STEM CELL-DERIVED EXOSOME THERAPY OF MICROBIAL DISEASES: FROM BENCH TO BED

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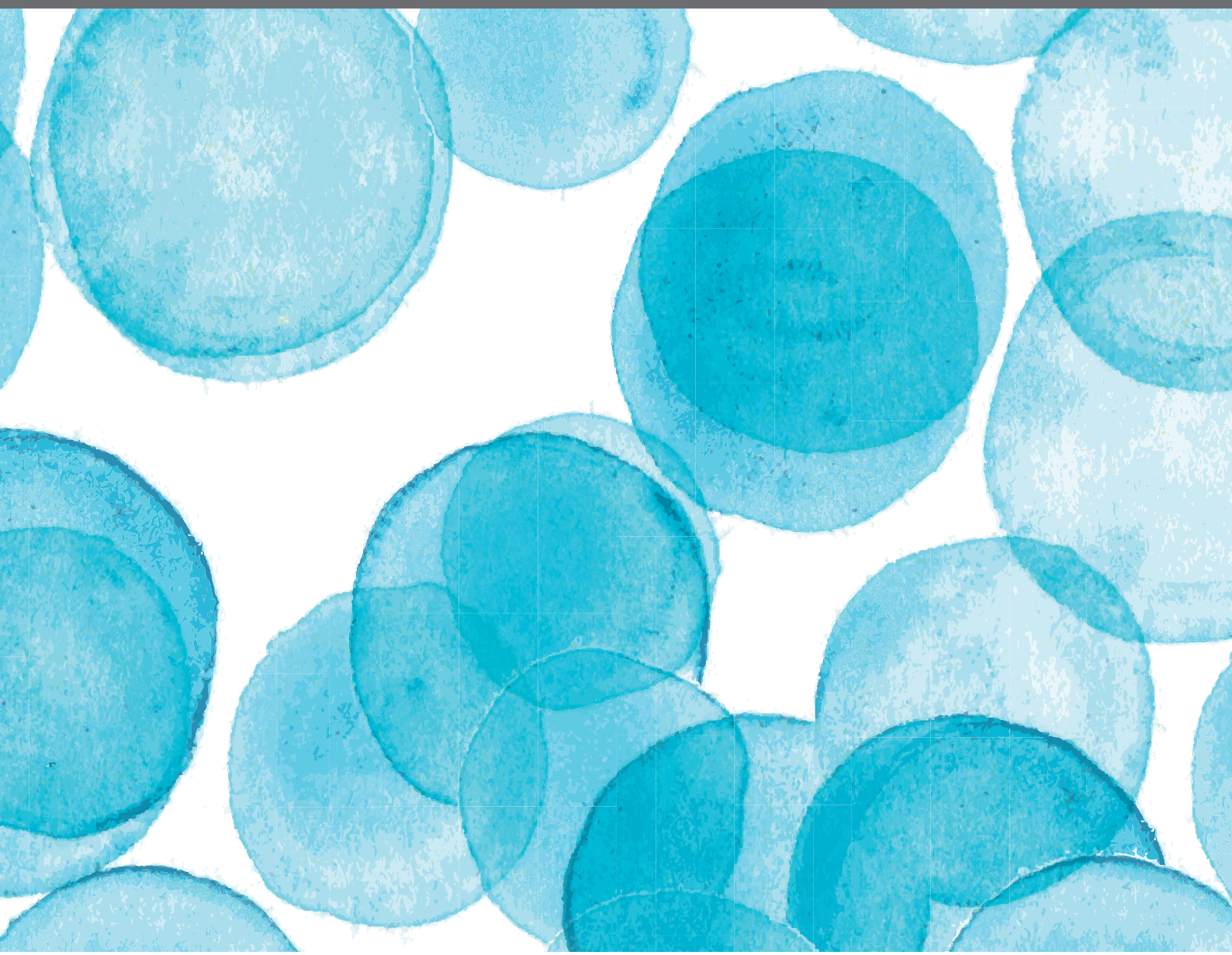

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STEM CELL-DERIVED EXOSOME THERAPY OF MICROBIAL DISEASES: FROM BENCH TO BED

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EDITORIAL
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Editorial: Stem Cell-Derived Exosome Therapy of Microbial Diseases: From Bench to Bed

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Keywords: stem cell, exosome (EXO), microbial diseases, therapy, extracellular vesicles

Editorial on the Research Topic

Stem Cell-Derived Exosome Therapy of Microbial Diseases: From Bench to Bed

Exosomes are nano-sized vesicles that play a mediator role in cell-to-cell communication. They are composed of unique proteins, lipids and nucleic acids, which replicate the composition of producer cells and can be used as cell-free therapeutics. Exosomes derived from stem cells have attracted great attention due to their immunomodulatory, regenerative and antimicrobial capabilities. These characteristics have been demonstrated in various *in vitro* and *in vivo* models. Furthermore, recent developments in the field of exosome therapy have resulted in elaboration of specific quality control methods and guidelines, which will facilitate the use of exosomes in clinical settings.

The leading cause of death in intensive care units (ICUs) is sepsis, with a mortality rate as high as 25% in severe cases (Fleischmann-Struzek et al., 2020). Microbial infections, which cause sepsis, involve complex interactions between microbial pathogens and the host immune system. Excessive induction of endogenous pro-inflammatory cytokines and coagulation pathways during the early phase of sepsis result in adverse effects in patients. Stem cells can modulate the expression of the corresponding genes in sepsis (Huang et al., 2017). Stem cells also enhance the clearance of pathogens and repair of injured tissues in sepsis. There are new insights for treatment of microbial disease using stem cell-derived exosomes which have been discussed in this Research Topic.

The researchers who contributed to this Research Topic presented 10 themed articles that highlighted the knowledge from recent advancements in the field of exosome therapy of microbial infections. For example, in the work by You et al., we learnt that the various mechanisms of stem cells-derived extracellular vesicles (MSC-EVs) treatment for infectious diseases in detail. The authors described MSC-EVs mechanisms for treatment of intestinal infections, sepsis, and respiratory infections. The authors also demonstrated challenges for implementing MSC-EVs from bench to bedside. In addition, Keshavarz Alikhani et al. verified biogenesis and the fate of EVs. They demonstrated EV-based therapy and current developments in understanding the potential application of stem cell-derived EVs on pathogenic microorganisms. They also highlighted the mechanisms by which EVs were exploited to fight against infectious diseases and the deriver challenges in translation of stem cell-derived EVs into the clinical arena. On the other hand, Keshtkar et al. described that most published studies on stem cell derived-exosomes are preclinical and are under way to reach clinical applications. They emphasized the challenges ahead of this cell-free therapeutic method that might be applied as a treatment alternative to stem cells. By the way, Wu et al. highlighted the latest progress in the clinical translation of the MSCs-derived exosomes therapy, by summarizing related clinical trials, routes of administration and exosome modifications.

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Four articles focused on the treatment of specific infectious diseases using MSCs-derived exosomes. Izadi et al. evaluated the studies about the potential therapeutic roles of MSCs-derived exosomes on sperm abnormalities and male infertility caused by sexually transmitted diseases (STDs) including Chlamydia infection. These investigators described that exosomes have potential properties for preventing the consequences of infection, such as reducing cell damage, preventing inflammation, and reducing scar formation by inhibiting fibrogenesis. The second review article by Zohrabi et al. discussed how MSCs-derived exosomes secrete different bioactive factors. These secretions can prevent infection and modulate the immune system. Thus, they reviewed the possible application of MSCs-derived exosomes in female reproductive system bacterial diseases. Furthermore, the other review by Raghav et al. summarized recent findings on the application of the cargo-loaded stem cell-derived exosomes in the treatment of diabetic foot ulcers (DFUs). They also categorized the different approaches for loading the desired cargo/drug inside exosomes. On the other hand, Jafari et al. described the ability of MSCs-derived exosomes as a therapeutic choice for controlling and treatment of orodental infectious diseases.

Considering to fungal disease, Ghasemian discussed applications of stem cell derived exosomes in in fungal diseases. In her review, the probable role of exosomes, limitations for clinical studies and mechanisms of action of exosomes in treating fungal diseases was explored. In a novel insight into exosome therapy of infectious diseases, Bayat et al. presented the first description that algae derived stem cells can produce EVs. They described properties of EVs extracted from this marine derived source and their antimicrobial effects.

In conclusion, this themed collection enhances our knowledge of exosome isolation methods from stem cell for anti-bacterial, antifungal, antiviral, or anti-parasitic applications. The papers particularly highlighted potential targets and methods for stem cell genome manipulation for improved production of antimicrobial agents and release-through exosomes and also summarized *in vitro* and *in vivo* studies evaluating stem cell-derived exosomes on pathogenic microbes. Nevertheless, introducing quality control measures and guidelines for production of stem cell-derived exosomes as antimicrobials in clinical settings needs further research and development.

AUTHOR CONTRIBUTIONS

AT drafted the Editorial while NT and RS contributed to editing. All authors conceived and designed the work and provided final approval of the version to be published.

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REVIEW
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Mesenchymal Stromal Cell-Derived Tailored Exosomes Treat Bacteria-Associated Diabetes Foot Ulcers: A Customized Approach From Bench to Bed

Alok Raghav[1†], Prashant Tripathi[2†‡], Brijesh Kumar Mishra[3†], Goo-Bo Jeong[4†], Shahid Banday[5], Kirti Amresh Gautam[1], Qazi Noorul Mateen[6], Prem Singh[7‡], Manish Singh[8], Akhil Singla[9] and Jamal Ahmad[10]*

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Exosomes are nano-vesicles of endosomal origin inherited with characteristics of drug delivery and cargo loading. Exosomes offer a diverse range of opportunities that can be exploited in the treatment of various diseases post-functionalization. This membrane engineering is recently being used in the management of bacteria-associated diabetic foot ulcers (DFUs). Diabetes mellitus (DM) is among the most crippling disease of society with a large share of its imposing economic burden. DM in a chronic state is associated with the development of micro- and macrovascular complications. DFU is among the diabetic microvascular complications with the consequent occurrence of diabetic peripheral neuropathy. Mesenchymal stromal cell (MSC)-derived exosomes post-tailoring hold promise to accelerate the diabetic wound repair in DFU associated with bacterial inhabitant. These exosomes promote the antibacterial properties with regenerative activity by loading bioactive molecules like growth factors, nucleic acids, and proteins, and non-bioactive substances like antibiotics. Functionalization of MSC-derived exosomes is mediated by various physical, chemical, and biological processes that effectively load the desired cargo into the exosomes for targeted delivery at specific bacterial DFUs and wound. The present study focused on the application of the cargo-loaded exosomes in the treatment of DFU and also emphasizes the different approaches for loading the desired cargo/drug inside exosomes. However, more studies and clinical trials are needed in the domain to explore this membrane engineering.

Keywords: exosomes, diabetes foot ulcers, diabetes mellitus, customized exosomes, bacterial infection

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INTRODUCTION

Extracellular vesicles (EVs), including exosomes, apoptotic bodies, and microvesicles, are secreted by various cell types. EVs showed diverse characteristics in size, function, indigenous cargo, and secretion pathway (Raghav et al., 2021). Exosomes are small-sized EVs formed by the process of inward budding in early endosomes and later form multivesicular bodies (MVBs) of average 100-nm dimensions (Raghav et al., 2021). These later released into the extracellular matrix/environment to deliver their indigenous cargo/components fulfilling their fate (Raghav et al., 2021). Cellular exosomes release involves various steps, i.e., formation of early endosomes, followed by fusion of the MVBs containing intraluminal vesicles (ILVs), with the plasma membrane by exocytosis and release of exosomes in the extracellular space (Than et al., 2017). Exosomes are present in all bodily fluids secreted by cells, including blood (Lewis et al., 2018), urine (Cavallaro et al., 2019), plasma (Yan et al., 2019), breast milk (Adriano et al., 2021), saliva (Kurian et al., 2021), bile, synovial fluid, semen, amniotic fluid, ascites fluid (peritoneal cavity), and bronchoalveolar and gastrointestinal lavage fluid (Kumar et al., 2019). The exosomal indigenous cargo is mostly rich in proteins, lipids, sugars, and nucleic acids [messenger RNAs (mRNAs), microRNAs (miRNAs), and mitochondrial DNA (mtDNA), etc.] (Jan et al., 2021; **Figure 1**). Exosomes' functions encompass an elaborative list depending on the origin of cell/tissue. Such functions include immune-modulatory, regeneration, antigen presentation programmed cell death (APPCD), inflammation, angiogenesis, and coagulation. The cargo imparts functionality to the exosomes for different cellular communications like paracrine, autocrine, endocrine, and/or juxtacrine signaling, while surface proteins provide identity to the exosomes for cargo delivery (Wei et al., 2021).

Authors of past studies exploited the exosomes as delivery vehicles for drugs and other desired cargo of interest (Bertrand and Leroux, 2012; Lai and Breakefield, 2012; El Andaloussi et al., 2013). These inbuilt characteristics of exosomes allow for tailoring "cargo of interest" for therapeutics and imaging purpose with an additional feature of prolonged circulation time, specific target cell recognition due to the presence of cell surface markers, negligible toxicity, and immune tolerance. Exosomes can be manipulated with more than one type of deliverables like drugs, proteins, and coding/non-coding nucleic acids, simultaneously. However, further studies are required to evaluate whether there exists any sort of allogeneic immune rejection among exosomes from different donors and recipients (Zhuang et al., 2011; Lee et al., 2012).

In one of the recently published studies, the protective effect of adipocyte stem cell (ADSC)-derived exosomes was investigated in a diabetic animal *in vitro* model and found that exosomes promoted angiogenesis and proliferation of cells in the hyperglycemic environment (Li et al., 2018). The study showed a significant reduction in diabetic ulceration/wound area in the animal group receiving the exosomes from ADSCs overexpressing the Nrf2 factor (Li et al., 2018). The study laid the foundation that the exosomes can be exploited for the healing of diabetic foot ulcers (DFUs). An et al. (2021) showed the therapeutic role of mesenchymal stem cell (MSC)-derived exosomes in the treatment of diabetes-induced ulcers and lower limb ischemia.

Diabetic foot ulcers are a severe complication associated with diabetes mellitus (DM) that impose economic burden ranges from US$9 to US$13 billion in the United States, along with additional cost for the management of DM (Raghav et al., 2018). DFUs are the cause of various complications including peripheral neuropathy, deformity in the foot, and peripheral arterial diseases' poor extremity perfusion (Noor et al., 2018). DFUs are characterized by the presence of bacterial pathogens that are responsible for wound microbiology and the development of the infection. Several microorganisms (fungi, aerobic, and anaerobic species) are responsible for the etiology of the DFUs, including *Staphylococcus*, *Streptococcus*, *Proteobacteria*, and *Pseudomonas aeruginosa* (Noor et al., 2015). In this review, first, we comprehensively focused on exosome biogenesis and factors affecting the biogenesis. In addition, we discussed the methods of isolation of exosomes and fabrication of the customized exosomes using various modification methods. This study discusses the idea that MSC-derived exosomes post-tailoring hold promise to accelerate the diabetic wound repair in DFU associated with bacterial inhabitant, along with the application of the cargo-loaded exosomes in the treatment of DFU, and this study also emphasizes the different approaches for loading the desired cargo/drug inside exosomes.

BIOGENESIS OF EXOSOMES

Biogenesis of exosomes is a constitutive mechanism that is initiated with plasma membrane inward invagination within cytosol generating early and late endosomes. These late endosomes further give rise to MVBs followed by ILV formation. It seems that during the ILV formation by inward budding, several essential proteins, growth factors, cytoskeleton components, nucleic acids, lipids, and other necessary cellular components get wrapped into it (Raghav et al., 2021). The key feature of biogenesis pathways includes internalization, fusion, and release (**Figure 2**). ILVs formed from MVBs fuse with the plasma membrane of the cells and released as exosomes into the extracellular environment by the mechanism of exocytosis.

In one of the recently published studies, it was quoted that the budding of the exosomes and their sorting are either endosomal sorting complex required for transport (ESCRT)-dependent or -independent (Raghav et al., 2021). The ESCRT-mediated exosomes sorting process involves screening, identification, and sequestration of ubiquitinated proteins specific for endosomal proteins. This ESCRT-mediated mechanism showed an association between subunits I, II, and III of ESCRT that terminate the exosome budding process (Raghav et al., 2021). Moreover, the ESCRT-independent mechanism of exosome budding involves proteins and lipids such as tetraspanins and ceramides (Raghav et al., 2021). The exosomes play a crucial role in intercellular communication *via* the transfer of the biomolecules loaded within them. Their biogenesis mechanism is governed by various factors including ESCRT

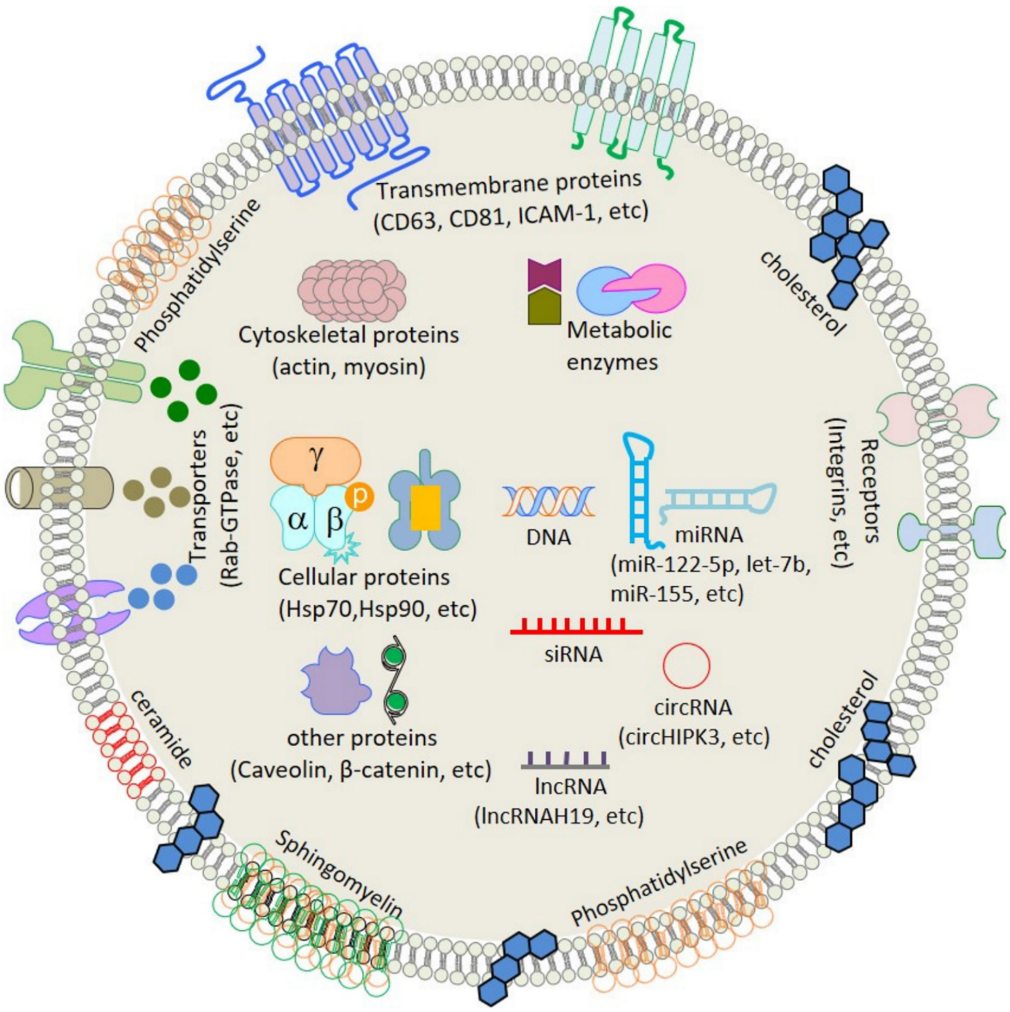

FIGURE 1 | Schematic structure and contents of exosome. ATPase, adenosine triphosphatase; CD, cluster of differentiation; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HSP, heat shock protein; ICAM-1, intercellular adhesion molecule-1; LAM 1/2, lysosomal-associated membrane protein 1/2; MHC, major histocompatibility complex; miRNA, microRNA; mRNA, messenger RNA; MVB, multivesicular body; PGRL, PG regulatory-like protein; pgk1, phosphoglycerate kinase 1. [Adopted from Jan et al. (2021) distributed under the Creative Commons Attribution Licens].

proteins, STAM1, VPS4, CHMP4, the Syndecan–syntenin–ALIX complex, nSMase2, CD9, and PLD2 (Gurunathan et al., 2021). Similar to the sorting mechanism, the exosome uptake process is mediated by either the clathrin-dependent or clathrin-independent events that involve micro-pinocytosis, phagocytosis, and lipid raft-mediated internalization. The exosomes are composed of several biomolecules including heat shock proteins, cell adhesion proteins, cell signaling proteins, tetraspanin membrane proteins, phosphatidylserine (PS), phosphatidic acid, sphingomyelin (SM), cholesterol, arachidonic acids, prostaglandins, and leukotrienes (Raghav et al., 2021). Besides these proteins and lipid components, exosomes are also rich in micro-RNAs, small nuclear RNAs, non-coding RNAs, long non-coding RNAs, piwi-interacting RNAs, rRNAs, and tRNAs (Raghav et al., 2021). Exosomes are considered to be the cocktail of these biomolecules that have therapeutic, diagnostic, and transmittance characteristics.

SOURCES OF EXOSOMES

Exosomes can be derived from various cell types and all have diverse clinical characteristics, depending on the source of cells from which they are derived. The various sources of exosomes include the following:

ADMSC-Derived Exosomes

Adipose tissue mesenchymal stem cells (ADMSCs) are abundantly distributed in the human body, compared to other exosome cell sources including umbilical cord mesenchymal stem cells (UCMSCs) and bone marrow mesenchymal stem cells (BMSCs). ADMSCs showed the highest degree of purification with high yield due to their abundance in nature (Tang et al., 2021). The extraction of ADMSCs is an easier and painless procedure, causing only a small episode of trauma (Tang et al., 2021). These cell-derived exosomes can be easily procured

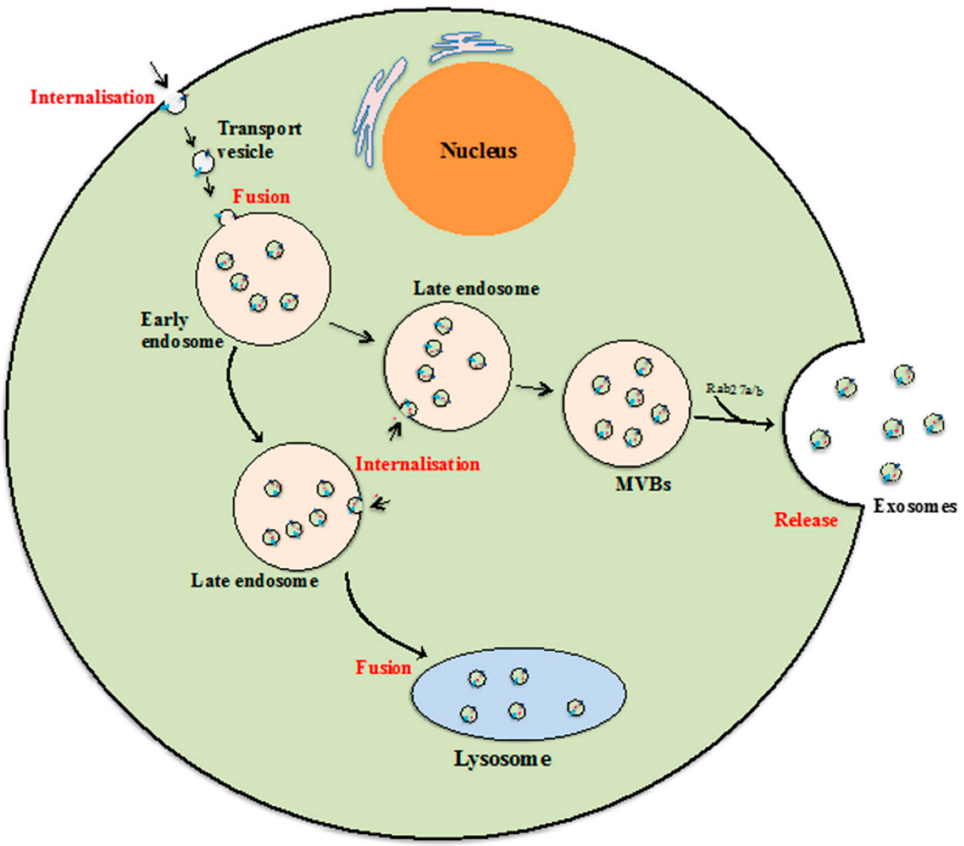

FIGURE 2 | Exosome biogenesis. Beginning from internalization of membrane proteins and lipid complexes by endocytosis, endocytotic vesicles are delivered to early endosomes, which fuse with each other resulting in formation of late endosomes/multivesicular bodies (MVB). [Adopted from Than et al. (2017) distributed under the Creative Commons Attribution License].

in clinics in the presence of plastic surgeons or medical aestheticians. ADMSC-derived exosomes require strict storage conditions, thereby possessing some restrictions in their clinical applications. Moreover, the lipid membrane structure is quite stable and has the properties to retain its contents for a long period and is therefore always a good choice for researchers (Tang et al., 2021).

The strict morphology of the adipose tissue is not fixed, and the primary source cells can be of any shape, either fusiform or circular. It was reported from *in vitro* cell culture observation that the primary cells get to adhere with other cells within 1–2 days of the cell culture (Tang et al., 2021). It was shown that after the fifth passage, these cells form a single layer, thereby showing a vortex or radial growth pattern (Tang et al., 2021). Later, their morphology changes to a single long spindle. The exosomes derived from ADMSCs have uniform cup shape morphology with average diameter ranges between 30 and 120 nm as evident from the scanning electron microscopy (Tang et al., 2021). The exosomes can be detected using flow cytometry, differential centrifugation, magnetic bead assay, and transmission electron microscopy. The presence of surface proteins CD9, CD10, CD13, CD29, CD44, CD63, CD73, CD90, CD105, enkephalin enzyme, and major histocompatibility complex MHC I molecules distinguishes these from other cell-derived exosomes. It is still a research lacuna that no single surface marker has been identified for these exosomes.

UCMSC-Derived Exosomes

The umbilical cord has a placental origin and is involved in giving nourishment and nutrition to the fetus from the mother during pregnancy. Human-derived MSCs can be broadly classified into (i) human umbilical cord mesenchymal stem cells (hUC-MSCs), (ii) human umbilical cord perivascular MSCs (HUCPV-MSCs), (iii) human umbilical cord Wharton's Jelly MSCs (HWJ-MSCs), and (iv) human amniotic membrane-derived MSCs (HA-MSCs) (Subra et al., 2010).

The morphology of the primary UCMSCs demonstrates a spindle shape with the absence of vortex growth. Later, the cells show vortex growth after day 1 of the direct adherent cell culture using the primary cell tissue mass culture method. Moreover, few cells during the fifth passage show vortex patterns from the second generation until the fifth generation (Tang et al., 2021). With the fifth passage, the cells become long, elongated, and fusiform with typical vortex growth. The exosomes derived from these primary cells show variation in size in the range between 30 and 100 nm, as revealed from electron microscopy

(Tang et al., 2021). The exosome morphology exhibits a round or elliptical membranous structure with clear and distinct boundaries (Vohra et al., 2020). The UCMSCs exhibit cell-specific surface markers including CD29, CD44, C/73 (SH3), CD90 (Thy-1), and CD105 (SH2) and negative for CD11b, CD34, and CD45, while their extracted exosomes demonstrate CD9, CD63, and CD81 and the multivesicular biosynthesis-related protein ALIX (Tang et al., 2021).

BMSC-Derived Exosomes

The BMSCs can be isolated from bone marrow with the inbuilt advantage of low infection rate of pathogenic microorganisms, efficient and stable biological role, low immune rejection post-transplantation, and good survival rate in higher passages (Tan et al., 2020). These cells exhibit diverse size and shape and become adherent after 1–2 days of the cell culture seeding in the appropriate culture medium. The adherent cell shows round morphology as demonstrated by electron microscopy. These cells begin to colonize after 4–5 days of the culture exhibiting a single fusiform shape forming a vortex growth pattern usually at the fourth passage (Tang et al., 2021). The exosomes derived from BMSCs are uniform with a size range between 30 and 100 nm in diameter and having a cup-shaped morphology with clear and distinct boundaries (Tang et al., 2021).

Western blotting and flow cytometry analysis of the BMSC-derived exosomes show expression of CD9, CD63, CD81, HSP70, syntenin-1, and multi-vesicular biosynthesis-related protein TSG101 (Tang et al., 2021).

MSC-DERIVED EXOSOME ISOLATION METHODS

The following exosome isolation methods are currently available worldwide: microfluidics, differential centrifugation, precipitation, antibody affinity capture, ultrafiltration, flushing separation, magnetic bead-based capture, and size-exclusion chromatography (SEC).

Microfluidics

Microfluidics provides highly efficient, precise control, and rapid methods for isolating the exosomes on a single chip with manipulated fluids at microscale levels. The basic principle of microfluidics is that it manipulates a small quantity of the fluid using specialized micro-dimension channels using capillary forces. Its manipulation characteristic with fluids in a micro/nanoscale environment makes it a highly preferred method of choice among researchers. The basic design of this method involves a single chip of a few square centimeters dimension with a scope of scaling up isolation and separation. This unique approach relies on interdisciplinary sciences that include physics, fluid chemistry, micro-processing, and bioengineering. In one published study, a microfluidics chip is coupled with acoustic, electrophoretic, and electromagnetic separations, which showed a fast and efficient way of exosome isolation and separation (Popovic et al., 2018). In another related study, the implication of silicon nanowires is engraved on the microchip pillar walls for trapping liposomes, and acoustic nanofiltration is used for isolation of exosomes within a size range of 100–1000 nm (Kurian et al., 2021).

One more study exhibited the use of viscoelastic microfluidics for the isolation and separation of the exosomes with an isolation efficiency of >80% and a purity degree of >90% (Kurian et al., 2021). Membrane of different pore sizes was also implicated for the separation of exosomes based on filtration using ExoTIC microfluidics chip (Lin et al., 2020). In another study, electric forces are applied along with a dialysis membrane of 30 nm pore size for the isolation of exosomes (Yang et al., 2017). Wu et al. (2017) used whole blood to isolate exosomes using the acoustic fluidics approach in combination with microfluidics. This system showed the unique feature of the cell removal module, which separates exosomes from microvesicles (Wu et al., 2017). The main advantages of this method are as follows: (i) it requires a lower amount of the sample volume, (ii) it is a time-saving approach, and (iii) it is a cost-saving and real-time process. The only disadvantage of this method is less sensitivity for the isolation of exosomes. So, a scale-up is required in this technology for the production of clinical-grade exosomes.

Differential Centrifugation

This is the most widely used method for the isolation of the exosomes (Momen-Heravi et al., 2013). Cell debris and apoptotic bodies shed exosomes during successive rounds of the centrifugation mechanism. This method is based on the density, size, and shape of the exosomes. This gold standard method for exosome isolation, however, exhibits low yield and insufficient purity due to similarity in sedimentation properties of the different types of EVs (Tauro et al., 2012; Witwer et al., 2013; Cvjetkovic et al., 2014; Lane et al., 2015).

The main advantages of this method include reduction of cost and contamination. Additionally, a large sample capacity can be easily handled with this technique followed by high yields of exosomes. In another study, researchers have added 30% sucrose in the first step and reported a high yield of the exosomes (Bajimaya et al., 2017). Moreover, the limitations of the present approach are that high-speed centrifugation can damage the exosomes and it needs a long runtime with labor-intensive work. In one of the studies, it was found that performing ultracentrifugation three times reduces the purity of the exosomes (Tang et al., 2021).

Precipitation

The precipitation of the exosomes depends on the principle of altering the solubility or dispersibility of the exosomes within a water-devoid medium. In this approach, the external solvent is implicated in the solution, which changes the polarity and solubility of the components present within the components, as a resultant, initiate the precipitation of desired molecules. It is a very simple approach for the isolation of the exosome. In one of the previously published studies, it was found that the precipitation approach is very effective in the separation of biological fluids (Maroto et al., 2017). Several commercial isolations and purification kits for exosomes are available, showing good yield and purity, including Serum^{TM},

the Exo-Q and Exo-Spin™ blood cell purification kits, the miRCURY Exosome Separation Kit, the Exo Quick-TC Exosome™ Precipitation Solution Kit, and the Total Exosome Isolation kit (Maroto et al., 2017; Zhao et al., 2017; Buschmann et al., 2018; Soares Martins et al., 2018). The advantages of the current methods are that they are easy to use, do not require sophisticated and specialized machines, do not put any harsh effect on exosomes, and can be used on large sample volumes. Some limitations of these methods include the co-precipitation of other contaminants like polymeric materials, proteins, and lipids and the fact that they additionally require a long runtime to complete the process.

Magnetic Bead-Based Capture

This process is also called an immunomagnetic bead-based assay. It is a recently developed technology that uses ExoCAS-2 charge-based ion exchange and magnetic beads for the isolation of exosomes from biofluids (Kim and Shin, 2021). This ExoCAS-2 implicates polycationic polymer-functionalized and -coated magnetic beads. The sample before the magnetic separation is filtered to exclude the large size impurities present. The mechanism of separation of the exosomes involves the binding of negatively charged exosomes with the positively charged poly-L-lysine-coated cationic beads *via* electrostatic interactions (Kim and Shin, 2021). Following the process of incubation and continuous stirring, the nano-sized exosomes bind to the surface of the coated beads and later eluted using an elution buffer with different ionic strengths that disrupts the electrostatic interactions. This efficient exosome separation and isolation approach yields exosomes of high purity grade, but the limitations associated with this technology are that it cannot be used in clinics, it has a high cost, and the rate of unspecific binding during the binding process is higher.

Ultrafiltration

This technique is based on the application of specific pore size diameter membranes for separation and isolation of the exosomes (Cheruvanky et al., 2007; Lobb et al., 2015; Konoshenko et al., 2018). This approach can be complementary with ultracentrifugation, although it can also be performed alone. Another improved version of ultrafiltration includes cross-flow filtration or tangential flow filtration (McNamara et al., 2018). This improvement helps in removing the protein contaminants from the exosomes containing samples if repeatedly passed from the exclusion filter of a defined diameter, thereby concentrating the exosomes. In one of the studies, it was claimed that a cellulose membrane with a pore size of 10 kDa is very efficient in the recovery of the exosomes using an ultrafiltration approach (Vergauwen et al., 2017). The advantages of ultrafiltration are that it does not require expensive equipment and consumes less time. The only associated limitation with the ultrafiltration method is exosome loss due to attaching with membranes as a result of shear stress and membrane clogging.

Size-Exclusion Chromatography

Size-exclusion chromatography depends on the separation of the exosomes' molecules based on their size. The sample containing the exosomes is passed through the column consisting of the beads with variant pore size. Each molecule is passed through the individual beads based on their size. The small-size molecules show delayed elution from the column, as they have to traverse the complete length of the column. In one of the studies, it was found that exosomes have large hydrodynamic radii, passing through the column faster, as they do not show penetration inside the beads (Feng et al., 2014). In another, a single-step SEC using a Sepharose CL-2B column was used for isolation of exosomes with 75 nm diameter effectively from body fluids (Böing et al., 2014). This method allows minimal harm to the isolated exosomes compared to other precipitation-based methods. The SEC approach for isolating exosomes can efficiently remove the plasma proteins from the biological samples, as claimed by one of the studies (Gámez-Valero et al., 2016). In one of the studies, the authors have isolated clean and non-aggregated exosomes with a size range of 50–200 nm (Hong et al., 2016). It is also evident that SEC in conjugation with an ultracentrifugation approach can be efficiently used for the isolation of the exosomes from the biological fluids, compared to alone itself. The main advantages associated with SEC are that it can be used for the separation of the small and large molecules in biological fluids without altering the exosomal structure. The only limitation is the requirement of a long runtime.

TAILORING APPROACHES FOR MSC-DERIVED EXOSOME MODIFICATIONS

Exosome-based delivery approaches showed promising benefits related to specificity, safety, and stability due to their inbuilt homing characteristics that exhibit effective delivery of desired cargo to specific target sites. Recent studies showed that exosomes can be used to deliver small interfering RNA (siRNA) or active pharmaceutical agents like drugs and vaccines to treat diseases (Aryani and Denecke, 2016). These nano-size envelopes tend to avoid phagocytosis and engulfment by lysosomes with a low immune response (Ha et al., 2016). Several tailoring approaches for modification of exosomes and loading of the desired cargo into the exosomes were studied, which can be broadly classified into two strategies: (i) exogenous tailoring of exosomes post isolation and (ii) endogenous tailoring during biogenesis of exosomes. Exogenous tailoring approaches can be further divided into an active and passive form; the active approach involves the sonication, extrusion, freeze–thaw cycles, electroporation, and chemical-based approach, while the passive form involves the incubation process. Moreover, the endogenous tailoring of exosomes involves the introduction of the cargo of interest into the cells producing exosomes, which commonly implies the application of transfecting cells with expression vectors as in genetic engineering for targeted therapy (Van der Meel et al., 2014). The following paragraphs provide a brief overview of the tailoring approach for modifications of exosomes.

Exogenous Tailoring of Exosomes

Incubation

This tailoring approach simply involves the incubation of exosomes with the desired interest of cargo, which can be referred to as passive loading. The potential difference created due to the interplay between the concentration of desired cargo inside and outside the exosomes drives the infusion of desired cargos through the lipid bilayer membrane of exosomes. In few cancer-related research studies, this method was used to load chemotherapeutic drugs like paclitaxel and doxorubicin into the exosomes and also to observe enhanced chemotherapeutic effects (Tian et al., 2014; Yang et al., 2015; Salarpour et al., 2019). This enhanced effect of drug-loaded exosomes is observed due to the ease of crossing the blood–brain barrier. In another study, exosomes loaded with enzymes were used in the treatment of Parkinson's disease (Haney et al., 2015). In one of the studies, the authors have co-incubated curcumin with exosomes and found that it gets self-assembled with the lipid bilayer of exosomes due to the interplay of hydroscopic interactions. The curcumin encapsulated exosome not only increased the target specificity but also enhanced the anti-inflammatory property of curcumin (Sun et al., 2010). Though proven to be useful for modifying exosomes for their enhanced functionality with the desired cargo, this method sometimes affects the size of exosomes resulting in low yield, low entrapment, and uncontrollable drug loading. The present method is simple, cost-effective, and effective in transporting hydrophilic cargos efficiently into the exosomes.

Sonication

Sonication provides an additional advantage of enhancing the loading of desired cargo inside the bilayer membrane of the exosomes. This approach utilizes sound waves generated from a sonicator machine to induce a shearing force effect upon the exosome membrane, which, in turn, increases the uptake of desired cargo inside the exosomes (Kim et al., 2016). Kim et al. (2016) successfully loaded paclitaxel and doxorubicin into the exosomes implicating this approach. It is believed that the sonication process decreases the micro-viscosity of the exosomal membrane that allows the passage of cargo inside (Kim et al., 2016). This cargo loading approach is healthy for biological molecules like small RNAs due to its high loading efficiency. Some limitations like the development of shearing forces, exosomal membrane deformation, heat generation during the sonication cycle, loss of exosomal surface proteins, and non-suitability for hydrophobic drug delivery are associated with this approach.

Extrusion

This tailoring method involves lipid bilayer membrane disruption of exosomes during extrusion through a small-size polycarbonate porous membrane. This reversible disruption in the membrane allows the entry of desired cargo of interest inside the exosomes (Haney et al., 2015). Le Saux et al. (2020) have reported that extrusion is an efficient and promising method for tailoring the exosomes and loading the desired cargo inside it for targeted delivery. In one previous study, exosomes were extruded with porphyrins (Fuhrmann et al., 2015). The extrusion mechanism reshapes and reforms the exosomal membrane extensively and thereby showed higher loading efficiency (Jamur and Oliver, 2010).

Freeze–Thawing

This tailoring approach involves freezing and subsequent thawing of the exosome sample in a vessel of desired cargo to be loaded. The mix is incubated at 37°C followed by rapid freezing at −80°C; the same steps were repeated several times depending on the efficiency of the system. It is always advisable to strictly monitor the freeze–thawing steps, because it may form the aggregates of these vesicles into large size particles (Haney et al., 2015; Le Saux et al., 2020). In one previously published study, catalase was loaded to exosomes using the freeze–thaw method (frozen at −80°C and thawed at RT) (Haney et al., 2015). It was also demonstrated that several freeze–thaw cycles resulted in a lipid dilution ratio that may be easily interpreted from fluorescence resonance energy transfer (FRET) assay (Sato et al., 2016). Though this method produces aggregates of exosomes with lower drug loading capacity compared to sonication or extrusion procedures, it is followed widely for cargo loading.

Electroporation

Tailoring of exosomes for loading cargo using electroporation is a commonly applied method that employs an electric field for cargo uptake. In the electric field, the phospholipid bilayer membrane is disrupted, thereby allowing the entry of hydrophilic compounds like small DNAs, miRNA, and siRNAs (Faruqu et al., 2018; Kobayashi et al., 2020; Lv et al., 2020; Orefice, 2020). A recent study has reported that exosomes tend to form aggregates during electroporation, although it did not affect the function of exosomes. However, certain refinements in the technique such as carrying out electroporation in an optimal buffer containing trehalose maintain the structural integrity of exosomes (Johnsen et al., 2016). In another study, fused exosomes were derived from αv-integrin-specific iRGD peptide with doxorubicin efficiently with electroporation and proved targeted tumor therapy (Gong et al., 2019). In one of the previously published studies, it was found that miRNA delivery to exosomes under mild electroporation protects miRNA from RNase degradation and showed efficient loading (Pomatto et al., 2019). In light of all the studies, it can be said that electroporation is a reliable method for cargo loading in exosomes that preserves the naïve cargo without compromising the structural integrity of exosomes.

Chemical Transfection

Chemical transfection is preferably used to incorporate siRNA into exosomes under the influence of the transfection agent Lipofectamine 2000. Wahlgren et al. used a liposome-based transfection reagent to incorporate MAPK-1-siRNA into the exosomes by incubating at 37°C for 10 min (Wahlgren et al., 2012). This method of loading desired cargo into exosomes achieves relatively high transfection efficiency using lipids. Cationic transfection agents are the preferred choice of researchers considering their high degree of success. These chemical transfection reagents

showed a high success rate in *in vitro* experiments; however, they have worse efficiency than electroporation. Immunogenicity and toxicity are some of the associated limitations of this approach.

Endogenous Engineering-Based Tailoring of Exosomes Producing Cells

Genetic engineering is another remarkable approach for the production of loaded exosomes with desired characteristics and functions. This approach involves transfection of the donor cells, thereby initiating the upregulation of specific genes, allowing the synthesis of specific gene-linked cargo-loaded exosomes during their biogenesis. The insertion of the desired "gene of interest" in the parent cell type is achieved by either viral/non-viral invasion/infection. The infection efficiency is optimized by the quantity and quality of the exosomal cargo. It is well reported that exosomes originate through the endosomal machinery of the cell membrane. Exosomal content reflects lineage and the original cell type; therefore, depending on the experimental requirement and/or therapeutic applications, the host cell selection should be performed. Genetic engineering for modification of the exosomal content from different cell types predominantly involves two types of viral vectors: (i) retroviral and (ii) adenoviral.

Jiang et al. (2020) observed the therapeutic effect of tumor necrosis factor (TNF)-stimulated gene-6 (TSG-6) modified MSC-derived exosomes in a wound model and found that tailoring of such exosomes prevents scar formation. In addition, several research studies demonstrated the therapeutic role of MSC-derived exosomes tailored with such methods carrying miRNA in improving treatment modalities (Xin et al., 2012). Wei et al. (2019) successfully engineered immature mouse dendritic cells, for exosome production, expressing Lamp2b fused to αγ integrin-specific iRGD peptide for breast cancer treatment *in vitro*. In one of the studies, engineered HEK293T was used for expression of Lamp2B along with a fragment of IL-3 and showed a reduction in tumor growth and was found to be effective in treating chronic myeloid leukemia (CML) (Bellavia et al., 2017). Rivoltini et al. (2016) transduced K562 cells with lentiviral human membrane TRAIL (TNF-Related Apoptosis-Inducing Ligand) for the production of TRAIL (+) exosomes. The authors reported apoptosis in cancer cells on treatment with TRAIL exosomes. Furthermore, the *in vivo* analysis revealed that engineered exosomes induced necrosis and vessel damage in melanoma tumor subjects (Rivoltini et al., 2016). In another study, exosomes enriched with miR-503 showed promising therapeutic potential for cancer treatment (Bovy et al., 2015).

"Omni Spirant" (patent pending) is a recently developed regenerative gene therapy for cystic fibrosis (CF) and involves the use of surface-engineered exosomes/bioengineered stem cell exosomes. The method involves mucus penetration of the exosomes and delivery of the gene therapy cargo for the effective treatment of CF (Health Europa, 2021). Bioengineering of cells for the production of engineered exosomes has gained significant attention in the past few years. However, further studies are mandatory for designing protocols with improved stability, drug solubility, and bioavailability, for the therapeutic application of engineered exosomes.

TABLE 1 | Clinical trials of BM-MSCs in DFUs.

Cellular type	Object	Delivery method	Duration of observation	Clinical parameters
Autologous BM-MSCs	24 patients with non-healing ulcers of the lower limb (diabetic foot ulcers and Buerger disease)	Autologous cultured BM-derived MSCs along with standard wound dressing	12 weeks	Decrease in wound size, increase in pain-free walking distance, maintain normal liver and renal function, improve leg perfusion sufficiently
Autologous BM-MSCs	51 patients with impending major amputation due to severe critical limb ischemia	Intramuscular transplantation	6 months	Improve leg perfusion sufficiently to reduce major amputations and permit durable limb salvage, reduce analgesics consumption, increase in pain-free walking distance
Autologous biograft composed of autologous skin fibroblasts on biodegradable collagen membrane (Coladerm) in combination with autologous BM-MSCs	Patients with diabetic foot	Directly to the wound and injected into the edges of the wound, finally covered with prepared autologous biograft, received two additional treatments with cultured MSC on days 7 and 17	29 days	Decrease in wound size and an increase in the vascularity of the dermis and in the dermal thickness of the wound bed
Autologous BM-MSCs	41 type 2 diabetic patients with bilateral critical limb ischemia and foot ulcer	Intramuscular injection	24 weeks	Increase in pain-free walking distance, improve leg perfusion, ankle-brachial index (ABI), transcutaneous oxygen pressure (TcO_2), magnetic resonance angiography (MRA) analysis
Autologous BM-MSCs	96 patients with critical limb ischemia and foot ulcer	Inject into the ischemic limb along the posterior and anterior tibial artery	120 days	79% limb salvage in patients

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THERAPEUTIC ROLE OF TAILORED MSC-DERIVED EXOSOMES IN BACTERIA-ASSOCIATED DFU

Mesenchymal stromal cell have a diverse role including multi-differentiation and immunomodulation that significantly contribute in reducing inflammation-related complications (Philipp et al., 2018). These MSCs show a contributory role in a paracrine manner mediating through secreted growth factors, cytokines, and exosomes (Phinney and Pittenger, 2017). One of the previously published studies quoted that MSC-mediated paracrine secretion promotes wound healing (Kourembanas, 2015). The advantage of using exosomes over cell-based therapies is that these vesicles may overcome the side effects associated with cell transplantation such as immune rejection. Pathogenesis of bacteria-associated DFUs is contributed by poor innervation and vascularization and chronic inflammation. In a recent study, it was observed that exosomes derived from MSCs inhibit M1 polarization and simultaneously promote M2 polarization that helps in the reduction of the inflammation (Cao et al., 2017). It is also found that these exosomes promote skin wound healing mediated by the regulation of M2 polarization (Cao et al., 2017). This dual nature of exosomes, i.e., anti-inflammatory and skin wound healing, can be explored in bacteria-associated DFUs.

Tailored MSC-derived exosomes possess promising result in the treatment of DFUs and diabetic wounds. In a recent study, exosomes derived after pre-treatment of MSCs with salidroside (glucoside of tyrosol) showed healing of diabetic wounds (Ariyanti et al., 2019). Similarly, fluoxetine and pretreated MSC exosomes managed diabetic neuropathy well (Abdelrahman et al., 2018).

It has been proved that these exosomes occupy the class of paracrine factor that mediates the therapeutic, tissue repair, and wound healing effects of MSCs (Joo et al., 2020). Several clinical trials showed the efficacy of BMSCs in the treatment of diabetic wound and ulcers (**Table 1**). In another research, tailored exosomes derived from pretreated BMSCs with atorvastatin (ATV) showed an acceleration in the healing of diabetic wound both *in vivo* and *in vitro* (Yu et al., 2020). It has been found that pretreated BMSCs with ATV secrete exosomes that activate the AKT/eNOS signaling mechanism that further initiates the angiogenesis of endothelial cells mediated through upregulation of miR-211-3p, thereby showing significant wound healing in the diabetic environment (Joo et al., 2020). In another study of exosome modification, it was found that exosomes derived from blue light-exposed human umbilical cord MSCs showed improved wound healing mediated through upregulation of MEF2C signaling (Yang et al., 2019).

