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Review

Regenerative therapy by using mesenchymal stem cells-derived exosomes in COVID-19 treatment. The potential role and underlying mechanisms

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ABSTRACT

COVID-19 disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), started in December 2019 in Wuhan, China, and quickly became the global pandemic. The high spread rate, relatively high mortality rate, and the lack of specific medicine have led researchers and clinicians worldwide to find new treatment strategies. Unfortunately, evidence shows that the virus-specific receptor Angiotensin-Converting Enzyme 2 (ACE-2) is present on the surface of most cells in the body, leading to immune system dysfunction and multi-organ failure in critically ill patients. In this context, the use of Mesenchymal Stem Cells (MSCs) and their secret has opened new therapeutic horizons for patients due to the lack of ACE2 receptor expression. MSCs exert their beneficial therapeutic actions, particularly anti-inflammatory and immunomodulatory properties, mainly through paracrine effects which are mediated by exosomes. Exosomes are bilayer nanovesicles that carry a unique cargo of proteins, lipids and functional nucleic acids based on their cell origin. This review article aims to investigate the possible role of exosomes and the underlying mechanism involved in treating COVID-19 disease based on recent findings.

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1. Introduction

The COVID-19 disease caused by the SARS-COV-2 is one of the most challenging global diseases that quickly became a major pandemic with a high mortality rate. The SARS-COV-2 is an enveloped single-stranded RNA-based virus with a diameter of 80–120 nm belonging to the *Nidovirals* order, *Orthocoronavirinae* subfamily, and Coronaviridae family [1]. With the assist of a specific Angiotensin-Converting Enzyme 2 (ACE-2) receptor available on the surface of most cells, the virus enters the body and exerts its destructive effects on target cells resulting in multi-organ failures in severe patients [2]. Among the various symptoms of the disease, respiratory problems, fever, and cough are most common in all patients [3]. At the same time, other severe complications such as Acute Respiratory Distress Syndrome (ARDS), immune system dysfunction, particularly cytokine stormcytokine storm formation, secondary infections, and cardiac, renal, or liver injuries are usually seen in critically ill patients [4]. Rapid disease progression, the emergence of new viral variants, and the lack of specific medicine have forced clinicians, researchers, and specialists worldwide to discover new treatment strategies. In this context, regenerative medicine using stem cells and their secretion, such as exosomes, is a promising approach to treat COVID-19 patients.

Exosomes are nanovesicles enclosed by a lipid bilayer via endocytosis and budding from the late endosomal membrane. Exosomes are released from various body cells in certain conditions such as systemic inflammation, disease, and imbalance in immune responses [5]. Exosomes have been used to treat many diseases to date [6–8], and there is ample evidence that exosomes have diagnostic, prognostic and therapeutic roles in COVID-19 infection [8–10].

Mesenchymal stem cells (MSCs) have been shown to be a rich source of exosomes [11]. Another significant advantage of using MSCs-exosomes in COVID-19 treatment is that MSCs, unlike many other cells in the body, do not express ACE2 receptors [12]. Currently, several clinical trials have been registered on the applications of exosomes [13] in the treatment of COVID-19 patients (https://clinicaltrials.gov), but only a limited number of them have been published [8]. The purpose of this review article is to explore the possible role of exosomes in the pathogenesis of COVID-19 and to discuss the potentials of MSC-derived exosomes in the treatment of COVID-19 patients.

2. Exosomes feature and performance

Exosomes are a subclass of extracellular vehicles (EVs) with a size range of 40–150 nm. They are formed by inward budding of the late endosomes and released following the multivesicular bodies (MVBs) fusion with the plasma membrane [14]. They were discovered during the investigation of sheep reticulocytes maturation in the 1980s and have rapidly attracted remarkable attention from researchers worldwide as a critical player of cell-to-cell communication [15]. Initially, they were considered to act as unwanted protein scavengers [16]. However, exosomes are much more attractive because of their ability to stimulate immune responses both *in-vivo* [17,18] and *in-vitro* [19,20]. It has been proved that exosomes to be secreted from almost all cells in the body; moreover, they are abundant in body fluids such as plasma, breast milk, serum, menstrual blood, saliva, urine, malignant ascites, cerebral, and amniotic fluids [21–25]. Ultracentrifugation and high-performance liquid chromatography (HPLC) are common methods for exosomes isolation [26], and western blotting, enzyme-linked immunosorbent assay (ELISA), flow cytometric analysis, nanoparticle tracking analysis, scanning electron microscopy as well as atomic force microscopy are common methods of identification and characterization of exosomes [26–28].

Based on their cellular origins, exosomes have an enriched and diverse cargo profile, including lipids, proteins, DNAs and RNAs, with specific functions such as regulating immune responses and cell-to-cell communications [29,30]. These specific components make exosomes distinguishable from other microvesicles. For example, having various membrane proteins such as Alix, TSG101, Rab5, Rab 27a, Rab27b, and multiple tetraspanins (e.g., CD37, CD63, CD81, CD82) differentiate exosomes from apoptotic bodies [31].

