Mitigation of Oxidative Stress in Idiopathic Pulmonary Fibrosis Through Exosome-Mediated Therapies

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Abstract: Idiopathic pulmonary fibrosis (IPF) poses a formidable clinical challenge, characterized by the thickening of alveolar septa and the onset of pulmonary fibrosis. The pronounced activation of oxidative stress emerges as a pivotal hallmark of inflammation. Traditional application of exogenous antioxidants proves inadequate in addressing oxidative stress, necessitating exploration into strategies to augment their antioxidant efficacy. Exosomes, nano-sized extracellular vesicles harboring a diverse array of bioactive factors, present as promising carriers with the potential to meet this challenge. Recent attention has been directed towards the clinical applications of exosomes in IPF, fueling the impetus for this comprehensive review. We have compiled fresh insights into the role of exosomes in modulating oxidative stress in IPF and delved into their potential as carriers for regulating endogenous reactive oxygen species generation. This review endeavors to bridge the divide between exosome research and IPF, traversing from bedside to bench. Through the synthesis of recent findings, we propose exosomes as a novel and promising strategy for improving the outcomes of IPF therapy.

Keywords: oxidative stress, idiopathic pulmonary fibrosis, exosomes, treatment

Introduction

Idiopathic pulmonary fibrosis (IPF) presents a complex and debilitating pulmonary pathology characterized by the unexplained scarring of lung tissue.¹ This chronic and progressive interstitial lung disease not only poses a significant threat to affected individuals but also imposes a substantial burden on global healthcare systems.² The insidious nature of IPF lies in its capacity to progressively diminish lung function, resulting in respiratory distress and compromised quality of life, particularly among individuals in their 50s and 60s.³ As the aging global population and persistent environmental risk factors contribute to an increasing prevalence of respiratory-related health concerns, urgent research efforts are warranted to develop innovative therapeutic approaches to mitigate the impact of this epidemic.⁴

The pathogenesis of IPF unfolds through an intricate cascade of events disrupting the delicate equilibrium of the lung’s cellular microenvironment.⁵ Commencing with recurrent injuries to the alveolar epithelium, the response triggers abnormal wound healing, initiating the involvement of oxidative stress as a pivotal factor in the disease’s progression.⁶ Characterized by an imbalance between the production of reactive oxygen species (ROS) and the body’s counteracting mechanisms, oxidative stress plays a crucial role in instigating and perpetuating fibrotic processes within lung tissue.⁷ Various sources contribute to ROS production, including environmental factors, inflammatory cells, and dysfunctional mitochondria, setting off a cascade that activates fibroblasts, the key contributors to tissue repair.⁸ These activated fibroblasts perpetuate the fibrotic cascade, generating excessive collagen and other matrix components, leading to scar tissue formation and the gradual distortion of lung architecture.⁹
Oxidative stress induces immune dysregulation, drawing immune cells into the inflamed and fibrotic lung milieu, while inflammation contributes to oxidative stress, establishing a self-sustaining loop that perpetuates lung tissue damage. Oxidative stress’s association with cellular senescence accelerates aging processes, further impairing cellular repair mechanisms, intensifying the cycle of injury and fibrosis. Mitochondrial dysfunction, another consequence of oxidative stress, amplifies ROS production and disrupts cellular energy balance, impacting normal physiological functions.

In the context of IPF, the conventional application of exogenous antioxidants proves insufficient in addressing overwhelming oxidative stress. This inadequacy prompts exploration into strategies to enhance the anti-oxidative capacity of antioxidants, presenting a potential therapeutic avenue for mitigating oxidative stress in IPF. Research efforts are ongoing to identify compounds that can modulate oxidative stress, either by targeting specific pathways or by promoting the overall antioxidant defense mechanisms of the body. Exosomes, nano-sized extracellular vesicles enriched with a diverse array of bioactive factors, emerge as innovative messengers with therapeutic potential. Their unique structure facilitates the delivery of a molecular toolkit, including proteins and nucleic acids, to target cells, presenting an opportunity to modulate redox homeostasis and counteract ROS. As research progresses, exosomes emerge as a novel and transformative strategy, offering a promising approach to combat the intricate molecular mechanisms underlying this challenging pulmonary disorder.

