

Review Article

COVID-19 and Extracellular Vesicles: An Intriguing Interplay

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Keywords

COVID-19 · SARS-CoV-2 · Extracellular vesicles · Pulmonary disease

Abstract

Background: The outbreak of severe acute respiratory syndrome β -coronavirus 2 (SARS-CoV-2) has the potential to become a long-lasting global health crisis. The number of people infected with the novel coronavirus has surpassed 22 million globally, resulting in over 700,000 deaths with more than 15 million people having recovered (<https://covid19.who.int>). Enormous efforts are underway for rapid vaccine and treatment developments. Amongst the many ways of tackling the novel coronavirus disease 2019 (COVID-19) pandemic, extracellular vesicles (EVs) are emerging. **Summary:** EVs are lipid bilayer-enclosed structures secreted from all types of cells, including those lining the respiratory tract. They have established roles in lung immunity and are involved in the pathogenesis of various lung diseases, including viral infection. In this review, we point out the roles and possible contribution of EVs in viral infections, as well as ongoing EV-based approaches for the treatment of COVID-19, including clinical trials. **Key Messages:** EVs share structural similarities to viruses and recent findings demonstrate that viruses exploit EVs for cellular exit and EVs exploit viral entry mechanisms for cargo delivery. Moreover, EV-virus interplay could be exploited for future antiviral drug and vaccine development. EV-based therapies, especially the mesenchymal stem cell-derived EVs, are being intensively studied for the treatment of COVID-19.

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Introduction

Coronavirus disease 2019 (COVID-19) will have a long-lasting impact on the biopharmaceutical industry. In particular, the urgent need both for a preventive vaccine and for advanced therapeutics will stimulate the growth of the post-COVID-19 bioindustry in ways that were unthinkable before. One of the initiatives to find swift solutions to challenges posed by COVID-19 is based on extracellular vesicles (EVs; Fig. 1).

COVID-19 is an infectious disease caused by the severe acute respiratory syndrome β -coronavirus 2 (SARS-CoV-2), first reported in Wuhan, China, in December 2019. It has

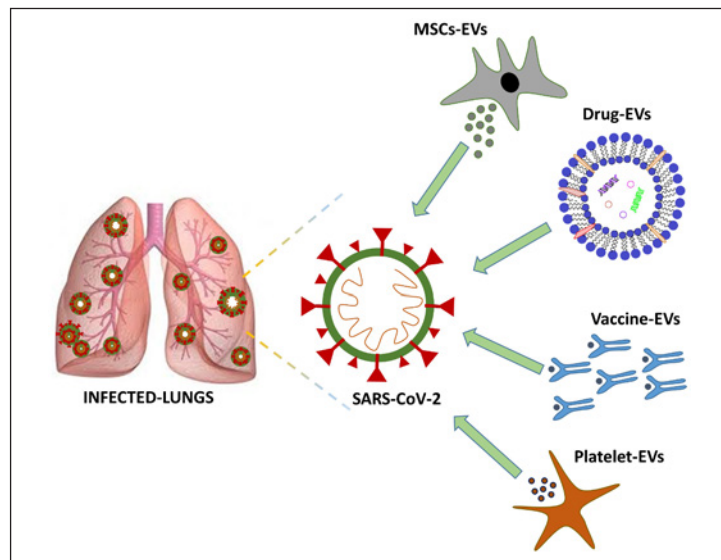


Fig. 1. Schematic representation of various EV-based therapeutic approaches against COVID-19.

