



Application of exosomes for the alleviation of COVID-19-related pathologies

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Abstract

The pandemic of COVID-19 caused worldwide concern. Due to the lack of appropriate medications and the inefficiency of commercially available vaccines, lots of efforts are being made to develop de novo therapeutic modalities. Besides this, the possibility of several genetic mutations in the viral genome has led to the generation of resistant strains such as Omicron against neutralizing antibodies and vaccines, leading to worsening public health status. Exosomes (Exo), nanosized vesicles, possess several therapeutic properties that participate in intercellular communication. The discovery and application of Exo in regenerative medicine have paved the way for the alleviation of several pathologies. These nanosized particles act as natural bioshuttles and transfer several biomolecules and anti-inflammatory cytokines. To date, several approaches are available for the administration of Exo into the targeted site inside the body, although the establishment of standard administration routes remains unclear. As severe acute respiratory syndrome coronavirus 2 primarily affects the respiratory system, we here tried to highlight the transplantation of Exo in the alleviation of COVID-19 pathologies.

KEYWORDS

COVID-19, exosomes, paracrine interaction, regenerative potential, stem cell, therapeutic effects

1 | BACKGROUND

The emergence and prevalence of the COVID-19 pandemic have challenged the healthcare community and led to serious socioeconomic impacts.^{1,2} The cause of COVID-19 is a virus from the Coronaviridae family namely severe acute respiratory syndrome coronavirus 2 (SARS-

CoV-2).^{3,4} This virus harbors a single-stranded positive-sense RNA enclosed by nucleocapsid. An envelope with three structural proteins including membrane, spike (S), and envelope surrounds the nucleocapsid (Figure 1).⁵ Of note, the attachment and viral entry into the target cells is done via a close interaction between S proteins with cell membrane-bound angiotensin-converting enzyme 2 (ACE2). From

molecular structure, S protein has two distinct regions S1 and S2. To maintain the physical connection between SARS-CoV-2 and epithelial cells, subunit S1 is activated in the early steps of infections. In later phases, the subunit S2 promotes the fusion of SARS-CoV-2 with the plasma membrane (Figure 1).⁵ The interaction between S subunits and ACE2 leads to trihomodimerization which, in turn, provides mechanical force to push the virus forward into the cytosol.⁵ It is thought that the occurrence of several mutations in the genomic structure of SARS-CoV-2 has changed the transmissibility and pathogenicity of the virus after nearly 3 years. From a molecular structure, the recently diagnosed variant Omicron (B.1.1.529) exhibits three deletions and one insertion mutation in S protein in comparison with the original SARS-CoV-2, leading to inefficiency of local and systemic neutralizing antibodies against Omicron S protein after vaccination or infection.^{6,7} Along with changes, the fusogenic activity of Omicron S protein is enhanced because of mutation in the vicinity of the furin cleavage site, leading to the prominent tropism toward epithelium in the upper respiratory tract.^{7,8} In support of this notion, the mutated S protein of Omicron has enhanced affinity to ACE2 compared to alpha and delta variants, leading to an accelerated viral entry to nasal epithelial cells.⁹ Recent data revealed the existence of an alternative Omicron entry into the target cells using ubiquitous endosomal pathways independent of transmembrane serine protease 2 (TMPRSS2) protease.⁹ Compared to other SARS-CoV-2 variants, Omicron can enter the host cells via TMPRSS2-dependent and independent manners.

To date, numerous therapeutic approaches have been used in COVID-19 patients to diminish the mortality rate.^{3,10} For example, the application of vaccines and transplantation of serum from infected individuals seems to be somewhat effective in the

Significance statement

Exosomes (Exo) are nanosized vesicles that are released from each cell type and can transfer therapeutic molecules to the injured sites. It has been shown that Exo therapy circumvents difficulties that are associated with whole cell-based therapies. Due to recent advances in the field of Exo-based therapies, the current review article highlighted recent data and the applicability of Exo during viral diseases such as COVID-19. The mechanism action and superiority of Exo were also debated in detail.

prevention of COVID-19.¹¹ It seems that local or systemic application of commercially available neutralizing antibodies cannot be an effective strategy to prevent breakthrough infections. Direct application of antibodies is difficult and effective just a few days after the onset of clinical manifestation.¹² Of note, based on recently conducted investigations, most of the clinically used monoclonal antibodies are inactive against Omicron.⁷ Even though, the existence of several variants during the COVID-19 pandemic can contribute to the prevention of several variants of SARS-CoV-2 with conserved S protein except for Omicron. Recent data have indicated the efficiency of whole mesenchymal stem cell (MSC) therapy and their exosome (Exo) alone or in combination with other therapeutic approaches in the alleviation of COVID-19-related pathologies.¹³ Here, we aimed to highlight the benefits associated with the application of Exo under the infection with SARS-CoV-2.