Therefore, in this review, we aim to elucidate the considerable potential of exosomes as an effective antioxidant for improving the therapeutic outcome of IPF. By thoroughly investigating the intricate biogenesis and multifaceted functional capacities inherent in exosomes, we aim to offer a comprehensive understanding of the latest state-of-the-art advances, the associated challenges, and the potential frontiers within advanced strategies for addressing IPF.

Exosomes: Biology and Therapeutic Potential

Origin and Secretion of Exosomes
Exosomes, minute extracellular vesicles, play a pivotal role in cellular communication, serving as dynamic messengers that convey bioactive molecules between cells. The biogenesis of exosomes is intricately linked to the endosomal pathway, a cellular process that begins with endocytosis within early endosomes. During this phase, the cell engulfs membrane proteins and extracellular materials, setting the foundation for the formation of intraluminal vesicles (ILVs). These ILVs encapsulate a selectively packaged cargo, comprising proteins, lipids, and nucleic acids originating from the cytoplasm of the cell. The maturation of early endosomes into multivesicular bodies (MVBs) marks a critical juncture in the exosome biogenesis pathway. MVBs can follow two distinct fates: fusion with lysosomes for degradation or movement towards the cells’ plasma membrane for exosome secretion. The latter results in the release of ILVs into the extracellular space through a process known as exocytosis. This intricate mechanism ensures that exosomes, upon release, carry a diverse and selectively packaged composition of bioactive molecules, including tetraspanins, heat shock proteins, and various nucleic acids (Figure 1).

Acting as potent messengers, exosomes can be transported to neighboring or distant cells, where they interact with target cells through surface receptor binding or direct fusion with the cell membrane. The cargo within exosomes, comprised of proteins, lipids, and nucleic acids, is then delivered to recipient cells, influencing various cellular processes. This functional cargo transfer plays a crucial role in the regulation of physiological functions and contributes to both homeostasis and the response to pathological conditions. The diverse composition of exosomes, coupled with their ability to traverse biological barriers and selectively target cells, positions them as valuable mediators in cell-to-cell communication. Understanding the intricacies of exosome biology not only sheds light on fundamental cellular processes but also opens avenues for innovative diagnostic and therapeutic applications.

Biological Functions of Exosomes
Exosomes are instrumental in orchestrating a broad spectrum of biological functions that profoundly impact intercellular communication and cellular homeostasis. One of the paramount roles of exosomes is their ability to facilitate...
intercellular communication. Exosomes act as carriers of a diverse cargo, encompassing proteins, lipids, and nucleic acids, that can be selectively delivered to target cells. This cargo transfer is a dynamic process that influences cellular signaling pathways, modulating various cellular processes. By binding to surface receptors or undergoing fusion with the cell membrane, exosomes can activate specific cellular responses, contributing to tissue development, immune regulation, and the maintenance of overall cellular homeostasis.

In the process of immune regulation, exosomes emerge as pivotal players. Exosomes derived from antigen-presenting cells participate in immune responses by presenting antigens to immune cells. This process is integral to the initiation and modulation of immune reactions. Moreover, exosomes exhibit immunomodulatory effects by influencing the activity of immune cells. Depending on the context, exosomes can either enhance or suppress immune responses, a feature with implications for autoimmune diseases, infections, and cancer. The intricate interplay between exosomes and the immune system underscores their role in sculpting the immune landscape, and ongoing research is elucidating the mechanisms that govern these immunoregulatory functions.

