International Journal of Nanomedicine

Dovepress
Taylor & Francis Group

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REVIEW

Stem Cell-Derived Exosomes: A Comprehensive Review of Biomedical Applications, Challenges, and Future Directions

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Abstract: Stem cell-derived exosomes (SC-Exos) represent an innovative therapeutic breakthrough that circumvents key limitations of direct stem cell transplantation, demonstrating significant therapeutic potential while offering distinct advantages including reduced ethical controversies, decreased immunogenicity responses, and minimized tumorigenicity risks. This review provides a systematic analysis of SC-Exos research, encompassing diverse aspects from fundamental biological mechanisms and isolation and characterization techniques to advanced engineering strategies and therapeutic applications. The review elucidates the biological foundations of exosomes, analyzes different SC-Exos types and their unique characteristics, and explores multiple functional optimization strategies to enhance SC-Exos performance. Comprehensive biomedical engineering applications of SC-Exos across diverse therapeutic domains are presented, covering tissue engineering, advanced drug delivery systems, and treatments for cardiovascular, neurological, oncological, immunological, inflammatory, reproductive, musculoskeletal, and dermatological diseases, as well as other emerging applications. Clinical translation status is evaluated through analysis of current trials, revealing favorable safety profiles and promising preliminary efficacy of SC-Exos across multiple therapeutic domains. Nevertheless, significant challenges remain in standardization of isolation and purification techniques, quality control measures, therapeutic heterogeneity, scalable production capabilities, and comprehensive biosafety evaluation protocols. Future research priorities include establishing unified isolation and purification standards, developing comprehensive functional evaluation systems, optimizing administration routes and dosing regimens, and conducting large-scale multicenter clinical trials. This review provides systematic guidance for advancing effective SC-Exos-based therapeutic solutions, ultimately facilitating their clinical translation and expanding applications across biomedical challenges.

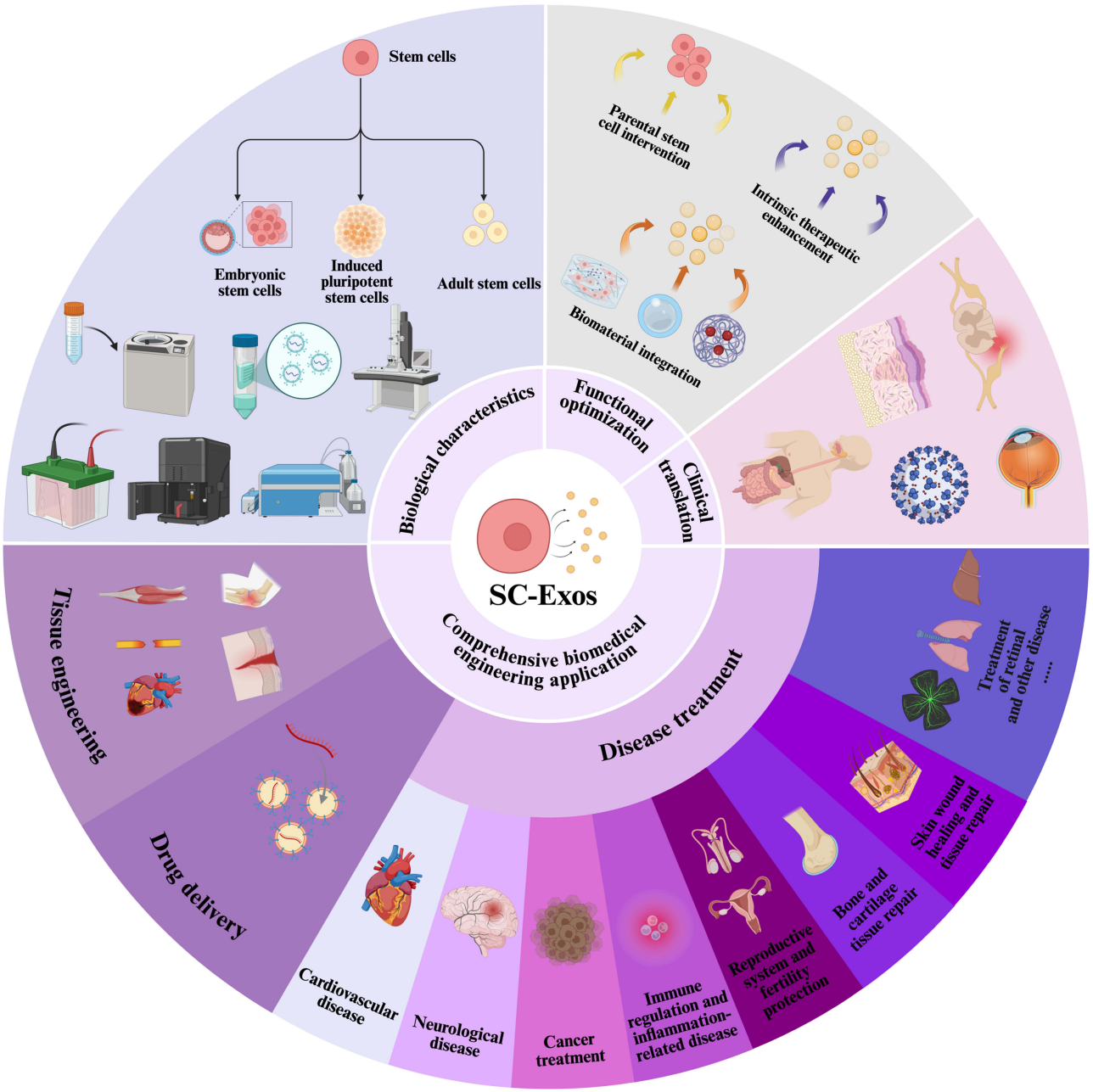
Keywords: stem cell-derived exosomes, biomedical applications, clinical translation, extracellular vesicles, biomedical engineering, nanomedicine

Introduction

The development of effective therapeutic strategies for complex diseases remains a fundamental challenge in modern biomedical research. Among emerging approaches, stem cell-based therapies have gained substantial attention due to their unique regenerative properties. Stem cells are undifferentiated cells characterized by their capacity for self-renewal and differentiation into specialized cell types, positioning them as valuable therapeutic tools for diverse pathological conditions, including cardiovascular diseases, metabolic disorders, and tissue injuries.[1–4] Studies have demonstrated that

Received: 9 March 2025
Accepted: 6 August 2025
Published: 4 September 2025

Graphical Abstract



stem cells possess therapeutic potential through promoting angiogenesis, inhibiting apoptosis, and modulating immune functions.[5] Despite their remarkable therapeutic promise, stem cell-based therapies face substantial clinical implementation challenges that have limited widespread adoption. Key barriers include ethical controversies, allogeneic immune rejection risks, potential for uncontrolled proliferation and tumorigenesis, technical difficulties in maintaining cell viability during storage and transport, and complex regulatory requirements.[6,7] Moreover, standardization of cell preparation protocols, quality control measures, and long-term safety monitoring remain significant challenges hindering

clinical translation.[8,9] These substantial limitations have necessitated the development of alternative therapeutic strategies that capitalize on the beneficial effects of stem cells while avoiding their inherent limitations.

To address these limitations, extracellular vesicles, particularly exosomes, have emerged as promising mediators of stem cell therapeutic effects. Exosomes are naturally secreted lipid bilayer-enclosed nanoscale vesicles (30–150 nm in diameter) that serve as crucial mediators of intercellular communication by transporting proteins, nucleic acids, lipids, and other bioactive molecules.[10–12] These vesicles play essential roles in maintaining cellular homeostasis, coordinating tissue responses, and regulating various physiological processes.[10] Stem cell-derived exosomes (SC-Exos) represent a particularly promising therapeutic approach, as they inherit the regenerative and immunomodulatory properties of their parent cells while offering distinct advantages over direct cell transplantation.[13,14] According to MISEV 2023 guidelines, "exosome" specifically refers to extracellular vesicles from endosomal compartments released via multivesicular bodies, while "small extracellular vesicles" represents a broader category including both exosomes and small ectosomes.[15] This review maintains the SC-Exos terminology used in the cited studies for consistency with the original literature, recognizing that these studies likely encompass diverse populations of stem cell-derived extracellular vesicles. Compared to traditional stem cell therapy, SC-Exos demonstrate reduced immunogenicity, decreased tumorigenicity risks, enhanced stability, improved ability to traverse biological barriers, and fewer ethical considerations.[13,16] These advantages position SC-Exos as promising candidates for therapeutic applications across multiple clinical domains.

The therapeutic potential of SC-Exos has been significantly enhanced through integration with biomedical engineering approaches.[17,18] This interdisciplinary framework provides essential tools and methodologies for optimizing SC-Exos production, characterization, modification, and delivery systems.[19] Engineering innovations have enabled the development of advanced isolation techniques, targeted delivery platforms, controlled-release systems using biomaterial scaffolds, and scalable manufacturing processes.[20–22] For example, integrating human placenta amniotic membrane mesenchymal stem cell-derived exosomes with laminin-derived peptide-modified hyaluronic acid hydrogels has demonstrated enhanced therapeutic efficacy in severe spinal cord injury (SCI) models through improved exosome retention, sustained release, and comprehensive microenvironment regulation.[23] Such biomedical engineering strategies are crucial for translating SC-Exos from laboratory research to clinical applications.

Leveraging these technological advances, SC-Exos have demonstrated therapeutic efficacy across diverse medical conditions, including wound healing, neurological disorders, immunomodulation, and various other therapeutic areas.[24] Despite this broad therapeutic potential, the clinical translation of SC-Exos faces ongoing challenges in standardization, quality control, scalable production, and comprehensive safety evaluation.[25,26] To advance the field toward widespread clinical implementation, systematic understanding of SC-Exos biology, optimization strategies, engineering approaches, their therapeutic applications, and the challenges hindering clinical translation is essential.

This comprehensive review provides a systematic and forward-looking analysis of SC-Exos research, applications, and clinical translation, addressing critical knowledge gaps and implementation challenges that currently limit their widespread clinical adoption. Unlike previous reviews, this study offers detailed analyses of SC-Exos across specific therapeutic domains while presenting multidimensional engineering approaches for functional optimization. Specifically, this review synthesizes the latest advances across the entire SC-Exos research pipeline, from fundamental biological mechanisms and isolation methodologies to sophisticated engineering strategies and diverse therapeutic applications. The review is structured to first establish the biological foundations of exosome biogenesis and comprehensive characterization of different SC-Exos types, followed by detailed examination of functional optimization strategies encompassing parental cell interventions, biomaterial integration approaches, and therapeutic efficacy enhancement techniques. Subsequently, this study presents comprehensive analyses of biomedical engineering applications across major therapeutic domains, including tissue engineering, advanced drug delivery systems, and treatment of cardiovascular, neurological, oncological, immunological, reproductive, musculoskeletal, and dermatological conditions, as well as other emerging therapeutic applications. Additionally, we provide detailed analysis of current clinical translation status, including clinical trial progress, regulatory pathways, and manufacturing standardization challenges. Notably, this review provides comprehensive evaluation of SC-Exos in emerging application areas such as reproductive health and fertility preservation, where SC-Exos overcome traditional therapy limitations through enhanced biocompatibility, reduced immunogenicity, and superior barrier penetration capabilities, broadening research horizons.[13,27,28] Finally, we critically

evaluate current challenges impeding clinical translation, including standardization of isolation and purification techniques, therapeutic heterogeneity, scale-up production barriers, regulatory frameworks, and safety considerations, while proposing potential solutions and future research directions. Through this comprehensive integration of current knowledge and future perspectives, this review provides systematic guidance for researchers and clinicians in developing effective SC-Exos-based therapeutic solutions, ultimately accelerating their clinical translation and expanding therapeutic applications across diverse biomedical challenges.

Exosome Biology and SC-Exos Characterization

Formation and Secretion Mechanisms of Exosomes

Exosomes, a crucial subset of extracellular vesicles, are nano-sized particles with a distinctive bilayer membrane structure released from multivesicular bodies (MVBs). These vesicles, typically 30–150 nm in diameter, are secreted through specific exocytotic processes.[29,30] Exosomes contain diverse bioactive molecules, including proteins, lipids, metabolites, and nucleic acids, which play pivotal roles in intercellular signaling.[31–33]

The biogenesis of exosomes initiates with primary endosome formation through plasma membrane invagination. Following cellular internalization through endocytosis, these primary endosomes undergo a series of dynamic transformations, progressing to late sorting endosomes and ultimately developing into MVBs. During MVB maturation, selective invagination of the limiting membrane generates numerous morphologically uniform intraluminal vesicles (ILVs).[34] These ILV-containing MVBs subsequently follow one of two distinct trafficking pathways, either fusion with lysosomes leading to cargo degradation or regulated exocytosis involving plasma membrane fusion, releasing ILVs as exosomes into the extracellular matrix.[35] This precisely regulated sorting mechanism ensures the orderly and specific nature of the exosome biogenesis.

To elucidate the molecular mechanisms underlying MVB formation and ILV sorting, current research on exosome formation mechanisms has predominantly focused on the Endosomal Sorting Complex Required for Transport (ESCRT)-dependent pathway.[36] The ESCRT machinery comprises four primary protein complexes (ESCRT-0, -I, -II, and -III) and auxiliary proteins, including vacuolar protein sorting (VPS)-associated protein 1, ALG-2-interacting protein X, and VPS4. These components work synergistically during MVB maturation to facilitate vesicular cargo delivery to lysosomes or secretion via exocytosis.[37] However, mounting evidence indicates the existence of ESCRT-independent mechanisms involving lipids (such as ceramides), tetraspanins (CD63 and CD81), and heat shock proteins in ILV formation and MVB sorting. For instance, CD63 can sort melanoma-associated proteins into human ILVs without ESCRT mediation,[38] while CD81 independently facilitates sorting various ligands into exosomes.[36] Moreover, ceramide, a crucial exosomal lipid component, directly participates in ILV formation, with neutral sphingomyelinase inhibition significantly reducing exosome secretion.[39]

Following ILV formation within MVBs, exosome secretion primarily relies on MVB-plasma membrane fusion, coordinated by the Ras-related GTPase (Rab) family and SNARE complexes. SNARE proteins mediate fusion events between exosomes and lysosomes or the plasma membrane, thereby facilitating extracellular transport. The MVB tethering mechanism, regulated by GTPases including Rab7A, Rab7B, Rab35, and RALA, is vital for exosome release.[40] Specifically, Rab11 and Rab35 primarily regulate endosome recycling pathways.[41,42] However, these Rab proteins exhibit distinct and specialized roles in determining MVB fate and exosome secretion efficiency. Rab7 serves as a pivotal molecular switch controlling MVB destiny, with its activation by Mon1a/b and neddylated Coro1a promoting dynein-dependent retrograde transport toward lysosomes for cargo degradation, while inactivation through the Rab31/TBC1D2B cascade favors kinesin-dependent antegrade transport toward the plasma membrane and exosome release.[43,44] Rab27a primarily controls MVB docking at the plasma membrane and determines exosome size, whereas Rab27b regulates MVB intracellular distribution and peripheral localization.[45] Additionally, Rab35 mediates MVB docking or tethering with the plasma membrane, with Rab11- and Rab35-regulated exosomal secretion appearing to operate through mechanisms distinct from the ESCRT-dependent pathways associated with Rab27.[46,47]

The molecular switches governing MVB trafficking fate between degradation and secretion directly impact exosome yield and cargo composition. Post-translational modifications serve as important regulatory mechanisms, with ISGylation

of ESCRT component TSG101 promoting lysosomal degradation of MVB proteins and reducing exosome secretion, while the ubiquitination status of key proteins like Rab27a and HRS modulates MVB fate decisions.[48,49] Lysosomal functional status also acts as a compensatory routing mechanism, where impaired lysosomal acidification or overloaded degradative capacity redirects MVBs toward secretory pathways, effectively increasing exosome production.[50] These regulatory mechanisms provide fundamental insights into exosome biogenesis control that are applicable across different cellular sources.

The comprehensive understanding of these formation, secretion, and regulatory mechanisms has opened new avenues for therapeutic intervention. Pharmacological modulation of key pathway components represents a promising strategy for controlling exosome secretion in pathological conditions. The compound GW4869, a noncompetitive neutral sphingomyelinase 2 (nSMase2) inhibitor with an IC_{50} of 1 μM, has been used to demonstrate the critical role of ceramide pathways in exosome formation.[51,52] More recently, DPTIP, a potent brain-penetrant nSMase2 inhibitor with significantly improved potency (IC_{50} = 30 nM), has shown therapeutic efficacy in reducing pathological exosome release in neuroinflammation models.[53] Additionally, the mevalonate pathway, which controls cholesterol synthesis essential for exosomal membrane formation, presents another therapeutic target. Simvastatin, an HMG-CoA reductase inhibitor, has been demonstrated to reduce exosome secretion through both cholesterol-dependent and independent mechanisms, offering a clinically approved approach for exosome modulation.[54]

In conclusion, exosome biogenesis and secretion represent highly coordinated cellular processes involving both ESCRT-dependent and ESCRT-independent pathways, including ceramide-mediated mechanisms, tetraspanin sorting, and lipid raft-associated processes. The Rab GTPase family serves as critical molecular switches determining MVB fate between lysosomal degradation and exocytotic release, with additional regulation provided by post-translational modifications and lysosomal functional status.[55] The identification of key metabolic checkpoints, particularly sphingomyelinase and mevalonate pathway components, has revealed clinically viable therapeutic targets for modulating exosome secretion in pathological conditions. This mechanistic complexity underscores the sophisticated regulatory networks underlying exosome biology and their potential for therapeutic intervention.

Mechanistic Diversity and Cellular Specificity in Exosome Biogenesis

While the fundamental mechanisms described above provide the molecular framework for exosome biogenesis, recent research has revealed that the relative utilization of ESCRT-dependent versus ESCRT-independent pathways is not uniform across cell types or physiological conditions. These ESCRT-independent pathways demonstrate distinct cargo sorting specificities. Ceramide-dependent mechanisms selectively sort specific membrane proteins such as proteolipid protein (PLP) through lipid raft microdomains and regulate nucleic acid packaging via recruitment of RNA-binding proteins like heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2B1). In contrast, tetraspanin-mediated sorting preferentially incorporates membrane-associated signaling molecules through direct protein-protein interactions, suggesting the generation of functionally distinct exosome subpopulations.[51,56]

The integration and interplay between ESCRT-dependent and ESCRT-independent pathways demonstrate significant cell-type specificity and condition-dependent preferences. These parallel mechanisms can operate simultaneously to generate functionally distinct exosome subpopulations, with the ESCRT machinery functioning independently of lipid raft microdomains while ceramide-dependent pathways rely on lipid raft organization.[57] Cell-type-specific variations in pathway utilization have been extensively documented. For instance, oligodendrocytes exhibit strong ceramide dependence for PLP sorting even when essential ESCRT components are depleted, whereas melanoma cells show ceramide-independent exosome secretion but require CD63 for ESCRT-independent sorting of melanosomal proteins.[37] Furthermore, exosomes from different cellular origins display distinct physical properties, with B cell-derived exosomes showing lower density (1.13 g/mL) compared to epithelial cell-derived exosomes (1.19 g/mL) on sucrose gradients, reflecting their different biogenic origins.[35] Additionally, Rab7 exhibits cell type-specific functions in controlling MVB transport to lysosomes,[45,46,58,59] further illustrating the cellular variability in exosome biogenesis regulation.

Pathway preference is dynamically regulated by cellular conditions and cargo requirements. Under stress conditions such as glucose starvation and hypoxia, mechanistic target of rapamycin complex 1 (mTORC1) negatively regulates exosome release, coordinating cellular waste management and recycling processes similar to autophagy regulation.[55]

Cancer cells frequently exhibit altered pathway utilization, with RAB31 overexpression promoting ESCRT-independent exosome biogenesis enriched in receptor tyrosine kinases (RTKs), while canonical ESCRT pathways preferentially sort ubiquitinated proteins for lysosomal degradation.[43] Additionally, ALIX provides an alternative ESCRT-III recruitment pathway that operates independently of canonical ESCRT-0/-I/-II machinery, specifically controlling tetraspanin sorting to exosomes through lysobisphosphatidic acid-dependent mechanisms.[60]

In summary, the pathway plasticity and cell-type specificity observed in exosome biogenesis underscore the need for tailored approaches in SC-Exos production and optimization. Different stem cell sources may exhibit distinct biogenic preferences that ultimately influence therapeutic outcomes, highlighting the importance of understanding these mechanistic variations for successful clinical translation. This pathway diversity ensures optimal cellular responses to varying physiological demands while maintaining cargo specificity and functional diversity essential for effective intercellular communication.

Stem Cell Types and Their Exosomal Characteristics

Stem cells represent undifferentiated primitive cells distributed throughout embryonic, fetal, and adult organisms, possessing the capacity to differentiate into multiple cell types and form tissues and organs.[6] These cells are classified into three primary categories based on their origin and characteristics: embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells (ASCs).[5] Each stem cell type exhibits distinct biological functions attributable to their unique structural and functional characteristics.[4] Consequently, SC-Exos inherit the biological properties of their parental cells, manifesting significant functional variations in their characteristics and applications (Table 1).[61]

Induced Pluripotent Stem Cell-Derived Exosomes (iPSC-Exos)

iPSCs are distinguished by their capacity to differentiate into multiple cell types through reprogramming technologies, establishing them as powerful tools for disease pathogenesis investigation and cross-disciplinary drug screening.[93] Unlike other stem cell types, iPSCs effectively circumvent ethical and practical limitations associated with restricted cellular

Table 1 Characteristics and Applications of Different SC-Exos

Classification	iPSC-Exos	ESC-Exos	ASC-Exos		
			BMSC-Exos	ADSC-Exos	NSC-Exos
Origin	iPSCs	ESCs	BMSCs	ADSCs	NSCs
Functions	Tissue repair,[62] Anti-apoptosis[63]	Anti-inflammation,[64] Tissue repair[65]	Anti-apoptosis,[66] Neuromodulation,[67] Immunomodulation,[68] Tissue regeneration[69]	Tissue regeneration,[70] Anti-inflammation,[71] Immunomodulation,[72] Metabolic regulation[73]	Neuromodulation[74]
Therapeutic Applications	Pulmonary diseases,[75] Cardiovascular diseases,[76] Dermatological conditions,[77] Neurological disorders,[62] Skeletal disorders[78]	Dermatological conditions,[79] Cardiovascular diseases,[80] Pulmonary diseases,[81] Skeletal disorders,[65] Hepatic diseases[82]	Neurological disorders,[83] Skeletal disorders,[84] Renal diseases,[85] and Immune system disorders[86]	Dermatological conditions,[87] Muscular disorders,[88] Hepatic diseases,[89] Skeletal disorders,[90] Pulmonary diseases[91]	Neurological disorders[92]
Advantages	Readily accessible, scalable production with high stability; suitable for personalized medicine	Sustainable production of high-quality exosomes capable of inducing similar biological responses in target cells	Broad applications with demonstrated therapeutic efficacy; promising clinical prospects	Simple procurement through routine liposuction; abundant stem cell yield	Promising potential in neurological disease treatment
Limitations	Complex isolation techniques with low-efficiency	Ethical concerns regarding embryonic collection	Lack of systematic therapeutic mechanisms; clinical safety and efficacy require further validation	Limited research on safety and applications; low yield and restricted procurement methods	Limited to neurological applications; challenges in purification and scale-up production

Note: Bold formatting represents table column headers and classification categories.