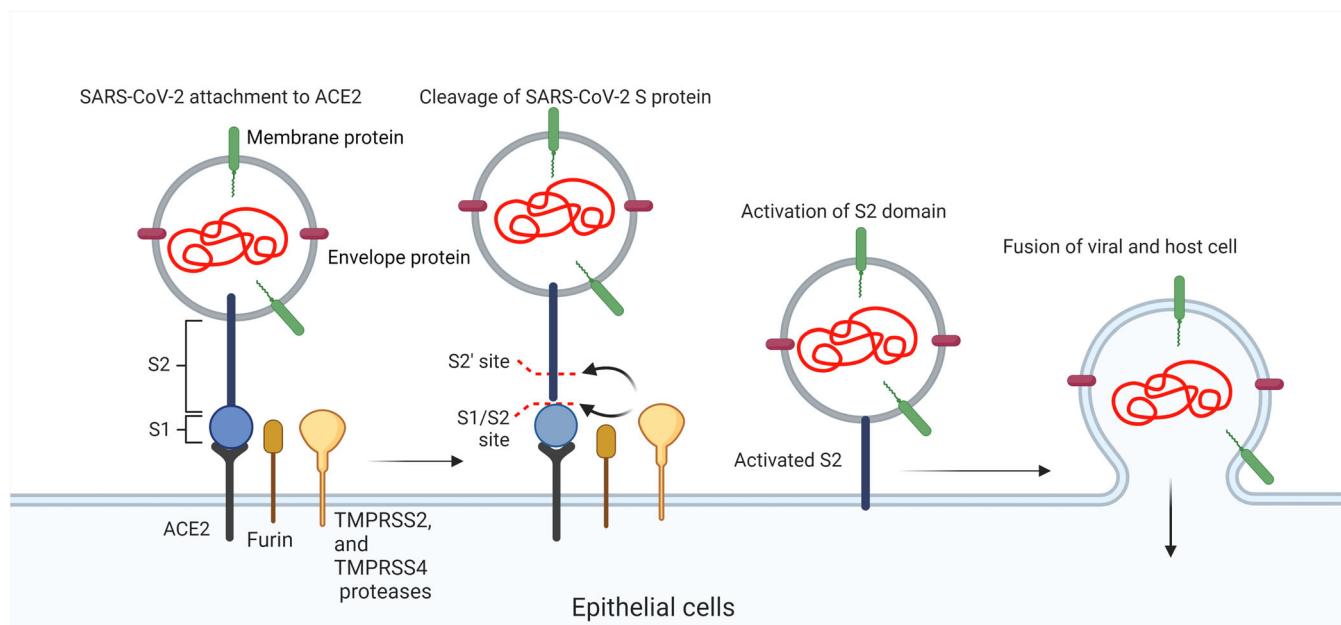


FIGURE 1 Mechanisms of SARS-CoV-2 entry into target cells. Using spike proteins (S1 and S2), this virus can fuse with the host cell membrane and inject genomic materials. ACE2, angiotensin-converting enzyme 2; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TMPRSS2 and 4, transmembrane serine protease 2 and 4.

Exo are extracellular vesicles (EVs) subset and secreted by almost all cell types under physiological and pathological conditions.¹⁴ These nanosized particles, 40–150 nm, distribute in various biofluids and participate in intercellular communication. Regarding transport activity, Exo transfer certain signaling biomolecules that may be involved in cellular hemostasis.¹⁵ Besides garbage disposal activity, accumulating data have indicated that Exo possesses specific protein and genomic fingerprints and reflects the metabolic status of origin cells.¹⁶ Exo are formed by the invagination of the membrane in early endosomes (Figure 2). In the next step, early endosomes generate multivesicular bodies (MVBs) where a lot of intraluminal vesicles (ILVs) are connected to the luminal surface of the lipid membrane. Later, MVBs and later endosomes are fused with the cell membrane and release ILVs into the extracellular matrix (ECM), hereafter known as Exo.¹⁷ Molecular investigation have suggested the existence of complex biogenesis machinery for Exo production within the cytosol.^{15,18} The process of Exo production inside the endosomal system can be done via endosomal sorting complexes required for transport machinery (ESCRT) or ESCRT-free mechanisms. The ESCRT system is a distinct cascade composed of several signaling effectors. It has been elucidated that several membrane bonded proteins such as ESCRT-0, I, II, III, Alix,

and VPS4/VTA1 in collaboration with other factors such as flotillin, and TSG101 actively participate in the formation of ILVs in the lumen of MVBs (Figure 2).¹⁹ In later phases, the close collaboration of other effectors such as SNARE and GTPase system leads to the fusion of later endosomes with the plasma membrane and release of ILVs into the ECM.¹⁷

