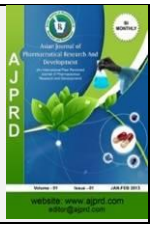
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Review Article

Autologous Blood-Derived Extracellular Vesicles (Exosomes) For Nebulized Respiratory-Tract Delivery: From Point-Of-Care Isolation to Upper-Airway and Pulmonary Regenerative Signaling

Dr Leroy Rebello, Dr Sreesudha Chepyala, Sahithi Polineni

Department of Research and Development, Biotex Life Solutions Pvt. Ltd., Plot No. 34, Bhavana Enclave-III, New Bowenpally, Secunderabad-500011, Telangana, India

ABSTRACT

Objective: To examine whether an autologous blood-derived extracellular vesicle (EV)/exosome-enriched fraction prepared at the point of care can be coupled to a closed medical nebulizer for delivery to the upper airway and lung, and to define the testing required before such a preparation could be evaluated clinically. **Data sources:** PubMed-indexed and publisher databases were searched for literature published between 2016 and September 2026 using combinations of terms covering extracellular vesicles, exosomes, serum- and platelet-derived EVs, nebulization, inhalation, aerosol deposition, intranasal delivery, and individual respiratory diseases. **Study selection:** Preference was given to primary preclinical studies of nebulized or intranasal EV delivery, human nebulized-EV trials, aerosol-science reports, and characterization-standard documents; systematic reviews and meta-analyses were used only as secondary, summarizing evidence. **Summary of contents of the article:** Fifty-eight sources are synthesized across vesicle biology and nomenclature, the manufacturer-defined kit workflow, nebulizer technologies, preservation of vesicle integrity during aerosolization, regional deposition and cellular uptake, regenerative and immunomodulatory signaling, preclinical evidence in the upper and lower airway, four human nebulized-EV studies, and a pre-/post-nebulization quality-control framework. **Conclusion:** Nebulization can preserve EV size, cargo, and potency, and locally delivered vesicles act on nasal, airway, and alveolar targets. All human evidence derives from allogeneic, culture-expanded MSC-EV products; no autologous whole-blood-derived fraction has been nebulized into a human airway. The workflow described is a development concept requiring product-specific validation and regulatory authorization.

Keywords: autologous exosomes, extracellular vesicles, nebulization, respiratory-tract delivery, upper airway, pulmonary delivery, allergic rhinitis, airway repair

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*Address for Correspondence:
Sahithi Polineni, Department of Research and Development, Biotex Life Solutions Pvt. Ltd., Plot No. 34, Bhavana Enclave-III, New Bowenpally, Secunderabad-500011, Telangana, India

INTRODUCTION

Exosomes are nanoscale membrane-enclosed extracellular particles that participate in intercellular communication through transfer of proteins, lipids, messenger RNAs, microRNAs, and other regulatory molecules. Within the respiratory system, extracellular-vesicle-mediated signaling occurs among airway and alveolar epithelial cells, endothelial cells, macrophages, fibroblasts, and other immune or stromal populations. These interactions can contribute to both pulmonary homeostasis and disease, and have generated interest in extracellular vesicles as potential cell-free therapeutics and delivery vehicles (1-4).

The term exosomes is retained in this review because exosome-associated vesicles are the principal translational focus. However, MISEV2023 emphasizes that size, morphology, or detection of commonly used tetraspanins alone does not prove endosomal biogenesis. For blood-derived preparations, where plasma contains heterogeneous

vesicles, lipoproteins, and abundant soluble proteins, the more conservative terms extracellular-vesicle-enriched or EV/exosome-enriched fraction are appropriate until vesicle identity and purity are sufficiently characterized (1,5).

Autologous blood-derived preparations are biologically distinct from culture-expanded MSC-derived EV products. Peripheral blood contains vesicles released from platelets, leukocytes, erythrocytes, endothelial cells, and other circulating populations, creating a patient-specific molecular pool that may carry complementary proteins, lipids, and regulatory RNAs. Platelet-derived EVs in particular have documented regenerative and drug-delivery relevance (6-8). Because the composition of circulating EVs reflects the physiological state of the individual, analytical characterization is important for defining the functional profile of each preparation and for supporting reproducible therapeutic development.

Local respiratory delivery is attractive because aerosolized or intranasally administered EVs can be placed directly near mucosal, epithelial, and immune-cell targets. In the lower respiratory tract, experimental studies show that inhaled or nebulized EVs can reach airway epithelium, alveolar epithelial cells, and macrophages while retaining functional molecular cargo (9-16). In the upper airway, intranasal EV studies have demonstrated delivery to nasopharynx-associated lymphoid tissue, restoration of nasal epithelial barrier function, and attenuation of allergic-rhinitis inflammation (17-20). These complementary findings support a broader respiratory-tract concept in which device and interface selection can be matched to the desired anatomical target.

The principal translational question is therefore how best to preserve and deliver the biological activity of an extracted autologous EV/exosome-enriched fraction across the respiratory tract. This review focuses on the exosome-nebulizer interface, preservation of vesicle integrity, upper- and lower-airway deposition, cellular uptake, regenerative signaling, clinical feasibility, quality assurance, and the specific position of a point-of-care autologous blood-derived workflow.

Objectives and Data Sources

The objective of this review is to establish whether an autologous blood-derived EV/exosome-enriched fraction prepared at the point of care can be delivered to the respiratory tract by a closed medical nebulizer with its biological activity intact, and to specify the characterization required before such a preparation could be considered for clinical evaluation. This is a narrative, evidence-focused review rather than a systematic review or meta-analysis. Peer-reviewed literature was prioritized from PubMed-indexed and publisher databases using combinations of the terms extracellular vesicle, exosome, serum-derived EV, platelet-derived EV, mesenchymal stromal cell extracellular vesicle, autologous EV, nebulization, inhalation, aerosol, intranasal delivery, nasal mucosa, allergic rhinitis, chronic rhinosinusitis, nasopharynx, pharynx, larynx, airway repair, pulmonary delivery, lung injury, pulmonary fibrosis, asthma, COPD, pneumonia, particle stability, and aerosol characterization. Emphasis was placed on literature from approximately 2016 through September 2026, while earlier studies were retained when directly relevant to aerosol deposition or upper-airway delivery. Primary preclinical and clinical reports were preferred; systematic reviews and meta-analyses were used as secondary sources, summarizing evidence only.

Biological Characteristics of Autologous Blood-Derived Ev/Exosome Preparations

Exosomes arise through the endosomal pathway, whereas other EV classes can bud directly from the plasma membrane. All EVs share a lipid-bilayer organization that can protect internal cargo and enable receptor-mediated or endocytic interactions with recipient cells. Commonly measured proteins include CD9, CD63, and CD81, but these markers are not exclusive to exosomes and should be interpreted as part of a multiparametric characterization strategy (1).