Abbreviations: ADSC-Exos, Adipose-derived mesenchymal stem cell-derived exosomes; ADSCs, Adipose-derived mesenchymal stem cells; ASC-Exos, Adult stem cell-derived exosomes; BMSC-Exos, Bone marrow mesenchymal stem cell-derived exosomes; BMSCs, Bone marrow mesenchymal stem cells; ESC-Exos, Embryonic stem cell-derived exosomes; ESCs, Embryonic stem cells; iPSC-Exos, Induced pluripotent stem cell-derived exosomes; iPSCs, Induced pluripotent stem cells; NSC-Exos, Neural stem cell-derived exosomes; NSCs, Neural stem cells; SC-Exos, Stem cell-derived exosomes.

tissue sources and post-mortem tissue degradation through reprogramming techniques, providing reliable cellular sources for diverse cell types in clinical applications with continuously improving reprogramming efficiency.[93,94]

iPSC-Exos demonstrate broad physiological effects encompassing cell proliferation, immune regulation, cytokine profile modulation, neural repair, and tissue regeneration.[95] Studies have illustrated that iPSC-Exos significantly inhibit vascular remodeling and right ventricular hypertrophy in monocrotaline-induced rat pulmonary hypertension models by targeting the hypoxia-inducible factor-1α (HIF-1α) and runt-related transcription factor 2 (Runx2) signaling pathways, effectively preventing and mitigating pulmonary hypertension progression by reducing abnormal proliferation and migration of pulmonary arterial smooth muscle cells.[75] Clinical applications span multiple therapeutic domains, including pulmonary diseases, cardiovascular diseases, dermatological conditions, neurological disorders, and skeletal disorders, demonstrating remarkable therapeutic versatility (Table 1).[62,75–78]

However, iPSCs exhibit inherent limitations, including preferential differentiation toward immature embryonic or fetal cell types rather than fully mature adult cells. Additionally, low induction rates and incompletely understood molecular mechanisms during differentiation remain significant obstacles for clinical applications, consequently limiting the clinical translation potential of iPSC-Exos.[96] While offering accessible and scalable production with high stability suitable for personalized medicine, iPSC-Exos face technical challenges including complex isolation procedures and low efficiency.

Embryonic Stem Cell-Derived Exosomes (ESC-Exos)

ESCs, derived from the inner cell mass of blastocysts, possess unlimited proliferation and self-renewal capabilities comparable to iPSCs' multipotent differentiation potential. However, ESCs achieve differentiation without requiring artificial intervention and exhibit significantly reduced uncertainty during differentiation processes, making them superior choices for progenitor cell generation.[97]

ESC-Exos have garnered considerable attention for their cardiovascular therapeutic potential. Their primary therapeutic mechanisms involve fibroblast growth factor 2-dependent signaling pathways that specifically promote myocardial angiogenesis. Research demonstrates that ESC-Exos promote myocardial angiogenesis through the fibroblast growth factor 2-dependent signaling pathway, effectively improving heart failure induced by transverse aortic constriction while maintaining ESCs' therapeutic efficacy and avoiding their potential adverse effects, with non-significant impact on myocardial fibrosis.[80] Further research demonstrates that ESC-Exos enhance cardiac function by delivering miR-294 to cardiac tissue, promoting neovascularization, enhancing cardiac progenitor cell proliferation, differentiation, and survival, and stimulating cardiomyocyte cell cycle entry.[98] Beyond cardiovascular applications, ESC-Exos also possess anti-inflammatory properties that contribute to their broader therapeutic efficacy.[64]

Nevertheless, the self-renewal, unlimited proliferation, and multipotent differentiation capabilities of ESCs present significant clinical challenges. The primary concern involves potential differentiation into tumor cells and subsequent proliferation following transplantation, thereby inducing tumorigenesis.[99] Additionally, ethical controversies surrounding embryonic stem cell utilization in clinical research and therapeutic applications constitute substantial barriers to large-scale implementation.[100] Consequently, while ESC-Exos inherit excellent therapeutic properties from their parental cells and effectively reduce tumorigenicity risks, clinical research remains limited due to persistent ethical constraints (Table 1).[65,79–82]

Adult Stem Cell-Derived Exosomes (ASC-Exos)

The pursuit of safer and more effective stem cell therapeutic approaches has intensified research focus on ASCs in recent years. ASCs effectively mitigate adverse effects including tumorigenicity and ethical controversies associated with cell transplantation while maintaining multipotent differentiation capacity.[101] ASC-Exos encompass various types derived from different adult stem cell populations, with mesenchymal stem cell-derived exosomes (MSC-Exos) being the most extensively studied due to their therapeutic versatility and accessibility. MSC-Exos can be further classified based on tissue origin, with bone marrow mesenchymal stem cell-derived exosomes (BMSC-Exos) and adipose-derived mesenchymal stem cell-derived exosomes (ADSC-Exos) being the most clinically relevant types. Additionally, neural stem cell-

derived exosomes (NSC-Exos) represent another important category of ASC-Exos with specialized therapeutic applications (Table 1).

BMSC-Exos contain bioactive molecules including transforming growth factor-β1 (TGF-β1)[102] and regulate the expression of inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-10 (IL-10),[103] which are essential for immune modulation mechanisms. Research demonstrates that BMSC-Exos inhibit microglial polarization toward the M1 phenotype while promoting M2 phenotype transformation and anti-inflammatory cytokine secretion, significantly improving neurological symptoms in experimental autoimmune encephalomyelitis rat models and reducing central nervous system inflammation and demyelination.[104] BMSC-Exos demonstrate broad therapeutic applications with proven efficacy in neurological disorders, skeletal disorders, renal diseases, and immune system disorders, although systematic therapeutic mechanisms require further validation.[83–86]

ADSC-Exos exhibit tissue regeneration, anti-inflammation, immunomodulation, and metabolic regulation functions,[70–73] with therapeutic applications spanning dermatological conditions, muscular disorders, hepatic diseases, skeletal disorders, and pulmonary diseases.[87–91] While adipose-derived mesenchymal stem cells (ADSCs) themselves offer advantages of simple procurement through routine liposuction with abundant stem cell yield,[105] the production of ADSC-Exos faces challenges including low extraction efficiency, standardization difficulties, and limited scalability for clinical applications.[106] NSC-Exos demonstrate specialized neuromodulation functions with promising potential specifically for neurological disease treatment; however, their applications remain limited to neurological conditions with significant challenges in purification and scale-up production.[74,92]

MSC-Exos possess substantial yield advantages compared to other ASC-Exos types, and their culture and purification methodologies are more established. A fundamental advantage of mesenchymal stem cells lies in their widespread tissue distribution, existing throughout human tissues including peripheral blood, dental pulp, bone marrow, adipose tissue, umbilical cord, amniotic fluid, and placenta, providing excellent accessibility suitable for large-scale clinical applications.[107] Compared to their parental cells, ASC-Exos further reduce infusion toxicity associated with stem cell transplantation while presenting minimal ethical concerns, demonstrating excellent therapeutic properties across multiple diseases.[25]

In summary, variations among SC-Exos types stem from distinct characteristics of their parental cells. While different stem cell types share capabilities for differentiating into multiple cell types for clinical disease treatment, they exhibit significant differences in differentiation potential and efficiency, differentiation pathways, scalable applications, transplantation-associated infusion toxicity, and ethical considerations. Therefore, selecting appropriate stem cell types and their corresponding exosomes based on specific therapeutic requirements, mechanistic considerations, and practical constraints is crucial for achieving optimal treatment outcomes (Table 1).

Selection Criteria for SC-Exos in Clinical Applications

The selection of appropriate stem cell sources for SC-Exos production currently lacks standardized guidance, with researchers predominantly relying on source accessibility and personal preference rather than evidence-based criteria.[108] While studies have demonstrated therapeutic efficacy of different SC-Exos types across various diseases (Table 1), systematic comparative analyses and standardized selection criteria remain limited. The inherent therapeutic heterogeneity among SC-Exos types requires systematic evaluation of disease-specific requirements, production scalability, regulatory pathways, and source accessibility.[101] To address this deficiency, we propose a systematic evaluation framework to guide evidence-based source selection for clinical applications.

Therapeutic efficacy and target specificity constitute the primary evaluation dimension. Stem cell sources possess distinct biological properties that determine their exosomal therapeutic potential. NSC-Exos demonstrate superior neurological specificity through inherent neurotropic properties but remain limited to neurological applications.[74,92] MSC-Exos exhibit broader therapeutic versatility across multiple organ systems due to multipotent regenerative capabilities and established paracrine signaling networks, making them optimal for systemic inflammatory conditions and multi-organ dysfunction.[109] ESC-Exos and iPSC-Exos show superior cardiovascular protective mechanisms compared to adult stem cell derivatives, particularly through enhanced angiogenic and anti-apoptotic pathways.[76,80]

Safety and regulatory considerations represent the second dimension. ASC-Exos demonstrate favorable safety profiles by inheriting parental cell advantages in mitigating tumorigenicity and ethical concerns while reducing infusion-related toxicities compared to cell-based therapies. This positions them as suitable candidates for clinical development. ESC-Exos, despite potent therapeutic efficacy, carry inherent tumorigenicity risks and face substantial ethical and regulatory barriers that may extend development timelines. iPSC-Exos present intermediate safety profiles with reduced ethical concerns but require comprehensive evaluation of reprogramming-related genetic modifications.

Production feasibility encompasses the third dimension, including source accessibility and scalability. ADSC-Exos benefit from minimally invasive procurement via routine liposuction but face production challenges including low extraction efficiency and standardization difficulties. BMSC-Exos require invasive bone marrow aspiration yet offer established purification methodologies and superior large-scale manufacturing scalability through well-characterized culture systems. iPSC-Exos provide theoretically unlimited expansion potential and enable personalized medicine approaches but require sophisticated quality control systems and face technical complexity in standardized production. ESC-Exos encounter substantial production limitations due to ethical restrictions and regulatory constraints on embryonic tissue procurement.

Clinical translation readiness defines the fourth dimension, determining practical timelines and development strategies. ASC-Exos offer immediate translational opportunities through established safety profiles, reduced regulatory complexity, and well-characterized production methods, making them ideal for proof-of-concept studies and early-phase trials. iPSC-Exos require extended development timelines but provide the greatest potential for precision medicine and patient-specific interventions. ESC-Exos, while demonstrating exceptional therapeutic potential, face prolonged regulatory pathways requiring resolution of ethical and safety concerns.

This systematic evaluation framework provides reference for SC-Exos source selection by systematically integrating therapeutic requirements with implementation conditions, facilitating more rational clinical translation strategies.

Isolation and Characterization of SC-Exos

Isolation and Purification Methods of Exosomes

Efficient isolation and purification methodologies are fundamental to the clinical implementation of SC-Exos. The selection of appropriate isolation techniques profoundly influences exosome yield, purity, functionality, and subsequent analytical applications. Contemporary methodologies can be categorized according to their underlying separation principles: physical properties (size and density), biochemical characteristics (surface markers), and chemical properties (solubility).[110,111]

Ultracentrifugation-based approaches represent the most extensively utilized methods for exosome isolation. Differential ultracentrifugation eliminates cellular debris through sequential centrifugation steps at progressively increasing speeds, ultimately isolating exosomes at 100,000–120,000 × g.[110,112] However, this technique may simultaneously precipitate other extracellular vesicles and protein complexes, thereby compromising purity.[113] To address these limitations, density gradient ultracentrifugation exploits buoyant density differences using sucrose gradients (1.08–1.22 g/mL) or iodixanol gradients (~1.11 g/mL) to achieve enhanced purity.[114,115] Similarly, size-exclusion chromatography (SEC) achieves separation based on particle size differentials while offering distinct advantages in both purity and yield with minimal effects on exosome structural integrity.[116] Recent advances have culminated in size exclusion fast-performance liquid chromatography (SE-FPLC), which demonstrates exceptional clinical translation potential through high-yield isolation (>88%), rapid processing (<20 min), and effective removal of albumin and lipoprotein contaminants.[117] SEC-based methods have demonstrated broad compatibility across multiple biological sources including cell culture media, serum, plasma, urine, and saliva.[118] These advantages have positioned SEC as the preferred method for clinical applications due to its superior balance of yield, purity, and standardization potential.[119,120]

In contrast, immunoaffinity-based approaches utilize biochemical specificity to achieve high-purity isolations through selective binding between exosomal surface markers (CD9, CD63, CD81) and corresponding antibodies.[121,122] Contemporary systems incorporate engineered antibody fragments and aptamers on diverse solid supports, providing enhanced specificity and multiplexed capture capabilities for simultaneous isolation of distinct exosome subpopulations.[123,124] Immunomagnetic separation represents a promising advancement, utilizing immunomagnetic

microbeads that provide enhanced specificity and faster processing compared to conventional ultracentrifugation while maintaining exosome integrity.[110,125] Recent innovations include Strep-tag II-based immunomagnetic isolation systems, which demonstrate rapid processing (38 min) with high isolation efficiency (82.5%) and mild release capabilities (62% release efficiency).[126] This approach combines the specificity of immunoaffinity capture with the operational advantages of magnetic separation, enabling automated processing and improved scalability.

Alternative methodologies offer practical advantages for specific applications but have inherent limitations. These include polyethylene glycol (PEG) precipitation, which is simple and scalable but may co-precipitate non-exosomal proteins;[110] ultrafiltration using molecular weight cutoff membranes, which can cause membrane clogging and sample loss;[119] and microfluidic separation technology, which enables rapid isolation but has limited sample processing capacity and requires specialized expertise.[110,127]

The optimal choice of isolation methodology depends on the intended application, required purity levels, sample volume, and downstream analytical requirements (Table 2). Based on comparative analysis, SEC demonstrates the highest clinical translation potential due to its excellent balance of yield, purity, and automated standardization capabilities. Traditional ultracentrifugation remains widely accepted as the established gold standard, while immunomagnetic separation shows promise for high-throughput clinical applications despite higher costs. In contrast, PEG precipitation, though highly scalable and cost-effective, requires additional purification steps that limit direct clinical use. Microfluidic approaches remain primarily research-focused due to equipment complexity and limited throughput. For immediate clinical implementation, automated SEC systems are increasingly favored for their reproducibility and standardization potential. However, the field as a whole still faces challenges in method standardization, cost-effectiveness, and scalability for widespread clinical adoption.

Characterization Techniques for Exosomes

Exosome characterization is crucial for validating isolation quality and biological properties. Transmission electron microscopy (TEM) serves as the gold standard for morphological characterization, directly revealing exosome morphology, size, and membrane integrity.[128] Western blotting detects specific marker proteins to effectively confirm exosome presence and assess sample purity.[29] Flow cytometry enables qualitative and quantitative analysis through fluorescent labeling of specific surface antigens. However, due to the small size of exosomes, traditional flow cytometry typically requires coupling exosomes to microbeads for multiple marker detection.[129,130]

Table 2 Comparison of Exosome Isolation and Purification Methods

Method	Principle	Yield	Purity	Cost	Scalability	Time	Advantages	Disadvantages	Clinical Translation Potential
Ultracentrifugation	Density-based separation	Moderate	Moderate	Low	High	4-6 h	Gold standard, Established protocols	Time-consuming, Potential damage	High
Density gradient ultracentrifugation	Density gradient separation	Low	Very High	Moderate	Moderate	16-24 h	Highest purity	Complex, time-Intensive	High
Ultrafiltration	Size-based membrane filtration	High	Low-Moderate	Low	High	0.5–2 h	Simple, Cost-effective	Membrane clogging, Contamination	Moderate
SEC	Hydrodynamic radius separation	High	High	Moderate	Moderate	20 min-2 h	Preserves integrity, High purity, Standardizable	Sample dilution, Equipment cost	Very High
Immunoaffinity capture	Antibody-antigen binding	Low	Very High	High	Low	2-4 h	Highest specificity	Expensive, Limited scalability	Moderate
Immunomagnetic separation	Magnetic separation + Immunoaffinity	Moderate	Very High	High	Moderate-High	0.5–1 h	Rapid, Highly specific	Expensive reagents	High
PEG precipitation	Polymer-induced aggregation	Very High	Low	Low	Very High	4-16 h	Simple protocol, Scalable	High contamination	Low
Microfluidics	Multiple mechanisms	Moderate	High	Very High	Very Low	10-60 min	High automation, Integration	Complex, Expensive equipment	Low

Note: Bold formatting represents table column headers and classification categories.
Abbreviations: H, Hours; Min, Minutes; PEG, Polyethylene glycol; SEC, Size exclusion chromatography.

Furthermore, nanoparticle tracking analysis (NTA) provides quantitative analysis of particle size distribution and concentration in exosome suspensions.[131] Dynamic light scattering can analyze particle size distribution but measures hydrodynamic radius, yielding results that differ from TEM and NTA measurements. This technique exhibits lower accuracy for heterogeneous samples.[132] Emerging technologies, including atomic force microscopy and super-resolution microscopy, have recently been applied to detailed exosome characterization, offering high-resolution insights into their structural and functional properties.[133] However, these advanced characterization methods face significant barriers for routine clinical adoption. Super-resolution microscopy techniques, while providing nanoscale resolution, require expensive specialized equipment, extensive operator training, and lengthy sample preparation protocols that limit throughput for high-volume clinical applications.[134–136] Similarly, atomic force microscopy offers detailed surface topology analysis but suffers from low throughput, complex sample preparation requirements, and susceptibility to environmental artifacts that compromise reproducibility in clinical settings.[137,138] The high capital investment costs and ongoing maintenance requirements present additional economic barriers for widespread implementation in routine diagnostic laboratories. Furthermore, the lack of standardized protocols and quality control measures across different platforms hinders the development of clinically validated assays using these technologies.[133,136]

For immediate clinical translation, conventional methods such as NTA, TEM, and flow cytometry remain more practical due to their established protocols, lower costs, and higher throughput capabilities, while emerging technologies may find specialized applications in research and development phases. These characterization techniques are collectively indispensable for ensuring the quality and reproducibility of exosome-based research and clinical applications.

Functional Optimization Strategies for SC-Exos

Although SC-Exos demonstrate promising biological properties and broad application potential, their limited natural yield and complex variable composition pose challenges for clinical translation.[139] Consequently, researchers have developed a series of optimization strategies for SC-Exos, including parental cell interventions to enhance exosome secretion and functionality, biomaterial integration to optimize physicochemical performance and delivery systems, and functional molecule loading and surface modifications to augment intrinsic therapeutic efficacy to meet clinical requirements.

Parental Cell Intervention Optimization

The secretion volume and functionality of SC-Exos can be effectively enhanced by modulating the biological state of parental stem cells through strategies including signaling pathway activation, hypoxic preconditioning, and specific factor stimulation.[140] Research has demonstrated that treating human umbilical mesenchymal stem cells (hUMSCs) with CHIR99021, a Wnt/β-catenin signaling pathway agonist, significantly increases exosome secretion (approximately 1.5-fold) and therapeutic efficacy, particularly in protein cargo loading (including proteins related to cell migration and wound healing). This enhancement significantly improves the therapeutic effects of human umbilical mesenchymal stem cell-derived exosomes (hUMSC-Exos) in diabetic chronic wound healing[141] (Figure 1A). Furthermore, hypoxia-preconditioned adipose-derived stem cells (ADSCs) significantly enhance the pro-angiogenic and osteogenic functions of their derived exosomes (Hypo-ADSC-Exos). These exosomes promote endothelial cell proliferation and migration by regulating targeting sprouly1 through miR-21-5p and activating the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway, demonstrating therapeutic efficacy in murine osteoporotic fracture healing[142] (Figure 1B). Moreover, nerve growth factor (NGF) stimulation of ADSCs significantly enhances the neurogenic effects of their secreted ADSC-Exos on neural and bone regeneration. Loading these ADSC-Exos onto three-dimensional (3D)-printed porous scaffolds enables sustained release, significantly promoting neurovascular structure formation and bone regeneration in rat models, offering a potential strategy for integrated neural and bone regeneration therapy (Figure 1C).[143]

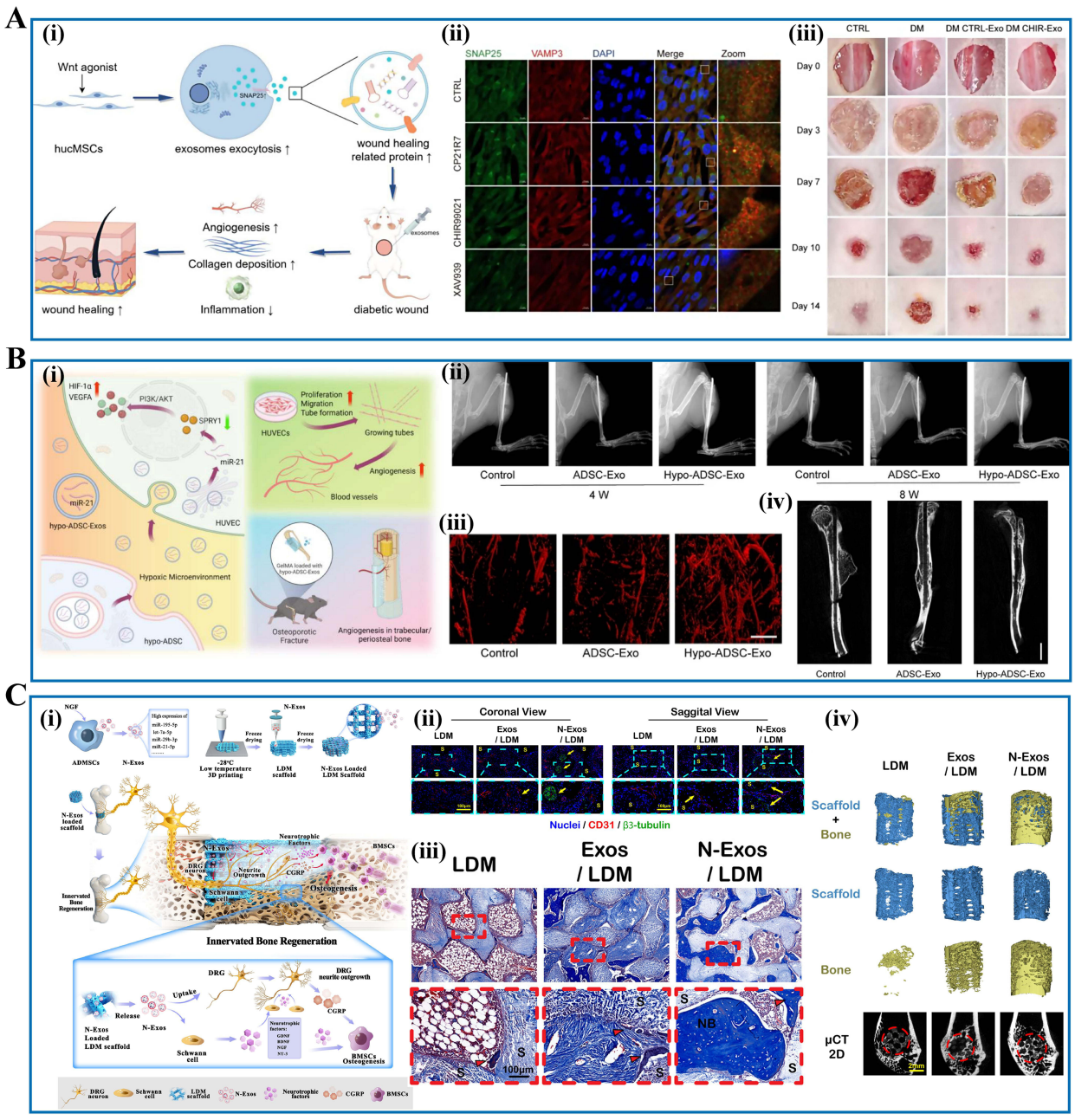


Figure 1 Optimization strategies for SC-Exos function through parental cell intervention. (**A**) CHIR99021 activates the Wnt/β-catenin pathway in hUMSCs, enhancing exosome secretion, cargo loading and promoting diabetic wound healing. Reproduced from Wang LM, Chen J, Song J, et al. Activation of the Wnt/β-catenin signalling pathway enhances exosome production by hucMSCs and improves their capability to promote diabetic wound healing. J Nanobiotechnol. 2024;22(1):373. © 2024. The Author(s). Creative Commons Attribution 4.0 International License.[141] (i) The Wnt agonist CHIR99021 activates hUMSCs, enhancing exosome secretion and accelerating wound healing in diabetic mice. (ii) Immunofluorescence staining exhibits that CHIR99021 promotes the colocalization of SNAP25 and VAMP3, driving exosome secretion (Scale bar = 20 μm). (iii) Wound images exhibit that exosomes derived from CHIR99021-pretreated hUMSCs accelerate diabetic wound closure within 14 days. (**B**) Hypoxia preconditioning of ADSCs enhances the function of the hypo-ADSC-derived exosomes (hypo-ADSC-Exos), promoting angiogenesis and osteoporotic fracture repair. Reproduced from Li XQ, Fang S, Wang SH, et al. Hypoxia preconditioning of adipose stem cell-derived exosomes loaded in gelatin methacryloyl (GelMA) promote type H angiogenesis and osteoporotic fracture repair. J Nanobiotechnol. 2024;22(1):112. Creative Commons Attribution 4.0 International License.[142] (i) Hypo-ADSC-Exos promote angiogenesis and osteogenesis via miR-21-5p-mediated activation of the PI3K/AKT pathway. (ii) X-ray imaging exhibits enhanced bone formation at fracture sites treated with Hypo-ADSC-Exos. (iii) 3D micro-CT angiography reveals improved vascular networks in fracture regions treated with Hypo-ADSC-Exos. (iv) Micro-CT analysis exhibits increased bone volume and density following Hypo-ADSC-Exos treatment. (**C**) ADSC-derived exosomes from NGF-preconditioned ADSCs enhance neurogenesis, angiogenesis, and bone regeneration through functionalized scaffolds. Reproduced from Nerve growth factor-preconditioned mesenchymal stem cell-derived exosome-functionalized 3D-printed hierarchical porous scaffolds with neuro-promotive properties for enhancing innervated bone regeneration. ACS Nano. 2024;18(10):7504–7520. Copyright © 2024 American Chemical Society.[143] (i) Schematic of scaffolds functionalized with ADSC-derived exosomes from NGF-preconditioned ADSCs and their neurogenic and osteogenic mechanisms. (ii) Immunofluorescence staining of CD31 and β3-tubulin exhibits new blood vessels and nerve formation in vivo, induced by ADSC-Exos from NGF-preconditioned ADSCs. Yellow S: scaffold; yellow arrows highlight the newly developed blood vessels and nerve fibers within the subcutaneously implanted scaffolds. (iii) Masson's staining demonstrates new bone and collagen formation in defects treated with scaffolds functionalized with ADSC-Exos from NGF-preconditioned ADSCs. S: scaffold; NB: newly formed bone tissue; red arrows highlight the newly formed immature woven bone. (iv) Micro-CT images exhibit enhanced bone regeneration in scaffolds functionalized with ADSC-Exos derived from NGF-preconditioned ADSCs. Red dashed circles: newly formed bone tissue areas around the defect regions.