Like other biological fluids, cumulative data have proved significant Exo levels in nasal discharge and airway mucus, indicating the putative key role of Exo in the pulmonary tract under physiological and pathological conditions.²⁰ Proteomic analysis of epithelium-derived Exo has revealed the alteration in exosomal cargo after the onset of nasal polyp.²¹

2 | THERAPEUTIC EFFECTS OF EXO DURING INFECTION WITH SARS-COV-2

Exo are nanosized vesicles released by several cell types in response to stimuli under pathological and physiological conditions.¹⁴ These particles can participate in intercellular paracrine activity via transferring several signaling biomolecules from donor to recipient

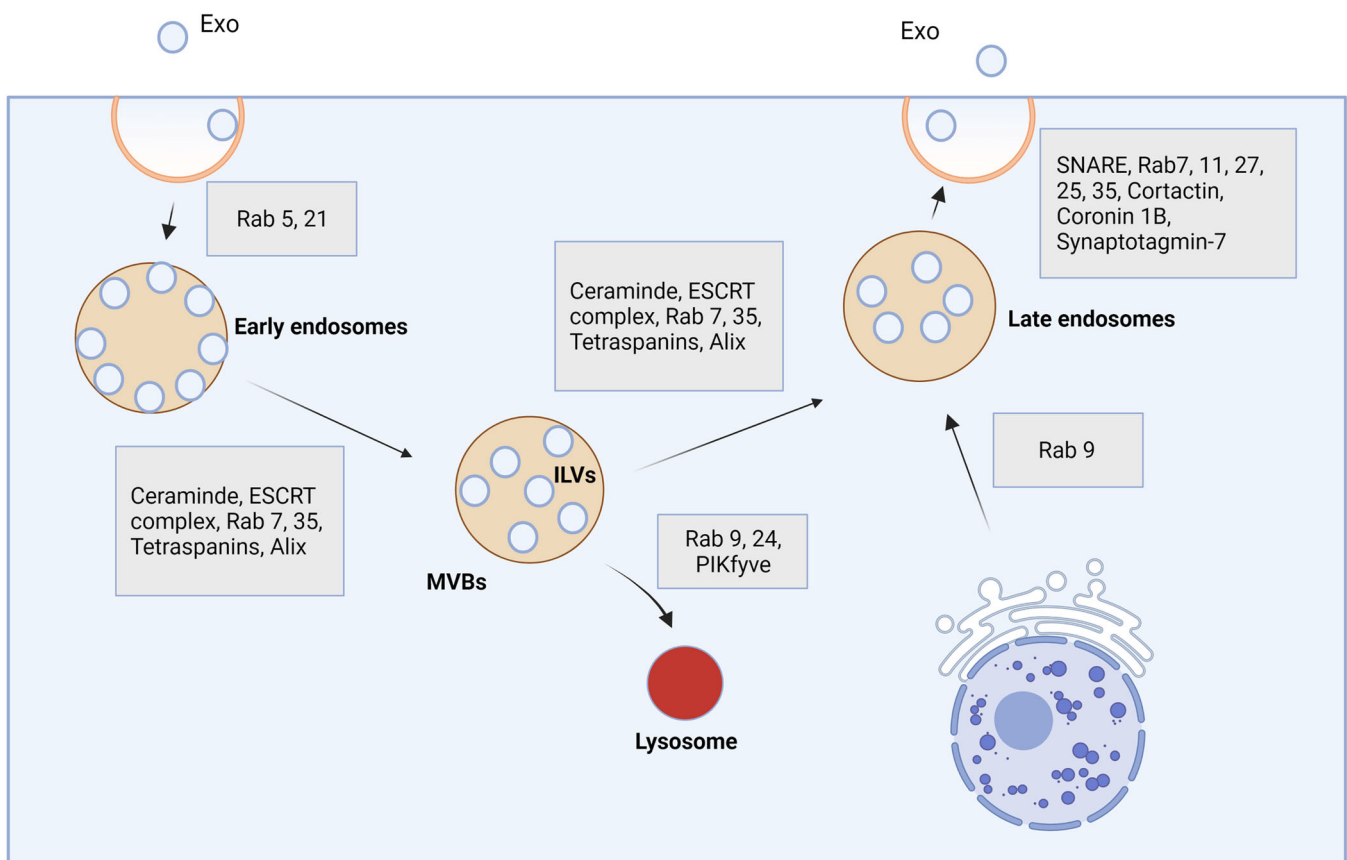


FIGURE 2 Exo biogenesis machinery system. These particles are produced using the endosomal system and several effectors. After the formation of early endosomes, the invagination of the vesicle membrane can produce numerous intraluminal vesicles (ILVs) which are attached. Multivesicular bodies are directed toward lysosomal degradation or formed late endosomes which can fuse with the plasma membrane and release ILVs into the ECM. ILVs hereafter are known as Exo. ECM, extracellular matrix; ESCRT, endosomal sorting complexes required for transport machinery; Exo, exosomes; MVBs, multivesicular bodies.

cells.¹⁵ Because of the existence of certain cargo types, Exo are potent enough to promote the regeneration of injured tissues, suppress the production of inflammatory cytokines, and modulate the activity of immune cells.²² Considering their unique physicochemical properties and suitable biodistribution in biofluids, Exo can be applied locally and/or systemically after the onset of pathological conditions. In this regard, Exo has been used for different purposes like the natural delivery vehicle, detection of pathological-associated biomarkers, and therapeutic purposes During the last decades.^{23,24} It has been found that Exo can diminish cell death and apoptosis via engaging several mechanisms. For instance, MSCs Exo can prevent detrimental effects of oxidative stress in degenerating cells via the regulation of reactive oxygen production.²⁵ The production of several inflammatory cytokines such as interleukin-1 (IL-1) and IL-6, tumor necrosis factor (TNF)- α in during COVID-19 can trigger the mitochondrial injury via the production of hydrogen peroxide and inducible nitric oxide synthase.