In addition, transported exosomal mRNAs or miRNAs from donor cells can potentially change the fate of recipient cells. For instance, glioblastoma cell-derived exosomes were taken up by recipient endothelial cells and led to elevated tubule formation potentials in them [32]. The miRNA profile of exosomes produced by regulatory T cells, downregulated the inflammatory pathways in recipient cells [33].

3. Exosomes-derived from mesenchymal stem cells (MSCs)

Mesenchymal Stem Cells (MSCs) are non-hematopoietic multipotent cells characterized by self-renewal potential and multilineage differentiation abilities. Researchers and clinicians have widely considered these cells in recent years due to their special biological features [34]. They are non- or low immunogenic, easily accessible, and have been isolated from various tissues such as bone marrow [35,36], menstrual-blood [24,25], umbilical cord [37], adipose tissues [38], periodontal ligament [39], and Wharton's Jelly [40]. MSCs home in the injured tissues and help the tissue regeneration by releasing abundant cytokines and mediators and/or through differentiation and replacement of the damaged cells. MSCs regulate the inflammatory responses [41]. MSC-derived exosomes represent the MSCs-paracrine effects and exert similar biological functions of MSCs [8,42]. For example, it has been shown that both MSCs and MSC-derived exosomes have neuroprotective effects [43]. MSC-derived exosomes could alleviate the endotoxin-induced acute lung injury in animal models [44], similar to MSCs [45]. In the same direction, another study conducted by Lee et al. demonstrated that MSCs-derived exosomes similar to MSCs have anti-tumor effects and could potentially inhibit tumor expansion and angiogenesis. These exosomes contained miR-16 that down-regulated the expression of vascular endothelial growth factor (VEGF) [46]. MSC-derived exosomes express the MSC-related antigens and adhesion molecules such as CD29, CD44, and CD73 [47]. Besides, it has been shown that the similar to MSCs, the MSC-derived exosomes contain a unique genetic information required for different signaling pathways such as angiogenesis or adipogenesis [48].

MSC therapy is a promising approach for treating various diseases. However, currently, cell-free therapy by using exosomes has attracted more attention of researchers because of multiple reasons, including DMSO independent storage because of their strong

membrane [49], the ability to pass the blood–brain barrier [50], coordination with the immune system even in immune-privileged sites [51], decreased risks of trapping in the lung due to their migration ability to the target cells which made them a good candidate for aerosol inhalation applications [52]), declined risk of tumor formation because of non-proliferative properties, and the lower membrane-bound protein contents with a lower risk of induction immune responses in comparison with their cell sources [51]. Indeed, it is now revealed that the beneficial therapeutic effects of MSCs are based on their soluble factors with paracrine and endocrine effects [53].

4. Exosomes-derived from stem cells and viral diseases

Viruses have many mechanisms to escape the immune system and to survive. Sequential mutations in the viruses' genome, similar to what is seen in the SARS-COV-2 today, and the multiple antigens expression are reasons that make it challenging to overcome viral diseases [54]. The applications of stem cells in viral disease are under investigation and results are promising. The antiviral activity of exosomes derived from human umbilical cord-MSCs (hucMSCs -Exo) was reported by Qian et al., in 2016 [55]. They found that hucMSCs-Exo was were able to target the hepatitis C virus replication and prevent the infection. They also suggested that these antiviral effects may have been mediated by a set of miRNAs transmitted by exosomes, particularly miR-221, miR-145, miR-199a, and let-7f [55]. It was also identified that exosomes derived from human vaginal fluid had an inhibitory effect on the early steps of the HIV-1 life cycle protecting women against HIV-1 infection. In fact, mimicking the function of reverse transcriptase by exosomes was considered as the mechanism involved in this post-entry blocking process [56]. In the same direction, in a recent clinical trial using exosomes derived from Bone Marrow-MSCs, it was found that exosomes alleviated the SARS-COV-2 disease through their immunomodulatory properties. The results showed that exosomes could reduce the inflammatory responses, particularly cytokine storm formation, and increase the lymphocyte counts against the virus attack [8]. These results suggest that exosomes could be a new beneficial therapeutic strategy against the COVID-19 disease.

