D. G. J., Borg, E. G. F., van 't Veld, E. M., Driedonks, T. A. P., Wubbolts, R., et al. (2019). Bystander T-Cells support clonal T-Cell activation by controlling the release of dendritic cell-derived immune-stimulatory extracellular vesicles. *Front. Immunol.* 10, 448. doi:10.3389/fimmu.2019.00448

Liu, S., Da Cunha, A.P., Rezende, R.M., Cialic, R., Wei, Z., Bry, L., et al. (2016). The host shapes the gut microbiota via fecal microRNA. *Cell Host and Microbe* 19 (01), 32–43. doi:10.1016/j.chom.2015.12.005

Liu, L., Davidorf, B., Dong, P., Peng, A., Song, Q., and He, Z. (2024). Decoding the mosaic of inflammatory bowel disease: illuminating insights with single-cell RNA technology. *Comput. Struct. Biotechnol. J.* 23, 2911–2923. doi:10.1016/j.csbj.2024.07.011

Loncke, J., Kaasik, A., Bezprozvanny, I., Parys, J. B., Kerkhofs, M., and Bultynck, G. (2021). Balancing ER-mitochondrial Ca^{2+} fluxes in health and disease. *Trends Cell Biol.* 31 (7), 598–612. doi:10.1016/j.tcb.2021.02.003

Luo, K., and Cao, S. S. (2015). Endoplasmic reticulum stress in intestinal epithelial cell function and inflammatory bowel disease. *Gastroenterol. Res. Pract.* 2015, 328791. doi:10.1155/2015/328791

Ma, X., Dai, Z., Sun, K., Zhang, Y., Chen, J., Yang, Y., et al. (2017). Intestinal epithelial cell endoplasmic reticulum stress and inflammatory bowel disease pathogenesis: an update review. *Front. Immunol.* 8, 1271. doi:10.3389/fimmu.2017.01271

Ma, Y. S., Wu, T. M., Ling, C. C., Yu, F., Zhang, J., Cao, P. S., et al. (2021). M2 macrophage-derived exosomal microRNA-155-5p promotes the immune escape of colon cancer by downregulating ZC3H12B. *Mol. Ther. Oncolytics* 20, 484–498. doi:10.1016/j.omto.2021.02.005

Mahadevan, N. R., Rodvold, J., Sepulveda, H., Rossi, S., Drew, A. F., and Zanetti, M. (2011). Transmission of endoplasmic reticulum stress and pro-inflammation from tumor cells to myeloid cells. *Proc. Natl. Acad. Sci. U. S. A.* 108 (16), 6561–6566. doi:10.1073/pnas.1008942108

Martinotti, S., and Ranzato, E. (2025). Targeting the unfolded protein response with natural products: therapeutic potential in ER stress-related diseases. *Int. J. Mol. Sci.* 26 (18), 8814. doi:10.3390/ijms26188814

Mazurov, D., Barbashova, L., and Filatov, A. (2013). Tetraspanin protein CD9 interacts with metalloprotease CD10 and enhances its release *via* exosomes. *FEBS J.* 280 (5), 1200–1213. doi:10.1111/febs.12110

Mekahli, D., Bultynck, G., Parys, J. B., De Smedt, H., and Missiaen, L. (2011). Endoplasmic-reticulum calcium depletion and disease. *Cold Spring Harb. Perspect. Biol.* 3 (6), a004317. doi:10.1101/cshperspect.a004317

Mennillo, E., Kim, Y. J., Lee, G., Rusu, I., Patel, R. K., Dorman, L. C., et al. (2024). Single-cell and spatial multi-omics highlight effects of anti-integrin therapy across cellular compartments in ulcerative colitis. *Nat. Commun.* 15 (1), 1493. doi:10.1038/s41467-024-45665-6

Mierke, C. T. (2024). Extracellular matrix cues regulate mechanosensing and mechanotransduction of cancer cells. *Cells* 13 (1), 96. doi:10.3390/cells13010096

Mitsuhashi, S., Feldbrügge, L., Csizmadia, E., Mitsuhashi, M., Robson, S. C., and Moss, A. C. (2016). Luminal extracellular vesicles (EVs) in inflammatory Bowel disease (IBD) exhibit proinflammatory effects on epithelial cells and macrophages. *Inflamm. Bowel Dis.* 22 (7), 1587–1595. doi:10.1097/MIB.0000000000000840