Physicochemical Performance Optimization of SC-Exos Through Biomaterial Integration

The post-isolation performance of SC-Exos can be enhanced by integrating them with biomaterials to optimize delivery systems, improving their in vivo stability and targeted delivery capabilities. These biomaterial integration strategies primarily focus on enhancing the physicochemical properties of SC-Exos, addressing challenges related to exosome stability, controlled release kinetics, and bioavailability while preserving their intrinsic molecular cargo. The emphasis lies in developing delivery platforms and carrier systems that maintain and optimize the inherent therapeutic properties of SC-Exos through external modifications.

Electrospun nanofibers serve as an effective platform for stable SC-Exos release. When hUMSC-Exos and mouse embryonic cortical NSC-Exos are loaded onto an Electrospun Nanofiber Platform (Duo-Exo@NF), they demonstrate significantly enhanced sustained release efficiency in traumatic brain injury (TBI) treatment, promoting axonal regeneration while reducing reactive glial cell accumulation (Figure 2A).[144] Encapsulating hUMSC-Exos within polyvinyl alcohol (PVA) hydrogels enable their stable release and localized action at alveolar bone defect sites. Through miR-21-5p-mediated inhibition of the WW domain-containing E3 ubiquitin-protein ligase 1 and plasma membrane calcium-transporting ATPase 4 (ATP2B4) pathways, this system significantly promotes osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) and angiogenesis in human umbilical vein endothelial cells, thereby enhancing bone regeneration and alveolar bone defect repair (Figure 2B).[145] Tympanic membrane perforation, a common cause of hearing loss, traditionally relies on graft repair surgery but is associated with high costs, procedural complexity, donor material scarcity, and potential complications.[146,147] In tympanic membrane repair, a trilayer composite scaffold comprising alginate hydrogel and electrospun polycaprolactone/gelatin fibers demonstrates stable, controlled release of human ADSC-Exos and excellent biocompatibility. This composite scaffold, optimized with ADSC-Exos concentration (100 μg/mL), significantly promotes fibroblast proliferation and migration, achieving rapid tympanic membrane perforation repair in rats within five days, offering an effective tissue engineering solution for non-surgical tympanic membrane regeneration (Figure 2C).[148]

Strategies for Enhancing SC-Exos Intrinsic Therapeutic Efficacy

The therapeutic efficacy of SC-Exos can be enhanced through functional molecule loading and improved delivery systems to optimize targeted therapeutic outcomes. Unlike biomaterial integration approaches that focus on external carrier systems, these strategies directly augment the intrinsic therapeutic capacity of SC-Exos through molecular engineering, surface functionalization, and targeted delivery optimization that fundamentally enhance the biological activity and therapeutic specificity of the exosomes themselves.

For instance, loading specific miRNAs such as miR-30c, miR-181b, and miR-613 into BMSC-Exos effectively inhibit non-small cell lung cancer proliferation and migration, offering a novel approach for targeted lung cancer therapy.[149] Moreover, an oxidized hyaluronic acid-polylysine composite hydrogel (OHA-PL) has been employed to load ADSC-Exos, effectively improving myocardial fibrosis and promoting angiogenesis by regulating oxidative stress and reducing the inflammatory microenvironment, offering a potential bioengineering therapeutic approach for post-myocardial infarction (MI) cardiac repair (Figure 3A).[150] Integrating ADSC-Exos into hyaluronic acid hydrogels using 3D printing technology and a 3D microfiber culture system demonstrates significantly enhanced cell proliferation, migration, and angiogenic capabilities compared to two-dimensional (2D) culture methods. This approach accelerates burn healing while optimizing collagen remodeling and re-epithelialization, providing an efficient and scalable strategy for burn repair (Figure 3B).[151] Furthermore, loading human endometrial MSC-derived exosomes (hEMSC-Exos) into conductive polypyrrole-chitosan (PPY-CHI) hydrogel promotes cardiomyocyte regeneration through the activation of the epidermal growth factor (EGF)/PI3K/AKT signaling pathway. Combined with PPY-CHI's conductive properties and sustained exosome release mechanism, this approach significantly enhances myocardial regeneration and electrical conduction in MI models, offering new perspectives for myocardial injury repair (Figure 3C).[152]

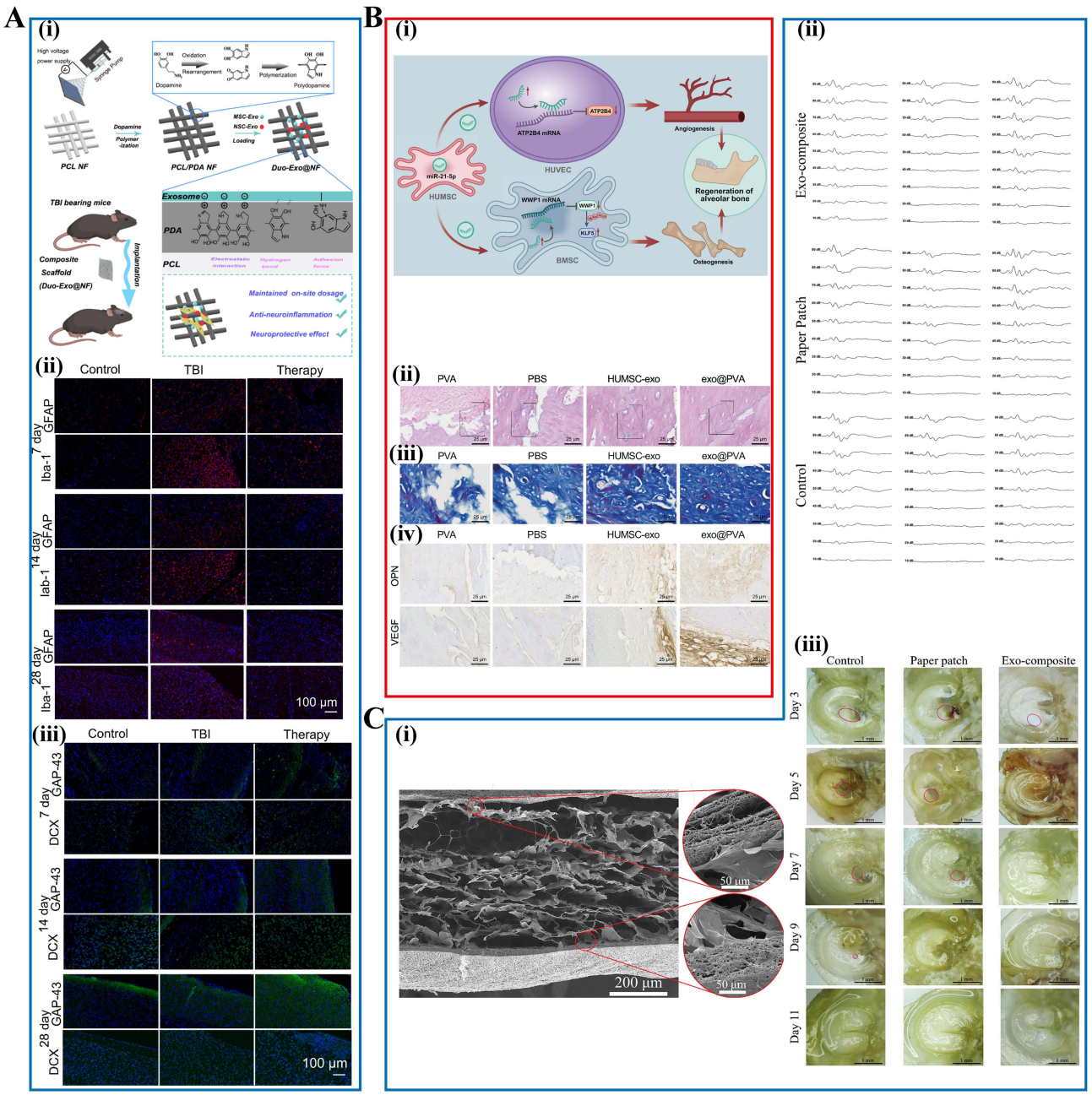


Figure 2 Optimization strategies for SC-Exos function through integration with biomaterials. (**A**) Duo-Exo@NF, integrating hUMSC- and NSC-Exos with optimized nanofibers, effectively suppresses neuroinflammation and promotes neuronal repair in TBI. Reproduced from Li JJ, Li XR, Li XY, et al. Local delivery of dual stem cell-derived exosomes using an electrospun nanofibrous platform for the treatment of traumatic brain injury. ACS Appl Mater Interfaces. 2024;16(29):37497–37512. Copyright © 2024 American Chemical Society.[144] (i) Duo-Exo@NF is fabricated by integrating hUMSC- and NSC-Exos with optimized nanofibers for synergistic TBI treatment. (ii) Immunofluorescence staining of GFAP and Iba-1 exhibits reduced activation of astrocytes and microglia with Duo-Exo@NF (hUMSC- and NSC-Exos). (iii) Immunofluorescence staining of GAP-43 and DCX demonstrates enhanced axonal regeneration and neuronal repair with Duo-Exo@NF (hUMSC- and NSC-Exos). (**B**) PVA hydrogel encapsulating hUMSC-Exos promotes alveolar bone repair by enhancing osteogenesis and angiogenesis through miR-21-5p delivery. Reproduced from He LL, Zhang HW, Zhao NB, Liao LF. A novel approach in biomedical engineering: the use of polyvinyl alcohol hydrogel encapsulating human umbilical cord mesenchymal stem cell-derived exosomes for enhanced osteogenic differentiation and angiogenesis in bone regeneration. Int J Biol Macromol. 2024;270:132116. Copyright 2024, with permission from Elsevier.[145] (i) Schematic of PVA hydrogel encapsulating hUMSC-Exos to deliver miR-21-5p, promoting osteogenesis and angiogenesis for alveolar bone defect repair. (ii) Hematoxylin & eosin (H&E) staining exhibits improved alveolar bone regeneration in rats treated with PVA hydrogel encapsulating hUMSC-Exos. Blue dashed boxes: original bone defect area; black dashed boxes: region of interest. (iii) Masson's staining highlights enhanced bone formation in rats treated with PVA hydrogel encapsulating hUMSC-Exos. (iv) Immunohistochemistry demonstrates enhanced osteogenesis and angiogenesis in the PVA hydrogel encapsulating hUMSC-Exos group. (**C**) Human ADSC-Exos composite scaffolds provide sustained exosome release, enhanced mechanical properties, rapid tympanic membrane regeneration, and hearing recovery. Reproduced from Chahsetareh H, Yazdian F, Pezeshki-Modaress M, et al. Alginate hydrogel-PCL/gelatin nanofibers composite scaffold containing mesenchymal stem cells-derived exosomes sustain release for regeneration of tympanic membrane perforation. Int J Biol Macromol. 2024;262:130141. Copyright © 2024 Elsevier B.V. All rights reserved.[148] (i) SEM image exhibits the sandwich-structured composite scaffold composed of alginate hydrogel and polycaprolactone/gelatin nanofibers. (ii) Auditory brainstem response demonstrates hearing restored to normal levels after tympanic membrane perforation repair using ADSC-Exos composite scaffolds. (iii) Surgical images exhibit complete closure of tympanic membrane perforations in rats within five days using ADSC-Exos composite scaffolds. Red circles: areas where regeneration was incomplete.

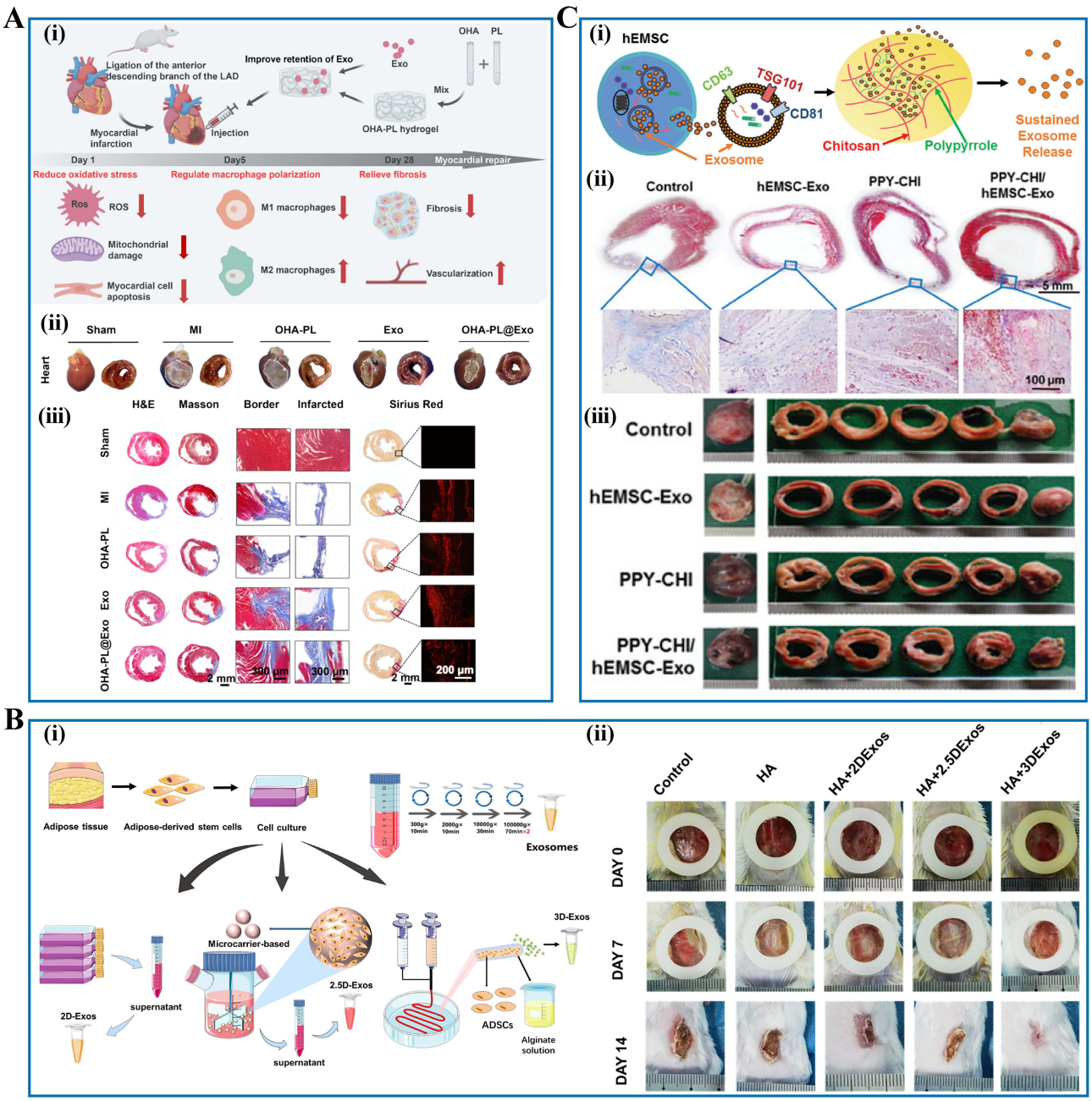


Figure 3 Optimization strategies for SC-Exos function through therapeutic efficacy enhancement. (**A**) OHA-PL hydrogel loaded with ADSC-Exos significantly improves myocardial repair by reducing fibrosis and promoting angiogenesis. Reproduced from Ren YC, Wang WT, Yu CJ, et al. An injectable exosome-loaded hyaluronic acid-polylysine hydrogel for cardiac repair via modulating oxidative stress and the inflammatory microenvironment. Int J Biol Macromol. 2024;275:133622. Copyright © 2024 Elsevier B.V. All rights reserved.[150] (i) Schematic representation of OHA-PL hydrogel-loaded ADSC-Exos for myocardial repair through modulation of oxidative stress, inflammation reduction, and tissue regeneration. (ii) Gross view of hearts and infarcted regions exhibits reduced infarct size and improved left ventricular wall thickness in the OHA-PL hydrogel-loaded ADSC-Exos group. White dashed areas: infarcted area; red dashed areas: left ventricular wall thickness of the infarcted heart cross-section. (iii) Histological staining reveals reduced fibrosis, decreased infarct size, and increased ventricular wall thickness in MI regions treated with OHA-PL hydrogel-loaded ADSC-Exos. (**B**) 3D microfiber-cultured ADSC-Exos in hyaluronic acid hydrogel accelerate burn wound healing. Reproduced from Zhu DL, Hu Y, Kong XK, et al. Enhanced burn wound healing by controlled-release 3D ADMSC-derived exosome-loaded hyaluronan hydrogel. Regen Biomater. 2024;11:rbae035. Copyright © 2024, © The Author(s) 2024. Published by Oxford University Press. license.[151] (i) Schematic of ADSC-Exos preparation via 2D, 2.5D, and 3D microfiber culture and their integration into a hyaluronic acid hydrogel for burn wound repair. (ii) Photographic images of burn wound healing over 14 days exhibit faster closure with hyaluronic acid-loaded 3D ADSC-Exos. (**C**) PPY-CHI/hEMSC-Exos hydrogel improves myocardial repair by promoting sustained exosome release, reducing fibrosis, and increasing ventricular wall thickness. Reproduced from Yan CP, Wang XZ, Wang Q, et al. A novel conductive polypyrrole-chitosan hydrogel containing human endometrial mesenchymal stem cell-derived exosomes facilitated sustained release for cardiac repair. Adv Healthcare Mat. 2024;13(10):14. © 2024 The Authors. Advanced Healthcare Materials published by Wiley-VCH GmbH. Creative Commons CC BY license.[152] (i) Schematic illustration of the PPY-CHI/hEMSC-Exos hydrogel exhibiting exosome incorporation and sustained release for myocardial repair. (ii) Masson's staining reveals a reduced fibrotic area in infarcted hearts treated with PPY-CHI/hEMSC-Exos. (iii) Morphological images of rat heart sections exhibit increased ventricular wall thickness with PPY-CHI/hEMSC-Exos treatment.

Comprehensive Biomedical Engineering Applications of SC-Exos

Tissue Engineering Applications

Therapeutic Applications in Tissue Regeneration

SC-Exos demonstrate significant therapeutic potential in various diseases through their multiple bioactive factors, emerging as a promising clinical treatment approach for skeletal muscle-tendon injuries, neural damage, cardiac dysfunction, and skin injuries (Table 3).

Table 3 Therapeutic Applications of SC-Exos in Tissue Repair and Regeneration

Application	Disease/ Condition	SC-Exos Type	Model	Key Mechanisms	Primary Outcomes	Reference
Skeletal	Osteoarthritis	ADSC-Exos	Mouse lumbar facet joint osteoarthritis	Coupled subchondral bone remodeling; H-type vessel formation regulation; Nociceptor nerve fiber reduction	Cartilage protection; Bone preservation; Pain reduction	[153]
	Rotator cuff tendon-bone healing	BMSC-Exos	Rat rotator cuff injury	VEGF/Hippo pathway activation; Angiogenesis promotion; M1 macrophage inhibition	Enhanced tendon-bone healing; Increased breaking load and stiffness	[68]
	Bone defect repair	hUMSC-Exos	Rat cranial defect	miR-21-mediated NOTCH1/DLL4 inhibition; VEGFA and HIF-1α upregulation	Enhanced bone regeneration and angiogenesis	[154]
Neural	Diabetic neuropathy	BMSC-Exos	Rat diabetic sciatic nerve injury	MEK/ERK pathway activation; M2 macrophage polarization via NF-κB	Enhanced axonal regeneration; Pain relief; Locomotor function restoration	[155]
	Peripheral nerve injury	Dental pulp stem cell-derived Exosomes	Rat sciatic nerve injury	miR-122-5p delivery; P53 suppression; Autophagy inhibition via miR-122-5p/P53 pathway	Myelin sheath regeneration; Enhanced Schwann cell function; Improved nerve fiber arrangement	[156]
	Optic nerve injury	hUMSC-Exos	Mouse optic nerve crush model	miR-222-3p and miR-22-3p delivery; mTORC1 activation	Axon regeneration; RGC survival; Visual electrophysiological recovery	[157]
Cardiac	Myocardial infarction	ESC-Exos	Mouse myocardial infarction	miR-294 delivery; CPC activation; Neovascularization; Cardiomyocyte proliferation	Enhanced cardiac function; Reduced infarct size; Angiogenesis	[98]
	Hypoxic cardiomyocyte injury	BMSC-Exos	Hypoxic H9C2 cardiomyocytes (rat, in vitro)	miR-144 delivery; PTEN inhibition; AKT pathway activation	Reduced cardiomyocyte apoptosis; Enhanced cardiomyocyte survival	[158]
	Myocardial infarction	iPSC-EC-Exos	Mouse myocardial infarction	miR-100-5p delivery; PP-1β targeting; PLB phosphorylation; Ca2+ homeostasis	Improved cardiac function; Enhanced cardiomyocyte survival; Ca^{2+} homeostasis	[76]
Skin	Atopic dermatitis	iPSC-derived mesenchymal stem cell exosomes	Mouse atopic dermatitis	IDO upregulation; TSLP/IL-25/IL-33 suppression; KRT1/KRT10/DSG1/CerS3 enhancement; T cell suppression	Improved skin lesions; Reduced TEWL; Restored skin barrier	[77]
	Diabetic wound healing	Epidermal stem cell-derived exosomes	Mouse diabetic wound	Functional miRNA delivery; M2 macrophage polarization; TGFβ and PI3K/AKT pathways	Accelerated wound closure; Enhanced fibroblast and macrophage proliferation; Angiogenesis; Reduced inflammation	[159]
	Wound healing and scar prevention	hUCBMSC-Exos	Rat skin wound	miR-21-5p/miR-125b-5p delivery; TGFBR1/TGFBR2 inhibition; Myofibroblast suppression; α-SMA and collagen I reduction	Accelerated wound closure; Reduced scar formation; Improved appendage/nerve/vessel regeneration	[160]

Note: Bold formatting represents table column headers and classification categories.

