


The Application and Challenges of Stem Cell-Derived Extracellular Vesicles in Inhalation Lung Injury

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Abstract: Inhalation-induced lung injury, caused by harmful factors like chemical fumes and dust, leads to acute and chronic inflammation and fibrosis. Traditional treatments, such as mechanical ventilation and anti-inflammatory drugs, can relieve symptoms but fail to promote tissue regeneration. Stem cells and their extracellular vesicles (EVs) offer new treatment possibilities due to their anti-inflammatory and regenerative properties. However, the specific pathological environment of these lung injuries limits the effectiveness and targeting of EVs, challenging their clinical use. This review outlines stem cell EVs' mechanisms in treating inhalation-induced lung injury, examines recent engineering advancements, and addresses challenges in moving from research to clinical application. It highlights the importance of interdisciplinary collaboration in carrier design, production, and regulation, offering a theoretical foundation for developing precision EV-based treatments.

Keywords: stem cells, extracellular vesicles, inhalation lung injury, optimization strategy, clinical translation

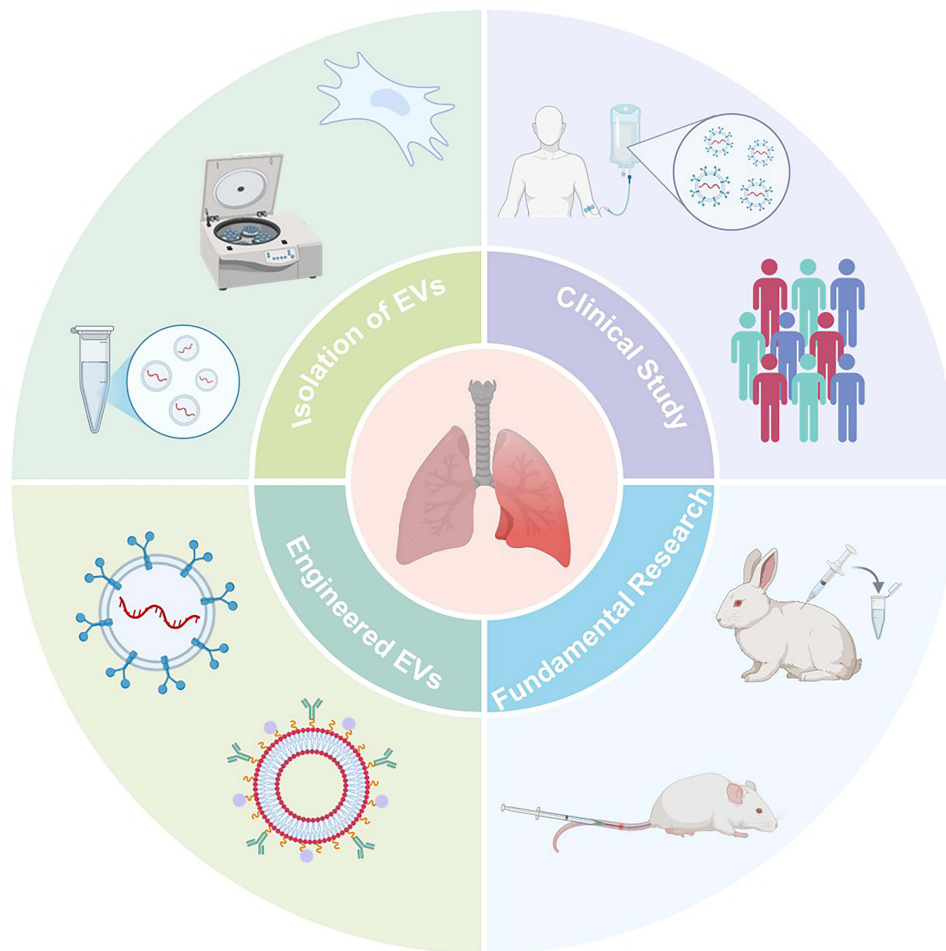
Introduction

Inhalation lung injury represents a prevalent and severe condition within medical practice, characterized by a complex pathogenesis and challenging treatment modalities. This condition may arise from incidents such as fires, industrial accidents, and exposure to hazardous environments, including chemical leaks, elevated dust concentrations, pathogen aerosols, and high-temperature gases or shock waves generated by explosions, all of which can result in varying degrees of respiratory tract damage (Figure 1). Pathological investigations have demonstrated that this disease follows a distinct biphasic progression pattern: the acute phase, occurring within 72 hours post-injury, is predominantly marked by uncontrolled inflammatory cascade reactions and the disruption of the alveolar-capillary barrier; the chronic phase, extending from several weeks to several months, is characterized by progressive pulmonary fibrosis and irreversible structural remodeling of the tissue.¹⁻³ Epidemiological data from the US National Burn Database indicate that among approximately 40,000 burn patients hospitalized annually, 5% also suffer from inhalation lung injury; the mortality rate for these patients increases by 60% after they develop pneumonia,⁴⁻⁶ underlining the serious clinical challenges posed.

Current clinical treatments for inhalation-induced lung injury mainly involve supportive measures like mechanical ventilation and broad-spectrum anti-inflammatory drugs.¹ While these approaches temporarily stabilize patients, they face two key limitations: first, they fail to regulate the pro-/anti-inflammatory balance during the acute phase; second, they cannot halt the vicious cycle of abnormal extracellular matrix deposition in chronic stages.^{7,8} Crucially, traditional therapies do not effectively promote alveolar epithelial regeneration or functional lung unit reconstruction, often resulting in long-term respiratory impairment and diminished quality of life for survivors.^{8,9}



Graphical Abstract



Recently, mesenchymal stem cells (MSCs) therapy has emerged as a promising approach for lung injury repair due to its notable tissue repair and immunomodulatory properties.^{10,11} While MSCs promote tissue regeneration via paracrine mechanisms, challenges including immune rejection, tumorigenic risk, and poor survival limit the efficacy of stem cell transplantation.^{12–15}

Research indicates that the therapeutic effects of stem cells are largely mediated by their secreted EVs, particularly exosomes. These EVs can replicate stem cell functions while offering advantages like high stability, low immunogenicity, and ease of storage/delivery, providing a novel cell-free approach for treating lung damage.¹⁶ MSC-derived EVs inherit their parent cells' therapeutic properties and possess three distinct advantages: 1) nanoscale size enhances tissue penetration;¹⁷ 2) a phospholipid bilayer protects cargo stability;¹⁸ 3) low immunogenicity minimizes rejection risks.^{15,19–21} The repair mechanisms of stem cell EVs in lung injury include: 1) regulating macrophage polarization to suppress excessive inflammation;²² 2) promoting alveolar epithelial and vascular endothelial cell proliferation while reducing apoptosis;²³ 3) alleviating oxidative stress damage;²⁴ 4) inhibiting fibroblast activation and slowing pulmonary fibrosis progression.²⁵ Enhancing vesicle targeting and efficacy can be achieved by genetic engineering or chemical modification.^{18,26}

Despite the therapeutic potential of stem cell-derived EVs in preclinical studies, their clinical translation faces significant challenges. These include standardizing large-scale production, determining optimal administration routes,

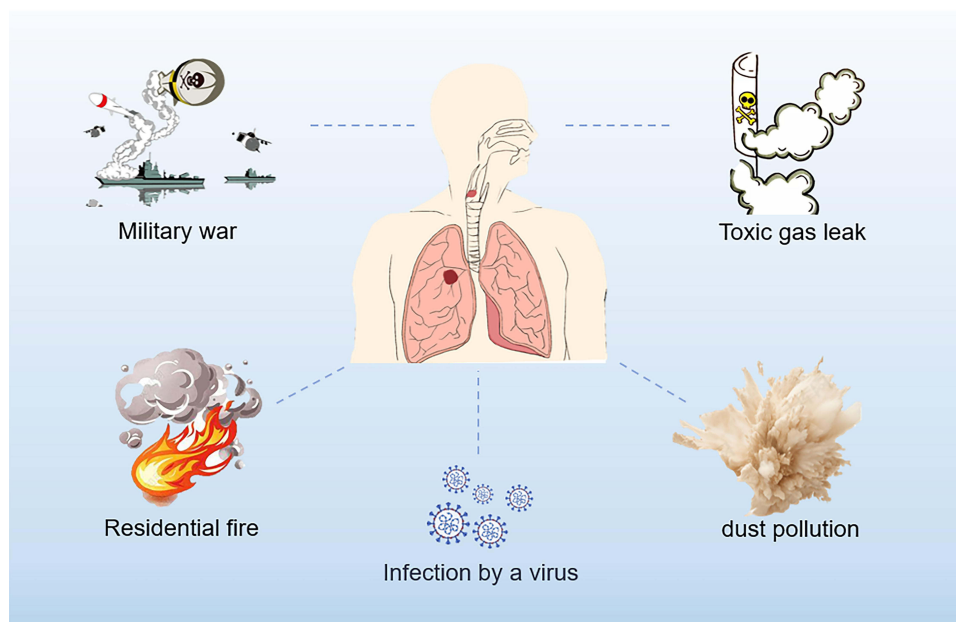


Figure 1 External factors contributing to inhalation lung injury.

and assessing long-term safety. Additionally, the distinct pathological microenvironment of inhalation-induced lung injury poses multiple barriers: during the acute phase, reactive oxygen species (ROS) can degrade EVs functional components, while the dense fibrotic matrix in chronic phases severely impedes targeted EVs delivery.^{4,27–29} To address these challenges, this review establishes a “spatiotemporal dynamic regulation” framework, systematically exploring: 1) EVs molecular mechanisms across pathological stages; 2) recent advances in engineered modification strategies; and 3) clinical translation bottlenecks. By integrating multi-omics data and advanced nanotechnology, we emphasize smart responsive EVs carrier design principles to inform precision treatment strategies. Finally, we advocate establishing a “basic-translational-clinical” R&D continuum to streamline EVs therapy translation from bench to bedside.