Exosomes also contribute significantly to tissue repair and regeneration. Stem cell-derived exosomes, for instance, harbor factors that influence the differentiation of target cells, thereby contributing to tissue repair processes. Additionally, exosomes play a role in angiogenesis, the formation of new blood vessels, which is crucial for supplying nutrients and oxygen during tissue repair. This regenerative potential positions exosomes as promising candidates for therapeutic interventions aimed at enhancing tissue repair and regeneration. Furthermore, the involvement of exosomes in cancer progression is noteworthy. Cancer-derived exosomes actively participate in remodeling the tumor microenvironment, influencing factors such as inflammation, angiogenesis, and immune responses. Additionally, these exosomes contribute to the formation of pre-metastatic niches, creating a supportive environment for the dissemination and metastasis of cancer cells. Unraveling the complexities of exosome-mediated processes in cancer is imperative for developing targeted therapeutic strategies that could impede disease progression.

Figure 1 Exosome biogenesis involves the interaction of these vesicles with target cells. These vesicles originate from the endocytic pathway, arising from the inward budding of the MVB membrane. Reproduced from Xie X, Xiong Y, Panayi AC, et al. Exosomes as a Novel Approach to Reverse Osteoporosis: a Review of the Literature. Front Bioeng Biotechnol. 2020;8:594247. Creative Commons.
In neuronal communication, exosomes play a pivotal role in synaptic plasticity—the ability of synapses to strengthen or weaken over time, critical for learning and memory. Neuronal-derived exosomes transport bioactive molecules that modulate synaptic function, contributing to the intricate network of neuronal communication. Furthermore, exosomes may confer neuroprotection by delivering factors that promote cell survival and reduce inflammation in the central nervous system. The involvement of exosomes in neuroprotective mechanisms raises intriguing possibilities for therapeutic interventions in neurodegenerative disorders. Beyond their physiological roles, exosomes hold diagnostic potential. The analysis of exosomal cargo, particularly in bodily fluids such as blood and urine, has unveiled diseasespecific biomarkers. This opens avenues for non-invasive diagnostic approaches, providing valuable insights into various diseases, including cancer and neurodegenerative disorders.

**Isolation and Engineering of Exosomes for Therapeutic Application**

**Methods for Exosome Isolation and Purification**

Exosome isolation and purification are pivotal steps in harnessing the therapeutic and research potential of these nanosized extracellular vesicles. Various methods have been developed to obtain highly enriched and purified exosome populations from complex biological fluids (Table 1).

**Engineering Exosomes for Enhanced Therapeutic Efficacy**

The engineering of exosomes for enhanced therapeutic efficacy represents a frontier in biomedical research, offering a versatile platform for targeted drug delivery and regenerative medicine. One pivotal aspect of exosome engineering involves the strategic loading of therapeutic cargo. Genetic modification of parent cells is a powerful strategy, allowing for the overexpression of specific therapeutic molecules within exosomes. This approach ensures that exosomes carry a predetermined payload, whether it be therapeutic proteins, RNA, or microRNAs. By harnessing the inherent cargo-loading capacity of exosomes, researchers can tailor these extracellular vesicles for precise therapeutic interventions. Additionally, exosomes can be loaded post-isolation through techniques like electroporation or sonication. This versatility allows for the customization of exosome cargo, enabling researchers to address the unique requirements of diverse therapeutic applications.

In a recent study, Xiong et al constructed engineering exosomes by the encapsulation of didymin into ginseng-derived exosomes to form G-sEVs for stable and sustained therapy of diabetic wound. The in vitro and in vivo results demonstrated the superior capacity of G-sEVs in promotion of neural regeneration and recovery.