Abbreviations: ADSC-Exos, Adipose-derived mesenchymal stem cell-derived exosomes; AKT, Protein kinase B; BMSC-Exos, Bone marrow mesenchymal stem cell-derived exosomes; Ca^{2+}, Calcium ion; CerS3, Ceramide synthase 3; CPC, Cardiac progenitor cells; DLL4, Delta-like ligand 4; DSG1, Desmoglein 1; ERK, Extracellular signal-regulated kinase; ESC-Exos, Embryonic stem cell-derived exosomes; HIF-1α, Hypoxia-inducible factor 1-alpha; hUCBMSC-Exos, Human umbilical cord blood mesenchymal stem cell-derived exosomes; Hippo, Hippo signaling pathway; hUMSC-Exos, Human umbilical mesenchymal stem cell-derived exosomes; IDO, Indoleamine 2,3-dioxygenase; IL-25, Interleukin-25; IL-33, Interleukin-33; iPSC, Induced pluripotent stem cell; iPSC-EC-Exos, Induced pluripotent stem cell-derived endothelial cell exosomes; KRT1, Keratin 1; KRT10, Keratin 10; MEK, Mitogen-activated protein kinase kinase; miR, microRNA; mTORC1, Mechanistic target of rapamycin complex 1; NF-κB, Nuclear factor kappa B; NOTCH1, Notch receptor 1; P53, Tumor protein p53; PI3K, Phosphoinositide 3-kinase; PLB, Phospholamban; PP-1β, Protein phosphatase 1β; PTEN, Phosphatase and tensin homolog; RGC, Retinal ganglion cell; SC-Exos, Stem cell-derived exosomes; TEWL, Transepidermal water loss; TGFβ, Transforming growth factor beta; TGFBR1, Transforming growth factor beta receptor 1; TGFBR2, Transforming growth factor beta receptor 2; TSLP, Thymic stromal lymphopoietin; VEGF, Vascular endothelial growth factor; VEGFA: Vascular endothelial growth factor A; α-SMA: α-smooth muscle actin.

Research indicates that ADSC-Exos obtained from hypoxia-preconditioned ADSCs can effectively alleviate lumbar facet osteoarthritis by protecting cartilage from degeneration, improving subchondral bone integrity, and reducing spinal pain.[153] Besides, Yang et al developed a conductive hydrogel neural dressing loaded with BMSC-Exos that alleviates inflammatory pain from diabetic peripheral nerve injury by modulating M2 macrophage polarization through the nuclear factor-κB (NF-κB) pathway. The dressing also promotes myelin axon regeneration via the mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway, improves denervation muscle atrophy, and ultimately achieves neural regeneration and functional recovery.[155] In cardiac function improvement, human ADSC-Exos significantly downregulate factors inhibiting HIF-1 through miR-31 delivery, inducing HIF-1α nuclear translocation and transcriptional activity. This mechanism effectively promotes mouse cardiac vascular repair and significantly alleviates MI symptoms.[161] In skin repair, ADSC-Exos from hypoxia-preconditioned ADSCs accelerate high-quality diabetic wound healing by activating the PI3K/AKT signaling pathway, promoting fibroblast proliferation, migration, and collagen metabolism, while regulating multiple growth factors, including TGF-β and vascular endothelial growth factor. This approach suppresses inflammation and enhances angiogenesis, offering a potential cell-free therapeutic strategy for chronic diabetic wounds.[162]

Integration with Biomaterial Scaffolds

Although exosomes have immense potential in regenerative medicine, their limited bioavailability and lack of controlled-release mechanisms in the free state restrict their practical applications. Integrating exosomes with biomaterials (such as hydrogels) to create exosome-loaded scaffolds has emerged as an effective solution, further advancing their application in specific disease repair.[163] The key to developing exosome-loaded scaffold materials is achieving efficient retention and sustained release at implantation sites while maximizing their biological activity. Various materials and their structural forms have been extensively studied to provide stable and safe in vivo delivery platforms. Current composite techniques primarily include chemical crosslinking, physical adsorption, and 3D printing.[164] Research has demonstrated that loading ADSC-Exos onto $CaCl_2$-crosslinked alginate hydrogels creates bioactive scaffold materials that significantly accelerate wound healing in rat full-thickness skin wound models, promoting collagen synthesis, angiogenesis, and re-epithelialization while reducing scar formation.[165] Similarly, Guan et al developed a composite hydrogel scaffold based on gelatin methacryloyl (GM) and aldehyde-functionalized chondroitin sulfate (OCS) by loading BMSC-Exos into extracellular matrix-mimicking hydrogels formed through crosslinking of OCS with GM. This scaffold system achieves controlled exosome release while modulating the local immune microenvironment, significantly promoting cartilage extracellular matrix synthesis and enhancing growth plate injury repair.[166] Fan et al developed a loading scaffold based on a double-network conductive hydrogel (GM/PPy), achieving effective exosome retention and controlled release through noncovalent hydrogen bonding, maintaining biological activity while suppressing inflammation, promoting neural stem cells (NSCs) recruitment, and regenerating neurons and myelin-related axons, ultimately significantly improving SCI repair by activating the phosphatase and tensin homolog (PTEN)/PI3K/AKT/mammalian target of rapamycin (mTOR) pathway.[167] Exosome-incorporated bone engineering scaffolds have exhibited potential for bone defect treatment, providing mechanical support, osteoconduction, osteoinduction, and osteogenic capabilities.[168] Research on bone engineering scaffolds primarily focuses on bioactive factor composition, characteristics, interface modification, and release control. Studies have demonstrated that human ADSC-Exos combined with mineral-doped polylactic acid porous scaffolds significantly enhance ADSCs' osteogenic differentiation and bone regeneration potential, with high mineral content scaffolds improving gene expression and bone tissue regeneration environment through active exosome release, providing a stable and efficient controlled delivery system for bone repair.[169] Recently, 3D printing has emerged as an advanced manufacturing technology that precisely controls exosome activity and structure through layer-by-layer construction or intelligent surface design, supporting 3D active tissue and organ generation.[170] For instance, reports have exhibited that ADSC-Exos functionalized with 3D-printed porous scaffold technology demonstrates significant advantages in regenerative medicine, enhancing cell communication and targeted delivery of key molecules to improve the efficiency of wound healing, bone tissue regeneration, and neural repair.[143,151]

Overall, exosome composite scaffold preparation techniques have gradually matured, with different composite scaffolds offering distinct advantages while maintaining appropriate porosity, good biocompatibility, and sustained exosome release capabilities that maximize their activity.[164]

SC-Exos for Therapeutic Drug Delivery

Cell-to-cell communication is vital for the development and homeostasis maintenance of multicellular organisms. Currently, common drug delivery carriers include liposomes and polymeric nanoparticles. However, liposomes exhibit significant limitations in long-term circulation ability, stability, and immune system evasion.[171] Although polymeric nanoparticles offer better stability than liposomes, they face challenges regarding biocompatibility and long-term safety.[172] Contrarily, exosomes demonstrate significant advantages as drug delivery carriers, particularly in biocompatibility and delivery efficiency.[173] MSC-Exos have garnered particular attention due to their small size, low immunogenicity, long circulation half-life, strong tissue penetration, and excellent biocompatibility, becoming a focal point in drug delivery research.[174]

Through engineering modifications such as cargo loading and surface functionalization, SC-Exos can be transformed into excellent drug carriers with multiple therapeutic properties, making them ideal drug-delivery matrices. Research demonstrates that a novel drug delivery system based on ADSC-Exos loaded with icariin effectively alleviates rheumatoid arthritis inflammation and cartilage destruction by inhibiting macrophage glycolysis through the ERK1/2/HIF-1α/glucose transporter 1 signaling pathway. This system modulates macrophage transformation from an M1 pro-inflammatory to an M2 anti-inflammatory phenotype, improves bone microstructure, and exhibits good safety profiles, providing an innovative strategy for rheumatoid arthritis treatment.[175] Another study developed a dual-drug delivery platform by loading galectin-9 siRNA into BMSC-Exos through electroporation and surface modification with oxaliplatin prodrug, achieving immunosuppression reversal and enhanced immunogenic cell death while significantly improving the pancreatic ductal adenocarcinoma tumor microenvironment and immunotherapy efficacy, demonstrating excellent targeting ability and biosafety.[176]

Engineering Strategies for Enhanced SC-Exos Targeting

As carriers of intercellular communication, exosomes naturally transport endogenous substances including proteins, mRNA, miRNA, and lipids to recipient cells.[177] Through engineering modifications, they can be loaded with exogenous drugs, therapeutic nucleic acids, and other bioactive molecules, leading to their widespread application in drug delivery.[178] However, natural exosomes face challenges in clinical applications due to poor targeting and rapid in vivo clearance, limiting their therapeutic effects. Consequently, researchers typically modify exosomes to enhance their targeting and therapeutic effects by creating engineered exosomes.[179] Engineering modifications of SC-Exos have been proven to significantly improve their yield, purity, targeting ability, and drug delivery efficiency, thereby enhancing therapeutic outcomes.[36,180]

Cargo loading in exosomes refers to the active or passive introduction of exogenous drug molecules into exosomes.[181] Electroporation, a common passive loading technique, creates temporary pores in the exosome membrane through electric field application, allowing drug molecule diffusion followed by membrane integrity restoration.[36] For example, research using electroporation to load miR-21-5p into human ADSC-Exos created an engineered exosome delivery platform that significantly promotes keratinocyte proliferation and migration through Wnt/β-catenin signaling pathway activation, accelerating epithelial regeneration, collagen reconstruction, angiogenesis, and maturation in diabetic rat wounds, providing an innovative cell-free transplantation strategy for diabetic wound healing.[182] In exosome engineering, surface modification is a key measure for enhancing targeting capability, primarily achieved through genetic engineering and chemical modification strategies.[181,183] Genetic engineering enhances exosome recognition of target cells by fusing specific guidance proteins or peptide gene sequences with exosome membrane protein genes, enabling more precise targeted therapy.[36] For instance, by employing genetic engineering techniques, researchers successfully conducted surface modification treatment on hUMSC-Exos by fusing the activated hepatic stellate cell-targeting peptide HSTP1 with exosome membrane protein Lamp2b and adding the glycosylation motif GNSTM at the N-terminus, conferring a high specificity targeting ability to activated hepatic stellate cells and significantly enhancing their

therapeutic effect on liver fibrosis.[184] Besides genetic engineering, chemical modification is another vital method for exosome modification, characterized by loading functional molecules onto exosome surfaces through noncovalent or covalent binding to confer targeting ability.[36] Research has demonstrated that constructing engineered exosomes enriched with netrin-1 by transfecting BMSCs with chemically modified messenger RNA encoding netrin-1 significantly reduces inflammation and pyroptosis in SCI while promoting axon regeneration through activating the uncoordinated-5b/PI3K/AKT/mTOR pathway, providing a novel strategy for SCI treatment.[185] Although chemical modification offers advantages such as rapid reaction, high specificity, and compatibility with aqueous environments, enabling various molecular loadings onto exosome surfaces through noncovalent or covalent interactions, research in this field remains relatively limited.[186,187] Membrane fusion technology is another essential approach for SC-Exos-targeting modification. Research has found that modifying rat BMSC-Exos with monocyte mimetics through membrane fusion significantly enhances their targeting ability to myocardial ischemia-reperfusion (I/R) regions. These biomimetic exosomes improve retention in damaged myocardium through interactions with key molecules, such as macrophage receptor 1/lymphocyte function-associated antigen-1, thereby promoting angiogenesis, regulating macrophage subgroup balance, and significantly improving cardiac function and tissue repair.[188]

SC-Exos as Advanced Drug Delivery Systems

SC-Exos as drug delivery carriers have gained increasing attention and validation for improving disease treatment and diagnostic efficiency. miR-29b plays essential roles by targeting extracellular matrix-related genes and key signaling pathways, such as C-X3-C motif chemokine ligand 1, transforming growth factor β receptor I, and Smad2/3, illustrating its significant effects, particularly in preventing and inhibiting fibrosis across various tissues and organs.[189–191] Liu et al's research demonstrates that BMSC-Exos delivering miRNA-29b-3p significantly reduces corneal injury-induced inflammation and fibrosis by activating autophagy through inhibition of the PI3K/AKT/mTOR signaling pathway while further reducing inflammatory responses through suppression of the NF-κB/interleukin-1β (IL-1β) pathway, thereby promoting corneal repair.[192] Additionally, the multifaceted influence of MSC-Exos on tumor development and strong tumor tropism make them promising platforms for antitumor drug delivery.[193] Research demonstrates that miR-99b-5p carried by human BMSC-Exos effectively inhibits colorectal cancer cell proliferation, migration, and invasion by targeting fibroblast growth factor receptor 3, thereby delaying tumor progression. At the same time, its plasma expression levels also exhibit potential as diagnostic markers for colorectal cancer.[194] Furthermore, MSC-Exos applications in immune system diseases have been extensively studied. Research indicates that MSC-Exos can suppress M1 macrophage polarization in systemic lupus erythematosus (SLE) by delivering the tRNA-derived fragment tsRNA-21109, reducing pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) and IL-1β expression levels while promoting M2 macrophage marker expression, thus playing key roles in inflammation regulation and immune balance, demonstrating its potential as a novel therapeutic strategy for SLE.[195]

Applications of SC-Exos in Disease Treatment

Applications in Cardiovascular Disease

SC-Exos demonstrates significant therapeutic potential in cardiovascular diseases, particularly in MI, myocardial I/R injury, cardiac remodeling, fibrosis treatment, and post-surgical applications. Recent studies have demonstrated that SC-Exos functions through multiple mechanisms, including anti-apoptotic, anti-inflammatory, and anti-fibrotic effects, promoting angiogenesis and regulating intracellular signaling pathways and metabolic states, significantly improving pathophysiological conditions following myocardial injury.

Studies reveal that iPSC-derived endothelial cell exosomes (iPSC-EC-Exos) enhance cardiac function by carrying miR-100-5p protein phosphatase 1β and promoting phospholamban phosphorylation. This mechanism enhances sarcoplasmic reticulum calcium ATPase 2a and ryanodine receptor-2 functions, maintaining cardiomyocyte calcium homeostasis, thereby improving energy metabolism, inhibiting apoptosis, and promoting cardiac function recovery. Animal models demonstrate significantly reduced infarct size and improved ventricular remodeling without increased arrhythmia risk (Figure 4A).[76] Furthermore, exosomes from iPSC-differentiated cardiomyocytes have been exhibited to regulate autophagy-related signaling pathways, such as inhibiting mTOR signaling and promoting autophagy flux, significantly

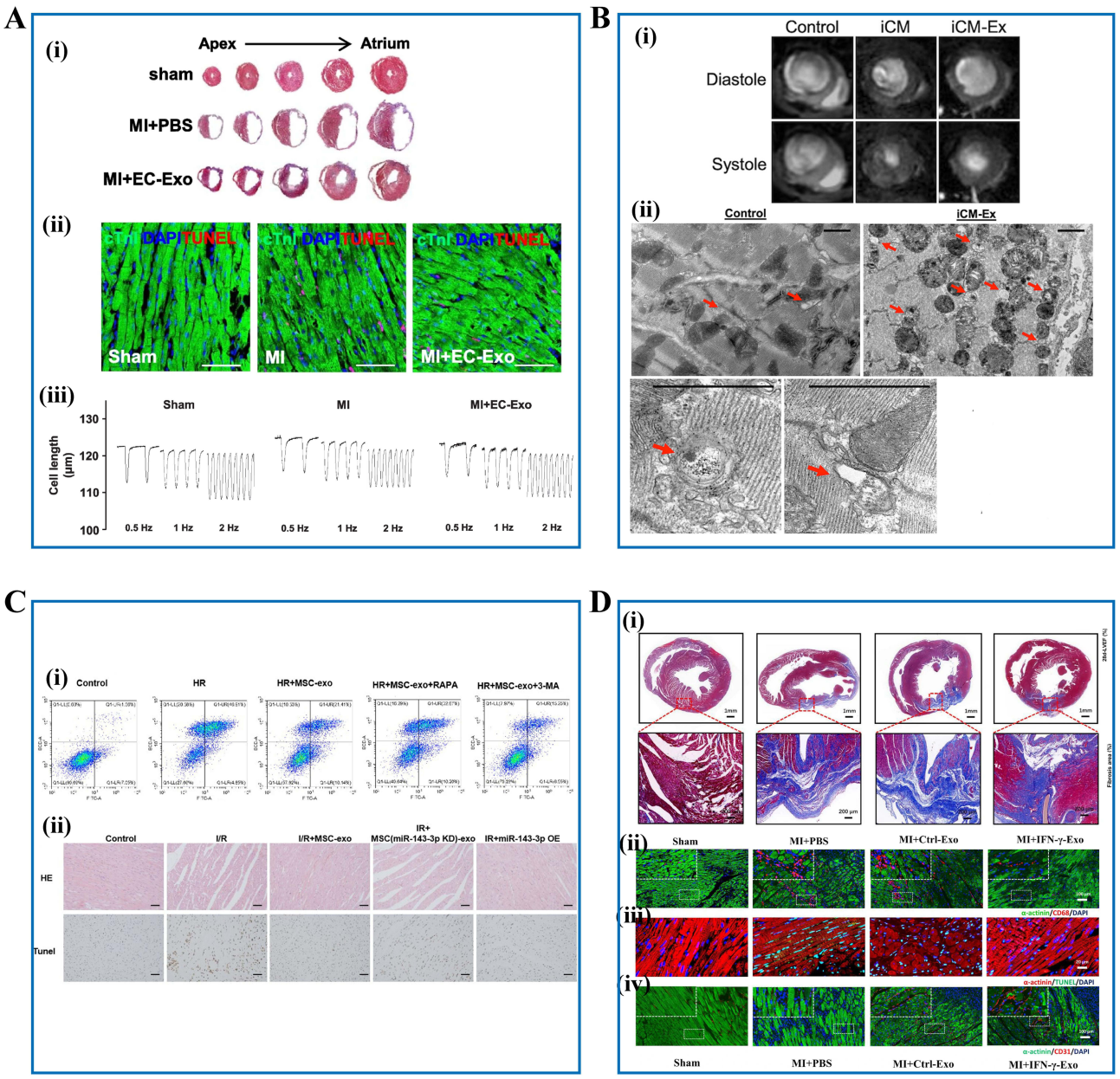


Figure 4 Therapeutic applications of SC-Exos in cardiovascular disease. (**A**) Therapeutic effects of iPSC-EC-Exos on infarct size, apoptosis, and cardiomyocyte contractility post-
MI. Reproduced from Li H, Wang L, Ma T, Liu ZM, Gao L. Exosomes secreted by endothelial cells derived from human induced pluripotent stem cells improve recovery from myocardial infarction in mice. Stem Cell Res Ther. 2023;14(1):278. © 2023. BioMed Central Ltd., part of Springer Nature.[76] (i) Masson's staining exhibits reduced infarct size 28 days post-MI with iPSC-EC-Exos treatment. (ii) TUNEL assay indicates reduced cardiomyocyte apoptosis in the infarct border zone three days post-MI with iPSC-EC-Exos treatment. (iii) Cell shortening traces exhibit improved cardiomyocyte contractility after iPSC-EC-Exos treatment. (**B**) Exosomes derived from iPSC-differentiated cardiomyocytes enhance autophagy and protect cardiac function post-
MI. Reproduced from Santoso MR, Ikeda G, Tada Y, et al. Exosomes from induced pluripotent stem cell-derived cardiomyocytes promote autophagy for myocardial repair. J Am Heart Assoc. 2020;9(6):e014345. © 2024 The Author(s). Journal of the American Heart Association is published by Wiley Periodicals LLC on behalf of the American Heart Association, Inc. Creative Commons CC-BY-NC-ND license.[196] (i) MRI images demonstrate preserved left ventricular function with exosomes derived from iPSC-differentiated cardiomyocytes treatment. (ii) TEM images reveal increased autophagosomes in peri-infarct regions with exosomes derived from iPSC-differentiated cardiomyocytes treatment. Red arrows: autophagosomes. (**C**) BMSC-Exos protects against myocardial I/R injury by reducing apoptosis and improving myocardial structure. Reproduced from Chen GC, Wang MX, Ruan ZB, Zhu L, Tang CC. Mesenchymal stem cell-derived exosomal miR-143-3p suppresses myocardial ischemia-reperfusion injury by regulating autophagy. Life Sci. 2021;280:119742. Copyright © 2021 Elsevier Inc. All rights reserved.[197] (i) Flow cytometry exhibits BMSC-Exos reduce hypoxia/reoxygenation-induced apoptosis. (ii) H&E staining and TUNEL assay demonstrate improved myocardial structure and reduced apoptosis with BMSC-Exos, regulated by miR-143-3p. (**D**) hUMSC-Exos activated by IFN-γ enhances cardiac repair by reducing inflammation, apoptosis, and fibrosis while promoting angiogenesis. Reproduced from Zhang J, Lu Y, Mao YM, et al. IFN-γ enhances the efficacy of mesenchymal stromal cell-derived exosomes via miR-21 in myocardial infarction rats. Stem Cell Res Ther. 2022;13(1):333. © 2022. The Author(s). Creative Commons Attribution 4.0 International License.[199] (i) Masson's staining demonstrates reduced fibrosis with hUMSC-Exos treatment. (ii) CD68 immunofluorescence staining displays reduced inflammation with hUMSC-Exos treatment. (iii) TUNEL assay presents fewer apoptotic cells after hUMSC-Exos treatment. (iv) CD31 immunofluorescence staining exhibits enhanced angiogenesis with hUMSC-Exos treatment.

improving cardiomyocyte survival, reducing apoptosis and fibrosis, and enhancing left ventricular ejection fraction and myocardial viability in MI models, providing a safe, effective, and clinically translatable cell-free therapeutic approach (Figure 4B).[196] In myocardial I/R injury models, studies found that BMSC-Exos regulate the CHK2-beclin1 signaling pathway through their carried miR-143-3p, significantly inhibiting excessive autophagy and apoptosis of cardiomyocytes, thereby improving myocardial function (Figure 4C).[197] Besides, researchers developed a targeted delivery system (DSPE-PEG-CMP-miR302-EXO) using mouse BMSC-Exos with cardiac myocyte-specific peptide (CMP) and loaded with miR302, effectively improving myocardial I/R injury through promoting cardiomyocyte proliferation, inhibiting inflammation and apoptosis, and activating the Hippo pathway yap signaling.[198] Notably, while these anti-apoptotic effects show consistency across different animal models, most BMSC-Exos cardiac studies focus on acute injury models, and chronic cardiac injury research remains an important area for future exploration.

In cardiac remodeling and fibrosis treatment, hUMSC-Exos activated by interferon-γ (IFN-γ) regulates miR-21 expression through signal transducer and activator of transcription 1, inhibiting BTG anti-proliferation factor 2 while promoting angiogenesis, anti-apoptotic, and anti-inflammatory effects, significantly improving cardiac function and fibrosis in MI models, demonstrating superior therapeutic potential compared to untreated MSC-Exos (Figure 4D).[199] In addition, another study demonstrated that hUMSC-Exos modified with macrophage migration inhibitory factor gene, by delivering miR-133a-3p to activate the AKT signaling pathway, significantly reduced the myocardial fibrosis area and improved cardiac function while promoting angiogenesis and inhibiting cardiomyocyte apoptosis.[200] Moreover, injectable photocurable Janus hydrogels carrying iPSC-differentiated cardiomyocyte exosomes demonstrate the dual functions of anti-oxidation and anti-pericardial adhesion, effectively reducing post-cardiac surgery tissue adhesion risk while improving exosome retention time and stability.[201]

Collectively, these cardiovascular applications demonstrate the methodological diversity of SC-Exos as engineered therapeutic platforms. The studies reveal distinct approaches through which molecular engineering via targeted miRNA cargo loading enables precise pathway modulation, exemplified by miR-100-5p-mediated calcium homeostasis regulation and miR-143-3p-controlled autophagy inhibition. Surface functionalization strategies, particularly peptide-mediated targeting systems such as CMP modification, represent significant advances in tissue-specific delivery. Integration with advanced biomaterial platforms, including photocurable hydrogel systems, further addresses critical challenges of exosome stability and controlled release. This methodological diversity illustrates the field's multifaceted approach toward precision-engineered solutions that harness the inherent biocompatibility of SC-Exos while incorporating sophisticated targeting and delivery mechanisms.