Circulating blood is an analytically difficult EV source because particle size and density overlap with lipoproteins and other non-vesicular extracellular particles. Karimi et al. demonstrated that size-exclusion chromatography alone did not fully separate plasma EVs from lipoproteins, and additional density-based separation substantially reduced contamination (5). More recent reviews reinforce the need to distinguish EVs from lipoproteins and potential EV-lipoprotein complexes when interpreting plasma or serum preparations (21).

Biological heterogeneity can also be viewed as a defining feature of autologous blood-derived EVs. Platelet-derived EVs carry molecules associated with haemostasis, angiogenesis, immune regulation and tissue repair and are being actively explored for regenerative applications (6-8). Other circulating cell populations contribute additional signaling vesicles, potentially broadening the molecular repertoire available in a patient-matched preparation. Serum-derived vesicles have themselves been shown to carry protective cargo in experimental lung injury, with miR-142-5p-enriched serum EVs limiting septic acute lung injury through PTEN/PI3K/Akt signaling (22). Heterogeneity is not uniformly advantageous, however: circulating myeloid-cell EVs isolated from endotoxaemic donor mice were sufficient to provoke pulmonary oedema and vascular inflammation when transferred into recipient lungs (23). A whole-blood-derived fraction therefore

cannot be assumed to be benign on the basis of its autologous origin. Because disease state, age, medication exposure, and platelet activation all influence EV composition, characterization of the actual recovered fraction - with a safety readout alongside a potency readout - is the most direct way to define its therapeutic profile.

For a point-of-care autologous product, the practical objective is therefore to recover a patient-matched EV/exosome-enriched fraction and then establish its identity, particle profile, molecular cargo, safety attributes and biological potency. This approach preserves the advantages of an accessible autologous source while providing the analytical framework required for reproducible respiratory delivery.

Biotex Autologous Exosome Kit: Manufacturer-Defined Point-Of-Care Workflow

The Biotex Autologous Exosome Kit is presented in this manuscript as the upstream preparation platform that generates the patient-derived material subsequently considered for pulmonary nebulization. According to the manufacturer-supplied workflow, peripheral blood is collected aseptically into eight proprietary gel-containing tubes. Each tube has an 8 mL capacity and is filled with approximately 5 mL of blood, corresponding to a total blood volume of approximately 40 mL.

The filled tubes are incubated at 38 °C for 30 minutes and are then centrifuged to separate cellular components from the plasma-containing fraction. The supplied workflow indicates recovery of approximately 12-15 mL of clarified plasma in total. This plasma is subsequently passed through a proprietary circular membrane assembly using a three-way connection, after which approximately 3 mL of processed material is collected as an autologous EV/exosome-enriched fraction.

The preparation is intended as a same-session, patient-specific workflow. For scientific reporting, the final material is appropriately described as an autologous EV/exosome-enriched fraction unless further analytical characterization supports a more specific identity. In the respiratory-delivery concept reviewed here, the approximately 3 mL fraction is transferred to a separate closed medical nebulizer for inhalational administration. The nebulizer is not a Biotex-manufactured component of the kit; its role is to convert the prepared biological fraction into a controlled aerosol suitable for local respiratory delivery.

This separation of functions is useful for product development: the kit defines the starting autologous biological material, whereas the nebulizer determines aerosol generation and regional deposition. Baseline characterization before nebulization and repeat testing after aerosolization can therefore be used positively as a quality-confirmation strategy to demonstrate that the extracted vesicles retain their key physical, molecular, and functional properties during delivery.

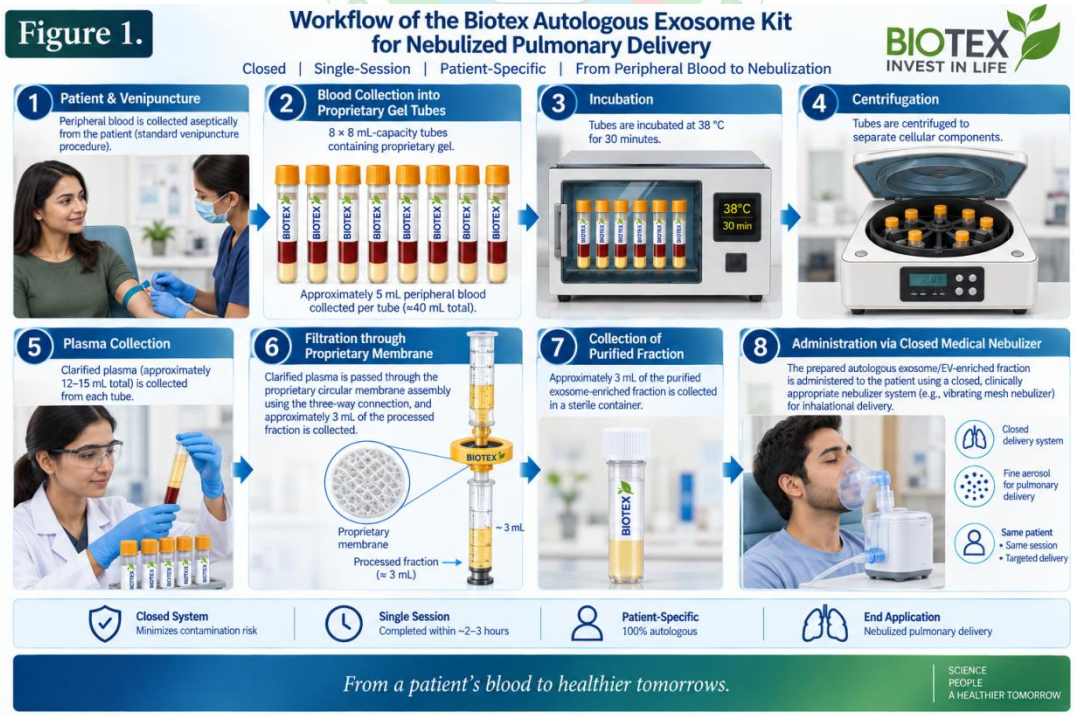


Figure 1: Manufacturer-supplied conceptual workflow of the Biotex Autologous Exosome Kit for same-session preparation of an autologous EV/exosome-enriched fraction followed by transfer to a separate closed medical nebulizer. The workflow illustrates the transition from patient blood to a locally administered respiratory aerosol; the nebulizer is a generic medical delivery device and is not manufactured by Biotex.