²⁶ Besides, nasal and pulmonary epithelium failed to produce and secrete antiviral factors such as interferon at early stages after infection with SARS-CoV-2.²⁷ One reason would be that viral particles are recognized via the activation of Toll-like receptors 3, 7, and cytoplasmic factors such as RIG-I and MDA5, leading to activation of nuclear factor- κ B and production of types I and III interferons.²⁸ Exo can also increase antiviral responses in infected cells using several mechanisms. Transfer of interferon-stimulated genes (ISGs) via Exo is touted as an effective way to increase the production and secretion of interferons.²⁹ A piece of evidence point to the fact that MSC-Exo harbors notable contents of anti-inflammatory cytokines such as IL-10 and transforming growth factor- β . These factors can reduce proinflammatory response and blunt uncontrolled immune cell recruitment into the pulmonary niche in response to local production of IL-6, IL-8, and TNF- α coincided with the activity of leukotriene A4 hydrolase.^{30,31} The secretion of certain growth factors such as vascular endothelial growth factor and basic fibroblast growth factor by MSC-Exo can restore the function of the alveolar–blood barrier which can per se limit the extravasation of immune cells into the infected sites.³² The activation of Janus kinase/signal transducer and activator of transcription 3 in inflamed cells received MSCs-Exo can lead to the stimulation of cell proliferation, migration, and suppression of apoptosis. Besides, this axis can promote the production of interferons during the infection of pulmonary epithelial cells with SARS-CoV-2.³³ The production of interferons in infected cells can stimulate the expression of ISGs in bystander cells in short and long distances, leading to relative immunity in recipient cells before the entry of the virus.³⁴ Shortly after the infection of epithelial cells in the pulmonary tract, superimposing and predominance of opportunistic bacteria can hamper immune cell response to a great extent.³⁵ Of note, postmortem biopsies from lungs infected with SARS-CoV-2 revealed the changes in the composition of bacteria and fungi.³⁶ In addition to the cytoprotective effects of Exo against viral infection, some experiments have shown considerable contents of antibacterial factors inside Exo. It is thought that Exo can exert antibacterial properties via inhibition of growth and promotion of phagocytosis by

