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REVIEW ARTICLE

Mesenchymal Stem Cell-Derived Exosomes for COVID-19 Therapy, Preclinical and Clinical Evidence

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Since the emergence of the novel coronavirus, named COVID-19, researchers are looking for a treatment to stop the devastating pandemic. During these efforts, mesenchymal stem cells (MSCs), the potential next generation of therapeutic methods with wide application for diseases, have successfully controlled cytokine storm following the virus infection. However, the use of MSCs has been limited due to the ethical issues, immunogenicity, and genetic modifications. Therefore, exosomes were introduced as a suitable substitute for the MSCs. In the case of COVID-19 treatment, both MSCs and exosomes exert their beneficial effect mainly through the management of the cytokine storm. This study provided the underlying mechanisms for the effect of exosomes on COVID-19 treatment and presented several preclinical and clinical studies of exosomes for COVID-19 treatment.

Keywords: COVID-19, Exosome, Stem cells, Cytokine storm

Introduction to COVID-19 and Treatments

In December 2019, a novel coronavirus, named SARS-CoV-2, COVID-19, or 2019-nCoV, was recognized in Wuhan city and turned into a pandemic at a fast rate. The danger of producing severe acute respiratory syndrome in many people infected with the highly contagious virus resulted in activity restrictions, economic recession, and the collapse of the health care systems around the world (1, 2). Despite some asymptomatic patients or those with mild symptoms, acute respiratory distress syndrome (ARDS) and ultimately multiple organ failure (MOF) was evident in the elderly, as well as cases with chronic lung disease, diabetes mellitus, and cardiovascular diseases comprising 10～34% of the whole virus-infected population (3, 4). Therefore, scientists have launched a big competition to find solutions for the management of the deadly coronavirus. Their attempts have been expanded from repurposing drugs that are already approved for other diseases to the invention of new drugs and therapeutic methods. Examples of effective drugs for COVID-19 treatment in animals that are used basically for other diseases

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are remdesivir (an antiviral), chloroquine (a malaria medication), lopinavir (an drug for human immunodeficiency [HIV] virus) combined to ritonavir, and a combination of lopinavir/ritonavir/interferon-beta (5, 6). Other options exist for the prevention and treatment of COVID-19 including vaccination and plasma transmission; however, the efficacy of these two methods requires stable viral epitopes while the RNA in this novel virus mutates rapidly and suppresses host T cell function that leads to frequent presentations of MOF in the setting of immunodeficiency even in healthy individuals (7).

As another therapeutic option, stem cell-based therapies have emerged for COVID-19 management. Despite significant progress, stem cell-based therapies are still accompanied by limitations including immunogenicity, limited cell source, and ethical concerns, rendering their clinical use (8). Additionally, it has been distinguished that the main mechanism of stem cell efficacy for COVID-19 treatment is the management of the cytokine storm through their paracrine effects (9-11). Therefore, stem cell-derived exosomes have been introduced as an alternative to stem cells considering their abilities to manage cytokine storm and not having stem cell-related problems (12, 13). In this study, the application of exosomes as a potential treatment for ARDS produced in COVID-19-infected patients is discussed. After presenting an introductory section on mechanisms of action of exosomes for ARDS management, different pre-clinical, and the most updated clinical practices of exosome therapy for COVID-19 treatment is discussed.

Cytokine Storm in COVID-19

The coronavirus structure is a non-segmented positive-sense single-stranded RNA, with 26 to 32 kb in length, covered with structural proteins consists of a spike (S), envelope (E), membrane (M), and nucleocapsid (N) protein. Among these structural proteins, the role of S protein in virus entry and replication in the host cell is more considerable. Binding this protein to the angiotensin-converting enzymes 2 (ACE2), a type of receptor expressed on the surface of human cells especially in the alveolar type II cells, results in virus entry and then virus replication with the help of the host machinery. Virus entrance into the lung causes massive alveolar damage, major histological changes, interstitial inflammation, intra-alveolar edema, granular tissue formation, fibrin and collagen deposition, bronchiolitis and leukocyte infiltration, pneumonia, and ultimately progressive respiratory failure (14-16). All these events take place following an excessive immune response induction, also known as a cytokine storm, that sharply increase inflammatory cytokines such as interleukin-2 (IL-2), IL-7, IL-10, granulocyte-colony stimulating factor (G-CSF), interferon-γ-inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 alpha (MIP-1A), and tumor necrosis factor-alpha (TNFα) (17).