Respiratory-Tract Delivery Rationale and Nebulizer Technologies

The respiratory tract provides a continuous mucosal surface extending from the nasal cavity and nasopharynx through the pharyngeal/laryngeal region, conducting airways, and distal alveoli. These compartments contain epithelial, endothelial, immune, and stromal cells that participate in barrier maintenance, inflammatory regulation, and tissue repair. EVs are naturally present in airway secretions, including nasal lavage, pharyngeal lavage, sputum, and bronchoalveolar lavage, supporting the biological compatibility of vesicle-mediated signaling throughout the respiratory tract (2-4,17,24).

A critical distinction must be maintained between vesicle diameter and aerosol aerodynamic diameter. EVs are nanoscale particles, whereas nebulizers generate micrometre-scale liquid droplets that can carry vesicles. Regional deposition can therefore be tuned by aerosol size, device/interface design and breathing pattern: larger droplets favor more proximal deposition, whereas smaller respirable droplets can reach peripheral bronchioles and alveoli (25-28). For a respiratory EV therapy, proximal deposition may be advantageous when the intended target is the nose, pharynx, or laryngeal region, while finer aerosols are preferred for distal-lung delivery.

Jet, ultrasonic, and vibrating-mesh nebulizers can all generate therapeutic aerosols, but current EV-specific evidence is particularly encouraging for vibrating-mesh systems. Serum-derived sEVs, MSC-derived EVs, and other vesicle preparations have retained measurable particle characteristics and biological function after controlled nebulization (9,11-13). Vibrating-mesh systems also offer practical advantages for small-volume biological formulations, including low residual volume and efficient aerosol generation. Device selection should therefore be based on the desired anatomical target and verified with the specific EV preparation rather than assuming that all nebulizers behave identically.

Upper-airway delivery adds a complementary opportunity. Intranasal EV studies demonstrate that vesicles can interact with nasal epithelium and nasopharynx-associated immune tissue, restore epithelial barrier function, and reduce experimental allergic-rhinitis inflammation (17-20). Aerosol science also shows that inhaled particles can deposit in the mouth, pharynx, and larynx depending on geometry and inhalation pattern (28). Thus, a closed nebulizer with an appropriate mask or interface can be conceptualized as a platform for regional respiratory delivery, while the strongest direct therapeutic nebulization evidence currently remains in the lower respiratory tract.

Table 1: Comparison of nebulizer technologies relevant to EV/exosome respiratory-tract delivery.

Parameter	Jet nebulizer	Ultrasonic nebulizer	Vibrating-mesh nebulizer
Aerosol-generation principle	Compressed gas produces droplets through a narrow orifice and baffle.	Piezoelectric acoustic energy generates droplets from the liquid surface.	A vibrating membrane drives liquid through microscopic apertures.
Potential EV stresses	High gas velocity, recirculation, impaction and air-liquid interfaces.	Acoustic energy, cavitation-related effects and possible warming.	Mesh passage, interfacial exposure, device adsorption and local deformation.
Residual volume	Often comparatively higher.	Device dependent.	Often comparatively low.
Small-volume biologic use	Possible, but residual loss can be important.	Possible, but temperature should be monitored.	Attractive for low-volume biologics if compatibility is demonstrated.
Direct EV/exosome evidence	Limited relative to vibrating-mesh literature.	Limited direct EV evidence.	Strongest direct evidence: multiple serum-, MSC-, and other EV studies demonstrate preservation and biological activity after controlled nebulization.
Key reporting parameters	Loaded volume, emitted dose, residual volume, MMAD, FPF, treatment time.	Temperature change, emitted dose, MMAD, FPF, vesicle stability.	Particle recovery, mesh/device retention, emitted dose, MMAD, FPF and residual volume.
Interpretation for autologous blood-derived preparations	Requires product-specific compatibility testing.	Requires thermal and structural evaluation.	Currently the most directly supported class, but still requires paired pre/post-nebulization validation.

Preservation of Ev/Exosome Integrity and Respiratory Functional Activity during Nebulization

Controlled nebulization can preserve the properties required for EV-mediated respiratory therapy. Direct studies show that extracellular vesicles tolerate aerosol generation while retaining measurable particle characteristics, molecular cargo, and biological activity. Han et al. recovered small EVs from murine serum, delivered them through a vibrating-mesh nebulizer, and found vesicle size and concentration were maintained while loaded small RNA was still functionally transferred in vivo (9).

Gonzalez et al. profiled their vesicles on both sides of the nebulizer - dimensions, surface antigens, and miRNA complement - and reported that neither the particles nor their immunomodulatory and wound-healing capacity were degraded by the process (11).

Popowski et al. showed that inhaled lung-derived exosomes carried mRNA and protein cargo into bronchiolar and parenchymal compartments more efficiently than liposomal comparators (13), and nebulized airway basal-stem-cell EVs have since been reported to support epithelial repair and lung function in an experimental COPD model (16).

Particle concentration and size distribution provide the first layer of evidence for successful preservation. NTA or TRPS can quantify concentration and size distributions, while DLS can complement assessment of larger aggregates. Published nebulization studies show that, under defined conditions, vesicle size profiles and concentrations can remain stable after aerosol generation (9,11). For an autologous preparation, the most informative approach is a paired comparison of the same sample before and after nebulization, allowing direct confirmation that the delivery step preserves the recovered vesicle population.

Membrane integrity is equally important because the lipid bilayer protects intravesicular RNA and proteins and contributes to recipient-cell interaction. Morphological assessment by TEM or cryo-EM can document preserved vesicular structure, while RNase-protection, cargo-retention, and membrane-permeability assays can provide additional confirmation.

The fact that nebulized EVs have retained functional RNA transfer, cellular uptake, and disease-modifying activity in multiple studies provides strong indirect evidence that biologically meaningful membrane integrity can be maintained during aerosolization (9,11-13,16).

Surface-associated proteins and molecular cargo provide a second level of preservation. EV-associated tetraspanins such as CD9, CD63, and CD81 can be followed across nebulization, while endogenous RNA/miRNA and selected protein cargo can be quantified before and after delivery. The rationale for tracking RNA in particular rests on earlier work showing that specific miRNAs are selectively enriched in exosomes and can be transferred to recipient lung cells with functional consequences (29,30).

Murine serum-derived sEVs delivered functional small RNA to lung macrophages and airway epithelial cells after nebulization (9), and nebulized MSC-EVs retained their miRNA profile alongside biological activity (11). For autologous blood-derived EVs, profiling naturally abundant cargo in the starting preparation and confirming its retention after aerosolization offers a direct way to document molecular continuity.

Functional preservation is the most meaningful endpoint because it links physical stability to therapeutic relevance. Suitable assays can include epithelial uptake, migration, and barrier recovery; macrophage inflammatory signaling; antioxidant responses; fibroblast-remodeling assays; or other target-specific readouts. The growing inhaled-EV literature shows that nebulized vesicles can remain biologically active after aerosolization, supporting post-nebulization potency testing as direct confirmation of retained biological function (9-13,16).