resident immune cells.³⁷ The secretion of several biomolecules, such as hepcidin, lipocalin 2, β -defensin 2, and so forth, activates clearance mechanisms. In circumstances when MSCs are directly exposed to bacteria, they can regulate the dynamic growth of T lymphocytes, increase T-regulatory cells, and trigger the phenotype shifting from M1 to M2 macrophages, leading to tissue regeneration and healing procedure (Figure 3).^{38,39} Considering the unique microanatomical features of nasal cavity with low temperature (about 33°C), a large number of viral particles can be replicated and released into the surrounding niche soon after exposure to viral particles. In addition, these features make the nasal cavity vulnerable to both viral and bacterial infections in the early steps.⁴⁰ Therefore, it seems that the intranasal application of Exo in the early steps of COVID-19 can reduce the replication of certain strains of SARS-CoV-2 and propagation into lower areas. Also, it should not be forgotten that SARS-CoV-2 infected epithelial cells per se produce Exo with certain proteomic signatures containing fibronectin, fibrinogen, P amyloid, and complement C1r subset which can be used as specific biomarkers for diagnosis and patients follow-up.⁴¹ In a recent experiment conducted by Barberis et al.,⁴¹ the interplay between the SARS-CoV-2 replication system and Exo biogenesis machinery was found, leading to the incorporation of viral genomic components into the Exo lumen and propagation infection to remote sites. Commensurate with these comments, one can hypothesize there is an apparent discrepancy, and the release of Exo from infected cells can lead to the rapid and progressive expansion of viral particles inside the body. The extension of SARS-CoV-2 to lower pulmonary sites such as lung parenchyma loosen the integrity of the alveolar–blood barrier and diminishes gas exchanges, leading to hypoxic condition and high-rate morbidity. To note, type II pneumocytes, and to less extent type I pneumocytes, are the main target cells infected by SARS-CoV-2 because of considerable contents of ACE2.⁴² It is shown that pulmonary vascular endothelial cells are less prone to infect directly with SARS-CoV-2. However, cytokine storm and vigorous proinflammatory response make them vulnerable to pathological conditions.⁴³ Along with these changes, in situ production of several cytokines, promotes diffuse immune cell extravasation and significant serum protein infiltration into the alveolar space.³ Vascular tissue injury predisposes COVID-19 patients to the formation of thrombi. Rahbarghazi et al. successfully revealed the protective effects of bone MSCs Exo on pulmonary endothelial cells by reducing the expression of adhesion molecules such as vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 in the rat model of asthma.⁴⁴ Physiological significance of human umbilical cord MSCs Exo has been previously proved on angiogenesis potential of endothelial cells in a rat model of vessel grafting. It was suggested that intravenous administration of Exo can promote the endothelialization process via the increase of CD31⁺ cells on the luminal surface. The activation of certain signaling cascades such as phosphoinositide 3-kinase/protein kinase B and mitogen-activated protein kinase/extracellular signal-regulated protein kinase 1/2 are critical in the angiogenesis potential of umbilical cord MSCs.⁴⁵ The precise cellular mechanisms that participate in the restoration of