Biological Characteristics of Stem Cell EVs

Stem cells possess the capability to self-renew, differentiate into multiple types, and regenerate tissues that are functional.³⁰ Based on their differentiation potential, they are classified into totipotent, pluripotent, multipotent, oligopotent, and unipotent stem cells. Pluripotent stem cells include embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), while adult multipotent stem cells comprise hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), neural stem cells (NSCs), and endothelial progenitor cells (EPCs).¹⁵ These subtypes are widely used in clinical trials for treating human diseases. This review focuses on EVs derived from MSCs for treating lung injuries caused by inhaled toxins.

MSCs are adult multipotent stem cells present in bone marrow, umbilical cord, placenta, adipose tissue, and dental pulp (Figure 2A). These non-terminally differentiated cells exhibit features of both mesenchymal cells and endothelial or epithelial cells. Their multipotent differentiation capacity enables them to stimulate growth, survival, and repair in other cells, making them a subject of significant interest.³¹ Nonetheless, certain trials have not demonstrated clinical advantages, possibly because of the intrinsic limitations of stem cell therapy, such as infusion toxicity, immune response, and the potential to cause tumors. Notably, MSCs-derived EVs can directly interact with target cells, acting as intercellular communication mediators, and can be engineered as drug carriers to deliver miRNAs and small-molecule drugs. Importantly, they are associated with minimal risks of immune reactions and tumor formation³² (Figure 2B). Through multiple pathways, these agents modulate the biological behavior of target cells, thereby regulating physiological homeostasis and/or the progression of human diseases.³³

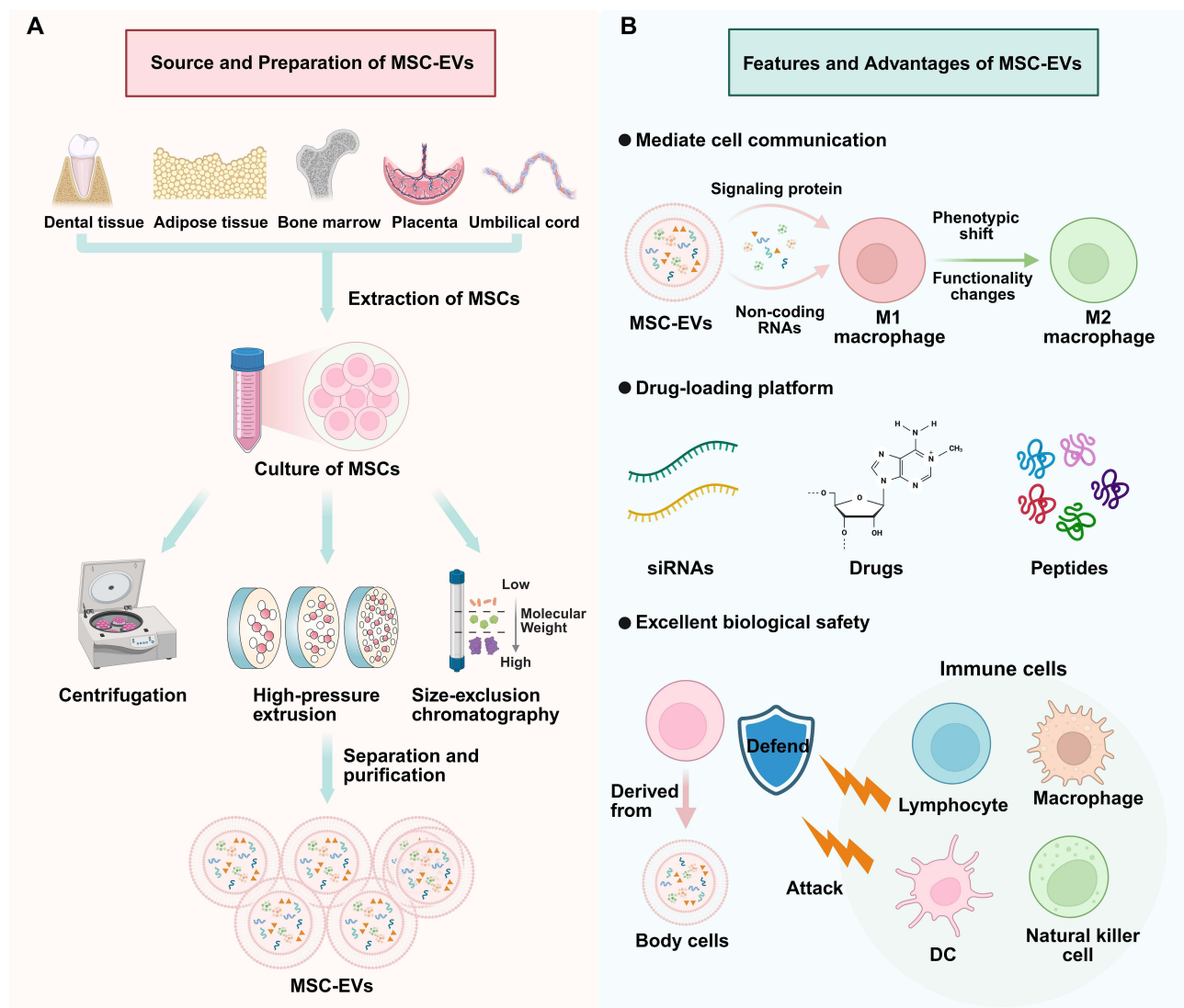


Figure 2 The origin and biological characteristics of extracellular vesicles. **(A)** The sources of EVs and their purification and separation preparation process; **(B)** the characteristics and advantages of EVs: extracellular vesicles have excellent biological safety and can be used as a medium for cell communication and a carrier platform for drug delivery.

MSC-EVs, which are small vesicles with biological activity, are secreted by MSCs and have a double-layer lipid membrane structure.³⁴ They carry numerous proteins associated with membrane transport and fusion, including adhesion proteins, ESCRT components, and Rab-GTPases. Additionally, EVs contain common membrane surface markers such as tetraspanins (CD9, CD63, CD81, CD82) and heat shock proteins (HSP70), along with various miRNAs/mRNAs, metabolites, growth factors, and cytokines.^{35,36} EVs are nanoscale particles actively secreted by cells. Based on their biogenesis pathways, size, contents, and functions, EVs are broadly categorized into three types: apoptotic bodies (50–2000 nm), microvesicles (50–1,000 nm), and exosomes (40–160 nm).^{37–39} The overlapping size ranges among these categories underscore the limitations of this classification system.⁴⁰

The vesicles cited in several studies are essentially referring to the broader category of EVs.⁴¹ As stated in the 2023 Minimal information for studies of EVs (MISEV2023),³⁹ EVs refers to particles that are released from cells, are delimited by a lipid bilayer, and cannot replicate on their own. Exosomes are a subtype of EVs that differ in their biogenesis from microvesicles and apoptotic bodies. They originate from the endosomal pathway, a process through which multivesicular bodies (MVBs) are formed and subsequently released from the cell. MVBs can either integrate with