5. COVID-19 as a new viral disease and immune system dysfunction

The pandemic caused by the SARS-COV-2, which causes COVID-19 disease, is undoubtedly one of the biggest challenges of the first half of the 21st century [41]. Unfortunately, it is about two years from the start of the COVID-19 pandemic; however, physicians and researchers worldwide are still looking for ways to save critically ill patients and no specific treatment has been found for it. Immune system dysfunction is common in COVID-19 disease, and the severity of uncontrolled immune responses is related to the infection rate. It is showed that the immune system of COVID-19 patients was inhibited in the early stages of the disease and became overactived, the conditioned called cytokine storm, in the final stages of the disease progression [57]. Indeed, in critically ill patients, cytokine storm leads to a vast range of morbidity and mortality. Activation of the inflammatory nuclear factor-kappa B (NF-kB) pathway and the subsequent secretion of large amounts of inflammatory cytokines (including Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), and Interferon-gamma (IFN-γ)) by the virus infected cells led to the formation of cytokine storm [58]. As a positive feedback, the cytokine storm caused more production of inflammatory cytokines and more activated immune cells [59]. The results from many clinical trials revealed that in severe COVID-19 patients, cytokine storm is followed by a lower number of T lymphocytes (both helper and suppressor T cells) [60,61]. T regulatory cells (T reg) and memory T cells are the most decreased populations among T cells, while Naïve T cells are highly present during the SARS-COV-2 attack [62]. It was shown that the CD3+, CD4+, and CD8+ T cells in severe COVID-19 patients were remarkably lower than normal peoples, whereas T h17 cells, as an inflammatory T subset, had an increased number [63]. Not only T cell but neutrophils cell number also increased in COVID-19 disease and changes in their structures and functions were observed [64,65]. It has been stated that as the disease progresses, the number of neutrophils increases that leads to severe inflammatory responses especially in lungs [66]. Excessive Neutrophil Extracellular Traps (NETs) formation or NETosis in response to viral attacks is another neutrophil-related exaggerated inflammatory response involved in the COVID-19 pathogenesis [67]. An overview of the mechanisms involved in the immune system dysfunctions following the SARS-COV-2 attack and the possible mechanisms by which MSCs or their exosomes help to treat the disease are been depicted in Fig. 1.

6. COVID-19, complication and multi-organ failure

As mentioned above, the SARS-COV-2 uses the ACE2 receptor to enter its target cells. On the other hand, the abundant expression of this marker on the surface of cells in various body organs such as kidneys, liver, testes, heart, and lungs [68] has given this opportunity to virus to invade multiple organs resulting in multiorgan failure and severe complications in critically COVID-19 patients. ARDS as a general lung injury in severely ill patients with COVID-19 is caused by ACE2-dysregulation, and cytokine storm [69]. Increased vascular permeability and the reduced compliance leading to alveolar inflammatory damages were the characteristic features of ARDS [70]. A systematic review reported a 39% correlation between ARDS and the death rate in COVID-19 patients [71].

Besides, it has been shown that SARS-COV-2 could affect cardiomyocytes both *in-vitro* [72] and *in-vivo* [73], leading to myocardial injuries and elevated levels of troponin T and N-terminal pro-brain natriuretic protein in most severely ill patients with COVID-19 [74]. A high level of ACE2 expression in the cardiac pericytes illustrates the susceptibility of the cardiac tissue to SARS-COV-2 infection [75]. Unfortunately, clinical trials have reported an increased mortality rate in COVID-19 patients with underlying cardiovascular diseases [76,77].

Liver dysfunction is another common complication in critically ill patients with COVID-19. Increased serum levels of aminotransferases enzymes such as Alanine amino-transferase (ALT) and Aspartate aminotransferase (AST) are indications of liver dysfunction [78]. Direct counteraction of the liver by the SARS-COV-2, excess systemic inflammation, mechanical ventilation, drug side effects, and ischemia-reperfusion injuries are the leading causes of liver impairment in COVID-19 patients [79]. Kidney is the second organ (after lung) affected in severe COVID-19 patients. This leads to severe problems such as Acute Kidney Injury (AKI) in up to 70% of COVID-19 patients [80]. Elevated serum creatinine and urea (BUN) are signs of renal involvement in COVID-19 patients [81,82]. Proteinuria and hematuria were also reported in 7–63% and 26.7% of COVID-19 patients respectively [83,84]. Many clinical trials have emphasized the relationship between kidney problems and high mortality rates in severe COVID-19 patients [84–86]. For example, an autopsy series of 18 dead patients with COVID 19 indicated the presence of viral genome sequence in the distal renal tubules [87].