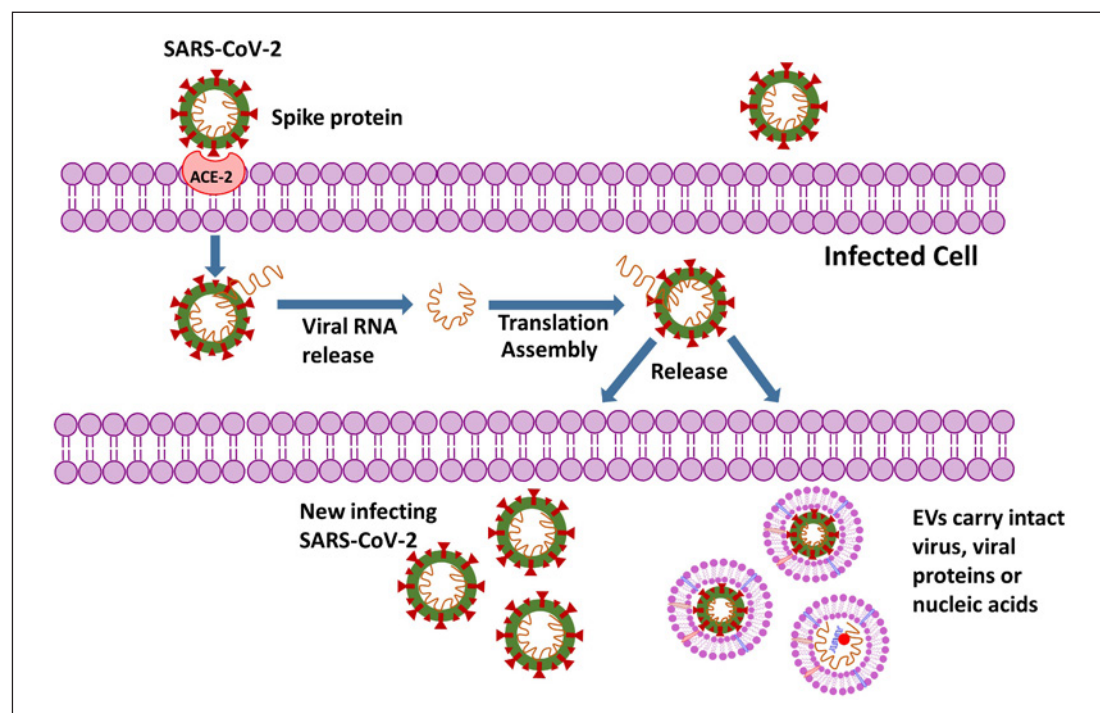


Fig. 2. Schematics of SARS-CoV-2 viral replication in host cells.

spread rapidly worldwide and has been declared a pandemic disease by the World Health Organization (WHO). Compared to other zoonotic coronavirus diseases that emerged earlier, like Middle East respiratory syndrome (MERS), swine acute diarrhea syndrome (SADS), and other related viruses like Ebola and H1N1, SARS-CoV-2 is more contagious due to its easy human-to-human transmission through air droplets resulting from sneezing, coughing, or even breathing and speaking, and its long incubation period (between 2–14 days) [1]. COVID-19 primarily affects the lungs and the infection may cause pneumonia, acute respiratory distress syndrome, acute lung injury, septic shock, multi-organ dysfunction, and even death. SARS-CoV-2 is a spherical or pleomorphic enveloped virus particle with a typical size in the range of 80–120 nm in diameter. It contains a positive single-stranded RNA of 30 kb, surrounded by a membrane with several viral proteins embedded, and of special interest is the spike (S) protein [2]. During virus internalization, S proteins bind to the angiotensin-converting enzyme 2 (ACE2) receptors in the host cell, then the serine protease TMPRSS2 primes the S protein for internalization by the fusion with the host membrane [3]. Viral genes are converted into polypeptides, which consequently turn into proteins that are required to form the viral core and surface S protein. The virus then matures, replicates, and leaves the host cells to infect new cells (Fig. 2).

Discussion

Recently, an increasing number of studies have reported the emerging role of EVs in inflammation [4, 5], injury [6], and viral infection of the lung and respiratory tract [7–9]. EVs are membrane-surrounded structures secreted by cells and involved in cell to cell communication by means of the horizontal transfer of molecules at short and long distances. EVs and viruses have similar physicochemical properties, like small size and heterogeneous size distribution [7], and share common mechanisms for biogenesis and cell entry. Viruses enter the uninfected cells by using the endocytic pathway and exit the host cell by direct budding through the membrane. During viral infections, EVs incorporate pathogen-derived nucleic acids, proteins, and lipids, and become a delivery vector of viral materials. This led Gould et al. [10] to propose the “Trojan exosome hypothesis” in which retroviruses utilize EVs to enter host cells, to promote viral spread, and to evade the immune response. EVs represent a new frontier in the field of viral infections. Pioneering work demonstrated that during viral infection the number of EVs secreted by infected cells increases markedly and that EVs play a central role in the pathogenesis of the disease [8]. In a recent review, Lanyu et al. [6] highlight the emerging role of EVs produced by pulmonary cells and alveolar epithelial cells during pulmonary injury and inflammation. EVs isolated from bronchoalveolar lavage fluid have been reported to have a role in fibrinogenesis during the pathogenesis of idiopathic pulmonary fibrosis by delivering signaling mediators such as WNT5A [11].