Applications in Neurological Diseases

SC-Exos demonstrate multiple therapeutic potential in neurological diseases, including reducing inflammatory responses, promoting neuroregeneration, and improving mitochondrial function. Recent research on stroke, brain trauma, TBI, SCI, and other neurodegenerative diseases has provided novel insights into the clinical application of SC-Exos.

Stroke is a major cause of long-term neurological dysfunction.[202] Studies indicate that MSC-Exos can improve neurological function recovery by delivering specific miRNAs. For instance, hUMSC-Exos modified with superparamagnetic iron oxide nanoparticles (Spion) under magnetic field (MF) guidance form a Spion-exosome system (Spion-Ex /MF), enhancing blood-brain barrier (BBB) penetration and brain tissue targeting capabilities. This system delivers miR-1228-5p to tumor necrosis factor receptor-associated factor 6 (TRAF6). It activates the TRAF6-NADPH oxidase 1 pathway, thereby improving neuronal mitochondrial function, oxidative stress, and cognitive deficits in middle cerebral artery occlusion (MCAO) mouse models (Figure 5A).[203] Additionally, ADSC-Exos effectively regulate the farnesoid X receptor-2/activating transcription factor 3/solute carrier family 7 member 11 pathway, inhibiting M2 microglial ferroptosis and ameliorating the inflammatory microenvironment in cerebral I/R, thereby promoting neuronal survival and functional recovery.[204] Mouse BMSC-Exos carrying highly expressed miR-132-3p effectively protect endothelial cells during I/R by modulating the Ras p21 protein activator 1/Ras/PI3K/AKT/endothelial nitric oxide synthase signaling pathway, reducing oxidative stress and apoptosis while maintaining tight junction integrity, thereby significantly improving cerebral ischemic injury.[205] In TBI research, SC-Exos demonstrates therapeutic potential through neuroregeneration promotion and inflammation reduction. For example, BMSC-Exos combined with degradable hyaluronic acid

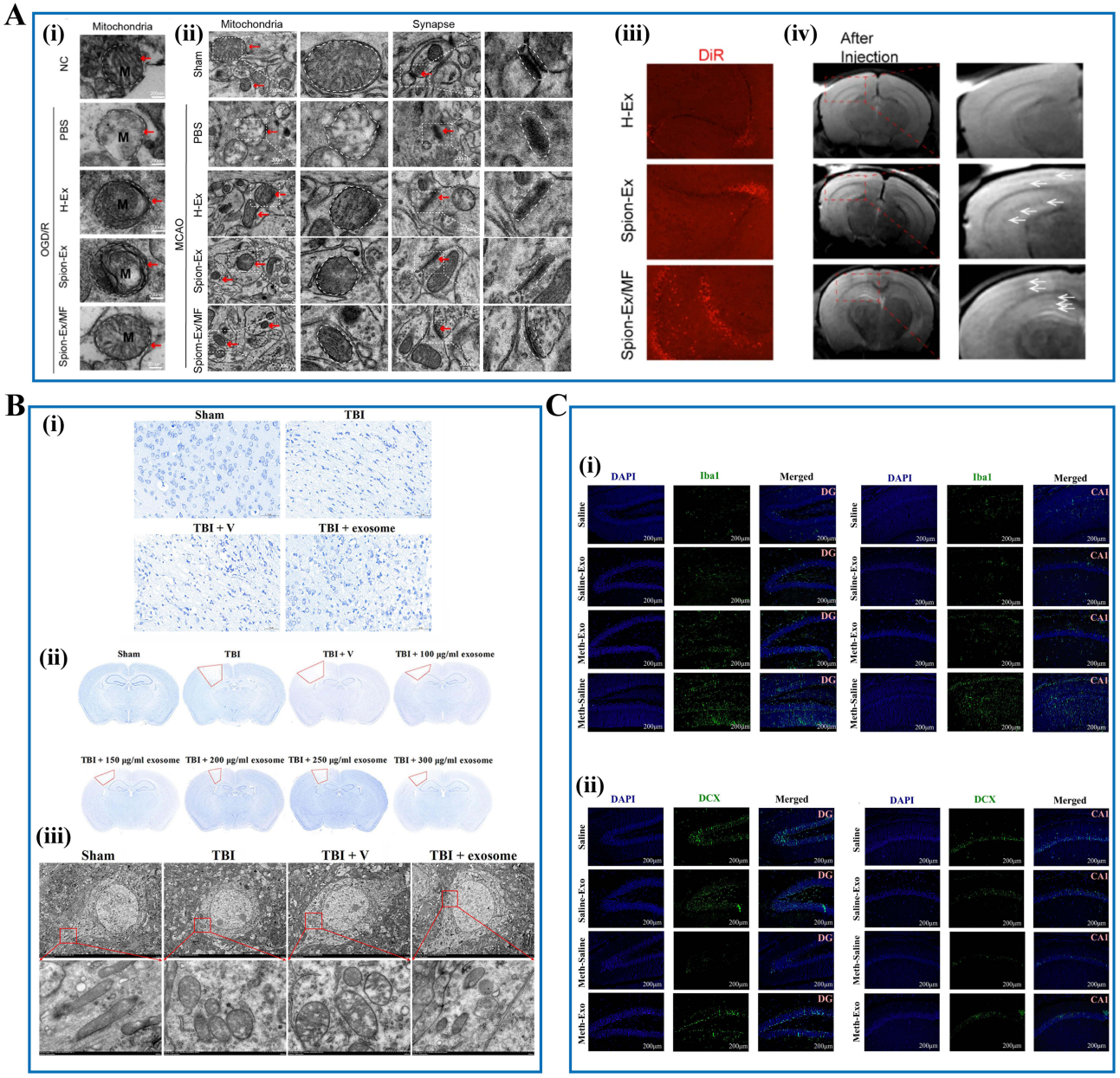


Figure 5 Therapeutic applications of SC-Exos in neurological diseases. (**A**) Spion-Ex/MF enhances brain targeting and restores mitochondrial and synaptic function in ischemic regions. Reproduced from Hu WJ, Wei H, Cai LL, et al, Magnetic targeting enhances the neuroprotective function of human mesenchymal stem cell-derived iron oxide exosomes by delivering miR-1228-5p. J Nanobiotechnol. 2024;22(1):665. Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.[203] (i) TEM demonstrates that Spion-Ex/MF reverses mitochondrial swelling and restores cristae. M: mitochondria; red arrows: mitochondrial membranes. (ii) TEM demonstrates restored mitochondrial integrity and improved synaptic structure in the hippocampal neurons of MCAO mice treated with Spion-Ex/MF. Red arrows: mitochondrial structures (in mitochondria images) and synaptic structures (in synapse images). (iii) DiR fluorescence imaging confirms Spion-Ex/MF targets ischemic brain regions. (iv) T2-weighted MRI exhibits enhanced Spion-Ex/MF accumulation in the ischemic regions. White arrows: hypointense areas indicating labeled Spion-Ex. (**B**) hUMSC-Exos reduce brain injury, neuronal damage, and mitochondrial dysfunction in TBI. Reproduced from Zhang L, Lin YX, Bai WS, Sun L, Tian M. Human umbilical cord mesenchymal stem cell-derived exosome suppresses programmed cell death in traumatic brain injury via PINK1/Parkin-mediated mitophagy. CNS Neurosci Ther. 2023;29 (8):2236–2258. © 2023 The Authors. CNS Neuroscience & Therapeutics published by John Wiley & Sons Ltd. Creative Commons CC BY license.[207] (i) Nissl staining demonstrates that hUMSC-Exos treatment reduces neuronal damage and preserves structural integrity. (ii) hUMSC-Exos significantly reduce brain tissue loss induced by TBI, demonstrating their neuroprotective effects. Red shapes: brain tissue loss areas. (iii) TEM images reveal that hUMSC-Exos alleviate TBI-induced mitochondrial damage, restoring mitochondrial morphology to normal. (**C**) BMSC-Exos improve memory, boosts neurogenesis, and reduces neuroinflammation in methamphetamine-induced neurotoxicity. Reproduced from Fallahi S, Zangbar HS, Farajdokht F, Rahbarghazi R, Ghiasi F, Mohaddes G. Mesenchymal stem cell-derived exosomes improve neurogenesis and cognitive function of mice with methamphetamine addiction: a novel treatment approach. CNS Neurosci Ther. 2024;30(5):e14719. © 2024 The Author(s). CNS Neuroscience & Therapeutics published by John Wiley & Sons Ltd. Creative Commons CC BY license.[211] (i) Immunofluorescence staining exhibits that BMSC-Exos reduce Iba-1 expression in the hippocampal dentate gyrus, suppressing neuroinflammation. (ii) Immunofluorescence staining demonstrates that BMSC-Exos increase DCX expression in the hippocampal dentate gyrus, enhancing neurogenesis.

collagen hydrogel provides a sustained neurogenic microenvironment at TBI sites, accelerating neurological function recovery through enhanced NSCs differentiation, vascularization, and brain structure remodeling.[206] Furthermore, hUMSC-Exos significantly suppress cellular apoptosis, pyroptosis, and ferroptosis in TBI models by activating PTEN-induced putative kinase 1/parkin ubiquitin ligase (Parkin)-mediated mitophagy while improving neurological function and reducing brain edema (Figure 5B).[207] In SCI treatment, SC-Exos exhibit significant neuroprotective and regenerative effects. Studies of genetically modified RGD-$CD146^{+}CD271^{+}$ hUMSC-Exos demonstrate specific targeting of neovascular endothelial cells through miR-501-5p/myosin light chain kinase axis regulation, significantly stabilizing the blood-spinal cord barrier, promoting angiogenesis, reducing inflammation, and enhancing axonal regeneration, thereby effectively improving neurological recovery in SCI mice.[208] Moreover, hUMSC-Exos combined with fibrin glue significantly promotes neurological repair, functional recovery, and urinary tract protection in acute SCI by optimizing the injury microenvironment through attenuating inflammation and oxidative stress.[209] Furthermore, SC-Exos exhibit promising progress in the treatment of Alzheimer's disease (AD) and drug addiction. hUMSC-Exos attenuate neuronal apoptosis in AD cell models through miR-223-mediated regulation of the PTEN-PI3K/AKT signaling pathway.[210] In methamphetamine addiction mouse models, BMSC-Exos effectively improve cognitive function and spatial memory by enhancing neurogenesis, suppressing neuroinflammation, and upregulating hippocampal doublecortin (DCX) and neuronal nuclei expression levels, thus providing a novel potential approach for methamphetamine neurotoxicity treatment (Figure 5C).[211]

Applications in Cancer Treatment

SC-Exos demonstrate significant potential for cancer treatment. These exosomes possess low immunogenicity, strong targeting capability, and efficient biological barrier penetration, enabling their use as drug delivery platforms and gene regulation tools, exhibiting promising therapeutic effects in various cancer models.

Studies have found that BMSC-Exos can regulate tumor signaling pathways through specific miRNA delivery to inhibit tumor growth and metastasis. For instance, BMSC-Exos significantly suppress non-small cell lung cancer proliferation and invasion by transporting miR-30b-5p to the target enhancer of Zeste Homolog 2 and inhibiting the PI3K/AKT signaling pathway while promoting tumor cell apoptosis and preventing tumor formation.[212] Additionally, human BMSC-Exos enriched with miR-29b significantly inhibits peritoneal metastasis formation in gastric cancer models by suppressing mesothelial-mesenchymal transition of peritoneal mesothelial cells, reducing cancer cell adhesion to mesothelial cells and inhibiting the expression of related migration and adhesion factors (Figure 6A).[213] SC-Exos exhibits superior targeting and efficacy in drug delivery applications. The loading of the chemotherapy drug doxorubicin (DOX) into mouse BMSC-Exos through electroporation and modifying their surface with mucin 1 aptamer achieves targeted delivery to colorectal cancer cells, significantly enhancing antitumor activity and targeting while reducing systemic toxicity.[214] Furthermore, mouse BMSC-Exos as nano drug carriers achieve targeted DOX delivery through the SDF1-CXCR4 axis for osteosarcoma treatment, significantly enhancing antitumor activity, inhibiting migration and proliferation while reducing cardiac toxicity (Figure 6B).[215]

In glioblastoma (GBM) treatment, research has verified that ESC-Exos can penetrate the BBB and inhibit GBM cell proliferation and survival, and engineered exosomes, specifically cyclic peptide c(RGDyK)-modified ESC-Exos loaded with Paclitaxel (cRGD-Exo-PTX), significantly improve drug targeting and antitumor efficacy while revealing that the reprogramming properties of ESC-Exos may be key to their antitumor effects (Figure 6C).[216] Furthermore, BMSC-Exos have a broad potential for breast cancer treatment. After DOX treatment, BMSC-Exos significantly enhance drug resistance by transporting miR-21-5p to upregulate resistance gene S100A6 expression in breast cancer cells while inhibiting exosomal miR-21-5p can reverse this resistance, providing new strategies for understanding tumor microenvironment-induced chemotherapy resistance mechanisms and overcoming related resistance.[217] Meanwhile, hUMSC-Exos loaded with miR-3182 significantly inhibits proliferation, migration, and invasion in triple-negative breast cancer models while inducing apoptosis by downregulating mTOR and ribosomal protein S6 kinase β-1 genes.[218]

In conclusion, SC-Exos applications in cancer treatment encompass multiple aspects, including gene regulation, drug delivery, and tumor microenvironment remodeling. These research findings reveal their potential in cancer treatment and

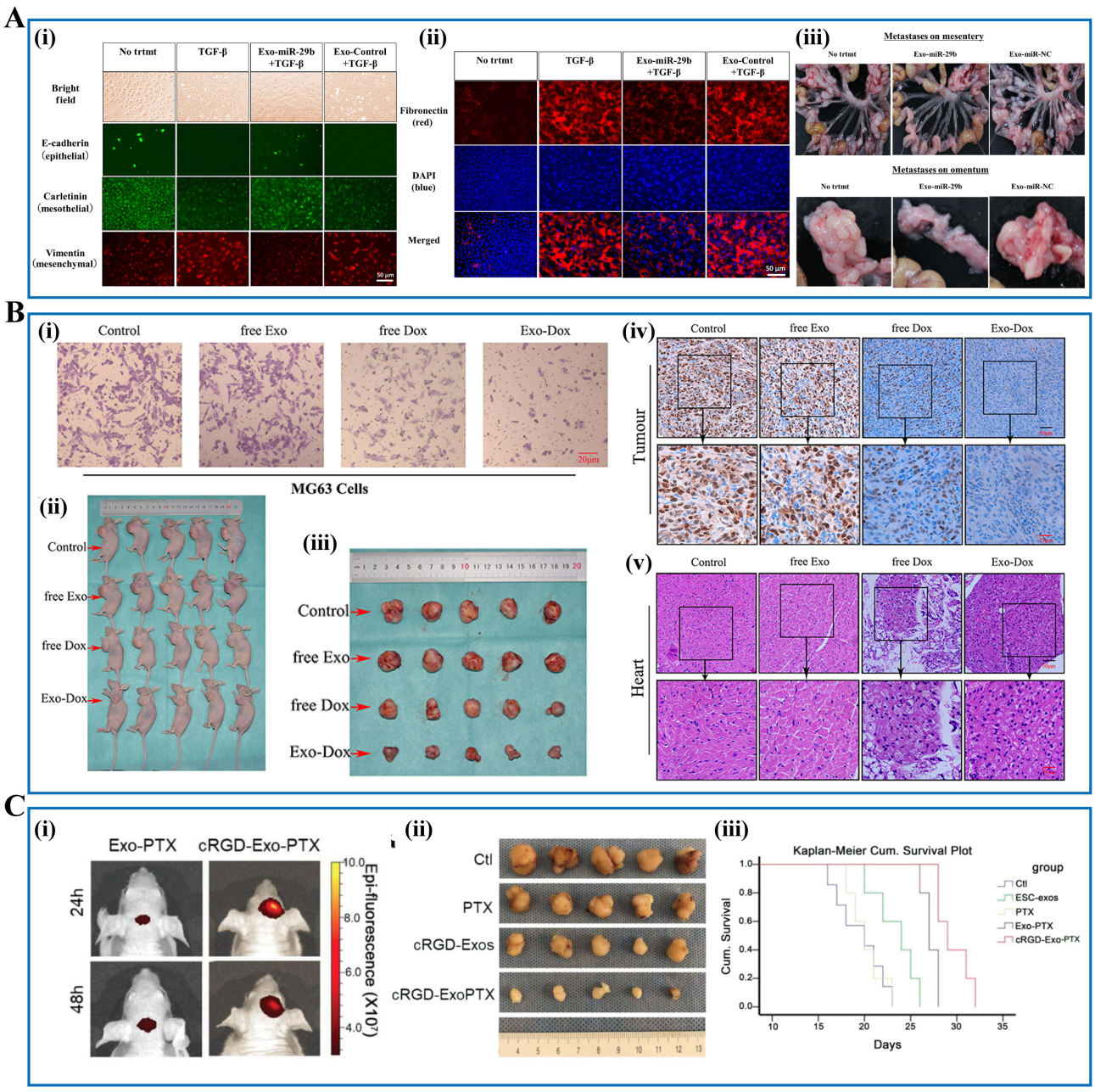


Figure 6 Therapeutic applications of SC-Exos in cancer treatment. (**A**) BMSC-Exos inhibit mesothelial-mesenchymal transition, reduce fibronectin, and suppress peritoneal metastases in gastric cancer. Reproduced from Kimura Y, Ohzawa H, Miyato H, et al, Intraperitoneal transfer of microRNA-29b-containing small extracellular vesicles can suppress peritoneal metastases of gastric cancer. Cancer Sci. 2023;114(7):2939–2950. © 2023 The Authors. Cancer Science published by John Wiley & Sons Australia, Ltd on behalf of Japanese Cancer Association. Creative Commons CC-BY-NC license.[213] (i) Immunofluorescence staining demonstrates BMSC-Exos inhibit TGF-β1-induced mesothelial-mesenchymal transition in human peritoneal mesothelial cells (HPMCs) by restoring E-cadherin and suppressing vimentin. (ii) Immunofluorescence staining displays BMSC-Exos suppress TGF-β1-induced fibronectin in HPMCs, disrupting tumor adhesion. (iii) Images display BMSC-Exos reduce peritoneal metastatic nodules in a gastric cancer mouse model. (**B**) BMSC-Exos loaded with Dox inhibit osteosarcoma cell migration and tumor growth while reducing cardiotoxicity. Reproduced from Wei HX, Chen F, Chen JY, et al, Mesenchymal stem cell derived exosomes as nanodrug carrier of doxorubicin for targeted osteosarcoma therapy via SDF1-CXCR4 axis. Int J Nanomed. 2022;17:3483–3495. © 2022 Wei et al,[215] (i) Transwell assay exhibits BMSC-Exos loaded with Dox significantly reduce MG63 osteosarcoma cell migration. (ii) Images of tumor-bearing mice illustrate that BMSC-Exos loaded with Dox markedly decrease tumor size in MG63 xenograft models. (iii) Excised tumor samples demonstrate that BMSC-Exos loaded with Dox significantly reduce tumor burden in osteosarcoma-bearing mice. (iv) Ki-67 antigen immunohistochemistry reveals reduced tumor cell proliferation in the BMSC-Exos loaded with the Dox group. (v) H&E staining of heart tissues indicates that BMSC-Exos loaded with Dox treatment decreases cardiotoxicity in treated mice. (**C**) cRGD-Exo-PTX effectively targets tumors and exhibits anti-cancer effects in GBM models. Reproduced from Zhu QW, Ling XZ, Yang YL, et al, Embryonic stem cells-derived exosomes endowed with targeting properties as chemotherapeutics delivery vehicles for glioblastoma therapy. Adv Sci. 2019;6(6):1801899. © 2019 The Authors. Published by WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. CCBY.[216] (i) Fluorescence imaging reveals enhanced tumor targeting and accumulation of cRGD-Exo-PTX in GBM tissue. (ii) Representative images show tumor growth suppression by cRGD-Exo-PTX in subcutaneous U87 xenograft models. (iii) Kaplan-Meier survival curves demonstrate prolonged survival in mice treated with cRGD-Exo-PTX in orthotopic GBM models.

provide a theoretical foundation for developing exosome-based precision medicine technologies. However, further research is needed to improve the exosome loading efficiency, targeting capability, and clinical safety.

Applications in Immune Regulation and Inflammation-Related Diseases

SC-Exos demonstrate significant therapeutic efficacy in various immune regulatory and inflammation-related diseases, particularly inflammatory bowel disease (IBD), systemic inflammation, autoimmune diseases, and acute graft-versus-host disease (aGVHD).