The studies have shown the effectiveness of MSCs in preventing the excessive release of cytokines by immune cells (14, 18-20). The mechanisms of stem cells' effect on cytokine storm have been attributed to 1) diminishing the level of chemokines and proinflammatory cytokines through IL-1 receptor agonist release, generating TNFα-stimulated gene-6 (TSG-6), and finally downregulating NF-κB signaling (21); 2) targeting T-lymphocytes and down-regulating their proliferation or preventing the infiltration of inflammatory cells into the lungs (22, 23); 3) increasing B lymphocytes, natural killer (NK) cells, and dendritic cells (DC) (24); and 4) releasing cytokines including tissue growth factor-beta (TGF-β), hepatocytic growth factor (HGF), prostaglandin E2 (PGE2), and IL-10 (25). As presented in Fig. 1, secretion of different factors from MSCs modulate the cytokine storm following COVID-19 infection (10).

The Role of Exosomes in the Management of Cytokine Storm

Exosomes with endosomal origin are small vesicles of 30～130 nm size rich in proteins like heat shock proteins, annexins, cytoskeletal proteins, signal transduction proteins, and multivesicular body synthesis proteins. The content of exosomes depends on the parent cell from which they are secreted. They also carry biological membrane proteins, cytosolic proteins, transcription factors, messenger RNA (mRNA), ribosomal RNA (rRNA), micro RNA (miRNA), various signal transduction molecules, and cell adhesion molecules on their surface for binding to the target cell (26-28).

MSCs, as one important cell types for the stem cell therapies application, manage the cytokine storm following COVID-19 infection through their paracrine effects. The research has shown that exosomes are as effective as MSCs in the management of cytokine storm so that in a pattern similar to MSCs, exosomes downregulate T-cells proliferation, induce auto-reactive lymphocytes for the apoptosis of activated T cells, and secrete anti-inflammatory cytokines such as IL-10 and TGF-β (15, 29, 30). Exosomes also induce polarization of M1 macrophages to M2 phenotype, an inhibitor of pro-inflammatory cyto-