Preservation of these structural and molecular characteristics enables nebulized EVs to engage epithelial, immune, and stromal targets throughout the respiratory tract. Evidence from intranasal and upper-airway studies supports nasal epithelial barrier restoration, local immune modulation, and allergic-rhinitis-related effects (17-20), while otorhinolaryngology and vocal-fold models support regenerative signaling in upper-airway tissues (24,31).

In the lower respiratory tract, nebulized or inhaled EVs have demonstrated epithelial and macrophage uptake, modulation of inflammatory and oxidative-stress pathways, epithelial repair, barrier support and regulation of fibrotic remodeling across models of COPD/emphysema, pneumonia, acute lung injury and pulmonary fibrosis (9-16,32-36). Together, these findings support a positive continuum in which preserved aerosolized EVs can deliver biologically active cargo locally from the nasal cavity and upper airway to the bronchi and alveoli.

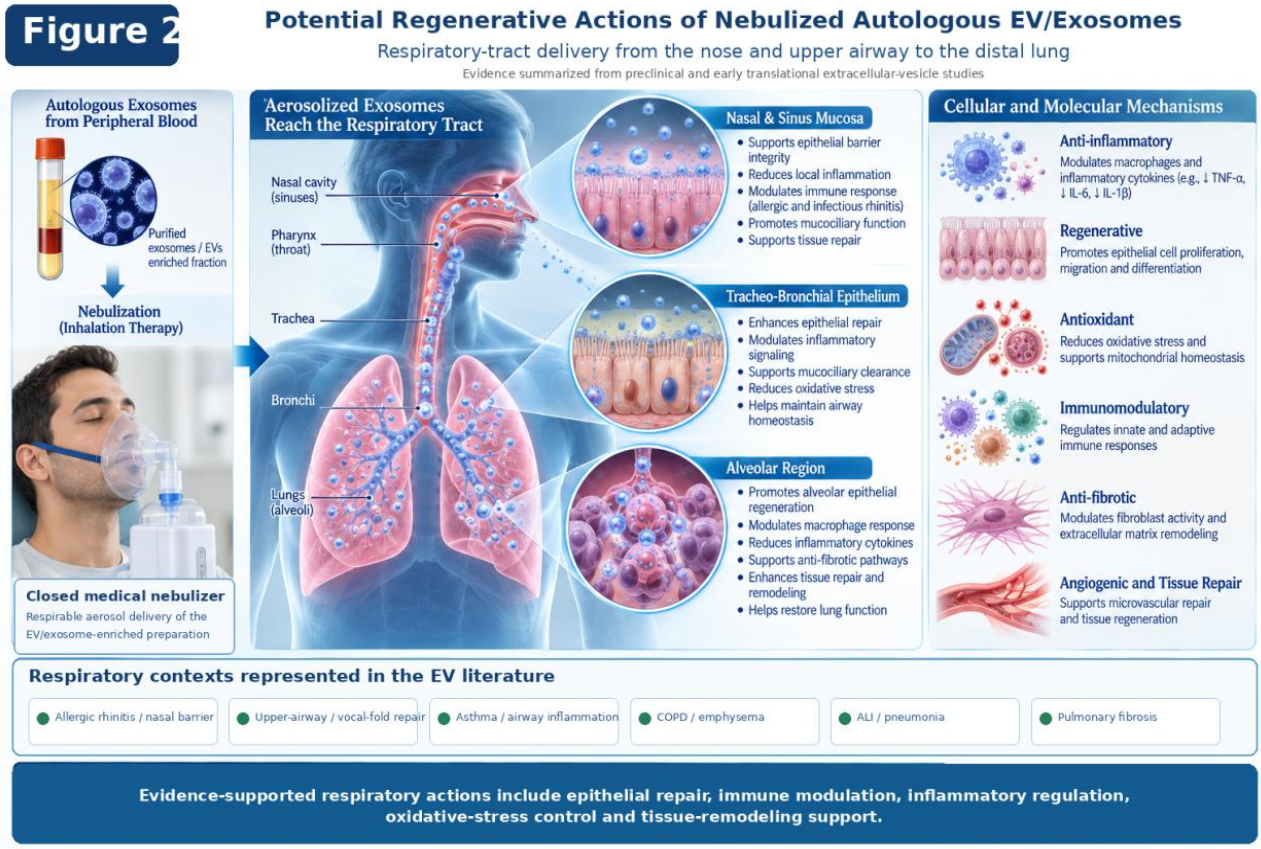


Figure 2: Potential regenerative actions of nebulized autologous EV/exosomes across the respiratory tract. The schematic summarizes respiratory regions and biological mechanisms represented in current EV literature, including nasal epithelial barrier support, upper-airway tissue repair, tracheobronchial epithelial recovery, alveolar epithelial repair, macrophage and immune modulation, inflammatory and oxidative-stress regulation, and tissue-remodeling support (9-20,24,28,31-36). These evidence-supported mechanisms provide the biological rationale for local respiratory delivery of a preserved autologous EV/exosome-enriched preparation.

Aerosol Characteristics, Upper-Airway/Pulmonary Deposition and Cellular Uptake

Once vesicle integrity is preserved, aerosol design determines where the preparation is delivered. Relevant variables include MMAD, GSD, fine-particle fraction, emitted dose, aerosol output, nebulization time, and residual volume (25-27). Larger droplets and specific inhalation patterns can favor deposition in the mouth, pharynx, and larynx, whereas smaller respirable droplets have a greater probability of reaching bronchioles and alveoli (28). Accordingly, proximal airway deposition should not automatically be viewed as loss when the intended therapeutic target includes nasal, pharyngeal, or laryngeal mucosa.

The upper airway is increasingly relevant to EV delivery. Intranasal EV administration has been reviewed as a promising non-invasive route for respiratory and mucosal applications (17). In allergic rhinitis, MSC-derived sEVs re-established tight-junction competence in human nasal epithelial cells, an effect mapped to a miR-143-GSK3B axis (18); adipose-stem-cell EVs instilled into the nasal cavity lowered inflammatory infiltration and Th2-associated responses (19); and sEVs co-loaded with antigen and a CpG adjuvant were taken up by CD11c-positive cells in nasopharynx-associated lymphoid tissue, shifting the antibody response and easing allergic signs (20). These studies indicate that EVs can remain biologically active in the nasal and nasopharyngeal environment and support the concept of targeted local respiratory administration.