The intriguing interplay between EVs and viruses led to the exploitation of EVs as a new perspective in therapeutic applications (Fig. 1) [12]. Kesimer et al. [13] demonstrated that EVs from human tracheobronchial epithelial cell culture media have a neutralizing effect on human influenza virus. An exponentially growing number of *in vitro*, *in vivo*, and clinical studies advance the progress in EV-based drug delivery, immunomodulatory, and regenerative therapies.

Currently, no specific antiviral treatment or vaccine is available for patients with COVID-19. However, there is an increasing number of clinical investigations to find therapeutic solutions to the various forms of the disease. Some approaches apply mesenchymal stem cells (MSCs) and/or EVs derived from them. MSCs produce many types of cytokines and paracrine factors that can interact directly with immune cells, including T cells, B cells,

Table 1. List of published studies on MSC therapy for COVID-19, and studies and ongoing clinical trials that apply EVs for the treatment of COVID-19 patients

Employed therapy	Source	Administration route	Patients, n	Outcome/aims (in the case of clinical trials)	Ref.
MSCs	Human umbilical cord	Intravenous administration	7 (+3 placebo)	Regulation of immune response by the increase of T cells and dendritic cells Reduction of pro-inflammatory cytokines and chemokines Promotion of tissue repair observed by the reduction of ground-glass opacities	15
MSCs	Human umbilical cord	Intravenous administration	1	Regulation of immune response Decrease of neutrophils and normal levels of T cells Reduction of pro-inflammatory cytokines and chemokines Injured tissues repaired and ground-glass opacities reduced significantly	16
MSCs	Human umbilical cord	Intravenous administration	12 (+29 placebo)	Patients treated with MSCs showed a faster improvement in clinical symptoms such as fatigue and weakness, shortness of breath, and oxygen saturation and lymphocyte counts Reduction of inflammatory markers, C-reactive protein, and interleukin-6 Ground-glass opacities reduced	17
MSCs and EVs	MSCs from human umbilical cord	Intravenous administration	30 MSCs + 30 MSCs + EVs (+30 placebo)	Clinical trial (ChiCTR2000030484) Aiming to investigate the safety and efficacy of treatment	24
EVs	MSCs from allogeneic adipose tissue	Aerosol inhalation	30	Clinical trial (NCT04276987) Aiming to explore the safety and efficiency of aerosol inhalation treatment with EVs	21
EVs	MSCs	Aerosol inhalation	13 (+13 control)	Clinical trial (ChiCTR2000030261) Aiming to inhibit inflammatory factors and enhance the immunity of the body, promoting early recovery and avoid complications	23
EVs (ExoFlo™)	MSCs from allogeneic bone marrow	Intravenous administration	24	Improvement in clinical symptoms within 3-4 days Regulation of the immune response Decrease of neutrophils and normal levels of lymphocyte count Reduction of acute markers, C-reactive protein, ferritin, and D-dimer	25
EVs (ExoFlo™)	MSCs from allogeneic bone marrow	Intravenous administration	60	Clinical trial (NCT04493242) Aiming to evaluate the safety and efficacy of intravenous administration of EVs as treatment for ARDS	26
EVs	Allogeneic COVID-19 specific T cells (CSTC)	Aerosol inhalation	60	Clinical trial (NCT04389385) Aiming to explore the safety and efficiency of inhaled CSTC-exosomes in the treatment of early-stage COVID-19 pneumonia	46
Product derived from HAF, containing EVs (Organicell™ Flow)	Human amniotic fluid (HAF)	Intravenous administration	10 (+10 placebo)	Clinical trial (NCT04384445) Aiming to evaluate the safety and therapeutic effect of HAF-derived acellular products against COVID-19	47
EVs (CAP-1002)	Allogeneic cardiosphere-derived cells	Intravenous administration	6	Clinical trial (NCT04338347) Aiming to investigate the safety and efficacy of treatment	48, 49
EVs	MSCs	Aerosol inhalation	30 EXO 1 + 30 EXO 2 (+30 placebo)	Clinical trial (NCT04491240) Aiming to evaluate the safety and efficiency of exosome inhalation in COVID-19 pneumonia	50