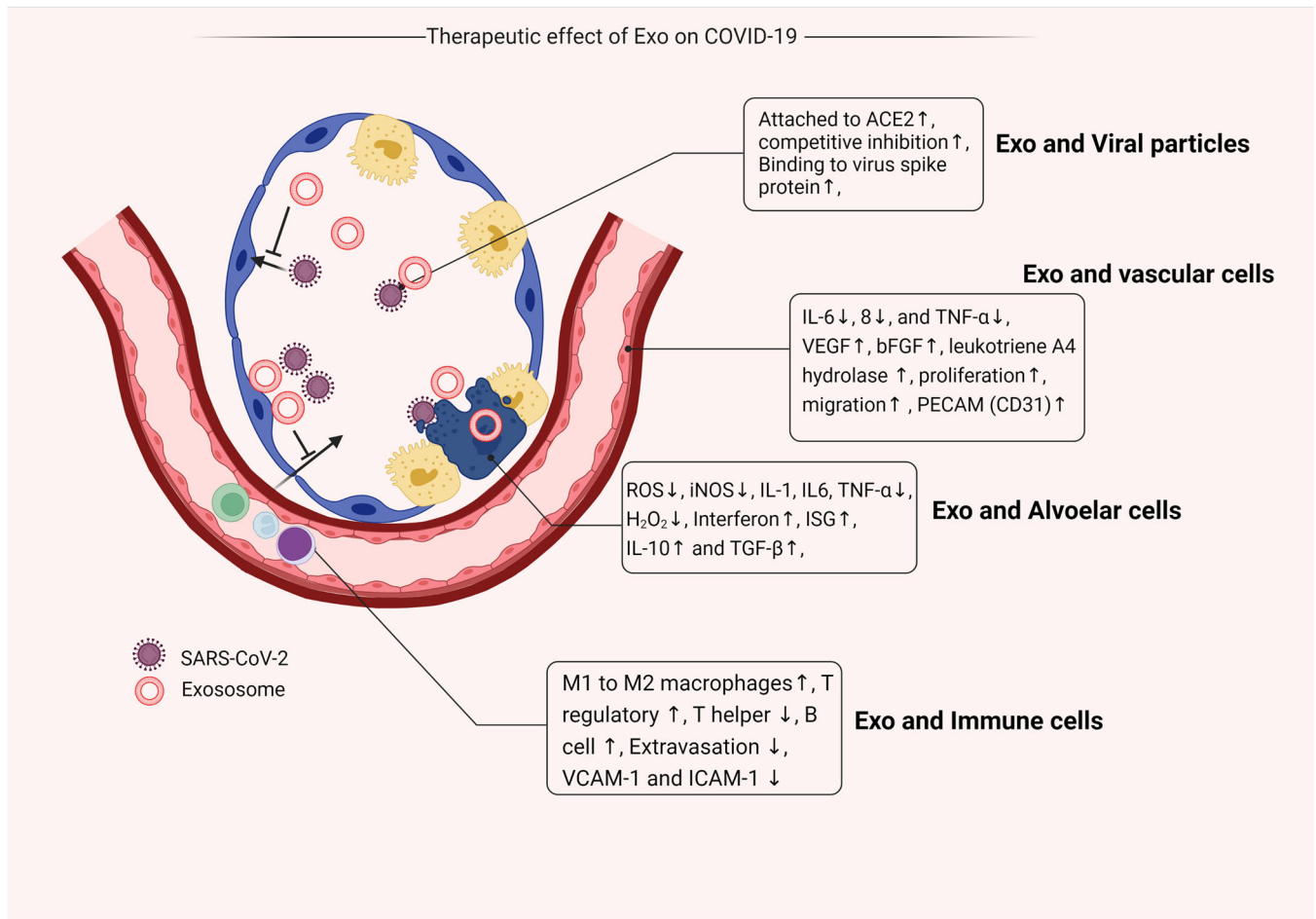


FIGURE 3 Different therapeutic effects of Exo in pulmonary tissue infected with SARS-CoV-2. ACE2, angiotensin-converting enzyme 2; bFGF, basic fibroblast growth factor; Exo, exosomes; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; ISG, interferon-stimulated gene; PECAM, platelet endothelial cell adhesion molecule; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

endothelial cell activity and vessel function are to determine in future studies. Whatever the reason, it seems the suppression of proinflammatory response and promotion of angiogenesis is the main underlying mechanisms involved in the restoration of vascular tissue function in COVID-19 patients.

3 | ENGINEERED EXO FOR THE INHIBITION OF VIRAL INFECTIONS

Transfer of certain viral components such as S protein can act as a natural vaccine and stimulate the production of neutralizing antibody titers.^{46,47} It has been shown that viral protein materials such as S protein can be packed and transferred by Exo in patients with mild and severe COVID-19.⁴⁷ These features can lead to proper activity of antigen-presenting cells and T lymphocyte function.⁴⁷ In support of this data, the development of engineered Exo-tagged with S protein increases the production of neutralizing antibodies in the mouse model.^{46,48} Interestingly, El-Shennawy et al.⁴⁸ showed the existence of

circulating Exo with high levels of ACE2 in COVID-19 patients which is closely associated with the severity of pathological conditions. In this scenario, these Exo possess a 135-fold binding capacity in comparison with engineered Exo tagged with recombinant ACE to neutralize circulating SARS-CoV-2 before reaching the target sites.⁴⁸ Noteworthy, recent data have revealed the shared molecular mechanisms between the virus replication system and Exo biogenesis machinery.⁴⁹ It is thought that ligand-receptor interaction is the main mechanism involved in Exo and virus entry into cells. Therefore, the application of ACE2⁺ Exo isolated from COVID-19 patients' serum can be touted as competitive inhibition therapy in terms of viral particles using ACE for entry.⁵⁰ Scott et al.⁵¹ designed anti-CoV-2-enriched EVs tagged with CD63 which can efficiently bind to SARS-CoV-2 S protein. They claimed that this sophisticated system is eligible enough to prohibit the virus expansion in the early stages of infection.⁵¹ In another experiment, engineered Exo with truncated CD9 and elevated ACE2 exhibited antiviral properties. These Exo successfully attached to viral S protein and inhibited the propagation of SARS-CoV-2 K18-hACE2 mice.⁵² The same strategy can be exploited to manipulate MSCs to