Momotyuk, E., Ebrahim, N., Shakirova, K., and Dashinimaev, E. (2025). Role of the cytoskeleton in cellular reprogramming: effects of biophysical and biochemical factors. *Front. Mol. Biosci.* 12, 1538806. doi:10.3389/fmolb.2025.1538806

Nail, H. M., Chiu, C. C., Leung, C. H., Ahmed, M. M. M., and Wang, H. D. (2023). Exosomal miRNA-mediated intercellular communications and immunomodulatory effects in tumor microenvironments. *J. Biomed. Sci.* 30 (1), 69. doi:10.1186/s12929-023-00964-w

Ocansey, D. K. W., Zhang, L., Wang, Y., Yan, Y., Qian, H., Zhang, X., et al. (2020). Exosome-mediated effects and applications in inflammatory bowel disease. *Biol. Rev. Camb. Philosophical Soc.* 95 (5), 1287–1307. doi:10.1111/brv.12608

Osada-Oka, M., Shiota, M., Izumi, Y., Nishiyama, M., Tanaka, M., Yamaguchi, T., et al. (2017). Macrophage-derived exosomes induce inflammatory factors in endothelial cells under hypertensive conditions. *Hypertens. Res.* 40 (4), 353–360. doi:10.1038/hr.2016.163

Otte, M. L., Lama Tamang, R., Papapanagiotou, J., Ahmad, R., Dhawan, P., and Singh, A. B. (2023). Mucosal healing and inflammatory bowel disease: therapeutic implications and new targets. *World J. Gastroenterol.* 29 (7), 1157–1172. doi:10.3748/wjg.v29.i7.1157

Park, S. M., Kang, T. I., and So, J. S. (2021). Roles of XBP1s in transcriptional regulation of target genes. *Biomedicines* 9 (7), 791. doi:10.3390/biomedicines9070791

Park, E. J., Shimaoka, M., and Kiyono, H. (2022). Functional flexibility of exosomes and MicroRNAs of intestinal epithelial cells in affecting inflammation. *Front. Mol. Biosci.* 9, 854487. doi:10.3389/fmolb.2022.854487

Park, J., Jeong, G. H., Song, M., Yon, D. K., Lee, S. W., Koyanagi, A., et al. (2023). The global, regional, and national burden of inflammatory bowel diseases, 1990–2019: a systematic analysis for the global burden of disease study 2019. *Dig. Liver Dis.* 55 (10), 1352–1359. doi:10.1016/j.dld.2023.04.003

Petrey, A. C., and de la Motte, C. A. (2017). The extracellular matrix in IBD: a dynamic mediator of inflammation. *Curr. Opin. Gastroenterology* 33 (4), 234–238. doi:10.1097/MOG.0000000000000368

Prakash, J., and Shaked, Y. (2024). The interplay between extracellular matrix remodeling and cancer therapeutics. *Cancer Discov.* 14 (8), 1375–1388. doi:10.1158/2159-8290.CD-24-0002

Qiao, D., Zhang, Z., Zhang, Y., Chen, Q., Chen, Y., Tang, Y., et al. (2021). Regulation of endoplasmic reticulum stress-autophagy: a potential therapeutic target for ulcerative colitis. *Front. Pharmacol.* 12, 697360. doi:10.3389/fphar.2021.697360

Rezaie, J., Feghhi, M., and Etemadi, T. (2022). A review on exosomes application in clinical trials: perspective, questions, and challenges. *Cell Commun. Signal.* 20 (1), 145. doi:10.1186/s12964-022-00959-4

Riaz, T. A., Junjappa, R. P., Handigund, M., Ferdous, J., Kim, H. R., Chae, H. J., et al. (2020). Role of endoplasmic reticulum stress sensor IRE1α in cellular physiology, calcium, ROS signaling, and metaflammation. *Cells* 9 (5), 1160. doi:10.3390/cells9051160

Rodrigues, B. L., Pascoal, L. B., Genaro, L. M., Warrak, L. S. C. A., Rodrigues, B. A. G., Coope, A., et al. (2025). *In vitro* inhibition of endoplasmic reticulum stress: a promising therapeutic strategy for patients with Crohn's Disease. *Cells* 14 (4), 270. doi:10.3390/cells14040270