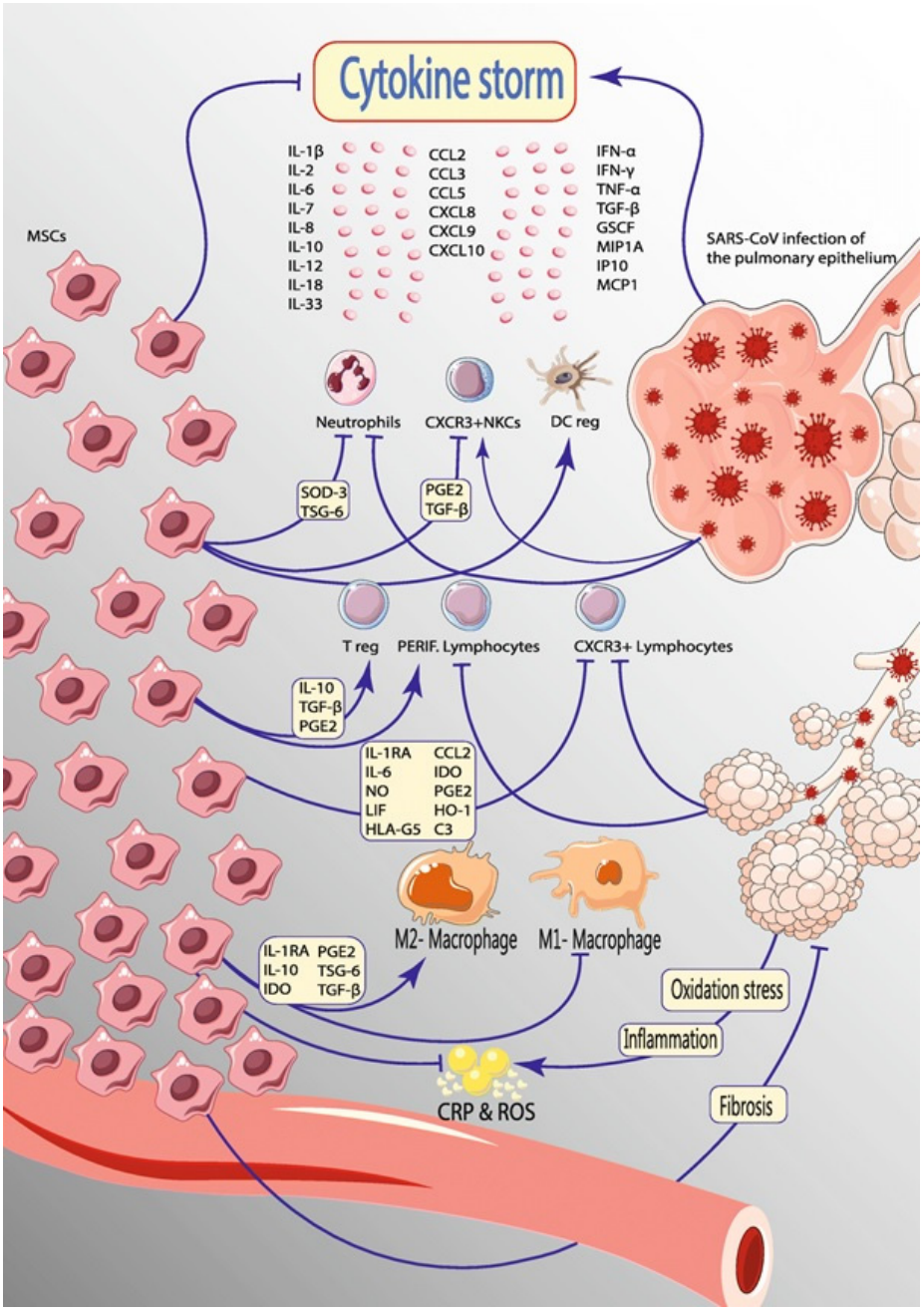

Fig. 1. Immunomodulation effect of mesenchymal stem cells on cytokines storm led by COVID-19. When SARS-CoV-2 enters the lungs, it attracts immune cells to infection areas and localizes inflammation. The lethal unchecked systemic inflammatory response is caused by the secretion of large levels of pro-inflammatory cytokines such as interleukin, interferons, chemokines, and other factors by immune effector cells in this infection. After MSC therapy, these cells reach the lung tissue and secrete factors that can modulate the immune system; they also can prevent ROS and even fibrosis of the lung tissue. Abbreviation: ARDS: acute respiratory distress syndrome, COVID-19: coronavirus disease 2019, CCL: chemokine (C-C motif) ligand, CXCL: chemokine (C-X-C motif) ligand, C3: Complement component 3, CRP: C-reactive protein, DC reg: regulatory dendritic cells, GSCF: granulocyte colony-stimulating factor, HO-1: Heme oxygenase-1, HLA-G5: human leukocyte antigen-G, IL: interleukin, IFN: interferon, IP10: IFN-γ-Inducible Protein 10, IL-1RA: interleukin-1 receptor antagonist, LIF: leukemia inhibitory factor, IDO: Indoleamine 2,3-dioxygenase, MSCs: mesenchymal stem cells, MIP1A: Macrophage Inflammatory Protein 1 Alpha, MCP1: *monocyte chemoattractant protein 1*, NKCs: natural killer cells, NO: nitric oxide, PERIF: peripheral, PGE2: Prostaglandin E_2, ROS: *reactive oxygen species*, SARS-CoV: severe acute respiratory-associated coronavirus, SOD-3: *superoxide dismutase*, TSG-6: TNFα-stimulated gene-6, TGF-β: transforming growth factor, Treg: regulatory T. With permission from Kavianpour et al. 2020 (10).