Epidermal growth factor (EGF) and human adipose cell-derived stem cell exosome-loaded microcapsules integrated with collagen hydrogel can effectively show tissue regeneration and also restoration of blood perfusion in diabetic wounds (Cao et al., 2017). In the previously published literature, it has been found that adipose-derived MSC exosomes incorporated in freeze–thaw-based polypeptide-based hydrogel possess self-healing, antibacterial, and exosome release characteristics (Shen et al., 2016). These properties are useful in promoting wound healing by enhancing cell proliferation, neovascularization, re-epithelialization, and collagen remodeling at the wound site (Wang et al., 2019). In another recent tailoring approach, the cells are genetically engineered with transfection and co-culture to synthesize exosomes containing long non-coding RNA H19 that helps promote wound healing in DFU mediated by upregulation of PTEN through miRNA-152-3p (Li et al., 2020). **Figure 3** demonstrates the paracrine effect of BMSCs in treatment of DFUs mediated *via* EVs. These tailoring approaches of exosomes may help provide promising results in the healing of DFUs associated with bacteria. The current work encourages the implication of differential centrifugation and ultracentrifugation method for isolation of EVs from spent media or any other sources. The reason for recommending these two methods is due to their low cost and easy installation in any lab/clinic. Moreover, the genetic engineering approach endogenous modification is suitable for modification of EVs if they are used for delivering genes of interest. The modified EVs can be easily used in the treatment of ulcers/wounds associated with the DM. For instance, DFUs associated with bacteria need antibacterial and regenerative therapy. EVs, if modified for gene delivery (for initiating regeneration of damaged skin) and drug (antibiotics/antibacterial), can fulfill the purpose of therapeutic intervention.

PATHOGENESIS OF BACTERIA-ASSOCIATED DFU

Diabetes mellitus is characterized by high blood glucose level and neuropathy that slow down the wound healing process. These slow-healing wounds are vulnerable to bacterial infections (Buch et al., 2019). These diabetic wounds and foot ulcers become chronic due to microbe habitat on the wound site (Bjarnsholt et al., 2008). This continuous growth of bacteria (both aerobes and anaerobes) on the wound site produces biofilm, which exhibits resistance toward antibiotics that in turn causes a problem in the treatment of these wounds (Shiau and Wu, 1998; Bridier et al., 2011). It has been observed that *Staphylococcus aureus* is among the most common bacteria that are prevalent in DFUs (Kalan et al., 2019). Moreover, other bacteria causing DFUs includes β-hemolytic streptococci, *S. aureus*, *S. saprophyticus*, *S. epidermis*, *Streptococcus pyogenes*, *S. mutans*, *P. aeruginosa*, *Bacillus subtilis*, *Proteus* species, *Escherichia coli*, and *Klebsiella pneumoniae*. The anaerobic bacteria include *Peptostreptococcus* species, anaerobic streptococci, *Bacteroides fragilis*, and *Clostridium* species (Lipsky et al., 2012; Richard et al., 2012; Kalan et al., 2019). Bacterial biofilms of diabetic wounds and DFUs are protected from various stresses, including antibiotics and immune responses. Biofilm production involves the uncontrolled growth of sessile and planktonic bacteria that grow continuously on themselves to form a layer that is termed biofilm. Treatment of biofilms is also a major health concern as emphasized by the World Health Organization (WHO), as it contributes to the development of antimicrobial resistance toward antibiotics. Clinicians and researchers are

TABLE 2 | Different aspects of exosomes.

Feature	Exosome	Apoptotic body	MV
Size	Homologous 30–100 nm	Heterogeneous 1–5 μm	Heterogeneous 100–1000 nm
Markers	Membrane impermeable (PI negative) CD63, TSG101, Alix, flottilin	Membrane permeable (PI positive) Annexin V, DNA, histones	Membrane impermeable (PI negative) integrin, selectin, flotillin-2
Density	1.13–1.19 g/mL	1.16–1.28 g/mL	1.25–1.30 g/mL
Contents	Protein, lipid, different RNA species, and DNA	Cytosolic content (protein, RNAs, fragmented DNA) and cellular organelles	Protein, lipid, different RNA species, and DNA
Determinant of controlled contents	The cellular origin and physiological state of the cell	The cellular origin and stimuli	No direct correlation
Lipids	A major sorting of lipidic molecules from the parental cells (include BMP)	Characterized by phosphatidylserine externalization	The lipid contents are primarily derived from plasma membrane, and resemble the parental cells (without BMP)
Origin	Multivesicular bodies fusion with plasmatic membrane	Cellular debris, plasma membrane blebbing during cell apoptosis	Direct outward budding or blebbing from the plasma membrane
Mechanism of release	Constitutive or inducible, depending on the cell type of origin	Rho-associated kinase I and myosin ATPase activity	Relocation of phospholipids to the outer membrane, cytoskeleton rearrangements, generation of membrane curvature, and vesicle release
Detection methods	Electron microscopy, Western blot for exosome enriched markers	Flow cytometry, electron microscopy,	Flow cytometry, electron microscopy
Isolation methods	Ultracentrifugation (100,000–200,000 × *g*) filtration, density gradient Immunoprecipitation, Immune affinity capture and ExoQuick precipitation methods	Ultracentrifugation (10,000–20,000 × *g*)	No standardized methods
Modification methods	Incubation, Sonication, Extrusion, Freeze thaw, Electroporation, Chemical transfection, Genetic engineering		
Size determination and quantification	Dynamic light scattering Nanoparticle tracking analysis Surface plasmon resonance		

MV, microvesicle; BMP, bone morphogenetic protein; PI, propidium iodide.
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focusing on the promising alternative treatment approaches to the use of antibiotics in reducing bacterial infections. Natural sources such as plant-derived extracts, polyphenols, anti-sense RNA, and stem cell-derived exosomes might be the prospective alternative therapies to manage DFUs and diabetic wounds. Several emerging technologies identify the risk assessment associated with DFUs, including laser Doppler flowmetry, infrared thermography, ultrasound indentation tests (elastography), and plantar pressure and pressure gradient system (Lung et al., 2020). These technologies may be helpful in the screening of risk in DFUs, so that treatment approaches may be customized accordingly.

PATHOPHYSIOLOGY OF CONTROLLING BACTERIA-ASSOCIATED DFU USING MSC-DERIVED EXOSOMES

Extracellular vesicles are the key component of cell-to-cell communication that facilitates transfer of internalized cargo, including proteins, nucleic acids, and other biological factors. EVs are known to play an active role in pathological conditions like kidney injury, inflammatory disorders, wound healing, and regeneration, along with several therapeutic and diagnostic characteristics. A previously published study demonstrated that EVs possess antimicrobial peptides (AMPs) (Hiemstra et al., 2014). EVs were also reported to contain lysozyme C, dermcidin, mucin-1, calprotectin, and myeloperoxidase and to have a bactericidal effect. In one of the recent studies conducted *in vitro* on urinary exosomes, it was found that these exosomes showed a bactericidal effect against *E. coli* (Francisca et al., 2017). The same research concluded that nasal lavage fluid-derived exosomes showed defense against pathogens and allergens (Francisca et al., 2017).

In another study, it was found that EVs released from biliary and intestinal epithelium luminal contain AMPs along with LL-37 and hBD-2 that activate the toll-like receptor (TLR)-4 signaling cascade and contribute toward antimicrobial defense (Hu et al., 2013). In the past few years, MSC-derived EVs have been explored for therapeutic, diagnostic, and anti-inflammatory roles in several pre-clinical trials. In one of the published reports, it was found that MVs secreted by BMSCs are efficient in the treatment of acute lung injury (ALI) caused by *E. coli* endotoxins *via* transfer of keratinocyte growth factor (KGF) mRNA from the MVs to damaged lung endothelium and alveolar epithelium (Zhu et al., 2014). In another animal study conducted on a

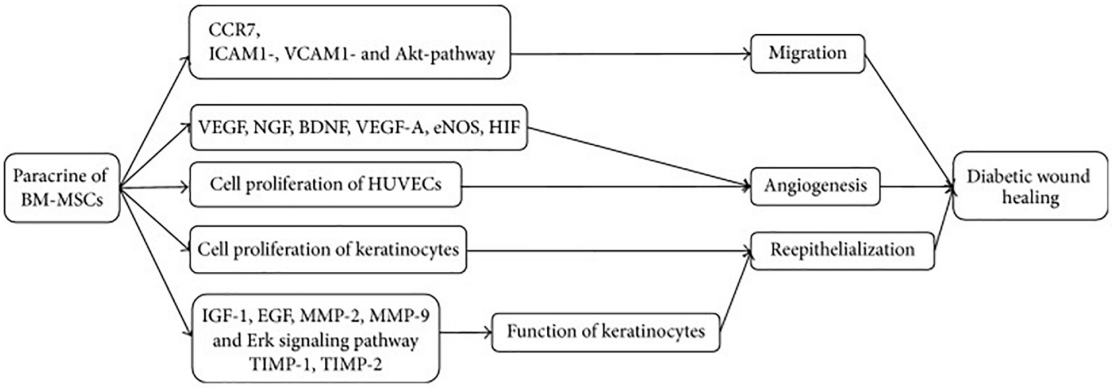

FIGURE 3 | Mechanism of BM-MSCs for treatment of DFU. BM-MSCs can migrate and adhere via CCR7, ICAM1-, VCAM1-, and Akt- dependent mechanism and enhance angiogenesis through increasing VEGF, NGF, BDNF, VEGF-A, eNOS, and HIF. Cell proliferation of HUVECs and keratinocytes plays significant role in angiogenesis and reepithelialization, respectively. Keratinocyte function is improved by regulating IGF-1, EGF, MMP-2, MMP-9, TIMP-1, TIMP-2, and Erk signaling pathway. CCR7, C-C chemokine receptor type 7; ICAM1, intercellular adhesion molecule 1; VCAM1, vascular adhesion molecule 1; VEGF, vascular endothelial growth factor; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; VEGF-A, vascular endothelial growth factor A; eNOS, endothelial nitric oxide synthase; HIF, hypoxia inducible factor; IGF-1, insulin-like growth factor 1; EGF, epidermal growth factor; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of metalloproteinase-1; and TIMP-2, tissue inhibitor of metalloproteinase-2. [Adopted from Cao et al. (2017) distributed under the Creative Commons Attribution Licens].

bacterial pneumonia mouse model, it was demonstrated that BMSC-extracted MVs showed significant survival and lessen the influx of inflammatory cells (Monsel et al., 2015). In another study, the antimicrobial effect of MSC-derived EVs was demonstrated, which is mediated by the transfer of mitochondria into the target cells that in turn increases the phagocytosis of macrophages (Islam et al., 2001). Several *in vivo* clinical trials demonstrated the antibacterial effect of MSC-derived EVs (Krasnodembskaya et al., 2010; Harman et al., 2017; Cortés-Araya et al., 2018). However, more studies and clinical trials are needed to establish the significant role of MSC-derived EVs as antimicrobial agent. This antimicrobial effect of EVs can be explored and serve as a prospective therapy for the treatment of diabetic wounds and DFUs.

SAFETY AND TOXICOLOGY CONSIDERATIONS OF EXOSOMES

Extracellular vesicles are known to be the safest therapeutic approach for both pre-clinical and clinical use. There were no signs of toxicity observed in previously published literature except that some human cell-derived EVs possess the potential to elicit an immune response, which is a positive sign for using EVs as cell-free therapeutic approach in DFUs (Zhu et al., 2017). In one study, C57BL/6 mice were given EVs for 3 weeks *via* intravenous and intraperitoneal administration, and no toxicity was observed with slight changes in expression of immune markers (Zhu et al., 2017). In another murine study, BMSC-derived engineered exosome (iExosomes) administration did not produce any toxicity and adverse immune reactions (Mendt et al., 2018). The engineered approaches for EVs mentioned in the present work suggest that EVs are a safe and non-toxic method for delivering cargo compared to cationic lipids, viral vectors, and polymer-based methods (Mendt et al., 2018). Moreover, long-term pre-clinical and clinical studies are needed to further evaluate the toxicological and immunological profile of engineered EVs (**Table 2**).

CONCLUSION

Extracellular vesicles are emerging as new therapeutics in the management of diseases, regeneration of tissue, and diagnostic markers. The heterogeneity and complexity with the ability of modification under a physiological and pathological environment make them interesting candidates for implication in the biological field. Exosomes have the potential to treat various diseases due to flexibility of loading diverse drugs and modifications. Exosomes can be used for detection, diagnosis, and treatment only because of their tendency of modification in the membrane. Moreover, MSC-derived exosomes are primarily exploited for regenerative medicine. Despite the fact that many advances in the modification approach of exosomes are currently being practiced; one of the most significant challenge with these vesicles is their inefficient production at a large scale for clinical use following GMP/GCP guidelines. MSC-derived exosomes are a rich source of AMPs along with other anti-bactericidal factors, which opens up the window of treating DFUs caused by microorganisms including *S. aureus*, *S. saprophyticus*, *S. epidermis*, *S. pyogenes*, *S. mutans*, *P. aeruginosa*, *B. subtilis*, *Proteus* species, *E. coli*, and *K. pneumoniae*. The potential bactericidal efficacy of the MSC-derived exosomes can be amplified through modification of cell conditioning medium and drug loading approach. AMP-encapsulated exosomes can be

exploited further for clinical trials to treat DFUs associated with microbes. Notable EV-based management therapies promote wound/ulcer healing along with minimal scarring without ethical issues and conflicts. Future studies including pre-clinical and clinical trials are required to explore the therapeutic and anti-microbial effect of the MSC-derived exosomes. These EVs can be exploited in designing wound dressings that might be prospectively used in the treatment of DFUs associated with bacteria.

AUTHOR'S NOTE

AR, PT, and KG are currently involved in COVID-19 testing duties.

AUTHOR CONTRIBUTIONS

AR, BM, SB, KG, QM, and AS are involved in manuscript writing, conceptualization, and data analysis. PT, G-BJ, PS, MS, and JA supervised and reviewed the manuscript. All the authors have read and agreed to the published version of the manuscript.

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Application of Exosomes-Derived Mesenchymal Stem Cells in Treatment of Fungal Diseases: From Basic to Clinical Sciences

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Fungal diseases such as candidiasis are some of the deadliest diseases among immunocompromised patients. These fungi naturally exist on human skin and throughout the digestive system. When the microbiota balance becomes upset, these fungi become pathogenic and potentially lethal. At the pathogenesis of fungal diseases, host immune system response is diverse. At the early stages of fungal pathogenesis such as *Candida albicans*, it was shown that these fungi use the immune cells of the host body and cause malfunction the early induction of proinflammatory cytokines of the host body leading to a reduction in their numbers. However, at some stages of fungal diseases, the immune response is severe. Despite many treatments already being available, it seems that one of the best treatments could be an immune-stimulatory agent. Some of the subsets of MSCs and exosome-derived cells, as a cell-to-cell communicator agent, have many roles in the human body, including anti-inflammatory and immune-modulatory effects. However, the TLR4-primed and IL-17+ subsets of MSCs have been shown to have immune-stimulatory effects. These subsets of the MSCs produce pro-inflammatory cytokines and reduce immunosuppressive cytokines and chemokines. Thus, they could trigger inflammation and stop fungal pathogenesis. As some biological activities and molecules inherit elements of their exosomes from their maternal cells, the exosome-derived TLR4-primed and IL-17+ subsets of MSCs could be a good candidate for fighting against fungal diseases. The applications of exosomes in human diseases are well-known and expanding. It is time to investigate the exosomes application in fungal diseases. In this review, the probable role of exosomes in treating fungal diseases is explored.

Keywords: fungi, exosome, mesenchymal stem cell, interleukin-17, toll-like receptor 4

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INTRODUCTION

Host-Fungi Interactions: Normal Flora or Pathogen?

There are fungi in the human body that are known as normal flora (Prasad, 2007). This population of fungi is called fungal microbiota or mycobiota (Limon et al., 2017). Knowing these microbiotas, including mycobiota, is an important factor in host diseases and health (Limon et al., 2017). For many reasons, when the balance of these mycobiota is upset they can become a pathogen. Fungal diseases effect a quarter of the human population worldwide (Brown et al., 2012). However, while

most of the fungal diseases are related to superficial skin conditions and can be treated locally, the systemic fungal infection could be so lethal (Brown et al., 2012; Vallabhaneni et al., 2016). These systemic fungal diseases usually occur because of diverse immune responses; especially in patients with immune system suppression (Pappas et al., 2018). There are lots of treatment option for systemic fungal diseases, but using them has limitations and usually brings poor outcomes (Scriven et al., 2017). It seems that one of the best choices to treat fungal diseases is reversing immune deficiency, which occurs in patients with immunosuppression (Scriven et al., 2017).

Pathogenesis of Fungi and Host Immunity

A previous study on *C. albicans* revealed that the host immune response to *C. albicans* is downregulated at early stages by pathogenic fungi (Halder et al., 2020). It was shown that the *C. albicans* attached to the C3 receptor of the monocytes by its β-glucan. Using this attachment to the monocytes, the fungi stimulate the monocytes to release extracellular vesicles contained transforming growth factor (TGF)-β. Using TGF-β-transporting vesicles, the fungi reduce immune response and cause anti-inflammatory effects at the early stages of fungi pathogenesis (Halder et al., 2020). Moreover, using TGF-β production, the fungi could reduce early production and induction of pro-inflammatory cytokines (Netea et al., 2002; Halder et al., 2020). This is how the fungi downregulate the host immune system in order to favor its existence and survival.

Mesenchymal Stem/Stromal Cells (MSCs), Immunosuppressive or Immune-Stimulator?

The MSCs are the progenitor/stem cells that have the capacity to differentiate into multilineage cells (Billing et al., 2016; de Castro et al., 2019). Due to their potential for differentiation, their immunomodulatory effect, and their regeneration capacity (Zhang et al., 2020a; Oh et al., 2021), they are widely used in treating injuries and some inflammatory disorders (Zhang et al., 2020a; Liao et al., 2021). Clinical studies have shown that because of the immunomodulatory function of some subsets of MSCs, MSC therapy could suppress the immune system and treat inflammatory and autoimmune diseases (Nauta and Fibbe, 2007; Yang et al., 2013). In detail, the MSCs, directly or indirectly, affect T cells and regulate them. The MSCs produce some chemokines and cytokines such as interleukin 10 (IL-10), prostaglandin E_2 (PGE_2), nitric oxide (NO), TGF-β, indoleamine 2,3-dioxygenase (IDO), tumor necrosis factor-inducible gene 6 (TSG-6), and chemokine ligand 2 (Batten et al., 2006; Nauta and Fibbe, 2007; Yang et al., 2013). These molecules affect $CD4^+CD25^+$ regulatory T (T reg) with positive transcription factor Foxp3 and T helper 17 (Th17) cells' population and regulate them (Batten et al., 2006; Park et al., 2011; Yang et al., 2013; Bi et al., 2020). That's how MSCs downregulate the immune system in inflammatory and autoimmune diseases.

However, some previous studies have shown that another type of MSCs has an immune-stimulatory effect, and this

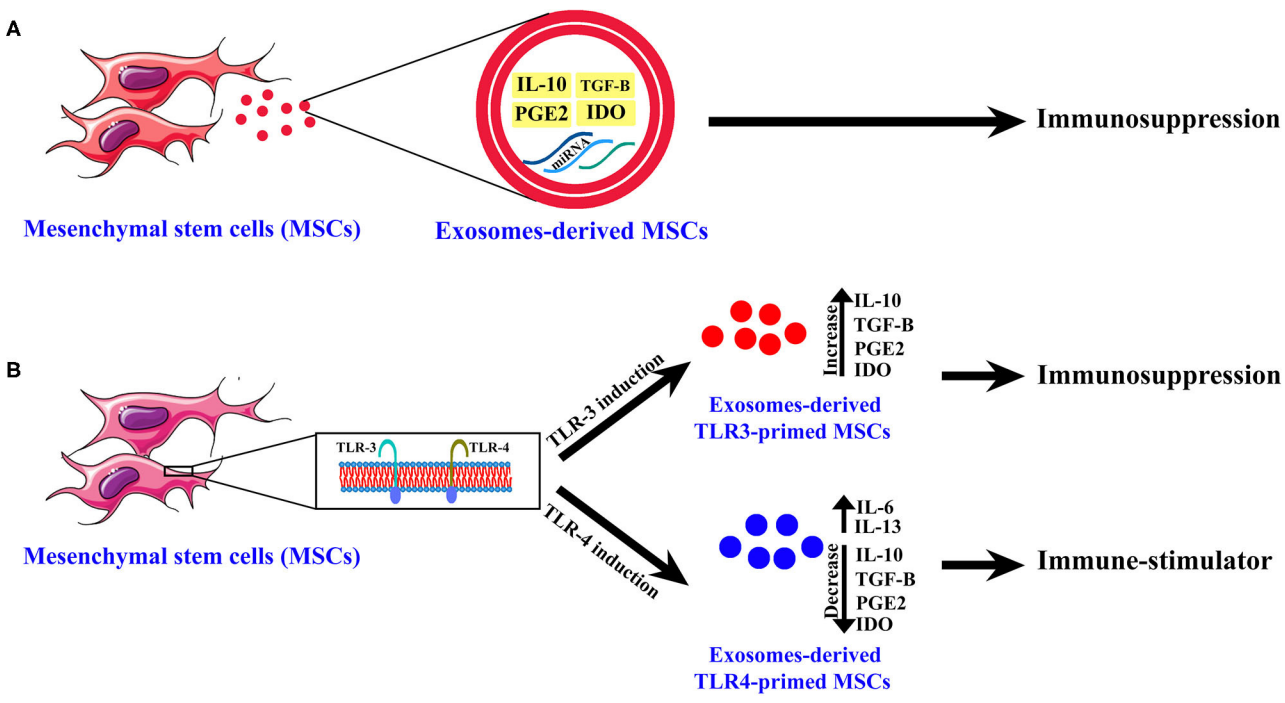

FIGURE 1 | The exosome-derived mesenchymal stem cells (MSCs) cytokines and chemokines content. **(A)** Normal MSCs. **(B)** TLR3-primed and TLR4-primed subtypes of MSCs. This figure shows chemokines and cytokines of exosomes-derived MSCs of different subtypes of MSCs and their biological activity.

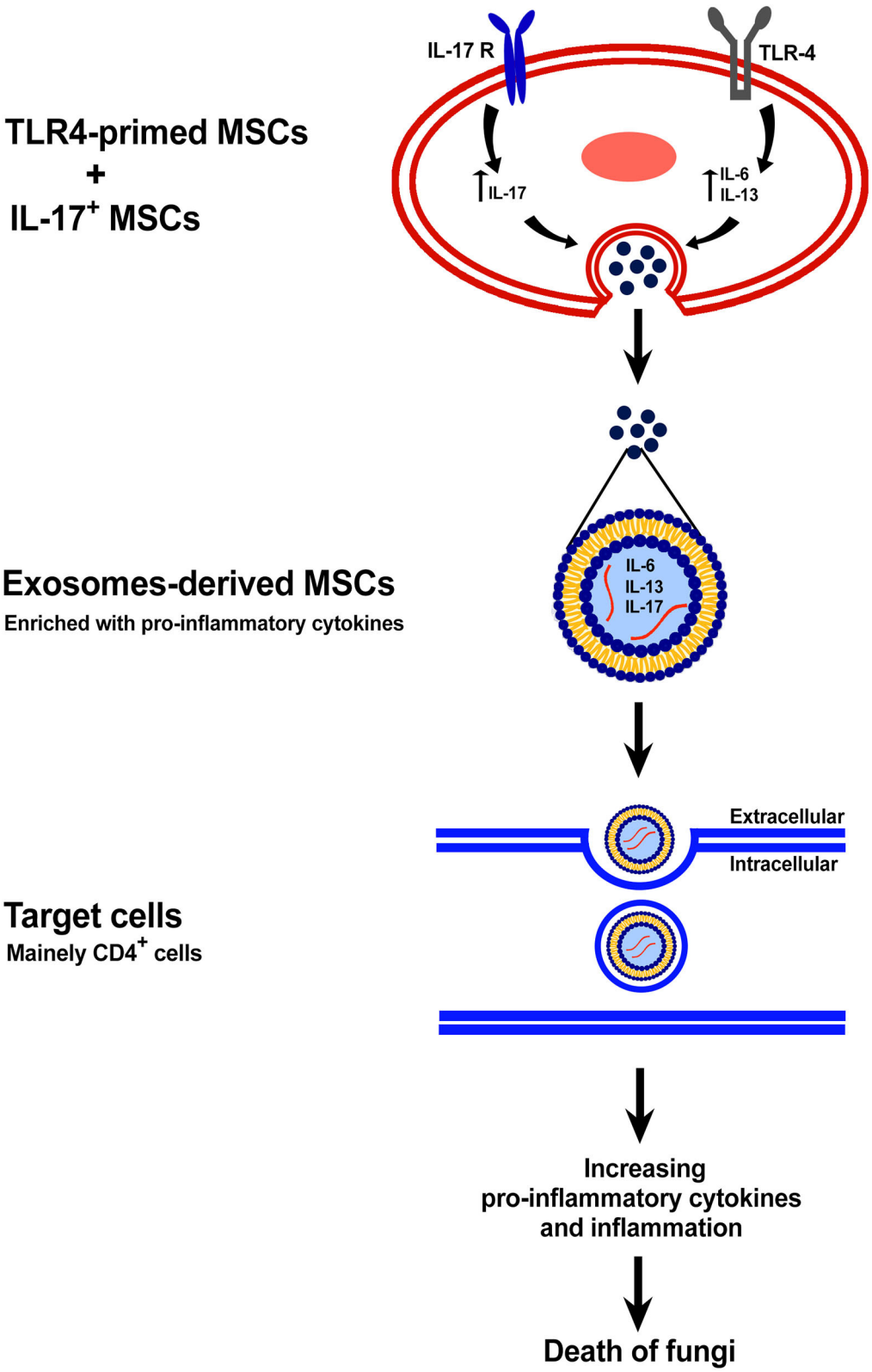

FIGURE 2 | The exosome-derived TLR4-primed and IL-17^{+} MSCs. This figure shows the mechanism of anti-fungal effects of exosomes-derived new subtypes of MSCs.

TABLE 1 | A list of companies producing various kinds of exosome-related products for therapeutic approaches.

Product application(s)	Company	Web site
Cancer detection	Exosomics	exosomics.eu
Cancer detection	Lonza	lonza.com
Carriers	Anjarium Biosciences	anjarium.com
Carriers	Codiak Biosciences	codiakbio.com
Carriers	Ilias Biologics Inc.	iliasbio.com
Carriers	MDimune	mdimune.com
Carriers	Tavec	tavecpharma.com
Exosome detection	NanoView Biosciences	nanoviewbio.com
Exosome isolation	Clara Biotech	clarabio.tech
Exosome isolation	EverZom	
Immunotherapy enhancer	EV Therapeutics	evtherapeutics.com
Inflammation therapy	The Cell Factory	esperite.com
Regenerative medicine	Aegle Therapeutics	aegletherapeutics.com
Regenerative medicine	Aruna Bio	arunabio.com
Regenerative medicine	Capricor Therapeutics	capricor.com
Regenerative medicine Vaccine	Ciloa	ciloa.fr
Regenerative medicine	Creative Medical Technologies Holdings	creativemedicaltechnology.com
Regenerative medicine	Direct Biologics	
Regenerative medicine	Evox Therapeutics	evoxtherapeutics.com
Regenerative medicine	Exocel Bio	exocelbio.com
Regenerative medicine	ExoCoBio	exocobio.com
Regenerative medicine	Exopharm	exopharm.com
Regenerative medicine	Exosome	exosomesciences.com
Regenerative medicine	Exogenus Therapeutics	exogenus-t.com
Regenerative medicine	Invitrx's	www.invitrx.com
Regenerative medicine	Kimera Labs	kimeralabs.com
Regenerative medicine	Oasis Diagnostics	4saliva.com
Regenerative medicine	OmniSpirant	omnispirant.com
Regenerative medicine	Organicell	organicell.com
Regenerative medicine	Percia Vista	perciavista.co
Regenerative medicine	Regen Suppliers	regensuppliers.com
Regenerative medicine	ReNeuron	reneuron.com
Regenerative medicine	RoosterBio	roosterbio.com
Regenerative medicine	Stem Cell Medicine Ltd.	stemcell-medicine.com
Regenerative medicine	Unicyte	unicyte.ch
Regenerative medicine	VivaZome Therapeutics	vivazome.com
Regenerative medicine	XOStem	xostem.com
Tumor exosome capture	Aethlon Medical	aethlonmedical.com

variety of the biological functions of MSCs depends on Toll-like receptors (TLRs) (**Figure 1**) (Waterman et al., 2010; Yang et al., 2013). It was shown that engagement of TLR-4 could enhance the production of pro-inflammatory mediators such as IL-17 and these MSCs are called TLR4-primed MSCs (**Figure 1**) (Waterman et al., 2010; Yang et al., 2013). In contrast, it was shown that TLR3-primed MSCs act as an immunomodulatory subset of MSCs (Waterman et al., 2010; Yang et al., 2013). The TLR4-primed MSCs, in contrast with TLR3-primed MSCs, was shown to increase expression of IL-6 and IL-13 as a pro-inflammatory cytokine and decrease IL-4, IDO, and PGE_2 as an immunomodulatory cytokine and chemokine (**Figure 1**) (Waterman et al., 2010; Yang et al., 2013). IL-17 is a pro-inflammatory cytokine that plays a crucial role in intracellular and extracellular pathogenic defense (Yang et al., 2013; Schinocca et al., 2021). It was shown that a subpopulation of IL-17^+ MSCs could inhibit *C. albicans* (Yang et al., 2013). Taken together, it might result that TLR4-primed and IL-17^+ subsets of MSCs

TABLE 2 | Animal studies of exosomes-derived MSCs.

Cell source	Therapeutics	Transplantation	Donor species	Recipient species	Biological effects	References
Embryonic MSCs	Exosome	Xenotransplant	Human	Rat	Osteochondral regeneration promotion	Zhang et al., 2016
Adipose tissue-derived MSCs	Exosome	Xenotransplant	Human	Mouse	Atopic dermatitis alleviation	Cho et al., 2018
Adipose tissue-derived MSCs	Exosome	Xenotransplant	Human	Rat	Evaluation of exosomes cell toxicity	Ha et al., 2020
Bone marrow- derived MSCs	Exosome	Xenotransplant	Rat	Mouse	Neuroprotective effect *via* inhibiting early neuroinflammation	Ni et al., 2019
Wharton's jelly-derived MSCs	Exosome	Xenotransplant	Human	Rat	Anti-inflammatory effects on microglia in perinatal brain injury	Thomi et al., 2019
Umbilical cord-derived MSCs	Exosome	Xenotransplant	Human	Mouse	Acute liver failure alleviation	Jiang et al., 2019
Bone marrow- derived MSCs	Exosome	Xenotransplant	Rat	Mouse	Inadequate promotion of bone regeneration in type 1 diabetes	Zhu et al., 2019
Bone marrow- derived MSCs	Exosome	Allotransplant	Rabbit	Rabbit	Regulation of injured endometrium repair	Yao et al., 2019
Umbilical cord-derived MSCs	Exosome	Xenotransplant	Human	Mouse	Inflammatory bowel disease treatment	Mao et al., 2017
Adipose tissue-derived MSCs	Exosome	Allotransplant	Rat	Rat	Promotion of endometrium regeneration in rats with intrauterine adhesion	Zhao et al., 2020
Placental- derived MSCs	Exosome	Xenotransplant	Human	Mouse	Enhancement of angiogenesis and improvement of neurologic function	Zhang et al., 2020b
Umbilical cord-derived MSCs	Exosome	Xenotransplant	Human	Mouse	Inhibition of silica-induced PF and improve lung function	Xu et al., 2020a
Bone marrow- derived MSCs Adipose tissue-derived MSCs	Exosome	–	–	Rat	Improvement of erectile dysfunction in bilateral cavernous nerve injury	Li et al., 2018
Bone marrow- derived MSCs	Exosome	Allotransplant	Rat	Rat	Rescuing myocardial ischaemia/reperfusion injury	Liu et al., 2017
Umbilical cord-derived MSCs	Exosome	Xenotransplant	Human	Rat	Inhibition of vein graft neointimal hyperplasia and acceleration of reendothelialization	Qu et al., 2020
Adipose tissue-derived MSCs	Exosome	Allotransplant	Mouse	Mouse	Exo-circAkap7, a potential treatment for cerebral ischemic injury.	Xu et al., 2020b
Bone marrow- derived MSCs	Exosome	Xenotransplant	Rat	Guinea pig	Reduction of demyelination and neuroinflammation in an immune-induced demyelination model	Li et al., 2019
Bone marrow- derived MSCs	Exosome	Allotransplant	Rat	Rat	Promotion of immunotolerance and prolong the survival of cardiac allografts	He et al., 2018

MSCs, mesenchymal stem cells.

could be good candidates for fighting against fungal diseases (**Figures 1**, **2**) (Waterman et al., 2010; Yang et al., 2013).

The Extracellular Vesicles (EVs) and Its Classification

EVs have the main role in cell-to-cell communications (Andaloussi et al., 2013),and have been observed in both eukaryotes and prokaryotes (Ellis and Kuehn, 2010; Andaloussi et al., 2013). Studies have shown that the EVs could transfer the proteins and nucleic acids by its bilayer membrane (Lee et al., 2012; Ratajczak et al., 2012). Due to their potential for transferring proteins and nucleic acids, EVs are used widely as drug delivery agents (Elsharkasy et al., 2020). In order to best discuss the biological roles of EVs, here we describe the classification of EVs. The EVs based on their cellular origin, biological function, biogenesis, and size classified into three main groups: exosomes, microvesicles, and apoptotic bodies (Andaloussi et al., 2013; Yáñez et al., 2015). The two first particles, the exosomes and microvesicles, have been shown to have therapeutic effects (Wang et al., 2015; Phinney and Pittenger, 2017). The exosomes, with 40–120 nm in size, are generated by the endolysosomal pathway. In contrast with exosomes, the microvesicles are generated by budding from the cell surface (Andaloussi et al., 2013; Raposo and Stoorvogel, 2013). The exosomes with their non-sized particles, composed of a bilayer membrane and cytoplasm, contained mRNA, miRNA, and other RNAs' generated from the parent cell (Andaloussi et al., 2013; Raposo and Stoorvogel, 2013).

The Exosomes-Derived MSCs and Their Biological Activity

Stem cells, especially mesenchymal stem cells, were used widely in past decades as a candidate for therapies of various diseases. In recent years, exosome-derived stem cells were substitutionally used for regenerative and immune-therapy as a cell-free therapy (Ji et al., 2019; Qiu et al., 2020). Previous studies have shown that the exosome-derived stem cells contained various bioactive molecules, especially proteins and microRNAs which originated

from maternal cells (Baharlooi et al., 2020; Ma et al., 2020). These exosomes were shown to have some biological effects inherited from their maternal cells (Baharlooi et al., 2020). For instance, the exosome-derived MSCs displayed angiogenesis, regeneration, and especially anti-inflammatory effects (Baharlooi et al., 2020). Moreover, it was shown that these exosomes could carry various cytokines and chemokines originated and produced by the maternal cell (Di Trapani et al., 2016; Baharlooi et al., 2020). So, here we can hypothesize that the TLR4-primed MSCs could pass their pro-inflammatory cytokines and chemokines into exosomes derived from them. Exosomes-derived TLR4-primed MSCs could trigger the host immune system to start inflammation against fungal pathogens and fight against the immunosuppressive path of fungi.

DISCUSSION

The MSCs have been used in the treatment of microbial diseases for the past decades (Zhou and Xu, 2020). In most microbial diseases, the host-microbe interactions cause inflammation, which damaged host tissues (Qiu et al., 2020). Some of the subsets of MSCs, using the production of anti-inflammatory and immunomodulatory cytokines and chemokines, serve to downregulate the host immune system and reduce host tissue damages (Waterman et al., 2010; Baharlooi et al., 2020). That is why the MSCs were widely used in past decades for inflammatory and autoimmune diseases treatment. Among all microbial diseases, the pathogenesis of fungal diseases is more complicated. The fungi pathogen at the first stages of pathogenesis downregulates the immune system of the host body using TGF-β-transporting vesicles produced by induced monocytes (Netea et al., 2002; Halder et al., 2020). Using immunosuppression, the pathogen could survive better.

In recent years, it was noticed that the different subtypes of MSCs could show different biological activities (Waterman et al., 2010; Yang et al., 2013; Baharlooi et al., 2020). It was shown that induction of TLR-4 of MSCs could enhance its immune-stimulatory activity using the production of pro-inflammatory cytokines and chemokines (Waterman et al., 2010; Yang et al., 2013). As is obvious, in contrast with other microbial pathogenesis (Nauta and Fibbe, 2007) the fungal pathogen stops inflammation and downregulates the host immune system; so to fight that, the immune system needs to be upregulated and made able to inflame (Waterman et al., 2010; Yang et al., 2013). It was shown that the TLR4-primed and IL-17^{+} subsets of MSCs could express pro-inflammatory cytokines and chemokines, which could lead to inflammation (Waterman et al., 2010; Yang et al., 2013). These subtypes of MSCs could be an agent for fungal diseases treatment.

As is known, cell therapy has some challenges for human diseases therapy (Choi and Lee, 2016). The exosomes, as a cell-free therapy, solve most of the problems of cell therapy (Choi and Lee, 2016). Unlike a cell therapy, the exosomes are capable of crossing the blood-brain barrier and traveling through capillaries, and owing to their small sizes they are safe from reticuloendothelial system clearing (Li and Huang, 2009; Choi and Lee, 2016; Baharlooi et al., 2020). Moreover, as the exosomes inherited some of the molecules and biological activity of their maternal cells, they could be a good substitute for cell therapy (Di Trapani et al., 2016; Baharlooi et al., 2020; Ma et al., 2020). The exosome-derived MSCs showed to have anti-inflammatory and regenerative effects, the same as their maternal cells (Baharlooi et al., 2020). Several companies are developing exosome-derived products to take advantage of these applications, which suggests that in the future exosomes and their derived applications will be a viable choice for various disease therapies (**Table 1**).

As the maternal cell produces anti-inflammatory cytokines and chemokines, these molecules could pass into the exosomes (Wang et al., 2015; Baharlooi et al., 2020). Based on previous results, it could be hypothesized that the TLR4-primed and IL-17^{+} subsets of MSCs could pass its produced pro-inflammatory cytokines and its immune-stimulatory activity into its exosomes. These exosomes could be a treatment for fungal pathogenesis.

During the past decade, many preclinical studies of exosomes have been conducted. Some of these studies have been shown in **Table 2**. These studies demonstrated that exosomes-derived MSCs could have anti-inflammatory, anti-atopic dermatitis, anti-neurodegenerative, anti-liver fibrosis biological activities, and so on (Li et al., 2013; Cho et al., 2018; Lee et al., 2018; Gowen et al., 2020). Despite many preclinical studies of exosomes, clinical studies of the MSCs-derived exosomes are few (Gowen et al., 2020). The MSCs-derived exosomes were used in previous clinical studies to treat diseases such as graft-versus-host disease (Kordelas et al., 2014), chronic kidney disease with grade III and IV (Nassar et al., 2016), type II diabetes (Sun et al., 2018), and prevention of the onset of type-1 diabetes *via* suppression of immune system and induction of beta cells regeneration (Ezquer et al., 2012). There are also several studies which have not yet been published.

However, stem cell-derived exosomes have some limitations for clinical studies. For instance, large-scale exosome production is lacking; large-scale exosome quantifications methods with rapid and accurate results, and determination of exosomes' contents with high accuracy also present dificulties (Gowen et al., 2020). Moreover, the pharmacokinetics, pathways, targets and mechanisms of action of the exosomes in the human body still remain unknown. Additionally, more studies are needed to evaluate the correct dosage of the exosomes for clinical studies in order to prevent possible toxicities (Gowen et al., 2020).

AUTHOR CONTRIBUTIONS

SOG: data collection, manuscript writing, idea conception, study design, and approved the final version.

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Mechanism and Potential of Extracellular Vesicles Derived From Mesenchymal Stem Cells for the Treatment of Infectious Diseases

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Extracellular vesicles (EVs) are nano-sized membrane vesicles secreted by cells. EVs serve as a mediator for cell-to-cell communication by regulating the exchange of genetic materials and proteins between the donor and surrounding cells. Current studies have explored the therapeutic value of mesenchymal stem cells-derived EVs (MSC-EVs) for the treatment of infectious diseases extensively. MSC-EVs can eliminate the pathogen, regulate immunity, and repair tissue injury in contagious diseases through the secretion of antimicrobial factors, inhibiting the replication of pathogens and activating the phagocytic function of macrophages. MSC-EVs can also repair tissue damage associated with the infection by upregulating the levels of anti-inflammatory factors, downregulating the pro-inflammatory factors, and participating in the regulation of cellular biological behaviors. The purpose of this mini-review is to discuss in detail the various mechanisms of MSC-EV treatment for infectious diseases including respiratory infections, sepsis, and intestinal infections, as well as challenges for implementing MSC-EVs from bench to bedside.

Keywords: mesenchymal stem cells, exosome, extracellular vesicles, acute lung injury, COVID-19, sepsis, infectious diseases

INTRODUCTION

Infectious diseases have been a significant cause of morbidity and mortality worldwide; respiratory infections and pneumonia are among the major causes of global death (Sharma et al., 2021b). With the increasing number of outbreaks of new infectious diseases and the lack of effective treatments, it is crucial to identify new therapeutic strategies to combat infections and restore infection-related organ and tissue damage.

Mesenchymal stem cells (MSCs) are among the most commonly employed cell types in tissue repair and homeostasis, which have become an attractive therapeutic option for treating infectious diseases and disease-related tissue injury (Kashte et al., 2018; Kotas and Matthay, 2018). The effects of MSCs include anti-inflammatory properties, immunomodulatory capabilities, and regeneration

(Fu et al., 2019). The efficacy of MSCs is mainly coming from the paracrine effect mediated by secreted growth factors, cytokines, and extracellular vesicles (EVs) (Liang et al., 2014; Paliwal et al., 2018).

MSC-derived extracellular vesicles (MSC-EVs) are identified to be the main components responsible for the paracrine effect. They transfer functional molecules, such as messenger RNA (mRNA), microRNA (miRNA), lipid, and protein, into tissue-specific cells that request repair (Taverna et al., 2017). Compared with MSCs, MSC-EVs possess hypoimmunogenic properties, have low tumorigenesis, and are more stable (Trounson and McDonald, 2015). In this mini-review, we briefly summarize the function of exosomes and discuss their potential role in therapeutic regimens in infectious diseases, including respiratory infections, sepsis, and intestinal infections in recent years.

EXTRACELLULAR VESICLES FROM MESENCHYMAL STEM CELLS

Almost all cells, including MSCs, can secrete EVs due to intracellular vesicle sorting (Kourembanas, 2015). EVs are nano-sized spherical bio-membrane structures, which were previously divided into three main categories based on their size and biosynthesis: smaller-sized exosomes (30–100 nm) from the endocytic pathway, medium-sized microvesicles (MVs) (100–1,000 nm) from the cell plasma membrane shedding, and larger-sized apoptotic bodies (1,000–5,000 nm) from the apoptosis (Raposo and Stoorvogel, 2013). The endocytosis of the cell membrane may form early endosomes, which then develop into late endosomes, namely, multivesicular bodies (MVBs). MVBs either combine with lysosomes or be released as exosomes through exocytosis (Joo et al., 2020). In terms of MVs, they can be secreted directly by budding from the plasma membrane (Abbaszadeh et al., 2020) (**Figure 1**).

Assigning an EV to a particular biogenesis pathway remains extraordinarily difficult because of the absence of specific surface markers for three EV categories and the overlap in their physical size (Carnino et al., 2021). Therefore, guidelines set by the International Society for Extracellular Vesicles (ISEV) suggest considering the use of operational terms for EV subtypes that are based on: (a) physical characteristics of EVs, such as size ["small EVs" (< 200 nm) and "medium/large EVs" (> 200 nm)] or density (low, middle, high, with each range defined); (b) biochemical composition ($CD63^+$/$CD81^+$-EVs, Annexin A5-stained EVs, etc.); or (c) descriptions of conditions or cell of origin (podocyte EVs, hypoxic EVs, large oncosomes, apoptotic bodies) (Théry et al., 2018).

Over 80% of researchers chose differential ultracentrifugation for EVs isolation (Tkach and Théry, 2016). Traditional identification ways for EVs usually involve nanoparticle tracking analysis (NTA) for size information, transmission electron microscope (TEM) for morphological details, and Western blotting for membrane protein makers (Théry et al., 2018). Kim et al. (2019) recently developed an atomic force microscope-infrared spectroscopy (AFM-IR) approach to probe the structural composition of a single EV. Their protocol involves incubating the EV sample on a suitable substrate and setting up the AFM-IR instrument, as well as collecting nano-IR spectra and nano-IR images. Recorded IR spectra for EVs showed characteristic peaks at specific wavenumbers; it is possible to determine the presence of DNA (1,050–1,290 cm^{-1}), RNA (1,250–1,380 cm^{-1}), proteins (1,500–1,700 cm^{-1}), and phospholipids (1,000–1,250 cm^{-1}, 1,730–1,750 cm^{-1}, 2,800–3,000 cm^{-1}) (Kim et al., 2019) that may contribute to the understanding of EV biology and the development of EV therapies. This method could improve the understanding of EV biology and the development of EV therapies.

EVs secreted from MSCs can deliver many functional molecules such as mRNA, miRNA, lipids, and protein into recipient cells (Yin et al., 2019). These biological components are considered stable and can modulate cell behaviors in recipient cells. EVs use specific receptors or membrane fusion to enter recipient cells. Once EVs are absorbed, the biomolecules of EVs can regulate gene expression, essential enzyme reactions, signal cascade pathways, or other mechanisms in recipient cells (Ranghino et al., 2017). Thus, MSC-EVs can promote tissue regeneration by reprogramming several pathophysiological pathways such as immunomodulation, proliferation, apoptosis, angiogenesis, and oxidative (Grange et al., 2019a,b).

THE THERAPEUTIC APPLICATION OF MESENCHYMAL STEM CELL-EXTRACELLULAR VESICLES IN INFECTIOUS DISEASES

The function of EVs is mainly dependent on their source cells (Keshtkar et al., 2018). The therapeutic use of MSCs was reported in lung injury, sepsis, and necrotizing enterocolitis (NEC) caused by bacteria or viruses (Krasnodembskaya et al., 2010; Sung et al., 2016; Rodrigues et al., 2019). MSC-EVs have similar functions to their parental cells, such as antimicrobial effects, immunomodulation property, and damage tissue repairability. Compared with MSCs, MSC-EVs keep the biological function of MSCs and are more stable and less easy to tumorigenesis, making them a promising candidate for the treatment of infectious diseases (Thirabanjasak et al., 2010).

For Respiratory Infection

Acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) is a heterogeneous syndrome characterized by diffuse epithelial and endothelial damage and a robust inflammatory response (Thompson et al., 2017). The most common risk factors of ARDS are infectious pneumonia caused by bacteria and viruses (Muraca et al., 2020; Meyer et al., 2021). Respiratory infections take more than 1.5 million lives a year. The number of deaths and disabled people is devastating in epidemic and pandemic outbreaks, such as the severe acute respiratory syndrome (SARS) outbreak in 2002, H1N1 flu in 2009, Middle East respiratory syndrome (MERS) outbreak in 2012, and coronavirus disease 2019 (COVID-19) outbreak in 2020 (Sharma et al., 2021b).

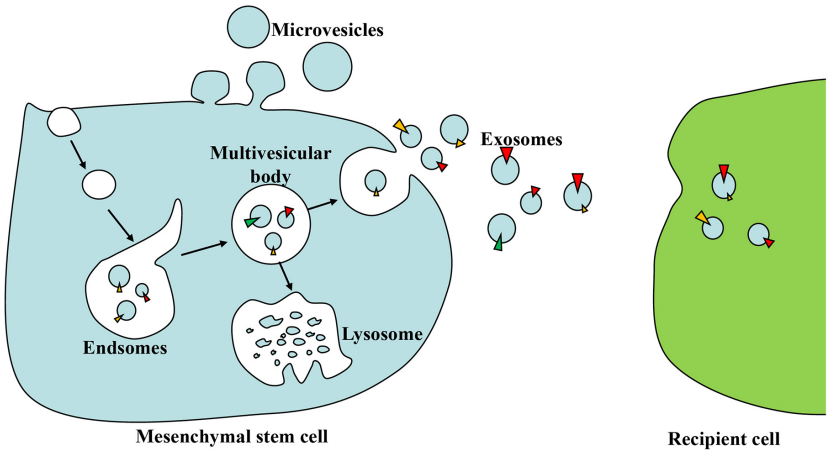

FIGURE 1 | The biogenesis and action of exosomes. Early endosomes are formed by the endocytosis of the cell membrane and then develop into multivesicular bodies (MVBs) in a budding manner. MVBs either combine with lysosomes and digest their contents or be released as exosomes through exocytosis. Exosomes can deliver lipids, proteins, and nucleic acid to recipient cells when circulating in the extracellular space.

Cell-based therapy with MSCs has been promising in ALI/ARDS in pre-clinical models for their immunomodulation and tissue repair properties (Laffey and Matthay, 2017). However, there were higher mean scores of Acute Physiology and Chronic Health Evaluation III (APACHE III) in models treated with MSCs than in those treated with placebo, but without difference of their 28-day mortality (Matthay et al., 2019). Since MSCs have limited engraftment and differentiation efficacy, high risk of tumorigenicity, and unstable ability (Eggenhofer et al., 2014), researchers paid more attention to MSC-EVs as a new candidate cell-free treatment for ALI/ARDS. Both other researchers and we demonstrated that intratracheal administration of MSC-EVs showed therapeutic effects in hyperoxia-induced lung injury, revealing that MSC-EVs could ameliorate impaired alveolarization in both short-term and long-term bronchopulmonary dysplasia (BPD) models and activate M2 macrophages (Porzionato et al., 2019, 2021; You et al., 2020). The anti-inflammatory and pro-regenerative properties of MSC-EVs are well established and have been exploited in a large number of studies (Phinney and Pittenger, 2017).