Registered clinical trials using MSCs are not reported as they are directly available at <https://clinicaltrials.gov/>.

dendritic cells, macrophages, and natural killer cells; this combination of actions gives MSCs an immunomodulation capacity. This helps to inhibit the over activation of the immune system. Moreover, they promote regeneration of tissue by secreting and attracting different growth factors such as VEGF, EGF, TGF, thus improving the microenvironment [14]. Apart from that and due to their immunomodulatory capacity, MSCs may prevent the cytokine storm induced by COVID-19. Recently, two clinical studies have been performed using the administration of umbilical cord-derived MSCs (HUMSCs) with promising results [15–17], and even though the subjects enrolled were only a few, the results are encouraging and have led to further clinical trials using MSCs to treat patients with COVID-19. However, there are some reported disadvantages of using MSCs; for example, the intravenous administration may cause aggregation that could lead to embolism, some MSCs, especially derived from embryonic tissue, entail the risk of mutagenicity and tumorigenicity. Therefore, MSC-derived products, like secretome [18] and EVs [19], have been proposed as alternative options to MSC therapy. Components of the secretome may interact with the target cells through ligand-receptor binding or by internalization to modulate cellular responses. Furthermore, secretome-based therapy has significant advantages over cell-based and monoclonal antibody treatments due to its low immunogenicity and tumorigenicity, easier manipulation, and lower cost. Additionally, it was reported that MSC secretome can be administered through inhalation and injection [18]. Preliminary studies suggest that EVs isolated from the cell secretome might also be efficient for the treatment of COVID-19 [20]. MSC-derived EVs were shown to produce similar effects as their parent cells; and they can be safely stored for long periods without losing function and have shown similar or better results than MSCs in animal models [19].

As of August 2020, there were 38 registered clinical trials using MSCs and 7 that use EVs for the treatment of COVID-19 (<https://clinicaltrials.gov/>). A search at the European Union Clinical Trials Register (<https://www.clinicaltrialsregister.eu/>) displayed a further 8 ongoing clinical trials applying MSCs for the treatment of COVID-19 (accessed August 20, 2020). Table 1 shows published studies that involve MSCs and ongoing clinical trials that apply EVs for the treatment of COVID-19 patients.

Clinical trial NCT04276987 was set up to evaluate the safety and efficiency of aerosol inhalation of allogenic adipose MSC-derived EVs in the treatment of severely ill COVID-19 pneumonia patients [21]. Participants will receive conventional treatment plus one dose of aerosol inhalation of MSC-derived EVs a day, in a dose of 2×10^8 nanovesicles/3 mL for 5 consecutive days. The same group aims to perform a tolerance clinical study (NCT04313647) to explore safety and efficiency, and to provide an optimal clinical dose of aerosol inhalation of MSC-derived EVs in the treatment of severe respiratory diseases. Healthy volunteers will receive up to a 10-times increased dose of aerosol inhalation of allogenic adipose MSC-derived EVs [22]. Another clinical trial (ChiCTR2000030261) will use atomization to deliver MSC-derived EVs directly into the lungs for the treatment of COVID-19 pneumonia [23]. They will investigate the reduction of inflammatory factors and will assess the immunity response of the body. In another clinical trial (ChiCTR2000030484), HUMSCs and EVs will be used to treat patients with lung damage caused by COVID-19 [24]. The participants will be divided into 3 groups: the first group will be subjected to intravenous infusion of HUCMSCs at a dose of 5×10^7 cells once a week, the second group will be subjected to the same dose of HUCMSCs plus an intravenous administration of EVs for 7 days, and the third, the control group, will receive the same amount of placebo. During the treatment, the study will evaluate the respiratory rate, frequency of respiratory exacerbation, time for the cough to become mild or absent, frequency of oxygen inhalation or non-invasive ventilation, frequency of mechanical ventilation, and other outcome measures. There is one published study that used bone marrow MSC-derived EVs (ExoFlo™) to evaluate the safety and effectiveness of the treatment of