kines, and upregulate miR-146a expression which is known to exert anti-inflammatory effects (31).

Several mechanisms underlying the effect of stem cell-derived exosomes in COVID-19 improvements in preclinical models of ARDS are suggested. For example, Zhu et al. (32) reported that the secretion of some soluble factors such as keratinocyte growth factor (KGF) following exosome therapy lead to the reduction of alveolar inflammation. In another case, Li et al. (33) employed a murine lung ischemia/reperfusion (I/R) model to show that bone marrow MSC-derived exosomes reduce lung I/R by transporting miR-21, an anti-apoptotic miRNA. They have demonstrated that intratracheal administration of either MSC-derived exosome or miR-21-5p alleviates lung edema and repress the secretion of high mobility group box protein 1 (HMGB1), IL-8, IL-1β, IL-6, IL-17, and TNF-α (33). Monsel et al. (34) also reported reduced secretion of inflammatory cytokines during an effort to study the effect of MSCs-derived exosomes on lung inflammation and bacterial clearance in mice suffering from severe *Escherichia coli* (*E. coli*) pneumonia. Exosomes also lowered inflammatory cytokines and increased intracellular ATP levels in the alveolar epithelial type II cells (34).

Other studies suggested the role of miRNA-126 in the alveolar-epithelial barrier and therefore affecting lung inflammation following exosomes administration (35). The important role of the CD44 receptors in the exosome effect and the necessity of this receptor for the exosome uptake has been evidenced by the co-administration of exosomes with anti-CD44 in human lungs (34, 36). In another work, Khatri et al. (37) collected exosomes from a conditioned medium of swine bone marrow MSCs by ultracentrifugation and then used them in *influenza* virus-induced acute lung injury (ALI) pig model. The inhibitory effect of a certain exosome concentration on hemagglutination activity of *influenza* viruses which resulted in a reduction of pro-inflammatory cytokines (TNF-α, CXCL10, IL-10) was considered as the reason behind the beneficial effects of exosome (37). Tang et al. (38) suggested that reduced inflammation and recovery of the alveolar-capillary barrier in a mouse model of ALI following exosome treatment is due to improved KGF and angiopoietin-1 (Ang-1) mRNA expression. Increasing Ang1 secretion following the release of MSC-derived exosomes restores lung protein permeability across Human Lung Micro Vascular Endothelial Cells (HLMVECs), as was reported by Hu et al. (39).

The Diverse Sources of MSC-Derived Exosomes as Therapeutic Approach for COVID

The source of MSC-derived exosomes is a determining factor in its therapeutic effect. Bone marrow has been the main source of MSC-derived exosomes in many studies while umbilical cord MSCs have been used for exosome extraction in a study (40). In this study, Varkouhi et al. (40) showed that exosomes derived from umbilical cord MSCs have a therapeutic effect on *E. coli*-induced ALI in rats. Bacterial phagocytosis by macrophages and endothelial nitric oxide synthase were ameliorated and alveolar-arterial oxygen gradient, protein leak, and alveolar TNF-α concentration were decreased (40). Zhu et al. (32) extracted exosomes from bone marrow-derived MSC using ultracentrifugation. After induction of ALI in mice by the intratracheal instillation of a nonlethal dose of endotoxin from *E. coli*, Zhu's team indicated that MSC-derived exosomes lead to improved indices of ALI after 48 hours and reduced extravascular lung water, and total protein levels in bronchoalveolar lavage fluid (32). Park et al. (41) also utilized exosomes derived from bone marrow MSCs in an ex vivo experiment in a human model of bacterial pneumonia induced by intra-bronchial insertion of *E. coli*. They observed increased alveolar fluid clearance, reduced protein permeability, and lower bacterial load in the injured alveolus (41).