The application of MSC-EVs on ALI/ARDS and severe pneumonia has been investigated in some pre-clinical studies. MSC-EVs' main effects on ALI/ARDS are reducing inflammation, promoting alveolar epithelial regeneration, and enhancing pulmonary endothelial repair (Shah et al., 2019). As a result, pro-inflammatory cytokine production was decreased, and alveolar fluid clearance was improved in ALI/ARDS models.

Two clinical trials are undergoing to determine the effects of MSC-EVs on COVID-19, a pandemic that lacks specific antiviral medicine. MSC-EVs will be administrated intravenously (NCT04798716) or by inhalation (NCT04276987). A prospective non-randomized open-label cohort study showed that allogeneic bone marrow MSC-derived exosomes (ExoFlo^{TM}) could be safe and effective in severe COVID-19 patients, which could restore oxygenation, downregulate cytokine storm, and reconstitute immunity (Sengupta et al., 2020). However, it is premature to draw any conclusion based on a single study, and it should be emphasized that there are no approved MSC-EV therapies for COVID-19 to date. The specific and scientific rationale for administering MSC-EV treatment in COVID-19 patients needs to be better understood and justified (Börger et al., 2020). In the meantime, the prevention and control of urgent COVID-19 should make efforts to test existing approved vaccines, antiviral therapeutics, and monoclonal antibodies (Sharma et al., 2021a).

miRNA, protein, mRNA, and mitochondria in MSC-EVs play vital roles in modulating immune responses and repairing lung damage of ALI/ARDS. miR-21-5p plays an essential role in alleviating ALI by reducing pro-inflammatory cytokine secretion and enhancing M2 polarization (Li et al., 2019). MSC-EVs are reported to ameliorate ALI *via* transferring miR-27a-3p to alveolar macrophages inhibiting NF-κB expression and inducing M2 polarization (Wang et al., 2020). MiR-145 mediated the antimicrobial effect of MSC-EV by suppressing the expression of multidrug resistance-associated protein 1 (MRP1) and increasing the levels of leukotriene B_4 (LTB_4) (Hao et al., 2019), a chemoattractant for immune cells including T cells, macrophages, and neutrophils, with the role of facilitating pathogen elimination (Saeki and Yokomizo, 2017).

EVs from interferon (IFN)-γ-primed MSCs more effectively attenuated *Escherichia coli*-induced lung injury *via* enhancing phagocytosis and killing of bacteria in macrophage (Varkouhi et al., 2019). MSC-EVs decreased the lipopolysaccharide (LPS)-induced permeability of microvascular endothelial cells partly through the presence of hepatocyte growth factor (HGF) (Wang et al., 2017). The expression of keratinocyte growth factor (KGF) (Zhu et al., 2014) and angiopoietin-1 (Ang1) (Tang et al., 2017) mRNA enclosed in EVs partly mediated the anti-inflammatory effects on *E. coli* endotoxin-induced ALI in mice models. The effectiveness of MSC-EVs has also been demonstrated in large animals and found that EVs from swine bone marrow-derived MSCs had anti-influenza and anti-inflammatory effects in influenza virus-induced pig ALI (Khatri et al., 2018).

TABLE 1 | The related exosomal cargo and mechanisms of mesenchymal stem cell-derived extracellular vesicles treatment in infectious diseases.

Related exosomal cargo	Disease model	Exosome source	MSC-EV isolation	Experimental outcome and related mechanism
miR-27a-3p (Wang et al., 2020)	LPS-induced ALI in mouse	hADMSCs	UC	Elevated miR-27-3a levels in alveolar macrophages, induced M2 polarization, and decreased alveolar macrophage expression of NF-κB
miR-145 (Hao et al., 2019)	*E. coli*-induced ALI in mouse	hBMSCs	UC	Suppressed MRP1 activity through transfer of miR-145, thereby resulting in enhanced LTB_4 production and antimicrobial activity through LTB_4/BLT1 signaling
Unknown (Varkouhi et al., 2019)	*E. coli*-induced ALI in rat	IFN-γ-primed hUCMSCs	UC	Enhanced macrophage phagocytosis and killing of *E. coli*
HGF (Wang et al., 2017)	*In vitro* LPS treatment of endothelial cells	mBMSCs	UC	Increased the expression of VE-cadherin and occluding, decreased endothelial apoptosis, induced endothelial cell proliferation
KGF (Zhu et al., 2014), Ang-1 (Tang et al., 2017)	*E. coli*/LPS-induced ALI in mouse	hBMSCs	UC	Demonstrated a reduction in pulmonary edema, lung protein permeability, and inflammation
RNAs (Khatri et al., 2018)	Influenza virus-induced ALI in pig	sBMSCs	UC	Reduced virus shedding in the nasal swabs, influenza virus replication, and pro-inflammatory cytokines in the lungs
miR-146a (Song et al., 2017), miR-21 (Yao et al., 2021)	CLP-induced sepsis in mouse	IL-1β primed hUCMSCs	UC	Exosomal miR-146a/miR-21 was transferred to macrophages, resulted in M2 polarization by modulating IRAK1, TRAF6, and IRF 5 signaling, or inhibited the effects of PDCD4.
miR-223 (Wang et al., 2015)	CLP-induced sepsis in mouse	mBMSCs	UC	Exosomal miR-223 was transferred to cardiomyocytes, inhibited the expression of Sema3A and Stat3, and reduced inflammation and cell death.
Unknown (Rager et al., 2016; McCulloh et al., 2018)	Premature and hypercaloric feeds-induced NEC in rat	rAFMSCs, rBMSCs, and mBMSCs	UC	Reduced the incidence and severity of experimental NEC and protected the intestines from NEC
miR-200b (Sun et al., 2020b)	*In vitro* TNF-α treatment of endothelial cells	HO-1-modified rBMSCs	Exosome separation kits	Targeted HMGB3 in intestinal epithelial cells to alleviate inflammatory injury
Let-7f, miR-145, miR-199a, and miR-221 (Qian et al., 2016)	*In vitro* HCV treatment of human hepatoma-7 cells	hBMSCs	UC	Suppression of HCV RNA replication, combined with INF-α or telaprevir, enhanced their anti-HCV ability
Unknown (Gu et al., 2020)	*In vitro* D-GalN/LPS treatment of hepatocytes	BMSCs	UC	Decreased the expression levels of the pro-apoptotic proteins Bax and cleaved caspase-3, upregulated the anti-apoptotic protein Bcl-2, reduced hepatocyte apoptosis
Unknown (Sun et al., 2020a)	CVB3-induced myocarditis in mouse	hBMSCs	UC	Activated AMPK/mTOR-mediated autophagy flux pathway to attenuate cardiomyocyte apoptosis

MSC-EV, mesenchymal stem cell-derived extracellular vesicle; hADMSCs, human adipose-derived MSCs; hBMSCs, human bone marrow-derived MSCs; hUCMSCs, human umbilical cord-derived MSCs; mBMSCs, mouse bone marrow-derived MSCs; sBMSCs, swine bone marrow-derived MSCs; rAFMSCs, rat amniotic fluid-derived MSCs; rBMSCs, rat bone marrow-derived MSCs; HCV, hepatitis C virus; HGF, hepatocyte growth factor; KGF, keratinocyte growth factor; Ang-1, angiopoietin-1; LPS, lipopolysaccharide; ALI, acute lung injury; E. coli, Escherichia coli; CLP, cecal ligation and puncture; NEC, necrotizing enterocolitis; D-GalN, D*-galactosamine hydrochloride; CVB3, coxsackievirus B3; HO-1, heme oxygenase-1; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; UC, ultracentrifugation; NF-κB, nuclear factor kappa B subunit 1; MRP1, multidrug resistance-associated protein 1; LTB_4, leukotriene (LT) B_4; HMGB3, high mobility group box 3.*

For Sepsis

Sepsis is a systemic inflammatory response to infection that leads to multiple organ dysfunction, and one out of four sepsis patients died during their hospital stay (Iskander et al., 2013; Fleischmann-Struzek et al., 2020). Sepsis is caused by the accumulation of various pro-inflammatory factors in the process of inflammatory response and immune dysfunction (Prescott and Angus, 2018). Even with the continuous development of intensive care and advances in the antibiotic application, the mortality of sepsis in intensive care units remains high (Angus and van der Poll, 2013). Therefore, a new therapy is urgent to improve the clinical outcomes.

Patients with sepsis had severe immunosuppression, leading to macrophage dysfunction and poor wound healing (Davis et al., 2019). Therefore, the new therapy strategy could be related to the immunoregulation of macrophages. Several studies have proven that MSC-EVs can improve the outcomes of sepsis in animal models. MiRNAs in MSC-EVs have been considered as a critical substance to exert efficacy in sepsis. For example, miRNA-146a was found to be strongly upregulated in MSC-EVs primed with interleukin-1β (IL-1β), which could more effectively induce M2 polarization by modulating IRAK1, TRAF6, and IRF5 signaling (Song et al., 2017). MiR-21 in MSC-EVs was abundantly upregulated in IL-1β-stimulated MSCs, which induced M2 polarization of macrophages *in vitro* and *in vivo* sepsis by inhibiting the effects of PDCD4, which can participate in multiple cellular biological behaviors, including apoptosis and transcription (Yao et al., 2021). Both studies supported

that pretreated MSCs with pro-inflammatory cytokines could enhance their immunomodulatory function of MSCs. The exosomal miR-223 was reported to contribute to MSC-mediated cardioprotection in sepsis by downregulation of Sema3A and STAT3 (Wang et al., 2015).

For Intestinal Infection

The balance between beneficial and harmful bacteria plays an important role in neonatal intestinal health (Rhoads et al., 2018). Bacterial infection is one of the most significant risk factors in NEC pathogenesis, a life-threatening disease in premature infants, with mortality as high as 30% (Neu and Walker, 2011; Markel et al., 2020). Full-thickness destruction of the intestine is the character of NEC, and inflammatory response is increased in infants affected by this disease, leading to intestinal perforation, peritonitis, bacterial invasion of the bloodstream, and systemic infection (Neu, 2014; Neu and Pammi, 2018). Survivors are faced with severe sequelae, including short gut syndrome and neurodevelopmental retardation (Neu, 2014). Despite decades of research on the pathophysiology of NEC, the treatment remains inadequate and supportive and desired a novel preventive and therapeutic intervention.

MSCs have great potential in NEC treatment, decreasing NEC incidence in rat models (Augustine et al., 2017; Thébaud, 2019). EVs from MSCs carry important biological components and can be utilized in disease prevention and treatment (Baglio et al., 2015). EVs from bone marrow-derived MSCs, heparin-binding EGF-like growth factor (HB-EGF) primed MSCs, and human umbilical cord MSCs have been reported to protect the integrity of the intestinal barrier and reduce the severity and incidence of NEC in an experimental model (Rager et al., 2016; McCulloh et al., 2018). Both miR-34 and miR-29 improved the intestinal epithelial barrier through the Snail/Claudins signaling pathway (Li et al., 2020). MiR-200b in heme oxygenase-1 (HO-1)-modified bone marrow MSCs-derived EVs was reported to target high mobility group box 3 (HMGB3) gene in intestinal epithelial cells to alleviate its inflammatory response (Sun et al., 2020b).

For Other Infectious Diseases

Qian et al. (2016) revealed that miRNAs, especially let-7f, miR-145, miR-199, and miR-221 from MSC-EVs, inhibited viral replication in hepatitis C virus (HCV)-treated cells. Hepatocyte injury model caused by D-galactosamine (D-GaIN) and LPS could be ameliorated by MSC-EVs through inducing autophagy and inhibiting apoptosis (Zhao et al., 2019). In addition, MSC-EVs had therapeutic effects on coxsackievirus (CVB3)-induced myocarditis in the mice model, which can shrink the production of pro-inflammatory cytokines and improve cardiac function *via* activating the AMPK/mTOR-mediated autophagy flux pathway to attenuate apoptosis (Gu et al., 2020).

CONCLUSION

MSC-EVs had outstanding prospects in treating infectious diseases, such as respiratory infections, sepsis, and intestinal infections. The therapeutic mechanisms included direct antimicrobial effects, immunomodulation, and tissue repair. MSC-EVs exert their effect through the transfer of mRNAs, miRNAs, and proteins (**Table 1**). MiRNA containing EV may be a new target for the development of new therapeutic drugs. The use of MSC-EVs has several benefits, namely, (a) small vesicles, readily circulating and penetrating biological barriers, like blood–brain; (b) low tumorigenesis; and (c) stable properties, MSC-EVs may achieve a higher "dose" than MSCs due to the poor viability and considerable death of engrafted MSCs in target tissues (Barbash et al., 2003). Importantly, EVs can maintain high activities at low temperatures. All the profits make MSC-EVs a promising agent in infectious diseases.

Despite the promising progress that has been made in the treatment of MSC-EVs on infectious diseases, several challenges are faced by the field in clinical translation: (a) there is wide variability of MSC-EVs preparations in the whole process (Börger et al., 2020), such as the different productions of cell sources, purification, and identification of the final product. Careful consideration of the optimal purity and rational clinical trial design of MSC-EVs is necessary to advance large-scale clinical trials (Muraca et al., 2018). Furthermore, lacking standardized quality parameters caused discrepancies and controversies about the biology and function of MSC-EVs. Members of four societies (SOCRATES, ISCT, ISEV, and ISBT) identified potential metrics of MSC-EVs to facilitate data sharing and comparison of MSC-EVs among different studies, including biological activity, vesicle integrity, the concentration of membrane lipid vesicles, the ratio of specific lipids, the ratio of membrane lipids to protein, and the ratio of MSC to non-MSC surface antigens (Witwer et al., 2019). Each metric needs to be quantified and validated in further studies. (b) How to determine reproducible and robust parameters to predict the therapeutic potency of MSC-EVs is unsolved. The therapeutic efficacy of MSC-EVs depends not only on the cell, such as the cell source and status of MSCs, delivery dose and route (Sun et al., 2020a), and half-life and *in vivo* biodistribution of MSC-EVs, but also on the disease condition, such as the disease microenvironment and the time window for intervention. (c) MSC-EVs from different sources have been reported to be efficacious in various kinds of infectious diseases; the therapeutic mechanism may be different and specific for each source and disease condition. To better understand the therapeutic activity, the mode of action needs to be studied further, trying to find out the key components in MSC-EVs, target cells in injured tissues, and the involved molecular signaling cascade.

AUTHOR CONTRIBUTIONS

LZ: conceptualization and review. ZF: supervision. JY: writing and editing. All authors contributed to the article and approved the submitted version.

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Application of Stem Cell-Derived Extracellular Vesicles as an Innovative Theranostics in Microbial Diseases

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Extracellular vesicles (EVs), as nano-/micro-scale vehicles, are membranous particles containing various cargoes including peptides, proteins, different types of RNAs and other nucleic acids, and lipids. These vesicles are produced by all cell types, in which stem cells are a potent source for them. Stem cell-derived EVs could be promising platforms for treatment of infectious diseases and early diagnosis. Infectious diseases are responsible for more than 11 million deaths annually. Highly transmissible nature of some microbes, such as newly emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), drives researcher's interest to set up different strategies to develop novel therapeutic strategies. Recently, EVs-based diagnostic and therapeutic approaches have been launched and gaining momentum very fast. The efficiency of stem cell-derived EVs on treatment of clinical complications of different viruses and bacteria, such as SARS-CoV-2, hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), *Staphylococcus aureus*, *Escherichia coli* has been demonstrated. On the other hand, microbial pathogens are able to incorporate their components into their EVs. The microbe-derived EVs have different physiological and pathological impacts on the other organisms. In this review, we briefly discussed biogenesis and the fate of EVs. Then, EV-based therapy was described and recent developments in understanding the potential application of stem cell-derived EVs on pathogenic microorganisms were recapitulated. Furthermore, the mechanisms by which EVs were exploited to fight against infectious diseases were highlighted. Finally, the deriver challenges in translation of stem cell-derived EVs into the clinical arena were explored.

Keywords: stem cells, infectious disease, antimicrobial agents, bacterial EVs, Viral EVs, MSC-derived EVs, extracellular vesicles

INTRODUCTION

What Are the Extracellular Vesicles?

Extracellular vesicles as "pro-coagulant dust" were identified by Wolf from blood platelets in Wolf (1967), and then in Pan and Johnstone (1983) were among the first scientists who described EVs (Pan and Johnstone, 1983). EVs are a heterogeneous group of vesicles containing different cargos (Abels and Breakefield, 2016). They can transfer different biomolecules such as lipids, proteins, and nucleic acids between different cells, distinguishing their significant role in cell-cell communications (Raposo and Stoorvogel, 2013). EVs are generated and released by almost all types of cells and are classified as exosomes, macrovesicles, and apoptotic bodies (Gurunathan et al., 2021; Pournaghi et al., 2021). Also, ectosomes, shedding vesicles, and microparticles are other types of EVs involved in inter/intra cellular communications (Gurunathan et al., 2019). EVs have attracted tremendous attention from both basic and clinical fields of study during the last decade due to their putative and significant role in several physiological and pathological processes (Jadli et al., 2020). EVs have been isolated from different body fluids such as blood, urine, tears, saliva, etc. (Akers et al., 2013). Some disorders such as inflammatory diseases can modify the EVs and change their numbers, content, composition, and function (Knijff-Dutmer et al., 2002). It has been shown that microbial infections can change the production and release process of EVs in infected cells (Rodrigues et al., 2018). Moreover, some EVs derived from immune cells can play a key role in induction of inflammation (Spencer and Yeruva, 2021).

Types of Extracellular Vesicles

Macrovesicles (MVs), also known as microparticles, are small membranous vesicles released from almost all cell types including mesenchymal stem cells, endothelial cells, some immune cells, etc. (Cocucci et al., 2009). Their size is ranging from 100 to 1000 nm, and they are formed by direct outward blebbing and pinching of the cell membrane. The production of EVs is regulated by physiological and/or pathological processes (Lynch and Ludlam, 2007; Sedgwick and D'Souza-Schorey, 2018). Initially, MVs are considered as cell debris, however, they recently were recognized as mediators of inter/intra cellular communication tools (Camussi et al., 2010). MVs can carry various bioactive molecules such as cytokines and chemokines, which highlight their antimicrobial potential and also their role in host defense against pathogenic microorganisms (Timár et al., 2013).

Apoptotic cell-derived EVs (ApoEVs) are another class of EVs released from apoptotic cells and contain cell organelles and nuclear materials (Gurunathan et al., 2019). ApoEVs are divided into two subtypes, including large apoptotic bodies (ApoBDs) with a diameter range of 1000–5000 nm and small apoptotic microvesicles (ApoMVs) with <1000 nm (Caruso and Poon, 2018). ApoEVs are important because they accelerate apoptotic cell clearance and also have a role in intercellular communication and immune modulation (Zitvogel et al., 2010; Poon et al., 2014). ApoEVs act as a key regulator of antigen presentation process, antimicrobial immunity against pathogens, and modulator of the dendritic cells' response against viral infections (Winau et al., 2006; Schiller et al., 2008; Caruso and Poon, 2018).

Exosomes (30-150 nm in diameter) are the third group of EVs isolated from a variety of body fluids and released by the fusion of multivesicular bodies (MVBs) with the plasma membrane (Simons and Raposo, 2009; Babaei and Rezaie, 2021; Mobarak et al., 2021). Some exosomes are generated and released from various cells in response to different stimuli, but others are continuously produced and released (Mathivanan et al., 2010). They contain various types of cargo molecules which are engaged in the biogenesis and transportation ability of exosomes (Zhang et al., 2019). Exosomes have been implicated in a variety of biological functions, including elimination of old and disused biomolecules (Harding et al., 2013), involvement in tumor progression especially in angiogenesis and metastases (Rak, 2010; Hood et al., 2011), antigen presentation (Bobrie et al., 2011), differentiation of some immune cells to modulate immune responses (Zhang and Grizzle, 2011), and facilitating the spread of some pathogenic microbes or elimination of microbes through interaction with recipient cells (Furuyama and Sircili, 2021; White et al., 2021).

Isolation Methods of Extracellular Vesicles

Several isolation methods are currently developed for the isolation and purification of EVs in bulk (Witwer et al., 2013). Sequential centrifugation and ultracentrifugation are the conventional methods to isolate EVs in cell culture media or body fluids (Théry et al., 2006). Gradient ultracentrifugation based on sucrose density is also used to minimize protein contamination (Tauro et al., 2012). Chromatography is another tool that can be employed to purify exosomes based on their size and dimensions or surface markers such as CD9, CD63, CD81, and EpCAM (Böing et al., 2014; Oksvold et al., 2015). Once isolated, the purified EVs are characterized. Currently, several methods are developed to analyze the EVs and their content for both research and clinical purposes. These methods include transmission and scanning electron microscopy (TEM and SEM), atomic force microscopy (AFM), dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), resistive pulse sensing (RPS), flow cytometry, fluorescence-activated cell sorting (FACS), enzyme-linked immunosorbent assay (ELISA), microfluidics, and electrochemical biosensor-based devices (Théry et al., 2006; Woo et al., 2016).

Contents of Extracellular Vesicles

The content, or cargo, of EVs varies and extremely depends on the parental cells and recently new databases including ExoCarta, Vesiclepedia, exoRBase, EVmiRNA, and EVpedia were developed to classify them (Liu et al., 2019). These databases provide information about the content of EVs such as lipids, proteins, miRNAs, and other components. Also they provide useful information about the isolation and characterization of

EVs (Jadli et al., 2020). The proteome of the EVs is affected by their biogenesis. For example, ESCRT proteins (Alix, TSG101, HSC70, and HSP90β) regulate the biogenesis and transportation of some EVs and thus these proteins are expected to be found in EVs regardless of the type of the originating cells (Théry et al., 2001; Van Niel et al., 2006). Therefore these proteins can be used as marker for the detection and characterization of EVs (Doyle and Wang, 2019). Some tetraspanin families of proteins such as CD63, CD9, and CD81 are commonly found in EVs and also used as marker proteins both for the detection and purification of EVs (Witwer et al., 2013). Along with the exosome and some EVs surface markers, EVs carry certain biomolecules such as mRNA, miRNA (Zhou J. et al., 2013), cytokines, and antigen presentation molecules (MHC-I, MHC-II) (Gutiérrez-Vázquez et al., 2013) which contribute to the physiological and pathological function of exosomes (Dini et al., 2020). Microbial EVs contain different cargoes based on their origin. The EVs of Gram-negative bacteria contain cytoplasmic proteins, nucleic acids, virulence factors (e.g., toxins), peptidoglycan, and inner membrane. The EVs of Gram-positive bacteria contain membrane-associated virulence proteins, fatty acids, lipoteichoic acid, phospholipids, and some components similar to the Gram-negative EVs (Yu et al., 2018; Bose et al., 2020).

Common Uses of Extracellular Vesicles

As mentioned above, EVs are mainly responsible for inter/intracellular communications. It was shown that EVs could interact with target cells and therefore they make an impact on cell physiology, phenotype, and function (Simons and Raposo, 2009; Mardpour et al., 2018). Also they can mediate the horizontal transfer of genetic materials (Natasha et al., 2014). Due to the widespread and cell-specific availability of some types of EVs, particularly exosomes, in almost all body fluids, they can be considered as biomarkers (Zhang et al., 2019). Moreover, EVs can be used as delivery vehicles for the efficient transfer of biological therapeutic agents across different biological barriers to desired cells (Haney et al., 2015). In addition, EVs can be applied in regenerative medicine, tissue engineering and cell homeostasis (Gurunathan et al., 2021). They play critical roles in immunoregulation, including antigen presentation, immune activation, immune suppression, and also immune tolerance via exosome-mediated inter/intracellular communications. They also play pivotal role in the host defense against viral and microbial infections (Gurunathan et al., 2021). Documented evidence has shown that host cells-derived EVs and even EVs derived from bacteria can mediate the crosstalk between pathogen and innate immune cells, and thus modulate the innate immune responses of the host (Munich et al., 2012).

In this review article, we first discussed the biogenesis and the fate of EVs. Then, EV-based therapy was described and recent developments in understanding the potential application of stem cell-derived EVs in infectious diseases were recapitulated. In addition, the mechanisms by which EVs were exploited to fight against infectious diseases were highlighted. Finally, the deriver challenges that exists in the translation of stem cell-derived EVs into the clinical arena were explored.

BIOGENESIS AND THE FATE OF EXTRACELLULAR VESICLES

Biogenesis

The biogenesis of the exosomes is well-defined as compared to the other types of EVs. The biogenesis of exosomes is a multistep biological process regulated through different signaling pathways (Abels and Breakefield, 2016). Biogenesis of exosomes initiates with the formation of early endosomes followed by second inward budding of the endosomal membrane which leads to the formation of the late endosomes. Late endosomes or intraluminal vesicles (ILVs) follow the endocytic pathway for the generation of exosomes (Sluijter et al., 2014). In the final stage, the generated ILVs are released as exosomes into the extracellular space via exocytosis (Jadli et al., 2020). Some endogenous molecules such as small GTPase Ral (Hyenne et al., 2018) and adiponectin/T-cadherin (Obata et al., 2018) and also some microbes including viral infections and Gram-positive and Gram-negative bacterial infections as extrinsic factors can influence the biogenesis of exosomes (Crenshaw et al., 2018). For the biogenesis of exosomes, different protein sorting mechanisms have been identified, among them endosomal sorting complex transport (ESCRT)-dependent pathway (Frankel and Audhya, 2018) and ESCRT-independent pathway (Babst, 2011) are two widely explained mechanisms (**Figure 1**).

Endosomal Sorting Complex Transport-Dependent Mechanism

The ESCRT-dependent mechanism is well-characterized and comprised of many proteins arranged into four proteins complexes including ESCRT-0, –I, –II, and –III. Some proteins such as VPS4, VTA1, and ALIX are associated with these protein complexes. The ESCRT-dependant exosome biogenesis is initiated by recognition and sequestration of ubiquitinated proteins via ubiquitin-binding subunits of ESCRT-0. Then, the ESCRT-0 interacts with the ESCRT-I and –II complexes and all of them will combine with ESCRT-III, a protein complex that is contributed to enhance budding processes. Finally, following cleaving the buds to form ILVs, the ESCRT-III complex disassociates from the MVB membrane with energy supplied by the sorting protein Vps4 (Ren et al., 2008).

Endosomal Sorting Complex Transport-Independent Mechanism

While ESCRT pathway is the most important mechanism for exosome formation, some EVs such as MBV and ILV are also formed in an ubiquitin-independent way called CRT-independent pathways (Babst, 2011). Heparan sulfate promotes exosome biogenesis through syntenin. Syntenin serves as an intermediate between ESCRT-I and ESCRT-III and is involved in the budding processes (Addi, 2019). Another ESCRT-independent exosome formation was described in the oligodendroglial cells. These cells secret exosomes containing proteolipid protein (PLP) which depends on the depletion of neutral sphingomyelinases (nSMase). Furthermore, tetraspanin-enriched microdomains (TEMs) full of CD81 particles are

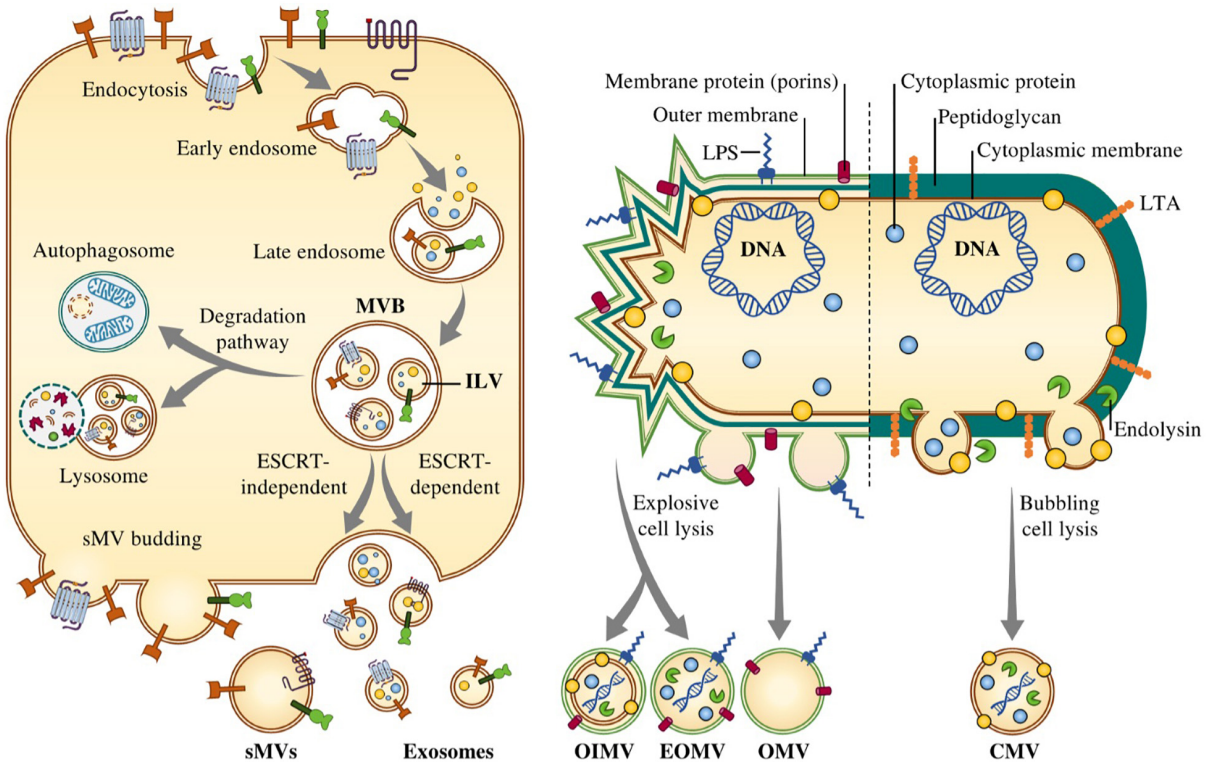

FIGURE 1 | Biogenesis of Extracellular vesicles (EVs) in eukaryotic cells and Gram-negative and –positive bacteria. **(A)** In eukaryotic cells, biogenesis of EVs consists of three consecutive steps, including (i) formation of early endosome by invagination of the plasma membrane; (ii) formation of late endosome and then MVBs; and finally (iii) fusion of MVBs with the plasma membrane and release of the vesicular contents by ESCRT-dependent and –independent mechanisms. **(B)** In the Gram-negative bacterial extracellular vesicles, known as outer membrane vesicles mainly originate from the outer membrane of bacterial envelope. Three potential biogenesis mechanisms have been suggested including the blebbing of the outer membrane of the bacterial envelope (OMVs), the formation of outer-inner membrane vesicles (OIMVs) and the formation of explosive outer membrane vesicles (EOMVs). Gram-positive bacteria **(B)** lack an outer membrane and also have a thick peptidoglycan cell wall outside of the cell membrane which convey the assumption that membrane-derived vesicles could not escape such large barriers but EVs may be forced through the wall by turgor pressure after release from the plasma membrane. In addition, cell wall-modifying enzymes facilitate the released of EVs.

regarded to be another ESCRT-independent pathway of protein sorting into ILVs (Garín et al., 2007).

Extracellular Vesicles Secretion

To release exosomes, multiple cellular steps are required to be completed including formation of MVBs, transportation of MVBs to the plasma membrane, and fusion of MVBs with the plasma membrane. Several molecules have been engaged in these processes (Rabinovich et al., 2000). MVB can either fuse with a lysosome to degrade their cargo or fuse with the plasma membrane, leading to exosome release. ISGylation, a post-translational ubiquitin-like modification, is one of the signals that regulates the MVBs' fate. ISGylation of MVB proteins promotes fusion of MVBs with lysosomes, which promoting the exosome release (Chen et al., 2003).

Mechanisms of Exosome Uptake

Following the release and secretion of EVs to the extracellular environment, the EVs' ability to interact with recipient cells and capacity to deliver their contents such as proteins, lipids, and nucleic acids into the recipient cells can determine the role of EVs in physiological and pathological processes (Jadli et al., 2020). Several mechanisms have been introduced for the uptake of exosomes, including phagocytosis, macropinocytosis, clathrin-mediated endocytosis (CME), caveolin-dependent endocytosis (CDE), and plasma membrane fusion (Tamura et al., 2016; Sun et al., 2018). Phagocytosis is in charge of the internalization of bacteria, EVs, and others components. Toll-like receptors (TLRs), scavenger, and complement receptors, as specific targets on the cell surface, participate in invaginations around the material intended for internalization (Wei et al., 2021). Macropinocytosis is another mechanism characterized by plasma membrane ruffling and is induced by growth factors or other signal stimulations. Membrane ruffles form a cup-like structure that seals at its distal tips to construct a relatively large endosome (Canton, 2018). The resulting vesicles contain extracellular fluid and small particles (Wei et al., 2021). CME is a receptor-mediated endocytic process used to transport a wide range of

cargo molecules from the cell surface to the interior. Clathrin and adaptor protein 2 (AP2) complexes are necessary for the formation of clathrin-coated vesicles and then the internalization of cargo molecules (Palmirotta et al., 2018). CDE requires the caveolin, a dimeric protein, in the plasma membrane to facilitate the raft-mediated endocytosis (Melo et al., 2015). CDE is another mechanism for the uptake of EVs, but the precise mechanism of internalization may differ depending on the type of EVs and recipient cells (Nanbo et al., 2013). Fusion is the last mechanism of EVs internalization, which enables EVs membrane to directly merge with the cell plasma membrane and transfer cargo molecules to recipient cells (Jadli et al., 2020).

EXTRACELLULAR VESICLES-BASED THERAPY OF MICROBIAL DISEASES

Role of Extracellular Vesicles in Bacterial and Viral Infections

Eukaryotic cells generate a heterogeneous group of EV subtypes, recognized by biogenesis mechanism and their size (Sedgwick and D'Souza-Schorey, 2018; Théry et al., 2018). EVs have been observed in all body fluids of humans (Raposo et al., 1996; Lässer et al., 2011; Street et al., 2012; Roberts and Kurre, 2013; Hiemstra et al., 2014) and are generated by various cell types (Raposo et al., 1996; Barry et al., 1998; Van Niel et al., 2001; Lai and Breakefield, 2012; Peinado et al., 2012). EVs are responsible for developing the functional range of the bioactive molecules secreted by cells, improving their stability, and boosting their ability to achieve better localized concentrations (Liu et al., 2019; Guo et al., 2021). EVs have an important role in delivery and orchestration of antimicrobial responses from host immune system. In infectious diseases, the cells such as epithelial cells and macrophages have the first contact with the EVs of pathogens that containing bioactive molecules and induce host pro-inflammatory responses (Hui Winnie et al., 2018; Li et al., 2018; White et al., 2021). The stimulated immune cells during infections may produce EVs that convey antimicrobial agents (Kesimer et al., 2009; Hu et al., 2013; Timár et al., 2013) or play decoy roles to protect host cells by binding and coating toxins secreted from bacterial pathogens (da Cruz et al., 2020) (**Table 1**). Production of EVs by a variety of host cells may be enhanced during infectious diseases (da Cruz et al., 2020).Different types of microorganisms and viruses have all been reported to directly stimulate host EVs secretion (Kim et al., 2012; Antwi-Baffour et al., 2020; Mehanny et al., 2020). The enhancement of host EVs secretion has been shown following an extracellular challenge of alveolar epithelial cells with heat-sacrificed bacteria, providing that the activator of this response was bacterial CpG DNA that bond to endosomal receptors of TLR9 (Keller et al., 2020). Host endothelial cells and macrophages infected by bacterial agents are similarly promoted to release EVs (Bhatnagar et al., 2007; Hui Winnie et al., 2018; Li et al., 2018). Besides increasing the production of EVs, infections can alter the contents of EVs produced by host cells (Bhatnagar et al., 2007). MSCs-derived EVs promote healing process in diabetic foot by loading some bioactive molecules including growth factors, nucleic acids, and proteins. Also, as a vehicle for non-bioactive substances like antibiotics can inhibit the bacterial growth and accelerate improvement in the diabetic wound repair in bacteria-associated diabetic foot ulcers (Raghav et al., 2021).

Bacterial Extracellular Vesicles

It is now well recognized that most bacteria produce soluble products such as metabolites, quorum sensing peptides, nucleic acids, proteins, and bacterial EVs (BEVs) that allow their communication with each other and host cells (Hughes and Sperandio, 2008; Tulkens et al., 2020). Interestingly, both beneficial and pathogenic bacteria release BEVs. BEVs carry various molecules such as proteins, peptidoglycan, enzymes, toxins, polysaccharides and DNA/RNA molecules (Riley et al., 2013; Kaparakis-Liaskos and Ferrero, 2015). Different BEVs have various structures and even various molecular cargo compounds. These differences may be due to various growth conditions, several biogenesis pathways, the unique membrane envelope structure of the parental bacterium which they emanate from, and also the genetic content of the paternal bacterial strain (Toyofuku et al., 2019). BEVs display high stability to different temperatures and treatments, and regarded safe because they are not able to replicate *in vitro* and *in vivo* conditions. They carry several immunogenic surface and membrane related components of their parental strains (Kaparakis-Liaskos and Ferrero, 2015). Based on the originating strains, BEVs can promote both humoral and cellular immunity and together with their nanoparticulate character, provide them with their own adjuvanticity, BEVs are capable to increase T-cell reactions to antigens (Chronopoulos and Kalluri, 2020).

Application of outer membrane vesicles and vaccine development

Gram-negative bacteria pursue two important routes for BEVs production. The primary pathway is blebbing of the outer membrane of the bacterial envelope, producing OMVs; and the other pathway requires explosive cell lysis forming outer-inner membrane vesicles (OIMVs) and explosive outer membrane vesicles (EOMVs) (**Figure 1**; Toyofuku et al., 2019; Tulkens et al., 2020). The blebbing process of the membrane giving rise to OMVs happens through a disruption of crosslinks between the outer membrane and the underlying peptidoglycan cell wall layer (Chronopoulos and Kalluri, 2020). Actually, the Gram-negative bacterial cell wall comprises a thin layer of peptidoglycan in the periplasmic environment between two membrane bilayers; the cytoplasmic and outer membranes (Toyofuku et al., 2019; Chronopoulos and Kalluri, 2020). The outer membrane includes lipopolysaccharides (LPS) or endotoxin on its outer leaflet and various membrane-bound channels and protein-like porins that simplify non-vesicle mediated transport. Reflecting this envelope structure, Gram-negative BEVs consist of an outer membrane with an interior leaflet of phospholipids and an exterior leaflet of LPS that is known to engage TLR4 (Toyofuku et al., 2019; Tulkens et al., 2020). BEVs of Gram-negative bacteria contain high concentration of different outer membrane proteins, such as ompA and encapsulated periplasmic luminal compounds. Nevertheless, the existence of cytoplasmic cargo

including virulence molecules and nucleic acids is debated and dependent on the certain biogenesis pathways of the OMV, OIMVs, and EOMVs (Toyofuku et al., 2019; Tulkens et al., 2020). The endotoxicity of BEVs can be simply modified through genetic engineering methods. Furthermore, BEVs from specific commensal, or beneficial and potentially probiotic bacterial species may excrete therapeutic effects. In future, BEVs may be applied in cancer immunotherapy to elicit durable antitumor immune response or act as anti-cancer vaccines. In a recent study, the applicability of BEVs in cancer immunotherapy or cancer vaccines was reported showing that systemic intravenous administration of Gram-negative BEVs from the genetically modified *Escherichia coli* msbB$^{-/-}$ has a directed tropism for tumor site and a notably capability of inducing long-term antitumor immune responses through the secretion of CXCL10 and INFγ that can completely eradicate tumors without considerable adverse consequences (Kim et al., 2017).

Application of membrane vesicles

Gram-positive bacteria also release nano-sized cytoplasmic membrane vesicles (CMVs), or so-called as MVs, through endolysin-triggered bubbling cell death into the extracellular environment either in a constitutive manner or in a regulated manner (Brown et al., 2015; Toyofuku et al., 2019). Gram-positive bacteria lack the entire outer membrane and possess a much thicker peptidoglycan layer or cell wall. The Gram-positive cell wall is connected to glycan polymers that can be covalently linked to peptidoglycan [as wall teichoic acids (WTAs)], or anchored in the cell membrane in the case of glycolipids such as lipoteichoic acids (LTAs) which can interact with TLR2 (Brown et al., 2015; Toyofuku et al., 2019; Tulkens et al., 2020). Similar antitumor effects were also seen for the Gram-positive BEVs originated from *Staphylococcus aureus* and *Lactobacillus acidophilus* (Chronopoulos and Kalluri, 2020). There is also an enormous interest in applying genetic engineering methods to manipulate bacteria and subsequently purify recombinant BEVs for utility as vaccines against some cancers (Chronopoulos and Kalluri, 2020).

Role of extracellular vesicles in antibiotic resistance and biofilm formation

The localization of chromosomal DNA in BEVs from different Gram-negative pathogenic bacteria such as *Salmonella typhimurium* is often extraluminal with smaller amounts settled in the intraluminal locations (Bitto et al., 2017). Sequencing of the intraluminal BEV DNA has been reported to be enriched in certain regions of the bacterial chromosome involved in pathogenicity and virulence capacity, stress response and antibiotic resistance as well as metabolic pathways. There is still a matter of controversy whether extraluminal or surfaces-associated BEV DNAs versus intraluminal ones render different types of actions. One can assume a probable role for external DNA in biofilm formation versus a role for internal BEV DNA in intercellular crosstalk and horizontal gene transfer (HGT) of virulence-associated markers and antibiotic resistance encoding genes (Bitto et al., 2017).

Bacterial extracellular vesicles contribute to immune response

Bacterial extracellular vesicles contain several microbe-associated molecular patterns (MAMPs) or pathogen-associated molecular patterns (PAMPs) such as peptidoglycan, lipoproteins, LPS and bacterial DNA/RNA. The MAMP content of BEVs enables them to interact with host pattern recognition receptors (PRR) in different types of host cells to induce immune tolerance, or confer protective immunity, and even host cell damage (Riley et al., 2013). The immunomodulatory effects of BEVs mainly depend on the particular parental bacterium and its association with the host. For example, BEVs from some pathogenic bacteria are capable of worsening the infection by suppressing host immune responses (Peek and Blaser, 2002; Lee Hannah et al., 2007), or induce overwhelming immune responses leading to sepsis (Shah et al., 2012). In opposite, BEVs from beneficial or commensal bacteria in the gut can promote immunological maturation and tolerance to confer protection from sepsis (Shen et al., 2012; Kang et al., 2013). In addition, a number of cell surface TLRs, prominently TLR2 and TLR4, can recognize extraluminal ligands of BEV such as LPS, lipoarabinomannan, peptidoglycan and LTA macromolecules (Prados-Rosales et al., 2011; Zhao et al., 2013; Athman et al., 2015; Gu et al., 2019). Also, both nucleotide-binding oligomerization domain-containing protein 1 (NOD1) and NOD2 are engaged in sensing peptidoglycans that are available in BEV contents produced by pathogenic and symbiotic bacterial strains (Kaparakis et al., 2010; Thay et al., 2014; Bitto et al., 2018; Cañas et al., 2018). In addition, intraluminal BEV DNA/RNA may be recognized via DNA/RNA sensing receptors. After endocytosis, BEV RNA cargo may be recognized via endosomal TLRs such as TLR3 and TLR7. In a similar fashion, RNAs rendered into the cytoplasm after fusion of BEVs with the cell plasma membrane may activate cytosolic RNA detectors like RIG-I-like receptors (Tsatsaronis et al., 2018). Similarly, BEV DNA cargo may be sensed by endosomal TLR9 or cytosolic DNA sensing cyclic GMP-AMP synthase stimulator of the interferon genes cascade. Overall, PRR stimulation promotes the activation of transcription factors and kinases that result in the secretion of chemokines and other cytokines leading to the immune cells recruitment and regulation of costimulatory factors normally associated with acquired immune response (Riley et al., 2013).

Extracellular vesicles as infection biomarkers

As mentioned above, EVs are found in various body fluids like blood, urine and saliva. EVs contain different biomolecules, which can be used as novel biomarkers for a variety of human diseases and cancers. Since they can be obtained by minimally invasive biopsy procedures, thus they would be very useful biomarkers for diagnosis (Lee et al., 2018; Min et al., 2019). As researchers begin to figure out the distribution pattern and composition of EVs during infectious diseases, new biomarkers can be introduced that can provide the possibility for the development of EV-based diagnostics (Tulkens et al., 2020). For instance, serum EVs can used to show biofilm-associated infections to support a rapid detection (Deng et al., 2020). Further understanding of the biology of EVs can provide possible clues to protect infectious diseases and early detection.

Viral Extracellular Vesicles

Viral EVs are generated by virus-infected cells and are considered to be engaged in inter/intracellular connection between infected and uninfected cells. Viral agents, especially oncogenic viruses and viruses that can develop chronic infections can regulate the EVs generation and the content. Viruses are defined by the virologists of last century as "submicroscopic infectious agents that can replicate only inside the living cells of an organism". EVs do not fall under this description, because contrary to their similarity to viruses in many aspects, they are basically distinct, as they are non-replicative particles. Nevertheless, current virology has distanced itself from such an out-dated description of virus by new terms of defective and non-infectious particles. Thus, EVs produced by virus-infected cells that contain different viral proteins and some parts of viral genomes can fall under the description of non-infectious viral agents (Nolte-'t Hoen et al., 2016). Moreover, there is a resemblance between biogenesis of virions and EVs. EVs and virus particles are altogether released by infected cells and share the routes for biogenesis at the plasma membrane (Colombo et al., 2014). Regardless of what described, it is essentially difficult to discriminate between EVs that deliver viral proteins, viral genomic fragments and host proteins and enveloped viral particles that carry the similar contents (Nolte-'t Hoen et al., 2016).

Effects of extracellular vesicles on viral pathogenesis

It was shown that cells infected with enveloped or non-enveloped viruses produce EVs that carry viral constituents. The preparation of viral particles may not be completely pure and are almost mixed with various types of EVs, and even some of these EVs may be either indiscernible from defective viruses. Due to their close biogenesis routes, EVs and viral particles may be near relatives, however only viruses can replicate inside the cells. Notably, EVs produced via infected cells are not neutral, as they may facilitate virus spread and viral infection or increase antiviral responses (Nolte-'t Hoen et al., 2016). For instance; numerous HIV RNAs and proteins have been identified in EVs produced from HIV-infected cells (Narayanan et al., 2013). The involvement of EVs in viral infection has already been described for many viruses, including rabies, coronaviruses, HCV, HIV, HPV, HSV, dengue, HTLV-1, Zika, West Nile Epstein Baar virus, influenza virus, and SFTS (Martins and Alves, 2020). Deciphering the EVs structure produced by infected cells, characterizing their cargo, and understanding the accurate strategy by which they affect viral infection are necessary for basic virology and therapeutic applications as well (Nolte-'t Hoen et al., 2016).

Effects of viral extracellular vesicles on host immunity

The process of infection may alter the contents of cells-derived EVs and change the ratios of host RNAs and proteins inside them (Nolte-'t Hoen et al., 2016). Upon infection process, EVs can intensify inflammatory responses and deflagrate antiviral activities (Urbanelli et al., 2019), also can mediate the crosstalk between immune cells and other cells (Isola and Chen, 2017; Rezaie et al., 2021). Those EVs which can be transferred between the immune cells may transmit signals affecting the chemokines or cytokine secretion level, and some EVs can directly activate antigen presentation (Lindenbergh and Stoorvogel, 2018). Also, EVs carry different cytokines and cytokine-associated RNAs that may trigger the generation of target molecules in recipient cells, contributing to antiviral responses (Urbanelli et al., 2019). Moreover, EVs produced by infected cells were able to trigger other cell types, as observed when EVs produced by U937 macrophages, contaminated with DENV-2, activated endothelial cells (Velandia-Romero et al., 2020). Also, EVs secreted from airway epithelial cells infected by respiratory syncytial virus (RSV) can enhance the expression of regulatory small RNAs and may activate chemokine and cytokine secretion in monocytes without being exposed to infective particles (Chahar et al., 2018).

Further, EVs are capable to mediate the severity of disease though increasing the secretion of pro-inflammatory cytokines associated with several infectious diseases. EVs derived from bronchoalveolar fluid of mice infected with H5N1 influenza virus displayed enrichment with miR-483-3p, which stimulates innate immunity in pneumocytes proposing the involvement of EVs in the inflammatory pathogenesis of H5N1 virus (Maemura et al., 2020). In addition, in dengue hemorrhagic fever, EVs were observed to play an important role in the disease development (Mishra et al., 2019).

Immune cell-derived extracellular vesicles and antiviral effects

Extracellular vesicles can interact with each other and with viruses *in vivo* either directly or indirectly though modulating the host responses, therefore they are engaged in a "War and Peace" scenario between host and viruses (Lisco et al., 2009; Bhattarai et al., 2015). In opposite, EVs containing viral proteins can be profitable to the host cells. For instance, they can present viral antigens to DCs to facilitate triggering activation of adaptive immunity. Therefore, EVs produced within viral infection may demonstrate either proviral or antiviral features. It has been shown that T cells could release EVs comprising HIV receptor CD4. Such EVs can directly bind to viral particles, thereby reducing the load of virions that would otherwise infect other $CD4^+$ T cells (de Carvalho et al., 2014). Thus, further investigations are required to uncover the exact roles of EVs in antiviral immune responses in order to direct EVs engineering that may exhibit robust antiviral potentials.