COVID-19 in infected patients (Table 1) [25]. The study reports an overall improvement in clinical symptoms and laboratory tests within 3–4 days after treatment without observed adverse effects. The neutrophil count returned to normal values, while an increase in lymphocyte count and a reduction of acute phase markers (C-reactive protein, ferritin, and D-dimer reduction) were reported. The same authors have registered a clinical trial (NCT04493242) for the treatment of COVID-19 patients with moderate and severe acute respiratory distress syndrome [26]. In this study, 60 patients will be enrolled. The participants will be divided into 3 groups: 2 groups will get different doses of bone marrow MSC-EVs (bmMSC-EVs) and the third group will receive a placebo for control. All-cause mortality and the median recovery time will be evaluated.

Due to the absence of a specific effective therapy, existing antiviral drugs with protease inhibition activity have been proposed, including chloroquine/hydroxychloroquine, remdesivir, lopinavir/ritonavir, corticosteroids, and baricitinib [27]. Because anti-HIV drugs have a strong interaction with the active site of SARS-CoV-2 protease, they could be useful in the treatment of COVID-19. However, high doses of protease inhibitor drugs could cause drug-drug interaction and toxicity. An EV-based drug delivery system was proposed to increase the drug concentration at the target cells and to inhibit off-target effects. Besides, there are some studies that employed EVs as an efficient delivery vehicle for anti-cancer agents like curcumin, doxorubicin, and paclitaxel to target tumors and support the above suggestions [28].

Another therapeutic option that has gained attraction is the use of convalescent blood products such as whole blood, plasma or serum, pooled human immunoglobulin IgG for IV or IM administration, and polyclonal and monoclonal antibodies [29]. The benefits are the improvement of clinical symptoms, increase of oxygen saturation, the same or increased neutralizing antibodies titers, a decrease in lung consolidation, an increase in IgG and IgM titers, and the reduction of the viral load [30, 31]. However, the mechanisms of action are not fully understood and some authors consider it an “empirical therapy” since most of the studies conducted are case series with few participants, and, although promising, the data could be biased due to the lack of a well-designed experimental set up. The presence of EVs in convalescent blood products may explain some of the benefits observed in this therapy by promoting immunomodulation and wound healing in the lung tissue. During the apheresis of plasma, neutralizing antibodies, growth factors, as well as EVs, are transferred to the patients. Some studies [32–34] have shown that plasma-derived EVs carry a large number of growth factors and can induce the activation of intracellular signaling pathways, alteration of vascular reactivity, and the induction of angiogenesis for tissue repair. In wound-healing models platelet-derived EVs were shown to improve cell proliferation, migration, and angiogenesis via phosphoinositide 3-kinase (PI3K)-Akt and mitogen-activated protein kinase (MAPK)-Erk signaling, and the interaction between TGF- β and yes-associated protein (YAP) [33], which could help to elucidate the mechanism by which convalescent plasma shows positive results. There is another study showing the effectiveness of engineered platelet-derived EVs loaded with TPCA-1 to treat pneumonia [35]. This treatment inhibited the inflammation and reduced the local cytokine storm in a mouse model targeting selectively inflammatory sites. The authors suggest that platelet-derived EVs can be useful for developing new treatment options for COVID-19 patients.