Preclinical Studies on MSC-Derived Exosome for COVID-19 Treatment

Several preclinical studies has shown the effectiveness of exosomes derived from MSCs for the management of ARDS and ALI as presented in Table 1. This suggests the potential use of exosomes for the management of ARDS in acute cases of COVID-19 infection.

Clinical Trials on MSC-Derived Exosome for COVID-19 Treatment and Their Limitations

Since the onset of the pandemic, only a research group has completed the study of the safety and effectiveness of MSC-derived exosome administration to the COVID-19 patients (18). In this prospective nonrandomized open-label cohort study, 15 ml exosomes derived from bone marrow MSCs delivered intravenously to 24 severe COVID-19 patients (18). The safety of the exosome was confirmed after observing no adverse effect within 72 h of injection. Improved clinical status evidenced by a 192% increase in the average pressure of arterial oxygen to fraction of in-

Table 1. MSC-derived exosomes in preclinical models of ARDS and ALI

PubMed	EVs-MSCs source	Disease model	Therapeutic EVs dose	Outcomes
23939814	Human bone marrow	Male C57BL/6 mice with *E. coli* endotoxin-induced ALI	EVs from 750,000 cells	· Reduced inflammatory cell influx · Reduced EVLW · Reduced alveolar MIP-2 protein · Expression of KGF mRNA · Increased KGF · Increased IL-10
30682335	Human bone marrow	Male C57BL/6 mice with lung ischemia/reperfusion Murine primary pulmonary endothelial cells	EVs from 2×10^6 cells	· Reduced lung edema · Reduced IL-8, IL-6, IL-17, IL-1β, TNF-α, HMGB1 · Reduced alveolar macrophage M1 polarization
30076187	Human bone marrow	Mice with *E. Coli* induced ALI	EVs from 1×10^6 cells	· Increased AFC rate · Increased antimicrobial effect · Decreased lung protein permeability · Decreased bacterial CFU
25847030	Human bone marrow	Ex vivo perfused human lung (rejected for the transplant)	100 μl EVs	· Increased AFC rate · Decreased lung weight gain · Increased lung compliance · Decreased pulmonary artery pressure and resistance · Increased NO in perfusate · Decreased pH of perfusate · Decreased elevation of lactate
28376568	Human bone marrow	*P. aeruginosa* induced mouse	10 μl EVs from 1×10^6 cells	· Decreased WBC influx · Decreased MIP-2 secretion · Restoration of pulmonary capillary · Expression of Ang-1 mRNA · Increased alveolar Ang-1 · Decreased TNF-α · Increased IL-10
26067592	Human bone marrow	Male C57BL/6 mice with *E. coli* induced ALI	30 or 60 μl (intratracheal) 90 μl (i.v. injection)	· Decreased total bacterial load · Decreased inflammation · Decreased lung protein permeability · Increased monocyte phagocytosis · Decreased TNF-α by LPS primed human monocytes · Increased COX2 and IL-10 mRNA expression · Increased IL-10 secretion by monocytes · Decreased bacterial CFU
29378639	Human bone marrow	*Influenza* virus-infected pig	EVs from 10×10^6 cells	· Decreased Haemagglutination activity of influenza viruses · Decreased virus replication · Decreased lung inflammation · Decreased virus replication · Decreased pro-inflammatory cytokine production · Increased IL-10
29737632	Human bone marrow	HLMVECs injured by cytomix (IL-1β, TNF-α, and IFN-γ)	20 μl EVs from 1×10^6 cells	· Restoring protein permeability across injured HLMVECs · Preventing actin stress fibers formation · Preventing reorganization of cytoskeleton protein F-actin into actin stress fiber in cytomix injured HLMVEC · Restoring the VE-cadherin (adherens junction) and ZO-1 (tight junction) in HMVEC injured by cytomix · Increased Ang-1 mRNA expression