Host Extracellular Vesicles as Antimicrobial Responses

Host EVs produced by immune cells have been demonstrated to elicit strong antimicrobial effects in *in vitro* and *in vivo* conditions (Timár et al., 2013; Wang C. et al., 2019). These antimicrobial potencies are contributed to various bacteriostatic and bactericidal compounds present in the EVs cargo (**Table 1**) (Timár et al., 2013; Hiemstra et al., 2014). For instance, EVs obtained from human urinary tract are usually enriched in proteins with immune functions, such as bacteriostatic proteins like mucin-1, fibronectin, CD14, and also bactericidal proteins such as calprotectin, dermcidin and lysozyme C (Hiemstra et al., 2014). Moreover, such EVs hindered the growth of probiotic and uropathogenic strains of *E. coli* as well as its laboratory-adopted strains, and mediated the bactericidal functions through

a lytic process (Hiemstra et al., 2014). Also, EVs are assumed to stabilize bioactive components like RNAs, and affect the non-lethal pathways of controlling microbial behaviors (Liu et al., 2016). EVs released from the host cells upon exposure to pathogenic microbes may also be able to safeguard cells from microbial assaults via efficient imitating the targets of their toxins and functioning as decoys (Keller et al., 2020).

Immunomodulatory Functions of Extracellular Vesicles Derived From Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are multipotent stem cells derived from mesoderm and have the ability to differentiate into a variety of cells. They can be easily obtained from many sources including mature tissues such as bone marrow (BM), peripheral blood (PB), adipose tissues (AT) and neonatal birth-related tissues including amniotic fluid (AF), Warton jelly (WJ), umbilical cord (UC), placenta (PL), and cord blood (CB) (Seo et al., 2019). These heterogeneous cells possess significant immunomodulatory and protective characteristics (Dabrowska et al., 2021). They are also modulate immune cell responses and produce various inflammatory mediators by which they can regulate both innate and adaptive immune responses. When the responses of some immune cells such as macrophages, natural killer (NK) cells, DCs, B and T cells are exaggerated, MSCs can repress their proliferation, differentiation, and activation to modulate the immune response (Ren et al., 2008). These immunomodulatory effects are exerted through the production of several soluble mediators such as nitric oxide (NO), indoleamine 2, 3-dioxygenase-1 (IDO-1), transforming growth factor-β1 (TGF-β1), interleukin-10 (IL-10), prostaglandin-E2 (PGE2), and hepatocyte growth factor (HGF) to the microenvironment (Puissant et al., 2005). Such immunomodulatory effects of MSCs might be associated with EVs which they release to the environment. It has been shown that EVs released from MSCs can inhibit the proliferation and differentiation of B lymphocytes in a dose-dependent manner (Budoni et al., 2013). Also, EVs produced by murine BM-MSCs inhibit the proliferation of T lymphocytes and modulate the adaptive immune system in mice via inducing of apoptosis in the activated T lymphocytes. This increases the number of regulatory T cells, and enhancing the secretion of anti-inflammatory cytokines such as IL-10 and TGF-β1 (Mokarizadeh et al., 2012). Notably, it has been reported that galectin-1 and programmed death receptor ligand (PD-L1) were present on the surface of EVs derived from MSCs (Garín et al., 2007). Endogenous galectin-1 induces apoptosis in the activated T lymphocytes and provoke the maturation of regulatory T lymphocytes (Rabinovich et al., 2000). PD-L1, on the other hand, is a ligand of the PD-1 receptor and induces the proliferation of regulatory T cells proliferation. TGF-β is another component of MSC-EVs, which also activates the formation of regulatory T cells (Chen et al., 2003).

The MSC-EVs have been shown to exhibit immunomodulatory properties *in vivo*. For example, it was shown that EV-treated mice demonstrated lower white blood cells (WBCs) counts and decreased neutrophil and monocyte influx into the hearts after myocardial ischemia reperfusion (MI/R) injury as compared to controls (Arslan et al., 2013). Also, MSC-EVs were able to switch the macrophages from a pro-inflammatory (M1 macrophages) to an anti-inflammatory (M2 macrophages) in the cardiomyopathy mice model and reduced the secretion of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α (Sun et al., 2018). The immunosuppressive effects of MSC-EVs on the liver injury animal models were also reported. It has been shown that the expression of pro-inflammatory cytokines such as IL-1, IL-2, TNF-α, IFN-γ was reduced while anti-inflammatory cytokines including TGF-β and HGF, and the number of T regulatory cells increased in the liver tissue following treatment with MSC-EVs (Tamura et al., 2016).

Mesenchymal Stem Cell Therapy for COVID-19 and the Other Viral Infections

Coronavirus disease 2019 (COVID-19) is a life-threatening infectious disease caused by a newly emerged coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Patients infected with this virus will experience mild to severe respiratory disease (Rezasoltani et al., 2020a). There are currently no specific antiviral treatments licensed for COVID-19, however many treatments are under investigation (Rezasoltani et al., 2020b; Shpichka et al., 2021). Hopefully, the management of severe acute respiratory infection form of COVID-19 significantly can decline the death rate, particularly within the high-risk people. Several preclinical and clinical studies have exhibited the effects of exosomes and MSC-EVs in decreasing cytokine storm-associated complications, such as alveolar inflammation, edema, and epithelial tissue regeneration in inflammatory diseases, such as chronic obstructive pulmonary disease (COPD), asthma, acute respiratory distress syndrome (ARDS), and acute lung injury (ALI) (Akbari and Rezaie, 2020; Keller et al., 2020). Recently, MSCs-based immunomodulation therapy has been suggested as an effective treatment option for COVID-19 and multiple clinical trials have been launched so far (Akbari and Rezaie, 2020; Golchin et al., 2020). Since it has been suggested that the therapeutic effects of MSCs are essentially due to their secreted EVs, clinical trials may begin to apply MSC-derived exosomes and their EVs to alleviate the cytokine storm in severe COVID-19 patients (Akbari and Rezaie, 2020). However, there are some concerns over the safety, efficacy, and scalability of clinical-grade MSC-EVs (Golchin et al., 2020).

Secretion of Antimicrobial Peptides and Proteins

Associated molecular patterns are a miscellaneous class of naturally occurring small effector molecules that play a key role as the first line of defense by all multicellular organisms. AMPs can have wide killing activity against different types of microorganisms and even cancer cells. These biomolecules can also be referred to as 'host defense peptides', highlighting their additional immunomodulatory functions. Such functions are diverse, unique to AMP type, and involve a number of growth factor-like and cytokine effects that are contributed to normal immune homeostasis status (Seyfi et al., 2020). Some studies showed that MSCs elicit potent antimicrobial activities via indirect and direct mechanisms, partially mediated by the production of antimicrobial peptides and proteins (AMPs) of members of the cathelicidins, defensins, hepcidin, or lipocalin

families as discussed below (Krasnodembskaya et al., 2010; Sung et al., 2016; Alcayaga-Miranda et al., 2017).

Cathelicidin LL-37

Cathelicidin LL-37 is the C-terminal part of the host cathelicidin, called human cationic antimicrobial protein (hCAP18), which is mostly produced by epithelial cells and neutrophils. The cathelicidin hCAP18/LL-37 is a multifunctional molecule that may regulate different human cellular and molecular processes such as epithelial cell activation, chemotaxis, bactericidal function, angiogenesis, and activation of cytokine and chemokine production. This antimicrobial peptide is produced from host cells upon infection of mycobacteria and exerts a bactericidal activity (Sandra Tjabringa et al., 2005). Besides a broad range of antimicrobial activity, LL-37 shows multiple immunomodulatory effects, anticancer functions, and also pro-angiogenic and chemotactic features. LL-37 has been found in many types of body fluids, tissues, and cells, and along with AMPs plays a critical role in host mucosal defense against microbial infections (Alcayaga-Miranda et al., 2017).

Human β-defensin-2

The hBD-2 is a cysteine-rich, cationic, low molecular weight antimicrobial peptide that is predominantly microbicidal against Gram-negative bacteria. It is expressed by many epithelial cells, granulocytes, and MSCs (Alcayaga-Miranda et al., 2017). The hBD-2 is a remarkable, inducible, antimicrobial peptide in a variety of epithelial cell types including skin cells, airways, kidney, oral mucosa, and gastrointestinal tract (Harder et al., 2000; Kumar et al., 2006). Its production is also induced by pro-inflammatory stimuli such as TNFα or microorganisms. The hBD-2 serves as a dynamic part of the local epithelial defense system of respiratory tract and skin which protect surfaces from infection. This is the reason why lung and skin infections caused by Gram-negative pathogens are rather rare (Li et al., 2016). Thus, based on significant antimicrobial and antiviral functions, modulating endogenous production of defensin by certain regulatory factors makes them promising therapeutic options against microbial infections.

Hepcidin

Hepcidin is a peptide encoded by the *HAMP* gene in human and is a natural host defense peptide found in urine (Nikfarjam et al., 2020) and plasma (Babaei and Rezaie, 2021). This peptide produces mainly by hepatocytes but other cells such as MSCs and myeloid leukocytes are also produce and release these peptides (Balhuizen et al., 2021). Two forms of hepcidin peptide, hep-20 and hep-25, exhibit antimicrobial properties (Nikfarjam et al., 2020) but hep-25 (LEAP-1) is also involved in the iron regulation (Chronopoulos and Kalluri, 2020). Beyond the iron regulatory effects, hepcidin has a broad spectrum of antibacterial and antifungal activity. For example, the antibacterial activity of this peptide against *Escherichia coli*, *S. epidermidis*, *S. aureus*, and group *B streptococci* has been shown previously which demonstrates its role as an antimicrobial peptide (Bitto et al., 2017). Incorporation of hepcidin into the EVs derived from the hepatocytes, MSCs, and myeloid leukocytes can be a mechanism for the prevention of microbial diseases.

Lipocalin-2

Lipocalin-2, also known as neutrophil gelatinase-associated lipocalin (NGAL), siderocalin, or 24p3, is a protein mainly secreted by neutrophils in response to infection and inflammation (Deng et al., 2020). Lnc2 blocks the siderophore iron-acquiring strategy of bacteria which leads to bacterial growth inhibition. It was shown that Lcn2-deficient ($Lcn2^{-/-}$) mice were more sensitive than wild-type mice to bacterial infection (Nolte-'t Hoen et al., 2016; Urbanelli et al., 2019). Moreover, Lcn2 is one of the components of the innate immune response against bacterial infection (Deng et al., 2020). MSCs are able to produce the Lnc2 protein and upregulation of this protein is directly corrected with bacterial clearance. Administration of antibodies against the Lnc2 protein have been found to block antimicrobial effects of MSCs (Riley et al., 2013).

Indoleamine 2, 3-dioxygenase

Mesenchymal stem cells secrete several soluble factors, including indoleamine 2, 3-dioxygenase (IDO). IDO is a tryptophan-degrading enzyme with antibacterial properties. IDO is involved in the antibacterial defense of some human cells and this was shown by using IDO specific inhibitors or by antagonizing the antibacterial effect with supplemental tryptophan. This enzyme acts against both intracellular (especially Chlamydia species) (Golchin et al., 2020; Dabrowska et al., 2021) and extracellular bacteria such as *Staphylococcus aureus*, *Streptococcus suis*, *enterococci*, and group *B streptococci* (Sandra Tjabringa et al., 2005; Alcayaga-Miranda et al., 2017). EVs derived from MSCs and containing IDO might be able to fight microbial diseases and reduce their growth rate *in vitro*.

Interleukin-17

Interleukin-17 (IL-17), a pro-inflammatory cytokine, contributes to host defense against both extracellular and intracellular pathogens. The antibacterial properties of this cytokine against *Aspergillus fumigatus*, *Cryptococcus neoformans*, and *Candida albicans* were demonstrated (Lombardi et al., 2015; Wang Q. et al., 2019). A variety of cells including $CD4^+$ Th17 cells, $CD8^+$ T cells (Tc17), natural killer T (NKT) cells, macrophages, and IL-17^+ MSCs have the capacity to produce IL-7 (Yang et al., 2013; Mardpour et al., 2018; Duong et al., 2019; Shpichka et al., 2021). The IL-17^+ MSCs are able to inhibit the growth of *C. albicans in vitro* and have a therapeutic effects on *C. albicans*-infected mice (Schroten et al., 2001). EVs derived from these MSCs, can inhibit bacterial growth.

Potential Application of Stem Cell-Derived Extracellular Vesicles on Pathogenic Microbes

Extracellular Vesicles as a Unique Drug Delivery System

Targeted drug delivery is among the most significant challenge in pharmacology and pharmaceutical sciences (Nolte-'t Hoen et al., 2016). Distinctive properties of EVs favor their utilization as novel DDSs over synthetic ones (**Figure 2**). These characteristics include their capability to cross physical barriers, their biocompatibility, their inherent targeting features, and also

their ability to exploit natural intracellular trafficking pathways (Elsharkasy et al., 2020). Interestingly, viruses incorporate specific binding proteins, and thus are highly targeted mostly due to their evolved and acquired high specificity toward their cellular targets. The EVs membrane can be engineered to incorporate with such specific viral proteins to facilitate EV-mediated transfer of drugs (György et al., 2015). Also, genetically manipulated cells from which EVs are originated, have been developed to provide distinct platforms for loading cargo and conjugation of targeting moieties to their EVs. However, further in-depth investigations into EV biogenesis, EV subpopulations, cargo sorting, internalization and trafficking routes in recipient cells are required to achieve translational applications of such engineered EVs. Furthermore, there are a number of obstacles that should be addressed toward clinical use and include scale-up of the EV production and isolation process, as well as standard protocols for proper banking (Elsharkasy et al., 2020).

Decoy Exosomes

Decoy exosomes represent a new class of therapeutic biologics that are generated by molecular engineering approaches to treat human diseases including inflammation, cancer, and cardiovascular disorders (Zhang et al., 2021). This type of exosomes functions as a biological sponge to absorb and antagonize detrimental factors such as bacterial toxins and inflammatory mediators particularly, TNFα, in host blood or tissues (**Figure 2**; Duong et al., 2019). Nonetheless, the scale-up production of decoy exosomes from more suitable producing cells is essential to obtain high-quality exosomes for therapeutic utilization against infections and inflammatory diseases.

Extracellular Vesicles as Vaccine Platform

Extracellular vesicles from pathogenic bacteria usually carry PAMPs and MAMPs which authorize them to activate the immune response, macking them the ability to be applied as vaccine candidates (**Figure 2**; Sharpe Samantha et al., 2011; Gorringe and Pajón, 2012; Bartolini et al., 2013; Mehanny et al., 2020). For instance, the BEVs from *Neisseria meningitides*, have been applied as the basis for a vaccine against meningococcal disease, as they induce antibacterial immune responses (Gorringe and Pajón, 2012). The outstanding outcomes of BEVs-based vaccines demonstrated a new avenue and proposed novel strategies to immunize individuals against pathogenic bacteria (Gorringe and Pajón, 2012; Fantappiè et al., 2014; Shkair et al., 2021). Moreover, EVs derived after viral inoculation may further be applied to develop more effective vaccines against viral infections by adding or expelling certain subpopulations of them. In contrast to utilizing pathogenic BEVs for vaccine targets, it has been proposed that BEVs originated from symbiotic bacteria may exhibit modulatory effects on host immune system. For instance, *Bacteroides fragilis* can selectively deliver capsular polysaccharide

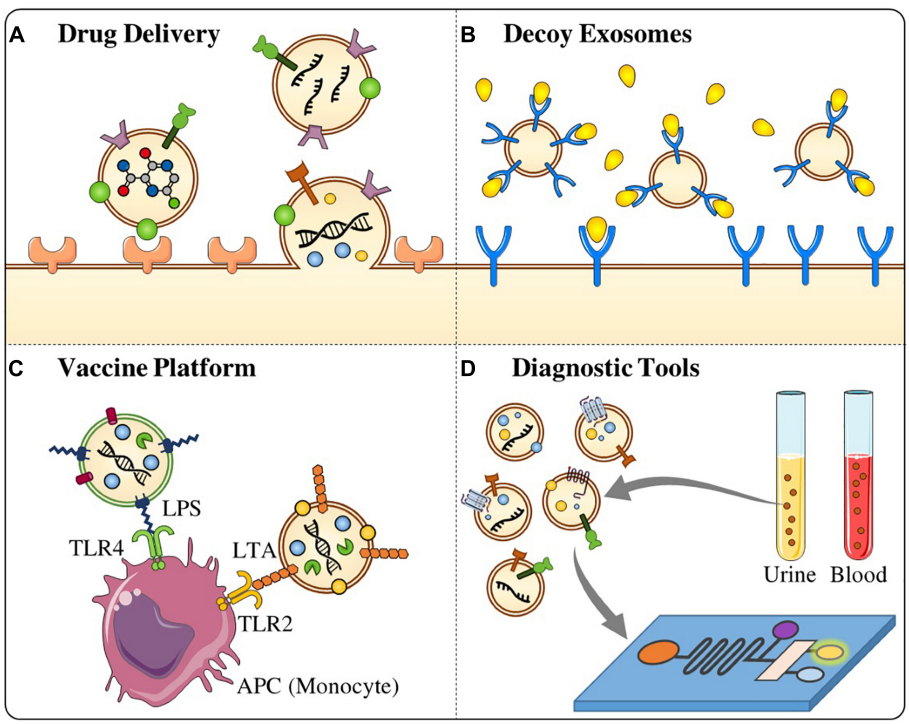

FIGURE 2 | Potential application of stem cell-derived EVs on pathogenic microbes. **(A)** Stem cell-derived EVs can be used as a targeted drug delivery tool against infectious microbes. **(B)** Decoy exosomes are a biological trap which can absorb and antagonize detrimental factors such as bacterial toxins and inflammatory mediators. **(C)** EVs derived from MSCs are also can be used as vaccine platform to activate the immune response and react against the infectious diseases. **(D)** MSCs-derived EVs are good candidate for the development of diagnostic tools as they are involved in several biological processes and isolated from different biofluids.

A (PSA) cargo in its BEVs that have been shown to induce immunomodulatory responses and prevent colitis in mice. These data support the rationale for designation of novel probiotic formulations based on specific beneficial BEVs which can be used for therapeutic purposes (Muraca et al., 2015; Choi et al., 2017).

Cell-Derived Extracellular Vesicles as Diagnostic Tools

Extracellular vesicles are involved in several biological processes and isolated from different biofluids which make them valuable biomarkers for the early diagnosis or prognosis of various diseases such as cancer, inflammatory diseases, and infections (**Figure 2**; Wei et al., 2021). Thus, these vesicles can be regarded as interesting and non-invasive biomarkers for the diagnosis of different diseases (Wei et al., 2021). EVs isolated from the blood have gained significant interest mainly in the context of tumor diagnosis, and their fluctuations are associated with tumor progression, metastasis, and immune evasion (Palmirotta et al., 2018; Nikfarjam et al., 2020; Salimi et al., 2020). Glypican-1 (GPC1), for instance, as a cell surface proteoglycan, is specifically expressed by exosomes isolated from the serum of pancreatic cancer patients, and it is used as an early biomarker. Further, it has been reported that levels of $GPC1^{+}$ exosomes are correlated with pancreas tumor burden and survival rate (Melo et al., 2015). EVs also can be used for the detection of early stages of metastasis. For example, in the exosomes of patients with metastatic melanoma, MDA-9 and GRP78 proteins have higher expression than those of patients without metastases (Guan et al., 2015). Moreover, EVs as urinary biomarkers have been introduced for the early diagnosis of a variety of kidney and genitourinary tract disorders (Street et al., 2017). Neutrophil gelatinase-associated lipocalin (NGAL) (Alvarez et al., 2013), polycystin-1 (PC1) (Hogan et al., 2015), transmembrane protein 2 (TMEM2) (Hogan et al., 2015), and WT-1 (Wilms' tumor-1) (Zhou H. et al., 2013) are some exosomal biomarkers that can be applied for the diagnosis of renal diseases. Some exosome/EV products are commercially available for the diagnostic purposes. For example, ExoDX Lung (ALK), the world's first exosome-based diagnostic kit, was developed and passed FDA certification in Wei et al. (2021).

As aforementioned, EVs have been considered as reliable biomarkers in the context of infectious diseases. From a diagnostic point of view, EVs carry antigens from parental cells and act as reporters of foreign agents (Yáñez-Mó et al., 2015). In the *M. tuberculosis*-infected patients, mycobacterial proteins responsible for *M. tuberculosis* intracellular survival were identified from their secreted exosomes (Kruh-Garcia et al., 2014). It was shown that the mRNA (Lv et al., 2017) and miRNA (Lyu et al., 2019) profiles of exosomes derived from the sera of healthy cases and patients with active and latent tuberculosis were different, which could be used as a diagnostic biomarker. Moreover, human macrophages infected with *Yersinia pestis* and *Bacillus anthracis* secrete particular miRNA-containing exosomes (Fleming et al., 2014). LPS induces the murine bone marrow-derived dendritic cells (BMDC) to secrete exosomes containing miR-146a and miR-155 (Jiang et al., 2019). *Helicobacter pylori* infection also exhibits an increase in miR-155 level in the exosomes derived from macrophages (Wang et al., 2016). These miRNAs are proper biomarkers for the rapid detection of such infectious diseases.

THE DRIVER CHALLENGES FOR THE APPLICATION OF STEM CELL-DERIVED EXTRACELLULAR VESICLES IN CLINIC

The process of manufacturing MSC-EVs in the clinic needs donor identification and screening. Donor identification and screening need a complete review of risk factors, relevant communicable disease agents, and diseases as outlined in FDA 21 CFR Donor Screening 1271.75. Common sources of MSCs and then EVs for the clinical application are those derived from bone marrow and adipose tissue. The EVs harvested from these tissues are then further characterized for their identity, purity, potency, and sterility. Currently, several challenges for manufacturing the clinical-grade EVs are ongoing, and many modifications and optimizations are needed to ensure the safety and reproducibility of the EVs as therapeutic agents (Wiest and Zubair, 2020). Some of the challenges are briefly described below (**Figure 3**).

Identity

Due to their nanometer to micrometer scale, detection of EVs by means of currently available lab equipment is challenging. Also, the tissue sources of the MSCs have an impact on the EVs characteristics. It was shown that the origin of the MSCs has an impact on the amounts and sizes of EVs. Exosome samples isolated by ultracentrifugation (UC) and tangential flow filtration (TFF) showed abundant expression of CD81 and CD9 markers and depletion of calnexin as compared to parental cells, but CD63 marker was only expressed on UC-isolated EVs (Haraszti et al., 2018). Therefore, different isolation methods produce different populations of EVs, and thus emphasize the need for standardized good manufacturing practices (GMPs). Because of different components of EVs, some databases such as ExoCarta, Vesiclepedia, and EVpedia have been established in recent years (Keerthikumar et al., 2016; Pathan et al., 2019). These databases have valuable resources for the identification of different EVs, but some specific and universal markers for each EV have not yet been provided. Therefore, further studies are required for the precise characterization of EVs and determining their identity.

Purity

Since EVs are derived from different cells, including MSCs, they may contain some impurities. For example, fetal bovine serum (FBS) is often added to the MSCs culture media. It was shown that FBS fractions contain RNA molecules and deep sequencing of these fractions showed that 13.6% and 21.7% of the RNA in cell pellet and supernatant mapped to the human genome, respectively (Wei et al., 2016). Some laboratories employed the UC for the removal of the majority of EVs and miRNAs found in the FBS (Shelke et al., 2014; Wei et al., 2016). Because of xenobiotic contents of FBS, some manufacturers used human

FIGURE 3 | Main challenges for the application of stem cell-derived EVs in clinic.

platelet lysate instead of FBS. Human platelet lysate is xenobiotic-free and fibrinogen-depleted and can be used for MSCs culture in GMP studies. It was shown that UC could be able to eliminate the serum-derived RNAs in the lysates (Pachler et al., 2017b). It should be noted that EVs isolated by UC and anion exchange chromatography have similar markers and size distribution, but EVs enriched by TFF are not similar (Heath et al., 2018). Other methods were developed to improve the EVs' purity include quantification of protein to particle ratio (Webber and Clayton, 2013) and protein to lipid ratio (Osteikoetxea et al., 2015); however, these methods still have some limitations.

Reproducibility

Many factors influence the content and amount of EVs released by cells and there are currently no standard protocols for the isolation and storage of EVs (Lener et al., 2015). Also, there is MSC donor-to-donor and batch-to-batch variation (Russell et al., 2018). It was shown that EVs are highly sensitive to cell stress, and their content may be changed in response to stress. For example, treatment of human placental cells with tunicamycin induces ER stress in these cells and leads to the release of EVs containing HSP70 and HMGB1 (Collett et al., 2018). The content of the cell culture media, the composition of the serum added to the media, and the drug interactions affect the EVs' integrity (Wiest and Zubair, 2020). Isolation methods also change the composition of EVs. Haraszti et al. showed that when MSCs were grown in 3D culture conditions and isolated by TFF, displayed a different protein content compared to other methods of culturing and isolation (Haraszti et al., 2018). Most studies focus on UC as a gold standard for the isolation of EVs. Busatto et al. (2018) reported that when they used the US for isolation, the size distribution and albumin purity of samples changed significantly from batch-to-batch, but using TFF, in contrast, showed less batch-to-batch differences. These studies highlighted the factors affecting the reproducibility of EVs and emphasized the need for the development and validation of isolation methods.

Sterility and Safety

Sterility tests are assays performed by manufacturers to determine the microbial contaminations. Strict donor eligibility criteria and screening methods for diseases are the first steps for the determination of sterility and safety of the products. The companies generating EVs must comply with FDA Title 21, Part 610 (Wiest and Zubair, 2020). Microbial contamination is important not only for the safety of the recipients, but also some microbes produce and secret EVs which might interfere with the EVs in the therapeutic product (Quah and O'neill, 2007; Giri et al., 2010). The size of some viral particles is similar to EVs which raises some challenges for EV isolation by size-dependent techniques such as TFF and other chromatography-based methods (Matthews, 1975). TFF is better than UC for reducing the risk of contamination because TFF method can be conducted in a closed system, but UC technique requires multiple steps and requires multiple transfer of the fractions to the new containers (Wiest and Zubair, 2020).

Due to cell-free nature, EVs are hypothesized to be safer than other products, but there are limited data about the safety profile of EV-containing compounds (Wiest and Zubair, 2020). A short-term safety study was conducted by Montaner-Tarbes et al. (2018), where they treated healthy pigs with EVs derived from pigs with the porcine syndrome. They found that healthy pigs treated with EVs have no signs of the disease (Montaner-Tarbes et al., 2018). Until recently, adverse events (AEs) or toxicity related to EVs-based treatments are rarely reported (Nassar et al., 2016; Saleh et al., 2019).

Storage

Extracellular vesicles are very sensitive to temperature and pH of the storage buffer (Wiest and Zubair, 2020). The ideal

temperature for the long-time storage of EVs is –80°C (Otrokocsi et al., 2014). It has been shown that the quantity of EVs decreases in a time-dependent manner when they are stored at room temperature or 4°C following isolation. Also, the results of the light-scattering analysis demonstrated a notable time-dependent increase in the structural changes of EVs when they stored for a long time at –20°C (Otrokocsi et al., 2014). Changes in the pH of the storage buffer can induce EVs' aggregation and loss of their functionality (Lener et al., 2015). The storage buffer is also an important factor for maintaining the EVs' functionality. The phosphate-buffered saline was exploited in the majority of published studies as the EVs storage buffer (Li et al., 2019; Marcoux et al., 2019), but others also have used sucrose buffer (Busatto et al., 2018), lactated Ringer's solution (Pachler et al., 2017b), and PBS supplemented with trehalose (Bosch et al., 2016) for the storage of EVs. The type of EVs and their application may require different storage buffers and maintaining conditions.

Potency

Despite the difficulties in identifying active components in the EVs, potency determination becomes more popular in the last few years in preclinical studies (Wiest and Zubair, 2020). Due to the pleiotropic effects of proteins and RNAs contained within EVs, identifying the active ingredients in exosome therapy is challenging. EVs' potency assays are promising methods to overcome the challenges of identifying an active ingredient. The basics of many potency assays is the release of pro-inflammatory cytokines by M1-phenotype macrophages. To do this, EVs must be added to the culture of M1-phenotype macrophages and the desired inflammatory marker is measured based on the dose of EVs (Pachler et al., 2017a; Willis et al., 2018). These methods are used frequently for the potency evaluation but further research is needed to validate their applications.

Large-Scale Production

Large-scale production of EVs for clinical applications needs scale-up culture of MSCs, but long-term passaging may result in losing clonal and differentiation capacity of cells (Raghav et al., 2021). Therefore, it is necessary to develop new methods for reliable expansion of MSCs to mass-produce EVs for clinical use. Also, large-scale culture of MSCs in bioreactor requires the addition of some ingredients such as fibronectin for cell adherence purposes. Fibronectin as an ingredient has some complications because this protein makes clogging in filter pores and interferes with the size-based selection of EVs using the TFF method. Therefore the conditioned media need an extra centrifugation step, which make the risk of contamination more (Wiest and Zubair, 2020).

Developing a Good Manufacturing Practice Protocol

For the production of EVs in large quantities, the companies demand a standardized manufacturing process which must comply with GMP regulations (Mobarak et al., 2021). The GMP-grade production of EVs is a process that depends on the cell type, culture media, cultivation, and purification methods. In the case of cell type, five cell types including bone marrow and adipose tissue-derived MSCs, monocyte-derived dendritic cells (DCs), human cardiac progenitor cells, and HEK293 cells have been used in GMP-grade for production of EVs. Cultivation methods employ both static and dynamic systems. Flask based systems are static but bioreactor systems are dynamic (Rezaie et al., 2021). In the GMP-grade production of EVs, bioreactor systems are preferred due to the dynamic monitoring system (Akbari and Rezaie, 2020). In the case of purification of GMP-grade products, a number of steps including filtration for the removal of cell debris, centrifugation for enrichment of the conditioned media, and isolation of EVs from the media

TABLE 1 | Current biomedical applications of Extracellular vesicles.

EVs in Biomedicine			References
Bacterial EVs	EVs as vaccine candidate (outer membrane vesicles (OMVs))		Balhuizen et al., 2021
	EVs as anticancer drugs (membrane vesicles (MVs))		Chronopoulos and Kalluri, 2020
	Role of EVs in antibiotic resistance and biofilm formation		Bitto et al., 2017
	EVs as immune modulator factors		Riley et al., 2013
	EVs as infection biomarkers		Deng et al., 2020
Viral EVs	EVs can facilitate viral infection		Nolte-'t Hoen et al., 2016
	EVs can intensify inflammatory responses and deflagrate antiviral activities		Urbanelli et al., 2019
MSCs-derived EVs	Immunomodulatory functions of EVs derived from MSCs		Dabrowska et al., 2021
	MSCs-derived EVs for treatment of COVID-19 and other viral infections		Golchin et al., 2020
	Secretion of antimicrobial peptides and proteins (AMPs) loaded in EVs	Cathelicidin LL-37	Sandra Tjabringa et al., 2005
		Human β-defensin-2 (hBD-2)	Alcayaga-Miranda et al., 2017
		Hepcidin	Lombardi et al., 2015
		Lipocalin-2 (Lcn2)	Wang Q. et al., 2019
		Indoleamine 2, 3-dioxygenase (IDO)	Schroten et al., 2001
		Interleukin-17 (IL-17)	Yang et al., 2013
	Decoy EVs provide protection against bacterial toxins		Keller et al., 2020

should be performed. Differential centrifugation, despite its complications, is the preferred method for concentrating the conditioned media (Salimi et al., 2020).

CONCLUSION

In recent years we have witnessed remarkable progress in the biology of EVs and their impact on microbial diseases. Now, a clear picture has emerged and showed that MSCs-derived EVs may play a crucial role in infectious diseases. MSCs-derived EVs retain the biological activity of parental MSCs and have a similar therapeutic potential. Evs derived from MSCs have potent antimicrobial activity by production of antimicrobial peptides and proteins (AMPs) such as hepcidin, lipocalin, defencins, etc. Also, MSCs-derived EVs are applicable in drug delivery systems, vaccine platform, and diagnostic tools to fight infectious diseases. In clinic, several challenges exist for the manufacturing clinical- and GMP-grade EVs which needs to be addressed. Further understanding of the manufacturing of EVs for clinical application, their biogenesis method as well as their optimization method can reduce many of the challenges in using MSCs-derived EVs in the clinic. Taken together, these data suggest that MSC-derived EVs could be promising therapeutic tool for the treatment of infectious diseases.

AUTHOR CONTRIBUTIONS

HK involved in drafting and editing. BS involved in drafting and figures. SR and NH-K involved in drafting. AY and MH involved in conceptualization, editing and proofreading. MV involved in conceptualization, reviewing and editing the draft, and final approval. All authors contributed to the article and approved the submitted version.

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REVIEW
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Algal Cells-Derived Extracellular Vesicles: A Review With Special Emphasis on Their Antimicrobial Effects

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Extracellular vesicles (EVs) originated from different cells of approximately all kinds of organisms, recently got more attention because of their potential in the treatment of diseases and reconstructive medicine. To date, lots of studies have been performed on mammalian-derived vesicles, but little attention has been paid to algae and marine cells as valuable sources of EVs. Proving the promising role of EVs in medicine requires sufficient resources to produce qualified microvesicles. Algae, same as its other sister groups, such as plants, have stem cells and stem cell niches. Previous studies showed the EVs in plants and marine cells. So, this study was set out to talk about algal extracellular vesicles. EVs play a major role in cell-to-cell communication to convey molecules, such as RNA/DNA, metabolites, proteins, and lipids within. The components of EVs depends on the origin of the primitive cells or tissues and the isolation method. Sufficient resources are needed to produce high-quality, stable, and compatible EVs as a drug or drug delivery system. Plant stem cells have great potential as a new controllable resource for the production of EVs. The EVs secreted from stem cells can easily be extracted from the cell culture medium and evaluated for medicinal uses. In this review, the aim is to introduce algae stem cells as well as EVs derived from algal cells. In the following, the production of the EVs, the properties of EVs extracted from these sources and their antimicrobial effects will be discussed.

Keywords: exosome, algae, stem cell, antimicrobial, marine

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INTRODUCTION

Algae have been recently interested by scientists from different aspects including their stem cells and extracellular vesicles (EVs). Unlike animals, which typically develop through division of small undifferentiated stem cells, algal development (apart from undifferentiated apical stem cells) often involves fully differentiated cells that have low capacity for division and then revert to a state where nuclear mitoses and cell division resume.

Most complex multicellular algae usually have well-defined meristems that generate a diversity of differentiated cell types and tissues. These tissues typically include outer layers with protective

epidermal cells and cells adapted for photosynthesis and interior cell layers that have more structural, reproductive, or transport functions. These meristems are niches for stem cells (Lenhard and Laux, 2003; Dodueva et al., 2017; Warghat et al., 2018). Stem cells are small morphologically undifferentiated cells with large nuclear/cytoplasmic ratios and a scant and poorly differentiated cytoplasm. Their main properties are a potential to go through numerous cycles of cell division while maintaining their undifferentiated state (self-renewal) and the potential to differentiate into one or more differentiated cell types (potency or potential). During tissue damage, stem cells take a main part in the maintenance of cell homeostasis and cell recovery. These stem cells need to be reached nearby or far away target cells during embryonic development and regeneration of adult tissues (Dodueva et al., 2017; Warghat et al., 2018). This communication is possible through soluble agents, direct cell-to-cell contact through long, thin tubular appendages, such as the cytonemes and cilia, or secretion of EVs (Jill Harrison, 2017). Recently, researchers have focused on plant's-derived EVs as a flexible and suitable alternative to mammalian's EVs regarding the role of EVs in cell-to-cell communications and its impact on medicine. This study was set out to evaluate the EVs of the algal cells and their possible antimicrobial effects.

STEM CELL IN PLANTS AND ALGAE

It has been accepted that within both animalia and plantae, there is a special space called stem cell niches where stem cells are located. Animal stem cells are classified based on their ability to generate either a wide or a restricted pool of descendant cell types (Terskikh et al., 2006). The zygotic cell is the only mammalian totipotent cell that generates all embryonic and extraembryonic cell types. During embryonic development, pluripotent stem cells give rise to embryonic germ layers but can no longer produce an entire embryo. Finally, multipotent stem cells can only yield a range of different cells belonging to a single tissue.

Laux (2003) defined plantae stem cells as those in which generated daughter cells can either maintain stem cells nature and generate new stem cells, or undergo differentiation, which can include apical cells in tip-growing plants as well as intercalary meristems located within or at the base of plant organs. The studies on the genetic labeling of stem cells revealed that the whole body of a mature plant descends from small groups of stem cells in their growing apices, which these stem cells are maintained by signals from other neighbor cells.

Activation of cell fate in plants is space-dependent. Every plant cell follows a developmental program, which is driven by the position of the cell concerning its surrounding, rather than by the lineage-based differentiation program seen in animals. For this reason, differentiated tissues in plants can regenerate a totipotent embryo or a callus. A set of events that follow cell division and differentiation performed by a stem cell and its daughter cells summarizes the three defining characteristics for a stem cell; self-renewal, possession of undifferentiated characteristics, and ability to differentiate into an array of specialized cells (Scheres, 2007). In terrestrial plant biology, stem cells are largely considered in the context of meristems.

Eukaryotic algae are a diverse polyphyletic assemblage assigned to the kingdoms Chromista, Plantae, and Protozoa (Guiry and Guiry, 2018). The algae have differences with land plants from the appearance to the reproduction process. However, due to the fact that algae and land plants are a sisters' group, they have some similarities, for instance, the existence of meristem, cell wall, totipotent cells, and etc. (McCourt et al., 2004). Due to these similarities, being a sister group with algae, and the reports of few studies on algal stem cells and EVs, the land plants were considered as an example for comparison with the plantae kingdom in order to more explain algae function and physiology. Moreover, because the algae have evolved multiple times independently of animals and land plants, they are natural experiments by which to explore the most diverse modes of cellular totipotency and stem cell ontogenies; algal multicellular body plans originated multiple times within diverse classes of Chromista and Plantae. Three algal lineages stand out for their complex morphologies and high diversity: brown algae (class: Phaeophyceae, with over 2,000 species); red algae (phylum: Rhodophyta, with over 7,500 species); and green algae (subkingdom: Viridiplantae in part the remainder being land plants).

Most complex multicellular algae usually have well-defined meristems generating a diversity of differentiated cell types and tissues. These tissues typically include outer layers with protective epidermal cells and cells adapted for photosynthesis and interior cell layers that have more structural, reproductive, or transport functions. Rigid cell walls constrain algal and land plant cells, including their stem cells, obscuring their functional homology with animal stem cells. Nevertheless, many of the properties of animal stem cells are also found in terrestrial plants, e.g., those associated with root and shoot apical meristems (Laux, 2003; Ivanov, 2007; Dodueva et al., 2017; Warghat et al., 2018), as it could be in the multicellular algae.

In multicellular algae, ontogeny generally can follow one of two developmental patterns: diffuse growth in which cell divisions can occur more or less throughout tissues of the organism, or division of dedicated stem cells, either solitary or in meristems, mostly apical, but sometimes intercalary. Diffuse growth, whether it occurs in multicellular filaments (e.g., the water silk *Spirogyra*) or multicellular sheets (e.g., the sea lettuce *Ulva*), results in little cell diversity and no identifiable set-aside cells, although the cells demonstrate virtual totipotency that is revealed through regeneration of a new thallus from thallus fragments or artificially created protoplasts.

It is not surprising that with the diversity of body plans, brown algae have a corresponding diversity of apical systems. Apical growth is considered ancestral in the class, as it is in land plants (bryophyte and vascular plants) and related green algae (Jill Harrison, 2017) and is typically generated by a single prominent apical cell at the apex of a filament or blade (e.g., *Dictyota*) or a band of apical cells at a blade apex (e.g., *Syringoderma* and *Padina*). In fucoids, brown algae, this apical cell is maintained in an apical pit and cuts-off derivatives from

a mostly three-sided apical cell analogous to that in primitive mosses and liverworts (Renzaglia et al., 2018), which gives these algae the ability to generate three-dimensional forms like those of land plants. The convergent evolution of fucoid and land plant apical systems results in similar regular patterns (phyllotaxy) of lateral-branch or lateral-organ placement around the main axis of the plant body below the central apical cell or meristem that conforms to the Fibonacci series (Peaucelle and Couder, 2016).

Brown algae have two kinds of intercalary meristems: (1) trichothallic meristems in which cell division occurs at the base of a multicellular hair to produce filamentous or syntagmatic thalli and (2) more elaborate meristems that give rise to parenchymatous systems in kelp, another type of brown algae (Kawai and Henry, 2017). The intercalary meristems in kelp (i.e., in the order Laminariales) are analogous to certain types of terrestrial plant meristems. With elaborate differentiation yielding multiple cell types including outer epidermal cells, photosynthetic cells, and interior structural and transport cells, kelps resemble vascular plants in their complexity of cells and tissues. In most kelp species, individual plants consist of a stipe or stem-like organ that supports a blade, a flattened leaf-like organ. An intercalary multicellular meristem region of stem cells at the junction of these organs produces the cells required for elongation of both the stipe and the blade. These meristems can remain active for years. Perennial temperate to arctic species can exhibit seasonal growth cycles in which the blades detach above the meristem and a new one is regenerated *de novo* (e.g., *Laminaria hyperborea*). The kelp intercalary meristem is analogous to the vascular cambium (an interior ring of stem cells found in the stems of vascular plants), where cell division on one side of the ring produces cells that differentiate into water-transporting xylem and on the other side into photosynthate-transporting phloem, although functionally, they most resemble the intercalary meristem at the base of hornwort sporophytes or at the base of grass leaves.

Plant biologists recognize that protoplasts could take a prominent role in plant and algal cell totipotency, so it has a noticeable impact on algae and plant biotechnology (Reddy et al., 2008; Baweja et al., 2009; Baweja and Sahoo, 2009). These wall-less cells generate artificial stem cells that can be used on other cells, or used to induce somatic cell embryo formation (plant cloning), hybridize somatic cells, and genetically transform cells. The totipotency of protoplasts obtained from red, green, and brown multicellular algae and has been evaluated in culture (Kevekordes et al., 1993; Reddy et al., 2006; Fukui et al., 2014). Therefore, it can be concluded that even differentiated cells can return to stem cells with full totipotency if their cell walls and adjacent cells are removed.

PLANTS AND ALGAL STEM CELL'S ABILITY TO EXCRETE EXTRACELLULAR VESICLES

Today, the presence of vesicles has been proven in most prokaryotic and eukaryotic organisms. The nano-sized membrane vesicles are secreted from different cells of these organisms and released into the extracellular environment (Brown et al., 2015).

During tissue damage, stem cells take a main part in the maintenance of cell homeostasis and cell recovery. These cells could be detected using immune-labeling *via* EdU staining. The EdU staining could detect proliferative cells which are one of the characteristics of stem cells. Using this method, the proliferative/stem cells could be isolated and used for further analysis (Hong et al., 2015). Moreover, these stem cells need to be reached nearby or far away target cells during embryonic development and regeneration of adult tissues. This communication is possible through soluble agents, direct cell-to-cell contact through long, thin tubular appendages, such as the cytonemes and cilia, or secretion of extracellular vesicles EVs (EVs). These vesicles with 30–3000 nm in diameters have free diffusion factor properties and a wide cell membrane and cytoplasmic organization. They have distinct biological compositions depending on size and origin and hence, their functions maybe vary (Aliotta et al., 2012; Camussi et al., 2013).

The composition of EVs consists of different molecules, which important components are metabolites, proteins, nucleic acids, and lipids (**Table 1**). The cargo of EVs is mainly dependent on the nature and origin of the primitive cells or tissues and the isolation technique (Kolonics et al., 2020; He et al., 2021). Some of these techniques are ultracentrifugation and chemical precipitation method *via* commercial EVs kit that both are very common in use (Afshar et al., 2021; Zhankina et al., 2021). The maintenance of tissue homeostasis is regarded as one of the most important functions of EVs. There is a mutual interaction between EVs secreted from damaged cells and stem cells, as EVs secreted from injured tissue affect stem cells, reciprocally splashed EVs of stem cells support injured tissue. Hence, the EVs extraction and purification methods highly affect EVs characteristics, the International Society for Extracellular Vesicles (ISEV) has determined standards of EVs purification (Théry et al., 2018; Russell et al., 2019).

As revealed so far, approximately all cells of different organisms can secrete EVs, although they might be different types, pose different functions depending on origin. Therefore, choosing the ideal cells to get EVs with the desired function needs to be concerned. Here, one of the most important sources of EV extraction and its therapeutic effects explain as an example of the therapeutic effects of EVs. The recent studies on mesenchymal stem cells (MSCs) revealed that they can be an effective branch of stem cells in therapeutic applications (Cui et al., 2018; Zhao et al., 2018; Riazifar et al., 2019). Exosomes derived from MSCs have either the advantages of exosomes, or the characteristics of MSCs, and their therapeutic effects have been proved in different diseases in recent studies (Bolivar-Telleria et al., 2018; Moon et al., 2019).

To use the advantages of exosomes in therapeutics, the optimized purification method to get a large amount of non-toxic homogenized exosomes, as well as efficient transfection strategies, is needed (Kooijmans et al., 2012; Yamashita et al., 2018).

Plant-derived exosomes, as one of the sister groups of algae, recently get great attention as a suitable alternative to mammal's

TABLE 1 | The components of EVs(EV) and their biological function (Alfieri et al., 2021).

Component		Biological function
Lipids	Sphingolipids	The high enrichment of GIPCs in plant EVs is suggestive of a signaling function of the EV membrane, especially in the extracellular ROS burst, as proven in Arabidopsis plants
	Glycosylinositolphosphoceramides (GIPCs)	
	phosphatidylethanolamine(PE)	PA is as an important class of lipid messengers involved in many cellular processes such as cytoskeletal organization, cell proliferation, and survival
	phosphatidylcholine (PC)	
	phosphatidylinositol (PI)	
	and phosphatidic acid (PA)	
Proteins	cytosolic proteins (e.g., actin and proteolysis enzymes)	vesicle stability in the case of plasma membrane vesicles purified from broccoli plants
	membrane channel/transporters (e.g., aquaporin and chloride channels)	
	Aquaporin	
	different hydrolases (ATPases, pectinesterase, phospholipases, amylases, _ galactosidases, and adenosylhomocystein hydrolyse),	
	enzymes (SODs, CATs, PODs, and GPXs)	
Nucleic Acids	mRNA, miRNA, DNA	play a role in inter-kingdom communication
Plant Metabolites	carbohydrates (glucose, fructose, sucrose)	Cell homeostasis
	amino acids (alanine, asparagine isoleucine, threonine, leucine)	
	organic acids (mainly glycolic and citric acids),	
	sugars and sugar derivatives	
	bioactive compounds, such as quinic acid, myo-inositol, and aucubin	

exosomes, because of their physiological, chemical, and biological characteristics, which make them a proper candidate to cope with the technical limitations of mammalian vesicles. Regente et al. in 2009 reported the presence of exosome-like vesicles with 50–200 nm in diameter in sunflower seeds (Regente et al., 2009). Far along, the isolation of vesicles by ultracentrifugation from different plant species like grape, grapefruit, ginger, and broccoli (Ju et al., 2013; Wang et al., 2014; Zhuang et al., 2015; Deng et al., 2017) has been reported that allows their effective and abundant production.

Facile large-scale production (Li et al., 2018), low toxicity, reduced immunogenicity (Deng et al., 2017), efficient cellular uptake (Wang et al., 2013), and high biocompatibility and stability (Zhang et al., 2016) make plant-derived EVs as promising therapeutic factors or drug deliver nanoparticles in medical applications in compared with MDEs or artificial nanoparticles.

Despite lots of studies on the bioactive content of the plant EVs, still, further studies are necessary to understand the bioactivities and applications of plant EVs. Besides plant-derived EVs, our knowledge on marine cell-derived EVs remains extremely limited, while they can be a more accessible source to produce a large amount of EVs very fast and easily. Algae as an important marine source for EVs are very economical compared to edible plants and can be grown in any place to get EVs within about 1 week, therefore can establish facile scaled-up production of pure EVs with high quality (Kuruvinashetti et al., 2020).

Therapeutic applications of EVs, in addition to their content, depend on their capability to cross barriers like the cytoplasmic membrane and blood/brain barrier. In mammalian and plant EVs, the mechanism of absorption is different, or they are absorbed either through endocytosis or through the fusion of vesicles and plasma membranes (Rome, 2019). Therefore, membrane properties of exosomes play an important role in crossing cellular barriers. In algae derived EVs, where the membrane is rich in beta proteins, the membranes are easier to attach. Thus, along with biocompatibility, no toxic effect on cells/tissues and organs, nano-nature, increasing circulatory stability, and low immunogenicity make algae a sustainable marine source for the production of exosomes for their potential use in medical and therapeutic applications (Kuruvinashetti et al., 2020).