**Table 1 Summary of the Methods for Exosome Isolation and Purification**

<table>
<thead>
<tr>
<th>Method</th>
<th>Principle</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Ultracentrifugation</td>
<td>Differential sedimentation</td>
<td>High purity, widely used</td>
<td>Time-consuming, may co-pellet contaminants</td>
</tr>
<tr>
<td>Density gradient centrification</td>
<td>Buoyant density separation</td>
<td>Highly pure exosome fractions, gentle, maintains exosome integrity</td>
<td>Time-consuming, requires optimization, may not yield highly concentrated exosomes</td>
</tr>
<tr>
<td>Size exclusion chromatography</td>
<td>Size-based separation</td>
<td>Selective isolation, specific for markers</td>
<td>Bias towards targeted markers, may be costly, limited purity, may require additional steps</td>
</tr>
<tr>
<td>Immunocapture affinity chromatography</td>
<td>Antibody-based capture</td>
<td>Rapid, scalable, suitable for pre-concentration</td>
<td>May co-precipitate contaminants, evolving technology, requires validation</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>Membrane-based size separation</td>
<td>Simple, quick</td>
<td>Cost, variable performance across kits</td>
</tr>
<tr>
<td>Precipitation methods</td>
<td>Chemical-induced precipitation</td>
<td>Automated, rapid, low sample volume</td>
<td>Increased complexity, potential for sample loss</td>
</tr>
<tr>
<td>Microfluidics-based isolation</td>
<td>Microfluidic devices</td>
<td>Convenient, standardized, reproducible</td>
<td></td>
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<tr>
<td>Commercial kits</td>
<td>Various (eg, precipitation, affinity)</td>
<td>Sequential use of multiple methods</td>
<td></td>
</tr>
<tr>
<td>Combination methods</td>
<td>Sequential use of multiple methods</td>
<td>Improved purity and yield</td>
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thereby enhance diabetic wound repair (Figure 2).\textsuperscript{58} Sun et al designed a clodronate (CLD)-loaded liposome and fibroblast-derived exosome (EL-CLD) hybrid drug delivery system with non-specific phagocytosis inhibition and fibroblast homing properties for the treatment of pulmonary fibrosis.\textsuperscript{59} That system preferentially accumulated in the

![Figure 2](image-url)

\textbf{Figure 2} (A) The augmented neurogenesis effect of engineered exosome-encapsulated hydrogel was examined through immunofluorescence staining. The scale bar is set at 50 µm. (B) The schematic representation of the tissue collection timeline. (C) General images depicting wound healing in various treatment groups. (D) Immunofluorescence images illustrating the expression of CGRP, NGF, and CRH in skin tissues on day 7 post-wounding, accompanied by corresponding statistical results. Scale bar: 100 µm. Reproduced from Xiong Y, Lin Z, Bu P, et al. A Whole-Course-Repair System Based on Neurogenesis-Angiogenesis Crosstalk and Macrophage Reprogramming Promotes Diabetic Wound Healing. \textit{Adv Mater}. 2023;35(19):e2212300. © 2023 The Authors. Advanced Materials published by Wiley-VCH GmbH.\textsuperscript{18}
fibrotic lung, and significantly increased penetration inside pulmonary fibrotic tissue by targeted delivery due to the specific affinity for fibroblasts of the homologous exosome. The surface modification is another key facet of exosome engineering, contributing to enhanced specificity and stability. The surface of exosomes can be modified with targeting ligands, such as antibodies or peptides, facilitating a more specific interaction with target cells or tissues. This targeted approach ensures that engineered exosomes reach their intended destination, minimizing off-target effects and enhancing therapeutic precision. For example, Huang et al employed engineering technology to synergize exosomes with long non-coding RNA (lncRNA) for targeted therapy in osteosarcoma (OS). They constructed c(RGDyK)-modified and MEG3-loaded exosomes (cRGD-Exo-MEG3). These tailored exosomes exhibit enhanced delivery efficiency to OS cells both in vitro and in vivo. As a result, cRGD-Exo-MEG3 significantly augment the anti-OS effects of MEG3, showing improved tumor-targeting therapy. This research underscores the potential therapeutic impact of engineered exosomes serving as targeted carriers for delivering lncRNA MEG3 in the context of OS treatment (Figure 3).

Fusion proteins represent an innovative approach in exosome engineering, involving the expression of fusion proteins that incorporate exosome-targeting peptides along with therapeutic agents. This strategy allows for the seamless integration of targeting and therapeutic functionalities, streamlining the engineering process. Engineered exosomes carrying these fusion proteins can be guided to specific cell types or tissues, ensuring a more targeted and efficient delivery of therapeutic cargo. Additionally, the loading of exosomes with small molecules, such as chemotherapeutic drugs, takes advantage of their natural membrane permeability. This intrinsic property allows exosomes to encapsulate hydrophobic compounds, providing protection during transport and facilitating their delivery to target cells. Moreover, the loading of exosomes with various forms of RNA, including small interfering RNA (siRNA) or messenger RNA (mRNA), offers a powerful means to modulate gene expression in target cells. This RNA cargo can be therapeutic by silencing specific genes or promoting the expression of therapeutic proteins, providing a versatile platform for gene-based therapies.