In IBD, hUMSC-Exos, enriched with miR-129-5p, can significantly alleviate disease symptoms by targeting ferroptosis-related pathways, reducing lipid peroxidation and inflammatory responses, and promoting intestinal barrier function restoration and epithelial cell regeneration (Figure 7A).[219] Additionally, hUMSC-Exos prevent inflammatory signal activation and slow colitis-associated cancer progression by regulating the miR-146a/small ubiquitin-like modifier 1 axis, providing new directions for treating IBD and its complications.[220] In systemic inflammation and sepsis treatment, IL-1β-pretreated BMSC-Exos enhance the ability to regulate endoplasmic reticulum stress and alleviate sepsis-induced lung injury and systemic inflammatory responses by activating the sirtuin 1/ERK signaling pathway.[221] Further research reveals that miR-27b in BMSC-Exos can significantly reduce pro-inflammatory factor production by targeting jumonji domain-containing protein 3 and the NF-κB signaling pathway, effectively alleviating multiple organ dysfunction in septic mouse models (Figure 7B).[222] In autoimmune diseases, BMSC-Exos improve SLE by promoting anti-inflammatory macrophage polarization. These exosomes significantly reduce inflammation and kidney lesions in SLE mouse models through miR-16 and miR-21-mediated macrophage function regulation.[86]

Moreover, olfactory mucosa mesenchymal stem cell-derived exosomes encapsulated in silk protein hydrogel effectively modulate the immune microenvironment in rheumatoid arthritis by inhibiting T follicular helper cell polarization and promoting regulatory T cell (Treg) generation, significantly alleviating inflammatory responses and joint damage.[224] In aGVHD treatment, human BMSC-Exos effectively alleviate disease symptoms and improve survival rates in aGVHD mice by regulating dendritic cell differentiation and T cell subgroup function, reducing pro-inflammatory factor levels while increasing Treg cell proportions (Figure 7C).[223] Furthermore, Gingiva-derived mesenchymal stem cell exosomes effectively reduce LPS/INF-γ and oxidized low-density lipoprotein-induced inflammatory macrophage responses in high-fat environments by significantly decreasing inflammatory factors such as TNF-α, IL-6, IL-1β, and CD86 expression, increasing anti-inflammatory IL-10 levels, inhibiting macrophage lipid accumulation, and promoting inflammatory M1 macrophage polarization to anti-inflammatory phenotypes.[225]

Applications in Reproductive System and Fertility Protection

SC-Exos demonstrate extensive therapeutic potential for male and female reproductive system disorders and fertility preservation. Compared to traditional hormone replacement therapies and invasive procedures, SC-Exos offer several advantages: they can be administered non-invasively, have minimal side effects, and provide targeted therapeutic effects without the risks associated with whole-cell transplantation.[13,226,227] By regulating inflammation, apoptosis, oxidative stress, and fibrosis, SC-Exos provide novel therapeutic approaches for ovarian function restoration, endometrial repair, protection against chemotherapy-induced reproductive organ damage, and male reproductive function improvement that address current limitations in reproductive medicine.[228,229]

SC-Exos demonstrate significant therapeutic potential in female reproductive system disorders, particularly ovarian function recovery, endometrial regeneration, and polycystic ovary syndrome. Research indicates that BMSC-Exos effectively improve chemotherapy-induced ovarian failure models by regulating oxidative stress and inflammatory responses, promoting ovarian function recovery, reducing ovarian tissue inflammation, and inhibiting granulosa cell apoptosis through miR-144-5p-mediated PTEN downregulation (Figure 8A).[230] Similarly, hUMSC-Exos ameliorate cyclophosphamide-induced primary ovarian insufficiency in rats by delivering miR-145-5p to target-inhibit X-box binding protein 1, reducing oxidative damage and apoptosis in ovarian granulosa cells.[231] Besides, BMSC-Exos have been used to treat premature autoimmune ovarian insufficiency. Studies demonstrate that in mouse models, BMSC-Exos loaded with miR-21-5p (miR-21-Exo) improve ovarian structure and function by regulating the Msh homeobox 1 (MSX1)-mediated Notch signaling pathway, inhibiting granulosa cell apoptosis and inflammation while enhancing

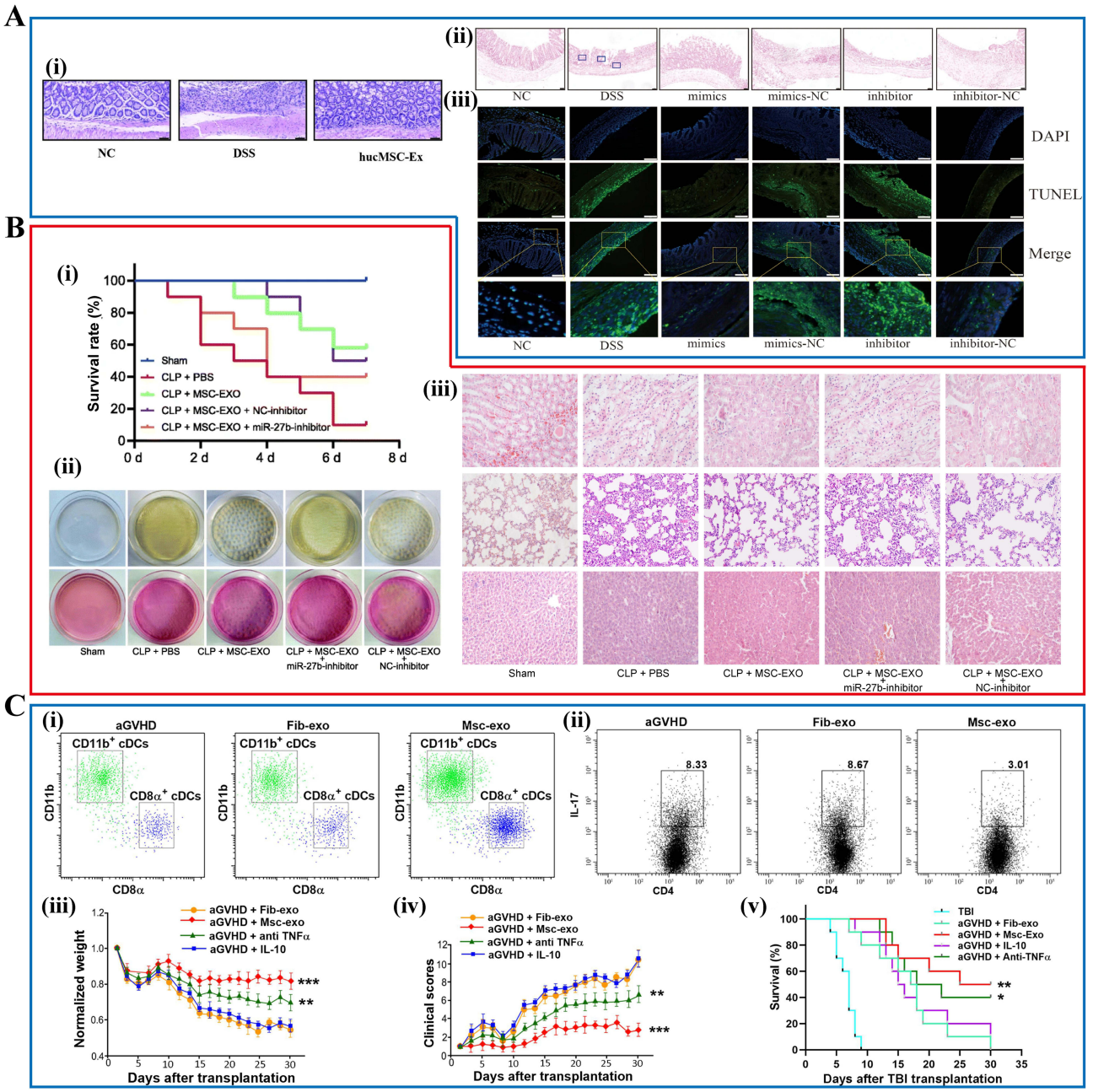


Figure 7 Therapeutic applications of SC-Exos in immune regulation and inflammation-related diseases. (**A**) hUMSC-Exos improve IBD by restoring colonic tissue integrity, reducing apoptosis, and suppressing iron deposition. Reproduced from Wei ZP, Hang SH, Ocansey DKW, et al. Human umbilical cord mesenchymal stem cells derived exosome shuttling mir-129-5p attenuates inflammatory bowel disease by inhibiting ferroptosis. J Nanobiotechnol. 2023;21(1):188. © 2023. The Author(s). Creative Commons Attribution 4.0 International License.[219] (i) H&E staining demonstrates restored colonic structure and reduced inflammatory infiltration after hUMSC-Exos treatment in IBD mice. (ii) Prussian blue staining reveals that miR-129-5p-loaded hUMSC-Exos markedly suppress iron accumulation in IBD mouse colonic tissues. Blue squares: small iron particle deposits. (iii) TUNEL assay indicates miR-129-5p-loaded hUMSC-Exos significantly reduce apoptosis in colonic tissues of IBD mice. (**B**) BMSC-Exos enhance survival, protect organs, and lower bacterial load in septic mice. Reproduced from Sun J, Sun X, Chen JH, et al. microRNA-27b shuttled by mesenchymal stem cell-derived exosomes prevents sepsis by targeting JMJD3 and downregulating NF-κB signaling pathway. Stem Cell Res Ther. 2021;12(1):14. © The Author(s) 2021. Creative Commons Attribution 4.0 International License.[222] (i) BMSC-Exos significantly improve the survival rate in septic mice over seven days. (ii) BMSC-Exos decrease bacterial colony counts in the peritoneal fluid and blood of septic mice. (iii) H&E staining demonstrates reduced liver, kidney, and lung injury in septic mice treated with BMSC-Exos. (**C**) Human BMSC-Exos alleviate immune dysregulation and inflammation, improving survival and clinical outcomes in aGVHD mice. Reprodued from Li KL, Li JY, Xie GL, Ma XY. Exosomes released from human bone marrow-derived mesenchymal stem cell attenuate acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation in mice. Front Cell Develop Biol. 2021;9:617589. © 2021 Li, Li, Xie and Ma. CCBY.[223] (i) Flow cytometric analysis demonstrates that human BMSC-Exos increase $CD8\alpha^+$ and $CD11b^+$ dendritic cells in the spleens of aGVHD mice. (ii) Flow cytometric analysis exhibits that human BMSC-Exos reduce $IL\text{-}17^+CD4^+$ Th17 cells in aGVHD mice. (iii) Human BMSC-Exos alleviate weight loss in aGVHD mice over time. (iv) Human BMSC-Exos decrease clinical scores, reflecting reduced disease severity in aGVHD mice. (v) Human BMSC-Exos significantly enhance survival rates in aGVHD mice. *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

hormone synthesis.[232] Furthermore, in endometrial repair, hUMSC-Exos effectively alleviate TGF-β-induced endometrial fibrosis by modulating the miR-140-3p/Forkhead Box P1/Smad signaling pathway,[233] while also improving ovarian function and reproductive capability in POI model mice through Hippo signaling pathway regulation.[226]

SC-Exos demonstrate significant therapeutic efficacy in male reproductive system disorders, particularly in testicular aging, testicular injury, and spermatogenesis restoration. Research exhibits that hUMSC-Exos effectively alleviate testicular aging by regulating macrophage polarization and reducing oxidative stress, promoting testicular cell proliferation and functional recovery while enhancing testosterone synthesis capacity (Figure 8B).[234] Additionally, in cyclophosphamide and busulfan-induced chemotherapy testicular injury mouse models, hUMSC-Exos significantly promote spermatogenesis and improve testicular function and serum testosterone levels by protecting testicular cells and restoring the spermatogonial stem cell microenvironment.[236] SC-Exos also exhibited potential in erectile dysfunction (ED) treatment. Studies demonstrate that multiple injections of human BMSC-Exos significantly improve ED in bilateral cavernous nerve injury (BCNI) rat models by enhancing the intracavernosal pressure (ICP)/mean arterial pressure ratio, increasing smooth muscle content, activating the nitric oxide/cyclic guanosine monophosphate signaling pathway, and promoting angiogenesis and cell proliferation through Ras homolog B (Figure 8C).[235]

Applications in Bone and Cartilage Tissue Repair

SC-Exos demonstrate exceptional bone and cartilage tissue repair potential, owing to their outstanding biological properties.[237] These nanoscale vesicles, carrying specific nucleic acids, proteins, and lipids, provide novel approaches to overcome the limitations of traditional treatments through cellular behavior regulation, tissue regeneration promotion, and immune microenvironment modulation.[238]

SC-Exos exhibit significant osteogenic capabilities for bone tissue repair. For example, a novel composite scaffold developed by combining osteogenically pre-differentiated human BMSC-Exos with GelMA hydrogel achieves sustained exosome release. It significantly enhances BMSC osteogenic differentiation and HUVEC angiogenic capacity, providing robust support for bone tissue regeneration.[239] Similarly, hUMSC-Exos combined with hyaluronic acid hydrogel and nano-hydroxyapatite/poly-ε-caprolactone scaffolds (nHP scaffolds) (hUMSC-Exos/Gel/nHP) significantly accelerate cranial defect repair through delivering exosomal miR-21 to upregulate vascular endothelial growth factor A and HIF-1α while inhibiting NOTCH1/delta-like ligand 4 signaling pathway (Figure 9A).[154] Moreover, BMSC-Exos derived from osteogenic induction culture significantly enhance osteogenic capacity through multiple miRNAs regulating bone morphogenetic protein receptor 2 and activin A receptor type 2B to activate the Smad1/5/9 signaling pathway competitively. When lyophilized onto mesoporous bioactive glass scaffolds, they maintain bioactivity and achieve sustained release, demonstrating exceptional bone-repair potential.[240]

SC-Exos also demonstrate significant advantages in cartilage tissue repair. CD56+ exosomes from hUMSCs combined with decellularized matrix hydrogels achieve sustained release and long-term effects in promoting meniscal tear healing, protecting articular cartilage, and inhibiting secondary cartilage degeneration.[243] Furthermore, research demonstrates that lithium-doped bioglass ceramics (Li-BGC) under lithium chloride (LiCl) stimulation significantly promote chondrocyte proliferation, migration, and matrix synthesis by upregulating miR-455-3p levels in BMSC-Exos, inhibiting histone deacetylase 2 expression, and enhancing histone H3 acetylation, effectively promoting cartilage regeneration (Figure 9B).[241] These results provide strong theoretical and practical support for the SC-Exos applications in cartilage repair.

Additionally, SC-Exos demonstrate multidimensional regulatory capabilities in bone-cartilage interface repair. Matrix metalloproteinase 1 (MMP1)-sensitive self-assembling peptide hydrogel (KLDL-MMP1/GelMA/Exo, KGE) microspheres enable spatiotemporally controlled release of BMSC-Exos, recruiting CD90+ stem cells and promoting bone formation during osteovascular genesis to accelerate bone defect repair and enhancing stem cell migration and polarization capabilities, providing an innovative solution for complex bone-cartilage interface injury treatment (Figure 9C).[242]

Applications in Skin Wound Healing and Tissue Repair

SC-Exos have emerged as a significant research focus in regenerative medicine, demonstrating a substantial potential for skin wound healing and tissue repair. Extensive research has validated that SC-Exos significantly accelerate skin wound

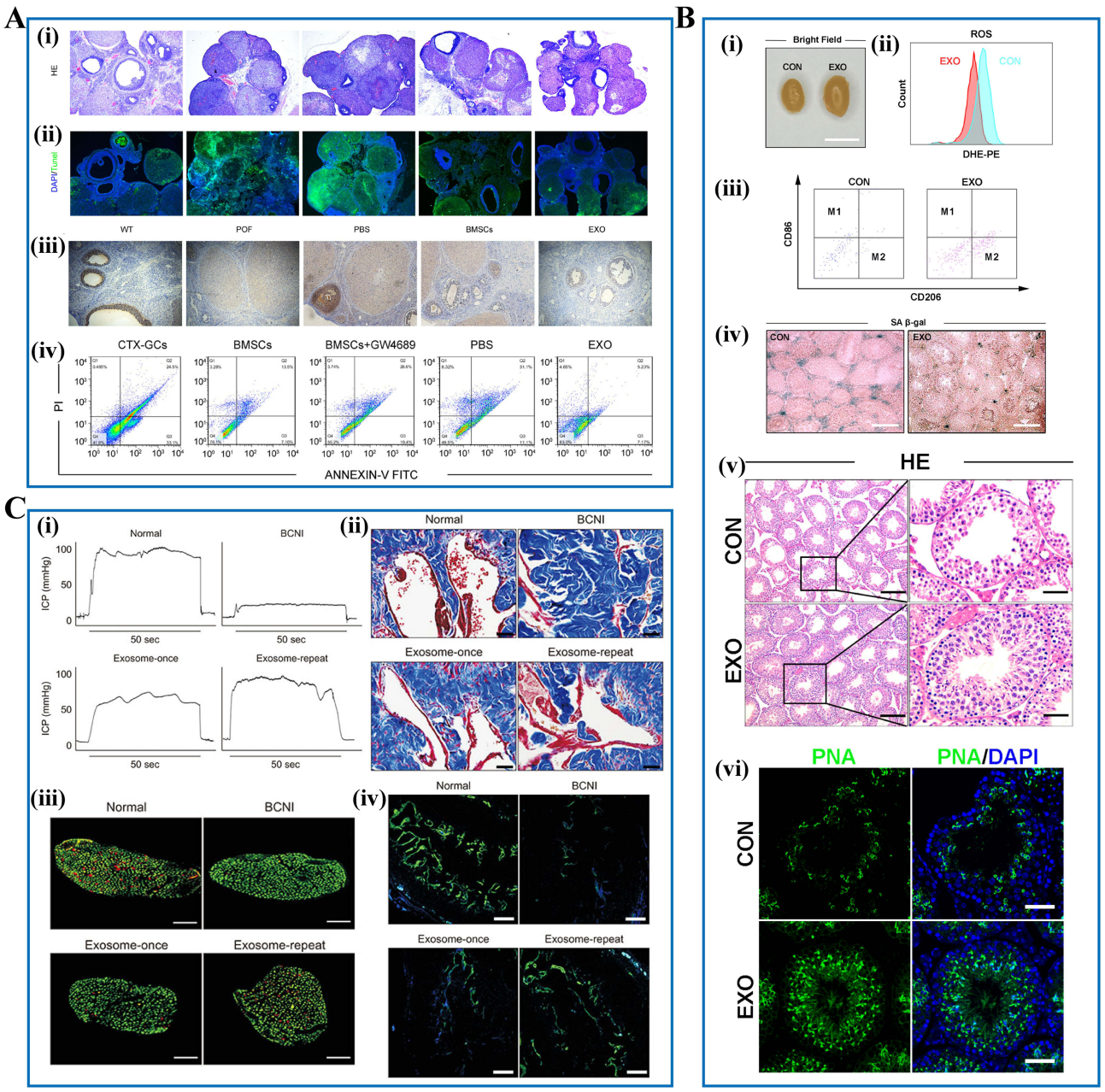


Figure 8 Applications of SC-Exos in reproductive systems and fertility protection. (**A**) BMSC-Exos improve ovarian function in chemotherapy-induced ovarian failure by reducing apoptosis and modulating PTEN expression. Reproduced from Yang ML, Lin L, Sha CL, et al. Bone marrow mesenchymal stem cell-derived exosomal miR-144-5p improves rat ovarian function after chemotherapy-induced ovarian failure by targeting PTEN. Lab Investigation. 2020;100(3):342–352. Copyright 2020, with permission from Elsevier.[230] (i) H&E staining displays improved follicular structure and reduced damage in ovaries following BMSC-Exos administration. (ii) TUNEL assay reveals reduced granulosa cell apoptosis in ovaries after BMSC-Exos administration. (iii) Immunohistochemistry presents lower PTEN expression in ovaries administered with BMSC-Exos. (iv) Flow cytometry exhibits decreased apoptosis of granulosa cells after the use of BMSC-Exos. (**B**) hUMSC-Exos mitigated testicular aging by reducing senescence, inflammation, and oxidative stress while restoring spermatogenesis. Reproduced from Luo P, Chen XR, Gao F, et al. Human umbilical cord mesenchymal stem cell-derived exosomes rescue testicular aging. Biomedicines. 2024;12(1):98. © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).[234] (i) hUMSC-Exos increase testis size and restore morphology in aged mice. (ii) hUMSC-Exos decrease reactive oxygen species (ROS) levels, mitigating oxidative stress in aged testes. (iii) Flow cytometry displays hUMSC-Exos promote M1-to-M2 macrophage polarization, reducing inflammation. (iv) hUMSC-Exos reduce senescence-associated β-galactosidase (SA-β-gal)-positive cells, alleviating senescence in aged testes. (v) H&E staining exhibits hUMSC-Exos enhance seminiferous tubule thickness and sperm production. (vi) PNA staining reveals improved spermatogenesis and sperm maturation with hUMSC-Exos. (**C**) Human BMSC-Exos improve erectile function by restoring smooth muscle, reducing fibrosis, and enhancing neuronal recovery in BCNI rats. Reproduced from Kim MY, Jo MS, Choi SG, Moon HW, Park J, Lee JY. Repeated injections of mesenchymal stem cell-derived exosomes ameliorate erectile dysfunction in a cavernous nerve injury rat model. World J Mens Health. 2024;10. Creative Commons Attribution Non-Commercial License.[235] (i) ICP tracings demonstrate enhanced erectile function after human BMSC-Exos treatment in BCNI rats. (ii) Masson's staining depict improved corpus cavernosa structure and reduced fibrosis after human BMSC-Exos treatment. (iii) Immunofluorescence staining reveals enhanced neuronal recovery and increased neuronal nitric oxide synthase-positive nerve fibers after human BMSC-Exos treatment. (iv) Immunofluorescence staining reveals enhanced α-smooth muscle actin expression and restored smooth muscle function in corpus cavernosum after human BMSC-Exos treatment.

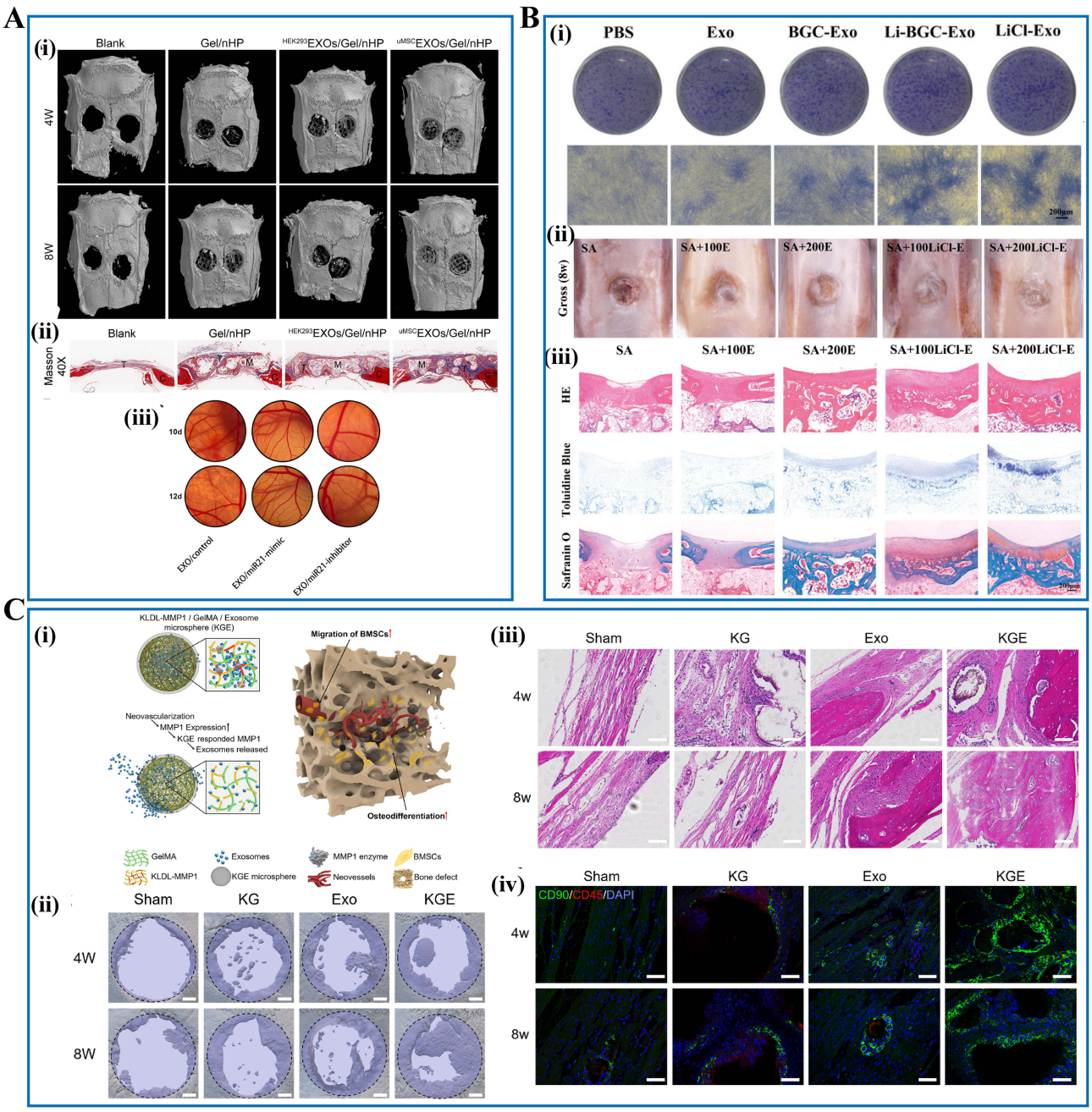


Figure 9 Therapeutic applications of SC-Exos in bone and cartilage tissue repair. (**A**) hUMSC-Exos/Gel/nHP scaffolds promote bone repair by enhancing collagen deposition, angiogenesis, and osteogenesis via miR-21 regulation. Reproduced from Zhang YT, Xie Y, Hao ZC, et al. Umbilical mesenchymal stem cell-derived exosome-encapsulated hydrogels accelerate bone repair by enhancing angiogenesis. ACS Appl Mater Interfaces. 2021;13(16):18472–18487. Copyright © 2021 American Chemical Society.[154] (i) Micro-CT 3D reconstructions exhibit enhanced bone formation in cranial defects treated with hUMSC-Exos/Gel/nHP at 4 and 8 weeks. (ii) Masson's staining reveals increased collagen deposition in defects treated with hUMSC-Exos/Gel/nHP scaffolds. C: cranium; M: materials; T: tissues. (iii) Chick embryo CAM assay demonstrates enhanced angiogenesis with hUMSC-Exos containing overexpressed miR-21, exhibiting more and longer blood vessels. (**B**) Li-BGC- or LiCl-treated BMSC-Exos enhance glycosaminoglycan secretion, cartilage matrix formation, and repair. Reproduced from Liu L, Yu F, Chen L, Xia LG, Wu CT, Fang B. Lithium-containing biomaterials stimulate cartilage repair through bone marrow stromal cells-derived exosomal miR-455-3p and histone H3 acetylation. Adv Healthcare Mat. 2023;12(11):12. © 2023 Wiley-VCH GmbH.[241] (i) Toluidine blue staining demonstrates increased glycosaminoglycan secretion after Li-BGC-BMSC-Exos treatment. (ii) Gross morphology reveals enhanced cartilage repair with LiCl-stimulated BMSC-Exos-loaded hydrogels. (iii) Histological staining demonstrates a mature cartilage matrix in defects treated with LiCl-stimulated BMSC-Exos. (**C**) KGE microspheres enable spatiotemporal release of BMSC-Exos to recruit stem cells and enhance bone regeneration. Reproduced from Yang Y, Zheng WH, Tan W, et al. Injectable MMP1-sensitive microspheres with spatiotemporally controlled exosome release promote neovascularized bone healing. Acta Biomater. 2023;157:321–336. Copyright 2023, with permission from Elsevier.[242] (i) Schematic illustration of enzyme-responsive KGE microspheres. (ii) Micro-CT imaging demonstrates enhanced bone regeneration induced by BMSC-Exos delivered via KGE microspheres. (iii) H&E staining depicts new bone formation promoted by BMSC-Exos released from KGE microspheres. (iv) Immunofluorescence staining indicates CD90+ stem cell recruitment by BMSC-Exos from KGE microspheres at defect sites.