Rozpedek, W., Pytel, D., Mucha, B., Leszczynska, H., Diehl, J. A., Majsterek, I., et al. (2016). The role of the PERK/eIF2α/ATF4/CHOP signaling pathway in tumor progression during endoplasmic reticulum stress. *Curr. Mol. Med.* 16 (6), 533–544. doi:10.2174/1566524016666160523143937

Ruivo, C. F., Adem, B., Silva, M., and Melo, S. A. (2017). The biology of cancer exosomes: insights and new perspectives. *Cancer Res.* 77 (23), 6480–6488. doi:10.1158/0008-5472.CAN-17-0994

Saez, A., Herrero-Fernandez, B., Gomez-Bris, R., Sánchez-Martinez, H., and Gonzalez-Granado, J. M. (2023). Pathophysiology of inflammatory bowel disease: innate immune system. *Int. J. Mol. Sci.* 24 (2), 1526. doi:10.3390/ijms24021526

Saleem, M., Shahzad, K. A., Marryum, M., Singh, S., Zhou, Q., Du, S., et al. (2024). Exosome-based therapies for inflammatory disorders: a review of recent advances. *Stem Cell Res. Ther.* 15 (1), 477. doi:10.1186/s13287-024-04107-2

Salminen, A., Kaarniranta, K., and Kauppinen, A. (2020). ER stress activates immunosuppressive network: implications for aging and Alzheimer's disease. *J. Mol. Med.* 98 (5), 633–650. doi:10.1007/s00109-020-01904-z

Schopf, F. H., Biebl, M. M., and Buchner, J. (2017). The HSP90 chaperone machinery. *Nat. Rev. Mol. Cell Biol.* 18 (6), 345–360. doi:10.1038/nrm.2017.20

Senft, D., and Ronai, Z. A. (2015). UPR, autophagy, and mitochondria crosstalk underlies the ER stress response. *Trends Biochem. Sci.* 40 (3), 141–148. doi:10.1016/j.tibs.2015.01.002

Shan, Y., Lee, M., and Chang, E. B. (2022). The gut microbiome and inflammatory bowel diseases. *Annu. Rev. Med.* 73, 455–468. doi:10.1146/annurev-med-042320-021020

Shao, J., Jin, Y., Shao, C., Fan, H., Wang, X., and Yang, G. (2021). Serum exosomal pregnancy zone protein as a promising biomarker in inflammatory bowel disease. *Cell. Mol. Biol. Lett.* 26 (1), 36. doi:10.1186/s11658-021-00280-x

Shapouri-Moghaddam, A., Mohammadian, S., Vazini, H., Taghadosi, M., Esmaeili, S. A., Mardani, F., et al. (2018). Macrophage plasticity, polarization, and function in health and disease. *J. Cell. Physiol.* 233 (9), 6425–6440. doi:10.1002/jcp.26429

Shen, Q., Huang, Z., Yao, J., and Jin, Y. (2021). Extracellular vesicles-mediated interaction within intestinal microenvironment in inflammatory bowel disease. *J. Adv. Res.* 37, 221–233. doi:10.1016/j.jare.2021.07.002

Shi, Y., Jiang, B., and Zhao, J. (2024). Induction mechanisms of autophagy and endoplasmic reticulum stress in intestinal ischemia-reperfusion injury, inflammatory bowel disease, and colorectal cancer. *Biomed. Pharmacother.* 170, 115984. doi:10.1016/j.biopha.2023.115984

Singh, R., Kaur, N., Choubey, V., Dhingra, N., and Kaur, T. (2024). Endoplasmic reticulum stress and its role in various neurodegenerative diseases. *Brain Res.* 1826, 148742. doi:10.1016/j.brainres.2023.148742

Siwecka, N., Rozpędek-Kamińska, W., Wawrzynkiewicz, A., Pytel, D., Diehl, J. A., and Majsterek, I. (2021). The structure, activation, and signaling of IRE1 and its role in determining cell fate. *Biomedicines* 9 (2), 156. doi:10.3390/biomedicines9020156

Smolková, K., Mikó, E., Kovács, T., Leguina-Ruzzi, A., Sipos, A., and Bai, P. (2020). Nuclear factor erythroid 2-Related factor 2 in regulating cancer metabolism. *Antioxidants Redox Signal.* 33 (13), 966–997. doi:10.1089/ars.2020.8024

Song, S., Lee, H., Kam, T. I., Tai, M. L., Lee, J. Y., Noh, J. Y., et al. (2008). E2-25K/Hip-2 regulates caspase-12 in ER stress-mediated Abeta neurotoxicity. *J. Cell Biol.* 182 (4), 675–684. doi:10.1083/jcb.200711066