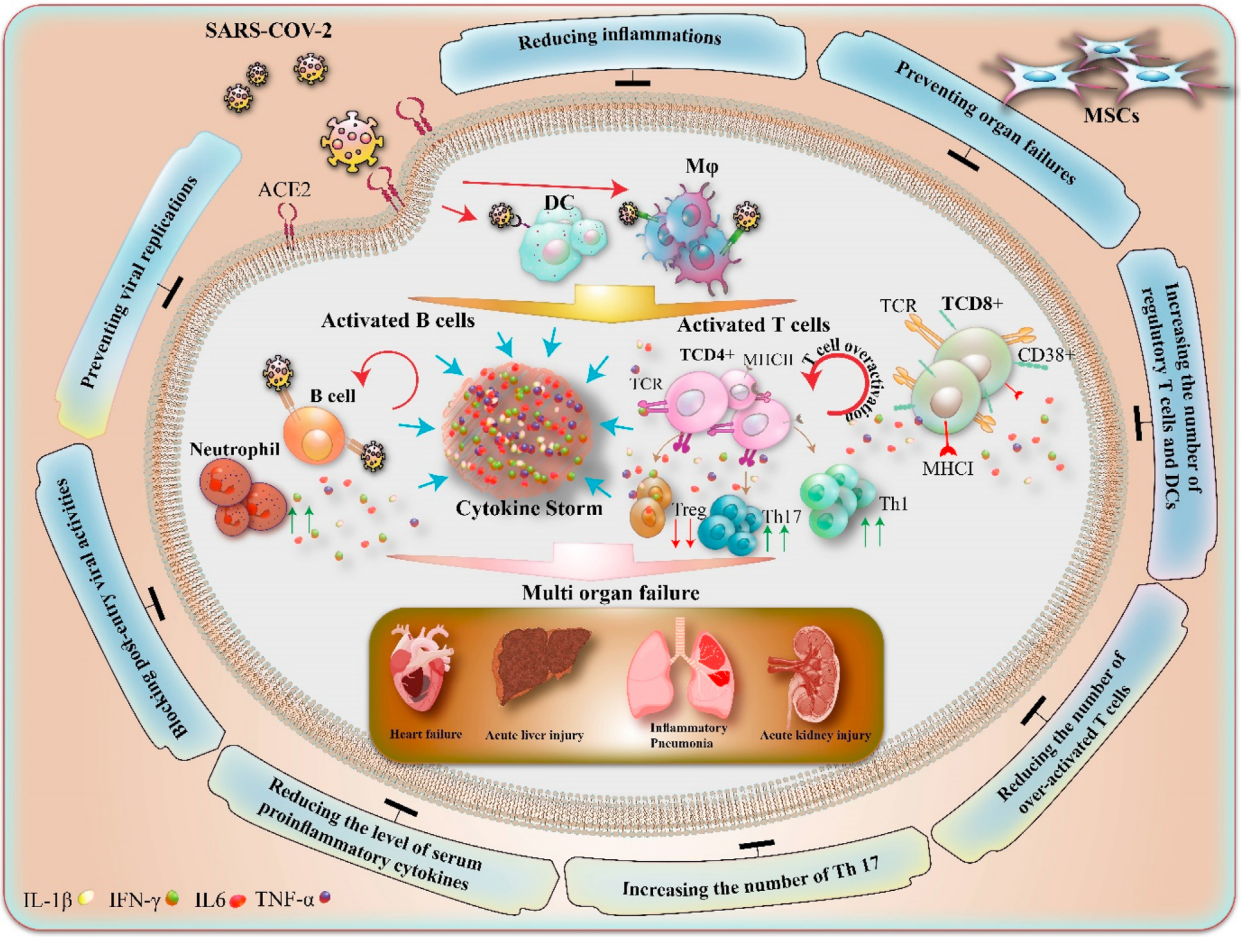

Fig. 1. Immune system dysfunction in COVID-19 disease and possible mechanisms underlying MSC- or exosome-based therapy (the figure is created using Adobe illustrator 2019).

7. Mesenchymal stem cell -therapy for COVID-19 disease

MSCs with immunomodulatory properties and differentiation abilities have been used in recent years to treat various diseases such as diabetes [88], rheumatoid arthritis [89], amyotrophic lateral sclerosis [36], systemic lupus erythematosus and musculoskeletal disorders [41,90]. The COVID-19 virus does not invade MSCs as they do not express ACE2 receptor [12]. Therefore, many physicians and researchers around the world have performed multiple clinical trials to treat COVID-19 disease by using MSCs [41]. Table 1 shows a summary of results from these studies.

Only one clinical study reported treatment related side effects such as heart failure and liver dysfunction, the rest of studies reported no treatment related serious side effects (Table 1). Most studies showed satisfactory clinical effects following MSC-therapy. For example, it was displayed that one dose of UMSCs (2×10^6 cell/kg BW) significantly reduced the inflammatory biomarkers such as C-Reactive Protein (CRP) and IL-6 levels, while improved the lymphocyte number, oxygen saturation level, and lung lesions in severe COVID-19 patients [99]. However, it is noteworthy that most of these studies lacked a control group (Table 1).

As mentioned before, MSCs can migrate to the injured tissues and exert their beneficial effects through the paracrine pathway. For example, in ARDS and lung injury, MSCs migrated and trapped in the lungs and secreted anti-inflammatory cytokines, antimicrobial agents and growth factors [101]. Keratinocyte growth factor secreted by MSCs led to alveolar epithelial cell repair and proliferation via induction of matrix metalloproteinase-9 enzyme, granulocyte-macrophage colony-stimulating factor, and IL-1 receptor antagonist [102,103]. Besides, MSCs regulated the overactive immune systems of COVID-19 patients via secretion of immune-modulators [104], inhibition of Th1 and Th17 proliferation [105] and suppression of dendritic cell activation [106]. Since exosomes are the major mediators of MSC effects, their application to treat COVID-19 syndrome sounds very promising.

8. Diagnostic and prognostic value of exosomes in COVID-19 disease

Based on their origin and environmental conditions, exosomes can have different functions in the body. In fact, exosomes have significant potentials in diagnosis and treatment of various diseases [107–110]. On the other hand, exosomes may be involved in the pathogenesis of diseases and act as a double-edged sword. For example, evidence showed that MSC-exosomes were actively involved in tumorigenesis, angiogenesis, and metastasis [111,112]. In contrast, the inhibitory role of these exosomes in tumor expansion have also been reported [113–115]. In viral diseases, exosomes can have a dual role in expanding or inhibiting viral infections. In the case of the HIV virus, for example, it has been shown that exosomes activated the latent reservoir of the virus. They facilitated the spread of the disease and affected the chronic inflammation [116,117]. In the same direction, studies showed that some body fluids-derived exosomes such as exosomes-derived from vaginal fluid and breast milk supressed the HIV infection through the inhibition of HIV infectivity for dendritic cells, prevention of reverse

Table 1
A summary of clinical studies that conducted MSC-therapy for COVID-19 patients.