Table 1. Continued

PubMed	EVs-MSCs source	Disease model	Therapeutic EVs dose	Outcomes
30870158	Human umbilical cord	Rat with *E. coli* induced ALI	EVs from 100×10^6 cells	· Increased survival · Reduced alveolar-arterial oxygen gradient · Reduced alveolar protein leak · Increased lung mononuclear phagocytes · Reduced alveolar TNF-α concentrations · Enhanced endothelial NOS production

Abbreviations: EVs: Extracellular Vesicles, HLMVECs: Human Lung Micro Vascular Endothelial Cells, ZO: Zonula Occludens, MSC: Mesenchymal Stem Cells, EVLW: Extravascular Lung Water, MIP-2: Macrophage Inflammatory Protein 2, KGF: Keratinocyte Growth Factor, IL: Interleukin, TNF: Tumor Necrosis Factor, HMGB 1: High Mobility Group Box 1, CFU: Colony-Forming Unit, WBC: White Blood Cell, LPS: Lipopolysaccharides, COX: Cyclooxygenase, NOS: nitric oxide synthase.

Table 2. Ongoing clinical trials of exosomes derived from MSCs or other cell types for COVID-19 therapy before February 26, 2021

Study ID	EVs source	Delivery mode	EVs dose	Study phase; participant (n)	Outcomes
NCT04602442	N/A	Inhalation	0.5-2$\times10^{10}$ exosome/ 3 ml, twice a day for 10 days	II; n=90	· Adverse events · TTCR · Blood biochemistry · SpO2 concentration changes · Chest imaging changes
NCT04491240	MSC	Inhalation	0.5-2$\times10^{10}$ exosome/ 3 ml, twice a day for 10 days	I & II; n=90	· Adverse events · TTCR · Blood gases changes · SpO2 concentration changes · Chest imaging changes
NCT04389385	T cells	Inhalation	2×10^8 exosome/3 ml; daily for 5 days	I; n=60	· Adverse events · TTCR · Rate of recovery without a mechanical ventilator
NCT04384445	Human amniotic fluid	Intravenous infusion	2-5$\times10^{11}$ exosome/ 1 ml, day 0, day 4, and day 8	I & II; n=20	· Adverse events · 60-day mortality · Survival rate · Levels of cytokines, D-dimer, CRP · Viral load · Improved organ failure · Chest imaging changes
NCT04493242	Bone marrow	Intravenous injection	N/A	II; n=60	· 28-day mortality · Median days to recovery
NCT04276987	MSC	Inhalation	2×10^8 exosome/3 ml, daily for 5 days	I; n=30	· Adverse events · TTCR · Number of patients weaning from mechanical ventilation · Days of ICU monitoring · Days of vasoactive agents' usage · Days of mechanical ventilation · Number of patients with improved organ failure · 28-day mortality

spired oxygen ratio (PaO2/ FiO2) showed the effectiveness of the therapy. Increased neutrophil and lymphocyte count, as well as reduced C-reactive protein, ferritin, and D-dimer, represent effective regulation of cytokine storm via exosome (18).