Stutzmann, G. E., and Mattson, M. P. (2011). Endoplasmic reticulum Ca(2+) handling in excitable cells in health and disease. *Pharmacol. Rev.* 63 (3), 700–727. doi:10.1124/pr.110.003814

Taha, E. A., Ono, K., and Eguchi, T. (2019). Roles of extracellular HSPs as biomarkers in immune surveillance and immune evasion. *Int. J. Mol. Sci.* 20 (18), 4588. doi:10.3390/ijms20184588

Tian, Y., Cheng, C., Wei, Y., Yang, F., and Li, G. (2022). The role of exosomes in inflammatory diseases and tumor-related inflammation. *Cells* 11 (6), 1005. doi:10.3390/cells11061005

Tkach, M., Kowal, J., Zucchetti, A. E., Enserink, L., Jouve, M., Lankar, D., et al. (2017). Qualitative differences in T-cell activation by dendritic cell-derived extracellular vesicle subtypes. *EMBO J.* 36 (20), 3012–3028. doi:10.15252/embj.201696003

Toribio, V., and Yáñez-Mó, M. (2022). Tetraspanins interweave EV secretion, endosomal network dynamics and cellular metabolism. *Eur. J. Cell Biol.* 101 (3), 151229. doi:10.1016/j.ejcb.2022.151229

Tsalikis, J., Pan, Q., Tattoli, I., Maisonneuve, C., Blencowe, B. J., Philpott, D. J., et al. (2016). The transcriptional and splicing landscape of intestinal organoids undergoing nutrient starvation or endoplasmic reticulum stress. *BMC Genomics* 17 (1), 680. doi:10.1186/s12864-016-2999-1

Tzng, E., Bayardo, N., and Yang, P. C. (2023). Current challenges surrounding exosome treatments. *Extracell. Vesicle* 2, 100023. doi:10.1016/j.vesic.2023.100023

van Vliet, A. R., and Agostinis, P. (2017). PERK and filamin A in actin cytoskeleton remodeling at ER-plasma membrane contact sites. *Mol. Cell. Oncol.* 4 (5), e1340105. doi:10.1080/23723556.2017.1340105

Vanhove, W., Nys, K., Arijs, I., Cleynen, I., Noben, M., De Schepper, S., et al. (2018). Biopsy-derived intestinal epithelial cell cultures for pathway-based stratification of patients with inflammatory bowel disease. *J. Crohn's Colitis* 12 (2), 178–187. doi:10.1093/ecco-jcc/jjx122

Verjan Garcia, N., Hong, K. U., and Matoba, N. (2023). The unfolded protein response and its implications for novel therapeutic strategies in inflammatory bowel disease. *Biomedicines* 11 (7), 2066. doi:10.3390/biomedicines11072066

Viaud, S., Terme, M., Flament, C., Taieb, J., André, F., Novault, S., et al. (2009). Dendritic cell-derived exosomes promote natural killer cell activation and proliferation: a role for NKG2D ligands and IL-15Ralpha. *PLoS One* 4 (3), e4942. doi:10.1371/journal.pone.0004942

Wang, S., and Shi, Y. (2022). Exosomes derived from immune cells: the new role of tumor immune microenvironment and tumor therapy. *Int. J. Nanomedicine* 17, 6527–6550. doi:10.2147/IJN.S388604

Wang, G., Jin, S., Ling, X., Li, Y., Hu, Y., Zhang, Y., et al. (2019). Proteomic profiling of LPS-induced macrophage-derived exosomes indicates their involvement in acute liver injury. *Proteomics* 19 (3), e1800274. doi:10.1002/pmic.201800274

Wang, H., Ye, C., Wu, Y., Yang, P., Chen, C., Liu, Z., et al. (2020). Exosomes in inflammatory bowel disease: what have we learned So far? *Curr. Drug Targets* 21 (14), 1448–1455. doi:10.2174/1389450121666200428102330

Wang, R., Li, Z., Liu, S., and Zhang, D. (2023). Global, regional, and national burden of inflammatory Bowel disease in 204 countries and territories from 1990 to 2019: a systematic analysis based on the global Burden of Disease Study 2019. *BMJ Open* 13 (3), e065186. doi:10.1136/bmjopen-2022-065186