Another proposed approach is the use of EVs as vaccines by exploiting their high stability in circulation, low toxicity, and immunogenicity [36]. One method is the use of a specific EV subpopulation with pro-inflammatory properties. For example, EVs generated by bone marrow-derived macrophages primed with lipopolysaccharide and adenosine triphosphate release EVs that contain IL-1 β , caspase-1, and inflammasome components. When these EVs are injected into naive animal models they produce an immune response. The other method is the use of viral vectors in combination with EVs. Interestingly, Kuate et al. [37] showed the

effective use of this approach for the treatment of SARS-CoV in a murine model. In this study, cells were transfected with an engineered chimeric S protein to produce EVs expressing the chimeric S protein, they also engineered an adenovirus vector expressing the chimeric S protein. They tested both approaches independently for comparison and then in combination. Their results showed that two injections of the S chimeric protein EVs without adjuvants were sufficient to induce neutralizing antibodies. However, they obtained the highest neutralizing activity when using the chimeric S protein EVs for priming followed by a second intervention with the adenovirus chimeric S protein vector. Furthermore, the use of EVs has proven to be more effective than using soluble protein subunit-based vaccines; this could be due to the expression of multiple copies of the same viral protein exposed on the surface of EVs facilitating the crosslinking between EVs and B-cell receptors [38].

We are still lacking information regarding the possible side effects of using EVs as therapeutics for COVID-19, and there is some concern among the community that needs to be addressed before implementing any EV-based therapy. One of them is the similarity between EVs and viruses. Both EVs and viruses interact with the endosomal-lysosomal system in charge of the production of exosomes. During cellular infection, some viruses may use this machinery by packing viral proteins and RNA into vesicles that are later released to the extracellular space, thereby contributing to the spread of the virus to non-infected cells [10]. This has been shown for HIV [39] but has not yet been elucidated for coronaviruses [40]. Another is their heterogeneity, since EVs are diverse in origin, size, composition, and functional characteristics; therefore, the choice of their source for therapeutic use should be carefully elaborated. For instance, Campanella et al. [41] demonstrated that the use of autologous exosomes in regenerative medicine is safer than those derived from allogeneic plasma. Soni et al. [42] reported that EVs from alveolar macrophages could have both pro-inflammatory and anti-inflammatory effects, depending on when EVs were isolated during the course of acute lung injury. Also, it was revealed that adipose-derived MSC-EVs have higher thrombogenic activities than bmMSC-EVs [43]. Recently, Inal et al. [44] drew attention to the possible role of specific subpopulations of EVs, specifically tissue factor-positive EVs, in the development of venous thromboembolism in COVID-19 patients that have comorbidities, such as hypertension and diabetes. They suggested that EVs might play a significant role in the poor prognosis of patients with such comorbidities, making them prone to developing thrombosis.

Conclusion

With their diverse properties, such as inherent immunomodulation and wound-healing attributes, as well as their drug delivery capacity, EVs represent an array of options for antiviral therapeutics, including COVID-19 (Fig. 1). However, it should be noted that no EV-based therapy has to date been approved. Despite the significant role of EVs in the pathogenesis of pulmonary diseases, several aspects remain unexplored. The heterogeneity of EVs due to their source, aging, isolation, and purification techniques may result in functional differences. Hence, some EVs could have an immunomodulatory and regenerative activity while others could contribute to the acceleration of viral infection by the transport of viruses inside EVs that let the viruses bypass the immune system. A recent statement from the International Society of Extracellular Vesicles (ISEV) and the International Society for Cellular and Gene Therapies (ISCT) encourages further development of the use of EVs from MSCs and other cells for treatments. Nonetheless, it also highlights the need for caution when using EVs [45]. All of the above indicates that further research, improvement of isolation, and characterization techniques will be fundamental to determine the benefits and safety of future efficient EV-based therapies against COVID-19.

Conflict of Interest Statement

Dr. Francesco Trepiccione, Associate Editor of *KBPR* and Prof. Capasso, Editor-in-Chief of the journal, declare no competing interest in anything that interferes with, or could reasonably be perceived as interfering with, the full and objective presentation, peer review, editorial decision-making, or publication of the manuscript.

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Author Contributions

A.P.R.J., R.M., R.B., and F.T. analyzed the current literature and clinical trials, provided critical feedback, and helped to draft the manuscript. R.B. designed the figures. G.P. and G.C. conceived the idea, carried out the implementation, and supervised the drafting.

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