An integral aspect of exosome engineering involves manipulating the parent cells that produce exosomes. Cell engineering through genetic modification ensures that these cells not only produce exosomes with enhanced cargo but also that they produce exosomes more efficiently. This approach guarantees a continuous and sustainable supply of

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Figure 3 Schematic illustration of preparation of engineered exosome as targeted lncRNA MEG3 delivery vehicles for OS therapy. Reprinted from J Control Release, volume: 343, Huang X, Wu W, Jing D, et al. Engineered exosome as targeted lncRNA MEG3 delivery vehicles for osteosarcoma therapy. 107–11, Copyright 2022, with permission from Elsevier.
engineered exosomes for therapeutic applications. Additionally, the integration of magnetic nanoparticles into exosomes allows for their magnetic guidance to specific target sites.\(^{69}\) This biophysical modification facilitates enhanced accumulation of exosomes at desired locations, improving therapeutic delivery and efficacy.\(^{69}\) Furthermore, the utilization of hybrid systems comprising exosomes and biocompatible nanoparticles offers synergistic advantages. These hybrid platforms can be designed to enhance cargo loading, stability, and targeting capabilities, combining the strengths of both exosomes and nanoparticles for superior therapeutic outcomes.\(^{70}\) Quality control measures, including the development of robust analytical tools, are crucial for characterizing engineered exosomes. These tools assess factors such as size distribution, cargo composition, and surface modifications, ensuring the reproducibility and safety of engineered exosomes for therapeutic applications.\(^{67,71}\)

### Exosomes in Mitigating Oxidative Stress

#### Mechanisms by Which Exosomes Counteract Oxidative Stress

Exosomes, being essential components of intercellular communication, exhibit a multifaceted repertoire of mechanisms by which they counteract oxidative stress-a condition characterized by an imbalance between ROS production and the cellular antioxidant defense system.\(^{72,73}\) (Table 2). One key mechanism involves the transfer of antioxidant enzymes, such as Superoxide Dismutase (SOD), through exosomes.\(^{74}\) SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen, playing a pivotal role in neutralizing one of the primary contributors to oxidative stress.\(^{75}\) This enzymatic activity, when transferred via exosomes, enhances the cellular capacity to scavenge harmful free radicals.\(^{76}\)

Non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), constitute another critical facet of exosome-mediated oxidative stress regulation.\(^{87}\) Exosomes encapsulate miRNAs that can modulate gene expression, particularly targeting genes involved in oxidative stress pathways.\(^{88}\) This regulatory function allows for fine-tuning the cellular response to oxidative challenges. Similarly, lncRNAs carried by exosomes play a role in cellular processes, including those linked to oxidative stress. The transport of these ncRNAs provides an additional layer of complexity in the regulatory network that influences redox balance and cellular responses to oxidative stress conditions.\(^{89}\)

The cargo within exosomes extends beyond genetic material to include antioxidant molecules such as glutathione (GSH), vitamins, and cofactors essential for the activity of antioxidant enzymes.\(^{90,91}\) GSH, a paramount cellular antioxidant, is transported by exosomes and contributes to the cellular redox status by scavenging free radicals.\(^{91}\) The transfer of antioxidant vitamins and cofactors further enhances the cellular defense against oxidative stress, providing a comprehensive antioxidant toolkit. Additionally, exosomes carry heat shock proteins (HSPs), including HSP70 and HSP90, which serve dual roles in protein folding and as antioxidants.\(^{92,93}\) These proteins contribute to cellular protection by preventing protein misfolding and by acting as molecular chaperones under conditions of oxidative stress.\(^{94}\)