Type of study	Severity of disease in patients	Dose & number of injections	Primary and main effector (efficacy outcomes)	Treatment-related Adverse effects	Refs
Uncontrolled clinical study	One critically ill case, four sever cases, and two cases with the common type of disease	1×10^6/kg of BW (once)	Primary outcomes: Patients symptoms of high fever, weakness, shortness of breath, and low oxygen saturation alleviated 2–4 days after transplantation. Main outcomes: • Decreased the number of overactivated $CD4^+$, $CD8^+$T cells and NK cells in the circulation. • Reduced inflammations. • Increased the number of peripheral $CD14^+CD11c^+CD11b^{mid}$ regulatory dendritic cells. • Improved lymphopenia. • Improved the level of biochemical indicators of liver and myocardium functions.	No acute infusion-related or allergic reactions were observed within 2 h after transplantation. Similarly, no delayed hypersensitivity or secondary infections were detected after treatment.	[12]
Case report	One severe case	1×10^6/kg of BW (once)	Primary outcome: Improved fever and shortness of breath within 2 days of transplantation. Main outcomes: • Increased number of $CD4^+$, $CD8^+$ T cells. • Reduced inflammation.	No acute reactions were observed within 2 h after transplantation. Similarly, no delayed hypersensitivity or secondary infections were detected after treatment.	[61]
Case report	One critically ill diabetic case	1.5×10^6/kg of BW (five times every 2 days)	Primary outcome: NM Main outcomes: • Increased number of peripheral leukocytes. • Improved kidney function. • PaO2/FiO2 ~ 200 • Improved respiratory function but not significant	No injection related febrile, allergic or hemolytic reactions occurred.	[91]
Case report	One critically ill case	5×10^7 cells (three times)	Primary outcome: NM Main outcomes: • Decreased serum CRP level • Increased number of lymphocytes (CD3+, CD4+ and CD8+). • Decreased number of WBC and neutrophils. • Decreased the ratio of neutrophil-to-lymphocyte and D-dimer levels. • Reduced pulmonary inflammation • Improved liver functions	No obvious side effects were observed	[92]
Case report	One critically ill case with multiple comorbidities (diabetes, hypertension, coronary heart disease)	5×10^7 cells (three times)	Primary outcome: less fatigue and listlessness, improved oxygen saturation Main outcomes: • Improved in lung CT scan images. • Decreased index of sequential organ failure assessment and pneumonia severity index • Decrease in serum inflammatory cytokine levels • Amelioration in multiple organ dysfunction syndrome and multiple comorbidities	No infusion-associated or allergic reactions were observed within 24 h after transplantation. Besides, no secondary infection, delayed hypersensitivity during the 30 days of follow up	[93]
Case report	Two severe cases	1×10^6 cells (three times)	Primary outcome: Improved fever and dyspnea Main outcomes: • Improved oxygen saturation • Increased number of lymphocytes (especially $TCD4^{+)}$	No injection-related reactions were observed.	[94]

(*continued on next page*)

Table 1 (*continued*)

Type of study	Severity of disease in patients	Dose & number of injections	Primary and main effector (efficacy outcomes)	Treatment-related Adverse effects	Refs
			• Decrease in CRP and Fio2 • Improved in lung CT scan images.		
Uncontrolled Clinical trial	11 Critically ill cases	200×10^6 cells (three times)	Primary outcome: Reliefs from cough and fever, improvements in dyspnea, and increased SpO2 were observed during 48–96 h after the first infusion. Main outcomes: • Improved in lung CT scan images. • Significant reductions in the serum proinflammatory cytokine levels	Slight shivering was observed after initial infusion. But, no acute reactions such as severe anaphylactic reaction or embolization were observed.	[95]
Uncontrolled Clinical trial	13 severe cases	$.98 \times 10^6$ /kg of BW (1–3 doses)	Primary outcome: 70% clinical improvement Main outcomes: • Decreased levels of D-dimer, CRP, and other inflammatory markers • Increased total lymphocytes counts	No adverse events were observed.	[96]
Phase 1 controlled clinical trial	10 moderate patients and 8 severe patients	3×10^7 cells (three times)	Primary outcome: Fewer patients in the treated group experienced dyspenea and the need of ventilation Main outcomes: • Healed lung lesions in treated groups • Improved respiratory functions • A higher reduction in serum biomarkers for inflammation (such as IL-6) and liver function in treated group • No difference in the duration from hospital admission to discharge between control and treated groups	No serious adverse events were observed. A transient facial flushing and fever in two patients and transient hypoxia in one patient were observed at 12 h post UC-MSCs transfusion.	[97]
Uncontrolled retrospective study	25 severe cases	1×10^6 mononuclear cells/kg of BW (1–3 times)	Primary outcome: NM Main outcomes: • No changes in inflammatory indexes including CRP after 48–72h of injection • Elevation in serum levels of lactate, cardiac troponin, and creatine kinase-MB • All patients recovered	Treatment related side effects, such as liver dysfunction, heart failure and allergic rash were observed in 3 cases	[98]
Randomized controlled trial	41 severe cases (12 cases in MSCs group and 29 cases in control group)	2×10^6 cells/kg of BW	Primary outcome: Improvements in symptoms such as weakness and fatigue, shortness of breath, and low oxygen saturation Main outcomes: in the treatment group: • No disease progression or mortality was observed • The time to clinical improvement was shorter • Decreased inflammatory indexes such as CRP and IL-6 • Faster lung inflammation absorption	No adverse reactions such as rash, allergic reaction, and febrile reaction after infusion were observed.	[99]
Randomized controlled phase 2 trial (double-blind)	100 severe cases (65 cases in MSCs group and 35 cases in control group)	4×10^7 cells (three times)	Primary outcome: NM Main outcomes: • Significant reduction in lung lesion volume • The 6-min walking distance was longer in the treated group • Improvement in the oxygen saturation level • No significant difference in lymphocyte counts (CD4+T cells, CD8+ T cells, B cells, NK cells) between groups	No treatment related adverse events	[100]