produce secretomes with high contents of ACE2.⁵³ Recently, Zhang et al.⁵⁴ found structural differences between ACE2 released by EVs and exomeres (subpopulation of Exo below 50 nm) from colorectal cancer cell lines namely LIM1215 and DiFi. They showed that EVs possess full-length glycosylated ectodomains of ACE2, while ectodomain fragments can be detected in exomeres.⁵⁴ Both short- and full-length ACE2 can be recognized using neutralizing antibodies. Likewise, full-length DPP4 ectodomain is sequestered onto EVs while Exo is equipped with the ectodomain of this enzyme.⁵⁴ These data showed that both EVs and exomeres can efficiently bind to the S protein of SARS-CoV-2. Alternatively, it was suggested the secretome of MSCs overexpressing ACE2 can blunt detrimental effects of lipopolysaccharide in mammary epithelial cells via the regulation of proinflammatory cytokines such as TNF- α , IL-1 β , and IL-6.⁵³ Results indicated that the promotion of inflammatory response in host cells can cause reduction of ACE2 presumably via an accelerated secretion of this factor. If so, ACE2-tagged Exo can act as a natural decoy to trap and neutralize circulating SARS-CoV-2.⁵⁴ The story gets more complicated when we acknowledge that TMPRSS2, TMPRSS4, and TNF- α converting enzyme is also secreted by EVs and exomeres.⁵⁴ Even though, the encapsulation of therapeutic agents and drugs into the Exo can also be touted as another modality to reduce or prohibit viral entry into the nasal or pulmonary tract epithelium.⁵⁵ Whether the existence of these factors can lead to viral particle priming and acceleration of infection is to be answered. Collectively, data indicate both stimulatory and inhibitory roles of Exo in the propagation of viral particles such as SARS-CoV-2. Whether and how Exo transfer SARS-CoV-2 from infected cells to bystander intact cells or exert blocking properties on circulating virus is the subject of area.

4 | APPLICATION OF EXO IN VIRAL INFECTIONS

The intranasal administration of therapeutic agents is touted as a reliable delivery route during several pathological conditions. In this approach, the therapeutic agents and drug can directly enter the brain parenchyma and circumvent the blood-brain barrier while the most fractions of injected compounds are not eliminated via the gastrointestinal tract or hepatic system.⁵⁶ Interestingly, intranasal administration of Exo isolated from stem cells and other lineages is touted as a suitable noninvasive delivery route for the alleviation of pathologies within the pulmonary tract and central nervous system.⁵⁷⁻⁵⁹ Unfortunately, the maximum fraction of Exo is eliminated a few hours after injection via intravenous route by trapping in the vascular bed of hepatic and pulmonary tissues.⁵⁷ Therefore, local administration of Exo into the nasal cavity during specific pathological conditions can provide sufficient Exo number to the site of injury. In terms of central nervous system disease, intranasal application of Exo is a promising therapeutic option to prohibit retrograde distribution of virus into the central nervous system and restore the function of the neurons a few days after administration.⁶⁰

Intranasal administration of Exo can lead to the reduction of repetitive behavior and improvement of mutual behavior in patients with an autism spectrum disorder.²² Nasal cavity is the entry point of several air-borne viruses such as SARS-CoV-2 and intranasal application of Exo in sensitive individuals exposed to this virus for a few hours could be an effective strategy to reduce the infection rate.⁴⁰ Along with intranasal injection of Exo, inhalation of Exo and/or stem cell secretome is an effective way to several types of lung injury and chronic pulmonary pathologies.⁶¹ In an experiment, it was suggested that Exo inhalation reduces fibrosis in a mouse model by restoring alveolar function and structure via the reduction of type I collagen deposition and myofibroblast proliferation.⁶¹ It is suggested that a runny nose and sore throat are the most clinical manifestation in Omicron patients. As a correlate, the application of Exo in Omicron patients seems easier and more effective because of local delivery of a low dose of Exo compared to the situation affecting lungs and bronchioles. Whether single or repeated doses of Exo are needed to alleviate Omicron-associated injury is the subject of debate. Like many pathological conditions, therapeutic effects of Exo have been proved in patients with COVID-19 in preclinical observations.³⁸ Regarding unique microanatomical properties, Exo application via intranasal dropping, and inhalation have been extensively considered in patients with lower and upper pulmonary tract inflammation. Based on an online survey related to the application of Exo in pulmonary disease recorded up to February 2022 (Table 1), Exo has been used as a biomarker, prophylactic (vaccination), and therapeutic agent for the alleviation of pulmonary disease and related cancers (available on <https://clinicaltrials.gov/ct2/results?cond=Pulmonary%2BDisease%26term=Exosomes%26cntry=%26state=%26city=%26dist=>). It seems that the number of clinical trials targeting COVID-19 patients increases by time.

Based on a great body of data, the application of crude or engineered Exo is an alternative approach to alleviate pathological changes along with conventional therapy following acute inflammatory response in the pulmonary niche. Although Exo administration is a noninvasive approach via different routes in specific types of COVID-19 especially Omicron local delivery has superiority compared to systemic administration.