Furthermore, exosomes play a pivotal role in maintaining mitochondrial quality and function—a crucial aspect of cellular resilience against oxidative stress.\(^{95}\) Exosomes have been shown to contain mitochondrial components, including

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<th>Mechanism</th>
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<tr>
<td>Antioxidant enzymes</td>
<td>SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen, enhancing the cellular capacity to neutralize harmful free radicals.</td>
<td>[77,78]</td>
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<tr>
<td>ncRNAs</td>
<td>Encapsulation of miRNAs and lncRNAs in exosomes. These molecules modulate gene expression, particularly targeting genes involved in oxidative stress pathways.</td>
<td>[79,80]</td>
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<tr>
<td>Antioxidant molecules</td>
<td>Inclusion of antioxidant molecules in exosomes, such as GSH, vitamins, and cofactors essential for the activity of antioxidant enzymes.</td>
<td>[81–83]</td>
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<tr>
<td>Maintenance of mitochondrial quality</td>
<td>Presence of mitochondrial components, including mtDNA, in exosomes. This cargo contributes to maintaining mitochondrial integrity and preventing the excessive generation of ROS within mitochondria.</td>
<td>[84,85]</td>
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<tr>
<td>DNA Repair Machinery</td>
<td>Transport of proteins involved in DNA repair mechanisms by exosomes. These proteins aid in the restoration of oxidative stress-induced DNA damage, supporting the repair of genomic instability caused by ROS.</td>
<td>[86]</td>
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mitochondrial DNA (mtDNA). This mitochondrial cargo contributes to the preservation of mitochondrial integrity and prevents the excessive generation of ROS within these cellular powerhouses. In tandem, exosomes influence cellular signaling pathways associated with oxidative stress responses. The Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) pathway, a master regulator of antioxidant gene expression, can be activated by exosomal cargo in recipient cells. This activation enhances the cellular antioxidant defense mechanisms, further emphasizing the diverse and integrated nature of exosome-mediated responses to oxidative stress.

The DNA repair machinery is another critical arm of exosome-mediated defense against oxidative stress. Exosomes may carry proteins involved in DNA repair mechanisms, aiding in the restoration of oxidative stress-induced DNA damage. By supporting the repair of genomic instability caused by ROS, these exosome-borne proteins contribute to the overall cellular resilience against oxidative stress-induced cellular damage. Moreover, metal ion chelation is facilitated by exosomes through the transport of metallothioneins, proteins capable of binding metal ions. This mechanism helps prevent the generation of ROS through Fenton-type reactions, presenting an additional layer of protection against oxidative stress-induced cellular damage.

**Application of Exosomes in Oxidative Stress Management in IPF**

The application of exosomes in the management of oxidative stress in IPF represents a promising frontier in the pursuit of effective therapeutic interventions. Exosomes serve as natural carriers of a diverse cargo, making them ideal candidates for targeted and multifaceted approaches to counteract oxidative stress in the lungs. One primary mechanism involves the delivery of antioxidant enzymes, such as SOD, by exosomes. SOD catalyzes the breakdown of superoxide radicals, a primary contributor to oxidative stress in IPF. By enhancing the delivery of antioxidant enzymes to the sites of oxidative stress, exosomes offer a localized and efficient strategy to neutralize harmful free radicals and mitigate oxidative damage.

Another facet of exosome-mediated oxidative stress management in IPF is the transfer of regulatory RNA molecules, including miRNAs and lncRNAs. Shao et al found that miR-454-3p may serve as an exosomal biomarker and may be developed into a novel treatment for glioma. By Valadi et al’s opinion, exosomes can deliver nucleic acids to cells at a distance and they are non-immunogenic, making them ideal candidates as vectors for gene therapy. These molecules play a pivotal role in modulating gene expression and signaling pathways associated with oxidative stress responses. In the intricate pathogenesis of IPF, where aberrant oxidative stress is a driving force, the transfer of these regulatory RNA molecules by exosomes provides a means to modulate the cellular response to oxidative challenges. The regulatory function of exosome-delivered miRNAs and lncRNAs adds a layer of complexity to the network that influences redox balance and cellular responses to oxidative stress conditions.