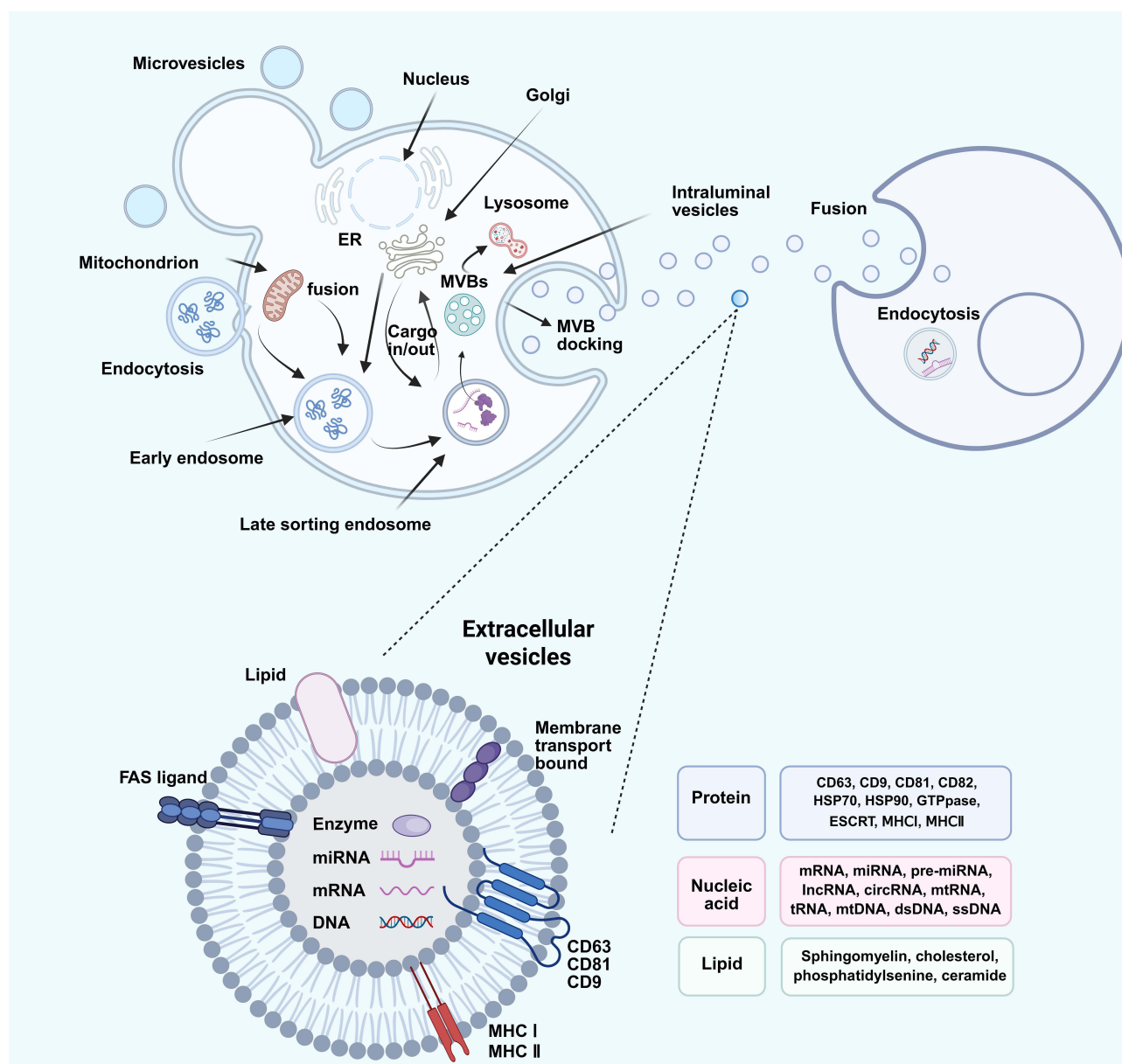


Figure 3 The generation process and molecular composition of EVs. Generation process: Various extracellular components, including proteins and lipids, are internalized to form early endosomes; early endosomes differentiate into late endosomes, forming multivesicular bodies (MVBs); MVBs fuse with microtubules and the cytoskeleton and then fuse with the plasma membrane to form EVs. The remaining MVBs are transported and fuse with lysosomes to degrade their cargo. EVs enter recipient cells through various pathways, including endocytosis, uptake, phagocytosis, and fusion.

lysosomes for degradation or with the cell membrane to release EVs into the extracellular space, depending on their final outcome^{16,42,43} (Figure 3).

Compared to conventional stem cell therapy, MSC-EVs exhibit superior clinical translation potential. Their nanoscale size and lipid bilayer structure enable efficient penetration of the pulmonary capillary network and blood-air barrier while avoiding the embolism and tumorigenesis risks associated with live cell infusion. Furthermore, MSC-EVs maintain biological activity during long-term storage at -80°C , providing critical assurance for clinical emergency use.^{44–46}

The Molecular Mechanism of Stem Cell EVs Repairing Inhalation Lung Injury

Although EVs derived from stem cells (particularly MSCs) exhibit significant advantages in treating inhalation lung injury, critical challenges remain in practical application. This progression occurs in two phases: the acute inflammation

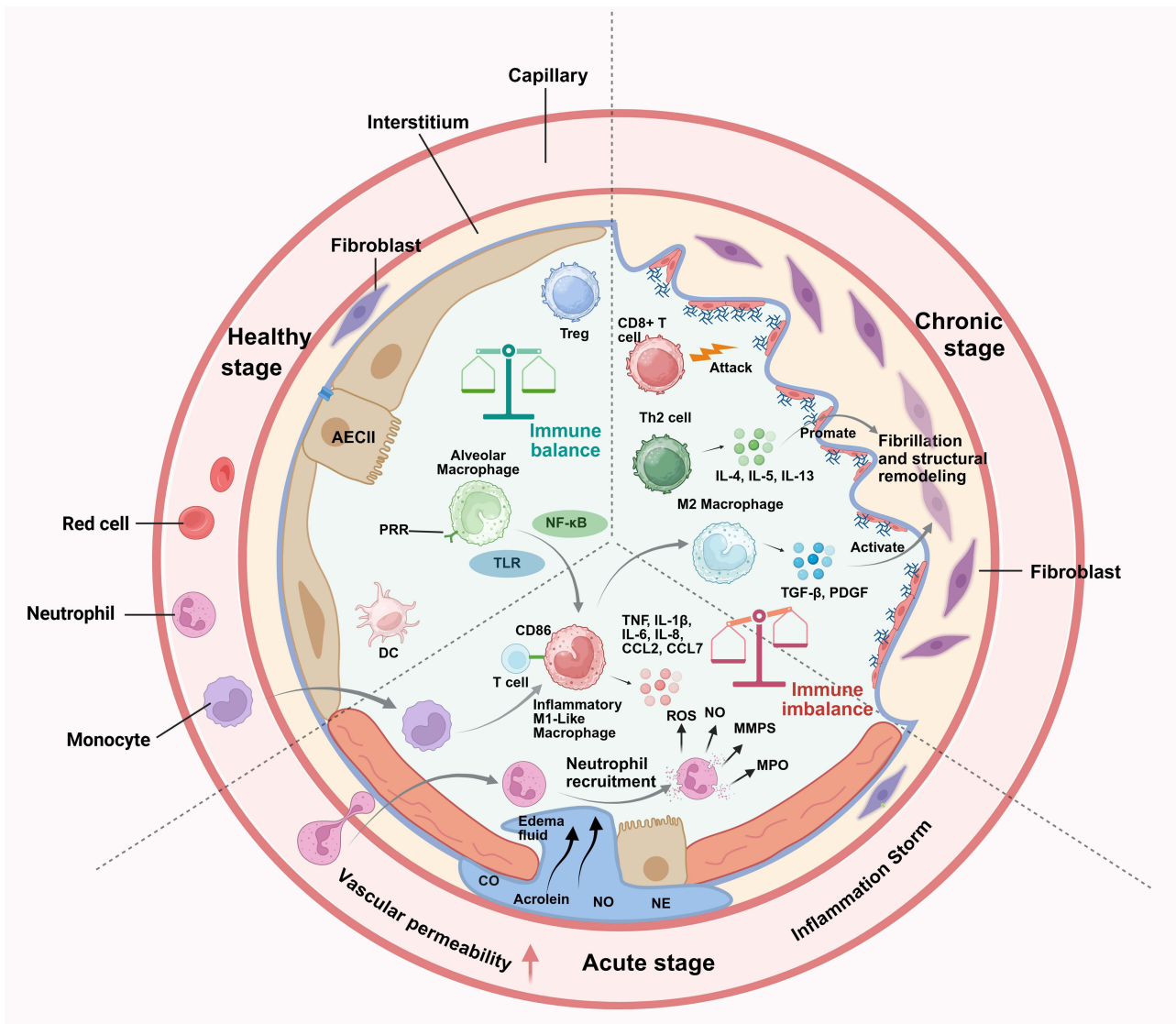


Figure 4 Schematic diagram of the therapeutic effects of EVs in the treatment of inhalation-induced lung injury. During the acute phase, EVs inhibit inflammatory cascades through multi-targeted interventions; during the chronic phase, they exert their effects by targeting and inhibiting signaling pathways such as TGF- β 1/Smad3d; eNOS: endothelial nitric oxide synthase.

stage and the chronic fibrosis stage. The pathological microenvironments of these two stages differ significantly, necessitating MSC-EVs to exert stage-specific reparative effects by regulating molecular mechanisms across multiple dimensions (Figure 4). Elucidating the mechanisms of MSC-EVs in distinct disease stages is fundamental to optimizing therapeutic strategies and advancing clinical translation.