Abbreviations and definition: NM: Not Mentioned/BW: Body Weight/PaO2: Partial pressure of Oxygen/FiO2: Fraction of Inspired Oxygen/CRP: C Reactive Protein/CT scan: computerized tomography. Primary outcomes show the effect of therapy on the immediate resolution of disease symptoms. Main outcomes show the main mechanisms of therapeutic effects.

transcription, and integration of viral genomes [56,118] Whereas, semen-derived exosomes activated the silence viruses through disrupting the NF-kB/Sp1/Tat pathway leading to the spread of infection [119]. Exosomes can also be used as diagnostic biomarkers of AIDS [120]. For example, results obtained from a recent study showed that plasma exosomal-derived miR-20a and miR-21 are promising biomarkers for the early detection of Classical Hodgkin Lymphoma in HIV infection [121]. Moreover, evidence from another study showed that exosomes probably were involved in retrovirus replication and infection [122].

In the case of COVID-19 disease, circulating exosomes were involved in the disease severity and could potentially induce different immune responses [10]. For example, in a recent clinical trial conducted by Barberis et al. the results of a proteomic analysis showed that the patient-derived circulating exosomes contained various molecules involved in the disease progression by modulating the coagulation, inflammation, and immune responses [123]. They also reported a significant association between exosomal protein cargos and patients' clinical indicators such as CRP and D-dimer levels. For instance, their findings showed that CRP (122-fold), alpha-1-acid glycoprotein 1 (38-fold), lysozyme C (13-fold), titin (12-fold), and zinc-alpha-2-glycoprotein (12- fold) were the five top up-regulated proteins while putative trypsin-6 (31-fold), coiled-coil domain-containing protein 34 (18-fold), C4b -binding protein alpha chain (18-fold), C4b-binding protein beta chain (15-fold), and pre-mRNA-processing factor 19 (14-fold) were the most down-regulated proteins in the plasma-derived exosomes of critical COVID-19 patients [123].

Moreover, the presence of viral RNA in the host plasma-derived exosomes justifies the hypothesis that the viruses' contents, such as functional viral RNAs or miRNAs, may transmit from the infected cells to the healthy cells through exosomes [123]. Another exciting result of this study was the presence of various biomarkers in the exosomal cargo (such as fibrinogen, fibronectin, complement C1r subcomponent, and serum amyloid P-component) to detect the severity of the disease, which can be used as diagnostic markers of COVID-19 disease [123]. Another case report study Analyzed the content of circulating exosomes in a COVID-19 patient and revealed the presence of SARS-COV-2 spike protein in host exosomes, suggesting a diagnostic role of exosomes for COVID-19 patients [124]. Similar results were obtained from a clinical trial in Wuhan, China. In this study, using the RT-PCR method SARS-COV-2 RNA (but not viral particles) was detected in the serum-derived exosomes of 4 out of 15 (26.6%) COVID-19 patients suggesting a prognostic potential for exosomes in COVID-19 disease. The level of viral nucleocapsid gene expression in patients also showed to be directly associated with the severity of the disease [9]. Another study on twenty COVID-19 patients showed that exosomes from COVID-19 patients contained tenascin-C and fibrinogen-β, two pro-inflammatory components that triggered distant organs inflammation [125].

Taken together, all of the above information points to the important diagnostic and prognostic roles of exosomes in COVID-19 disease.

9. Multiple therapeutic effects of exosomes considering their possible role in COVID-19 complications

A couple of studies have shown the therapeutic effects of exosomes in the treatment of COVID-19 disease [8,126]. For example, in a clinical study conducted by Milrani et al. on three severely COVID-19 patients, it was found exosomes derived from the amniotic fluid are highly effective in treating the respiratory failure of COVID-19 patients [126].