Direct nebulization evidence is strongest in the lower respiratory tract. Han et al. tracked nebulized serum-derived EV-associated RNA to lung macrophages and airway epithelial cells, with the signal confined to the lung (9). Xuan et al. found that nebulized human platelet-derived EVs distributed to alveolar regions, where they were internalized by type I and type II pneumocytes and by macrophages (10). Lung-derived exosomes carried mRNA and protein cargo into bronchiolar and parenchymal tissue (13), while nebulized airway basal-stem-cell EVs supported epithelial repair, new vessel formation, and better lung function in experimental COPD (16). Together, these studies trace a coherent pathway from aerosol generation to respiratory deposition, cellular uptake, and biological response.

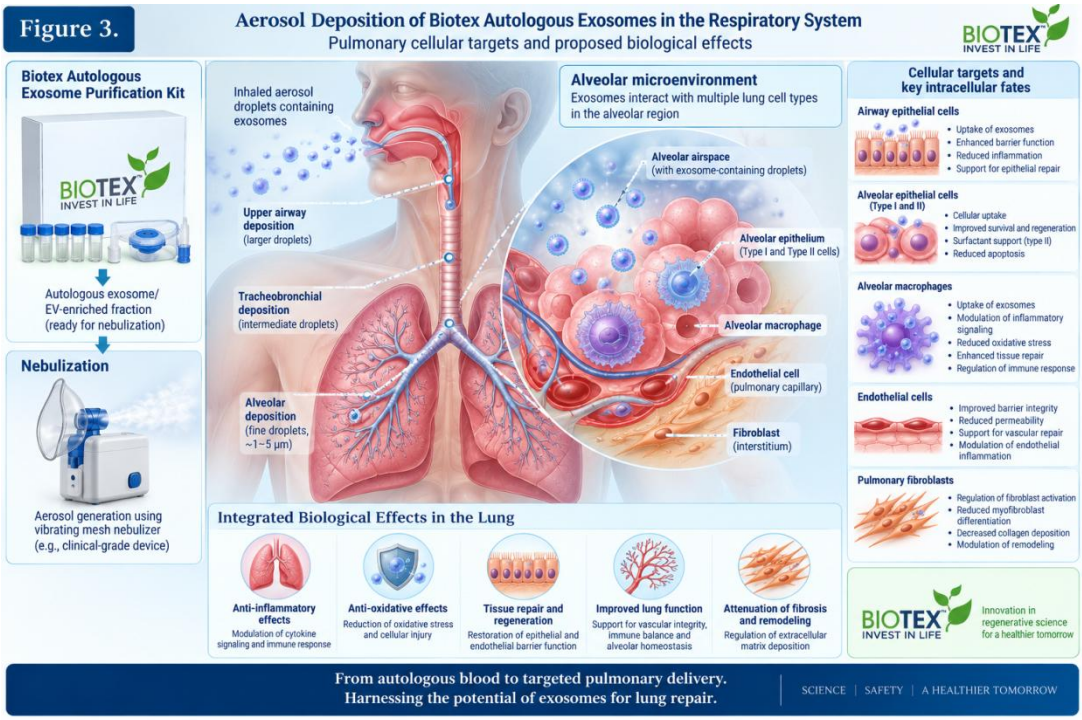


Figure 3: Schematic representation of aerosol deposition and pulmonary cellular targets relevant to nebulized autologous EV/exosome-enriched preparations. The figure emphasizes lower-airway delivery, where the direct evidence base is currently strongest; complementary nasal and upper-airway evidence is discussed in the text (9,10,13,16-20).

For the throat and laryngeal region, aerosol deposition is physiologically achievable and can be influenced by mouthpiece geometry and inhalation technique (28). EV-based regenerative work in vocal-fold fibroblast systems also supports the biological plausibility of local laryngeal tissue responses (31). Direct therapeutic studies of nebulized EVs specifically targeting the human pharynx or larynx remain limited, so these sites are best described as promising extensions of the broader respiratory-delivery concept rather than established indications.

Respiratory Epithelial, Upper-Airway and Pulmonary Regenerative Signaling

EV biology across the respiratory tract is best understood as a multicellular signaling network. In the nasal cavity, intact epithelial tight junctions are central to defence against allergens and pathogens, and MSC-derived sEVs have reversed junctional disruption and reduced allergic-rhinitis pathology in experimental systems (18,19). In the conducting airways and alveoli, epithelial survival, migration, and repair matter equally. Engineered Wnt3a-loaded EVs promoted alveolar epithelial regenerative responses after lung injury (33), and nebulized airway basal-stem-cell EVs have recently been shown to enhance epithelial repair and lung function in experimental COPD (16).

Immune modulation is another recurring mechanism. In the upper airway, intranasal EV systems can influence dendritic-cell and Th1/Th2-related responses (19,20). In the lung, alveolar and interstitial macrophages are prominent EV recipient populations, and EVs are now recognized as both mediators and candidate treatments of acute lung injury (37). MSC-derived EVs have altered macrophage inflammatory phenotypes in models of acute lung injury, pulmonary fibrosis, lung ischaemia-reperfusion injury and bronchopulmonary dysplasia (15,32,34,35,38,39), and comparable macrophage-directed effects have been described in asthma models (12,40); the same vesicle class has also been assessed in a multifactorial neonatal lung-injury model carrying both pulmonary and neurological endpoints (41). Endogenous epithelial- and endothelial-derived exosomes also regulate macrophage behavior through miRNA-dependent signaling, illustrating that EV-mediated immune crosstalk is part of normal respiratory biology (42).

Oxidative-stress regulation, vascular support, and matrix remodeling provide additional regenerative mechanisms. Nebulized human platelet-derived EVs attenuated oxidative injury, inflammatory signaling, and apoptosis in cigarette-smoke-induced emphysema (10). Lung spheroid cell-derived exosomes and MSC-derived exosomes have reduced collagen accumulation, myofibroblast proliferation, and fibrosis-associated inflammatory programmes (14,32). In the upper airway, experimental nasal EV therapies have also lowered local inflammatory and oxidative responses (18,19).

Taken together, the literature supports a favorable respiratory biological profile for appropriately selected

and characterized EV preparations: epithelial-barrier support, immune modulation, antioxidant activity, cargo delivery, and remodeling control recur across nasal, airway, and pulmonary models. Source-specific differences remain important, but they do not diminish the central translational opportunity; instead, they provide a rationale for characterizing the actual autologous blood-derived fraction and matching its potency profile to the intended respiratory target.

Preclinical Evidence Across The Upper And Lower Respiratory Tract

The preclinical evidence now spans both upper- and lower-respiratory applications. For the nose and nasopharynx, allergic rhinitis is the best-developed model. MSC-derived sEVs repaired nasal epithelial barrier dysfunction in human nasal epithelial cells and in vivo, acting through a miR-143-GSK3B axis (18). Human adipose-stem-cell EVs given into the nasal cavity lowered inflammatory infiltration, IgE- and IL-4-associated responses, and Th1/Th2 imbalance in allergic-rhinitis mice (19). Intranasal sEVs co-loaded with ovalbumin and CpG DNA were taken up by CD11c-positive cells of nasopharynx-associated lymphoid tissue, raised antigen-specific IgG, lowered serum IgE, and eased allergic signs (20). These findings show that locally delivered EVs can act on upper-airway mucosal and immune targets, although in the last study the vesicles served as an antigen and adjuvant carrier rather than as a regenerative agent in their own right.