Acute Phase of Inhalation Lung Injury

Acute lung injury (ALI) is defined as damage to the alveolar epithelium and capillary endothelium resulting from direct or indirect injury, which leads to diffuse pulmonary edema and acute hypoxic respiratory failure. Its pathological hallmarks include bronchial mucosal edema, increased permeability of the alveolar-capillary barrier, prominent neutrophil infiltration, extensive release of inflammatory mediators, and severe oxidative stress. Inhalation lung injury occurs following exposure to toxic agents, with the acute phase typically developing within days. However, the time to onset may vary depending on the type and concentration of the inhaled toxin; highly toxic agents at high concentrations can induce injury within hours.⁴ Toxic substances in smoke, such as carbon monoxide, can directly damage airway epithelial

cells and activate Toll-like receptors (TLRs) and the nuclear factor kappa-B (NF- κ B) signaling pathway. This activation leads to the massive release of pro-inflammatory factors, including IL-6 and TNF- α . These factors recruit neutrophils, triggering a “respiratory burst” that produces ROS and myeloperoxidase (MPO) proteases, thereby exacerbating tissue damage.⁴⁷

At this stage, EVs inhibit inflammatory cascades through multi-targeted interventions. Bone marrow mesenchymal stem cell-derived EVs deliver miR-146a and miR-181c, which target and suppress the TLR4/NF- κ B pathway. This reduces IL-8 and TNF- α expression while decreasing neutrophil chemotaxis and infiltration.^{48–51} Additionally, EVs transport enriched superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), enzymes that directly scavenge ROS and thereby alleviate oxidative stress-induced damage.^{52,53} Zhao et al demonstrated that inhalation of MSC-EVs in LPS-stimulated RAW264.7 cells and an ALI mouse model contributes to the efficacy of MSC-EVs in treating LPS-induced ALI, macrophage polarization, and Nrf2-mediated antioxidant signaling. The study showed that MSC-EVs significantly downregulated pro-inflammatory pathways (eg, TLR4/NF- κ B) and upregulated the Nrf2/HO-1 antioxidant axis, reducing oxidative damage markers including 8-OHdG. These findings suggest that MSC-EVs exert dual therapeutic effects through modulating inflammation and oxidative stress⁵⁴ (Figure 5). In a mouse model of silica-induced lung injury, EVs promote macrophage polarization toward the M1 phenotype via miR-223-3p delivery, inhibit IL-1 β and TNF- α secretion, and reduce inflammation and pulmonary fibrosis severity.⁵⁵

Chronic Phase of Inhalation Lung Injury

Long-term exposure to harmful substances can induce persistent lung tissue inflammation. Repeated inflammatory stimulation and aberrant repair processes drive continuous fibrous tissue proliferation, advancing to the chronic phase. The fibrotic stage is characterized by fibroblast activation, excessive extracellular matrix (ECM) deposition, and substantial alveolar structural damage.^{4,56} Pulmonary fibrosis is primarily driven by persistent activation of the TGF- β 1/Smad signaling pathway, which induces fibroblast-to-myofibroblast transformation and enhances collagen and fibronectin synthesis.⁵⁶ The TGF- β /Smad pathway exerts bidirectional effects in EV-mediated fibrosis regulation: low TGF- β 1 concentrations promote tissue repair, whereas high concentrations exacerbate fibrosis, warranting further validation of EV dose-dependency.^{57–59}

EVs counteract this vicious cycle through the delivery of anti-fibrotic molecules. Studies demonstrate that EVs derived from bone marrow mesenchymal stem cells (BMSCs-EVs) significantly suppress fibroblast activation and proliferation, mitigating pathological changes associated with pulmonary fibrosis. Zhou and his team discovered that BMSC-EVs are loaded with miR-186, which is markedly lower in the lung tissues of patients diagnosed with idiopathic pulmonary fibrosis (IPF). EV-delivered miR-186 is internalized by pulmonary fibroblasts and acts by directly targeting and suppressing the transcription factor SOX4. This effectively blocks fibroblast activation, migration, and invasion while promoting apoptosis, ultimately attenuating histological damage and collagen deposition in a mouse model of pulmonary fibrosis⁶⁰ (Figure 6). In another study, three-dimensionally cultured human umbilical cord mesenchymal stem cell exosomes (UCMSC-EVs) were shown to effectively inhibit pulmonary fibrosis progression in a silicosis model. Xu et al showed that UCMSC-EVs target lung tissue and internalize into fibroblasts, significantly reducing collagen I (COL1A1) and fibronectin (FN) expression at both gene and protein levels, and improving lung function. The mechanism likely involves exosome-carried active components regulating downstream fibrotic signaling in the TGF- β /Smad pathway, providing experimental evidence for EV-based anti-fibrotic therapy.⁶¹

Dynamic Regulation: Integrated Effects from Acute to Chronic Conditions

The therapeutic benefit of EVs stems from their capacity to modify target sites in response to the changing microenvironment of injured tissues.^{62,63} During the acute phase of high oxidative stress, CD44 and integrins on EVs surfaces selectively bind to injured endothelial cells, rapidly releasing antioxidant enzymes;^{62,64} while in the chronic phase, EVs target fibrotic regions and regulate fibroblast metabolism by delivering miRNAs.⁶⁵ This spatiotemporal specificity arises from the heterogeneous nature of EVs cargo and their microenvironment-responsive release. Recent studies also indicate that engineering EVs surface ligands (eg, CXCR4) can enhance their targeting efficiency to injured lung tissue,