Wei, S., Zhang, J., Wu, X., Chen, M., Huang, H., Zeng, S., et al. (2023). *Fusobacterium nucleatum* extracellular vesicles promote experimental colitis by modulating autophagy *via* the miR-574-5p/CARD3 axis. *Inflamm. Bowel Dis.* 29 (1), 9–26. doi:10.1093/ibd/izac177

Wei, Z., Hang, S., Wiredu Ocansey, D. K., Zhang, Z., Wang, B., Zhang, X., et al. (2023). Human umbilical cord mesenchymal stem cells derived exosome shuttling mir-129-5p attenuates inflammatory bowel disease by inhibiting ferroptosis. *J. Nanobiotechnology* 21 (1), 188. doi:10.1186/s12951-023-01951-x

Wozniak, A. L., Adams, A., King, K. E., Dunn, W., Christenson, L. K., Hung, W. T., et al. (2020). The RNA binding protein FMR1 controls selective exosomal miRNA cargo loading during inflammation. *J. Cell Biol.* 219 (10), e201912074. doi:10.1083/jcb.201912074

Wu, D. M., Wen, X., Han, X. R., Wang, S., Wang, Y. J., Shen, M., et al. (2018). Role of circular RNA DLEU2 in human acute myeloid leukemia. *Mol. Cell. Biol.* 38 (20), e00259-18. doi:10.1128/MCB.00259-18

Wu, Q., Zhang, H., Sun, S., Wang, L., and Sun, S. (2021). Extracellular vesicles and immunogenic stress in cancer. *Cell Death Dis.* 12 (10), 894. doi:10.1038/s41419-021-04171-z

Wu, Q., Kan, J., Fu, C., Liu, X., Cui, Z., Wang, S., et al. (2024). Insights into the unique roles of extracellular vesicles for gut health modulation: mechanisms, challenges, and perspectives. *Curr. Res. Microb. Sci.* 7, 100301. doi:10.1016/j.crmicr.2024.100301

Xia, B., Liu, Y., Wang, J., Lu, Q., Lv, X., Deng, K., et al. (2023). Emerging role of exosome-shuttled noncoding RNAs in gastrointestinal cancers: from intercellular crosstalk to clinical utility. *Pharmacol. Res.* 195, 106880. doi:10.1016/j.phrs.2023.106880

Xie, S., Zhang, Q., and Jiang, L. (2022). Current knowledge on exosome biogenesis, cargo-sorting mechanism, and therapeutic implications. *Membranes* 12 (5), 498. doi:10.3390/membranes12050498

Xu, M., Ji, J., Jin, D., Wu, Y., Wu, T., Lin, R., et al. (2022). The biogenesis and secretion of exosomes and multivesicular bodies (MVBs): intercellular shuttles and implications in human diseases. *Genes Dis.* 10 (5), 1894–1907. doi:10.1016/j.gendis.2022.03.021

Xu, Y., Le, H., Wu, Q., Wang, N., and Gong, C. (2025). Advancements in CRISPR/Cas systems for disease treatment. *Acta Pharm. Sin. B* 15, 2818–2844. doi:10.1016/j.apsb.2025.05.007

Yamamoto, K., Sato, T., Matsui, T., Sato, M., Okada, T., Yoshida, H., et al. (2007). Transcriptional induction of mammalian ER quality control proteins is mediated by single or combined action of ATF6alpha and XBP1. *Dev. Cell* 13 (3), 365–376. doi:10.1016/j.devcel.2007.07.018

Yang, C., Chen, J., Zhao, Y., Wu, J., Xu, Y., Xu, J., et al. (2024). Salivary exosomes exacerbate colitis by bridging the oral cavity and intestine. *iScience* 27 (11), 111061. doi:10.1016/j.isci.2024.111061

Yang, S., Liu, H., and Liu, Y. (2025). Advances in intestinal epithelium and gut microbiota interaction. *Front. Microbiol.* 16, 1499202. doi:10.3389/fmicb.2025.1499202

Ye, J., and Liu, X. (2022). Interactions between endoplasmic reticulum stress and extracellular vesicles in multiple diseases. *Front. Immunol.* 13, 955419. doi:10.3389/fimmu.2022.955419

Yin, Y. B., de Jonge, H. R., Wu, X., and Yin, Y. L. (2019). Mini-gut: a promising model for drug development. *Drug Discov. Today* 24 (9), 1784–1794. doi:10.1016/j.drudis.2019.06.006