5 | LIMITATIONS RELATED TO THE APPLICATION OF EXO IN THE CLINICAL SETTING

Although therapeutic intervention using Exo has led to splendid progress in the alleviation of several pathological conditions this era faces some limitation that needs further attention.⁶² The lack of standard protocols and characterization systems can lead to the restricted application of Exo in *in vivo* conditions.¹⁴ For example, it was shown that the morphology and integrity of Exo are changed using available purification methods.¹⁴ In samples isolated by

TABLE 1 List of Exo-based clinical trials recorded up to February 2022

Status	Study title	Conditions	Clinical phase
Recruiting	Omics sequencing of exosomes in body fluids of patients with acute lung injury	Acute lung injury	ND
Active, not recruiting	Early diagnosis of lung cancer using blood plasma-derived exosome	Lung cancer	ND
Unknown	Combined diagnosis of CT scan and exosome in early lung cancer	Early lung cancer	ND
Recruiting	Molecular profiling of exosomes in the tumor-draining vein of early-staged lung cancer	Non-small cell lung cancer	ND
Completed	Serum exosomal long noncoding RNAs as potential biomarkers for lung cancer diagnosis	Lung cancer	ND
Unknown	Clinical study of ctDNA and exosome combined detection to identify benign and malignant pulmonary nodules	Pulmonary nodules	ND
Completed	A pilot clinical study on inhalation of mesenchymal stem cells (MSCs) exosomes treating severe novel coronavirus pneumonia	Coronavirus	I
Active, not recruiting	Randomized, double-blind, placebo-controlled study to evaluate the safety and efficacy of exosomes overexpressing cd24 to prevent clinical deterioration in patients with moderate or severe covid-19 infection	COVID-19 disease	II
Not yet recruiting	The use of exosomes for the treatment of acute respiratory distress syndrome or novel coronavirus pneumonia caused by covid-19	COVID-19 disease	I and II
Recruiting	Efficacy and safety of exosome-MSC therapy to reduce hyper-inflammation in moderate covid-19 patients	COVID-19 disease	II and III
Recruiting	Immune modulation by MSCs-derived exosomes in covid-19	COVID-19 disease	ND
Recruiting	Circulating exosome RNA in lung metastases of primary high-grade osteosarcoma	Lung metastases and osteosarcoma	ND
Recruiting	Evaluation of the safety of cd24-exosomes in patients with covid-19 infection	COVID-19	I
Active, not recruiting	Covid-19 specific T cell-derived Exo	COVID-19	I
Recruiting	A clinical study of MSC exosomes nebulizer for the treatment of acute respiratory distress syndrome	Acute respiratory distress syndrome	I and II
Enrolling by invitation	Safety and efficiency of the method of Exo inhalation in covid-19 associated pneumonia	COVID-19	II
Completed	Evaluation of safety and efficiency of the method of exosome inhalation in SARS-CoV-2-associated pneumonia	COVID-19	I and II
Recruiting	Safety and efficacy of exosomes overexpressing cd24 in two doses for patients with moderate or severe covid-19	COVID-19	II
Not yet recruiting	Exosomes detection for the prediction of the efficacy and adverse reactions of anlotinib in patients with advanced non-small-cell lung cancer	Non-small cell lung cancer	ND
Completed	Trial of vaccination with tumor antigen-loaded dendritic cell-derived exosomes	Non-small cell lung cancer	II
Completed	Extracellular vesicle infusion treatment for covid-19 associated acute respiratory distress syndrome	COVID-19	II

Abbreviations: CT, computed tomography; ctDNA, circulating tumor DNA; Exo, exosomes; ND, nondetermined; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

ultracentrifugation, contamination with serum proteins, lipoproteins, and other EVs is possible.⁶³ Besides, the metabolic status, culture system setting, route of injection, and proper Exo dosage are fundamental factors that can affect therapeutic efficiency.⁶⁴ Systemically administrated Exo, especially from allogeneic sources, are

eliminated by the activity of splenic and hepatic macrophages, leading to a lack of appropriate Exo delivery to the target sites.⁶⁴ Sterility and the possibility of infectious agents such as viral particles and mycoplasma are other critical issues that must be carefully considered during the purification of Exo.⁶⁵

6 | CONCLUSION

Administration of Exo from different cell sources, especially MSCs or development of engineered Exo has paved the way for efficient control of viral infection in the pulmonary tract. For better therapeutic outcomes, it is mandatory to redefine the dose and time of injection in the COVID-19 patients. Despite numerous advantages associated with the Exo application, the unwanted side effects should be also considered in the context of COVID-19.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data generated or analyzed in the manuscript are included in this article.

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