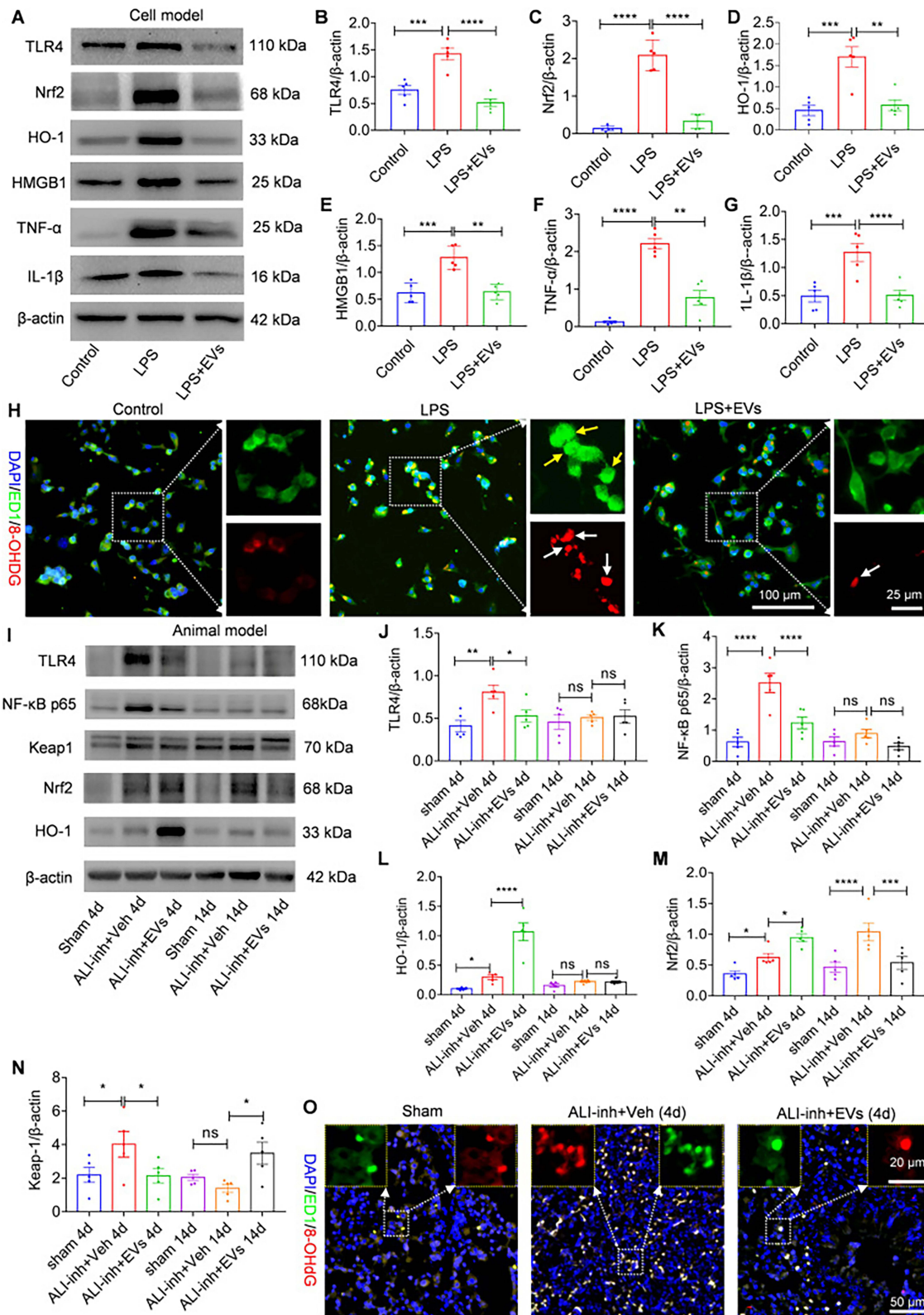


Figure 5 The influence of MSC-EVs on macrophage polarization in LPS-stimulated cell and animal models. ns: no significance. ^{ns} P > 0.05, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. (A) Regulation of Key Proteins by MSC-EVs. (B–G): Inflammatory Factors Suppressed, Antioxidant Proteins Activated. (H) MSC-EVs Reduce Oxidative Stress. Representative images display double immuno-staining with ED1 (macrophage marker, green) and 8-OHdG (oxidative stress marker, red) in each group. Left image of each group, bar = 100 μm; right image of each group, bar = 25 μm. (I): Dynamic Changes in Proteins In Vivo. (J–N): MSC-EVs Inhibit TLR4/NF-κB and Activate Nrf2 Pathway. (O): MSC-EVs mitigate oxidative damage in vivo, Bottom image of each group, bar = 50 μm; top image of each group, bar = 20 μm. (A–O). Come from Ruijing Zhao, Lina Wang, Tian Wanget al, Inhalation of MSC-EVs is a noninvasive strategy for ameliorating acute lung injury. J Control Release. 2022; 345: 214–30. © 2022 The Authors. Published by Elsevier B.V. Creative Commons CC-BY-NC-ND.⁵⁴

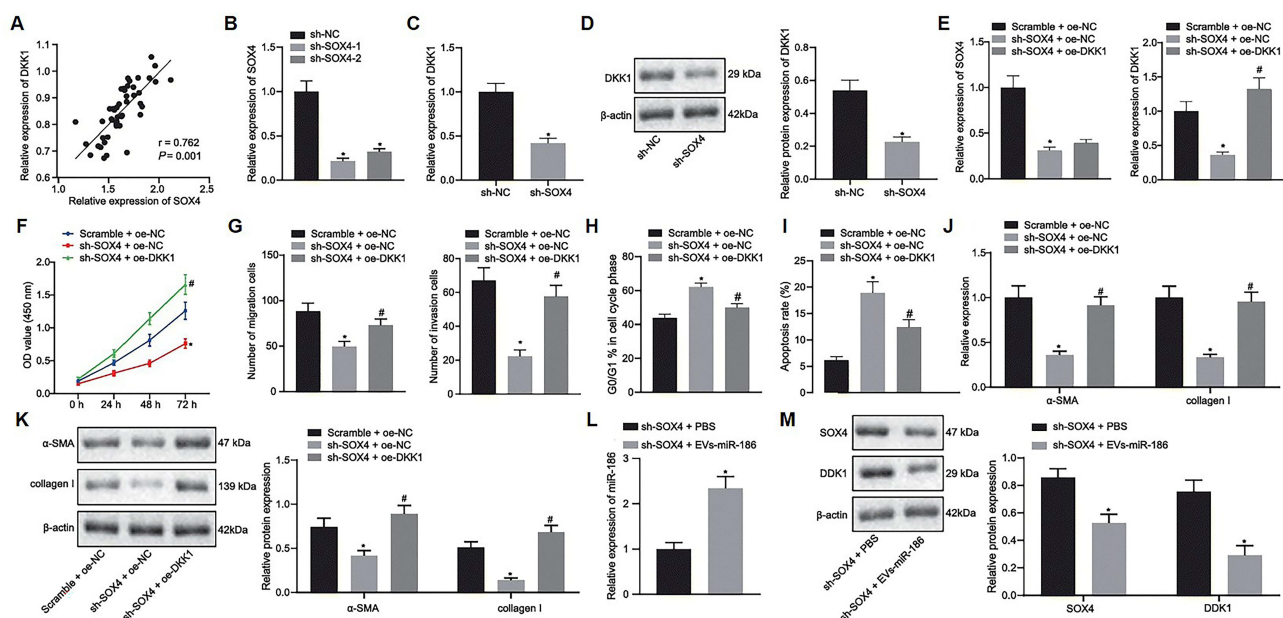


Figure 6 EV-delivered miR-186 decreases the expression of DKK1 through targeting SOX4 to impair fibroblast activation. *p < 0.05 vs the fibroblasts co-transfected with scramble and oe-NC; #p < 0.05 vs the fibroblasts co-transfected with sh-SOX4 and oe-NC. **(A)** DKK1 expression positively correlates with SOX4 expression. **(B-E)** Validates the regulatory axis of SOX4-DKK1. **(F-K)** Reveals its functional role in fibroblast activation. **(L-M)** Exosome-delivered miR-186 mediates this axis. **(A-M)** come from Zhou J, Lin Y, Kang X, Liu Z et al, *Microrna-186 in extracellular vesicles from bone marrow mesenchymal stem cells alleviates idiopathic pulmonary fibrosis via interaction with sox4 and dkk1*. *Stem Cell Res Ther.* 2021; 12: 96. © 2023 The Authors. This work is published and licensed by Springer Nature limited. Minor adjustments have been made to the order of the content. The full terms of this license are available at <http://creativecommons.org/licenses/by/4.0/>.⁶⁰

improving therapeutic outcomes.⁶⁶ Table 1 summarizes studies on the application of stem cell-derived EVs for treating inhalation lung injuries.

EVs exhibit broad repair capabilities across pathological stages through the synergistic action of multiple components. Inhalation-induced lung injury is characterized by pathological changes, including a thickened mucous layer and

Table 1 Research on EVs Derived from Stem Cells in the Treatment of Inhalation Lung Injury

Inhalation Lung Injury	Sources	Key Molecules/Mechanisms	Target	Function	References
Wood smoke-induced ALI	BMSC-EVs	Inhibits the NF-κB pathway; downregulates proinflammatory factor levels	Macrophages, alveolar epithelial cells	Alleviate inflammation and repair alveolar barrier function.	[51]
LPS-ALI	UCMSC-EVs	miR-124-3, Ang-1 mRNA	Macrophages, pulmonary vascular endothelial cells	Reduce alveolar permeability and improve oxygenation	[67]
LPS-ALI	UCMSC-EVs	Reduce pro-inflammatory factors and enhance anti-inflammatory factors	Alveolar macrophages	Mitigate histopathological damage in pulmonary tissues	[54]
COPD (elastase-induced emphysema)	Human AD-MSC-derived artificial nanovesicles	FGF2-dependent pathway	Alveolar epithelial cells	Induce proliferation of alveolar epithelial cells and have regenerative effects at lower doses	[68]

(Continued)

Table 1 (Continued).

Inhalation Lung Injury	Sources	Key Molecules/ Mechanisms	Target	Function	References
Silica-induced pulmonary fibrosis	UCMSC-EVs	miR-26a-5p	Alveolar epithelial cells, fibroblasts	Attenuates silica-induced lung inflammation and fibrosis	[69]
Phosgene-induced ALI	MSC-EVs	Inhibits MMP-9 and upregulates SP-C levels	Type II alveolar epithelial cells	Alleviate pulmonary edema and facilitate the repair of alveolar epithelial cells.	[70]
Silica-induced lung injury	UCMSC-EVs	miR-223-3p	Macrophage	Alleviate silica-induced lung inflammation and fibrosis	[55]
LPS-ALI	BMSC-EVs	Mitochondrial transfer	Alveolar epithelial cells, endothelial cells	Rescue mitochondrial dysfunction, improve alveolar-capillary barrier	[71]
E. coli pneumonia-induced ALI	BMSC-EVs	Enhance monocyte phagocytosis promote M2 polarization via COX2/PGE ₂	Alveolar epithelial cells, monocytes/macrophages	Reduce inflammation and lung permeability, decrease bacterial load	[72]
LPS-ALI	EPC-EVs	miR-126	Endothelial cells	Improved arterial oxygenation and lung histology, decreased lung edema	[73]
LPS-ALI	EPC-EVs	miR-126	Endothelial cells	Protects lung microvascular endothelial cells, reduces lung inflammation and edema	[74]
Hyperoxia-induced ALI	IPSC secretome	α Klotho, Nrf2 activation	Alveolar epithelial cells	Antioxidative protection, reduced oxidative damage	[75]
Bleomycin-induced pulmonary fibrosis	UCMSC-EVs	miR-486-5p	Alveolar macrophages, fibroblasts	Ameliorates fibrosis, restores lung volume, improves lung function	[76]
LPS-ALI	BMSC-EVs	ACE2, TLR4/MyD88, caspase-1	Alveolar epithelial cells, immune cells	Anti-inflammatory, reduces cell damage, improves barrier function	[49]
Silica-induced silicosis model	UCMSC-EVs	Downregulation of COL1A1 and FN expression	Pulmonary fibroblasts	Ameliorated silica-induced pulmonary fibrosis and respiratory dysfunction	[61]
Bleomycin-induced pulmonary fibrosis	BMSC-EVs	miR-186	Pulmonary fibroblasts	Inhibits fibroblast activation, migration, and invasion; promotes apoptosis; reduces collagen deposition	[60]
LPS-ALI	MSC-EVs	miR-23a-3p, miR-182-5p	Alveolar epithelial cells	Attenuate lung injury and pulmonary fibrosis	[77]