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healing through multiple biological mechanisms, including cell migration and proliferation promotion, inflammatory response regulation, angiogenesis acceleration, and extracellular matrix remodeling enhancement.

MSC-Exos have attracted considerable attention because of their potential to address the clinical challenges of diabetic chronic wound healing. Recent research has developed a multifunctional hydrogel by integrating 3D-cultured ADSC-Exos with dynamic Schiff base-crosslinked chitosan-dihydroxycinnamic acid, pluronic F127-CHO, and tannic acid. This innovative hydrogel, featuring self-healing, antibacterial, antioxidant, and anti-inflammatory properties, and sustained exosome release capabilities, significantly promotes diabetic wound healing through enhanced angiogenesis, collagen deposition, and macrophage polarization, providing rapid hemostasis and tissue regeneration, thus offering a novel therapeutic strategy for chronic wounds.[244] In burn treatment, exosomes derived from iPSC-differentiated keratinocytes have demonstrated significant promotion of keratinocyte and endothelial cell migration through miR-762 regulatory mechanisms, accelerating wound angiogenesis and re-epithelialization in deep second-degree burn wounds (Figure 10A).[245] Furthermore, MSC-Exos ameliorate hypertrophic scar formation by modulating scarring fibroblast proliferation, migration, fibrosis, and inflammatory responses through inhibiting the tumor necrosis factor superfamily member 13/heparan sulfate proteoglycan 2 signaling pathway and its activated NF-κB pathway.[246]

ADSC-Exos applications in skin regeneration have garnered widespread attention. Combined with extracellular matrix hydrogels, ADSC-Exos achieve sustained release at wound sites, effectively reducing inflammatory responses while promoting angiogenesis, collagen deposition, and cell migration, accelerating wound healing and improving skin tissue regeneration quality.[70,249] Researchers have explored novel delivery carrier combinations to optimize the therapeutic efficacy of SC-Exos. An exosome delivery system based on thermosensitive pluronic F-127 (PF-127) hydrogel effectively prolongs hUMSC-Exos retention time and bioactivity at wound sites. It significantly promotes angiogenesis, cell proliferation, and granulation tissue regeneration in diabetic chronic wound models through sustained exosome release. This system upregulates VEGF and TGFβ-1 expression, thereby achieving complete skin regeneration.[250] Similarly, innovative gelatin silver nanoparticle microneedles incorporating hUMSC-Exos and antibacterial silver nanoparticles accelerate diabetic chronic wound repair and effectively inhibit bacterial infections.[251]

Furthermore, hUMSC-Exos demonstrates exceptional potential for alleviating oxidative stress-induced skin damage. Research has demonstrated that hUMSC-Exos repair oxidative stress-induced skin damage by regulating the nuclear factor erythroid 2-related factor 2 defense system, reducing ROS generation and DNA damage, improving calcium signaling and mitochondrial function, and enhancing antioxidant capacity, thereby demonstrating significant efficacy in UV-induced skin damage and other oxidative stress-related diseases (Figure 10B).[247] Besides, under external magnetic field guidance, hUMSC-Exos labeled with magnetic Fe_3O_4 nanoparticles demonstrate significantly enhanced targeted migration to damaged areas, promoting endothelial cell proliferation, migration, and angiogenesis, thereby accelerating skin burn wound healing, reducing scar formation, and enhancing collagen synthesis and epidermal regeneration effects (Figure 10C).[248]

Applications in Other Disease Treatments

Beyond their extensive applications in cardiovascular diseases, neurodegenerative disorders, and cancer therapy, SC-Exos demonstrate unique advantages and potential for various other disease treatments. Notable progress has been made in retinal diseases, liver fibrosis, acute kidney injury (AKI), oral tissue regeneration, fat graft survival, pulmonary fibrosis, and corneal repair, further validating their crucial roles in diverse pathological environments.

In retinal disease research, hUMSC-Exos carrying highly expressed miRNA-22-3p significantly protect RGCs and improve retinal structure and function in N-methyl-D-aspartate (NMDA)-induced retinal ganglion cell (RGC) injury mouse models by inhibiting MAPK pathway-mediated cell apoptosis, offering a safe and effective potential therapeutic strategy for glaucoma and RGC injury-related diseases (Figure 11A).[252] For liver fibrosis, studies using CCl_4-induced mouse models demonstrate that hUMSC-Exos deliver miR-148a-enriched molecules to hepatic macrophages, targeting Krüppel-Like factor 6 and modulating the signal transducer and activator of transcription 3 (STAT3) signaling pathway, thereby promoting macrophage transformation from pro-inflammatory M1 to anti-inflammatory M2 phenotype, improving inflammatory microenvironment and fibrosis, and significantly alleviating liver fibrosis (Figure 11B).[253] In AKI treatment, recent studies demonstrate that human BMSC-Exos effectively alleviate hypoxia-induced AKI within a precise

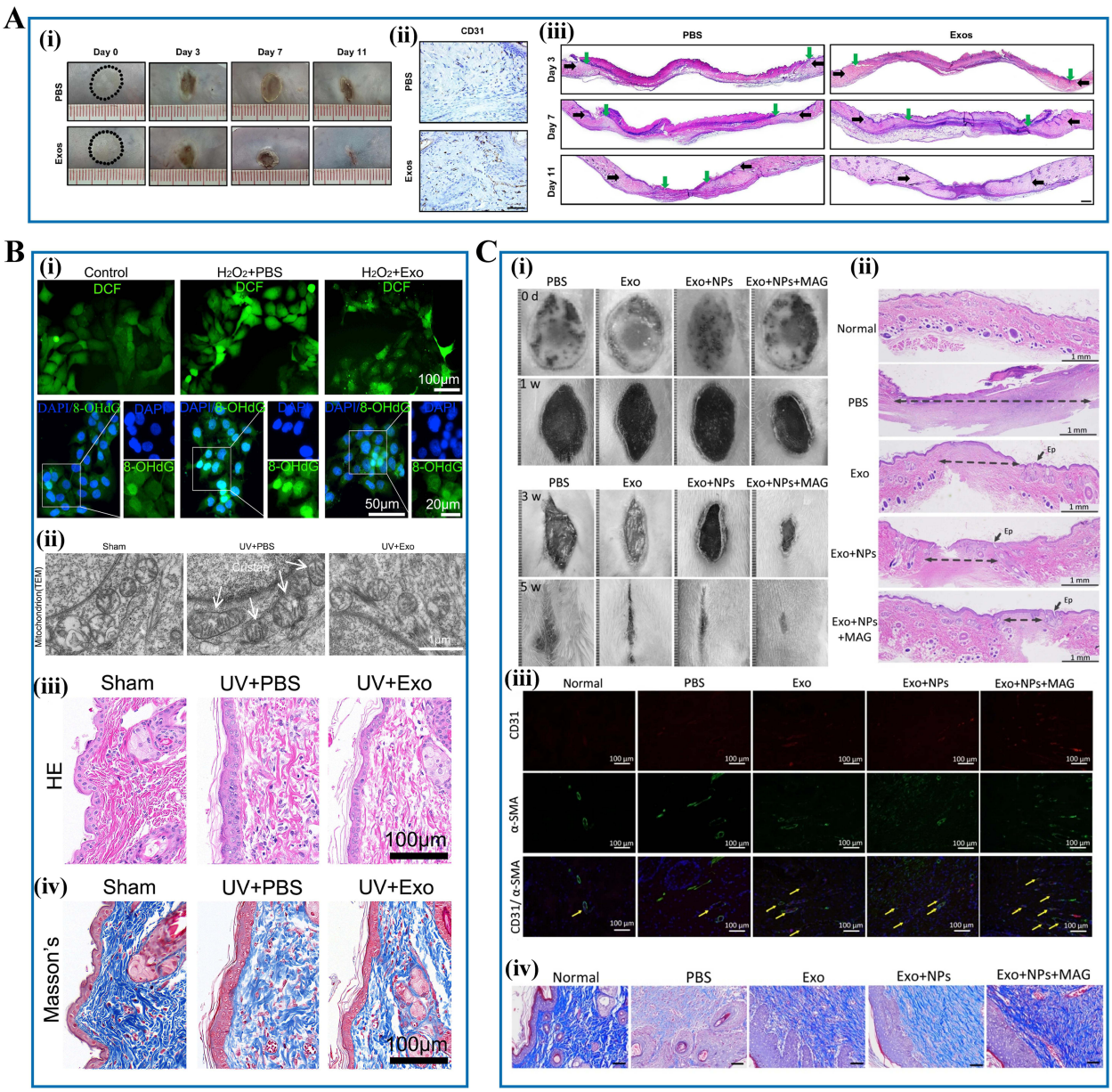

Figure 10 Therapeutic applications of SC-Exos in skin wound healing and tissue repair. (**A**) Exosomes derived from iPSC-differentiated keratinocytes enhance burn wound healing by promoting re-epithelialization, angiogenesis, and tissue repair. Reproduced from Bo YY, Yang LJ, Liu BT, et al. Exosomes from human induced pluripotent stem cells-derived keratinocytes accelerate burn wound healing through miR-762 mediated promotion of keratinocytes and endothelial cells migration. J Nanobiotechnol. 2022;20 (1):291. Creative Commons Attribution 4.0 International License.[245] (i) Wound images exhibit accelerated closure with exosomes derived from iPSC-differentiated keratinocytes. Black dashed circles: initial burn wound areas. (ii) CD31 immunohistochemistry demonstrates increased angiogenesis with exosomes derived from iPSC-differentiated keratinocytes. (iii) H&E staining reveals enhanced re-epithelialization and dermal repair with exosomes derived from iPSCs-differentiated keratinocytes. Black arrows: dermal border; green arrows: epidermal margin. (**B**) hUMSC-Exos mitigate oxidative stress, restore tissue structure, and promote repair in keratinocyte and UV-irradiated mouse skin models. Reproduced from Wang T, Jian Z, Baskys A, et al. MSC-derived exosomes protect against oxidative stress-induced skin injury via adaptive regulation of the NRF2 defense system. Biomaterials. 2020;257:120264. Copyright 2020, with permission from Elsevier.[247] (i) hUMSC-Exos treatment reduces ROS levels (DCF staining) and alleviates DNA damage (8-OHdG immunofluorescence) in H_2O_2-stimulated keratinocytes. (ii) TEM images demonstrate restored mitochondrial cristae structure in UV-irradiated skin following hUMSC-Exos treatment. White arrowheads: mitochondrial cristae. (iii) H&E staining reveals reduced epidermal thickening in UV-irradiated mouse skin treated with hUMSC-Exos. (iv) Masson's staining demonstrates enhanced collagen regeneration in UV-irradiated skin after hUMSC-Exos treatment. (**C**) hUMSC-Exos with Fe_3O_4 magnetic nanoparticles and magnetic guidance promote wound healing by enhancing re-epithelialization, collagen deposition, and angiogenesis. Reproduced from Li XY, Wang Y, Shi LY, et al. Magnetic targeting enhances the cutaneous wound healing effects of human mesenchymal stem cell-derived iron oxide exosomes. J Nanobiotechnol. 2020;18(1):113. Creative Commons Attribution 4.0 International License.[248] (i) Gross view of wound healing exhibits significant closure in the hUMSC-Exos with Fe_3O_4 nanoparticles and magnetic guidance group. (ii) H&E staining demonstrates reduced scar width and re-epithelialization in the hUMSC-Exos with Fe_3O_4 magnetic nanoparticles and magnetic guidance group. Double-headed arrows: scar edges; Ep: epithelium. (iii) immunofluorescence staining presents increased blood vessel formation in the hUMSC-Exos with Fe_3O_4 magnetic nanoparticles and magnetic guidance group. Yellow arrows: mature blood vessels. (iv) Masson's staining reveals enhanced collagen deposition and maturity in wounds treated with hUMSC-Exos with Fe_3O_4 magnetic nanoparticles and magnetic guidance.

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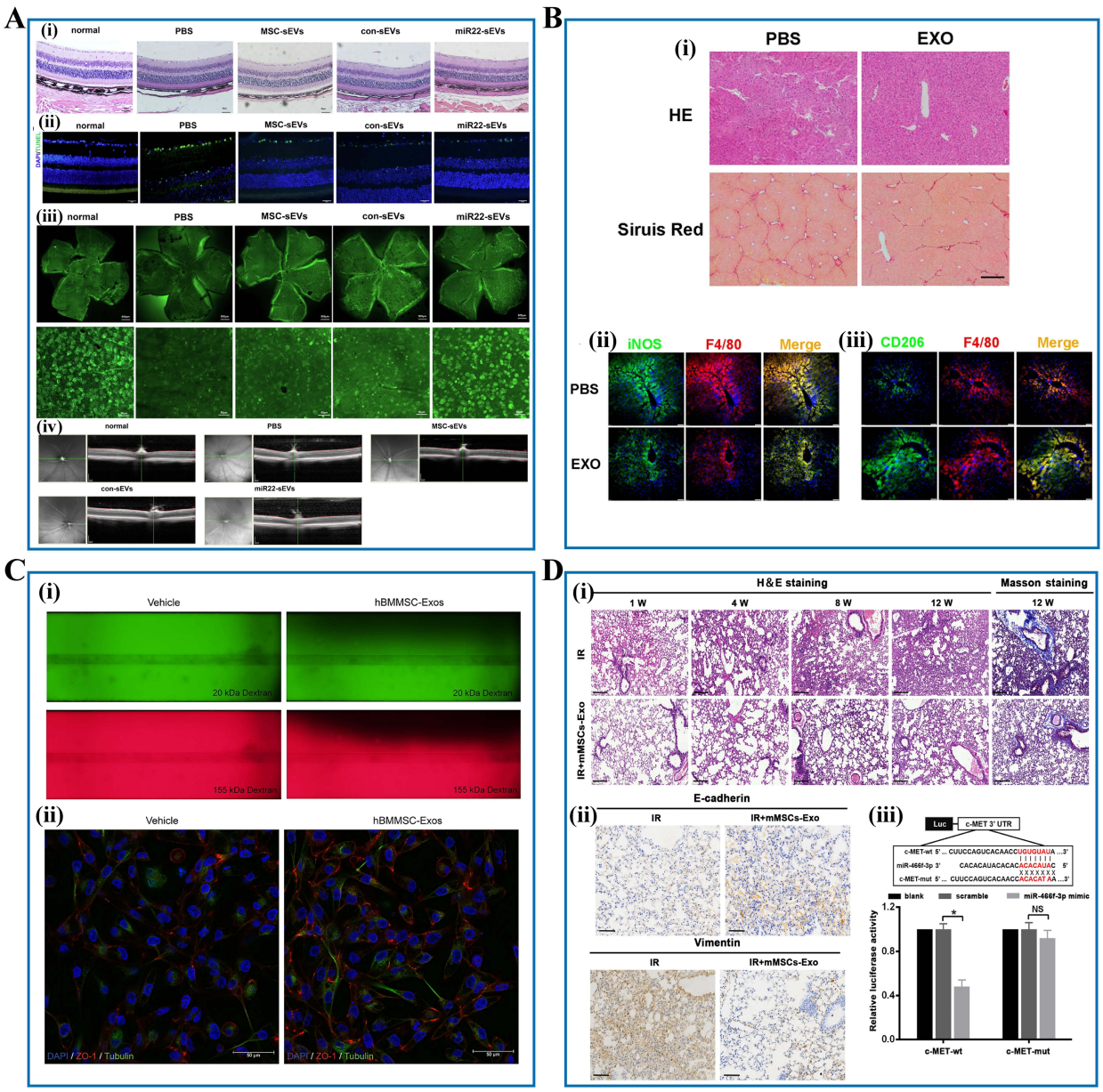

Figure 11 Therapeutic applications of SC-Exos in the treatment of other diseases. (**A**) hUMSC-Exos with miRNA-22-3p overexpression protect against NMDA-induced retinal injury by preserving structure, reducing apoptosis, and enhancing cell survival. Yu B, Wang K, Hao HJ, et al. Small extracellular vesicles derived from microRNA-22-3p-overexpressing mesenchymal stem cells protect retinal ganglion cells by regulating MAPK pathway. Commun Biol. 2024;7(1):807. Creative Commons Attribution 4.0 International License.[252] (i) H&E staining displays preserved ganglion cell layer density after hUMSC-Exos treatment with miRNA-22-3p overexpression (Scale bar = 50 μm). (ii) TUNEL assay exhibits reduced apoptosis in the ganglion cell layer after hUMSC-Exos treatment with miRNA-22-3p overexpression (Scale bar = 50 μm). (iii) RGC staining represents improved retinal ganglion cell survival in flat-mounted retinas after hUMSC-Exos treatment with miRNA-22-3p overexpression. (iv) OCT images depict increased retinal thickness after hUMSC-Exos treatment with miRNA-22-3p overexpression. (**B**) hUMSC-Exos alleviate liver fibrosis by reducing inflammation, improving liver structure, and modulating macrophage polarization. Reproduced from Tian SY, Zhou X, Zhang M, 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(1):330. Creative Commons Attribution 4.0 International License.[253] (i) H&E and Sirius Red staining demonstrate that hUMSC-Exos improve liver structure and alleviate fibrosis. (ii) Immunofluorescence staining demonstrates that hUMSC-Exos reduce pro-inflammatory (M1) macrophages, marked by inducible nitric oxide synthase and F4/80 co-expression (Scale bar = 100 μm). (iii) Immunofluorescence staining demonstrates that hUMSC-Exos increase anti-inflammatory (M2) macrophages, characterized by CD206 and F4/80 co-expression (Scale bar = 100 μm). (**C**) Human BMSC-Exos protect against hypoxic injury by restoring epithelial integrity, polarity, and barrier function. Reproduced from Çam SB, Çiftci E, Gürbüz N, Altun B, Korkusuz P. Allogeneic bone marrow mesenchymal stem cell-derived exosomes alleviate human hypoxic AKI-on-a-Chip within a tight treatment window. Stem Cell Res Ther. 2024;15(1):105. © 2024. The Author(s). Creative Commons Attribution 4.0 International License.[254] (i) Human BMSC-Exos restore epithelial barrier integrity by reducing the permeability to FITC- and TRITC-dextran probes after hypoxic injury. (ii) immunofluorescence staining reveals human BMSC-Exos restore PT epithelial polarity (acetylated α-tubulin) and tight junctions (ZO-1) disrupted by hypoxia. (**D**) BMSC-Exos alleviate radiation-induced lung injury and epithelial-mesenchymal transition via miR-466f-3p targeting c-MET to inhibit AKT/GSK3β signaling. Reproduced from Li Y, Shen ZF, Jiang X, et al. Mouse mesenchymal stem cell-derived exosomal miR-466f-3p reverses EMT process through inhibiting AKT/GSK3β pathway via c-MET in radiation-induced lung injury. J Exp Clin Cancer Res. 2022;41(1):128. Creative Commons Attribution 4.0 International License.[255] (i) H&E and Masson's staining exhibit BMSC-Exos reduce lung damage, collagen deposition, and inflammation post-radiation. (ii) Immunohistochemistry demonstrates BMSC-Exos reverse radiation-induced epithelial-mesenchymal transition by restoring E-cadherin and reducing vimentin. (iii) Luciferase assay confirms miR-466f-3p directly targets c-MET. Red text: miR-466f-3p binding sites and mutated sequences. NS, not significant; *$p < 0.05$.

therapeutic window by restoring proximal tubular epithelial cell polarity, repairing barrier integrity, and significantly promoting cell proliferation, providing an innovative strategy for precise renal repair therapy (Figure 11C).[254]

In oral tissue regeneration, dental pulp stem cell-derived exosomes effectively reduce periodontal ligament stem cell inflammation by inhibiting the IL-6/Janus kinase 2/STAT3 signaling pathway while modulating macrophage polarization from M1 to M2 type, optimizing the immune microenvironment, and significantly promoting alveolar bone repair and periodontal tissue regeneration, exploring a novel therapeutic strategy for precise treatment of periodontitis.[256] For fat graft survival challenges in soft tissue repair, ADSC-Exos combined with PF-127 thermosensitive hydrogel significantly improve autologous fat graft survival rates by activating the HIF-1α/VEGF signaling pathway to promote angiogenesis, providing vital support for clinical applications of fat grafting.[257] In pulmonary fibrosis treatment, mouse BMSC-Exos carrying enriched miR-466f-3p target c-MET and inhibit the AKT/glycogen synthase kinase 3 β (GSK3β) signaling pathway, thereby reversing radiation-induced alveolar epithelial cell epithelial-mesenchymal transition and significantly reducing radiation-induced pulmonary fibrosis and inflammation, exhibiting great potential as a therapy for radiation-induced lung injury (Figure 11D).[255] Meanwhile, in corneal repair research, iMSC-Exos combined with thermosensitive chitosan hydrogel effectively reduce excessive type I and V collagen deposition in the transparent extracellular matrix by inhibiting translocating chain-associating membrane protein 2 expression through the sustained release of miR-432-5p-containing exosomes, promoting corneal epithelial and stromal repair while reducing scar formation, providing new clinical technical directions for corneal injury and related diseases.[258]

Furthermore, exosome labeling and tracking technologies are important supporting tools for promoting clinical translation.[259] Research demonstrates that hUMSC-Exos labeled with glucose-functionalized polymer dots (Pdots-Glu) featuring second near-infrared window (NIR-II) fluorescence properties achieve high-brightness, long-term in vivo tracking and imaging and accelerate liver function recovery in post-hepatectomy mice by inhibiting inflammation, promoting cell proliferation, and anti-apoptotic effects.[260] This technology provides possibilities for precise monitoring and optimization of therapeutic effects in various disease applications of exosome therapy.

Clinical Translation Status of SC-Exos

The therapeutic potential of SC-Exos is increasingly being realized through clinical implementation, with numerous documented clinical trials investigating their safety and efficacy across diverse medical conditions (Table 4). This systematic analysis encompasses a broad spectrum of therapeutic applications, ranging from dermatological conditions and wound healing to complex systemic diseases, including IBD, SCI, and severe COVID-19 pneumonia. The majority of these investigations represent early-phase studies (Phase I and Phase II) and pilot trials, with primary emphasis on safety evaluation and proof-of-concept efficacy assessment. The current clinical trial portfolio demonstrates remarkable diversity in both therapeutic targets and SC-Exos sources. Investigations employ multiple stem cell-derived platforms, hUMSC-Exos, adipose mesenchymal stem cell exosomes (AMSC-Exos), ADSC-Exos, human placenta mesenchymal stem cell-derived exosomes, and human Wharton's Jelly mesenchymal stem cell-derived exosomes, reflecting the versatility of different cellular origins. Clinical applications span multiple medical specialties, including gastroenterology (refractory perianal fistulas in both Crohn's disease[261,262] and non-IBD patients[263]), neurology (subacute SCI[264]), dermatology (psoriasis,[265] facial aging,[266,267] acne scarring,[268] androgenetic alopecia[269]), pulmonology (COVID-19 pneumonia[270,271]), and ophthalmology (Sjögren's syndrome-related dry eye syndrome)[272] Diverse administration methodologies have been employed, including direct injection,[261–263,265] intrathecal delivery,[264] intradermal administration,[265] topical application,[268,272] nebulization therapy,[270,271] and microneedling-assisted delivery,[266,267,269] demonstrating the platform flexibility and adaptability of SC-Exos therapeutics.