THE ANTIMICROBIAL EFFECTS OF PLANT'S EVs

Plant-derived EVs because of their biological characteristics got more attention in recent years, many studies emphasize their role in the immune response against invading pathogens (Rybak and Robatzek, 2019; Kolonics et al., 2020). Actually, involving EVs in pathogenesis is two-sided, and some pathogens like bacteria, fungi, and parasites also depend on EVs cargo to exploit their host (Kuipers et al., 2018; Liu et al., 2018; Bielska et al., 2019; Ofir-Birin and Regev-Rudzki, 2019). Therefore, it is accepted that Evs have a key role in plant-pathogen interactions and many studies have been proved it (**Figure 1**; Boevink, 2017; Hansen and Nielsen, 2017; Rutter and Innes, 2018). The first evidence of antimicrobial nature of plant-derived EVs was showed in barley against powdery mildew fungus *Blumeria graminis* (An et al., 2006), later in sunflower against phytopathogenic fungus, *Sclerotinia sclerotiorum* (Regente et al., 2017), and in Arabidopsis against bacterial plant pathogen, *Pseudomonas syringae (*Rutter and Innes, 2016*).*

EVs involving in plant-pathogen interactions as well as the proteome of EV derived from uninfected Arabidopsis rosettes and apoplastic fluids pathogen-infected have been recently analyzed (Rutter and Innes, 2016). EVs derived from extracellular fluids of tomato (De Palma et al., 2020) and sunflower seedlings (Regente et al., 2017) as well as

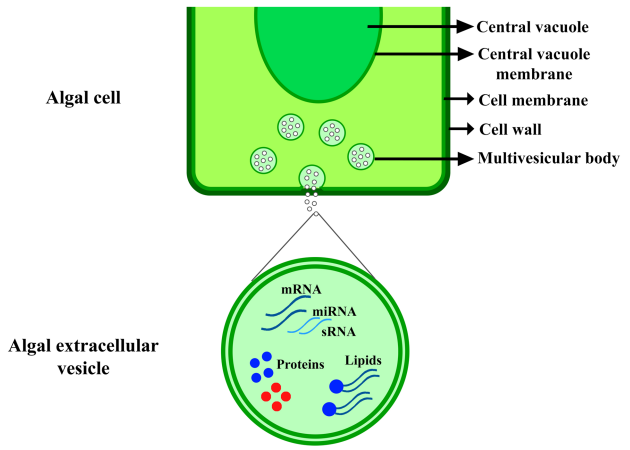

FIGURE 1 | EVs production in algal cell. The extracellular vesicle includes nucleic acids, such as mRNA, microRNA and small RNA (sRNA), proteins, and lipids, which were explained in **Table 1**.

those derived from leaf apoplastic fluid of *N. tabacum* and *C. plantagineum* (Woith et al., 2021) have been known to be involved in plant-microbe interactions. In most cases, EV secretion increases by pathogen invading and raises the severe role of EV in plant defense mechanisms. The proteome analysis of EVs derived from these mentioned plants showed that these EVs are enriched in proteins involved in signal transmission in response to biotic and abiotic stresses, immunity responsible proteins, cell wall remodeling enzymes as well as a protein involved in plant-microbe interactions. **Table 2** lists some of these proteins raised from proteome analysis of plant-derived exosomes.

The proteome analysis is still in the early days and to get a clear clue large amount of replication, precise, and continuous processing of high-quality data and reference genomes are needed; however, the results to date provide candidate logical components in the interactions between plants and pathogens.

Cross-kingdom RNA interference can be explained as one of the possible mechanisms involved in plant-derived EVs-pathogen interaction in plants immunity responses (**Figure 2**). The study of the gray mold caused by *Botrytis cinerea* in *A. thaliana* and *Solanum lycopersicum*, revealed small RNAs (sRNAs) of *B. cinerea* where they were revealed to be transferred from the fungus to the host to silence plant immunity genes (Cai et al., 2019). In response to pathogens, plants deliver sRNAs into the fungus using exosomes to limit the virulence potential of the organism upon knockdown (Lu et al., 2018). These mechanisms are widespread in other pathogens infected plants, such as cotton plants infected by *Verticillium dahliae* (He et al., 2016), and wheat plants for suppressing the invasion of *Fusarium graminearum* (Jiao and Peng, 2018). At some point, additional studies will be needed to better explain the subsets of EVs involving in the transfer of sRNAs into invading pathogens. It appears probable that both

TABLE 2 | Proteins list from proteome analysis of some plant-derived exosomes.

Plant	Description	References
N. tabacum and *C. plantagineum*	annexin D5-like clathrin heavy chain 1-like coatomer subunit alpha-1-like coatomer subunit beta-1 coatomer subunit beta'-2-like coatomer subunit gamma patellin-3-like isoform X2 tetraspanin-3-like tetraspanin-8-like endochitinase EP3-like G-type lectin S-receptor-like serine/threonine-protein kinase At1g34300	Woith et al., 2021
Arabidopsis	RABD2a/ARA5 (Golgi/TGN/EE/secretory vesicles) Plasmodesmata RABG3f (LE/MVB/tonoplast) RABF1/ARA6 (LE/MVB) PM CLC2 (clathrin-coated vesicle pits) GOT1 (Golgi) VAMP711 (tonoplast)	Rutter and Innes, 2016
Tomato	endochitinase patatin-like protein 2 glucan endo-1,3-beta-glucosidase B precursor hypersensitive-induced response protein 1 calmodulin 5,460,408,499 trypsin inhibitor 1-like probable linoleate 9S-lipoxygenase 5 annexin p34 lysM domain-containing GPI-anchored protein 2 ethylene-responsive proteinase inhibitor 1 putative late blight resistance protein homolog R1A-10 putative late blight resistance protein homolog R1A-3 NDR1/HIN1-like protein 3-like isoform X2 putative LRR receptor-like serine/threonine-protein kinase At4g00960 putative late blight resistance protein homolog R1A-3 basic 30 kDa endochitinase germin-like protein subfamily 1 member 19 CASP-like protein PIMP1 probable LRR receptor-like serine/threonine-protein kinase At1g06840 hypersensitive-induced response protein 1 monocopper oxidase-like protein SKU5 wound/stress protein precursor MRLK1 serine/threonine-protein kinase, partial	De Palma et al., 2020

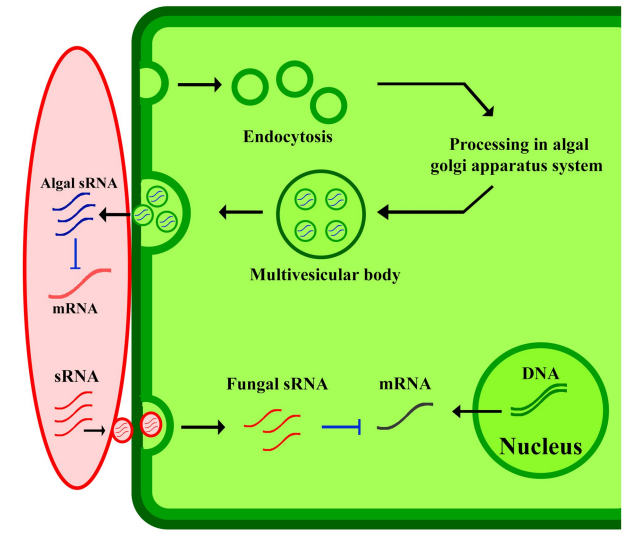

FIGURE 2 | EVs-pathogen interaction in plants immunity responses. The algal sRNA in EVs suppressed fungal mRNA production in algal-fungal interaction.

EV-dependent and -independent mechanisms will be discovered for facilitating this transfer.

THE ANTIMICROBIAL EFFECTS OF ALGAL EVs

Microorganism's communication in the marine environment has a great impact on trophic level interactions and population substitution, awareness of EVs importance in cell-to-cell communication raises the question of how EVs participate in these processes. Early evidence from marine EVs came from model alga *Emiliania huxleyi*, studies demonstrated that vesicles generated over viral infection by this organism act as a pro-viral signal, through accelerating infection and increasing the half-life of the virus in the extracellular milieu (Schatz et al., 2021). Later authors profiled the sRNA cargo of vesicles generated by *E. huxleyi* over bloom succession and concluded that *E. huxleyi*-derived vesicles modulate host-virus dynamics and other components of the microbial food webs, so highlighting the importance of EVs to microbial interactions in the marine environment.

Generally, plant-derived vesicles reveal a broad therapeutic potential, which can help patients, and may establish the future generation of therapeutics.

FUTURE ASPECT OF ANTIMICROBIAL PRODUCTS OF PLANT EVs

To get clear insights on the exact role of EVs originated from different cells, more studies on their biological characteristics and interactions are needed. Studies on plants' EVs revealed similar intrinsic therapeutic materials as mammal's EVs, while there are some advantages on plants' EVs compared to mammal's EVs. First, they can be obtained from a variety of renewable sources; moreover, allowing researchers to select their desired EVs with precise effects on disease, also facilitates its large-scale production. Second, EVs' component seems to be evolved naturally in plant cells which makes them biocompatible and non-toxic. The EVs' lipid membrane stability helps them to be simply adapted to target specific ligands, gives them the potential use as drug delivery nanocarriers. Moreover, plant-derived vesicles can be examined in a comparably short time through eco-friendly protocols (Zhang et al., 2016; Pocsfalvi et al., 2018; Wiklander et al., 2019). Besides these advantages, there are still some concerns on plant EVs to be solved. The standard isolation techniques with low cost and complexity and increase purity should be established for mass-production of high-quality exosomes for the use in therapeutic applications (Ludwig et al., 2019). Primary, the exact content and functionality of the miRNA, mRNA, proteins, and lipids in the exosomes have been unknown so far (Lee et al., 2012). Second, in spite of the developments in exosome isolation methods, a gold standard has not been yet presented (Ludwig et al., 2019). The isolation process cost and difficulty should be decreased, while the exosome purity should be enhanced. Third, mass-production of high-quality exosomes should be probable for the therapeutic applications.

A multi-functional system with a highly efficient isolation technique and real-time quantification and analysis technology is needed for efficient applications. Also, to keep EVs components, such as proteins and RNAs, storing below −70°C (Huang et al., 2020), or freeze-drying is recommended (Charoenviriyakul et al., 2018). Though, long-term preservation using these approaches is still not clarified to be applied in the diagnosis and therapeutic applications (Li et al., 2018). In addition, optimization of isolation approaches for should be performed to obtain uniform nanovesicles. Additionally, a detailed evaluation of their morphological features, the quantitative aspects, and chemical components should be performed to attain evidence on their functional roles. Lastly, exosomes might be the important element in the medicine in future.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Mesenchymal Stem Cell-Derived Exosome Therapy of Microbial Diseases: From Bench to Bed

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Microbial diseases are a global health threat, leading to tremendous casualties and economic losses. The strategy to treat microbial diseases falls into two broad categories: pathogen-directed therapy (PDT) and host-directed therapy (HDT). As the typical PDT, antibiotics or antiviral drugs directly attack bacteria or viruses through discerning specific molecules. However, drug abuse could result in antimicrobial resistance and increase infectious disease morbidity. Recently, the exosome therapy, as a HDT, has attracted extensive attentions for its potential in limiting infectious complications and targeted drug delivery. Mesenchymal stem cell-derived exosomes (MSC-Exos) are the most broadly investigated. In this review, we mainly focus on the development and recent advances of the application of MSC-Exos on microbial diseases. The review starts with the difficulties and current strategies in antimicrobial treatments, followed by a comprehensive overview of exosomes in aspect of isolation, identification, contents, and applications. Then, the underlying mechanisms of the MSC-Exo therapy in microbial diseases are discussed in depth, mainly including immunomodulation, repression of excessive inflammation, and promotion of tissue regeneration. In addition, we highlight the latest progress in the clinical translation of the MSC-Exo therapy, by summarizing related clinical trials, routes of administration, and exosome modifications. This review will provide fundamental insights and future perspectives on MSC-Exo therapy in microbial diseases from bench to bedside.

Keywords: microbial diseases, exosomes, mesenchymal stem cells, cell-free therapy, antibiotic resistance

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INTRODUCTION

Microbial diseases, known as infectious diseases, refer to the clinical manifestation of damage that results from a host-microbe interaction (Casadevall and Pirofski, 2000). Infections can be classified into four broad categories based on phylogenetic groupings of microbes, bacteria, viruses, parasites, and fungi. In Global Risks Reports 2021 from World Economic Forum, infectious diseases rank first by impact among top global risks, and are a leading cause of morbidity and mortality worldwide. The predicament in microbial disease treatments is a consequence of three simultaneous factors. Firstly, antibiotic resistance presents an acute threat

to the effectiveness of available antimicrobial therapies. Original pathogenic microorganisms occasionally reappear in drug-resistant forms, as exemplified by multidrug-resistant *Mycobacterium tuberculosis* (Laxminarayan et al., 2020). Secondly, the eradication of microbes is not equivalent to the termination of clinical symptoms, since immunological damages to the host may persist following a successful antipathogen response (Kaufmann et al., 2018). Finally, new pathogenic microbes keep emerging, for which no therapy exists. Since the outbreak of coronavirus disease 2019 (COVID-19) in late December 2019, it has brought tremendous casualties and economic losses to over 200 countries and regions. Although the pathogenesis of COVID-19 has been fully elucidated, there is no specific therapy for the disease at present (Tsang et al., 2021).

The invention of antimicrobial agents is a remarkable victory in the pathogen-directed therapy (PDT) to treat infectious diseases. However, the efficacy of existing antimicrobials is losing sustainability, as antimicrobials constantly pose selective pressure on mutations in the genes of drug targets (Holmes et al., 2016). It is important to come up with a novel therapy that does not exacerbate antimicrobial resistance. The host-directed therapy (HDT) is a choice, which functions by regulating host cell factors to negatively influence survival or proliferation of microorganisms (Zumla et al., 2016). The application of mesenchymal stem cell-derived exosomes (MSC-Exos) in treatments of microbial diseases is an explorative and promising HDT. Exosomes are double lipid layer vesicles ranging from 30 to 150 nm, basically composed of lipids, proteins, and nucleic acids (Doyle and Wang, 2019). MSC-Exos are commonly used as a source of acellular therapy due to their immunomodulatory, pro-reparative, and drug delivery properties. They have been studied deeply for the application in treatments of several kinds of diseases, such as neurodegenerative diseases (Guy and Offen, 2020), cancers (Vakhshiteh et al., 2019), and injuries in heart (Suzuki et al., 2017; Babaei and Rezaie, 2021), kidney (Nargesi et al., 2017), and nerve (Zhang et al., 2021b). Exosome-based cell-free vaccines are also in development against HIV-1 associated diseases (Rezaie et al., 2021) and cancers (Nikfarjam et al., 2020). Modification of exosomes *via* pre-loading or post-loading approaches can further boost the therapeutic efficacy (Madrigal et al., 2014).

In this review, we mainly focus on the application of MSC-Exos in microbial diseases. Specifically, we update current understandings of MSC-Exo therapy in periodontitis, pneumonia, sepsis, and diabetic foot ulcer (DFU) infection. Then, progresses in clinical translation of exosome therapy are summarized, with the discussion in routes of administration and exosome modification to enhance therapeutic effects of MSC-Exos. Finally, we give perspectives in the future direction of MSC-Exo therapy.

MICROBIAL DISEASE THERAPEUTICS

Based on the target difference, microbial disease therapeutics can be categorized into two strategies: PDT and HDT (Nisini et al., 2020). In this section, we make a detailed introduction to methods involved in these two strategies, and discuss how they complement each other to improve outcomes of microbial diseases. Thereinto, MSC-Exo therapy, originating from MSC therapy, is brought up as a promising HDT candidate.

Pathogen-Directed Therapy

Pathogen-directed therapy, as the name suggests, interacts directly with pathogens (bacteria, viruses, fungi, and parasites) to interrupt their intrusion, survival, and proliferation (Shang et al., 2020). Anti-infective drugs, as the representative, combine directly with the components of pathogens, causing death of pathogens or inhibiting their replication. Among antibacterial, antiviral, antifungal and antiparasitic drugs, antibacterial drugs are by far the most used. They function by interrupting essential bacterial activities, such as destructing cell wall integrity, depolarizing cell membrane potential, suspending DNA replication, and inhibiting protein synthesis (Kohanski et al., 2010; Leekha et al., 2011).

Although the use of antibiotics saved billions of lives in the past over half a century, shortcomings have gradually surfaced (Clardy et al., 2009). Firstly, nonstandard medication accelerates the progress of antimicrobial resistance. Broad-spectrum antibiotics abuse in common infections, which are indications for narrow-spectrum antibacterial agents, raises concerns about their effectiveness in the long term (Holmes et al., 2016). Genes encoding antibiotic resistance are continuously evolving, and are distributed to numerous bacterial species in a plasmid-mediated way (Laxminarayan et al., 2020). Protective mechanisms against antimicrobial agents include expressing drug efflux systems, modifying drug target sites, producing enzymes to destroy drugs, or producing an alternative metabolic pathway to bypass the action of the antimicrobials (Tenover, 2006). The emergence of multidrug-resistant bacteria become increasingly prevalent, such as Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*, which are the most common antibiotic-resistant bacteria (Vivas et al., 2019). Secondly, physicochemical properties of current antimicrobials hold back their efficacy. Hydrophilic antibiotics are inactive against intracellular pathogens, with narrow bio-distribution limited at the extracellular space. They show low permeability toward biological barriers, thus hard to achieve minimum inhibitory concentration at specific sites (e.g., ocular fluid, cerebrospinal fluid, and abscess cavity; Pea et al., 2005).

Other pathogen-directed strategies mainly include antimicrobial peptides (AMPs), antibodies, antimicrobial nanoparticles (NPs), and the CRISPR-Cas system. AMPs are small effector molecules produced naturally or synthetically (Tan et al., 2021), such as cathelicidins, defensins, and hepcidin (Alcayaga-Miranda et al., 2017). They exhibit direct bactericidal properties by physically destroying microbial lipid bilayers to release cell contents (Zhang et al., 2021a). Antimicrobial NPs (metal NPs, semi-conductive NPs, and organic NPs) are promising antimicrobial agents, the underlying basic mechanism of which is related to reactive oxygen species-induced interruption of bacteria membranes (Reshma et al., 2017; Calabrese et al., 2022). Antimicrobial NPs can be applied in the coating for implantable devices (Wang et al., 2017; Fernando et al., 2018) and treatments of superficial infections

(Paladini and Pollini, 2019), whereas the potential toxicity of NPs should not be neglected (Xu et al., 2020). Targeting antimicrobial-resistant plasmid or bacteria genome, the CRISPR-Cas system can induce DNA damage to program bacterial death (Bikard and Barrangou, 2017; Vila, 2018). Nevertheless, the major obstacle lies in the development of specific and efficient delivery approach (Fagen et al., 2017; Fage et al., 2021). It is obvious that new remedies should be exploited to compensate drawbacks of PDT.

Host-Directed Therapy

There is no doubt that antimicrobial therapy is the mainstream treatment for most infectious diseases. However, when confronted with complicated situations such as drug-resistant microbes, biofilm-associated infections, existing antimicrobials lose their efficacy. To counteract the emergence of antimicrobial resistance, a novel anti-infectious therapy focused on the modulation of host response (i.e., HDT) has been proposed. HDT is aimed at improving innate or adaptive protective immune response to control pathogens and/or limit immunopathology. Conventional HDT includes the application of immunomodulators, therapeutic vaccines, repurposed drugs, micronutrients, and stem cell therapy (Zumla and Maeurer, 2016).

Immunomodulatory drugs play important roles in HDT, as they not only promote protective immune responses in acute phase, but also attenuate constant, excessive inflammation in chronic stage (Kilinc et al., 2021). For example, NSAIDs are administrated in the treatment of late-stage multidrug-resistant tuberculosis, to promote phagocytosis and bacterial killing by inhibiting the production of prostaglandin E2 (PGE2) in macrophages (Kroesen et al., 2017). Therapeutic vaccines refer to the injection of pathogen antigens (proteins, nucleic acids) into patients with persistent, recurrent, or chronic infectious diseases. They aim at reducing the severity of the disease or preventing complications, by stimulating the immune defense response (Moingeon et al., 2003; Autran et al., 2004). Drug repurposing is a strategy for identifying new uses of existing drugs for non-communicable diseases (Pushpakom et al., 2019). Cholesterol-lowering drugs, asthma drugs, diabetes drugs, and anticonvulsants are common candidates (Zumla and Maeurer, 2016). Repurposed drugs outrun new drug development in terms of efficiency, lower costs, and safety. Supplementing micronutrients, such as vitamin D, zinc, and probiotics, helps build up immunity (Zumla et al., 2016). Thereinto, probiotics are a novel HDT, in which adequate amounts of probiotic bacteria or bacterial products are administrated to confer health benefits to the host. Underlying mechanisms include competitive colonization with pathogens, promotion of beneficial immune modulation, and suppression of excessive inflammation (Chibbar and Dieleman, 2015).

Stem cell therapy stands out among other host-directed therapies for its unique capability in multi-lineage differentiation and immunomodulation. Its applications in the treatment of various kinds of bacteria and virus infections are supported by solid experimental researches, meanwhile clinical trials are going through to further validate its safety and efficacy (Al-Anazi and Al-Jasser, 2015; Marrazzo et al., 2019; Sleem and Saleh, 2020). Recently, the attention on mesenchymal stem cell (MSC) therapy rockets, with more than 50 clinical trials in progress to evaluate its application on COVID-19-associated acute respiratory distress syndrome (ARDS)/pneumonia (Meng et al., 2020; Shetty et al., 2020). Other hot research fields include septic shock (McIntyre et al., 2018; Schlosser et al., 2019; Laroye et al., 2020), human immunodeficiency virus infection (Allam et al., 2013; Zhang et al., 2013), influenza-associated pneumonia (Darwish et al., 2013), hepatitis B virus-induced liver failure/cirrhosis (Peng et al., 2011; Kantarcioglu et al., 2015; Lin et al., 2017), mycobacterium tuberculosis-induced bone defects (Zhang et al., 2021c), and refractory cytomegalovirus infection (Meisel et al., 2011). MSC therapy has huge potential in adjuvant anti-infectious treatments *via* immunomodulation and tissue repair.

Early studies mainly attribute the therapeutic effects to the homing and differentiation ability of MSCs (Li et al., 2020c). However, recent researches have revealed that MSCs had short survival time after transplantation and only a small proportion of MSCs succeeded in arriving at injured sites (Burst et al., 2010; Xu et al., 2016). It is demonstrated that the essential of therapeutic effects might lie in the secretome of MSCs, which exerts immunomodulatory and reparative properties (Xie et al., 2021). MSCs have active paracrine actions, releasing large amounts of growth factors, cytokines, immunomodulators, and extracellular vesicles (EVs). EVs that are classified into apoptotic vesicles, microvesicles (MVs), and exosomes, play an important role in intercellular and even interorganismal communications. Compared to apoptotic vesicles, MVs and exosomes are the more widely investigated. Assembled in composition and functions, major differences between MVs and exosomes lie in the biogenesis pathway and size. MVs are plasma membrane-derived relatively large EVs, ranging from 100 to 1,000 nm; while exosomes are endosome-origin small EVs with a diameter of 30–150 nm (Cocucci and Meldolesi, 2015; Thery et al., 2018). Due to high similarities in constituent and limitations in available purification methods, some reports have interchangeably used the terms "exosomes" and "MVs" (Lee et al., 2012). In this review, we mainly focus on MSC-Exos, but studies on MSC-EVs or MSC-MVs are also included in consideration of comprehensiveness. Proteomic (Pierce and Kurata, 2021), metabolic, lipidomic (Showalter et al., 2019), and miRNA-sequence analysis (Shao et al., 2017; Zhao et al., 2019a) and experimental studies have indicated that MSC-Exos inherit similar biological properties from their parent cells, in aspect of immunomodulation (Willis et al., 2018), tissue repair promotion (Shao et al., 2017) and homing capacity (Shao et al., 2017; Guo et al., 2019), which are important properties for treatments of microbial diseases. What is more, exosome therapy is superior to stem cell therapy in biosafety. Reports about adverse events of MSC therapy are not uncommon and concerns about MSC therapy have never ceased. There are worries about tumorigenesis, disease transmission, undesired immune responses, replantation on unwanted sites, and administration site reactions (Prockop et al., 2010; Barkholt et al., 2013; Casiraghi et al., 2013; Arango-Rodriguez et al., 2015). In contrast, few serious adverse events are reported in MSC-Exo

therapy. Taken comparable biological properties and superior biosafety, MSC-Exo therapy might be a better choice for HDT.

MESENCHYMAL STEM CELL-DERIVED EXOSOMES

As described in the previous section, MSC-Exos have received increasing attentions for therapeutic administration. Furthermore, a variety of clinical trials are underway for the application of MSC-Exos as a novel, safe and efficacious cell-free therapy in microbial diseases. Below, we give a global perspective regarding biogenesis, isolation, and characterization, as well as their molecular composition of exosomes.

Biogenesis of Exosomes

Exosomes originate in the endosome system, and this process is mediated by several molecules, as illustrated in **Figure 1**. Early endosomes are formed by invagination of the plasma membrane during endocytosis (Huotari and Helenius, 2011). Then they mature into late endosomes, which turn into multivesicular bodies (MVBs), when exosomes are generated as intraluminal vesicles (ILVs) by invagination of late endosome membrane. The MVBs can either be degraded by lysosomes or released into extracellular matrix as exosomes *via* exocytosis (Kowal et al., 2014; Hessvik and Llorente, 2018; Zhang et al., 2019). It has been established that exosomes are actively secreted by almost all cell types especially MSCs, as we describe in detail later. MSCs can be derived from different tissues, such as bone marrow, adipose tissue, dental pulp, and menstrual blood (Shi et al., 2021b). The secreted exosomes could be taken up by recipient cells *via* endocytosis, phagocytosis, or direct membrane fusion, then the contained bioactive cargos are transferred to modify gene expression, signaling, and overall functions and behaviors of recipient cells (Fujita et al., 2015; Rani and Ritter, 2016).

Isolation and Characterization of Exosomes

Exosomes can be directly isolated from cell culture medium or biological fluids, such as urine, breast milk, and amniotic fluid (Thery et al., 2018). The isolation strategies include ultracentrifugation, polymer precipitation, size-exclusion chromatography, and immunoaffinity capture. Ultracentrifugation is the most commonly-used isolation method in basic researches (Gardiner et al., 2016). The typical ultracentrifugation protocol

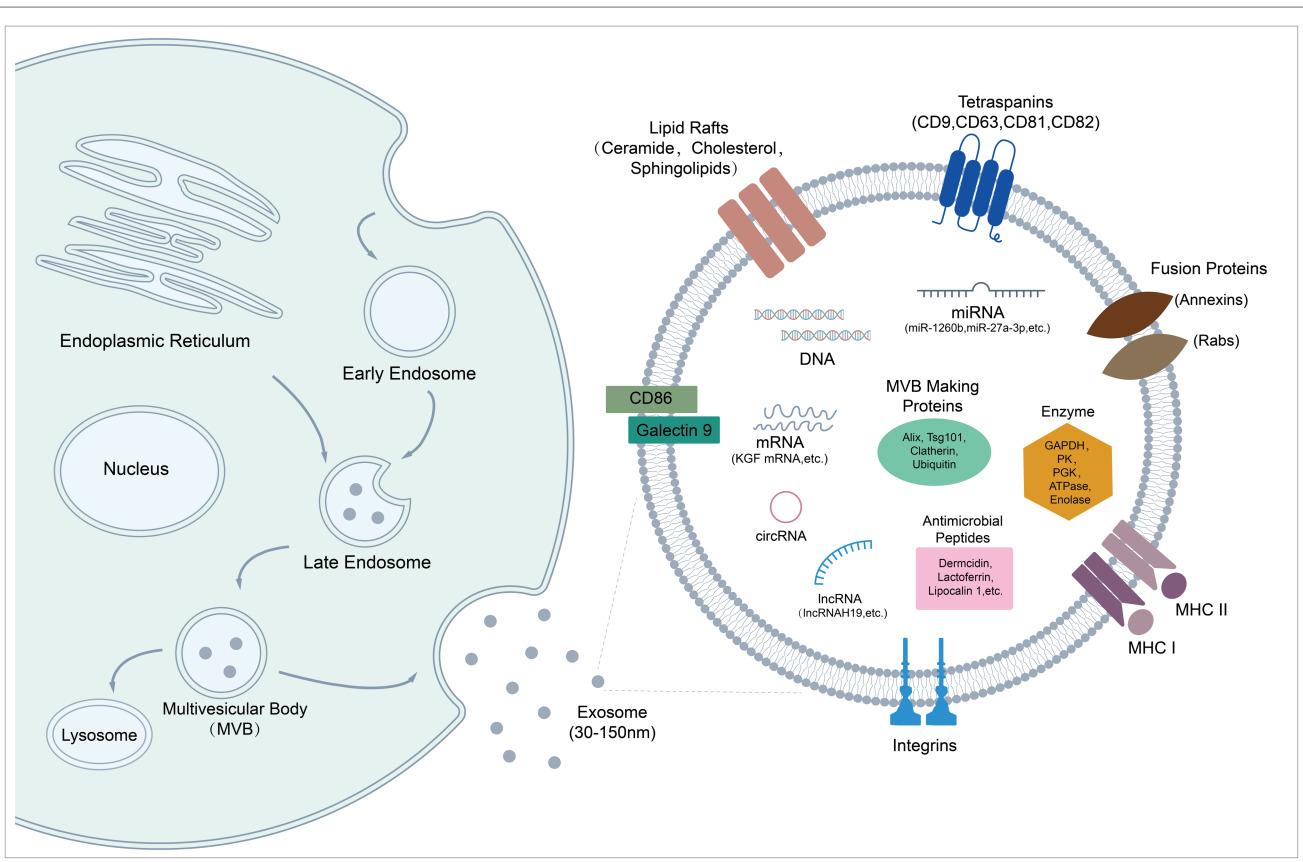

FIGURE 1 | Biogenesis and molecular composition of exosomes. Schematic diagram shows exosome formation and biological cargoes. Exosomes originate in endosome system, and are released from cells as particles (30–150 nm) with a lipid bilayer. They are endowed with therapeutic potential by carrying various kinds of bioactive molecules, such as peptides, microRNAs, and mRNAs.

includes: (i) low-speed (300 × *g* and 2,000 × *g*) centrifugation to remove cells and dead cells respectively; (ii) higher-speed centrifugation (10,000 × *g*) to remove cell debris; and (iii) high-speed centrifugation (100,000 × *g*) to pellet exosomes (Thery et al., 2006). Time consumption and complex operation procedures remain the main disadvantages of ultracentrifugation. Isolation with proprietary polymer precipitation and centrifugation is convenient for small volume samples, but the biggest problem lies in the low purity of exosomes with the contamination of miscellaneous proteins and polymers (Coughlan et al., 2020). Size-exclusion chromatography can screen exosomes of high purity and integrity, in which molecules filtrate through gels at different speeds depending on size difference (Sidhom et al., 2020). In addition, immunoaffinity capture can recover exosomes from complex and viscous fluids, making it a good choice for clinical diagnosis with small-volume plasm (Yang et al., 2017).

Followed by isolation, characterization of exosomes is necessary before therapeutic administration and mechanistic explanation. Various techniques have been developed to confirm the biochemical, biophysical, and biomechanical properties of exosomes. Western blotting is the main tool for general biochemical characterization. MISEV2018 guidelines require the identification of at least three positive protein markers of EVs: (i) transmembrane proteins or GPI-anchored proteins (e.g., CD63, CD81, and CD9); (ii) cytosolic proteins recovered in EVs (e.g., TSG101, ANXA, and HSPA8); and (iii) major components of non-EV co-isolated structures for purity control (e.g., albumin and ribosomal proteins; Thery et al., 2018). For biophysical characterization of single vesicles, electron or atomic force microscopy is necessary to provide both close-up and wide-field images. Apart from that, other techniques are available as a supplement to estimate size, light scattering, and fluorescence properties of exosomes. Tunable resistive pulse sensing provides reliable and fast particle-by-particle measurement of EV size and concentration distribution (Vogel et al., 2016). Nanoparticle tracking analysis can visualize and track the Brownian motion of individual vesicles by light scattering, and make calculation of size distribution and total concentration (Sokolova et al., 2011). High resolution flow cytometry is applicable for exosome immunophenotyping (Nolan and Duggan, 2018).

Molecular Composition of Exosomes

Exosomes are vesicles with a diameter of 30–150 nm, mainly composed of lipids, proteins, and nucleic acids (**Figure 1**; Doyle and Wang, 2019). Exosomes inherit similar but different substances and biological properties from their parent cells. Compared to parent cells, the double membrane structure of exosomes contains a higher content of unsaturated phospholipids and a higher ratio of lipid/protein, which increases the rigidity of exosomes, ensuring relative stability of exosomes in biologic fluids. Furthermore, integrin-associated proteins on the surface protect vesicles from phagocytosis of mononuclear phagocytic system (MPS) to certain extent (Record, 2018). Currently, nucleic acids and proteins are considered as main participants of exosome treatments (Tan et al., 2015). MSC-Exos are enriched in miRNAs with different functions, such as anti-inflammatory miRNAs, anti-apoptotic miRNAs, and immunoregulatory miRNAs (Schultz et al., 2021). Some studies report therapeutic roles of exosomal mRNA and other non-coding RNA (lncRNA, cirRNA, and piRNA) in microbial diseases (Zhu et al., 2014; Li et al., 2020a; Shi et al., 2020; Yu et al., 2020a). In addition, protein profiling of MSC-EVs reveals that exosomal proteins are related to biological process such as innate immunity, antimicrobial, host-virus interaction, cellular oxidant detoxification, and complement and coagulation cascades. Several AMPs were identified, including dermcidin, lactoferrin, lipocalin 1, lysozyme C, neutrophil defensin 1, S100A7 (psoriasin), S100A8/A9 (calprotectin), and histone H4 (Pierce and Kurata, 2021). AMPs partially account for the antimicrobial effects of MSCs' secretome, which may also work in terms of MSC-Exos (Alcayaga-Miranda et al., 2017).

MSC-EXOS FOR THERAPEUTIC APPLICATIONS IN MICROBIAL DISEASES

The idea of bringing MSC-Exos into HDT for infectious diseases is explorative. In this section, we mainly focus on summarizing experimental proves for the efficacy of MSC-Exos in the treatment of some persistent or refractory infectious diseases. We first start with a topical disease, periodontitis, and then discuss multiple systematic diseases, including bacteria/viruses-associated pneumonia, sepsis, and bacteria-associated DFUs (**Figure 2**).

MSC-Exo Therapy for Periodontitis

Periodontitis refers to the inflammatory destruction of the periodontal supportive tissue (gingiva, periodontal ligament, and alveolar bone) as a result of polymicrobial colonization on tooth surfaces in the form of biofilms. Periodontitis has been recognized and treated for at least 5,000 years, and the classification of which has been changed and evolved with the development of new knowledge. Several microbes are associated with specific types of periodontal diseases, such as *Aggregatibacter actinomycetemcomitans* with aggressive periodontitis, and *Porphyromonas gingivalis* with severe or progressive periodontitis. The presence of the microbial biofilm might not be sufficient to directly cause periodontal disease. Periodontitis occurs when the balance between microbial biofilms and immune responses of the host is lost (Kinane et al., 2007; Kinane and Hajishengallis, 2009; Hajishengallis and Lamont, 2012). As pathogens invade periodontium, immune cells release anti-inflammatory cytokines and antibacterial molecules to fight against pathogens, upsetting the homeostasis of alveolar bone at the same time. Mechanistically, a cascade of events activates osteoclastogenesis leading to subsequent alveolar bone loss *via* the receptor activator of nuclear factor-kappa B (RANK)- ligand (RANKL)-osteoprotegerin (OPG) axis (Cochran, 2008; Barbato et al., 2015). Moreover, periodontitis is a disease of high morbidity and recurrence (Frencken et al., 2017). Progressive alveolar bone loss ultimately leads to loss of teeth, posing negative influences to oral function and aesthetics. The harm of periodontitis exceeds teeth loss, but also

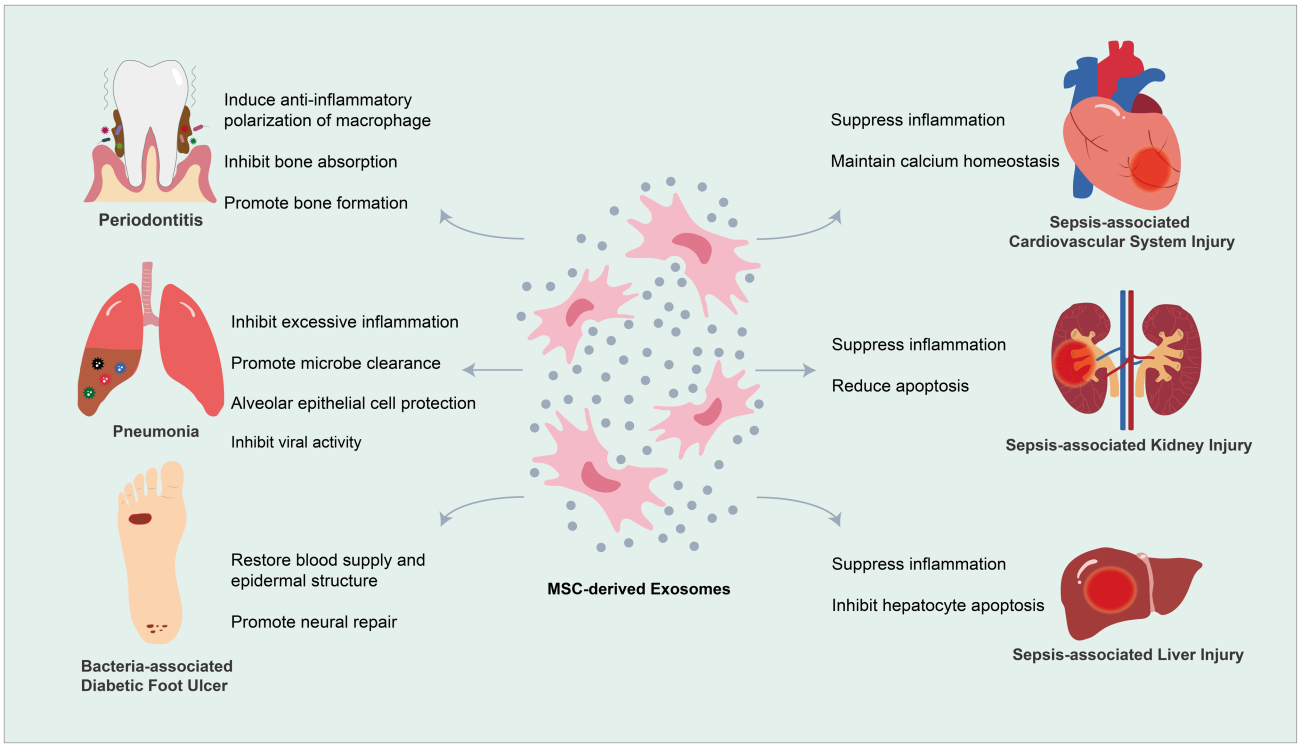

FIGURE 2 | Mesenchymal stem cell-derived exosomes (MSC-Exos) for therapeutic application in microbial diseases.

involves a higher risk of systematic diseases, such as cardiovascular disease (Donders et al., 2021), oral squamous cell carcinoma (Hu et al., 2021), and rheumatoid arthritis (Choi and Lee, 2021).

Routine treatments for periodontal diseases include mechanical approaches, scaling and root planning, to remove microbial biofilms. *In situ* or systematic antibiotics are applied as adjunctive therapies when periodontal infection is hard to control. Yet, the recurrence of periodontal disease is high, and no mechanical techniques rescue the loss of alveolar bone (Kinane et al., 2017). MSC-Exo therapy stands out in the treatment of periodontitis for its ability in suppressing excess inflammation and promoting tissue regeneration simultaneously. In treatment of periodontitis, MSC-Exos, often co-assembled with tissue engineering scaffolds, are implanted into periodontal bone defects to promote the regeneration of periodontal supportive tissues. Abundant studies have demonstrated that the regulation of MSC-Exos involves several kinds of cells, such as macrophages, osteoclasts, and periodontal ligament cells in periodontium (Yu et al., 2020b; Gegout et al., 2021).

Macrophages are crucial immunomodulators of the periodontal disease and account for both initiation and resolution of inflammation and osteoclastogenesis (Darveau, 2010; Hienz et al., 2015). Macrophages can be polarized into pro-inflammatory phenotype (M1 macrophage) and anti-inflammatory phenotype (M2 macrophage), to mediate inflammation and maintain tissue homeostasis, respectively (Shapouri-Moghaddam et al., 2018; Jin et al., 2019). MSC-Exo therapy inhibits excessive inflammation in periodontium by converting M1 macrophages into M2 macrophages. Shen et al. (2020) injected dental pulp stem cell-derived exosomes (DPSC-Exos) and DPSC-Exo-incorporated chitosan hydrogel (DPSC-Exos/CS) respectively into periodontal pockets of ligature-induced periodontitis mice. Both DPSC-Exos and DPSC-Exo/CS rescued alveolar bone loss and periodontal epithelial lesion to some degree, with the chitosan hydrogel one performing better (**Figure 3**). Mechanistically, it was demonstrated that DPSC-Exos delivered miR-1246 to induce anti-inflammatory polarization of macrophage, and downregulated NF-κB p65 and p38 mitogen-activated protein kinase (MAPK) signaling pathways to alleviate periodontal inflammation (Shen et al., 2020). In another research, Nakao et al. (2021) locally injected human gingiva-derived MSC-derived exosomes (GMSC-Exos) into periodontal pockets of mice, and observed reduced bone resorption and the number of tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts in periodontal tissue, and these effects were further enhanced by pretreating GMSCs with TNF-α. Delivery of exosomal miR-1260b accounts for the anti-osteoclastogenic ability of GMSC-Exos, which targets Wnt5a-mediated RANKL pathway (Nakao et al., 2021). Analogously, decreased RANKL/OPG ratio and number of TRAP-positive cells indicate inhibition of osteoclastogenesis by bone marrow mesenchymal stem cell-derived exosomes (BMSC-Exos) in periodontitis rats (Liu et al., 2021).

In addition to the immunomodulation of MSC-Exos in the treatment of periodontitis, other studies indicate MSC-Exos rescue the osteogenic ability of stem cells in periodontal ligaments. Wei et al. (2020) indicated human exfoliated deciduous

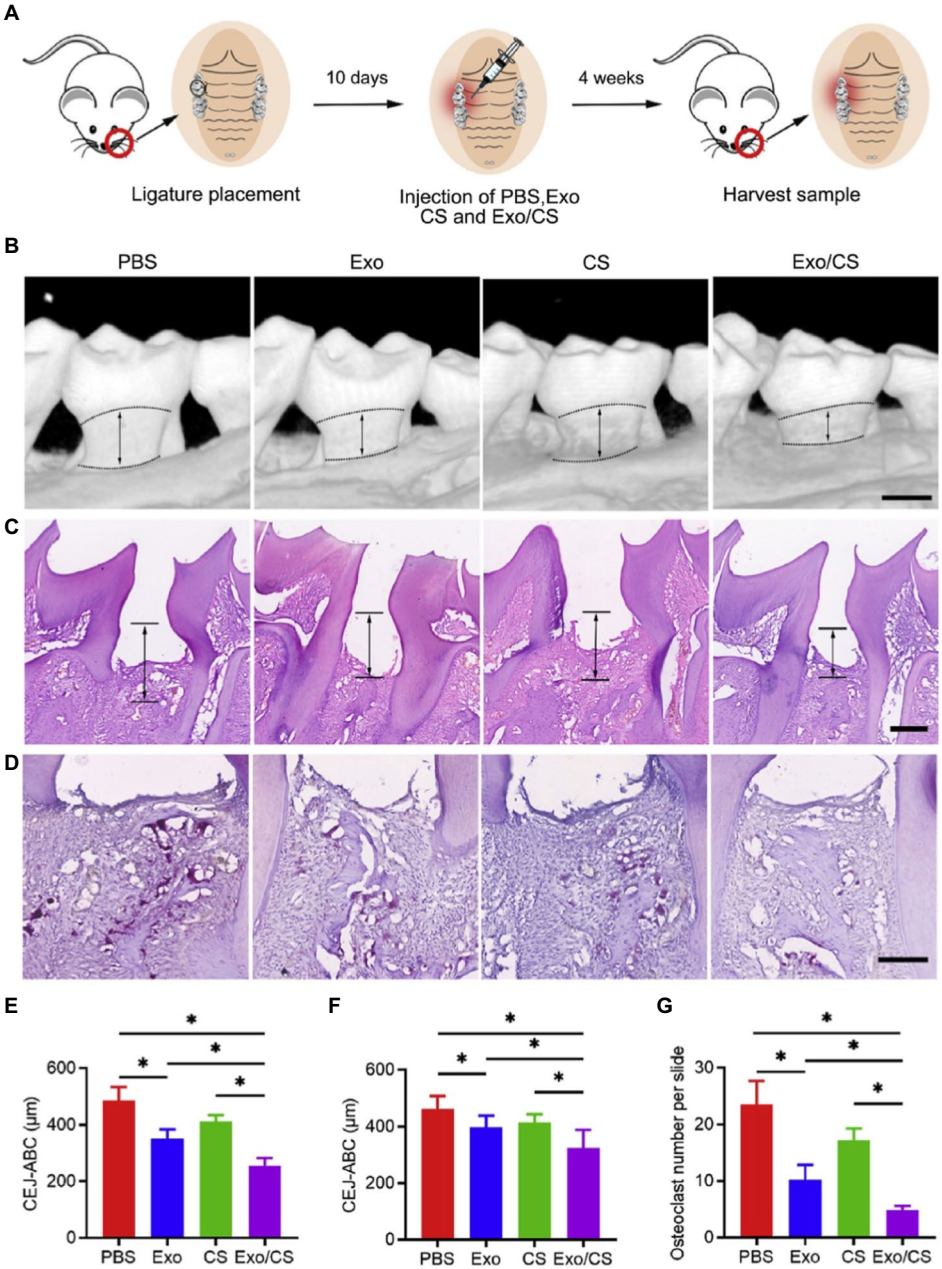

FIGURE 3 | Dental pulp stem cell-derived exosomes (DPSC-Exos)-incorporated chitosan hydrogel (DPSC-Exo/CS) rescues epithelial lesion and alveolar bone loss in mice with experimental periodontitis. **(A)** Schematic illustration. **(B)** 3D micro-CT reconstructions of maxillae of PBS-, CS-, DPSC-Exo- and DPSC-Exo/CS-treated groups ($n = 6$ per group). **(C)** Histological H&E-stained sections of the periodontium from each group. **(D)** Histological tartrate-resistant acid phosphatase (TRAP)-stained sections of the periodontium from each group. The number of osteoclasts was quantified in each microscope field of view. **(E,F)** Statistical analysis of the CEJ-ABC distance in each group ($n = 6$ per group) as determined by micro-CT and H&E staining, respectively. **(G)** Statistical analysis of the number of osteoclasts in each group ($n = 6$ per group) as determined by TRAP staining. Error bar represents SEM. $^*p < 0.05$ (Adopted from Shen et al., 2020 distributed under the CC BY-NC-ND license).

teeth (SHED-Exos) promoted BMSCs osteogenesis, differentiation, and bone formation *via* Smad5 signaling in a ligature-induced periodontitis mouse model. Consistently, Wang et al. (2020c) demonstrated the role of SHED-Exos in enhancing the osteogenic differentiation of periodontal ligament stem cells (PDLSCs) *via* Wnt and BMP signaling *in vitro*. Enhancing angiogenesis is another important strategy in promoting delayed bone healing, as vascular system supplies nutrients, oxygen, and serves as a niche for osteoprogenitor cells during bone repair (Liu and Castillo, 2018). In the study of Wu et al. (2019), SHED-Exos/β-tricalcium phosphate targeted AMPK signaling pathway to promote the coupling of human umbilical vein

endothelial cells angiogenesis and BMSC osteogenesis in a rat periodontal defect model. Chew et al. (2019) reported MSC-Exo-loaded collagen sponge promoted regeneration of periodontal defects by enhancing viability, proliferation, and migration of periodontal ligament cells through CD73-mediated adenosine receptor activation of pro-survival AKT and ERK signaling pathways. The above researches indicate that the latent ability of MSC-Exos to regulate inflammation and bone remodeling paves the way for the establishment of a therapy for periodontitis.

MSC-Exo Therapy for Pneumonia

Pneumonia, an inflammation of lung parenchyma, usually caused by infections, remains a heavy global burden on health (Watkins and Sridhar, 2018). According to Global Burden of Disease Study 2019, lower respiratory infections ranked fourth in leading causes of all ages, which pose severe health threat on people with a weak immunity system, especially children younger than 10 years and the elderly aged more than 75 years (Vos et al., 2020). Pneumonia starts with the pathogen invasion into the lower respiratory tract, which induces alveoli and interstitium inflammation, and pulmonary vascular congestion. As pulmonary permeability increases, transudate fluid and debris in the alveolar sacs compromise gas exchange (Brooks, 2020). Pneumonia can develop into ARDS and acute lung injury (ALI), the mortality rate of which is as high as 43% (Zambon and Vincent, 2008). Novel pharmacologic therapies for the treatment of ARDS/ALI including surfactant, vasodilators, prostacyclin, anti-inflammatory, and anti-oxidant reagents, have not yet proven to be effective (Cepkova and Matthay, 2006). In terms of promising new therapies, MSC-Exos have been explored in both preclinical and clinical studies. Accumulating evidence has demonstrated that MSC-Exo therapy is effective in attenuating excessive inflammation, restoring pulmonary function, and reducing mortality, verified in several typical ARDS/ALI animal models (Kannan et al., 2009; Knapp, 2009; Islam et al., 2012; Zhu et al., 2014; Hraiech et al., 2015; Monsel et al., 2015; Ogata-Suetsugu et al., 2017; Hao et al., 2019; Domscheit et al., 2020; Metcalfe, 2020; Wang et al., 2020a; Kaspi et al., 2021; Shi et al., 2021a; Tieu et al., 2021). Herein, the following portion aims at providing a comprehensive understanding of therapeutical mechanisms of MSC-Exos on bacteria/viruses-induced pneumonia.