Zhang, M., Viennois, E., Prasad, M., Zhang, Y., Wang, L., Zhang, Z., et al. (2016). Edible ginger-derived nanoparticles: a novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer. *Biomaterials* 101, 321–340. doi:10.1016/j.biomaterials.2016.06.018

Zhang, H., Wang, L., Li, C., Yu, Y., Yi, Y., Wang, J., et al. (2019). Exosome-induced regulation in inflammatory bowel disease. *Front Immunol.* 10, 1464. doi:10.3389/fimmu.2019.01464

Zhang, R., Li, M., Li, H., Ran, X., Jin, F., Tan, Q., et al. (2024). Immune cell-derived exosomes in inflammatory disease and inflammatory tumor microenvironment: a review. *J. Inflamm. Res.* 17, 301–312. doi:10.2147/JIR.S421649

Zhang, Y., Zhuang, H., Chen, K., Zhao, Y., Wang, D., Ran, T., et al. (2025). Intestinal fibrosis associated with inflammatory bowel disease: known and unknown. *Chin. Med. J.* 138 (8), 883–893. doi:10.1097/CM9.0000000000003545

Zhao, T., Huang, Y., Zhu, J., Qin, Y., Wu, H., Yu, J., et al. (2025). Extracellular matrix signaling cues: biological functions, diseases, and therapeutic targets. *MedComm* 6 (8), e70281. doi:10.1002/mco2.70281

Zheng, H. B. (2023). Application of single-cell omics in inflammatory bowel disease. *World J. Gastroenterol.* 29 (28), 4397–4404. doi:10.3748/wjg.v29.i28.4397

Zheng, T., Huang, K. Y., Tang, X. D., Wang, F. Y., and Lv, L. (2025). Endoplasmic reticulum stress in gut inflammation: implications for ulcerative colitis and Crohn's disease. *World J. Gastroenterol.* 31 (13), 104671. doi:10.3748/wjg.v31.i13.104671

Zhou, J., Liu, J., Gao, Y., Shen, L., Li, S., and Chen, S. (2021). miRNA-based potential biomarkers and new molecular insights in ulcerative colitis. *Front. Pharmacol.* 12, 707776. doi:10.3389/fphar.2021.707776

Zhou, S., Zhou, Z., Chen, S., Wang, M., and Wang, L. (2025). PERK and IRE1α promote exosome secretion *via* blocking lysosomal degradation of multiple vesicular body. *J. Biol. Chem.* 301 (8), 110504. doi:10.1016/j.jbc.2025.110504

Zubair, M., Abouelnazar, F. A., Iqbal, M. A., Pan, J., Zheng, X., Chen, T., et al. (2025). Mesenchymal stem cell-derived exosomes as a plausible immunomodulatory therapeutic tool for inflammatory diseases. *Front. Cell Dev. Biol.* 13, 1563427. doi:10.3389/fcell.2025.1563427

Glossary

AMPK	AMP-Activated Protein Kinase
ATF4	Activating Transcription Factor 4
ATF6	Activating Transcription Factor 6
BDNs	Broccoli-Derived Nanoparticles
CD	Crohn's Disease
CHOP	C/EBP Homologous Protein
DAMPs	Damage-Associated Molecular Patterns
DCs	Dendritic Cells
DNA	Deoxyribonucleic Acid
dsDNA	Double-Stranded DNA
DSS	Dextran Sulfate Sodium
ER	Endoplasmic Reticulum
ERAD	ER-Associated Degradation
EVs	Extracellular Vesicles
eIF2α	Eukaryotic Initiation Factor 2 alpha
ESCRT	Endosomal Sorting Complex Required for Transport
GRP78	Glucose-Regulated Protein 78
HSP	Heat Shock Protein
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
IECs	Intestinal Epithelial Cells
IRE1	Inositol-Requiring Enzyme 1
MAPK	Mitogen-Activated Protein Kinase
MHC	Major Histocompatibility Complex
MSC	Mesenchymal Stem Cells
MUC2	Mucin 2
NF-κB	Nuclear Factor kappa B
ORMDL3	Orosomucoid-like protein 3
PERK	Protein kinase RNA-like Endoplasmic Reticulum Kinase
ROS	Reactive Oxygen Species
TNFα	Tumor Necrosis Factor-alpha
TLR	Toll-Like Receptor
UPR	Unfolded Protein Response
XBP1	X-box Binding Protein 1

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