In the lower respiratory tract, the evidence base is broader. Pulmonary fibrosis is among the most developed inhalational EV applications: Dinh et al. found that inhaled lung spheroid cell secretome and exosomes limited collagen deposition and myofibroblast expansion while preserving lung architecture (14), and Mansouri et al. reported antifibrotic effects of MSC-derived exosomes linked to monocyte and macrophage reprogramming (32). The miRNA cargo implicated in these effects has been reviewed in detail (43), and more recent engineered inhalable systems support the feasibility of delivering therapeutic vesicular cargo to fibrotic lungs (44,45).

For COPD and emphysema, Xuan et al. supply the most directly relevant blood-source evidence: nebulized human platelet-derived EVs reached alveolar targets and attenuated oxidative stress, inflammation, apoptosis, and emphysematous injury (10). Nebulized airway basal-stem-cell EVs have since reproduced much of the reparative benefit of the parent cells in an elastase-induced murine COPD model, restoring alveolar architecture, promoting new vessel formation, and improving arterial blood-gas indices, with proteomic enrichment for lung-development proteins and PI3K-Akt activation offered as the mechanism (16). In asthma, nebulized hypoxia-conditioned hUCMSC-EVs reduced inflammatory infiltration, Th2-associated cytokines, goblet-cell change, collagen deposition, and remodelling (12); systematic reviews have separately surveyed MSC-EV therapy in asthma and the part exosomes play when asthma and COPD exacerbate (40,46). Inhaled MSC-EVs have also attenuated lung ischaemia-reperfusion injury in rodent and orthotopic lung-transplant models by promoting macrophage polarization through a miR-22-3p/NLRP3/IL-1beta axis, with inhalation outperforming intravenous delivery in a head-to-head comparison (38). In bacterial pneumonia, Gonzalez et al. found the vesicles physically and molecularly unchanged by aerosolization and still immunomodulatory in culture; in rats instilled with E. coli, MSC-EVs given either intravenously or by direct nebulization reduced bacterial burden and oedema, raised blood oxygenation, and improved histological injury scores (11).

Upper-airway regenerative biology is also expanding beyond rhinitis. A 2026 otorhinolaryngology review mapped where EVs are currently being tested across ENT practice - sinonasal inflammatory disease, loss of smell, and mucosal healing - and concluded that almost all of that work is still preclinical (24). In vitro, Wharton's jelly MSC-derived sEVs delivered from a genipin-crosslinked gelatin hydrogel raised vocal-fold fibroblast proliferation and viability at an optimized dose (31); this is a scaffold-based culture study rather than an aerosol study and supports biological plausibility only. These observations complement the aerosol-deposition literature showing that the pharynx and larynx receive inhaled particles (28).

Table 2: Representative preclinical evidence for local respiratory-tract EV/exosome therapy.

Study	EV/exosome source	Model/indication	Delivery	Main findings	Relevance to autologous blood-derived concept
Han et al., 2022 (9)	Serum-derived small EVs	Pulmonary small-RNA delivery	Vibrating-mesh nebulization	Measured size/concentration preserved under tested conditions; functional RNA delivery to macrophages and airway epithelial cells.	High: blood (serum) source with direct vibrating-mesh nebulization data; murine serum, not human.
Xuan et al., 2024 (10)	Human platelet-derived EVs	Cigarette-smoke emphysema	Nebulized	Alveolar epithelial and macrophage uptake; reduced oxidative stress, inflammatory signaling, and	Very high mechanistic relevance: blood-derived source.

				apoptosis.	
Gonzalez et al., 2023 (11)	BM/UC-MSC EVs	E. coli pneumonia	IV or direct nebulization (head-to-head)	EV size, markers, and miRNA preserved across nebulization; reduced bacterial load and oedema, improved oxygenation and histology.	Strong model for stability-plus-function testing.
Xu et al., 2023 (12)	Hypoxic hUCMSC-EVs	Asthma	Nebulized inhalation	Reduced airway inflammation and remodeling; functional miRNA delivery.	Mechanistic support; source differs.
Dinh et al., 2020 (14)	Lung spheroid cell exosomes	Pulmonary fibrosis	Inhalation	Reduced collagen and myofibroblast proliferation; improved architecture.	Supports local antifibrotic/regenerative concept.
Zhao et al., 2022 (15)	MSC-EVs	Acute lung injury	Inhalation	Attenuated injury with macrophage/antioxidant-related mechanisms.	Supports pulmonary immunomodulatory pathways.
Mansouri et al., 2019 (32)	MSC exosomes	Pulmonary fibrosis	Experimental delivery	Prevented/reversed fibrosis with monocyte/macrophage modulation.	Mechanistic support for antifibrotic responses.
Gao et al., 2023 (33)	Wnt3a-loaded EVs	Alveolar epithelial injury	EV-based delivery	Promoted alveolar epithelial regeneration.	Supports cargo-driven regenerative signaling.
Liu et al., 2024 (35)	MSC exosomes	Acute lung injury	Experimental EV therapy	Reduced alveolar macrophage pyroptosis and inflammation.	Supports macrophage-targeted mechanism.
Zhou et al., 2019 (36)	EPC exosomes	LPS-induced ALI	Intratracheal	Reduced permeability, inflammatory mediators, and edema; miRNA-126 implicated.	Supports barrier-restoration mechanisms.
Xu et al., 2025 (18)	MSC-derived sEVs	Allergic rhinitis / nasal epithelial barrier	Local nasal/experimental	Restored nasal epithelial barrier integrity; miR-143/GSK3B implicated in tight-junction and barrier effects.	High upper-airway relevance: direct nasal epithelial repair evidence.
Yang et al., 2025 (19)	Human adipose stem-cell EVs	Allergic rhinitis	Nasal cavity administration	Reduced nasal inflammatory infiltration, IgE/IL-4-related responses and Th1/Th2 imbalance; improved rhinitis symptoms.	High upper-airway relevance: local nasal EV administration.
Liu et al., 2022 (20)	Antigen/adjuvant-loaded sEVs	Allergic rhinitis	Intranasal	Taken up by CD11c+ cells in nasopharynx-associated lymphoid tissue; raised antigen-specific IgG, lowered serum IgE, and eased allergic signs.	Supports nasopharyngeal targeting and functional cargo delivery.
Zou et al., 2026 (16)	Airway basal stem-cell-derived EVs	Experimental COPD	Nebulized	Restored alveolar architecture, promoted new vessel formation, and improved arterial blood-gas indices; PI3K-Akt signaling implicated.	Strong direct nebulization evidence for regenerative airway/lung repair.
Zamlus et al., 2025 (31)	WJMSC-derived sEVs	Vocal-fold fibroblast regeneration (in vitro)	Local biomaterial delivery model	Hydrogel-delivered sEVs raised vocal-fold fibroblast proliferation and viability at an optimized dose (in vitro only).	Mechanistic support for potential local laryngeal regenerative applications; not a nebulization study.