However, more results are available on MSC-derived exosomes therapy in conditions other than COVID-19, showing the possible effectiveness of MSC-derived exosomes to treat COVID-19 related complications. Some of these preclinical and clinical studies that applied MSC-derived exosomes for therapeutic purposes are summarized in Table 2.

As shown in Table 2, exosomes are effective in lung repair. Results obtained from an animal study revealed that MSC-derived exosomes potentially could alter the phenotype of alveolar macrophages (M1) toward an anti-inflammatory state (M2) with enhanced phagocytic activities. Macrophages treated with MSC-derived exosomes were able to reduce the alveolar inflammation in mice bearing the endotoxin-induced lung injuries [140]. Furthermore, another study has stated that MSC-exosomes acted as a TLR signaling mediator and enhanced the transfer of regulatory miR-451 to the macrophages, thereby ameliorating the TNF-α secretion and suppressing the excess inflammatory responses to the lung injury [141]. Exosomes derived from human amnion epithelial cells could suppress the activated neutrophils and T cells proliferation and decrease the percentage of interstitial macrophage in the lungs [127].

MSC exosomes are also able to improve liver injuries via several mechanisms. Inhibition of collagen (type I/III) productions and the suppression of epithelial−mesenchymal transition of hepatocytes were two possible beneficial effects of hucMSCs-exosomes in promoting the liver functions and reducing the serum AST levels in mice with the CCL4-induced liver injury [130]. MicroRNA-125b overexpressed exosomes isolated from chorionic plate-derived mesenchymal stem cells promoted the liver functions by inhibiting the hedgehog signaling pathway [142]. In this regard, findings from another study similarly showed that hucMSCs-exosomes with glutathione peroxidase-1 activity inhibited the oxidative stress-induced apoptosis in liver cells and enhanced their proliferation and functions [143]. An animal study showed that exosomes derived from adipose stem cells significantly declined hepatic inflammation and necrosis [144]. Since liver complications are common in critically COVID-19 ill patients, exosomes with the above-mentioned potentials may offer a promising treatment for COVID-19 patients.

Cardioprotection is another crucial feature of exosomes demonstrated by various preclinical studies, making them good candidates for treating heart problems caused by SARS-COV-2. For instance, Wang et al. using a rat model of myocardial infarction, found that specific miRNAs, particularly miR-21 released by exosomes isolated from endometrium-derived MSCs, could have cardioprotective effects by enhancing cell survival and angiogenesis [145]. Exosome therapy in animals with ischemia-reperfusion injuries decreased the cardiomyocyte apoptosis and improved the heart functions [135]. In another study, using exosomes derived from bone marrow-MSCs, it was found that exosomes reduced the inflammation of myocardial tissue and induced M2 macrophage polarizations through the inhibition of the NF-kB pathway and activation of AKT1/AKT2 signaling pathways [134].

Exosomes are also capable of ameliorating renal complications. Exosomes home in the damaged kidney and exert beneficial biological actions [137]. Intravenous injections of 100 μg hucMSCs-exosomes alleviated the ischemic injuries in a murine AKI model. Treatment decreased the tubular epithelial cell apoptosis through miR-125b-5p-mediated inhibition of P53 expression [137]. Results from another study also showed that hucMSCs-exosomes were effective in recovering AKI induced by cisplatin, both *in-vivo* and *in-vitro*. Decreasing the oxidative stress levels, preventing cellular apoptosis, and enhancing cell proliferations were mentioned as underlying mechanisms [138].

Table 2
Multiple therapeutic effects of MSC-exosomes in animal and human studies.