heightened oxidative stress. This unique pathological microenvironment can impair the targeting efficiency and functional stability of EVs.^{78,79} Elevated ROS concentrations degrade EVs surface proteins, compromising their stability; increased collagen components in the fibrotic matrix may impede EVs diffusion,⁸⁰ limiting bioavailability. Thus, optimizing EVs targeting efficiency and functional stability through engineering strategies is essential for refining therapeutic approaches and enabling clinical translation.

Optimization Strategies for EVs in Inhalation Injury Scenarios

The repair efficacy of EVs is closely linked to their intrinsic biological characteristics and targeting efficiency. Targeting the pathological features of inhalation-induced lung injury – such as oxidative stress, inflammatory cascade reactions, and

disruption of the air-blood barrier – a systematic treatment system can be constructed through three approaches: stem cell pretreatment, engineered modification of EVs, and optimization of drug delivery routes.

Stem Cell Pretreatment

Pre-treatment strategies for stem cells represent a key approach for enhancing EVs secretion and modulating their functions.^{81,82} Exposing progenitor cells to hypoxia, inflammatory factors (such as TNF- α or IL-1 β), or ROS significantly upregulates the expression of antioxidant enzymes (SOD3 and GPX4) and anti-inflammatory factors (TSG-6 and IL-10) within EVs.^{83,84} Studies have demonstrated that hypoxia pretreatment significantly elevates the content of HIF-1 α -mediated pro-repair proteins in EVs derived from MSCs.⁸⁵ Furthermore, advances in gene editing technology have enabled precise regulation of EVs contents. Introducing miRNA overexpression vectors into stem cells via lentiviral transfection yields EVs whose miRNAs effectively inhibit NF- κ B pathway activation in alveolar epithelial cells.^{86,87}

Modification of EVs

EVs can enhance disease treatment outcomes through two strategies: internal modification (eg, drug loading) and external modification (eg, surface modification).⁸⁸ For instance, genetic engineering to overexpress the CXCR4 receptor on EV surfaces enables homing to lung injury sites with elevated SDF-1 α levels, thereby mitigating lung damage.⁶⁶ Similarly, CD38 antigen receptor-modified MSCs-EVs improved targeting efficiency toward type II alveolar epithelial cells, alleviating pulmonary fibrosis.⁸⁹ However, these strategies present a critical trade-off: the introduction of exogenous proteins can fundamentally alter the natural EVs membrane composition and topology. This may disrupt the function of endogenous targeting ligands (eg, integrins) that mediate natural tissue tropism, potentially compromising the inherent homing capabilities of EVs even as new ones are introduced.^{90,91}

Biomaterial integration techniques, such as liposome fusion or surface ligand anchoring, can endow EVs with targeting capabilities for damaged sites. Conjugating peptides and nanobodies to EVs via enzymatic reactions can mitigate non-specific phagocytosis and increase in vivo circulation.^{92–94} A pivotal consideration here is the application of “stealth” coatings like polyethylene glycol (PEG) to evade immune clearance.⁹⁵ While PEGylation effectively prolongs circulation half-life by reducing opsonization and mononuclear phagocyte system uptake, it acts as a double-edged sword.^{96,97} The polymer chains create a steric barrier that can physically mask not only immunogenic epitopes but also the very surface proteins and glycans responsible for the EV’s innate targeting specificity.⁹⁶ This trade-off between enhanced bioavailability and diminished natural targeting efficiency necessitates a delicate balance in carrier design.⁹⁸ Furthermore, the chemical crosslinkers or synthetic lipid components used in these processes introduce novel immunogenic risks, such as complement activation or the generation of anti-PEG antibodies upon repeated administration.^{99,100}

Internally, EVs can be loaded with therapeutic cargo like hyaluronic acid for sustained-release.¹⁰¹ Nonetheless, the intrinsic cargo capacity of EVs is physically limited. Techniques to enhance loading (eg, electroporation, sonication) often compromise EVs membrane integrity, leading to aggregation and reduced yield.¹⁰² Consequently, achieving therapeutic drug concentrations frequently requires high EVs doses, making loading efficiency a significant bottleneck that can offset the benefits of engineered delivery.¹⁰³

From a translational perspective, scalability remains a paramount challenge. Techniques like lentiviral transfection, though precise, are labor-intensive and prone to batch-to-batch variability, hindering consistent, large-scale production of clinical-grade engineered EVs.¹⁰⁴ A critical comparison of strategies reveals that while genetic engineering offers high precision, it faces scalability hurdles; chemical methods provide flexibility but carry higher immunogenic risk.^{103,105} Therefore, the choice of modification must be guided by a holistic view of the intended application, weighing the imperative for enhanced efficacy against the constraints of biocompatibility, manufacturing feasibility, and regulatory pathways.

Optimizing the Route of Administration

Currently, EVs are primarily administered intravenously. However, intravenous delivery faces multiple biological barriers, including adsorption of EVs’ surface-targeted molecules by serum proteins in the bloodstream and mechanical entrapment in the pulmonary capillary bed.^{106,107} In patients with inhalation-induced lung injury, enhanced mucociliary clearance and

airway mucosal edema result in shorter retention times for EVs and reduced targeting efficiency.^{54,68,108–110} Conversely, nebulized inhalation allows for direct delivery to the airways, accessing damaged tissue.¹¹¹ It significantly enhances EVs' penetration through the mucus layer, increasing their deposition rate in the bronchi and lungs.^{112–114}

While unmodified EVs possess inherent therapeutic potential, their efficacy can be significantly enhanced through engineering for specific cell targeting. Research demonstrates that in chronic obstructive pulmonary disease models, unmodified EVs exhibit substantially shorter airway retention times compared to modified counterparts.^{90,115}

In summary, while engineering modifications have greatly enhanced the therapeutic potential of EVs, their clinical translation still faces three major challenges. Safety concerns involve the potential impact of exogenous components on biocompatibility, as well as the need for systematic evaluation of immunogenicity and in vivo metabolic behavior. Technologically, scaling up from laboratory to GMP-grade production poses challenges in reproducibility, purity, and cost control. Strategically, the absence of head-to-head comparisons among different modification approaches in terms of targeting, drug loading, stability, and efficacy impedes the selection of optimal solutions for specific applications.^{116,117} Moving forward, AI-driven design and high-throughput screening using organ-on-a-chip technologies could help balance functionality and safety, while also facilitating the development of scalable and standardized protocols.¹¹⁸

Preclinical Research and Challenges

In preclinical studies of EVs for inhalation-induced lung injuries, selecting relevant animal models and understanding the biological properties of EVs from different sources are crucial for clinical translation. However, current research challenges are primarily manifested in two key aspects: First, insufficient standardization of model systems creates a “species gap” in efficacy assessment, hindering the direct translation of findings from different species models to human clinical settings. Second, heterogeneity in cellular sources causes “batch variability” in therapeutic effects, compromising the consistency and reliability of EV treatment outcomes.

Diversity Analysis and Translational Applicability of Animal Models

In preclinical studies of inhalation lung injury, the choice of animal model directly influences the clinical translational value of experimental outcomes. The pathological mechanisms of smoke inhalation injury are complex, involving airway thermal injury, toxic gas-induced inflammatory cascades, oxidative stress, and tissue repair dysfunction. Consequently, selecting animal models with anatomical structures and physiological responses closely resembling humans' is crucial for addressing specific research questions.