Beyond direct antioxidant mechanisms, exosomes contribute to the reduction of inflammation and fibrosis, both of which are intertwined with oxidative stress in IPF. Certain exosomes possess anti-inflammatory and anti-fibrotic properties, delivering bioactive molecules that modulate immune responses and inhibit fibrotic processes in the lungs. By targeting the underlying mechanisms that drive inflammation and fibrosis, exosomes indirectly alleviate oxidative stress. For instance, the Cheng et al group introduced a series of studies employing the lung spheroid cell-secretome (LSC-Sec) and exosomes (LSC-Exo) delivered through inhalation to address various models of lung injury and fibrosis. They demonstrated that treatments with LSC-Sec and LSC-Exo can alleviate and resolve fibrosis induced by bleomycin and silica. This therapeutic effect is achieved by restoring normal alveolar structure and reducing both collagen accumulation and myofibroblast proliferation. Notably, LSC-Sec and LSC-Exo surpass their mesenchymal stem cell-derived counterparts in certain efficacy measures. The inhalation-based administration of secretome and exosomes presents a promising therapeutic approach for lung regeneration in experimental models of pulmonary fibrosis (Figure 4). This research sheds light on a potential avenue for developing treatments that may enhance lung function and alleviate the impact of pulmonary fibrosis.

Furthermore, the maintenance of mitochondrial quality is crucial in conditions associated with oxidative stress, and exosomes play a role in this aspect as well. Mitochondrial components, including mtDNA, transported by exosomes contribute to preserving mitochondrial integrity. This preservation helps regulate the production of ROS within mitochondria, addressing a significant source of oxidative stress in IPF. Moreover, the potential for exosomes to stimulate endogenous antioxidant defense mechanisms in recipient cells adds a layer of adaptability to their therapeutic impact.
The cargo within exosomes, encompassing antioxidant molecules and signaling factors, may activate cellular pathways involved in antioxidant responses. This activation not only addresses existing oxidative stress but also promotes sustained cellular resilience against future oxidative challenges. Additionally, the prospect of engineered exosomes for targeted drug delivery is a frontier with transformative potential. By designing exosomes to carry specific antioxidant compounds or drugs targeting oxidative stress pathways, researchers can ensure that therapeutic agents reach the affected lung tissue efficiently, minimizing off-target effects and maximizing the efficacy of oxidative stress management in IPF.

Recent reports on studies of EVs/exosomes role in IPF
Recent studies have increasingly shed light on the role of EVs, particularly exosomes, in the pathogenesis of IPF. These tiny membrane-bound vesicles, released by various cell types including epithelial cells, fibroblasts, endothelial cells, and immune cells, carry a cargo of proteins, lipids, and nucleic acids that can influence cellular communication and modulate the microenvironment within the lungs.

In IPF, epithelial cells play a central role in the disease process, undergoing aberrant activation and apoptosis, leading to the formation of fibroblastic foci and excessive deposition of extracellular matrix. Recent research suggests that...
exosomes released by injured or activated epithelial cells may contribute to the fibrotic response by promoting fibroblast activation, myofibroblast differentiation, and collagen production.\textsuperscript{118,119} These exosomes contain pro-fibrotic mediators such as TGF-β, PDGF, and various microRNAs that can induce fibroblast proliferation and collagen synthesis.\textsuperscript{118,119}

Moreover, fibroblasts themselves are potent producers of exosomes, which can propagate fibrotic signaling cascades in an autocrine and paracrine manner. Studies have demonstrated that fibroblast-derived exosomes carry fibrogenic cargoes, including TGF-β, Wnt proteins, and connective tissue growth factor (CTGF), which can exacerbate the fibrotic phenotype and promote the persistence of myofibroblasts.\textsuperscript{120}
Endothelial cell-derived exosomes have also emerged as key players in IPF pathogenesis, contributing to vascular remodeling, endothelial dysfunction, and the perpetuation of fibrosis. These exosomes may carry angiogenic factors, inflammatory cytokines, and microRNAs that influence endothelial cell behavior and contribute to the dysregulated angiogenesis observed in IPF.\textsuperscript{121}