Safety evaluations across completed trials consistently reveal favorable profiles, with the majority of studies reporting no serious adverse events attributable to SC-Exos administration. Documented adverse effects were predominantly mild and transient, including injection site discomfort,[265] mild erythema,[267–269] localized tingling sensations,[269] and transient pain[267] that resolved within one week. Notably, no treatment discontinuations due to adverse events were reported across any of the trials, underscoring the excellent tolerability profile of SC-Exos therapeutics. Therapeutic outcomes demonstrate significant promise across multiple domains, including tissue regeneration, immune modulation, and organ function

restoration. Notable efficacy findings include substantial fistula closure rates (60–81%), improved neurological function scores, enhanced skin quality parameters, increased hair density, and ameliorated dry eye symptoms.

However, several significant limitations constrain the current evidence base and warrant careful consideration. These include relatively small patient cohorts (5–30 participants per study), variable follow-up periods (ranging from 12–24 weeks, with most studies employing 3–6 month follow-up), and substantial heterogeneity in SC-Exos sources, preparation methodologies, administration routes, and dosing regimens. Dosing strategies varied considerably across studies, ranging from single injections to multiple treatment sessions, with exosome concentrations spanning from 10 μg to 2.0×10^9 particles per treatment. These limitations underscore the critical need for standardized protocols, harmonized manufacturing processes, and larger-scale confirmatory trials to establish optimal therapeutic approaches for each clinical indication. Furthermore, the global distribution of clinical trials, evidenced by registrations across American (NCT), Iranian (IRCT), and Chinese (ChiCTR) registries, reflects worldwide interest in SC-Exos therapeutics while highlighting the imperative for harmonized regulatory frameworks and coordinated international development strategies.

The regulatory classification and approval pathways for SC-Exos therapeutics present unique challenges that significantly impact clinical translation timelines and commercial viability. In the United States, the Food and Drug Administration (FDA) regulates SC-Exos as biological medicinal products under the oversight of the Center for Biologics Evaluation and Research (CBER), requiring comprehensive regulatory applications that include preclinical safety studies, manufacturing and quality control information, clinical protocols, and investigator qualifications.[273] As biological medicinal products, SC-Exos must comply with Current Good Manufacturing Practice (cGMP) standards and meet stringent requirements outlined in 21CFR312.23(a)(7) for Chemistry, Manufacturing, and Control Information, as well as 21CFR312.23(a)(8) for Pharmacology and Toxicology Information.[274] A critical challenge in regulatory approval lies in defining clear mechanisms of action for SC-Exos, given their heterogeneous cargo containing multiple effector molecules with pleiotropic downstream effects.[275,276] This mechanistic complexity necessitates comprehensive quality control requirements to ensure consistent biological, molecular, and physical characteristics across batches. The regulatory pathway ultimately requires progression through systematic clinical testing phases followed by submission of a Biologics License Application (BLA) to demonstrate safety, quality, purity, and potency.[274]

Currently, limited FDA-approved SC-Exos products are available, reflecting the evolving regulatory landscape governing these novel therapeutics,[277] although early clinical trials have demonstrated promising safety and efficacy profiles across multiple therapeutic domains (Table 4). Analysis of the US direct-to-consumer market reveals substantial regulatory non-compliance, with 68% of businesses marketing exosome therapies providing no explicit representations about regulatory status, highlighting widespread confusion about appropriate regulatory pathways and the urgent need for clearer guidance.[277] Manufacturing and standardization challenges represent critical bottlenecks in clinical translation. Current obstacles include the absence of established upstream and downstream manufacturing processes, lack of universally accepted potency assays, and inconsistent batch-to-batch evaluation criteria.[278] The development of scalable production requires compliance with cGMP standards, adequate bioreactor platforms, and established quality management systems.[279] Significant regulatory harmonization challenges persist across different jurisdictions, with varying classification frameworks that may categorize SC-Exos as biologics, drugs, or medical devices depending on intended use and manufacturing processes, creating uncertainty for developers and inconsistent approval timelines. These regulatory complexities must be addressed through comprehensive guidance documents and standardized manufacturing protocols to facilitate the safe and effective translation of SC-Exos from experimental interventions to approved therapeutic modalities.

Challenges in Clinical Applications of SC-Exos

Although SC-Exos demonstrate broad therapeutic potential across various diseases, their clinical application faces multiple challenges.

First, regarding preparation and quality control, standardized protocols for SC-Exos isolation and purification have not been established. Currently, different laboratories employ various techniques (such as ultracentrifugation and SEC), leading to significant differences in product characteristics and therapeutic efficacy. Although ultracentrifugation is considered the "gold standard" for exosome isolation, it still faces challenges, including expensive equipment, lengthy

Table 4 Clinical Trials of SC-Exos

Trial ID/Phase	SC-Exos Type	Indication	Patient Population (n)	Study Design	Intervention	Primary Outcome	Key Findings	Safety Profile	Reference
NCT05499156/Phase II	hUMSC-Exos	Refractory perianal fistula in Crohn's disease	23 patients enrolled, 20 completed	Single-center, nonrandomized study	5 mL exosome solution (0.5×10^{10} particles/mL), 3 injections at 2-month intervals	Fistula tract closure (clinical/MRI)	Complete closure in 60% of patients (12/20); 69.7% tracts (30/43) healed	No AEs reported	[261]
IRCT20200413047063N3/ NCT05499156/Phase I	hUMSC-Exos	Refractory perianal fistula in Crohn's disease	5 patients, median age 35 years	Phase I, open-label study	5 mL exosome solution (50 μg/mL), single injection	Safety assessment	4 of 5 patients (80%) responded to treatment; 3 patients showed complete healing	No AEs reported	[262]
ChiCTR2000030261/Pilot	hUMSC-Exos	COVID-19 pneumonia	7 patients (2 severe, 5 mild cases)	Single-arm pilot study	Nebulization 7.0×10^{7} to 7.66×10^{8} particles/mL, twice daily for 10 min	Safety assessment	Improved pulmonary lesion absorption; Reduced hospitalization duration (15.3 ± 1.33 days); Faster lesion absorption (16.00 ± 5.23 vs 20.85 ± 3.57 days, p = 0.033)	No acute allergic reactions or AEs	[270]
IRCT20200502047277N1/ Phase I	hUMSC-Exos	Subacute SCI	9 patients	Single-arm study	Intrathecal injection of 300 μg total protein exosomes in 5 mL PBS, single dose	Safety assessment and incidence of AEs	Significant improvements in ASIA sensory scores, SCIM III total score, NBD score at 12 months	7 AEs in 5 patients, All treatment-unrelated	[264]
Not provided	ADSC-Exos	Acne scars	25 patients, age 19–54 years	12-week double-blind, randomized, split-face study	ADSC-Exos gel + fractional CO_2 laser vs control gel + laser, 3 sessions	Change in total ECCA score	Greater ECCA score reduction (32.5% vs 19.9%); Milder erythema, shorter downtime	Milder erythema, shorter recovery time; No AEs reported	[268]
Not provided	ADSC-Exos	Androgenetic alopecia	30 patients enrolled, 29 completed (14 males, 15 females), mean age 47.1 ± 7.6 years	24-week open-label, prospective study	ADSC-Exos (20 mg) + microneedling, biweekly for 3 months then every 3 weeks for 3 months	Total hair counts in target area	Significant increase in total hair density (158.03 to 166.14/cm^2); Improved photographic assessments, subjective satisfaction	5 patients reported slight tingling, mild erythema; No treatment discontinuation	[269]
Not provided	ADSC-Exos	Facial photoaging	28 patients completed	12-week randomized, split-face study	ADSC-Exos solution (5×10^{9} particles) + microneedling vs control, 3 sessions	GAIS score	Higher GAIS scores vs control; Improved wrinkles, elasticity, hydration, pigmentation	No serious AEs; Transient erythema, edema, petechiae resolved within 1 week	[266]
IRCT20080901001159N2/ Phase I/II	AMSC-Exos	Mild to moderate plaque psoriasis	12 patients enrolled, 10 completed	Phase I/II study	Single intradermal injection of 50, 100, 200 μg per cm^2 of lesion	Safety and efficacy assessment	Optimal dose 200 μg; Reduced erythema, induration; Decreased IL17, CD3; Increased FOXP3	Mild pain and burning in 2 patients; No serious AEs	[265]
NCT04276987/Phase IIa	AMSC-Exos	Severe COVID-19 pneumonia	7 patients, median age 57 years	Single-arm interventional study	Daily nebulization of 2.0×10^{8} exosomes for 5 consecutive days (total 1.0×10^{9} per patient)	Safety assessment	Well tolerated; Increased lymphocytes (1.61 vs 1.78×10^{9}/L); Improved pulmonary lesion resolution in 4 of 7 patients	No prespecified AEs; Well tolerated	[271]
Not provided	AMSC-Exos	Facial photoaging	15 patients enrolled, age 44–68 years	Investigator-blind, split-face, non-inferiority study	Three RFMN treatments at 4-week intervals; PRP vs exosomes (split-face)	Skin quality assessment	Both treatments equally improved skin quality parameters; Histological analysis confirmed increased collagen I, GAGs	No AEs reported; Mild side effects (pain, erythema, edema, crusting) resolved within 1 week	[267]
IRCT20200413047063N3/ NCT05402748/Phase I	Human placenta mesenchymal stem cell-derived exosomes	Complex perianal fistula (non-Crohn's)	11 patients	Phase I, open-label study	5 mL exosome solution (0.5×10^{10} particles/mL), weekly injections, 3 sessions	Safety assessment	81% of patients experienced complete or partial healing; Discharge stopped in 6 patients	No AEs reported	[263]
IRCT20211102052948N1	Human Wharton's Jelly mesenchymal stem cell-derived exosomes	Sjögren's syndrome dry eye	8 patients (16 eyes), females, age 34–56 years	Triple-blind, randomized controlled trial	10 μg exosomes twice daily for 2 weeks vs PBS (split-eye)	Changes in OSDI score	Improved OSDI, tear secretion, TFBUT; Reduced fluorescein scores; Increased EGF, THBS1; Decreased IL-6, MMP-9	No local or systemic complications reported	[272]

Note: Bold formatting represents table column headers and classification categories.

Abbreviations: ADSC-Exos, Adipose-derived stem cell exosomes; AEs, Adverse events; AMSC-Exos, Adipose mesenchymal stem cell exosomes; ASIA, American Spinal Injury Association; CD3, Cluster of Differentiation 3; ChiCTR, Chinese Clinical Trial Registry; CO_2, Carbon dioxide; COVID-19, Coronavirus disease 2019; ECCA, Échelle d'évaluation clinique des cicatrices d'acné; EGF, Epidermal growth factor; FOXP3, Forkhead box P3; GAGs, Glycosaminoglycans; GAIS, Global Aesthetic Improvement Scale; hUMSC-Exos, Human umbilical mesenchymal stem cell-derived exosomes; IL-6, Interleukin-6; IL17, Interleukin-17; IRCT, Iranian Registry of Clinical Trials; MMP-9, Matrix metalloproteinase-9; MRI, Magnetic resonance imaging; NBD, Neurogenic bowel dysfunction; NCT, National Clinical Trial; OSDI, Ocular Surface Disease Index; PBS, Phosphate-buffered saline; Phase, Clinical trial phase; PRP, Platelet-rich plasma; RFMN, Radiofrequency microneedling; SC-Exos, Stem cell-derived exosomes; SCIM III, Spinal Cord Independence Measure version III; TFBUT, Tear film break-up time; THBS1, Thrombospondin-1.

processing time (4–6 hours), limited recovery rates, and purity concerns due to co-precipitation of other extracellular vesicles and protein complexes.[280] Other isolation methods also have limitations, such as operational complexity, high contamination risk, and low yield.[281] Therefore, the current lack of an economically efficient and standardized isolation method represents a major bottleneck hindering the clinical application of SC-Exos. The existing quality control methods primarily rely on marker protein detection (such as CD63 and CD81). However, these markers cannot comprehensively reflect SC-Exos biological functions, limiting quality assessment accuracy.[282] Moreover, exosome identification and analysis are prerequisites and guarantees for clinical research.[283] However, current detection technologies (including transmission electron microscopy, nanoparticle tracking analysis, and flow cytometry) still face numerous challenges when analyzing size, morphology, and concentration, including contamination from other particles, technical inefficiency, limited reproducibility, and insufficient resolution.[284–286] Simultaneously, the lack of unified evaluation standards for bioactivity and functional molecules further hinders direct comparisons between research results.

Second, therapeutic heterogeneity represents a key challenge in SC-Exos applications. Stem cell type, donor differences, and culture conditions significantly influence SC-Exos composition and function, resulting in considerable variations in therapeutic effects among different types of SC-Exos for the same disease.[287] A critical aspect of this heterogeneity stems from variability in functional inheritance from parent cells under different conditions. Environmental stresses, disease states, and in vitro expansion conditions can significantly influence the stability of functional properties inherited from parent cells. While hypoxic preconditioning has been shown to enhance the therapeutic potential of SC-Exos by modulating the expression of specific molecules, including microRNAs, and improving their regenerative capacity,[288,289] prolonged in vitro expansion and multiple passages can lead to cellular senescence, which potentially affects the characteristics of parent cells and their secreted exosomes, although the direct impact on exosomal functional integrity requires further investigation.[290] However, systematic studies examining the robustness of functional inheritance under various stress conditions, disease states, and extended in vitro culture remain limited, representing a critical knowledge gap in the field. The heterogeneity of natural exosomes and their sensitivity to production conditions restricts their clinical applicability.[291] This heterogeneity increases the uncertainty in clinical applications and highlights the necessity for in-depth exploration of their mechanisms and applicable scope.

Challenges in scaling-up production are equally significant. Stem cell cultivation requires strict sterile conditions while maintaining SC-Exos characteristics and activity stability. Researchers attempt to increase SC-Exos yield in industrial production through genetic engineering, parental cell pretreatment, 3D culture, and culture material optimization to reduce batch variation and contamination risk.[292] However, these techniques might alter SC-Exos biological functions in ways not fully understood, presenting potential risks. Consequently, the actual effects of these methods in scale-up production and clinical applications remain difficult to predict.

Regarding administration routes and dose optimization, different delivery methods (such as intravenous injection, local injection, and intranasal inhalation) and dosages significantly affect SC-Exos distribution and metabolism in vivo.[293–295] Studies depict that in SOD1(G93A) mutant mouse models of amyotrophic lateral sclerosis, intravenous injection and intranasal administration of ADSC-Exos significantly improve motor function, protect lumbar spinal motor neurons, neuromuscular junctions, and muscle tissue while reducing neuroglial cell activation levels. However, compared to intravenous injection, intranasal inhalation exhibits clear advantages in reducing astrocyte activation and targeting damaged areas, enabling more concentrated delivery of ADSC-Exos to lesion areas, with noninvasive and simple operation characteristics, demonstrating higher clinical translation potential.[295] Nevertheless, systematic research on the effects of different administration routes on SC-Exos efficacy is lacking, hindering in-depth exploration in this field. Additionally, regarding SC-Exos dosage, significant differences exist in dose calculation methods across studies, challenging direct efficacy comparisons between doses.[25] Accordingly, rational design of administration routes and dosing strategies is crucial for ensuring SC-Exos safety and efficacy.

Finally, biosafety issues require particular attention. Although SC-Exos are widely considered to have high biosafety, some studies indicate their dual role in cancer treatment. They may promote tumor growth, angiogenesis, and metastasis by delivering specific miRNAs (such as miRNA-21 and miRNA-34a) and proteins while potentially inhibiting tumor growth through signaling pathway regulation. Their effects closely relate to the culture conditions and source tissues, necessitating further standardized research to clarify their mechanisms and application strategies.[296] For instance, human

BMSC-Exos have been found to significantly promote osteosarcoma (MG63) and gastric cancer (SGC7901) cell migration and proliferation through activation of the Hedgehog signaling pathway.[297] Moreover, a critical knowledge gap exists regarding the long-term safety and immunogenicity of repeated SC-Exos administrations, particularly for chronic conditions requiring multiple treatments such as cardiovascular diseases, neurological disorders, autoimmune diseases, cancer, reproductive disorders, and other chronic inflammatory conditions. Reproductive applications present additional complexity due to potential off-target effects on hormonal regulation and gamete quality, requiring specialized safety assessments to evaluate impacts on fertility and long-term reproductive health. Although short-term studies have demonstrated the safety of repeated dosing in preclinical models, with daily BMSC-Exos administration for 14 consecutive days showing no adverse effects,[298] clinical data on repeated human exposure remains extremely limited. Despite some clinical studies involving repeated dosing, comprehensive long-term safety data remains extremely limited, with most studies having follow-up periods ranging from a few weeks to 6 months, which is insufficient for evaluating chronic treatment regimens.[25] Critically, no studies have specifically examined the potential for adaptive immune responses, neutralizing antibody formation, or immune memory development against SC-Exos surface proteins and cargo molecules that could compromise treatment effectiveness in subsequent administrations. Therefore, systematic evaluation of SC-Exos mechanisms under different pathological backgrounds and comprehensive long-term safety studies are essential to reduce the risks associated with clinical use.

In conclusion, while SC-Exos demonstrate enormous potential in cell therapy, their clinical translation faces significant challenges across preparation and quality control, therapeutic heterogeneity, scale-up production, administration routes and dose optimization, and biosafety assessment. Addressing these challenges requires multicenter large-scale clinical trials with standardized protocols, comprehensive safety evaluation, and long-term follow-up studies to ensure the safe and effective implementation of SC-Exos in clinical practice.

Summary and Perspectives

This comprehensive review has systematically examined the current state of SC-Exos research, revealing significant advances in understanding their biological mechanisms, engineering optimization strategies, therapeutic applications, and clinical translation progress across diverse biomedical domains. This analysis demonstrates that SC-Exos represent a transformative therapeutic platform that inherits the regenerative properties of parent stem cells while circumventing key limitations associated with direct cell transplantation, including reduced immunogenicity, enhanced stability for storage and transport, and circumvention of many safety concerns associated with whole-cell therapies.

The biological foundation of SC-Exos has been substantially elucidated through examination of exosome biogenesis mechanisms, which revealed the operation of both ESCRT-dependent and ESCRT-independent pathways with significant cell-type specificity. Comparative analysis of different SC-Exos types, including iPSC-Exos, ESC-Exos, and ASC-Exos, demonstrates distinct therapeutic profiles that enable targeted clinical applications. Specifically, ASC-Exos, particularly MSC-Exos, emerge as the most clinically viable option due to their established safety profiles, scalable production methods, and broad therapeutic versatility across cardiovascular, neurological, and regenerative medicine applications. In contrast, while ESC-Exos and iPSC-Exos show superior cardiovascular protective mechanisms, their clinical translation faces regulatory challenges and safety concerns that extend development timelines.

Functional optimization strategies have emerged as crucial approaches for enhancing SC-Exos therapeutic potential, addressing limitations of limited natural yield and complex variable composition that pose challenges for clinical translation. These strategies encompass three complementary approaches with distinct optimization objectives. Parental cell intervention strategies focus on modulating the biological state of stem cells through signaling pathway activation, hypoxic preconditioning, and specific factor stimulation to enhance both exosome secretion volume and functionality.[141–143] Physicochemical performance optimization through biomaterial integration addresses challenges related to exosome stability, controlled release kinetics, and bioavailability while preserving intrinsic molecular cargo.[144–148] Intrinsic therapeutic efficacy enhancement strategies directly augment the biological activity and therapeutic specificity of SC-Exos through molecular engineering, surface functionalization, and targeted delivery optimization. These approaches include specific miRNA loading (miR-30c, miR-181b, miR-613) for targeted cancer therapy and integration with conductive biomaterials for enhanced tissue regeneration through optimized cellular signaling pathways.[149–152] Building upon these

optimization strategies, the comprehensive biomedical engineering applications of SC-Exos demonstrate remarkable therapeutic versatility across diverse medical domains. Through systematic integration of tissue engineering scaffolds, advanced drug delivery platforms, and targeted therapeutic interventions, SC-Exos have shown significant efficacy in cardiovascular diseases, neurological disorders, cancer treatment, immune regulation, inflammation-related diseases, reproductive health, musculoskeletal repair, dermatological applications, and numerous other therapeutic areas including retinal diseases, liver fibrosis, and pulmonary disorders.

The clinical translation landscape reveals promising progress with documented trials across multiple therapeutic domains, including gastroenterology,[261–263] neurology,[264] dermatology,[265–269] pulmonology,[270,271] and ophthalmology.[272] These trials consistently demonstrate favorable safety profiles with only mild, transient adverse events and no treatment discontinuations, supporting the therapeutic potential of SC-Exos. However, current clinical evidence is limited by small patient cohorts, variable follow-up periods, and substantial heterogeneity in preparation methods and dosing regimens. Paralleling these clinical developments, the expanding commercial interest is reflected in robust patent activity across multiple domains, indicating the field's transition from basic research toward clinical translation and commercialization. Patent analysis reveals innovation activity across therapeutic applications such as neurodegenerative diseases, cardiovascular disorders, oncological treatments, wound healing, orthopedic conditions, and various other clinical indications. Manufacturing and optimization patents focus on improving exosome production efficiency and quality, including optimized cell culture conditions, large-scale bioreactor systems, and novel purification techniques. Advanced delivery technologies represent a major innovation focus, encompassing targeted SC-Exos with surface modifications, hydrogel-based sustained release systems, and nanoparticle hybrid systems. Patents encompass diverse stem cell sources, with mesenchymal stem cells and iPSCs being most prominent, suggesting movement toward more standardized and scalable cell sources. This extensive patent landscape demonstrates strong commercial momentum with particular emphasis on anti-aging therapies, wound healing products, and neurological disease treatments.

Critical challenges that must be addressed for successful clinical implementation reflect those identified through detailed analysis of current limitations. In isolation and purification, the lack of standardized methods and quality control systems leads to considerable variations in yield and purity, with limited comparability between studies. In functional characterization, existing technologies struggle to comprehensively reflect SC-Exos biological functions, and the evaluation standards for functional molecules remain unclear. Moreover, therapeutic heterogeneity across different donor sources, production conditions, and disease models increases clinical application uncertainty. In scale-up production, high technical requirements for stem cell cultivation, batch variation, contamination risks, and biological activity stability issues have hindered the development of mature industrial production systems. Furthermore, significant exploration remains needed in administration routes, dose optimization, and targeting improvement, while biosafety concerns, particularly potential tumor-promoting effects as demonstrated by human BMSC-Exos significantly promoting osteosarcoma and gastric cancer cell migration, pose elevated requirements for clinical applications.[297]

To advance SC-Exos clinical translation, future research must address several key scientific challenges through systematic approaches. First, unified standards for isolation and purification should be established to develop efficient, low-cost, and high-purity isolation techniques with comprehensive quality control systems to improve product consistency and comparability. Second, comprehensive functional evaluation platforms should be constructed with clear assessment standards for key functional molecules, accompanied by systematic studies evaluating immune responses and long-term biosafety. Additionally, deeper exploration of SC-Exos mechanisms and the effects of different stem cell sources and culture conditions is essential to optimize production processes and improve therapeutic consistency. Industrial-scale stem cell cultivation and exosome isolation platforms must be developed for scale-up production to ensure product characteristics and functional stability. Optimization of administration routes and dosing represents a crucial requirement that necessitates systematic comparison of the efficacy and safety of different delivery methods. Finally, multicenter large-scale clinical trials with rational design are essential for validating SC-Exos efficacy and safety across diverse patient populations.

The convergence of advancing biotechnology, regulatory pathway clarification, and growing clinical evidence positions SC-Exos as a promising therapeutic platform for biomedical applications. As this novel therapeutic strategy continues to demonstrate significant potential, SC-Exos research and clinical applications are expected to expand rapidly

alongside technological breakthroughs and advancing fundamental research. Current challenges in the field may be addressed through interdisciplinary collaboration and continued technological innovation, ultimately enabling widespread clinical implementation of SC-Exos therapies and providing innovative solutions for complex medical conditions.

Data Sharing Statement

No data was used for the research described in the article.

Ethics Approval and Consent to Participate

No ethical approvals or consent forms are needed.

Acknowledgments

We thank Home for Researchers editorial team (www.home-for-researchers.com) for language editing service. Graphical abstract Created in BioRender. Zhang, J. (2025) https://BioRender.com/xmv4jj3

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the National Natural Science Foundation of China (Grant No.: 82274325) and the Sichuan Science and Technology Program (Grant No.: 2024NSFSC0684).

Disclosure

The authors report no conflicts of interest in this work.

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