Human Evidence and Clinical Feasibility of Nebulized Ev/Exosome Administration

Human evidence is still early but provides an important translational foundation: nebulized EV administration has already been performed in healthy volunteers and in patients with serious respiratory disease. Shi et al. combined preclinical work with an early safety investigation in 24 healthy volunteers who received a single nebulized dose of clinical-grade adipose-MSC-derived EVs across escalating particle-dose groups, with no serious short-term treatment-related safety signal reported under the study conditions (47).

Repeated administration has also been demonstrated clinically. Zhu et al. evaluated nebulized allogeneic adipose-MSC-derived exosomes in seven patients with severe COVID-19 and reported feasible repeated dosing with clinical and laboratory improvement signals (48). Chu et al. likewise conducted a seven-patient pilot study of nebulized umbilical-cord MSC-derived exosomes in COVID-19 pneumonia (49). Although these studies were small, together they show that multi-dose EV nebulization can be implemented in acutely ill respiratory patients.

The 2025 investigation by Li et al. further advanced the field by including a randomized, single-blind, placebo-controlled phase I component in 24 patients with pulmonary fibrosis. Nebulized hUCMSC-EVs were reported as tolerable, with exploratory pulmonary-function and symptom outcomes (50). Collectively, these human studies support route feasibility and short-term tolerability and justify continued development of carefully characterized respiratory EV products. They do not yet establish efficacy of the specific Biotex autologous preparation, but they provide a clinically relevant precedent for the nebulized delivery route.

Table 3: Human evidence for nebulized extracellular-vesicle/exosome administration.

Study	Source	Population	Design/sample	Nebulized intervention	Main observation	Major limitation
Shi et al., 2021 (47)	Allogeneic adipose MSC-EVs	Healthy volunteers	Early safety study; n=24	Single nebulized administration across 2 x 10^8 to 16 x 10^8 particles	No serious short-term safety signal reported under study conditions.	Healthy volunteers; short follow-up; not an efficacy trial.
Zhu et al., 2022 (48)	Allogeneic adipose MSC exosomes	Severe COVID-19	Open-label pilot; n=7	2 x 10^8 nanovesicles daily for 5 days	Repeated nebulization feasible; clinical/laboratory changes reported.	Very small uncontrolled study.
Chu et al., 2022 (49)	Umbilical-cord MSC exosomes	COVID-19 pneumonia	Pilot clinical trial; n=7	Nebulized administration	Feasibility/tolerability reported.	Small cohort and exploratory design.
Li et al., 2025 (50)	hUCMSC-EVs	Pulmonary fibrosis	Randomized, single-blind, placebo-controlled phase I; n=24	Nebulized hUCMSC-EVs plus routine care	Tolerability reported; exploratory lung-function and symptom outcomes.	Early-phase sample; correction notice should be consulted.

Quality Assurance for Preserving EV Activity during Nebulization

A major strength of an EV-nebulizer development program is that preservation of the biological product can be measured directly. Sterility and endotoxin testing support safe mucosal administration, while particle concentration, morphology, membrane integrity, EV-associated proteins, molecular cargo, and potency assays establish that the extracted fraction remains suitable after aerosolization. Monitoring soluble plasma proteins, lipoproteins, and aggregates further improves product definition and strengthens interpretation of biological effects.

The most informative analytical design is paired within the same patient-specific preparation: pre-nebulization starting material, aerosol-collected post-nebulization fraction, and residual device fraction. This approach minimizes the confounding effect of inter-individual EV variability and directly quantifies the performance of the delivery step. Particle-number-normalized functional assays can then confirm that retained biological activity reflects true potency rather than simply differences in recovered dose.

Same-session processing is especially attractive because it can reduce prolonged storage and repeated freeze-thaw exposure, both of which are known to influence EV characteristics (51,52); where fresh same-session use is not possible, stabilized and dried EV formulations have been developed as an alternative to ultra-low-temperature storage, including inhalable dry powders (53). For a fresh autologous workflow, recording pH, osmolality, loaded volume, nebulization time, temperature, residual volume, emitted dose, and respirable aerosol fraction provides a practical quality framework for reproducible delivery. These measurements convert nebulization from a simple administration step into a controlled, documentable component of the therapeutic process.

Table 4: Recommended pre- and post-nebulization quality-control framework for an autologous blood-derived EV/exosome-enriched preparation.

Quality attribute	Pre-nebulization	Post-nebulization	Suggested method	Purpose
Particle concentration	Required	Required	NTA and/or TRPS	Quantify recovery/loss.
Size distribution	Required	Required	NTA/TRPS; DLS complementary	Detect aggregation/fusion/fragmentation.
Morphology	Required	Required	TEM or cryo-EM	Assess gross structural preservation.
Membrane integrity	Required	Required	Permeability/leakage and RNase-protection approaches	Detect membrane compromise.
EV-associated proteins	Required	Required	CD9/CD63/CD81 within a broader panel	Assess preservation of EV-associated features.
Non-vesicular contaminants	Required	As appropriate	Albumin, ApoA1/ApoB and preparation-specific markers	Assess plasma-protein/lipoprotein contamination.
RNA/miRNA cargo	Baseline	Repeat	RT-qPCR/sequencing; free vs vesicle-associated RNA	Assess cargo retention/leakage.
Protein cargo	Baseline	Repeat	Immunoassay/proteomics as appropriate	Assess molecular preservation.
Cellular uptake	Reference	Repeat	Validated uptake assay	Functional interaction.
Biological potency	Reference	Required	Disease-relevant cell assay	Integrated functional preservation.
Sterility/endotoxin	Required	Process-dependent	Validated microbiological/endotoxin testing	Pulmonary safety.
Loaded/residual volume	Record	Residual required	Volumetric/gravimetric	Quantify device-associated loss.
MMAD/GSD/FPF	-	Required for aerosol validation	Cascade impactor or equivalent	Define aerodynamic deposition profile.
Emitted dose	-	Required	Validated aerosol collection	Quantify material leaving device.
Nebulization time/temperature	Baseline	Record during/end	Direct recording/sensor	Identify duration and thermal stress.