In addition to their role in disease pathogenesis, EVs, particularly exosomes, hold promise as novel biomarkers for IPF. The cargo of circulating exosomes reflects the pathological processes ongoing in the lungs, offering insights into disease activity and progression. Recent studies have identified specific exosomal microRNAs, proteins, and lipids that show differential expression patterns in IPF patients compared to healthy individuals or those with other lung diseases.\textsuperscript{122} These findings suggest that exosomal biomarkers could potentially aid in early diagnosis, prognostication, and monitoring of IPF patients, providing valuable clinical tools for disease management.\textsuperscript{123}

**Future Directions**

In the pursuit of harnessing the therapeutic potential of exosomes, researchers are increasingly focused on precision targeting of oxidative stress pathways implicated in IPF. This targeted approach aims to neutralize ROS and interrupt the oxidative stress cascade integral to IPF progression. Future research should try to elucidate the optimal cargo composition within exosomes, ensuring their payload is not only effective in mitigating oxidative stress but also tailored to the unique molecular characteristics of individual patients. By understanding the intricacies of redox imbalance in IPF, researchers can design exosome-based interventions that act as finely tuned molecular tools, disrupting the fibrotic process at its core.

To augment the therapeutic efficacy of exosome-based interventions, innovative loading strategies have emerged as a focal point of exploration.\textsuperscript{124} This involves refining techniques such as pre-conditioning donor cells or leveraging nanotechnology for cargo loading. The goal is to enhance the delivery and stability of exosomes, ensuring their payload reaches target cells with precision. In the context of oxidative stress mitigation, researchers are investigating the most effective combination of antioxidants and anti-fibrotic agents within exosomes.\textsuperscript{125} This synergistic approach may hold the key to a more potent and comprehensive therapeutic impact, disrupting not only the oxidative stress pathways but also intervening at multiple points in the fibrotic cascade. By refining loading strategies, researchers aim to harness the full potential of exosomes as therapeutic vehicles, offering a multifaceted and targeted approach to address the complexities of IPF pathophysiology.

As the field advances, researchers are increasingly recognizing the importance of tailoring exosome characteristics for optimal delivery. This involves understanding the biodistribution, pharmacokinetics, and stability of exosomes to enhance their ability to traverse the pulmonary barrier and reach target cells effectively. Surface modification and size control are emerging as critical parameters in this pursuit, offering avenues to fine-tune exosomes for enhanced therapeutic delivery.\textsuperscript{126} Simultaneously, the integration of exosome-based therapies with emerging technologies presents an exciting frontier in IPF research. Gene editing tools such as CRISPR/Cas9 can be harnessed within exosomes to modulate oxidative stress-related genes, providing a level of precision and specificity previously unimaginable.\textsuperscript{127} This integration of exosomes with cutting-edge technologies represents a convergence of biological and engineering principles, opening new dimensions for the development of highly targeted interventions in the realm of pulmonary fibrosis.

Recognizing the heterogeneity of IPF, there is a growing emphasis on personalized medicine approaches in the development of exosome-based therapies.\textsuperscript{110,128,129} This entails identifying biomarkers that can guide the customization of treatments based on the unique oxidative stress profiles of individual patients. By utilizing patient-derived exosomes for therapy, researchers aim to tailor interventions to the specific molecular characteristics of each IPF patient, ushering in a new era of precision medicine. As we continue to unravel the complexities of exosome biology and oxidative stress mechanisms, the translation of these insights into clinical applications may offer new hope for patients battling this devastating disease.

**Conclusion**

In conclusion, the future of exosome-based therapies in IPF, particularly in mitigating oxidative stress, holds immense promise. The integration of advanced technologies, refinement of delivery strategies, and a personalized medicine
approach collectively position exosome-based therapies at the forefront of innovative and targeted interventions for IPF. The journey from bench to bedside is ongoing, but with each discovery, we inch closer to a new era in IPF therapy—one where the complexities of the disease are met with equally sophisticated and tailored solutions.

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Disclosure
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