Source of exosomes	Exosomal characterization method	Type of disease	Type of study		Main results	Ref
			Animal	Human		
BM-MSCs	Western blot analysis (CD81 $^{+}$, CD63^{+}, CD9^{+}) and transmission electron microscopy	Acute Lung Injury	rat	–	• P2X7 mediated decrease in inflammatory responses • ↓oxidative stress	[6]
hAEC	Western blot and bead-based flow cytometry analysis (CD81 $^{+}$, CD9^{+}, Alix^{+} and HLA-G protein) & transmission electron microscopy	Lung fibrosis	C57Bl/6 mice	–	• ↑Macrophage phagocytosis • ↓lung fibrosis • ↓T cell proliferation • ↓neutrophil myeloperoxidases	[127]
BM-MSCs	Western blot analysis (CD81 $^{+}$, CD63^{+}, TSG101^{+}) and transmission electron microscopy	Acute Lung Injury	rat	–	• ↓Lung cell apoptosis and inflammation through down-regulation of TLR4 and NF-κB expression	[128]
hEPC	Western blot analysis (CD81 $^{+}$, CD63^{+}, CD9^{+}) and nanoparticle Tracking analysis (NTA)	Acute Lung Injury	mice	–	• ↓Myeloperoxidase (MPO) activity • ↓lung injury score • pulmonary edema	[129]
hucMSCs	Western blot analysis (CD81 $^{+}$, CD9^{+}) and transmission electron microscopy	Liver fibrosis	Kunmingbai strains mice	–	• ↓Expression of collagen type I & III, and TGF-B • ↓phosphorylation Smad2 expression	[130]
hucMSCs	Western blot analysis (CD81 $^{+}$, CD63^{+}, TSG 101 $^{+}$) and transmission electron microscopy	Acute liver injury	C57Bl/6 mice	–	• ↓Macrophage infiltration, • ↓local liver damage • ↓serum levels of inflammatory factors	[131]
ADSCs	Western blot analysis (CD81 $^{+}$, CD63^{+}) and transmission electron microscopy	Liver fibrosis	C57Bl/6 mice	–	• miR-181−5p mediated anti-fibrotic effects	[132]
BM-MSCs	Bead-based flow cytometry analysis (CD63 $^{+}$, CD9^{+}) and transmission electron microscopy	Liver injury	C57Bl/6 mice	–	• ↓Liver necrosis and apoptosis • ↑Anti-inflammatory cytokines • ↑Treg cell number	[7]
hucMSCs	Flow cytometry analysis (CD63 $^{+}$, CD9^{+}) and transmission electron microscopy	Liver injury	BALB/c mice	–	• Exo mediated antioxidant and hepatoprotective effects.	[133]
BM-MSCs	Western blot analysis (CD81 $^{+}$, CD63^{+}, HSP-70 $^{+}$, TSG101^{+}) and transmission electron microscopy	Myocardial injury	rat	–	• ↑M2 macrophage polarization • ↓M1 macrophage polarization • ↓Inflammatory signaling pathways	[134]
BM-MSCs	Tunable resistive pulse sensing analysis by qNano	Myocardial Ischemia	rat	–	• ↓Apoptosis and the • ↓Myocardial infarct size • ↑Heart function	[135]
ADSCs	Western blot analysis (CD81 $^{+}$, CD63^{+}, CD9^{+}, HSP70 $^{+}$) and transmission electron microscopy	Myocardial Ischemia/ Reperfusion	rat	–	• Wnt/β-catenin mediated protection of ischemic myocardium	[136]
hucMSCs	Flow cytometry analysis (CD63 $^{+}$, CD9^{+}, TSG101^{+}, Alix $^{+}$) and transmission electron microscopy	AKI	C57Bl/6 mice	–	• Promoted tubular repair • miR-125b-5p/p53 pathway mediated amelioration of ischemic AKI	[137]
hucMSCs	Western blot analysis (CD81 $^{+}$, CD63^{+}, CD9^{+}) and transmission electron microscopy	AKI	Sprague−Dawley rats	–	• ↓Blood BUN, Cr levels • ↓apoptosis & necrosis of proximal kidney tubules	[138]
hCBMSCs	Flow cytometry analysis (CD63 $^{+}$, CD9^{+}, CD45 $^{+}$, CD73^{+}, HLA-1 $^{-}$, HLA-II^{-}) and transmission electron microscopy	Chronic kidney disease	–	Human	• Significant improvement of eGFR and BUN, Cr and UACR levels • ↑TGF-β1, IL-10 • ↓TNF-α	[139]
BM-MSCs	NM	Covid-19	–	Human	• ↓CRP, IL-6, D-Dimer, neutrophil counts • ↑Lymphocyte counts	[8]

Abbreviations: (BM-MSCs: Bone Marrow-Mesenchymal Stem Cells/P2X7: purinergic receptor P2X ligandgated ion channel 7/hAEC: Human amnion epithelial cell/TLR4: Tool Like Receptor 4/NF-κB: Nuclear Factor-kappa B/hEPC:human Endothelial Progenitor cell/hucMSCs: human umbilical cord-MSCs/TGF-B: transforming growth factor B/ADSCs: adipose-derived mesenchymal stem cells/Treg: T lymphocyte regulatory cells/Exo: Exosomes/ AKI: Acute kidney Injury/BUN: Blood Urea Nitrogen/Cr: creatinine/hCBMSCs: Human Cord Blood-derived mesenchymal stem cells/eGFR: estimated Glomerular Filtration Rate/UACR: Urinary Albumin Creatinine Ratio/NM: Not Mentioned/CRP: C Reactive Protein/IL-6:Interlukine-6).

10. Conclusion

COVID-19 disease caused by the SARS-COV-2 quickly became a global pandemic. Unfortunately, available treatments are complementary and there is no specific treatment for the disease so far. Thus, the discovery of new treatment strategies is a necessity of medical science at this juncture. As a subclass of extracellular vesicles, exosomes have various roles in regulating the immune system and restoring injured organs. Studies have shown that exosomes are promising candidates for COVID-19 treatment as well as their applications in the diagnosis and prognosis of the disease. Although limited clinical trials are underway, more studies are required to evaluate the efficacy of exosome therapy for COVID-19 disease.

Author contributions

Dr. Elahe Mahdipour had the idea for the article. Najmeh kaffash Farkhad performed the literature search and provided the first draft of the manuscript. Ali Mahmoudi made the first draft of art works. Dr. Elahe Mahdipour updated the literature search and critically revised the whole work including art works. All authors read and commented on the final draft of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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