Inhibiting Excessive Inflammation

Mesenchymal stem cell-derived exosomes exhibit immunomodulatory properties by directly targeting innate immune system. Innate immunity cells (monocytes, macrophages, and neutrophils) protect the host against infections by secretion of antimicrobial molecules and phagocytosis. However, excessive activated macrophages and neutrophils can damage alveolar epithelium and lung endothelium *via* secretion of proinflammatory cytokines, oxidants, and proteases. Recovery of intact epithelium and endothelium depends on the cessation of inflammatory injury (Matthay and Zemans, 2011). The anti-inflammation effect of MSC-Exos is repetitively proved in a lipopolysaccharide (LPS)-induced ALI mouse model, which is the most widely used and simplified model for ARDS/ALI, simulating the pulmonary response to bacterial endotoxin [7].

Mesenchymal stem cell-derived exosomes attenuate inflammation development and progression by regulating macrophage polarization by targeting intracellular signaling pathways or cellular metabolic pathways. In one aspect, MSC-Exos target the downstream pathway of pattern-recognition receptors (PRRs), such as NF-κB signaling pathway (Liu et al., 2017). MiR-27a-3p from MSC-EVs downregulated the expression of nuclear factor kappa B subunit 1 to promote M2 macrophage polarization, evidenced by elevated expressions of M2 markers arginase-1, interleukin (IL)-10, and decreased levels of M1 marker inducible nitric oxide synthase. Significantly reduced proinflammatory cytokines including IL-1β, IL-6, and TNF-α in the bronchoalveolar lavage (BAL) were observed (**Figure 4**; Wang et al., 2020a). MVs from Toll-like receptor 3 preactivated-MSCs further decreased TNF-α and increased IL-10 secretion of monocytes, which might be involved with the transfer of cyclooxygenase 2 (COX2) mRNA from MSC-MVs to monocytes. The increase in COX2, the key enzyme in PGE2 synthesis, shifted monocytes toward an anti-inflammatory phenotype by promoting PGE2 secretion (Monsel et al., 2015). In another aspect, MSC-Exos control the activation state and function of macrophages by reprogramming intracellular metabolisms. M1 and M2 macrophages exhibit different metabolic patterns. The former relies more on aerobic glycolysis, whereas the latter mainly employ mitochondrial oxidative phosphorylation (Zhu et al., 2015). BMSC-exos attenuated M1 macrophage polarization through inhibiting glycolysis, proved by decreased levels of end-products of aerobic glycolysis (adenosine triphosphate and lactic acid). Specifically, BMSC-Exos functioned by downregulating hypoxia-inducible factor 1 to inhibit the expression of rate-limiting proteins of glycolysis (Zhong et al., 2019; Deng et al., 2020). Morrison et al. (2017) reported the role of functional mitochondrial transfer through MSC-EVs in the conversion of macrophages into an anti-inflammatory phenotype *via* augmented oxidative phosphorylation (Morrison et al., 2017).

Moreover, MSC-Exos facilitates the resolution of inflammation by intermitting neutrophil migration towards lung epithelia. Hao et al. (2019) reported BMMSC-EVs reduced infiltration of white blood cells, neutrophils, and levels of TNF-α by elevating the level of extracellular leukotriene A_4 hydrolase (LTA_4H), proved in both *Escherichia coli* endotoxin-induced acute lung injury and *E. coli* pneumonia mouse models. LTA_4H reduced inflammation by degrading the matrikine proline–glycine–proline, a neutrophil chemoattractant (Patel and Snelgrove, 2018). Similarly, in a *Pseudomonas aeruginosa*-induced pneumonia mouse model, nebulized human adipose-derived MSC-EVs (ADSC-EVs) reduced the inflammatory cell counts, and levels of IL-6, and TNF-α in BAL fluid. The researchers reported a dose-response effect of ADSC-EVs. Within 2×10^5 to 2×10^6 particles per administration, mice survival rates and EV

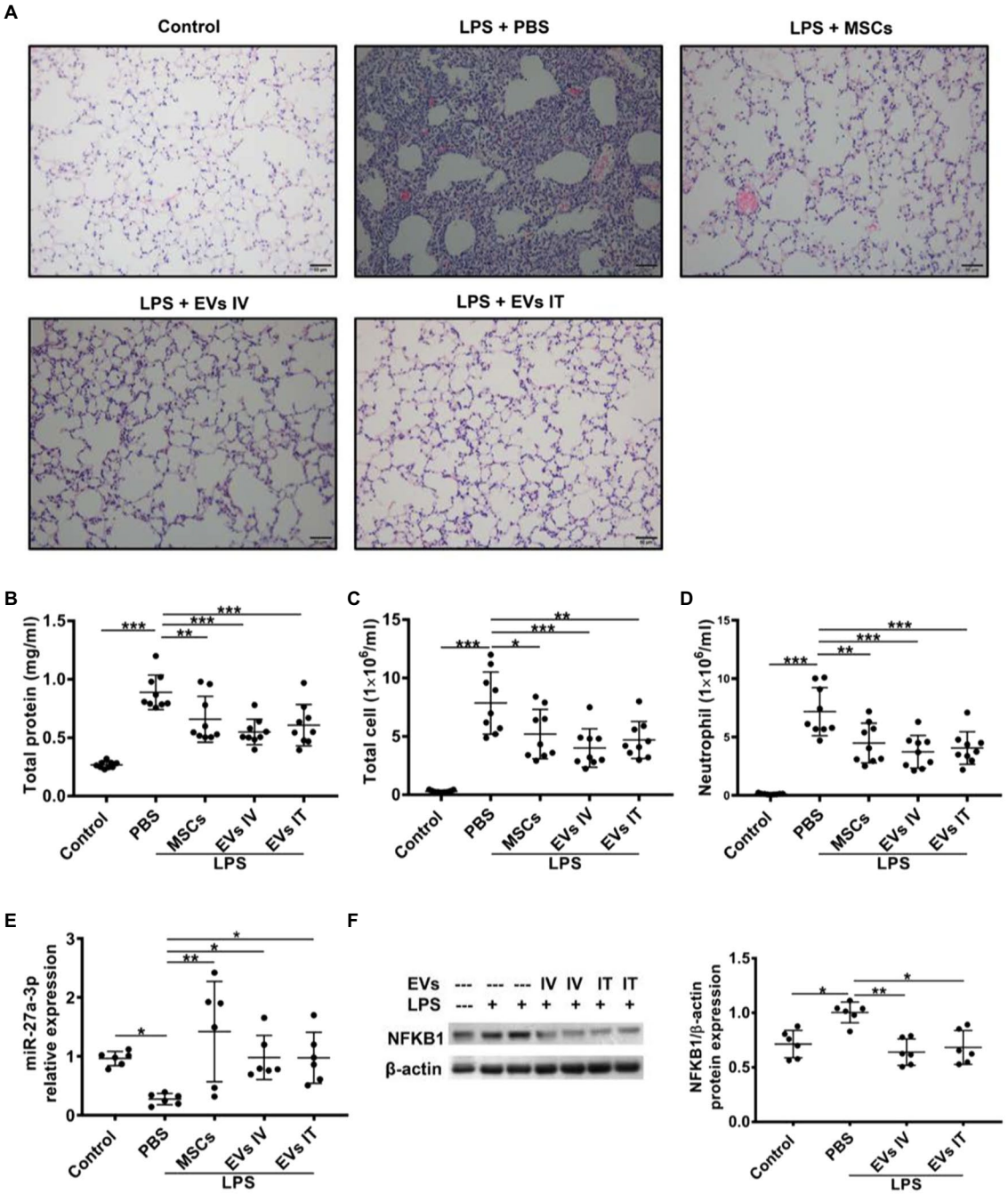

FIGURE 4 | Both IV and intratracheal (IT) administration of mesenchymal stem cell-extracellular vesicles (MSC-EVs) alleviate lipopolysaccharide (LPS)-induced lung injury, elevate miR-27a-3p levels, and decrease NFKB1 levels. **(A)** Similar to the effects of MSCs, administration of EVs *via* both IV and IT dramatically improved lung injury as shown in histology. Both EVs IV and IT decreased protein concentrations **(B)**, total cell counts **(C)**, and neutrophil counts **(D)** in the bronchoalveolar lavage (BAL) harvested at 48 h after LPS insult. **(E)** Alveolar macrophages were separated 48 h after LPS insult and assayed for miR-27a-3p expression *via* quantitative real-time PCR. Results are presented relative to control group. **(F)** Alveolar macrophages were separated from BAL 48 h after IT LPS insult and assayed for NFKB1 expression *via* Western blot analysis. Data are expressed as mean ± sd; n = 6. One-way analysis of variance with Bonferroni post hoc test **(B-D)** or Kruskal-Wallis test with Dunn post hoc test **(E,F)** was used for the analysis. $^{*}p < 0.05$; $^{**}p < 0.01$; and $^{***}p < 0.001$. (Adopted from Wang et al., 2020a distributed under the creative commons CC BY license).

dosage were positively correlated. However, once exceeding the dose of 2×10^6 particles, ADSC-EVs posed an adverse effect on the survival rate (Shi et al., 2021a). A numerically lower influx of neutrophils was also seen in an *ex vivo* perfused human lung injured with severe *E. coli* pneumonia, after MSC-MV treatment (Park et al., 2019).

Promoting Microbe Clearance

Phagocytes (e.g., monocytes, macrophages, and neutrophils) safeguard lung tissue against infectious insult through the ingestion and phagocytosis of microbes (Kaufmann and Dorhoi, 2016). It has been reported that MSC-MV treatment dramatically increased bacterial phagocytosis *via* freshly isolated human alveolar macrophages (Park et al., 2019). Pro-bacterial killing effects of MSC-Exos were demonstrated in intratracheal instillation of bacteria-induced ALI mouse models, which better mimicked immune response of pneumonia patients (Knapp, 2009). In an *E. coli* pneumonia mouse model, miR-145 from BMSC-EVs decreased the activity of multidrug resistance-associated protein 1 (MRP1) in monocytes, an ATP-binding cassette transporter, to increase Leukotriene B4 production, which exerts antimicrobial effects by augmenting phagocytosis and the release of antimicrobial agents (Hao et al., 2019). Besides, Monsel et al. (2015) demonstrated increased monocyte bacterial phagocytosis after administration of MSC-MVs on *E. coli* pneumonia in mice. It is attributed to the upregulated protein level of keratinocyte growth factor (KGF) in the alveolus, which promoted bacterial clearance by decreasing apoptosis of monocytes through AKT phosphorylation (Lee et al., 2013).

Alveolar Epithelial Cell Protection

Mesenchymal stem cell-derived exosomes can also restore function of injured alveolar epithelial type II cells, which play an important role in the maintenance of alveolar integrity and activation of immune defense (Kannan et al., 2009). Lee et al. (2009) made deep explorations into the detailed mechanisms of epithelial cell protection effect of MSC-MVs (Zhu et al., 2014; Park et al., 2019). In an LPS-induced ALI mouse model (Zhu et al., 2014) and severe *E. coli* pneumonia *ex vivo* human lung model (Park et al., 2019), MSC-MVs dramatically improved alveolar fluid clearance and decreased lung protein permeability by the delivery of KGF mRNA to alveolar epithelial type II cells. KGF was proved effective in upregulating the key epithelial sodium channel in alveolar epithelial cells to increase fluid absorption (Lee et al., 2009). Injured alveolar epithelial type II cells benefited from the restoration of ATP levels, which might be attributed to the transfer of key metabolic enzymes (such as glyceraldehyde 3-phosphate dehydrogenase and pyruvate kinase) or mRNA for key mitochondrial genes carried by MVs in an *E. coli* pneumonia mouse model (Monsel et al., 2015). Bioenergetics reprogramming of epithelial cells can also be mediated by BMSC-EVs mitochondria transfer, reported by Islam et al. (2012).

Inhibiting Viral Activity

In case of viral pneumonia, apart from inhibition of cytokine storm, suppression on viral replication and attack on viruses are underlying mechanisms of MSC-Exo therapy. Exosomal microRNAs derived from MSCs might target viral genome to interfere with viral RNA transcription or protein translation essential for viral replication (Qian et al., 2016; Demirci and Adan, 2020; Sardar et al., 2020). Khatri et al. (2018) reported that MSC-EVs attenuated influenza virus-induced ALI in pigs by inhibiting viral replication, evidenced by significantly decreased virus loads in both lung lysates and nasal swabs. Meanwhile, *in vitro* experiment proved reduced virus activity in hemagglutination, replication, and pro-apoptosis. The anti-influenza property is attributed to the transfer of exosomal RNAs to epithelial cells, as therapeutic effects were reversed by pre-incubation of MSC-EVs with RNase enzyme (Khatri et al., 2018). It is presumable that miRNAs might prevent viral replication by targeting viral genes (e.g., reducing the spike protein) or inhibiting the expression of host cells receptors to avoid virus-cell interaction (Chauhan et al., 2021). Similar inhibition in viral replication was observed in the application of exosomes/microvesicles derived from murine hypothalamic neural stem/progenitor cells (htNSC) on pseudotyped SRAS-CoV-2-infected human respiratory cells *in vitro*. Furthermore, NSC-Exos exerted inherent antiviral ability by attacking and degrading viruses independent of cells. TEM imaging confirmed direct exosome-virus interaction in a cell-free environment, suggesting exosomes functioned by surrounding, engulfing, and breaking down viruses. Pretreated viruses with NSC-Exos led to degradation of spike glycoprotein and lessened infection ability toward cells (Yu et al., 2020a). However, the underlying molecular mechanisms remain to be explored.

Taken together, MSC-Exo therapy has great potential in treatment of infectious pneumonia with the ability to modulate protective immune response, provide epithelial cell protection, and inhibit viral-cell inhibition. Multiple relevant clinical trials are in progress, which will be discussed in the Section "Clinical Translation of Exosome Therapy."

MSC-Exo Therapy for Sepsis

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infections, according to the third international consensus definition (Singer et al., 2016). Sepsis-related death accounts for 19.7% of all global death in 2017, remaining a major public health problem (Rudd et al., 2020). Common causative microorganisms include Gram-positive bacterial pathogens (e.g., Staphylococcus aureus, *Streptococcus pneumoniae*), and Gram-negative pathogens (e.g., *E. coli*, *Klebsiella* spp., and *P. aeruginosa*; Opal et al., 2003; Umemura et al., 2021). The pathophysiologic mechanisms of sepsis can be generally concluded into three aspects: inflammation, microcirculatory dysfunction, and metabolic reprogramming (Peerapornratana et al., 2019). The possibility of polymicrobial infections adds difficulties to the early-stage diagnosis of sepsis. Empiric combined broad-spectrum antimicrobial therapy is recommended at the initial stage (Rhodes et al., 2017). However, it acts as a double-edged sword, with side effects such as increased risks of multidrug-resistant infections, organ damages, and anaphylaxis (Klompas et al., 2018; De Waele and Dhaese, 2019). In absence of such side effects, MSC-Exos may complement as an adjuvant therapy in sepsis, with their indistinctive host protection against a broad range of microorganisms and reparative effects on injured organs. MSC-Exo therapy in remedy of sepsis-induced acute injuries in liver, kidney, and cardiovascular system will be discussed in detail as follows.

Liver

The incidence of liver failure is relatively low in sepsis because of its ability in clearance of endotoxins and self-regeneration

(Weiss et al., 2001). However, once intestinal barrier compromised under sustained systematic inflammation, bacterial translocation from the gut lumen through circulation can result in severe liver dysfunction (Sun et al., 2020). MSC-Exos show hepatic protection in condition of acute liver injury, demonstrated by improved hepatic function indicators (lower serum alanine aminotransferase and aspartate aminotransferase levels), histological characteristics changes (lower degree of hepatocellular necrosis and inflammation), and survival rates in D-GalN/LPS-induced acute liver injury mouse models (Lou et al., 2017; Hu et al., 2020; Zhang et al., 2020b). In one aspect, therapeutic effects of MSC-Exos lie in the modulation of innate immune system. Chen et al. (2017) reported exosomes from human menstrual blood-derived stem cells (MenSC-Exos) inhibited recruitment of NK cells, macrophages and release of inflammatory cytokines including TNF-α, IL-6, and IL-1β in liver. Liu et al. (2018) reported miR-17-containing ADSC-Exos suppressed the activation of thioredoxin-interacting protein-mediated NLRP3 inflammasome in hepatic macrophages, indicated by reduced cleaved-Caspase-1, IL-1β, and IL-18 expressions. Similar inhibition effects on the activity of the NLRP3 inflammasome by human umbilical cord mesenchymal stem cell (hucMSC)-Exos were observed (Jiang et al., 2019). Furthermore, Shao et al. (2020) confirmed that exosomes from IL-6 preconditioned-hucMSCs targeted phosphatidylinositol-3-kinase (PI3K) signaling pathways to suppress monocyte/macrophage activation and inflammatory cytokine secretion by transfer of miR-455-3p. In another aspect, MSC-Exos participate in the maintenance of hepatocyte hemostasis by inhibiting cell apoptosis. MenSC-Exos suppressed apoptosis by downregulation of Caspase-3, an important apoptosis-associated protein (Chen et al., 2017). Zhao et al. (2019b) reported BMSC-Exos reduced apoptosis of hepatocytes by inducing autophagy, evidenced by increased levels of autophagy marker proteins, microtubule-associated protein 1A/1B-light chain 3, Beclin-1, and the number of autophagosomes. Autophagy is a self-protection mechanism that attenuates liver cell death, functioning by removing damaged organelles and alleviating intracellular stress (Ni et al., 2012).

Kidney

Sepsis-associated acute kidney injury (S-AKI) is of high morbidity in severely ill patients, with high risk of developing into chronic kidney diseases and death (Peerapornratana et al., 2019). Multiple studies have provided histological and laboratory tests evidence to validate the capability of MSC-Exos in rescuing renal function in sepsis (Gang et al., 2021). In cecal ligation and puncture (CLP)-induced sepsis mice, the kidney morphology was more intact after intervention of MSC-Exos. Decreased kidney interstitial edema, higher integrity of brush borders and reduced inflammatory cell infiltration were observed in HE staining kidney tissues (Blanco et al., 2020; Gao et al., 2020). Blood tests further confirmed renal function restoration. In serum, the levels of blood nitrogen urea, serum creatinine and various inflammation indicators were downregulated after the treatment of MSC-Exos, indicating increased glomerular filtration rates (Nassar et al., 2016; Li et al., 2020b). The protective function is associated with the upregulation of SIRT1, which regulates NF-κB and apoptotic pathway (Gao et al., 2020). In another research, Shen et al. (2021) reported ADSC-Exos inhibited ROS accumulation and M1 polarization by downregulating Kelch Like ECH Associated Protein 1 and activating Transcription factor nuclear factor-E2-related factor 2 (Nrf2)/Heme Oxygenase-1 (HO-1) pathway (Shen et al., 2021). Other molecular mechanisms involve upregulation of miR-146b level in kidney tissue by hucMSC-Exos, which targets IL-1 receptor-associated kinase (IRAK1) and inhibits NF-κB activity (Zhang et al., 2020a).

Cardiovascular System

The pathogenesis of septic cardiomyopathy has not been fully revealed, and the current understanding of pathogenic mechanisms includes increased capillary permeability, oxidative stress, and calcium dyshomeostasis (Kakihana et al., 2016; Ehrman et al., 2018). MSC-Exos provide cardioprotection under septic conditions by suppressing inflammation and maintaining calcium homeostasis. Intravenous injection of MSC-Exos improved septic mice survival and inhibiting cardiomyocytes death by attenuating excess inflammation *via* miR-233, which downregulated Sema3A and Stat3 (**Figure 5**; Wang et al., 2015). Pink1 mRNA-containing hucMSC-Exos rescued injured cardiomyocytes by activating PINK1-PKA-NCLX axis, which alleviated cardiomyocyte mitochondrial Ca^{2+} efflux disorder (Zhou et al., 2021b). Anti-apoptotic effects on cardiomyocytes may associate with miR-21a-5p (Luther et al., 2018), miR-19a (Yu et al., 2015), miR-451 (Zhang et al., 2010), and miR-211 (Yu et al., 2013).

Taken together, MSC-Exos may represent a promising novel and efficacious cell-free therapeutic modalities in treatment of sepsis, depending on its orchestrated ability in anti-inflammation and anti-apoptosis.

MSC-Exo Therapy for Bacteria-Associated Diabetic Foot Ulcers

Diabetic foot ulcers, characterized as micro-vascular dysfunction and peripheral neuropathy, is highly susceptible to secondary infections, as impaired immune function slows down wound healing and suppressed proprioception potentially leads to injuries. The biofilm on the wound site, produced by aerobic and anaerobic bacteria, exhibits resistance toward antibiotics that causes difficulties to the treatment of DFU (Raghav et al., 2021). Prevalence of polymicrobial or multidrug-resistant bacteria infections increased the difficulties to control infections, and the risk of amputation (Pitocco et al., 2019). In this part, we place particular emphasis on the mechanisms of action by which MSC-Exos confer the ability to accelerate wound healing and strengthen biological barriers against microbes in treating bacteria-associated DFU.

Mesenchymal stem cell-derived exosomes rescue function of epithelial progenitor cells (EPC) and promote angiogenesis by delivering RNAs (microRNAs, lncRNAs, and circRNAs) and proteins (Dalirfardouei et al., 2021). Several studies demonstrated hydrogels combined with MSC-Exos promoted wound healing and skin regeneration under diabetes conditions *via* the transfer

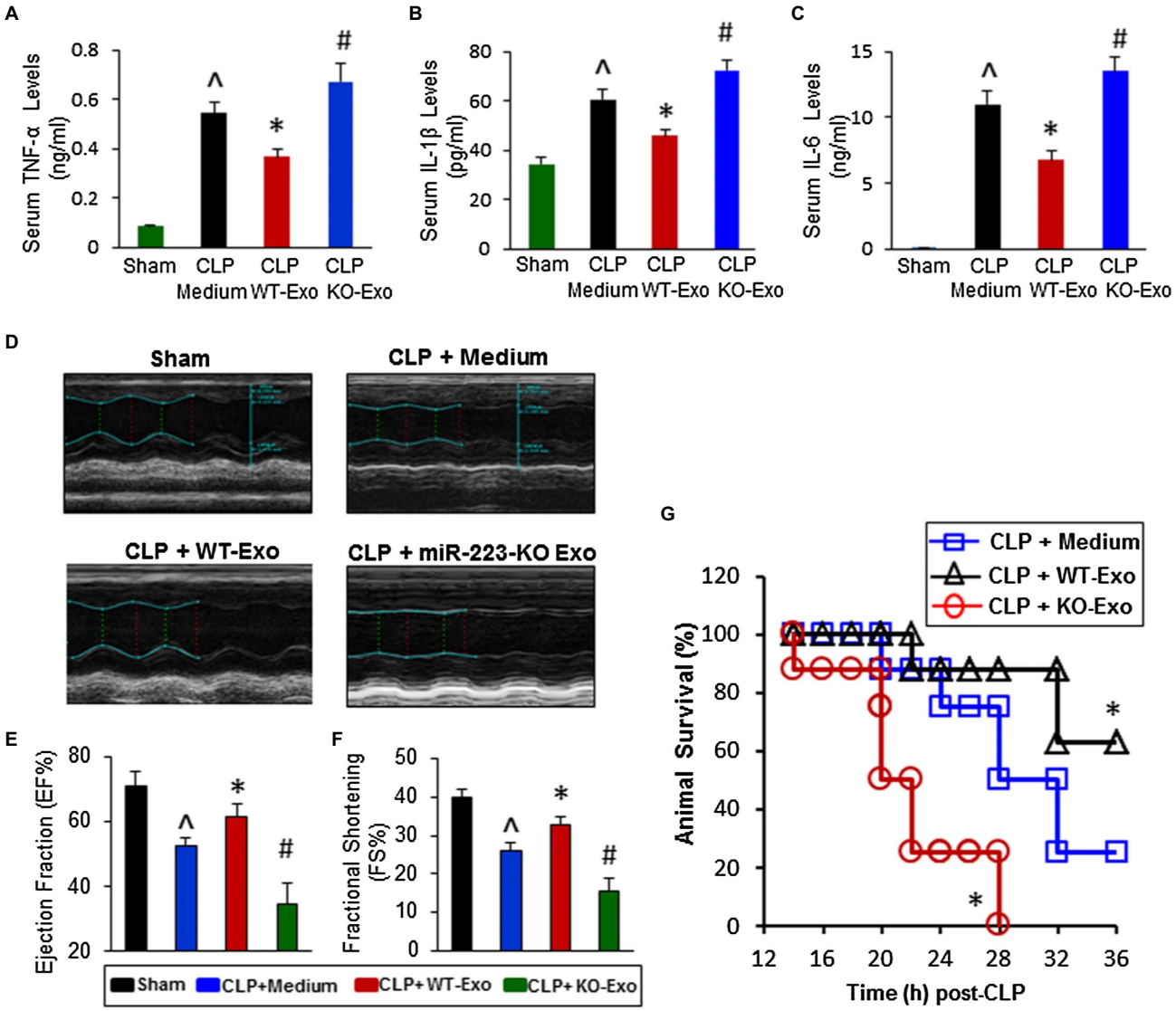

FIGURE 5 | The effects of WT-exosomes and miR-223-KO exosomes on cecal ligation and puncture (CLP)-induced inflammatory response, cardiac dysfunction and animal mortality. **(A–C)** CLP-mice treated with WT-exosomes ($n = 11$) showed lower levels of serum TNF-α **(A)**, IL-1β **(B)**, and IL-6 **(C)**, whereas CLP-mice injected with KO-exosomes ($n = 11$) exhibited higher levels of circulating TNF-α **(A)**, IL-1β **(B)**, and IL-6 **(C)**, compared with those treated with incomplete DMEM medium ($n = 10$; $^{\wedge}p < 0.05$ vs. shams; $^{*}p < 0.05$ vs. CLP + medium; $^{\#}p < 0.05$ vs. CLP + medium). **(D)** Results of echocardiography measurement showed that values of the left ventricular ejection fraction (EF%, **E**) and the fractional shortening (FS%, **F**) were significantly decreased in CLP mice injected with incomplete DMEM medium ($n = 10$), compared with shams ($n = 8$). Remarkably, the reduction of EF% and FS% was attenuated in WT-exosome-treated CLP mice ($n = 11$); whereas it was aggravated in CLP mice administrated with miR-223-KO exosomes ($n = 11$; $^{\wedge}p < 0.05$ vs. shams; $^{*}p < 0.05$ vs. CLP + medium; $^{\#}p < 0.05$ vs. CLP + medium). **(G)** The survival of CLP-mice was significantly improved by WT-exosome treatment, whereas it was worse by miR-223-KO exosome injection ($n = 8$, $^{*}p < 0.05$ vs. CLP + medium; Adopted from (Wang et al., 2015) distributed under the creative commons CC BY license).

of RNAs and proteins (Wang et al., 2019a,b). MiR-126 in exosomes from deferoxamine-pretreated BMSCs activated PI3K/AKT signaling pathway to promote angiogenesis in diabetic rat wounds (Ding et al., 2019). Synovial MSCs overexpressing miR-126 accelerated angiogenesis, re-epithelization, and maturation of collagen (Tao et al., 2017). ADSC-Exos overexpressing linc00511 enhanced proliferation, migration, and angiogenesis of EPC, by inhibiting Progestin and adipoQ receptor family member III (PAQR3) expression, and increasing Twist homolog 1 protein level by reducing its degradation (Qiu et al., 2021). ADSC-Exos were found to suppress apoptosis of EPC induced by high glucose through stimulating autophagy. The transmit of mmu_circ_0000250 inhibited miR-128-3p and upregulated expression of SIRT1, which promoted autophagy in EPCs (Shi et al., 2020). Protein cargoes also contribute to the improvement of EPC function. ADSC-Exos overexpressing Nrf2 alleviated senescence and oxidative stress of EPC under high glucose conditions, evidenced by improved levels of

Senescence Marker Protein 30, and decreased levels of oxidative stress-related proteins (NADPH oxidase 1, NADPH oxidase 4; Li et al., 2018). Deleted in malignant brain tumors 1 (DMBT1), a pro-angiogenic protein, from human urine-derived stem cells (USCs) accounts for the pro-angiogenic effects of USC-Exos (Chen et al., 2018). Other activated signaling pathways include MAPK (Li et al., 2016) and NF-kB (Dalirfardouei et al., 2019) pathways.

The wound healing of DFU requires the collaboration of multiple types of cells. MSC-Exos also target fibroblasts and keratinocytes for the acceleration of re-epithelialization, collagen deposition, and remodeling *via* regulating MAPK/ERK (Li et al., 2016), PI3K/AKT (Li et al., 2015; Zhang et al., 2018; Sears et al., 2021), and Wnt/β-catenin (Lv et al., 2020) pathways. Engineered hADSC-Exos overexpressing miR-21 significantly strengthened the migration and proliferation of keratinocytes by upregulating Matrix Metallopeptidase 7 (Lv et al., 2020). Modified MSC-Exos transferred lncRNA H19 to fibroblast, which impaired miR-152-3p-mediated phosphatase and tensin homolog (PTEN) inhibition and thus suppressed PI3K/AKT pathway, leading to increased migration, proliferation, and decreased apoptosis of fibroblast (Li et al., 2020a).

Mesenchymal stem cell-derived exosomes also benefit the treatment of DFU by promoting neural repair to regain peripheral sensation. Shi et al. (2017) reported the combination of exosomes from GMSCs and chitosan/silk hydrogel increased nerve density, compared with the hydrogel group, indicating that GMSC-Exos might facilitate neuronal ingrowth into the wound bed (Shi et al., 2017). MiR-146a-overexpressing MSC-Exos constructed by Fan et al. (2021) promoted axon remyelination, and improved intraepidermal nerve fiber density in hind paw plantar skin of diabetic mice. In a word, MSC-Exos prevent infections of DFU by restoring blood supply, epidermis structure, and peripheral neuropathy.

CLINICAL TRANSLATION OF EXOSOME THERAPY

Clinical Trials

Clinical trials using stem cell-derived exosomes as intervention were searched by using the terms "exosomes" or "extracellular vesicles" in the ClinicalTrials.gov database,[1] the European Union Clinical Trials Register[2] and the World Health Organization International Clinical Trials Registry Platform (Chinese Clinical Trial Registry).[3] Then selected studies went through manual screening to sort out microbial disease-related clinical trials. Eventually, 13 interventional studies and 2 expanded access are included, with observational studies excluded (**Table 1**). These clinical studies mainly aim at investigating the safety and efficacy of MSC-Exos in the treatment of infections. Some try to explore optimal dosage of exosomes by setting up different dosage intervention groups.

[1]https://clinicaltrials.gov
[2]https://www.clinicaltrialsregister.eu
[3]http://www.chictr.org.cn

Most studies investigate systematic diseases, and only two of 15 studies (NCT04270006, ChiCTR1900027140) focus on a topical disease, periodontitis. Noteworthily, those two studies applied different routes of administration: One (NCT04270006) locally injects autogenous adipose stem cell exosomes into the periodontal pockets; while in another study (ChiCTR1900027140), the mixtures of DPSC-Exos, DPSCs, and Bio-Oss bone meal are applied during guided tissue regeneration. Changes in bone defect depth, pocket depth, clinical attachment loss, and gingival inflammation are measured to evaluate the degree of periodontal tissue regeneration in both studies. However, the lack of control group or blinding method may increase bias and compromise the reliability of outcomes.

The majority of trials (11 out of 15) are registered to investigate MSC-Exo treatment in COVID-19-associated pneumonia (one trial for Phase I, four for Phase I/II, two for Phase II, two for expanded access, and two for unclear phase). And one study (Phase I/II) focuses on drug-resistant pneumonia. Most studies are controlled, randomized, parallel, double-blinded trials, in which only NCT04493242 is conducted in multi-center. The parent cells sources are diverse, including bone marrow, adipose tissue, umbilical cord, amniotic fluid. Administration routes are roughly evenly split between intravenous injection and aerosol inhalation. Among 12 registered studies, only three (NCT04491240, NCT04493242, and NCT04276987) are completed. Results information of NCT04276987 is currently not publicly available. In NCT04491240, no adverse event was reported during both exosome solution inhalation procedure and the whole trial, indicating the safety of intranasal administration of MSC-Exos. Regarding therapeutic efficacy, it seems that no data (time to clinical recovery, SpO2 concentration, C-reactive Protein, and Lactic Acid Dehydrogenase level in serum) indicated a significant difference between exosome treatment groups and placebo group. NCT04493242 has been published to report the safety and efficacy of allogeneic BMSC-Exos (ExoFlo™) for severe COVID-19 infections. No adverse events were observed 72 h after exosome therapy. After one course of treatment, PaO_2/FiO_2 (an oxygenation indicator), neutrophils and lymphocytes ($CD3^+$, $CD4^+$, and $CD8^+$) counts had significant increases. Meanwhile, acute phase reactants (C-reactive protein, ferritin, and D-dimer) declined. The results indicated ExoFlo™ therapy's capacity in restoring lung function and promoting protective immune response (Sengupta et al., 2020). Although the study demonstrated a promising future of MSC-Exo therapy in COVID-19 infections, doubts and uncertainties remained. The study was lack of crucial details in terms of exosome production, characterization, biological properties, and dosage. Furthermore, standards in evaluating the correlation between adverse events and exosome therapy were also questioned. More data are needed to allow proper assessment of the medical value and deeper exploration of the molecular mechanisms of ExoFlo™ (Lim et al., 2020). Only one not-yet-recruiting study (NCT04850469/ChiCTR2100044280) is to explore the administration of MSC-Exos in sepsis. And it is also the only study that chooses children as test subjects, with the rest of studies only involving adult participants.

TABLE 1 | Published clinical trials of mesenchymal stem cell-derived exosome (MSC-Exo) therapy in microbial diseases.

Trial ID number	Target disease	Stage	Study design	Sample volume	Exosome source	Route	Frequency
NCT04270006	Periodontitis	Early phase 1	Single group, open label	10	Adipose-derived stem cells	Location injection	-
ChiCTR1900027140	Chronic Periodontitis	N/A	Randomized parallel controlled study	48	Autologous dental pulp stem cells	Loaded on scaffold	-
NCT04602442	Covid19 pneumonia	Phase 2	Randomized, parallel, and double-blinded	90	Mesenchymal stem cells	Aerosol inhalation	Five times (every 2 days)
NCT04491240	Covid19 pneumonia	Phase 1, Phase 2	Randomized, parallel, and double-blinded	30	Mesenchymal stem cells	Aerosol inhalation	Five times (every 2 days)
NCT04657406	Mild to moderate COVID-19	Treatment IND	Expanded access	-	Amniotic stem cells and epithelial cells	Intravenous administration	Three times at day 0, 4, and 8
NCT04384445	Moderate to severe COVID-19	Phase 1, Phase 2	Controlled, randomized, parallel, and double-blinded	20	Amniotic stem cells and epithelial cells	Intravenous administration	Three times at day 0, 4, and 8
NCT04493242	Moderate-to-severe ARDS in patients with severe COVID-19	Phase 2	Multi-center, double-blinded, placebo-controlled, and randomized controlled	120	Bone marrow mesenchymal stem cells	Intravenous administration	-
NCT04276987	Covid19 pneumonia	Phase 1	Single group, open label	24	Allogenic adipose mesenchymal stem cells	Aerosol inhalation	Five times (each day)
NCT04657458	COVID-19 associated moderate to severe ARDS	Intermediate-size population	Expanded access	-	Bone marrow mesenchymal stem cells	Intravenous administration	-
NCT04798716	COVID-19 with moderate to severe NCP or ARDS	Phase 1, Phase 2	Open-label for the first 15 patients; RCT for the final 40 patients	55	Mesenchymal stem cells	Intravenous administration	Three times (every other day)
ChiCTR2000030484	Lung Injury following novel coronavirus pneumonia	N/A	Randomized parallel controlled study	90	Human umbilical cord mesenchymal stem cells	Intravenous administration	14 times (every day)
ChiCTR2000030261	Novel coronavirus pneumonia (COVID-19)	N/A	Randomized parallel controlled study	26	Mesenchymal stem cells	Aerosol inhalation	-
2021-002184-22	COVID-19 disease	Phase 2	Controlled, randomized, and single blinded	90	-	Aerosol inhalation	-
NCT04544215	Drug-resistant pulmonary infection	Phase 1, Phase 2	controlled, randomized, parallel, and double-blinded	60	Allogeneic human adipose-derived mesenchymal progenitor cells	Aerosol inhalation	Seven times (every day)
NCT04850469/ ChiCTR2100044280	Sepsis (in children)	-	-	200	Mesenchymal stem cells	-	-

Routes of Administration

Biodistributions and biological properties of exosomes vary depending on the application form. Therefore, it is of great importance to figure out optimal ways to deliver exosomes based on disease characteristics. Generally, there are two strategies for exosome administration, systematic administration, and topical administration. Systematic administration involves intravenous injection and aerosol inhalation, appropriate for multi-system diseases or internal organs-affected diseases, sepsis and novel corona pneumonia as typical examples. Conversely,

topical administration is suitable for limited infections, with the hope that exosomes are constraint in the focus of infections and exert maximum curative effects to local cells.

Systematic Administration

Intravenous Administration

Intravenous administration might be the most common exosome delivery method in basic research or clinical trials. Generally, biodistributions and pharmacokinetics of exosomes vary depending on parent cell sources and patients' pathologic conditions. Grange et al. (2014) performed intravenous injection of DiI-labeled MSC-EVs in healthy mice, and detected the fluorescent signal from freshly dissected tissues after 5 and 24 h. It showed that fluorescence intensity peaked in liver, spleen, and lung successively. While in an acute kidney injury mouse model, the accumulation of exosomes in the kidney increased and extended (Grange et al., 2014). Choi et al. (2021) observed over 80% of HEK293T cell-derived exosomes were cleared out from the circulation in 1 h after intravenous injection, with most of the rest tentatively accumulating in liver and then translocated to the intestine from 8 h post-injection. While in sepsis mice model, clearance speed significantly slowed down, indicating that liver dysfunction in later stage of sepsis may delay biliary excretion of exosomes (Choi et al., 2021). After intravenous injection, exosomes showed a short half-life (several minutes) in the circulation of healthy animals, most of which were captured by peripheral macrophages and neutrophils. Later, the remaining exosomes mostly accumulated in liver and spleen for more than 24 h. Rapid clearance from blood imposes restriction on the proportion of exosomes arriving target tissue, thus lessening therapeutic effects (Morishita et al., 2017; Yang et al., 2021).

Intranasal Administration

Intranasal exosome delivery refers to the transformation of exosome solution into aerosol which is inhaled directly into the lung (Pires et al., 2009). In this way, exosomes target lung tissue first, and go through lung air-blood barrier to target remote organs. It attracts increasing attentions in the treatment of COVID-19 associated pneumonia (Tsuchiya et al., 2020). Exosome nebulization results in a more homogeneous spread with deeper penetration to the distal lung lobules. Furthermore, it is non-invasive, almost painless, and convenient to conduct without the need for sterilization. More importantly, intranasal delivery may improve on-target effect in central neural system, as it overcomes difficulties in crossing blood-brain barrier by passing through neuroepithelium in nasal olfactory region to get direct access to the brain (Haney et al., 2015; Guo et al., 2019). Guo et al. (2019) labeled exosomes with glucose-coated gold nanoparticles (GNP), and tracked them by *in vivo* neuroimaging, giving a hint about the difference of intravenous and intranasal administration in brain accumulation and whole-body biodistribution. It turns out that the latter one made it easier to pass blood-brain barrier, leading to superior brain targeting, while the former one resulted in higher accumulation within the liver (Guo et al., 2019). However, consideration should be taken that pathological conditions in airway may affect nasal mucociliary clearance and influence drug absorption.

Topical Administration

Topical administration directs exosomes to sites of injection, suitable for superficial injuries or localized infections. For example, in open fractures, local administration of antibiotics leads to higher concentrations within the wound cavity, meanwhile minimizing systemic toxicity. In contrast, systemically administered antibiotics are hard to access avascular wound cavities (Lawing et al., 2015). The application forms are diverse, including local injection, smearing, exosome-loaded scaffolds, etc.

Gong et al. (2021) performed intramyocardial injection of gold nanoparticle-labeled exosomes in a myocardial infarction mouse model. *In vivo* CT imaging showed that majority of MSC-Exos remained in the MI area for up to 24 h after injection, and only few spread to other organs, indicating local injection as an effective way to deliver exosomes to limited treatment areas (Gong et al., 2021). Mohammed et al. (2018) studied the use of ADSC-Exos as adjunctive therapy to nonsurgical periodontal treatment. By local injection into periodontal pockets, the ADSC-Exo group revealed the best results with significantly higher area % of newly formed tissues in ligature-induced periodontitis rat model demonstrated by histologic study (Mohammed et al., 2018). Zhou et al. (2021c) compared two administration routes, smearing and subcutaneous injection, in the efficiency of delivering human ADSC-Exos to promote cutaneous wound healing. The results showed that the ADSC-Exo-smearing group significantly shortened healing time and narrowed scars on full-thickness wounds in mice. Specifically, HE staining and Masson staining illustrated better re-epithelialization and well-reorganized collagen fibers in the ADSC-Exo-smearing group than the subcutaneous injection group. The authors attributed this to the loss of exosomes during the local injection and direct injection may disturb the hierarchy of wound. Smearing might be an optional treatment options for clinical patients with exposed surface wounds accompanied with chronic infections (Zhou et al., 2021c).

The application of exosome-scaffold complexes is expected to repair tissue defects and release therapeutic exosomes at a controlled and sustained speed. Qian et al. (2020) created a multi-functioned chitosan-silk fibroin dressing with silver nanoparticles-loaded exosomes for infected wounds healing. The CTS-SF/SA/Ag-Exo dressing showed a sudden burst of exosome release at the beginning, and maintained release in low speed for up to 48 h, exerting constant antimicrobial and healing promotion effect. Shiekh et al. (2020) developed ADSC-Exo-embedded oxygen releasing cryogels as wound dressing, in which exosomes were released gradually for up to 6 days. The exosome-laden wound dressing improved the wound healing in *Staphylococcus aureus*, and *P. aeruginosa* infected diabetic wound ulcers, with enhanced collagen I deposition and mature epithelial structures observed (Shiekh et al., 2020). In other novel wound dressing, ADSC-Exos were encapsulated in the FHE hydrogel (F127/OHA-EPL) through electrostatic interaction and exhibited a representative long-term pH-responsive sustained release behavior. The exosome laden FHE hydrogel showed great potential in promoting chronic diabetic wound healing and complete skin regeneration (Wang et al., 2019a). Similarly, GMSC-derived exosome-chitosan/silk hydrogel sponge complex accelerates wound healing in a diabetic rat skin defect

model (Shi et al., 2017). Hydrogel promoted better healing of rat bone defects with the addition of hucMSC-derived exosomes (Wang et al., 2020b).

Exosome Modifications

Exosome modifications are an essential process to endow exosomes with more powerful therapeutic effects. Efforts are made with the purpose of increasing therapeutical components loadings and enhancing delivery efficiency.

Increasing Loading of Therapeutical Components

To improve the therapeutic capacity of exosomes, exosome modifications can be divided into pre-loading and post-loading two strategies, depending on the timing of intervention (de Jong et al., 2020).

Promoting Expressions of Bioactive Molecules

In the pre-loading approach, parent cells are pretreated with biochemical or biophysical stimulations. Endogenous bioactive molecules are then packaged into exosomes in the process of vesicle biogenesis. Alterations in extracellular environments change the synthesis patterns of proteins and nucleic acids in stem cells (Katsuda et al., 2013). Preconditioning with pro-inflammatory cytokines or virulence factors simulates the environment of early infections and tissue damages, in which MSCs adapt and prepare themselves to survive in harsh conditions, by releasing soluble factors or extracellular vesicles to regulate microenvironment and accelerate tissue repair (Munir et al., 2020). The activation of toll-like receptors (TLRs)/pathogen-associated molecular patterns (PAMPs) signaling pathway plays an important role in stimulating immune responses and tissue repair in MSCs (Shirjang et al., 2017). PAMPs, such as LPS (Ti et al., 2015), or synthetic ligands, such as Poly (I:C; Pierce and Kurata, 2021), can interact with TLRs in MSCs to initiate downstream pathways to enhance the antimicrobial and immunomodulatory proteomic profile of secreted EVs, which can promote M2 polarization and enhance pathogen phagocytosis of macrophages/monocytes. Similar enhancements in EVs' biological properties can be acquired by preconditioning with pro-inflammatory cytokines, TNF-α (Nakao et al., 2021) and IFN-γ (Varkouhi et al., 2019). Apart from enhancing expressions of therapeutic substances, stimulations of pro-inflammatory cytokines or virulence factors also induce a larger amount of exosome secretion (Ti et al., 2015; Varkouhi et al., 2019; Nakao et al., 2021). Upregulation of specific microRNA expressions in MSCs endows exosomes with properties such as pro-angiogenesis (Li et al., 2016; Tao et al., 2017; Ding et al., 2019), immunomodulation (Fan et al., 2021), and anti-apoptosis (Yu et al., 2015; Shi et al., 2020). What is more, researchers have found that biophysical stimuli, such as hypoxia and ionizing radiation (Jabbari et al., 2019), can alter biomolecules composition of EVs to increase the pro-angiogenic property. Gorgun et al. (2021) reported inflammatory cytokines (TNF-α, IL-1α) and hypoxia exerted synergistic effects on improving the pro-angiogenic property of secreted MSC-EVs. Similarly, in another research, hypoxia-treated human ADSCs released exosomes with a greater pro-angiogenesis property in grafted tissue *via* regulating VEGF/VEGF-R signaling (Han et al., 2019).

Incorporation of Therapeutic Drugs

The post-loading approach refers to primitive exosome processing, in which therapeutic agents are internalized or attached to the isolated exosomes. Conventional post-loading methods include passive incubation, electroporation, sonication, and transfection (de Jong et al., 2020). Antimicrobial or immunomodulatory substances, such as antibiotics (Yang et al., 2021), antimicrobial nanoparticles (Qian et al., 2020), microRNA mimics (Lv et al., 2020), or other therapeutic drugs (Sun et al., 2010) can be loaded into exosomes. For instance, Yang et al. (2021) improved cell permeability of vancomycin, a hydrophilic antibiotic, by loading it into exosomes *via* sonication. With such modification, vancomycin was able to target and eradicate intracellular *Methicillin-resistant S. aureus*. Furthermore, exosomes as vectors helped the accumulation of antibiotics in liver and spleen, where infected macrophages were predominantly distributed. Encapsulating antibiotics with exosomes contributes to alleviating toxicity and possibility of antibiotic resistance by lowering the dosage of antibiotics (Yang et al., 2021). In another research, Qian et al. (2020) observed synergistic effects between exosomes and silver nanoparticles (AgNPs) in repairing infected wounds. The combination of exosomes and AgNPs formed a protein corona around the nanoparticles, which stabilized the nanoparticles and prevented agglomeration. When AgNP-Exos were administered in the infection site, AgNPs and bioactive molecules of exosomes were released by the lysis of exosome membranes *via* the phospholipase secreted by *P. aeruginosa*. AgNPs exhibited antimicrobial activity by interrupting the integrity of bacterial cell walls, meanwhile exosomes promoted epithelial, vascular, and nerve fiber regeneration (Qian et al., 2020). Analogously, the incorporation of curcumin into exosomes greatly improved the solubility, stability, and bioavailability of curcumin, which is an insoluble, hydrophobic polyphenol compound. Moreover, exosomes increased delivery of curcumin to activated monocytes because of target specificity. In an LPS-induced septic shock mouse model, exosomal curcumin group dramatically surpassed curcumin group in terms of downregulating the $CD11b^{+}Gr\text{-}1^{+}$ cell population, which was characteristics in acute lung inflammation (Sun et al., 2010). Generally, exosomes as drug delivery system help improve the solubility, stability, and bioavailability of therapeutic drugs. Moreover, the drug-exosome combination also benefits from the target specificity of exosomes.

Increasing Delivery Efficiency

There are two strategies in enhancing the on-target effect, minimizing sequestration by MPS and increasing tissue target specificity, which can be summarized as "eat me/do not eat me" strategy.

Exosomes are mainly cleared by MPS, which attributes to the short half-life of exosomes in blood circulation (Morishita et al., 2015). Camouflaging exosomes with anti-phagocytotic molecules is a feasible strategy to avoid MPS uptake, and thus extend exosomes' half-life in circulation. Anti-phagocytotic candidate molecules that can be inserted or expressed on the surface of exosomes include CD47, CD24, CD44, CD31, β2M,

PD-L1, App1, and DHMEQ (Parada et al., 2021). The time EVs stayed in the plasma doubled after CD47 modification, and improved biodistribution in targeted tissue was observed (Wei et al., 2021). When EVs were recognized by macrophages, the activation of CD47-SIRPα pathway initiated immune evasion and reduced the phagocytosis of EVs (Chao et al., 2012). Some evidence indicated clathrin heavy chain (Cltc) plays an important role in mediating endocytosis of exosomes in the liver and spleen. Wan et al. (2020) encapsulated siCltc into exosomes *via* electroporation to block endocytosis by mononuclear phagocyte system. *Via* such modification, exosomes' biodistribution pattern was altered with less exosome detained in liver and spleen and more arriving target organs (Wan et al., 2020). In the treatment of lung cancer, Belhadj et al. (2020) developed a dosing scheme: First they saturated macrophage receptors with cationized mannan-decorated extracellular vesicles, and then injected chemotherapy drugs-loaded exosomes, which were functionalized with CD47, to further avoid sequestration in liver and target lung tissue. The combined strategy induced a 123.53% increase in tumor distribution compared to conventional nanocarriers (Belhadj et al., 2020).