Translational Opportunities and Product-Development Considerations

For translation, the biological preparation and nebulizer can be developed as a coordinated delivery system even though they are manufactured separately. The kit provides a same-patient EV/exosome-enriched starting material, while the nebulizer determines emitted dose and regional aerosol deposition. This modular structure is advantageous because device parameters can be optimized for the intended target - nasal/upper airway versus distal lung - while the biological preparation is characterized independently.

Dose standardization is an important development opportunity. EV studies report particle number, protein quantity, preparation volume or parent-cell equivalents, but inhaled delivery allows dose to be linked more directly to loaded particle number, emitted dose, respirable fraction and deposited dose. Two independent head-to-head comparisons have found inhaled delivery of the same vesicles superior to intravenous administration, in acute lung injury and in lung ischaemia-reperfusion injury respectively (15,38), which argues for developing the aerosol route deliberately rather than treating it as merely a convenient alternative. This framework is particularly useful for a small-volume autologous preparation because it enables efficient use of patient-derived material and quantitative comparison across devices and treatment sessions.

Patient-to-patient molecular variability is intrinsic to autologous manufacturing, but the processing workflow can still be standardized through controlled collection, incubation, centrifugation, membrane processing, formulation, release criteria, and nebulizer settings. MISEV-aligned characterization, potency testing, sterility assurance, and route-specific dose definition provide a rational pathway for translating the concept while preserving the personalized nature of the biological material (1,17,54,55).

Discussion: Current Evidence Boundaries and Interpretation

The respiratory EV literature is expanding rapidly, but the evidence is not uniform across anatomical regions or vesicle sources. Direct nebulization data are strongest for lower-airway and pulmonary delivery, whereas upper-airway therapeutic studies are predominantly intranasal rather than nebulizer-based. Most disease-modifying studies use MSC-, platelet-, serum-, or airway-cell-derived EVs rather than same-patient whole-blood EV/exosome-enriched fractions. Systematic reviews and meta-analyses of preclinical EV therapy in lung injury report consistent directional benefit but also substantial heterogeneity in EV source, dose definition, timing, and outcome measurement, together with incomplete reporting of risk of bias (56-58); in this review, such pooled analyses are treated as secondary evidence summarizing the field rather than as primary support for any individual claim. These distinctions should guide interpretation without obscuring the recurring cross-study observation that locally delivered EVs can retain biological activity and engage respiratory epithelial and immune targets.

Differences in isolation method, dose definition, device type, and aerosol reporting further limit direct numerical comparison between studies. Even so, the convergence of findings across nasal-barrier models, allergic-rhinitis models, nebulized RNA-delivery studies, platelet-EV emphysema studies, MSC-EV lung-injury studies, and early human nebulization trials provides a mechanistic platform for testing an autologous respiratory EV strategy (9-20,47-50).

Three boundaries deserve explicit statement. First, no published study has administered a point-of-care autologous whole-blood-derived EV/exosome-enriched fraction to the human respiratory tract by nebulization; the Biotex workflow described here is a development concept rather than an evaluated intervention, and nothing in this review should be read as evidence of clinical efficacy or safety for that preparation. Second, the human data that do exist derive from allogeneic, culture-expanded, clinical-grade MSC-EV products manufactured under controlled conditions (47-50), and their tolerability profile cannot be transferred to a differently sourced and differently processed autologous material. Third, inhaled administration of a biological product is a regulated activity; any clinical application of the workflow described here would require the applicable regulatory authorization, independent ethics approval, and prospective safety monitoring.

The figures in this manuscript are conceptual. Figure 1 represents the manufacturer-supplied Biotex workflow; Figures 2 and 3 synthesize preservation, aerosol-deposition, and pulmonary mechanisms from the broader EV literature. They are intended to illustrate a biologically supported development pathway rather than to represent completed clinical outcome data for the Biotex preparation.

CONCLUSION

Local respiratory delivery is emerging as one of the most attractive applications of extracellular-vesicle and exosome biology. Controlled nebulization can preserve vesicle size, membrane-associated characteristics, molecular cargo, and biological activity, allowing functional EVs to reach airway epithelium, alveolar cells, and macrophages (9-13,16). In parallel, intranasal studies show that the same class of particles can act at the nasal mucosa and the nasopharyngeal immune interface, repairing junctional disruption and damping allergic inflammation in experimental models (17-20). Aerosol deposition science further supports access to the pharyngeal and laryngeal regions when device and breathing conditions are selected appropriately (28).

These findings support a unified respiratory-tract concept in which an extracted autologous EV/exosome-enriched fraction can be characterized, transferred to a closed medical nebulizer, and delivered locally according to the desired anatomical target. For the Biotex point-of-care workflow, the most valuable next analytical step is demonstration that the kit-derived patient-matched fraction retains particle recovery, membrane integrity, cargo, and potency after nebulization and produces a reproducible aerosol. The published literature provides a consistent mechanistic basis for EV-mediated epithelial repair, immune modulation, antioxidant activity, antifibrotic signaling, and cargo delivery across respiratory models, almost entirely in preclinical systems and with allogeneic or culture-derived vesicles. Product-specific validation can therefore build on an established biological and delivery rationale, but that validation remains to be performed, and no claim of clinical benefit for the Biotex preparation is made or implied here.

CONFLICTS OF INTEREST

The authors are employees of Biotex Life Solutions Pvt. Ltd., the developer/manufacturer of the Biotex Autologous Exosome Kit discussed in this review. This affiliation represents a potential conflict of interest and should be considered when interpreting product-specific sections.

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ETHICS STATEMENT

This review synthesizes published literature and manufacturer-supplied workflow information and does not report a new human or animal experiment. No patient data were collected or analysed.

REGULATORY NOTE

The Biotex Autologous Exosome Kit is described here as an investigational point-of-care preparation workflow. Neither the kit-derived EV/exosome-enriched fraction nor its nebulized respiratory administration is presented as an approved therapy, and no therapeutic, preventive, or disease-modifying claim is made for it. The nebulizer referred to throughout is a separate, generically available medical device and is not manufactured or supplied by Biotex Life Solutions Pvt. Ltd.

AUTHOR CONTRIBUTIONS

All authors contributed to the conception and critical review of the manuscript and approved the final version.

DATA AVAILABILITY

No new datasets were generated. All literature cited is publicly available through the DOIs listed in the References section. Manufacturer-supplied process information relating to the Biotex Autologous Exosome Kit is summarized in Section 4 and is available from the corresponding author on reasonable request.

DECLARATION ON THE USE OF ARTIFICIAL-INTELLIGENCE TOOLS

Large language model assistants were used for literature organization, reference verification, language editing, and structural drafting. All scientific content, source interpretation, claim calibration, and final wording were verified by the authors, who accept full responsibility for the accuracy and integrity of the manuscript.

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