Besides, a handful of clinical studies are performed on exosome therapy in COVID-19 patients. After searching the words "exosome" and "COVID-19' in clinical trial da-

Table 2. Continued

Study ID	EVs source	Delivery mode	EVs dose	Study phase; participant (n)	Outcomes
NCT04747574	T-REx™-293 cells	Inhalation	1×10^9 exosome/ 2 ml, daily for 5 days	I; n=30	· Adverse events · Survival at Day 5 · SpO2 saturation measurement · Respiratory rate measurement · Proportion of patients with no artificial ventilation · Change in the absolute lymphocyte count from baseline · Change in neutrophil-to-lymphocyte ratio
ISRCTN 33578935	Placental MSC	Intravenous infusion	0.2 mg/kg, 15 ml, on day 1 and day 3	II; n=64	· Adverse events · PaO2/FiO2 ratio
IRCT20200510047385N1	Condition media of SVF and blood	Injection into the patient's lungs through the trachea	10 ml daily for 3 days	II & III; n=60	· SOFA score · Lung damage score · Inflammatory status (IL1, IL6) · Oxidative status (TAC)
ChiCTR 2000030261	MSC	Inhalation	N/A	N/A; n=26	· Lung CT · Nucleic acid detection of pharyngeal test · Leukocytes and lymphocytes count
ChiCTR 2000030484	N/A	Intravenous injection	180 mg/day, for 7 days	N/A; n=90	· Adverse events · PaO2/FiO2 or respiratory rate (without oxygen) · Frequency of respiratory exacerbation · Physical signs and symptoms · TTCR · The number and range of lesions indicated by CT and X-ray of the lung · Time for the cough to become mild or absent · Time for dyspnea to become mild or no dyspnea · Frequency of oxygen inhalation or noninvasive ventilation · Frequency of mechanical ventilation · Inflammatory cytokines (CRP/PCT/SAA, etc.)

Abbreviations: EVs: Extracellular vesicles, TTCR: Time to clinical recovery, SVF: stromal vascular fractions, CRP: C-reactive protein, PCT: Procalcitonin, SAA: serum amyloid A, HAF: human amniotic fluid, TAC: total antioxidant capacity, SOFA: Sequential Organ Failure Assessment.

tabases including ClinicalTrials.gov, ISRCTN registry, the International Clinical Trials Registry Platform (ICTRP), EU Clinical Trials Register, Cochrane Central Register of Controlled Trials (CENTRAL), trials listed in Table 1 were identified before February 26, 2021 (Table 2).

Although the clinical use of exosomes for the treatment of different diseases has shown promising results, there are still challenges for entering exosome into the clinic. The most important challenge is the differences in the source of exosomes (MSCs from different origins or other cells types such as T cells), different isolation and purification protocols that result in different yields, exosome function, and the possibility of exosome contamination with other types, all hinder acquiring homogenous exosomes and standardization of clinical practices (42). Suggesting harmonized methods of purifying and characterizing exosomes by members of societies including the society for clinical research and translation of extracellular vesicles Singapore (SOCRATES), the international society for extracellular vesicles (ISEV), the international society for cellular and gene therapies (ISCT), and the international society of blood transfusion (ISBT) is an effective movement for obtaining and sharing comparable data (43).

The time-consuming process of exosome extraction and acquiring a low yield due to exosome damage during this process is also problematic (44). Since the exosome characteristics are dependent on several factors including iso-

lation method, culture conditions, the type of cell treatments, etc., batch-to-batch variations of exosomes are highly probable that might produce different results in patients during a clinical study (45).

It worth noting that the physiological state of patients and the existence of some underlying diseases such as cancer or *HIV* might alter molecular cargos of exosomes (46, 47). Exosome usage may also be contraindicated when the patients use specific drugs (35). Therefore, inclusion and exclusion criteria should be selected with care.

Conclusions

The success of exosome in controlling cytokine storm and reducing ARDS in acute cases of COVID-19, as well as the benefits of using exosomes instead of stem cells, suggest that exosomes have the potential to be used clinically for improving the well-being of patients infected with COVID-19 and developed respiratory syndromes. However, exosomes extracted from cells with different sources have shown different therapeutic effects on COVID-19 improvement. Before the widespread use of exosomes, it is necessary to control not only the source of exosomes but also the isolation, purification, and characterization methods precisely to prevent uncontrolled effects on different patients.

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Potential Conflict of Interest

The authors have no conflicting financial interest.

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