Surface decorations that promote exosome-target cell interaction enhance precise delivery. Zhou et al. (2021a) strengthened the therapeutic effect of MSC-EVs in bacteria-associated ALI, by co-incubation of MSC-EVs with high molecular weight hyaluronic acid (HMW HA; 1.0 MDa). It seems that HMW HA played a role as the connecter between target cells and MSC-EVs, thus promoting trafficking, adhesion, and internalization of EVs. This process was mediated by the interaction between HA and CD44, a surface receptor enriched in both MSC-EVs and immune cells. Such modification boosted the therapeutic potency of EVs in *P. aeruginosa* pneumonia (Zhou et al., 2021a).

FUTURE PERSPECTIVES

Considering the breadth of research into exosome therapies, as well as the vast need for new therapeutic modalities for infectious diseases, this area of medicine is a growing field. Based upon these studies, we suggest that a combination of both host-directed and pathogen-directed therapeutic approaches may represent a valuable and exploitable strategy, over single therapies, to (i) control multidrug-resistant infections, (ii) minimize the risk of emergence of drug resistance, and (iii) reduce the time of therapy. There are many new approaches to improving the efficacy of exosome therapies, such as enhancing the on-target effect of exosome therapies or combining exosomes with existing drugs for synergistic effects. There are also many new research developments that will expand the possibilities for exosome therapies.

Although several clinical trials have preliminarily demonstrated the safety and efficacy of MSC-Exos in patients, because of cell source difference, the heterogeneity of MSCs is in the way of exosome quality control and comprehensive evaluation of different studies. For example, the consistency and uniformity of MSC-Exo quality cannot be quantified or guaranteed, when MSCs are extracted from different fat donors. A rigorous quality control system of MSC-Exo production is critical to reduce batch-to-batch variation. Therefore, overcoming the heterogeneity of stem cells is one of the most pressing issues in the process of clinical translation of exosome therapy. To tackle these challenges, MSCs can also be generated from pluripotent stem cells (PSCs) to overcome many limitations of above MSC sources (Lian et al., 2010). These MSCs can be derived from the same parental PSC to avoid disadvantages of adult MSCs i.e., batch-to-batch variations in MSC quality, stem cell senescence, and limited proliferative potency (Lian et al., 2016). Most recently, GMP-grade MSCs derived from human induced PSCs have been used in refractory graft-vs.-host-disease (GVHD) in clinical trials (Bloor et al., 2020). Exosomes produced from PSC-MSCs may provide another putative therapeutic tool to overcome many limitations of adult MSC-Exos or EVs (Thakur et al., 2021). Future work should focus on establishing international standards in quantifying the quality and consistency of MSC-Exo therapies. Proper completion and data sharing of existing clinical trials are needed in convenience of comprehensive evaluation and further study. Furthermore, technological breakthrough in industrial mass production of clinical-grade MSC-Exos is a prerequisite for extensive clinical applications.

AUTHOR CONTRIBUTIONS

YL, SJ, XW, and CD are involved in manuscript writing, conceptualization, and figure drawing and data analysis. YW and DH supervised and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Mesenchymal Stromal/Stem Cells-Derived Exosomes as an Antimicrobial Weapon for Orodental Infections

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The oral cavity as the second most various microbial community in the body contains a broad spectrum of microorganisms which are known as the oral microbiome. The oral microbiome includes different types of microbes such as bacteria, fungi, viruses, and protozoa. Numerous factors can affect the equilibrium of the oral microbiome community which can eventually lead to orodental infectious diseases. Periodontitis, dental caries, oral leukoplakia, oral squamous cell carcinoma are some multifactorial infectious diseases in the oral cavity. In defending against infection, the immune system has an essential role. Depending on the speed and specificity of the reaction, immunity is divided into two different types which are named the innate and the adaptive responses but also there is much interaction between them. In these responses, different types of immune cells are present and recent evidence demonstrates that these cell types both within the innate and adaptive immune systems are capable of secreting some extracellular vesicles named exosomes which are involved in the response to infection. Exosomes are 30–150 nm lipid bilayer vesicles that consist of variant molecules, including proteins, lipids, and genetic materials and they have been associated with cell-to-cell communications. However, some kinds of exosomes can be effective on the pathogenicity of various microorganisms and promoting infections, and some other ones have antimicrobial and anti-infective functions in microbial diseases. These discrepancies in performance are due to the origin of the exosome. Exosomes can modulate the innate and specific immune responses of host cells by participating in antigen presentation for activation of immune cells and stimulating the release of inflammatory factors and the expression of immune molecules. Also, mesenchymal stromal/stem cells (MSCs)-derived exosomes participate in immunomodulation by different mechanisms. Ease of expansion and immunotherapeutic capabilities of MSCs, develop their applications in hundreds of clinical trials. Recently, it has been shown that cell-free therapies, like exosome therapies, by having more advantages than previous treatment methods are emerging as a promising strategy for the treatment

of several diseases, in particular inflammatory conditions. In orodental infectious disease, exosomes can also play an important role by modulating immunoinflammatory responses. Therefore, MSCs-derived exosomes may have potential therapeutic effects to be a choice for controlling and treatment of orodental infectious diseases.

Keywords: exosomes, mesenchymal stromal/stem cells, dental infection controls, dentistry, orodental

INTRODUCTION

The oral cavity is the second most diverse microbial community in the human body after the gut (Caselli et al., 2020). Numerous microorganisms including fungi, viruses, protozoa, and over 700 species of bacteria in this community are called "microbiome" (Deo and Deshmukh, 2019). The microbiome is a term that was coined by Joshua Lederberg, a Nobel Prize laureate, to explain the ecological community of symbiotic, commensal, and pathogenic microorganisms that share human body space (Kilian et al., 2016). Orodental infections are caused by changes in the balance of microbial populations or the dynamic relationship between them and the oral cavity (Cho and Blaser, 2012; Marsh et al., 2015). In addition, the oral cavity is exposed to external environmental microorganisms that can cause oral diseases (Gerba, 2015).

The host immune system plays an important role in defending against pathogens (Dunkelberger and Song, 2010). At first, It fights against pathogens through innate immunity and then through adaptive immunity (Cerny and Striz, 2019). Although the innate immune system response is general, non-specific, and does not directly target a single pathogen, it provides a defense barrier against all infectious agents (Aderem and Ulevitch, 2000). The skin and mucosal membranes act as a mechanical barrier against pathogens, also epithelial cells contain peptides that have antimicrobial properties (Ganz, 2003; Oppenheim et al., 2003). If the pathogens can get past the primary defense, the second line of defense becomes active (Frank, 2000). In the infected area, an inflammatory response begins due to stimulation of high blood pressure, the blood vessels dilate, and white blood cells leave the veins during diapause to fight the pathogen (Chen et al., 2018). The vessels diameter increase, because of the secretion of "histamine" from mast cells. Mast cells are a type of white blood cell and phagocytes that draw in pathogens and kill them. During the inflammatory response, the infected area becomes red, swollen, and painful (Janeway et al., 2001b; Csaba et al., 2003) and, the immune system may release substances that raise the body temperature and cause fever. An increase in temperature can decelerate the growth of pathogens and the immune system fights against infectious agents more quickly (Evans et al., 2015). Some phagocytic cells detect pathogenic cells and other kill cells in the body and digest them (Bain, 2017). In the human body, some proteins are normally inactive and activated in infection conditions. They create pores in the membrane of pathogenic cells and destroy them. These proteins are unable to distinguish different pathogens from each other and attack all pathogens non-specifically (Janeway et al., 2001a).

Acquired or specific immunity is activated when a pathogen can cross the innate or non-specific immune mechanism (McDade et al., 2016). The cells of the body have signs that the immune system distinguishes them from other foreign cells (Rich and Chaplin, 2019). When the immune system encounters cells that do not have these signs, it recognizes them as aliens and attacks them through specific or acquired mechanisms, using lymphocytes and producing antibodies (Elgert, 2009). This mechanism develops during the growth of the human body. In this way, with the development of the human body and exposure to pathogens and various vaccinations, a library of antibodies from the cells of the immune system related to various pathogens is created in the body. This process is sometimes called "Immunological Memory" because immune cells remember their former enemies (Crotty and Ahmed, 2004). The acquired mechanism produces antibodies to protect the body against foreign agents, for example, if previous pathogens attack the body, it will produce antibodies more quickly and eliminate the infection (Jerne, 1973). Acquired immunity is caused by the presence of antigens. Antigens are usually located on the surface of pathogen cells, and each pathogen has its antigen (Lamm, 1997). The immune system responds to antigens by certain cells or by producing antibodies (**Figure 1**). Antibodies attack antigens and produce a signal that attracts phagocytes or other killer cells (Davies and Cohen, 1996). In the immune system, cells like mast cells (Raposo et al., 1997), epithelial cells (van Niel et al., 2001; Lin et al., 2005), antigen-presenting cells (Zitvogel et al., 1998), T lymphocytes (Anel et al., 2019), B lymphocytes (Kato et al., 2020), neutrophils (Vargas et al., 2016), and macrophage (Singhto et al., 2018) release small extracellular vesicles (EVs) which called "exosomes."

THE ROLE OF EXOSOMES IN MICROBIAL INFECTIONS

EVs are made and secreted in normal and diseased states by most types of cells and have an essential role in intercellular communication and facilitate the immunity process They contain a wide range of lipid-bound nanoparticles that vary in size (Yanez-Mo et al., 2015; Maas et al., 2017; Herrmann et al., 2021). There is no certain agreement on markers or specific naming for EV subtypes, and EVs are usually classified according to their biogenesis pathway or their physical properties used for isolation (Théry et al., 2018). In fact, differences in size help to separate different types of EVs. Microvesicles, exosomes, and apoptotic bodies are the three main subtypes of EVs which are distinguished by their biogenesis, size, content, release pathways, and function (**Figure 2**; Karpman et al., 2017; Doyle and Wang, 2019; Ståhl et al., 2019).

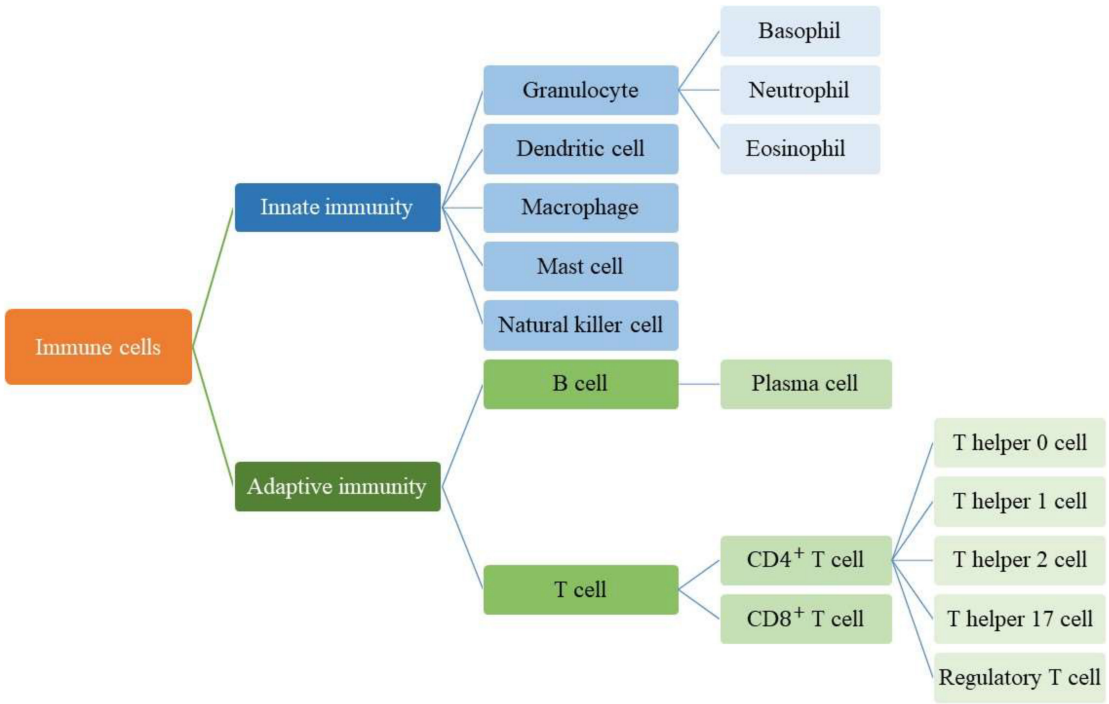

FIGURE 1 | Immune system cells in innate and adaptive immunity responses.

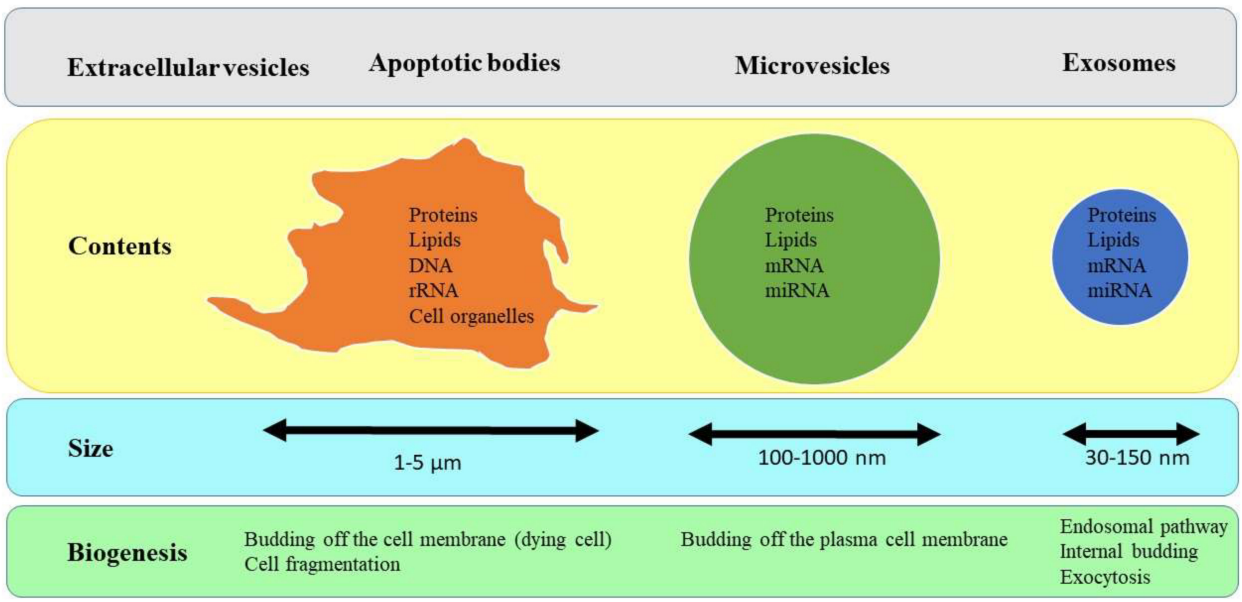

FIGURE 2 | Three main subtypes of EVs and their properties.

In the late 1960s, for the first time, Bonucci (1967) and Anderson (1969) described small, secreted vesicles as small, 100-nm-diameter vesicles secreted by chondrocytes. A special subset of small EVs, between 30 and 150 nm in diameter, are known as exosomes that appear through endosomal biogenesis pathways (Willms et al., 2018; Tschuschke et al., 2020). A wide range of cell types can secrete exosomes, and the size of exosomes can vary even for exosomes secreted from a single cell line (Zhang et al., 2019). Exosomes consist of approximately 4,400 proteins, 194 lipids, 1,639 mRNAs, and 764 miRNAs and as secretory vesicles, the possibility of their physiological function has been defined (Mathivanan et al., 2012;

Kim et al., 2013; Zhang et al., 2019; O'Brien et al., 2020). They can regulate the immune system and also interfere with biological processes. Pathogenic infections alter the number of exosomes, their contents, and membrane structure (Li et al., 2006; Zhang et al., 2018).

Infectious diseases like lower respiratory infections, malaria, diarrhea, tuberculosis (TB), human immunodeficiency virus (HIV) infection, and malaria are major reasons for morbidity and mortality worldwide and their treatment is challenging (Murray et al., 2014; Kirtane et al., 2021). Exosomes can interfere with the processes of infectious diseases. On the one hand, they can contribute to the pathogenesis of microorganisms, be effective in the progression of infection, and can fight against pathogens and infections. This functional variation of exosomes depends on the source of cells and their contents. To confirm this, **Tables 1**, **2** provide examples of the role of exosomes in infectious diseases. Briefly, **Table 1** provides examples of the effects of exosomes on the pathogenicity of various microorganisms so that they cause and promote infections, and **Table 2** lists several antimicrobial and anti-infective functions of exosomes in microbial diseases.

ORODENTAL INFECTIOUS DISEASE

Orodental infectious diseases are caused by both pathogenic microorganisms and the loss of balance in the ecological community of symbiotic microorganisms in the oral cavity. Oral microbial diseases include a wide range of different diseases such as periodontitis and caries. If proper measures are not taken

TABLE 1 | The role of exosomes in the development of infections caused by various pathogens.

Microorganisms	Pathogens	Exosomes' effects in promotion of infection	References
Bacteria	*Staphylococcus aureus*	*S. aureus*-derived exosomes spread the infection in the body by transmission of bacterial pore forming molecule α-toxin to distant cells.	Husmann et al., 2009
	Bacillus anthracis	Exosomes from *B. anthracis*-infected cells transport the lethal toxin virulence factor to sites distal to the infection.	Abrami et al., 2013
	Helicobacter pilori	Exosomes in *H. pylori* infection are secreted from cytotoxin-associated gene A (CagA)-expressing gastric epithelial cells enter the circulation and deliver CagA, a virulence factor, to distant organs and tissues.	Shimoda et al., 2016
Viruses	Human T-cell leukemia virus-1 (HTLV-1)	Exosomes produced by HTLV-1-infected T-cell lines deliver the viral transactivator (Tax) protein which can activate transcription in target cells.	Jaworski et al., 2014
	HIV-1	Exosomes derived from HIV-1-infected cells contain proteins of viral and cellular origin that inhibit target cell migration as well as dsRNA/ssRNA which can increase nuclear gene expression and promote infection.	Barclay et al., 2017
	Human herpesvirus 6 (HHV-6)	Exosomes derived from HHV-6-infected cells contain mature virions; therefore, they help spread infection more efficiently	Mori et al., 2008
	Hepatitis A virus (HAV)	Vacuolar protein sorting 4 homolog B (VPS4B) and ALG-2-interacting protein X (ALIX) play an important role in cloaking the HAV released from cells in host-derived membranes so protecting the virion from antibody-mediated neutralization. These enveloped viruses resemble exosomes and can escape the host immune system.	Feng et al., 2013
	Hepatitis B virus (HBV)	Exosomes derived from HBV-infected hepatocytes transport miR-21, miR-29a, and other miRs to Tamm-Horsfall Protein 1 (THP-1) macrophages, which results in suppressing Interleukin 12p35 (IL-12p35) mRNA expression and limitation of host innate immune response.	Kouwaki et al., 2016
	Hepatitis C virus (HCV)	*In vitro* study has shown that hepatic exosomes by protecting HCV against antibody neutralization can help transmit HCV infection.	Cosset and Dreux, 2014
	Hepatitis E virus (HEV)	HEV RNA-containing particles in an exosome fraction are infectious and cannot be neutralized by anti-HEV antibodies so they protect from the immune response.	Chapuy-Regaud et al., 2017
	Epstein-Barr virus (EBV)	EBV escapes immune responses by sequestering immune effectors like caspase-1, interleukin 1b (IL-1b), interleukin 18 (IL-18), and interleukin 33 (IL-33), in exosomes which are continuously secreted.	Ansari et al., 2013
	HIV type 1 (HIV-1)	Exosomes derived from HIV-1-infected cells allow HIV-1 to replicate inside resting human primary CD4 + T lymphocytes.	Arenaccio et al., 2014
Yeast	*Saccharomyces cerevisiae*	Cytosolic Sup35 NM prions are packaged into exosomes which are able to transmit the prion phenotype to neighboring cells.	Liu et al., 2016
Parasites	*Trypanosoma brucei*	*T. brucei rhodesiense* EVs mediating non-hereditary virulence factor transfer by containing the serum resistance-associated protein (SRA) and causing host erythrocyte remodeling, inducing anemia. Also, these EVs by transferring the SRA to *T. brucei* gain the ability to evade innate immunity.	Szempruch et al., 2016
	Toxoplasma gondii	Exosomes secreted by *T. gondii*-infected host cells. L6 cells could change the host cell proliferation and alter the host cell cycle and slight enhancement of S phase in L6 cells.	Kim et al., 2016
	Trypanosoma cruzi	*T. cruzi*-derived have been shown to increase the secretion of interleukin 4 (IL-4) and interleukin 10 (IL-10) and a diminished inducible nitric oxide synthase expression in CD4 + T cells and macrophages.	Trocoli Torrecilhas et al., 2009

TABLE 2 | The function of different sources of exosomes in infectious disease.

Source of exosomes	Role of exosomes	References
Adipose tissue-derived MSCs	Combined with melatonin, an anti-inflammatory hormone, could limit inflammation caused by colitis *in vivo*.	Chang et al., 2019
Colonic lumen of IBD patients	Contribute to IBD diagnosis by containing significantly higher mRNA and protein levels of IL-6, IL-8, IL-10, and TNF-α compared with those from healthy individuals.	Larabi et al., 2020
Dendritic cells	Stimulate the responses of IL-4 and TNF-α and increase the IL-4 production in CD14 in *Malassezia sympodialis* infection.	Gehrmann et al., 2011
Dendritic cells	Stimulate the production of IgM, IgG3, and IgG1 types of anti-Cps14 responses in *Streptococcus pneumoniae* type 14 infection.	Colino and Snapper, 2007
Dendritic cells	Promote intestinal barrier function by activating NF-κB *via* the exosomal miR-146b in a murine model of colitis.	Nata et al., 2013; Alexander et al., 2015
HBV-infected hepatocytes	Stimulate MyD88, Toll-IL-1 receptor-containing adaptor molecule-1 (TICAM-1), and mitochondrial antiviral signaling (MAVS)-dependent pathways to induce NKG2D ligand expression and evoke NK cells.	Kouwaki et al., 2016
Healthy human semen	Prevent the spread of HIV-1 and reduce the intravaginal proliferation of AIDS in mice as well as the systematic spread of virus and viremia.	Madison et al., 2015
Human vaginal secretions	Have inhibitory properties against HIV-1 infection and protect women against HIV-1 infection as a female innate defense.	Smith and Daniel, 2016
Macrophages	Suppression of IFN-γ stimulated MHC class II and CD64 expression on BMMØ dependent on lipoproteins, TLR2 and MyD88 and also increase secretion of chemokines and stimulate migration of macrophages and splenocyte in *Mycobacterium tuberculosis* infection.	Singh et al., 2011, 2012
Macrophages	Induce Pro-inflammatory responses dependent on TLR 2, TLR4, and MyD88 in *Mycobacterium avium* infection.	Bhatnagar et al., 2007
MDSC	Reduce the severity of colitis by inhibiting Th1 proliferation and promoting Treg cell expansion.	Wang et al., 2016
MSCs	Inhibit inflammatory cytokine production by colonic macrophages stimulated with LPS and promote the polarization of these macrophages into M2 phenotype *in vitro* and also, alleviate colitis by inhibiting expression of IL-7 and iNOS in mouse colonic macrophages *in vivo*.	Mao et al., 2017; Cao et al., 2019
Mycoplasma-infected tumor cells	Activate the splenic B cells and increase the production of splenocytes cytokines.	Yang et al., 2012
Plasmodium yoelii-infected reticulocytes	Decrease period of parasitemia and increase clearance of parasites, reticulocytosis, immune modulation, elicits IgG2a and IgG2b, and promoted survival time and protect mice from lethal infections.	Martin-Jaular et al., 2011
uMSCs	Contain some small RNAs (let-7f, miR-145, miR-199a, and miR-221) can prevent HCV replication by detecting specific cellular factors or binding directly to the virus genome and intercede the antiviral process.	Qian et al., 2016

IBD, Inflammatory bowel disease; IgM, Immunoglobulin M; IgG3, Immunoglobulin G3; IgG1, Immunoglobulin G1; Cps14, capsular polysaccharide of S. pneumonia type 14; NF-κB, Nuclear factor- κB; MyD88, Myeloid differentiation primary response 88; NKG2D, Natural killer group 2 member D; NK cells, natural killer cells; AIDS, acquired immune deficiency syndrome; IFN-γ, Interferon gamma; BMMØ, bone marrow derived macrophage; TLR 2, toll like receptor 2; TLR 4, toll like receptor 4; MDSC, myeloid-derived suppressor cells; LPS, Lipopolysaccharides; IL-7, interleukin 7; iNOS, inducible nitric oxide synthase; IgG2a, Immunoglobulin G2a; IgG2b, Immunoglobulin G2b; uMSC, umbilical mesenchymal stem cells.

to control and treat mouth-infectious diseases, it can lead to whole-body systemic diseases (**Table 3**).

Periodontitis

The periodontium contains the supporting tissues around the structure of the teeth, such as the gingiva, cementum, junctional epithelium, periodontal ligament, and alveolar bone (Taba et al., 2005). Periodontal diseases are a result of periodontal structure destruction (Nanci and Bosshardt, 2006). The prevalence of periodontal disease is very high and more than 90% of adults worldwide suffer from it (Pihlstrom et al., 2005). There are two main categories of periodontal disease: gingivitis and periodontitis (Dorfer et al., 2004). Gingivitis is a milder form of periodontitis and is limited to gum tissue, but periodontitis occurs when the inflammation spreads to deeper tissues and causes loss of supporting connective tissue and alveolar bone (Kononen et al., 2019). The structure and texture of the periodontium can provide a suitable environment for the growth of various microorganisms (Cobb and Killoy, 1990). Microorganisms such as *Porphyromonas gingivalis*, *Tannerella forsythensis*, and *Treponema denticola* play an important role in the development of periodontal disease (Mineoka et al., 2008). *T. forsythensis*, *T. denticola*, and *Treponema lecithinolyticum* can be present in all phases of periodontal disease (Scapoli et al., 2015). *Porphyromonas endodontalis* and *p. gingivalis* are more specifically associated with periodontitis and *Capnocytophaga ochracea* and *Campylobacter rectus* associated with gingivitis (Scapoli et al., 2015).

Dental Caries

Tooth decay is the most common chronic infectious disease which deals with the chronic and progressive destruction of hard tooth tissue (Ozdemir, 2013; Rathee and Sapra, 2020). In this disease, the hard tooth tissue (enamel and dentin) loses calcium and phosphorus minerals due to acid secretion from cariogenic bacteria (mainly *Streptococcus mutans*) (Moynihan and Petersen, 2004; Selwitz et al., 2007; Krzysciak et al., 2014). There are various causes for caries, but in general, the four main factors of tooth-adherent specific bacteria, time, susceptible tooth surface, and fermentable carbohydrates play a role in tooth decay (Tahir and Nazir, 2018). These four factors always cause caries, and if each one is not present, the tooth will not decay (Fejerskov, 1997;

TABLE 3 | Systemic diseases associated with oral microbiome and orodental infection.

The human body systems	Disease	References
Gastrointestinal system diseases	IBD	Read et al., 2021
	Gastrointestinal cancer risk increases	Meurman, 2010
	Pancreatic cancer	Fan et al., 2018
Nervous system diseases	Alzheimer's disease	Miklossy, 1993; Riviere et al., 2002; Poole et al., 2013
Endocrine system diseases	Diabetes mellitus	Cianciola et al., 1982; Rylander et al., 1987; Emrich et al., 1991; Thorstensson and Hugoson, 1993; Casarin et al., 2013
	Adverse pregnancy outcomes (APOs)	Han et al., 2004, 2010; Madianos et al., 2013
	Obesity	Goodson et al., 2009
	Polycystic ovary syndrome (PCOS)	Lindheim et al., 2016
Human immune system diseases	Rheumatoid arthritis (RA)	Zhang et al., 2015
	HIV infection	Dang et al., 2012; Li et al., 2014a; Heron and Elahi, 2017
Cardiovascular system diseases	Atherosclerosis	Koren et al., 2011

Sheiham, 2001; Wade, 2013; Kidd and Fejerskov, 2016; Tahir and Nazir, 2018). Tooth decay, in addition to its high prevalence, affects a wide range of age groups, and from children to the elderly, they are at risk for tooth decay (Smith and Szuster, 2000). The most harmful type of caries occurs in childhood and is named "early childhood caries" which has become a common public health problem among preschool children worldwide (Colak et al., 2013; Alazmah, 2017). Numerous factors, including the oral microbiome, affect the incidence of tooth decay in children (Dzidic et al., 2018). Bacteria are considered the main pathogen in tooth decay (Dzidic et al., 2018). Different lactobacilli promote the development of dental caries, but the most important microorganism in the development of dental caries is *S. mutans* (Loesche, 1996).

Oral Leukoplakia

In 1877, oral leukoplakia was described for the first time by Schwimmer (1877) Oral leukoplakia is one of the most common diseases of the oral mucosa which has malignant potential (van der Waal et al., 1997). According to the Pindborg study, leukoplakia is a white patch on the oral mucosa that cannot be removed and there is no other clinical diagnosis (Mehta et al., 1969; Bánóaczy, 1983). Different microorganisms like *Fusobacterium*, *Leptotrichia*, *Campylobacter*, and *Rothia* species were detected in oral leukoplakia (Amer et al., 2017).

Oral Squamous Cell Carcinoma

Oral squamous cell carcinoma is the eighth most common cancer worldwide and is the most common oral malignancy (Scully and Bagan, 2009). Numerous hypotheses have been proposed for the association of microorganisms and their products with oral cancer (Perera et al., 2016). Acetaldehyde converted from ethanol, reactive oxygen species, reactive nitrogen species, and volatile sulfur compounds by bacteria are some examples of carcinogenic substances which can cause oral cancer (Meurman and Uittamo, 2008). The metabolization of alcohol to acetaldehyde can be happened by *Streptococcus gordonii*, *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus salivarius*, *Streptococcus sanguinis,* and Candida by the using of alcohol dehydrogenase enzyme (Mantzourani et al., 2009; Marttila et al., 2013). Also, hydrogen sulfide (H2S), methyl mercaptan (CH3SH), and dimethyl sulfide [(CH3)2S] are produced by *P. gingivalis*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum* (Nakamura et al., 2018; Suzuki et al., 2019).

APPLICATION OF STEM CELLS-DERIVED EXOSOMES IN ORODENTAL INFECTIONS

Mesenchymal stromal/stem cells (MSCs) are adult pluripotent stem cells with self−renewing potential that have been administered in different types of diseases (Undale et al., 2009; Fitzsimmons et al., 2018). The unique biomedical characteristic of MSCs is their stemness by stimulating their proliferation and differentiating into multi-lineage cells (da Silva Meirelles et al., 2006). MSCs are immunologically safe. Low expression of major histocompatibility complex (MHC) class I molecules and expression of only a few MHC class II molecules make MSCs low immunogenicity cells (Hass et al., 2011; Lee et al., 2014). Immunomodulatory and regenerative functions of MSCs have been shown in various types of diseases (Zappia et al., 2005; Corcione et al., 2006; Wang et al., 2013; Forbes et al., 2014; Le Blanc and Davies, 2015). MSCs-derived exosomes also have angiogenic potential that can improve ischemic diseases (Babaei and Rezaie, 2021). Senescence of MSCs during *in vitro* expansion makes the cells less productive and can increase disease severity by causing inflammaging (Lee and Yu, 2020). Also, weak engraftment of infused MSCs, and donor-dependent variations are some limitations of application MSCs in clinical trials (Karp and Leng Teo, 2009; Siegel et al., 2013; Li et al., 2016). An alternative method to improve MSC-based therapy is to use exosomes (Zavatti et al., 2020). Being free of immunogenic problems and not being trapped in the lung or liver like infused MSCs, and keeping the therapeutic functions of their cells of origin make MSC exosomes more suitable for clinical application than MSCs (**Table 4**; U.S. National Library of Medicine clinicaltrials.gov, 2021). The immunomodulatory function of MSCs and MSC-derived exosomes is the most important clinical feature of their application (Kang et al., 2020). Recent studies show that MSCs can inhibit T cells, B cells, natural killer cells, and dendritic cells and result in immune suppression (Bocelli-Tyndall et al., 2007; Li et al., 2012). Regarding MSCs properties, they have been used in clinical trials over several decades (Kabat et al., 2020). The MSCs mainly modulate the activity of the immune system by paracrine agents and exosomes, and the exosomes play an important role in cellular communication

TABLE 4 | Some applications of MSCs-derived exosomes in recent clinical trials (U.S. National Library of Medicine clinicaltrials.gov, 2021).

Disease type	Official study title	Condition or disease	Intervention/ treatment	Last update	ClinicalTrials.gov Identifier
Cancer	Phase I study of mesenchymal stromal cells-derived exosomes with KrasG12D siRNA for metastatic pancreas cancer patients harboring KrasG12D mutation	KRAS NP_004976.2:p.G12D Metastatic pancreatic adenocarcinoma Pancreatic ductal adenocarcinoma Stage IV pancreatic cancer AJCC v8	Mesenchymal stromal cells-derived exosomes with KRAS G12D siRNA	April 29, 2021	NCT03608631
Cardiovascular diseases	Safety and efficacy of allogenic mesenchymal stem cells derived exosome on disability of patients with acute ischemic stroke: a randomized, Single-blind, Placebo-controlled, Phase 1, 2 trial	Cerebrovascular disorders	Exosome	January 25, 2021	NCT03384433
COVID-19 treatment	A Pilot clinical study on aerosol inhalation of the exosomes derived from allogenic adipose mesenchymal stem cells in the treatment of severe patients with novel coronavirus pneumonia	Coronavirus	MSCs-derived exosomes	September 7, 2020	NCT04276987
	A tolerance clinical study On aerosol inhalation of mesenchymal stem cells exosomes in healthy volunteers	Healthy	Biological: 1X level of MSCs-Exo Biological: 2X level of MSCs-Exo Biological: 4X level of MSCs-Exo Biological: 6X level of MSCs-Exo Biological: 8X level of MSCs-Exo	August 4, 2021	NCT04313647
	A phase I/II randomized, double blinded, placebo trial to evaluate the safety and potential efficacy of intravenous infusion of zofin for the treatment of moderate to SARS related to COVID-19 infection vs. placebo	Corona virus infection COVID-19 SARS Acute respiratory distress syndrome	Biological: Zofin Other: Placebo	February 23, 2021	NCT04384445
	Bone marrow mesenchymal stem cell derived extracellular vesicles infusion treatment for COVID-19 associated acute respiratory distress syndrome (ARDS): A phase II clinical trial	COVID-19 ARDS Pneumonia, Viral	Biological: DB-001 Other: Intravenous normal saline	July 14, 2021	NCT04493242
	Mesenchymal stem cell exosomes for the treatment of COVID-19 positive patients with acute respiratory distress syndrome and/or novel coronavirus pneumonia	COVID-19 Novel coronavirus pneumonia Acute respiratory distress syndrome	MSC-exosomes delivered intravenously every other day on an escalating dose: (2:4:8) MSC-exosomes delivered intravenously every other day on an escalating dose (8:4:8) MSC-exosomes delivered intravenously every other day (8:8:8)	July 21, 2021	NCT04798716
	The protocol of evaluation of safety and efficiency of method of exosome inhalation in SARS-CoV-2 associated two-sided pneumonia	COVID-19 SARS-CoV-2 pneumonia COVID-19	EXO 1 inhalation EXO 2 inhalation Placebo inhalation	November 4, 2020	NCT04491240
	The extended protocol of evaluation of safety and efficiency of method of exosome inhalation in COVID-19 associated two-sided pneumonia	COVID-19 SARS-CoV-2 pneumonia COVID-19	EXO 1 inhalation EXO 2 inhalation Placebo inhalation	October 26, 2020	NCT04602442
Immune diseases	Phase 1 study of the effect of cell-free cord blood derived microvesicles On β-cell mass in type 1 diabetes mellitus (T1DM) patients	Diabetes mellitus type 1	MSC exosomes	May 14, 2014	NCT02138331

(Continued)

TABLE 4 | (Continued)

Disease type	Official study title	Condition or disease	Intervention/ treatment	Last update	ClinicalTrials.gov Identifier
	Effect of umbilical mesenchymal stem cells derived exosomes on dry eye in patients with chronic graft vs. host diseases	Dry eye	Umbilical mesenchymal stem cells derived exosomes	February 21, 2020	NCT04213248
	Effect of adipose derived stem cells exosomes as an adjunctive therapy to scaling and root planning in the treatment of periodontitis: A human clinical trial	Periodontitis	Adipose derived stem cells exosomes	February 17, 2020	NCT04270006
	Exosome of mesenchymal stem cells for multiple organ dysfuntion syndrome after surgical repaire of acute type A aortic dissection: a Pilot Study	Multiple organ failure	MSC exosomes	May 6, 2020	NCT04356300
Neurological diseases	Focused ultrasound delivery of exosomes for treatment of refractory depression, Anxiety, and Neurodegenerative dementias	Refractory depression anxiety, Disorders neurodegenerative diseases	Exosomes	March 5, 2021	NCT04202770
	The use of exosomes In craniofacial neuralgia	Neuralgia	Exosomes	March 5, 2021	NCT04202783
	Open-label, Single-center, Phase I/II clinical trial to evaluate the safety and the efficacy of exosomes derived from allogenic adipose mesenchymal stem cells in patients with mild to moderate dementia Due to Alzheimer's disease	Alzheimer's disease	Biological: Low dosage MSCs-Exos administrated for nasal drip Biological: Mild dosage MSCs-Exos administrated for nasal drip Biological: high dosage MSCs-Exos administrated for nasal drip	June 25, 2021	NCT04388982
Wound healing	Mesenchymal stem cells derived exosomes promote healing of large and refractory macular holes	Macular holes	Exosomes derived from mesenchymal stem cells (MSC-Exo)	April 6, 2021	NCT03437759
	A safety study of the administration of mesenchymal stem cell extracellular vesicles in the treatment of dystrophic epidermolysis bullosa wounds	Dystrophic epidermolysis bullosa	AGLE 102	June 24, 2021	NCT04173650

(Xu et al., 2016). MSCs-derived exosomes have a role in tissue regeneration, infection treatment, and inflammation control (Afshar et al., 2021; Zhankina et al., 2021).

Periodontitis is an inflammatory and destructive disease that has a relationship with several factors such as the pathogens, host inflammation, and immune responses, and the imbalance of multiple T helper cells 17 (Th17)/regulatory T cell (Treg) related cytokines (Wang et al., 2014; Silva et al., 2015; Pan et al., 2019). Bacterial infection is a primary factor in the development of periodontitis, but what ultimately causes periodontitis is improper regulation of the host immune system and inflammatory response (Hajishengallis, 2014, 2015). Th17 cells play a destructive role in the immune balance of periodontitis (Zhao et al., 2011). Over-regulation of Th17 and improper regulation of Treg may lead to periodontal disease through immune-mediated tissue destruction (Zhao et al., 2011; Yang et al., 2014; Karthikeyan et al., 2015). Periodontal ligament stem cells (PDLSCs)-derived exosomes have a similar role with exosomes from MSCs and PDLSCs-derived exosomes contain microRNA−155−5p and regulate Th17/Treg balance by targeting sirtuin−1 in chronic periodontitis (Zheng et al., 2019).

Interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) are pro-inflammatory cytokines that are needed for periodontal inflammation and alveolar bone resorption (Delima et al., 2001; Grauballe et al., 2015). Macrophages that are activated by bacteria can release many inflammatory cytokines, causing gingiva destruction and alveolar bone resorption (Spiller and Koh, 2017; Dutzan et al., 2018; Garaicoa-Pazmino et al., 2019). Macrophages can be divided into two groups which are known as pro-inflammatory macrophages and anti-inflammatory macrophages and periodontal destruction occur following the imbalance of pro-inflammatory/anti-inflammatory macrophages (Gonzalez et al., 2015; Wynn and Vannella, 2016; Zhuang et al., 2019). Pro-inflammatory macrophages play an important role in the production of many inflammatory cytokines such as interleukin 1 beta (IL-1β) and TNF-α. Also, they can stimulate T cells and neutrophils, which cause the destruction of alveolar bone, and they can increase the local expression of receptor activator of nuclear factor ligand (RANKL), which causes osteoclast differentiation in the periodontium (Darveau, 2010; Hienz et al., 2015). In contrast, anti-inflammatory macrophages by

secreting the anti-inflammatory mediators play a significant role in the elimination of inflammation and tissue regeneration and contribute to efferocytosis of the apoptotic osteoblastic cells so that mediating bone formation (Zhang et al., 2012; Shapouri-Moghaddam et al., 2018).

Dental pulp stem cells (DPSCs) as a population of dental−derived mesenchymal stem cells have easy accessibility and minimal ethical concerns for use (Mahdiyar et al., 2014; Potdar and Jethmalani, 2015; Mehrabani et al., 2017). The DPSCs have beneficial immunomodulatory and anti-inflammatory properties and have a regulating effect on macrophages of the immune system (Lee et al., 2016; Omi et al., 2016; Galipeau and Sensebe, 2018). Since the therapeutic effects of stem cells are mainly related to the release of paracrine agents, stem cell-derived exosomes, as one of the most important paracrine mediators, show therapeutic effects through immunomodulation (Sun et al., 2018; Riazifar et al., 2019). DPSC-derived exosomes containing miR-1246 can facilitate the conversion of pro-inflammatory macrophages to anti-inflammatory macrophages in the periodontium of mice with periodontitis and accelerate the healing of alveolar bone and the periodontal epithelium (Shen et al., 2020).

In connection with the issue of infectious diseases, exosomes, in addition to treatment, can also help in the diagnosis of infectious diseases. For instance, hand, foot, and mouth disease (HFMD) is a common acute viral infection that has spread worldwide (Guerra et al., 2017). Human enterovirus 71 (EV71) and coxsackie virus A16 (CVA16) are the two main causes of HFMD (Yan et al., 2001; Osterback et al., 2009). HFMD has mild and severe forms which are known as mild HFMD and extremely severe HFMD (Jia et al., 2014), EV71 can cause extremely severe HFMD in which severe neurological symptoms occur and significant mortality (Huang et al., 1999). Many children with extremely severe HFMD die before a definitive diagnosis. There are no effective and reliable methods and tools for diagnosing (Li et al., 2014b; Hossain Khan et al., 2018). A study has shown that patients with different HFMD conditions express a specific type of exosomal miRNA profile (Jia et al., 2014). In fact, these exosomes provide a supplemental biomarker for differential infection stage at an early stage. Therefore, by examining the exosomal content, the disease can be diagnosed, and its different forms can be distinguished from each other (Jia et al., 2014). The immunomodulatory properties of exosomes have enhanced their use in the field of cancer biology. For example, dendritic cells-derived exosomes called "Dexosomes" can be used as a cell-free vaccine for cancer immunotherapy (Nikfarjam et al., 2020). Also, homeostasis and metastasis of tumor cells can change by exosomal and autophagy pathways (Salimi et al., 2020). Radiotherapy may affect the mechanism of paracrine intercellular communication within irradiated tumor tissue and surrounding cells (Jabbari et al., 2019).

TABLE 5 | Advantages and limitations of exosomes therapy in clinical applications (Tian et al., 2010; Takahashi et al., 2013; Lötvall et al., 2014; Yu et al., 2014; Théry et al., 2018; Xing et al., 2020; Babaei and Rezaie, 2021).

Advantages	Limitations
Efficient cellular entry	Controversies in defining exosome dosage
Excellent immune-compatibility	Difficulty in identification of isolation and purification strategy in order to produce optimal results
Exerting different therapeutic mechanisms simultaneously	Lack of reliable methods for distinguishing them from other EVs
Free of ethical issues	Lack of standardized methods for large-scale production
Good stability and protection by having bilayer lipid membrane	Needing appropriate, safe, and confident cell sources of exosomes based on their intended therapeutic use
High diagnostic sensitivity and specificity by having multiple diagnostic parameters	Needing considerable attention of stability and storage strategies for clinical and commercial success as off-the-shelf diagnostic and therapeutic tools
Intrinsic ability to traverse biological barriers	Short half-life and quick clearance
Lower toxicity	Uptake capacity of target cells
Minimal trauma than other diagnostic methods in diagnosis of disease	
Modification ability	
Not immunogenic	
Potential targeting ability by the surface-specific domain	
Safe and non-tumorigenic	
Wide availability in various bodily fluids	

FUTURE PERSPECTIVE OF EXOSOME THERAPY

Over the last decades, the knowledge about biogenesis, molecular content, and biological function of exosomes have significant progress and a considerable amount of manuscripts have been published in this field. Exosome therapy as a cell-free therapy is emerging as a promising strategy for the treatment of several diseases, in particular inflammatory conditions. The characteristic properties of exosomes, including the transmission of exosomal competent, protecting it from extracellular degradation, and delivering it in a highly selective manner to target cells, have led to their numerous uses in various fields of treatment. The use of exosomes in clinical applications as well as in the treatment of diseases has both advantages and challenges, some of which are listed in **Table 5**. Despite the existing limitations, the use of exosomes as a new method in various fields of medical science is phenomenal and inspiring that need more data collection.

CONCLUSION

The oral cavity as a part of the digestive system which is in close contact with the external environment of the body and also by having its special microbiome is prone to a wide range of infectious diseases. In infectious diseases, the pathogenic mechanism of the microorganism is significantly affected by a

special type of EVs called exosomes. In this way, these exosomes can be effective in the process of disease development and progression, as well as in the face of preventing and limiting the disease. Exosomes also play an important role in microbial infections by regulating the host immune system. In addition, exosomes can be used in the diagnosis of infectious diseases. Due to the importance of treating oral infectious diseases as well as the ease of using non-cellular therapies, mesenchymal stromal/stem cells-derived exosomes can be considered as a suitable and available option for the treatment of orodental infectious diseases that require more and more extensive studies in the future.

AUTHOR CONTRIBUTIONS

NJ wrote the manuscript with support from AK and RM. MS helped supervise the project. All authors reviewed the manuscript and approved the final version of the manuscript.

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REVIEW
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Mesenchymal Stem-Cell Derived Exosome Therapy as a Potential Future Approach for Treatment of Male Infertility Caused by *Chlamydia* Infection

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Some microbial sexually transmitted infections (STIs) have adverse effects on the reproductive tract, sperm function, and male fertility. Given that STIs are often asymptomatic and cause major complications such as urogenital inflammation, fibrosis, and scarring, optimal treatments should be performed to prevent the noxious effect of STIs on male fertility. Among STIs, *Chlamydia trachomatis* is the most common asymptomatic preventable bacterial STI. *C. trachomatis* can affect both sperm and the male reproductive tract. Recently, mesenchymal stem cells (MSCs) derived exosomes have been considered as a new therapeutic medicine due to their immunomodulatory, anti-inflammatory, anti-oxidant, and regenerative effects without consequences through the stem cell transplantation based therapies. Inflammation of the genital tract and sperm dysfunction are the consequences of the microbial infections, especially *Chlamydia trachomatis*. Exosome therapy as a noninvasive approach has shown promising results on the ability to regenerate the damaged sperm and treating asthenozoospermia. Recent experimental methods may be helpful in the novel treatments of male infertility. Thus, it is demonstrated that exosomes play an important role in preventing the consequences of infection, and thereby preventing inflammation, reducing cell damage, inhibiting fibrogenesis, and reducing scar formation. This review aimed to overview the studies about the potential therapeutic roles of MSCs-derived exosomes on sperm abnormalities and male infertility caused by STIs.

Keywords: exosomes, mesenchymal stem cells, *Chlamydia trachomatis*, infectious diseases, male infertility

INTRODUCTION

A prominent etiological factor in male infertility is genital tract infection. The infertility may be induced by various mechanisms, such as damage to gametogenic cells, decrease in the quality of sperm, and obstruction of the male reproductive tract (Keck et al., 1998; Sanocka-Maciejewska et al., 2005). The most common sexually transmitted microorganisms are *Chlamydia trachomatis* (*C. trachomatis*) (Nieschlag et al., 1997; Keck et al., 1998; Ombelet et al., 2008). There are controversial opinions on the role of *C. trachomatis* in male infertility (Dehghan Marvast et al., 2017). Several studies have shown that male infertility induced by chlamydial infection occurs in different forms of sperm abnormalities such as loss of mitochondrial membrane potential, increase in apoptosis through the activation of caspase 3 (Sellami et al., 2014) and DNA damage (Dehghan Marvast et al., 2018), and changes in sperm quality (Bezold et al., 2007; Sellami et al., 2011, 2014). Also, other studies have claimed that this microorganism infection causes an inflammatory reaction which leads to seminal tubes occlusion (Dohle, 2003; Dehghan Marvast et al., 2016; Zhou et al., 2021). Many sexually transmitted infections (STIs) pathogens such as *C. trachomatis* are asymptomatic in subfertile men (Sharma and Agarwal, 1996; Bezold et al., 2007; Geisler, 2010; Hakimi et al., 2014; Bai et al., 2021). Screening and treatment should be performed to prevent the detrimental effect of *C. trachomatis* on male fertility (Geisler, 2010; Bryan et al., 2019). Widespread antibiotics are currently the most common treatment for chlamydial infection (Murray and McKay, 2021), and this treatment can effectively alleviate the infection and ameliorate sperm quality (Gallegos et al., 2008; Hamazah and Al-Dahmoshi, 2021). However, antibiotic resistance is one of the remaining challenges for this treatment, especially in patients with multidrug resistance (Hamazah and Al-Dahmoshi, 2021; Vanić et al., 2021).

The new experimental methods of the infertility treatment are stem cell and exosome applications. Because of the limitations using live cells injections and also the therapeutic effect of their paracrine substances (Janockova et al., 2021), MSC–derived exosomes containing bioactive molecules have been recently used in studies of infertility treatment. Exosome therapy as a noninvasive approach has shown promising results on the ability to regenerate damaged sperms and treating asthenozoospermia by their repairing molecules and counteracting with the reactive oxygen species (ROS) (Kharazi and Badalzadeh, 2020). These experimental methods may be helpful in the novel treatments of male infertility. This review aimed to overview the studies about the therapeutic potentials of the MSCs-derived exosomes on sperm abnormalities and male infertility caused by STIs.