Rodents, particularly mice and rats, are widely employed in mechanistic studies and gene-targeted therapy research owing to their highly editable genomes.^{119,120} Mouse models offer particular value for elucidating regulatory mechanisms of inflammatory signaling pathways such as NF- κ B and MAPK. However, they lack bronchial mucous glands and respiratory bronchioles, limiting their applicability in studies of mucus hypersecretion or airway remodeling.^{121–123} Conversely, guinea pigs exhibit a respiratory tract branching pattern closer to humans, with tracheal epithelium rich in goblet and ciliated cells, making them well-suited for modeling chronic airway disease pathology.^{124,125}

The anatomical similarity of sheep and pig lungs to human lungs makes them ideal models for assessing the clinical feasibility of treatment methods.^{126,127} Sheep bronchial mucus secretion characteristics, alveolar-capillary barrier function, and inflammatory response patterns closely resemble the pathological features observed in human burn patients with inhalation injuries. These similarities make sheep commonly used to assess airway obstruction, pulmonary edema, and the efficacy of mechanical ventilation interventions.¹²⁸ Pigs are commonly used to test drug delivery systems or biomaterial scaffolds because their thoracic cavity volume and cardiopulmonary parameters closely resemble those of humans.^{129,130} Non-human primates (eg, monkeys) exhibit tissue structures, physiological functions, and immune systems most similar to humans, making them ideal models for studying respiratory diseases and evaluating inhaled drugs or toxins (Table 2). However, large animal studies entail high costs and scarcity of targeted immunological reagents, limiting their utility in high-throughput screening.¹³¹ Ethical concerns and technical complexities further constrain their application in preclinical research.^{2,132–134}

Beyond species selection, model diversity is also evident in the methods used for model construction. Simple smoke exposure models enable control over the concentration of toxic components, such as acrolein and carbon monoxide, by

Table 2 Comparison of Respiratory System Compatibility Between Different Animal Models and Humans

Animal Model	Similarity to Human Respiratory System	Advantages	Main Limitations	References
Mouse/Rat	Low to Moderate	<ul style="list-style-type: none"> • Highly editable genome; • Suitable for mechanistic and genetic studies 	<ul style="list-style-type: none"> • Lack bronchial mucous glands and respiratory bronchioles • Limited for mucus/remodeling studies 	[119–123]
Guinea Pig	Moderate	<ul style="list-style-type: none"> • Tracheal branching pattern closer to humans; • Rich in goblet and ciliated cells 	<ul style="list-style-type: none"> • Less commonly used; • Limited genetic tools 	[124,125]
Sheep	High	<ul style="list-style-type: none"> • Mucus secretion and inflammatory responses resemble humans; • Good for ventilation studies 	<ul style="list-style-type: none"> • High cost; • Limited immunological reagents 	[126–128]
Pig	High	<ul style="list-style-type: none"> • Lung anatomy and cardiopulmonary parameters closely resemble humans; • Ideal for drug delivery tests 	<ul style="list-style-type: none"> • Expensive; • Ethical and practical constraints; 	[129,130]
Non-human Primate	Very High	<ul style="list-style-type: none"> • Tissue structure, physiology and immune system most similar to humans 	<ul style="list-style-type: none"> • Very high cost; • Ethical concerns; • Limited availability 	[2,131–134]

adjusting combustion materials.¹³⁵ Composite injury models combining smoke inhalation with skin burns or bacterial infections better simulate the systemic inflammatory response observed in critically ill patients.¹³⁶ Model diversity necessitates clear experimental objectives: mechanistic exploration requires standardized, controllable models, while translational research demands pathologically complex models and comprehensive therapeutic interventions.

Basic research on inhalation injuries requires selecting appropriate animal models based on causative factors. Pathological characteristics of smoke inhalation models primarily involve alveolar epithelial cell apoptosis and persistent neutrophil infiltration. Thermal injury models center on protein denaturation and increased vascular permeability induced by high-temperature gases, necessitating the use of Evans blue dye for enhanced vascular barrier assessment.⁴⁷ It is noteworthy that the dose-response relationship of EVs is nonlinear across different models: in smoke-induced injury models, low doses reduce inflammatory factors, whereas chemical injury models require doses 2–3 times higher than low doses to inhibit collagen deposition.¹³⁷ This discrepancy underscores the need for model-specific dose optimization criteria in preclinical studies.

The Influence of Cell Origin

Variations in EVs production, leading to heterogeneity and diverse therapeutic outcomes, are attributable to multiple factors. These include differences in gene expression between cell sources, fluctuations in gene expression and transcription due to culture conditions, the genomic integrity of the cells, changes in cytoplasmic and membrane protein concentrations, and the limited cargo capacity of EVs.^{42,138} Bone marrow mesenchymal stem cells (BMSCs) release EVs enriched with immune-regulating factors such as TSG-6 and IL-10, which significantly reduce inflammation; however, their ability to target lung tissue is limited.^{139–141} In contrast, EVs secreted by lung-resident stem cells, such as lung spheroid cells (LSCs), preferentially home to damaged alveolar cells and directly target alveolar type II epithelial cells. Additionally, LSC-EVs transport lung-specific markers, including AQP5, and anti-fibrotic miRNAs. These miRNAs activate the miR-30a/WISP1 axis, inhibiting TGF- β -mediated myofibroblast activation and upregulating MMP-2 to promote extracellular matrix remodeling. This process collectively reduces the severity of pulmonary fibrosis.¹⁴²

Moreover, the composition of EVs from different tissues also differs. BMSCs-EVs contain a wealth of bioactive molecules that oversee cellular processes such as proliferation, differentiation, apoptosis, and inflammatory responses.

Table 3 Summary of Comparison of Different MSC Sources for EVs Therapy in Inhalation Lung Injury

MSC Source	Strengths	Limitations	Status	Level of Evidence
BMSCs (Bone Marrow-MSCs)	Potent immunomodulation (eg, TSG-6, IL-10); Most extensively studied.	Limited lung tissue targeting.	Advancing	Robust preclinical data. ^{51,60,71} Clinical trials up to Phase 3 (eg, NCT05354141).
UCMSCs (Umbilical Cord-MSCs)	Strong tissue repair capability; Accessible source, low; immunogenicity.	Standardization challenges; Potential oncogenic risks.	Early Clinical	Substantial preclinical evidence. ^{54,55,61} Early-phase trials (Phase I, eg, NCT05808400).
ADMSCs (Adipose Tissue-MSCs)	Easily accessible; Immunoregulatory function.	Lower lung targeting specificity; EVs heterogeneity.	Preliminary Clinical	Established preclinical profile. ^{143–145} Limited clinical data (Phase I, eg, NCT04313647).
LSCs (Lung Spheroid Cells)	Superior lung homing; Carries lung-specific markers (eg, AQP5); Direct targeting of alveolar cells.	Source is limited and invasive; Early-stage research.	Preclinical	Promising animal data. ¹⁴² No clinical trials to date.

EVs derived from adipose tissue mesenchymal stem cells (ADMSC-EVs) play an important role in regulating the immune system. EVs from umbilical cord-derived mesenchymal stem cells (UCMSC-EVs) demonstrate particular efficacy in repairing tissue damage.^{143–145} However, ethical concerns and potential cancer risks restrict their clinical research. Therefore, selecting the appropriate stem cell source and tailoring EVs for specific inhalation injuries is crucial to enhance therapeutic effectiveness. To facilitate a clear comparison of the therapeutic potential of EVs derived from different MSC sources, their key characteristics, limitations, and current translation status are summarized in [Table 3](#).

Investigating the similarities and differences in the molecular mechanisms underlying EVs derived from different species and stem cell sources could provide a more accurate scientific basis for clinical translation in repairing inhalation-induced lung injuries. Additionally, developing AI-based functional prediction models for EVs through deep learning and analysis of large-scale experimental data could enable precise prediction and optimized design of EVs therapeutic effects. This would represent a significant step in advancing clinical translation and holds potential for improving EV-based therapies for inhalation-induced lung injury.

Bottlenecks in Clinical Translation and Paths to Breakthroughs

Stem cell-derived EVs have been widely investigated, but clinical trials remain scarce, with many studies still in the preclinical phase. This indicates persistent bottlenecks in clinical translation, including production standardization, long-term safety assessment, and clinical trial design. Addressing these challenges would require collaboration across multiple disciplines, including enhancing production processes, conducting biosafety checks, and innovating intelligent drug delivery mechanisms. [Table 4](#) summarizes clinical trials involving EVs for treating inhalation lung injury.

Table 4 Clinical Trials of EVs for the Treatment of Inhalation Lung Injury

Disease	Medicines	Method	Country	Phase	Status	Number
ARDS (Moderate to severe)	BMSC-EVs	Injection	USA	Phase 3	In progress	NCT05354141
Health volunteers	ATMSC-EVs	Inhalation	China	Phase I	Completed	NCT04313647
ARDS	BMSC-EVs	Inhalation	China	Phase I/2	Completed	NCT04602104

(Continued)

Table 4 (Continued).

Disease	Medicines	Method	Country	Phase	Status	Number
Acute respiratory failure	MSC-EVs	Injection	Brazil	Phase 1/2	Not yet recruited	NCT06002841
ARDS (mild to moderate)	MSC-EVs	Injection	Iran	Phase 2/3	Unknown	NCT04366063
COVID-19 (pneumonia)	MSC-EVs	Inhalation	China	Early Phase I	Unknown	NCT05787288
ARDS	BMSC-EVs	Injection	USA	Phase 2	Completed	NCT04493242
ARDS	BMSC-EVs	Injection	Unknown	Phase 1/2	Withdrawn	NCT05127122
COVID-19	UCMSC-EVs	Inhalation	China	Early Phase I	Unknown	NCT05808400
COVID19 (lung fibrosis)	MSC-EVs	Blood testing	Germany	Observatio-nal	Recruitment	NCT05191381
Lung fibrosis	UCMSC-EVs	Inhalation	China	Phase I	Unknown	MR-46-22-004531/ ChiCTR2300075466

Standardization and Quality Control in EVs Production

Production standardization and quality control form the foundation for clinical translation of EVs, yet heterogeneity-induced efficacy variability remains a major obstacle. While engineering modifications can enhance functionality, they may introduce biosafety risks. Thus, establishing a tripartite assessment system—encompassing quality control, safety verification, and clinical adaptation—is essential for advancing EVs from laboratory to clinical application.

The core challenge in exosome isolation lies in source selection—certain biological fluids or tissues are rich in EVs, while others are not. The primary methods for separating and purifying EVs include ultracentrifugation, ultrafiltration, size-exclusion chromatography, tangential flow filtration, and polymer precipitation (Figure 2A).^{146,147} Among these, ultracentrifugation is most commonly used due to its low reagent consumption and ease of operation. However, its limitations include time-consuming procedures, high costs, and the risk of vesicle loss during phase separation, thereby restricting its large-scale application. Consequently, exploring alternative methods that combine high yield with good reproducibility is essential for the large-scale production of EVs.^{148,149} Polymer precipitation yields high quantities but is prone to contamination by protein aggregates.¹⁵⁰ Size-based separation methods, such as ultrafiltration and size-exclusion chromatography, are rapid and ideal for large-scale applications. However, their widespread adoption is limited by pore blockage, extracellular vesicle loss, and low purity.^{151–153} Kekoolani S Visan and co-researchers assessed a protocol for extracting small EVs from different cancer cell lines using tangential flow filtration (TFF) and compared it with ultracentrifugation methods. Their study demonstrated TFF's superiority over ultracentrifugation regarding yield, reproducibility, processing time, and cost.¹⁵⁴ While each technology has limitations, combining this approach with others, such as microfluidic technology, may enhance the results.

In standardized production, strict quality control is essential. According to the MISEV guidelines published by the International Society for EVs, the combined use of transmission and scanning electron microscopy is recommended for the precise identification and quantification of EVs. Additionally, nanoparticle tracking analysis (NTA) is recommended for assessing vesicle size. Compared to electron microscopy, NTA offers cost advantages and requires no specialized expertise. Such integrated strategies are essential for achieving standardized production and quality control.^{39,43}

Long-Term Safety Assessment of EVs

For clinical translation, assessing the long-term safety of EVs is essential. Animal studies indicate that high intravenous doses of MSC-EVs can activate the complement system and cause acute lung injury.¹⁵⁵ The inhalation route may reduce systemic exposure, but repeated nebulization can result in heightened phagocytosis of EVs by alveolar macrophages, causing damage due to lysosomal accumulation.⁵⁴ Additionally, engineering modifications of EVs can enhance targeting, though residual agents—such as chemical crosslinkers used during modification—may pose a risk of local

inflammation.²¹ Current studies indicate that oncogenic miRNAs transported by EVs promote epithelial-mesenchymal transition in fibrotic microenvironments, enhancing tumor cell metastasis and invasion.¹⁵⁶ Thus, longitudinal monitoring models should be established, alongside single-cell sequencing, to track the metabolic fate and potential genetic toxicity of EVs in lung tissue.

Critical Appraisal of Ongoing Clinical Trials

As shown in Table 4, numerous clinical trials have been initiated to assess the therapeutic potential of stem cell-derived exosomes in inhalation lung injury and related diseases,⁵⁰ representing a pivotal transition from laboratory research to clinical practice. However, a critical evaluation of these trials reveals both the current state of research and notable challenges. Most studies are still in early stages (Phase I or I/II), involve limited sample sizes, and primarily focus on safety and feasibility—making it too early to draw definitive conclusions regarding efficacy. Moreover, considerable heterogeneity exists in trial designs, including differences in EVs sources, isolation techniques, and routes of administration. This lack of standardization hinders the comparability of results across studies.¹⁵⁷

Notably, various delivery routes are being explored. Nebulization, as a direct and non-invasive approach, aligns with advantages observed in preclinical studies, yet requires further validation of its safety, dosage accuracy, and compatibility with delivery devices in clinical settings. Another prominent concern is the substantial number of trials listed as “Unknown” or “Not Recruiting,” which may reflect challenges in patient enrollment, manufacturing consistency, or regulatory approval processes. Despite these limitations, the completion of several early-phase trials marks an important milestone, as their outcomes will offer the first evidence regarding the safety and preliminary efficacy of EV-based therapies in humans.

To advance the field, future efforts should prioritize large-scale, randomized, placebo-controlled Phase III trials that employ standardized EVs products and well-defined patient populations, thereby establishing a solid foundation for clinical approval.

Conclusions and Perspectives

EVs represent a promising therapeutic platform for inhalation lung injury, demonstrating an inherent ability to regulate multiple pathological targets. However, their clinical translation faces significant hurdles. Current research relies heavily on simplified animal models that fail to recapitulate the complex and dynamic microenvironment of human disease, where factors such as oxidative stress and inflammatory cascades may alter EVs bioactivity.^{158,159} Furthermore, while engineering strategies can enhance targeting specificity, such modifications risk disrupting native vesicle structures and incurring unforeseen immunological consequences.^{72,160} Thus, a central challenge is to balance functional enhancement with the preservation of biocompatibility.

To address these bottlenecks, future work should prioritize two fronts. First, in EVs engineering, focus must be on optimizing targeting ligands for lesion-specific accumulation and developing “smart” EVs with microenvironment-responsive (eg, ROS/pH) release mechanisms.¹⁶¹ Parallel development of standardized protocols—covering isolation, potency assays, and storage stability—is critical for reproducibility. Second, bridging the preclinical-clinical gap requires establishing collaborative multicentre frameworks. This includes defining universal core outcome sets, creating centralized EVs biorepositories, and employing human organ-on-a-chip platforms for predictive validation.

Looking further ahead, next-generation EVs therapeutics should progress toward enhanced intelligence and integrated functionality. This entails engineering responsive nanocarriers for targeted release and constructing EV-eluting biomaterial scaffolds to reduce dosing frequency.^{162–164} Mechanistically, single-cell spatial transcriptomics and dynamic metabolomics can delineate EV-mediated recovery processes and metabolic reprogramming.¹⁶⁵ Leveraging AI to decode EVs composition and predict functional molecules will further accelerate rational design.

In conclusion, the pathological complexity of inhalation injury demands strategies that synergize multi-target regulation with spatiotemporal precision. A concerted effort integrating multi-omics technologies, intelligent material design, and standardized collaborative frameworks is essential to propel EV-based treatments from empirical management into a new era of precision medicine.

Abbreviations

CAT, catalase; EVs, extracellular vesicles; ECM, extracellular matrix; ESCs, embryonic stem cells; EPCs, endothelial progenitor cells/stem cells; EMT, epithelial-mesenchymal transition; GPx, glutathione peroxidase; HSCs, hematopoietic stem cells; IL, interleukin; iPSCs, induced pluripotent stem cells; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; TGF- β , transforming growth factor β ; TNF, tumor necrosis factor; TIMP, tissue inhibitor of metalloproteinase; TLRs, activate Toll-like receptors; MSCs, mesenchymal stem cells; MVBs: multivesicular bodies; MPO, myeloperoxidase; MIP-2, macrophage inflammatory protein-2; NSCs, neural stem cells; ARDS, acute respiratory distress syndrome; LPS-ALI, lps-induced acute lung injury; COPD, Chronic obstructive pulmonary disease; ACE2, Angiotensin converting enzyme 2; TLR4, Toll like receptors 4; MyD88, Myeloid Differentiation Primary Response Gene 88; MMP-9, matrix metalloprotein-9; SPC, surfactant protein C; COL1A1, collagen 1; FN, fibronectin.

Disclosure

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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