C. TRACHOMATIS: CELL BIOLOGY

Chlamydia is a gram negative bacterium, an obligate intracellular parasite, divided into 18 serovars (A-C, D-K, and L1-L3) distinguished by the antigen named the Major Outer Membrane Protein. This antigen gives the pathologic properties to the serovars D-K and may play an essential role in genital tract infection (Murray and McKay, 2021). Unlike other microorganisms, *C. trachomatis* has two distinct developmental cycles, the infectious type or elementary body (EB) and intracellular replicative type or reticulate body (RB). Both types of this bacterium are metabolically active, although their energy sources are different (Omsland et al., 2012). Expressions of different antigens during the cell cycle lead to difficulties in eradicating the bacterium (Paavonen and Eggert-Kruse, 1999; Murray and McKay, 2021). EB form attaches to the host cell and enters it and protects itself from host cellular defense by formation of vacuoles and inclusions (Hosseinzadeh et al., 2000).

PATHOPHYSIOLOGICAL MECHANISMS

Approximately 50% of *C. trachomatis* infections in men are asymptomatic, but it can cause epididymitis, epidiymo-orchitis, urethritis, and prostato-vesiculitis (Eley et al., 2005; Rana et al., 2016). Because of wide range of pathological changes and tissue injuries in the urogenital tract, it is necessary to briefly review the pathophysiology of *C. trachomatis*. This bacterium first attaches to the epithelial cells in the urogenital tract, and this is where immunological reactions are initiated. The infected non-immune cells recognize different invaded pathogens such as *C. trachomatis* by their PRRs (pathogen recognition receptors) (Mackern-Oberti et al., 2013). The interaction between non-immune host cell and bacterium leads to secretion of many cytokines (IL-1, IL-8, IL-6) (Al-mously and Eley, 2007; Redgrove and McLaughlin, 2014) and tumor necrosis factor alpha (TNFα); these, in turn, recruit natural killer (NK) cells, DCs, neutrophils, macrophage, T cells, and B cells (Redgrove and McLaughlin, 2014). One of the most substantial cellular immune reactions against chlamydia infection is mediated by antigen-specific IFN-γ secreting $CD4^+$, $CD8^+$ T cells, and NK cells. Also, elimination of chlamydial infection depends on IFN-γ secreting $CD4^+$ Th1 cells (Cain and Rank, 1995; Perry et al., 1997). Immune cells also generate chronic inflammation by increasing the production of ROS and releasing molecules with degradative properties including defensins, elastase, collagenase, cathespins, and lysozyme. Finally, the immune reactions lead to tissue remodeling and scarring in the reproductive system (Redgrove and McLaughlin, 2014).

EFFECTS OF *C. TRACHOMATIS* ON SPERM AND MALE INFERTILITY

Infertility in men is caused by various reasons such as genetic abnormalities, testicular damage, varicocele, immunological subjects, systemic diseases, environmental factors, endocrine disorders, and exposure to gonadotoxic agents (Dohle et al., 2004; Jungwirth et al., 2012). In addition to the above-mentioned factors, male genital tract infection and inflammation play a devastating role in 8–35% of male infertility. Infectious

factors such as fungi, parasites, viruses, and several other microorganisms including *C. trachomatis*, *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, and *Trichomonas vaginalis* are involved in these disorders, which can affect the testis, epididymis, accessory sex glands, sperm cell function, and finally fertility (Isaiah et al., 2011). The most common cause is *C. trachomatis*, which leads to infertility by affecting both the sperm and the male reproductive tract (Nieschlag et al., 1997; Keck et al., 1998; Ombelet et al., 2008).

Some studies have regarded the relationship between *C. trachomatis* infection and semen quality. Semen of *C. trachomatis* infected patient indicates reduced volume, decrease in sperm motility, change in sperm concentration, and pH alteration (Veznik et al., 2004; Rana et al., 2016). It seems that aforementioned effects on the sperm can be due to Chlamydia lipopolysaccharide (LPS) which interacts with CD14 on the sperm membrane and leads to elevating production of ROS and eventually induced apoptosis (Harris et al., 2001). Another study demonstrated that *C. trachomatis* infection can cause rising in the mitochondria membrane potential, caspase 3 activation, and finally apoptosis induction in spermatozoa (Sellami et al., 2014). Moreover, externalization of phosphatidylserine (PS) in sperm membrane and DNA fragmentation has been reported as a negative impact of *C. trachomatis* on sperm function and fertility (Satta et al., 2006). In addition, several studies have reported infections of the reproductive system can cause leukocytospermia, and the leukocytes are able to produce oxidative damage of the sperm plasma membrane and DNA through the release of cytokines, free oxygen radicals, and reactive nitrogen (Anderson and Hill, 1988; Aitken and West, 1990; Hamada et al., 2011).

CURRENT TREATMENT

Current treatment includes azithromycin 1 g single dose or doxycycline 100 mg orally twice daily for 7 days (Stamm et al., 1995; Dieterle, 2008; Mishori et al., 2012). Timely management of sexual intercourse and sex partner treatment are also necessary to reduce the re-infection risk (Centers for Disease Control and Prevention, 1998a,b; Workowski and Berman, 2011). Approximately 50% of *C. trachomatis* infections in men are asymptomatic and can cause many complications (Pacey and Eley, 2004; Eley et al., 2005; Rana et al., 2016). Thus, screening programs are necessary to prevent long-term complications of *C. trachomatis* infection such as epididymitis, accessory sex glands inflammation, testicular atrophy, tubular tract occlusion, and male infertility (Paavonen and Eggert-Kruse, 1999). While treatment with antibiotics significantly clears sexually transmitted patients, this treatment has its limitations (Kong et al., 2014). First, screening programs to identify chlamydia infected individuals are costly and impractical, so they are limited to symptomatic patients who are following their diseases (World Health Organization, 2016). Antibiotic therapy may also impair the production of a sustained protective immune response to chlamydia (Patton et al., 2014).

Vaccines have long been designed to treat chlamydia infection. Despite numerous successes in this field, there are still issues that have limited human access to deliver effective vaccines without complication. Biological characteristics, two-phase life cycle, and especially the ability of this bacterium to hide from the view of the immune system are the main reasons for this limitation in vaccine production. Providing a reliable and effective vaccine for *Chlamydia* prophylaxis is still awaiting further research and possibly shifting from whole-cell based vaccines to subunit-based vaccines, especially considering the role of MOMP (Murray and McKay, 2021).

Importantly, in some cases in which the complications still remained following antibiotic therapy, a new therapeutic approach is necessary for treatment. In this regard, MSCs-derived exosomes have been shown to have critical roles such as anti-inflammatory, antioxidant, regenerative and fibrogenesis inhibiting, and wound and fracture healing (Janockova et al., 2021), which can be considered a novel approach in the male infertility complications of *C. trachomatis* infection.

EXOSOME: GENERAL ASPECTS

In different multicellular organisms, the intercellular communication occurs through cell-to-cell contact or through the secretion of molecules (Lai, 2004). Two decades ago, another mechanism was considered in the intercellular communication, which involves the transfer of extracellular vesicles that release from the plasma membrane into the intercellular space under physiological and pathological events and influence the other cells in paracrine and endocrine manners (György et al., 2011). Based on biosynthesis pathways and their size, the extracellular vesicles are divided into three categories: micro vesicles (50–3,000 nm), exosomes (40–100 nm), and apoptotic bodies (800–5,000 nm) (Yamamoto et al., 2016). Other studies have also mentioned other sizes for exosome: (30–100) (Wang et al., 2017), (50–150 nm) (Théry et al., 2018), (40–160 nm) (Kalluri, 2016), and (50–100 nm) (Gould and Raposo, 2013). Recently exosomes have attracted huge attention from researchers due to their genetic material and protein shuttling ability to other cells with various contents according to their origin (Han et al., 2016). Exosomes secrete from T cells (Nolte-'t Hoen et al., 2009), B cells (Clayton et al., 2005), macrophages (Bhatnagar et al., 2007), epithelial cells (Skogberg et al., 2015), endothelial cells (Song et al., 2014, 2015), as well as MSCs (Yeo et al., 2013). The vesicles with exosomal characteristics have been also founded in the various body fluids such as semen (Fabiani et al., 1994; Arienti et al., 1999; Park et al., 2011; Aalberts et al., 2012), blood (Blanc et al., 2005; Caby et al., 2005; Yunusova et al., 2016), breast milk (Admyre et al., 2007; Qin et al., 2016; Miyake et al., 2020), ascites fluid (Andre et al., 2002; Navabi et al., 2005; Runz et al., 2007), saliva (Ogawa et al., 2008; Michael et al., 2010), amniotic fluid (Asea et al., 2008; Zhang et al., 2021), urine (Gonzales et al., 2010; Street et al., 2017), and bile (Masyuk et al., 2010; Sagredo et al., 2017). Because exosomes are in nano sized range, they spread through body fluids and easily penetrate through tissues and affect targeted cells (Phinney and Pittenger, 2017),

even if those cells are far away (François et al., 2006). The synthesis, secretion, and effects of the extracellular vesicles were intensively considered in the past few decades so that it led to the creation a scientific association named the International Society for Extracellular Vesicles (ISEV) (Kowal et al., 2014). Various techniques for isolation and detection of exosomes have been reported in recent studies. Isolation techniques include differential ultracentrifugation (Parolini et al., 2009), density gradient (Beyer and Pisetsky, 2010), size exclusion chromatography (Livshits et al., 2015), ultrafiltration (Greening et al., 2015), immunological separation (Beyer and Pisetsky, 2010), isolation by sieving (Taylor and Shah, 2015), cell sorting (Peterson et al., 2015), polymer-based precipitation (Grant et al., 2011), and microfluidic technologies (Oves et al., 2018). Exosome identification techniques include electron microscopy, western blot, flow cytometry, and nanosight tracking analysis (Crenshaw et al., 2018). The latest methods and techniques are RNA-seq techniques (Jeppesen et al., 2019).

EXOSOME BIOGENESIS

Exosome generation, which was conserved during evolution, is a continuation of the extracellular ligands internalization and endocytosis process, which is carried out by the curvature of the plasma membrane and budding inside the intracellular endosome that leads to the formation of multivesicular bodies (MVB). Later, the MVB, which contains intraluminal vesicles (ILVs) that can be the precursors of the exosome, either leads to fusion with lysosomes and degradation, or undergoes exocytic merging with plasma membranes and exosome secretion (Stoorvogel et al., 2002; Février and Raposo, 2004; Colombo et al., 2014; Kowal et al., 2014; Meldolesi, 2018; Xunian and Kalluri, 2020). Molecular mechanisms of ILV generation depend firstly on the endosomal sorting complex required for transport (ESCRT), a molecular apparatus comprised of four sets including ESCRT-0 which consists of two subunits HRS (hepatocyte growth factor-regulated tyrosine kinase substrate) and STAM1/2 (signal transducing adaptor molecule1/2) (for cargo clustering and sorting), ESCRT-I and ESCRT-II (induce membrane curvature and vesicle budding), and ESCRT-III (membrane deformation and vesicle detachment) (Henne et al., 2011; Meldolesi, 2018; Xunian and Kalluri, 2020). The subordinate proteins (Vps4-Vta1 complex, Tsg101, Vps24, Vps37, Vps2, and Alix) are also critical for exosome biogenesis pathway (Henne et al., 2011). ESRT apparatus is also involved in the deubiquitination of some proteins that are ubiquitinated in ILVs (Henne et al., 2011; Meldolesi, 2018). The deubiquitination is mediated by the protein tyrosine phosphatase HD-PTP, which is an essential process for exosome function (Meldolesi, 2018). The subordinate proteins (class I AAA ATPase Vps4) can cause the ESCRT apparatus recycling (Xunian and Kalluri, 2020). In addition to the ESRT pathway, there are other independent pathways, for example, ceramide derived from sphingomyelin can cause membrane deformation and vesicles budding within the MVB (Trajkovic et al., 2008; Henne et al., 2011).

EXOSOME COMPOSITION

Exosomes are extra cellular vesicles that are secreted from different cells under both normal and disease conditions and represent cells function or even as diagnostic markers of diseases. Existence of mRNA and miRNA within the exosomes has led to more studies in recent years, making this field more attractive (Valadi et al., 2007). The exosomes carry bimolecular content such as protein (membrane proteins, cytosolic and nuclear proteins, and extracellular matrix proteins), lipid, and nucleic acid which are different between cells (McAndrews and Kalluri, 2019). This content can be verified and accessed in the Exocarta,[1] a manually curated web-based database. The current Exocarta is based on about 286 studies on exosomes and contains about 41,860 proteins, 1,116 lipid, and more than 7,540 RNAs from 10 various species (Keerthikumar et al., 2016). Several most common proteins on the exosomal surface such as tetraspanins (CD63, CD81, CD82, and CD9) are known as membrane scaffolds (Ma et al., 2020); in addition to the above-mentioned tetraspanins, in the MSC-derived exosomes, there are expressions of CD73, CD44, and CD90 (Ramos et al., 2016). Exosomes present antigen proteins such as major histocompatibility complex (MHC) I and II, flotillin-1, and integrins. Other proteins include MVB biogenic proteins such as ESCRT complex 0,-1,-II,-III, Alix, syntenin, TSG101, membrane transporters, and fusion proteins such as RAB protein, RAP1B, RhoGDIs and annexins (Ma et al., 2020), several enzymes such as glyceraldehydes- 3-phosphate dehydrogenase (GAPDH), phosphoglycerate kinase 1 (PGK1) (Van Niel et al., 2011; Charrin et al., 2014), and alanylaminopeptidase N (Ma et al., 2020), a number of chaperones such as heat shock protein 70 (HSP70), heat shock cognate 70 (HSC70) (Van Niel et al., 2011; Charrin et al., 2014), HSP90, HSP60, and HSP8 (Ma et al., 2020), adhesion proteins such as L1 cell adhesion molecule (L1CAM), and lysosomal associated membrane protein 2 (LAMP2) (Urbanelli et al., 2013).

Exosomes are also rich in genetic materials. Different types of RNAs including mRNAs and miRNAs, vault RNAs (vtRNAs), Y-RNAs, ribosomal RNAs (rRNAs), and transfer RNA (tRNAs) (Squadrito et al., 2014; Vojtech et al., 2014; Shurtleff et al., 2017). Also, various types of DNAs in exosomes are double-stranded DNAs (dsDNA) (Thakur et al., 2014), mitochondrial DNAs (mtDNAs) (Guescini et al., 2010), and single-stranded DNAs (ssDNAs) (Balaj et al., 2011).

Other exosome contents are lipid compositions including cholesterol, phosphatidylserine (PS), sphingomyelin, ceramide, lysobisphosphatidicacid, and phosphatidylethanolamine (PE), which play an important role in membrane structure and exosome formation and are secreted in the extracellular environment (Skotland et al., 2019).

Exosomes with lipid bilayer membrane can protect genetic material and other contents through transportation to the targeted cell (Fu et al., 2019). MSC-derived exosomes transmit their composition to the targeted cells either *via* plasma

[1] http://www.exocarta.org

membrane fusion or membrane receptor function which lead to the exosome internalization (Harrell et al., 2019a).

MESENCHYMAL STEM CELLS-DERIVED EXOSOMES

MSCs which are mainly tissue specific stem cells can be isolated from adult (Akyash et al., 2020) and fetal (Hoseini et al., 2020) sources. MSCs can be also be produced from pluripotent human embryonic stem cells (hESCs) (Javidpou et al., 2021). Different cells secrete exosomes that have similar protein molecules and biological activities. Immune modulation, regeneration, tissue repair, and promotion of angiogenesis are the similar *in vivo* and *in vitro* therapeutic effects of MSC-derived exosomes. These similar activities may be related to the presence of common protein signature in all MSCs-derived exosomes (van Balkom et al., 2019). However, MSCs are a massive source for production of exosomes, more accessible, and highly proliferative (Cheng et al., 2017) and that makes them more suitable for different fields of research. Moreover, exosomes derived from specific types of MSCs have unique properties (Tang et al., 2021). Additionally, different specific cells secrete exosome containing unique protein molecules and exert biological activity (Simpson et al., 2008). For example, in a recent study, amelioration of the spermatogonia injuries by Sertoli cell-derive exosome was revealed (Salek et al., 2021).

ROLE OF MESENCHYMAL STEM CELLS-DERIVED EXOSOMES IN INFLAMMATION AND CELLULAR DAMAGE

Numerous studies have shown the potential of MSC-derived exosomes for treatment of diseases, which can be used as vaccines (prophylaxis), treatment, disease biomarkers, and drug delivery (Wang et al., 2017; Janockova et al., 2021).

It has been demonstrated that MSC-derived exosomes exhibit a crucial role in repair of the epithelium damage and re-epithelialization (Zhang et al., 2015a), angiogenesis (Shabbir et al., 2015; Zhang et al., 2015b), and prevention of the scar formation by suppressed myofibroblast differentiation (Fang et al., 2016). Studies have also reported that MSC-derived exosomes containing miRNAs can reduce inflammation by transforming the pro-inflammatory macrophage M1 to anti-inflammatory phenotype M2. The phenotype M2 reduces local interleukin-1β, interleukin-6, and tumor necrosis factor alpha (TNF-α) and increases the secretion of anti-inflammatory factors such as IL-10 as well as immune regulation (Wei et al., 2019; Zhao et al., 2019). Recent study demonstrated that MSC-derived exosomes can cause suppression of $CD4^+$ Th1 and Th17 and induction of T regulatory cells (Treg) expansion which it in turn regulates and suppresses the immune system (Harrell et al., 2019b). Also, the protective effects of MSC-derived exosomes have been mediated *via* oxidative stress suppression and maintain balance of cellular redox state (Yang et al., 2015).

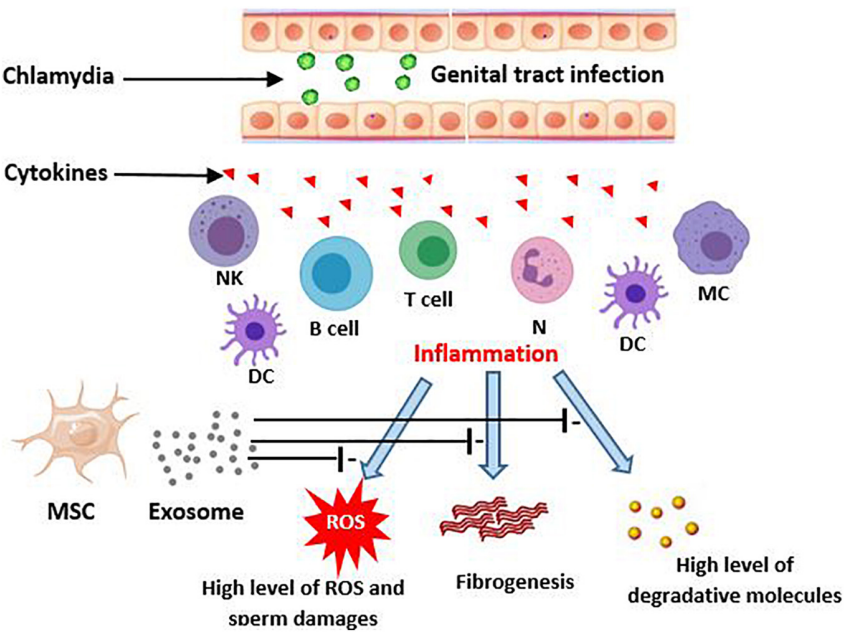

FIGURE 1 | Potential effects of MSCs-derived exosomes on consequences of chlamydia infection in the genital tract. Genitalia tract infection with chlamydia evokes an inflammatory immune response by epithelial and local immune cells. This, in turn, produces the high level cytokins that initiate a more severe immune reaction. The responses may result in male genital inflammation and fibrosis. On the other hand, the inflamed tissue can lead to creation of ROS production and then sperm damages. MSC derived exosomes potentially improve these consequences of chlamydia induced inflammation. DC, Dendritic cells; MC, Macrophage; NK, Natural killer; MSC, Mesenchymal stem cell; ROS, Reactive oxygen species.

Studies have also shown the important role of MSC-derived exosomes in tissue repair after injury, the effect that is mediated by inducing cell differentiation, proliferation, and prevention of apoptosis. The miRNAs such as miR-21-5p, miR-144, and miR-19a are the factors that inhibit apoptosis in the MSC-derived exosomes and reduce apoptotic proteins such as caspase 3, caspase 8, and caspase 9 after tissue injury (Yu et al., 2015; Li et al., 2019; Wen et al., 2020).

In the inflammatory response of colitis it has been reported that MSC-derived exosomes attenuate inflammation through decrease in TNF-α, nuclear factor kappaBp65 (NF-κBp65), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), interleukin-1β (IL-1β), and increase in expression of IL-10. Alleviation of LPS-induced inflammation and acute respiratory distress syndrome (ARDS) by MSC-derived exosomes has been demonstrated (Deng et al., 2020). Another study on premature ovarian failure reported that MSC-derived exosomes with miR-644-5p can cause apoptosis inhibition *via* impressing p53 and recover normal function in ovarian granulosa cell (Sun et al., 2019). Considering the male infertility caused by *C. trachomatis* has inflammation-based pathology (Lotti and Maggi, 2013; Redgrove and McLaughlin, 2014), exosome therapy may be a beneficial technique to attenuate the cell injuries and the tissue remodeling such as occurrence of fibrosis and scar formation (**Figure 1**).

ROLE OF MESENCHYMAL STEM CELLS-DERIVED EXOSOMES IN INFECTION

The antimicrobial properties of MSC-derived exosomes have been reported by several clinical trials (Krasnodembskaya et al., 2010; Harman et al., 2017; Cortés-Araya et al., 2018). Studies also showed that exosomes contain antimicrobial peptides (AMPs) and the proteins that have bactericidal effect (Gläser et al., 2005; Krasnodembskaya et al., 2010; Allen and Stephens, 2011; Alcayaga-Miranda et al., 2017). MSC-derived exosomes indicated the therapeutic effect on lung injury that induced by *E. coli* (Zhu et al., 2014). Also, enhancing anti-microbial function of immune cells infiltration in lung by MSC-derived exosome has been reported in an animal study (Hao et al., 2019). A previous study revealed that exosomes can protect the brain against sepsis induced in an experimental model (Chang et al., 2018). MSC-derived exosomes enhanced the bacterial phagocytosis capability of the monocytes in severe bacterial pneumonia (Monsel et al., 2015) and enteric infections (Islam et al., 2001). Moreover, immunoregulatory properties of monocytes and decrease in inflammatory cytokine secretion were observed after use of the exosomes (Monsel et al., 2015). There is evidence that MSC-derived exosomes with their immunomodulatory, pro-angiogenesis, and anti-inflammatory activities can prevent inflammatory responses and alleviate COVID-19-induced pneumonia and lung injury (Raghav et al., 2021). In sum, these evidences about the role of exosomes in infections, especially their effects in increase of phagocytosis by monocytes, generate promising reasons to give them a potential property for eradication of the micro-organisms.

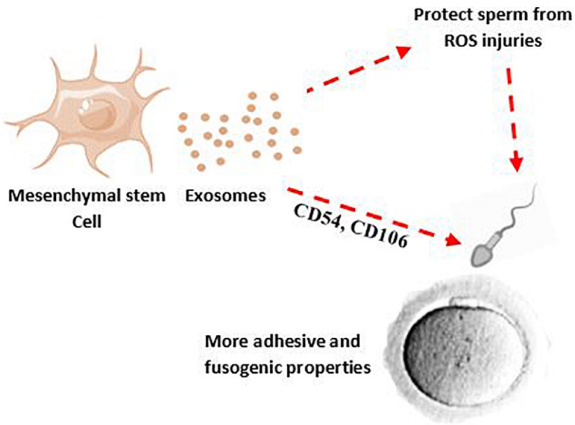

FIGURE 2 | MSC-derived exosomes may decrease ROS production after chlamydia infection and their effects on sperm membrane and DNA. Therefore, MSCs-derived exosomes can potentially improve quality and adhesive properties of sperm.

MSC-derived exosomes, as a natural carrier, possess a capability of embedding and delivering antibiotics and drugs. The use of exosomes as carriers leads to reduction of drugs that metabolize, targeted drug delivery, and thus overcome drug resistance (Bartolini et al., 2013; Yeo et al., 2013; Batrakova and Kim, 2015; Gao et al., 2018; Oves et al., 2018; Herrmann et al., 2021). However, exosome modifications change the functions and therapeutic effects of these vehicles (Ma et al., 2017; Tamura et al., 2017).

POTENTIAL THERAPEUTIC ROLE OF MESENCHYMAL STEM CELLS-DERIVED EXOSOME IN SPERM ABNORMALITY

To achieve proper male fertility, safe sperm manipulation is important. Recently, new methods such as the use of nanoparticles have been used to develop non-invasive techniques for treating and manipulating sperm (Feugang, 2017). The effectiveness and non-invasiveness of the nanoparticles such as exosome for mammalian sperm have been proven (Vilanova-Perez et al., 2020). According to animal studies, exosomes appear to be a promising avenue to restore spermatogenesis and sperm regeneration; a study has shown that amniotic fluid-derived exosome can restore sperm parameters such as motility, concentration, as well as the number of spermatogonia, spermatocytes, and ultimately male fertility (Mobarak et al., 2021). The protective effect of exosomes against sperm cryoinjuries (such as cell membrane injury, DNA damage) and oxidative stress produced by cryopreservation process and improvement of the post-thaw sperm parameters has been reported (Qamar et al., 2019; Mahiddine et al., 2020). Interestingly, treatment of spermatozoa with MSC-derived exosomes, in addition to improving sperm parameters after frozen-thawed, can increase sperm adhesive and fusogenic properties by adhesion molecules shuttling such as CD44, CD29, CD54, and CD106 (Mokarizadeh et al., 2013; **Figure 2**).

Exosomes contain different molecules such as RNAs that can be incorporated into immune or host cells. RNA sequencing analysis showed that microRNAs were the most frequent in exosomes (Huang et al., 2013). MSC-exosomes can play a role in injury repair and preventing apoptosis after injury through the miRNAs (e.g., miR-19a, miR-144, and miR-21-5p). The potential role of the miRNAs in improvement of chlamydial-induced sperm damages may confer a therapeutic application to the exosome. In addition, there are several clinical trials that demonstrated loading of exosomes with drugs or bioactive molecules (NCT01294072, NCT03608631, NCT01159288) for therapeutic proposes (NCT04602442, NCT04213248, NCT03437759, NCT04276987) (Herrmann et al., 2021). Therefore, it seems that exosomes can be used for treatment of sperm damage.

CONCLUSION

There are reported evidences demonstrated regenerative, anti-microbial, and anti-inflammatory and anti-oxidant activities of exosomes. It is worthwhile to investigate and challenge the identity and effectiveness of the exosomes in the treatment and control of the consequences of male genitalia tract infections, especially chlamydia. MSC-derived exosomes therapy can lend itself as the potential treatment of male infertility caused by microbial infections in the near future.

AUTHOR CONTRIBUTIONS

MI: study design, investigation, and writing original draft. LD: validation of data and revising the manuscript. MR and MZ: helping on writing the manuscript. SM: validation of data. AA: helping on writing the first draft of the manuscript. BA: supervisor, validation of data, and revising the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Potential of Mesenchymal Stem Cell-Derived Exosomes as a Novel Treatment for Female Infertility Caused by Bacterial Infections

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Neisseria gonorrhoeae and *Chlamydia trachomatis* are the most common causes of bacterial sexually transmitted diseases (STDs) with complications in women, including pelvic inflammatory disease (PID), ectopic pregnancy, and infertility. The main concern with these infections is that 70% of infected women are asymptomatic and these infections ascend to the upper female reproductive tract (FRT). Primary infection in epithelial cells creates a cascade of events that leads to secretion of pro-inflammatory cytokines that stimulate innate immunity. Production of various cytokines is damaging to mucosal barriers, and tissue destruction leads to ciliated epithelial destruction that is associated with tubal scarring and ultimately provides the conditions for infertility. Mesenchymal stem cells (MSCs) are known as tissue specific stem cells with limited self-renewal capacity and the ability to repair damaged tissues in a variety of pathological conditions due to their multipotential differentiation capacity. Moreover, MSCs secrete exosomes that contain bioactive factors such as proteins, lipids, chemokines, enzymes, cytokines, and immunomodulatory factors which have therapeutic properties to enhance recovery activity and modulate immune responses. Experimental studies have shown that local and systemic treatment of MSC-derived exosomes (MSC-Exos) suppresses the destructive immune response due to the delivery of immunomodulatory proteins. Interestingly, some recent data have indicated that MSC-Exos display strong antimicrobial effects, by the secretion of antimicrobial peptides and proteins (AMPs), and increase bacterial clearance by enhancing the phagocytic activity of host immune cells. Considering MSC-Exos can secrete different bioactive factors that can modulate the immune system and prevent infection, exosome therapy is considered as a new therapeutic method in the treatment of inflammatory and microbial diseases. Here we intend to review the possible application of MSC-Exos in female reproductive system bacterial diseases.

Keywords: antimicrobial effects, mesenchymal stem cells, MSC-derived exosomes, antibacterial properties, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, female infertility

INTRODUCTION

Today, with the huge concern regarding antibiotic resistance and due to absence of an effective vaccine, researchers are looking for suitable alternatives to solve this problem (Hosseiniyan Khatibi et al., 2020; Russell et al., 2020). Mesenchymal stem cells (MSCs) are defined as undifferentiated renewable cells. These cells can be isolated from different tissues including bone marrow, cord blood, skin, fallopian tube, liver, lungs, endometrium, testis, amnion, ovary, and adipose tissue (Akyash et al., 2016a,b; Sadeghian-Nodoushan et al., 2016; Zhang et al., 2016; Akyash et al., 2020; Hoseini et al., 2020). Moreover, there are reports indicating the generation of MSCs from pluripotent human embryonic stem cells (hESCs) (Akyash et al., 2016a; Javidpou et al., 2021). The therapeutic potentials of MSCs are accomplished through three mechanisms. The first is differentiation into multiple cell types, which provides the condition for repairing and replacing damaged tissues. The second is that MSCs migrate to injured tissues due to chemical gradients. The third mechanism is the most important mechanism due to secretion of bioactive factors (Vizoso et al., 2017). Moreover, MSCs are able to secrete nanoparticles called exosomes that from by fusion of the cell membrane of multivesicular and are considered as extracellular vesicles (EVs). EVs according to *International Society for Extracellular Vesicles* (ISEV) are divided into three classes based on their size and origin, which include exosomes, microvesicles (MVs), and apoptotic bodies; (a) exosomes with various in size of 30–150 nm originate from multivesicular bodies (MVBs), (b) microvesicles in size of 150–1000 nm, (c) apoptotic bodies with a wide size distribution of 50–2000 nm (Gurunathan et al., 2019; Akbari and Rezaie, 2020; Rezaie et al., 2021). Studies show that MSCs exert their paracrine effects by secreting exosomes which are known by other names including nanoparticles, exosome-like vesicles, dexosomes, prostasomes, and tolerosomes (Zhang et al., 2020; Rezaie et al., 2021). Exosomes are transitional vesicles that release into the extracellular space through fusion with the cell membrane, which can reach distance target cells and affect their function and activity (Kowal et al., 2014). MSC-derived exosomes (MSC-Exos) are able to secrete cytokines, chemokines, and growth factors, proteins, mRNA, non-coding RNA, and bioactive lipids that could elicit a wide range of physiological activities (Harrell et al., 2019; Adib et al., 2020b,a; Yuan et al., 2020). Moreover MSC-Exos are considered as an innovative therapeutic tool to treat bacterial infections, consistent with their unique properties (Park et al., 2019). *Neisseria gonorrhoeae* (*N. gonorrhoeae*) and *Chlamydia trachomatis* (*C. trachomatis*) are gram-negative bacteria that are both considered as obligate human pathogens (Chen et al., 2018). Due to the pathogenesis of *N. gonorrhoeae* and *C. trachomatis* and the ability of these bacteria to cause chronic infections and, on the other hand, considering the side effects of antibiotic resistance and the absence of effective vaccines, new treatment strategies are needed to repair damaged epithelial cells of fallopian tube (FT) in these infections. As regards conditioned medium (CM) or MSC-Exos contain growth factors, antimicrobial peptides/proteins (AMPs) and cytokines have immunosuppression properties on innate and adaptive immune responses *via* direct and indirect mechanisms. In addition, CM and MSC-Exos have other therapeutic potentials including anti-apoptotic activity, wound healing, tissue repair, antiscarring, and angiogenesis regulation (Burlacu et al., 2013; Williams et al., 2013; Vizoso et al., 2017; Adib et al., 2020b,a), Here, we intend to review the application of MSC-Exos in female reproductive system bacterial diseases.

ANTIMICROBIAL EFFECTS OF MESENCHYMAL STEM CELLS

Many studies have shown that MSCs display antimicrobial features by secretion of AMPs and regulation of immune responses (Krasnodembskaya et al., 2010; Koniusz et al., 2016). These antimicrobial effects of MSCs are mediated *via* direct and indirect mechanisms (Russell et al., 2020). MSCs directly interact with pathogens by secreting AMPs, including lipocalin 2, cathelicidin, β-defensin 2, and hepcidin, thereby playing an important role in increasing bacterial clearance (Marrazzo et al., 2019; Chow et al., 2020). While MSCs are exposed to pathogenic factors, including pathogen-associated molecular patterns (PAMPs), lipopolysaccharide (LPS), and damage-associated molecular patterns (DAMPs) *via* toll-like receptors (TLRs), caused a change in their proliferation, differentiation, migration, and secretory factors (Marrazzo et al., 2019; Hosseiniyan Khatibi et al., 2020). The immunomodulatory effects of MSC-Exos are mainly due to inhibition of T cells proliferation and conversion of these cells to regulatory T cells (Tregs) and also through reprogramming of M1 macrophage cells to M2 phenotype that these immunomodulatory and anti-inflammatory effects of MSC-Exos lead to tissue repair and healing (Xie et al., 2020; Arabpour et al., 2021).

Direct Mechanisms

Antimicrobial peptides and proteins secreted from the MSC directly play important roles in the bacteria clearance from different pathways, including inhibition in the synthesis of DNA and RNA, disruption of membrane integrity, and inhibition of bacterial growth through disruption in iron uptake (Brogden, 2005; Hosseiniyan Khatibi et al., 2020). AMPs are produced as the first line of defense of innate immunity against a wide range of microorganisms, including bacteria, viruses, and fungi (Diamond et al., 2009). Families of MSCs-derived AMPs listed in **Figure 1** are mainly studied including cathelicidin, β-defensin-2, lipocalin 2, and Hepcidin (Alcayaga-Miranda et al., 2017; Russell et al., 2020).

Cathelicidin

One of the important antibacterial peptides is the cathelicidin family, which recruits monocytes, neutrophils, and macrophages (Krasnodembskaya et al., 2010). LL-37 is a factor of this family that is an essential part of the innate immune system that exerts its antibacterial effect by disrupting the integrity of the bacterial membrane and neutralizing LPS (Krasnodembskaya et al., 2010; Thennarasu et al., 2010). This factor has also been shown to play an important role in regulating inflammatory responses, inducing tissue repair and healing as well as anti-apoptotic and

angiogenic effects (Oliveira-Bravo et al., 2016; Yang et al., 2020), and in a mouse model of septicemia provided protection against endotoxin shock (Yagi et al., 2020). Johnson et al. (2017), in a study of the antibacterial effects of MSC administration in chronic infections associated with biofilms in mouse and dog models, stated that i.v. administration of activated MSCs induce the killing of bacteria by secretion of cathelicidin, and this effect was increased by antibiotics.

β-Defensin 2

β-defensin 2 play important roles in innate and adaptive immunity against microbial and exert its antibacterial effect by creating pores in the bacterial membrane and destroying the integrity of the membrane and leaking intracellular contents, as well as inhibiting protein, DNA, and RNA syntheses (Laverty et al., 2011; Méndez-Samperio, 2013). A study showed that MSCs secrete the antimicrobial peptide of β-defensin 2 through the TLR-4 signaling pathway after exposure to *Escherichia coli* (Sung et al., 2016). The bacteriostatic potential of this peptide is mainly against gram-negative bacteria and with a lower antibacterial potential against gram-positive bacteria (Harder and Schröder, 2005).

Lipocalin 2

Lipocalin 2 is secreted by various cells including neutrophils, macrophages, epithelial cells, and MSCs in response to inflammatory conditions, which plays an important role in the antibacterial defense of the innate immunity (Dahl et al., 2018). After exposure of MSCs to pathogenic factors lead to the secretion of a large amount of lipocalin 2, this peptide binds to siderophore, as an iron chelator of bacteria, which in turn prevents iron uptake and subsequently reduces bacterial growth (Goetz et al., 2002; Flo et al., 2004). Harman et al. (2017) reported that MSCs derived from the peripheral blood of healthy horses by the secretion of AMPs, including Cystatin C, elafin, lipocalin, and cthelicidin, through disturbance in membrane integrity, the growth of bacteria (*E. coli* and *Staphylococcus aureus*) was inhibited .

Hepcidin

Hepcidin is secreted by hepatocytes, renal epithelial cells, as well as by macrophages and MSCs in inflammatory conditions, which plays an important role in the systemic regulation of iron homeostasis (Kulaksiz et al., 2004, 2005; Esfandiyari et al., 2019). Hepcidin is an antibacterial peptide of the innate immune system that is primarily induced by the IL-6, LPS, and TLR-4 which sequesters bacterial siderophores, and therefore restricts iron availability and as a result inhibits bacterial growth (Ganz and Nemeth, 2012; Michels et al., 2017). Two isoforms of hepcidin are known, including hepcidin 20 and 25, both of which have antibacterial properties (Maisetta et al., 2010).

Inducible Nitric Oxide Synthase Pathway

Mesenchymal stem cells and macrophages activated by LPS, pro-inflammatory cytokines, and interferons (IFN) cause the expression of inducible nitric oxide synthase (iNOS), which in turn iNOS produces nitric oxide (NO) from the amino acid L-arginine inside these cells. Production of NO in this way halts the growth of microorganisms inside macrophages and MSCs (Bogdan, 2015; Yang et al., 2016).

Cysteine Proteases

Studies show that MSC-Exos contain a variety of proteases, including cysteine proteases, which impact the stability of bacterial biofilms by degrading extracellular proteins, and thereby provide conditions for antimicrobials penetration into biofilms and also increase the effectiveness of antibiotics tolerated by biofilms previously (Matsumoto et al., 1999; Marx et al., 2020).

Indoleamine 2,3 Dioxygenase

Indoleamine 2,3 dioxygenase (IDO) is the most important enzyme in the kynurenine pathway (KP), which is primarily responsible for the degradation of the tryptophan amino acid, which MSCs mainly express this enzyme in response to the stimulatory effect of INFγ (Däubener et al., 2009; Croitoru-Lamoury et al., 2011). Depletion of tryptophan in microorganisms due to IDO impairs protein synthesis and disrupts cell division (Frumento et al., 2002; Meisel et al., 2011). IDO has also been shown to induce immunomodulatory effects by inhibiting T cells proliferation and modulating the function of B, T cells, and natural killer (NK) cell (Poormasjedi-Meibod et al., 2013).

Indirect Mechanisms

The antibacterial effects of MSCs can be indirectly mediated by increasing phagocytic activity of macrophages and neutrophils (Hosseiniyan Khatibi et al., 2020). These cells can also induce immunomodulatory effects mentioned in **Figure 2** by modulating immune responses and regulating cytokine homeostasis and reducing immune cells transfer into the damaged organ, and thereby provide the conditions for tissue remodeling and healing. Moreover MSC-Exos perform their major immunomodulatory effects by inhibiting T cell proliferation and converting these cells to Tregs as well as reprogramming M1 macrophages to the M2 phenotype (Riazifar et al., 2019; Hoseini et al., 2020; Hosseiniyan Khatibi et al., 2020; Liu W. et al., 2020). These cells and their exosomes can also inhibit the proliferation and function of B cells, natural killer cells (NKC), and dendritic cells (DC). MSCs can induce both bacterial clearance and immunomodulatory effects, which are dependent on inflammatory signals in the environment (Fan et al., 2019; Xie et al., 2020). MSCs increase immune responses during the early phases of inflammation such that in addition to the migration of neutrophils to sites of inflammation, MSCs induce lymphocyte and M1 macrophages, through the production of chemokines. In fact, the stimulatory effects of mesenchymal cells on immune cells occur when these cells encounter insufficient levels of proinflammatory cytokines such as TNF and IFN-γ, while MSCs and MSC-Exos provide conditions for immunosuppressing during exposure to high levels of inflammatory cytokines through polarization to anti-inflammatory cells, M2 macrophages, and Tregs (Raicevic et al., 2010; Bernardo and Fibbe, 2013; Song et al., 2017; Xie et al., 2020). Thus, MSCs can activate both phenotypes of macrophages and provide a balance between inflammatory and anti-inflammatory

TABLE 1 | Antibacterial and Immunomodulatory Effects of MSCs and MSC-Exos in *in vitro* and *in vivo* studies.

Study type	Source of MSC	Outcomes	References
***In vivo*:** Mouse and dog models of chronic infections	AT-MSC	↑ Cathelicidin secretion ↑ Clearance of bacteria ↑ Monocyte recruitment ↑ M2 phenotype ↑ Neutrophil bacterial Phagocytosis	Johnson et al., 2017
***In vivo*:** Murine Cystic fibrosis	BM-MSC, AT-MSC	↑ Enhance antibiotic sensitivity ↑ Capacity to kill bacteria (*Pseudomonas aeruginosa*, *Staphylococcus aureus*) ↑ LL-37	Sutton et al., 2017
***In vitro*:** Bacterial growth in Equine model	BM-MSC, AT-MSC, EM-MSC	↓ Growth of *E. coli* ↑ Lipocalin-2 expression ↑ MCP-1, IL-6, IL-8, and CCL5	Cortés-Araya et al., 2018
***In vitro* and *In vivo*:** Murine sepsis model	BM-MSC	↓ Genes expression of apoptosis ↓ Genes expression of Pro-inflammatory cytokine ↑ Antibacterial peptides ↑ Anti-inflammatory cytokines ↑ Animal survival rates ↑ Bacterial clearance (Staphylococcal enterotoxin B)	Saeedi et al., 2019
***In vitro*:** Chronic skin wounds in Equine model	PB-MSC	↓ Growth of *E. coli* and *S. aureus* biofilms ↑ Cystatin C, elafin, lipocalin, cthelicidin	Harman et al., 2017
***Ex vivo*:** Acute Lung Injury in Mice	HU-MSC	↑ Keratinocyte growth factor (KGF) ↓ Influx of neutrophils ↓ Lung protein permeability ↓ Pulmonary edema	Zhu et al., 2014
***In vivo*:** Chronic inflammation (*Staphylococcus aureus*) of the ovaries in mice	BM-MSC	↓ Leukocyte infiltration in ovaries ↓ Number of atretic follicles ↑ Ovary morphological parameters ↓ Apoptotic oocytes ↑ Pregnancy rate	Volkova et al., 2017
***In vivo*:** Chronic salpingitis (*E. coli*) model in rabbits	WJ-MSC	↓ TNF-α ↑ Oviductal glycoprotein ↑ Repaired the structure of the tubal epithelium ↑ Pregnancy rates	Li et al., 2017
***In vivo*:** Chronic salpingitis (*Chlamydia trachomatis*) murine model	hUC-MSC	↓ Macrophage infiltration ↑ IL-10 ↓ FT cell apoptosis (Caspase-3) ↑ Pregnancy rate	Liao et al., 2019
***In vitro*:** Human Fetal Liver	FL-MSC-Exos	↓ Proliferation, activation, and cytotoxicity of NK cells *via* TGFb	Fan et al., 2019
***In vivo*:** Intrauterine adhesions in a female rat model	UC-MSCs-EVs	↓TNF-α, ↓TGF-β ↓IL-1, ↓IL-6 ↓RUNX2, ↓Fibrosis ↓collagen-I ↓VEGF ↓IUA	Ebrahim et al., 2018
***In vivo*:** Premature ovarian insufficiency model mice	hU-MSC-Exos	↑Restored ovarian phenotype and function ↑ovarian cells proliferation ↑exosomal miR-17-5P ↓SIRT7 expression	Ding et al., 2020
***In vitro*:** inflammation in endometrial cells of equine models	A-MSC- MVs	↓Apoptosis rate ↓Pro-inflammatory gene expression ↓Pro-inflammatory cytokines secretion	Perrini et al., 2016

(Continued)

TABLE 1 | (Continued)

Study type	Source of MSC	Outcomes	References
***Ex vivo*:** Lung injury models in mice	BM-MSC-EV	↑M2 macrophage marker expression ↑Phagocytic macrophage Phenotype ↑Mitochondrial transfer to macrophage ↓Inflammation and lung injury	Morrison et al., 2017
***In vitro*:** Asthma in human	BM-MSC-Exos	↑IL-10 ↑TGF-β1 ↑Immunosuppression capacity of Tregs	Du et al., 2017

MSC, mesenchymal stem cell; AT-MSC, adipose tissue-MSC; HU-MSC, human-MSC; BM-MSC, bone marrow-MSC; EM-MSC, endometrium-MSC; PB-MSC, peripheral blood-MSC; FL-MSC, fetal liver-MSC; WJ-MSC, wharton's jelly; hUC-MSC, human umbilical cord-MSC; A-MSC, amniotic-MSC; MCP-1, monocyte chemoattractant protein-1; CCL5, chemokine ligand-5; FT, Fallopian tube; IUAs, intrauterine adhesions.

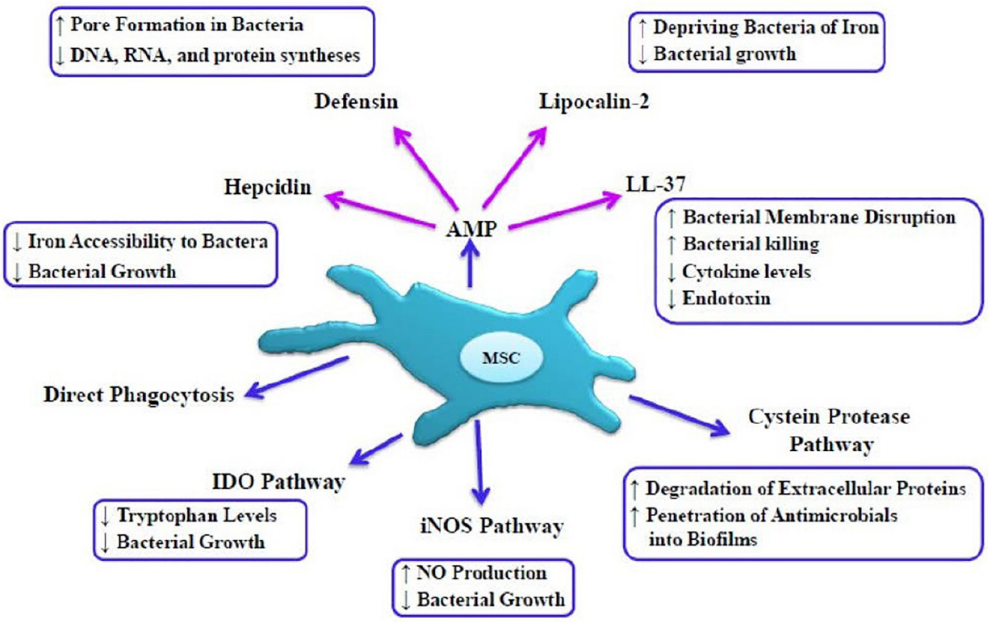

FIGURE 1 | Direct mechanisms of MSC-mediated bacterial killing, MSC exert direct antibacterial effects due to host defense peptides or AMPs. MSCs can mediate bacterial killing by disrupting the integrity of the bacterial membrane, creating pores in the bacterial membrane, preventing of iron uptake, inhibiting biofilm formation, depleting tryptophan in microorganisms, and halting the growth of bacteria. IDO pathways, Indoleamine 2,3 dioxygenase; iNOS pathways, inducible nitric oxide synthase; NO, nitric oxide.

responses through interaction with the immune system and thereby provide the condition for maintaining integrity and homeostasis of tissue (Liu W. et al., 2020; Xie et al., 2020). MSCs inhibit proliferation and function of T cells by secreting factors such as nitric oxide (NO), IDO, prostanglandin-E2 (PGE2), transforming growth factor (TGF)-β, and interleukin (IL)-10 (DelaRosa and Lombardo, 2010).

Kol et al. (2014) in the study of effects of adipose-derived MSCs on intestinal microbes (*Salmonella typhimurium* and *Lactobacillus acidophilus*) concluded that these cells could increase the expression of key immunomodulatory genes including COX2, IL-6, and IL-8, as well as increase the secretion of PGE2, IL-6, and IL-8, and they also found that exposure of MSCs to *S. typhimurium* increased the capacity of these cells to inhibit T cell proliferation *via* PGE2. MSC-Exos also exert their immunomodulatory effects through their RNA and proteins (Lo Sicco et al., 2017). Song et al. stated that exosomal miR-146a is an anti-inflammatory micro-RNA that is transferred into macrophages and leads to polarization to M2 phenotype and ultimately increases survival in sepsis models of mice (Song et al., 2017).

It has also been shown that MSCs inhibit T cell activity by inhibiting the function, differentiation, and maturation of dendritic cells (DCs) (Aggarwal and Pittenger, 2005). DCs are the main cells of the immune system which present antigens to T cells and are able to express high levels of co-stimulatory molecules and thereby effectively induce immune responses; thus MSCs and MSC-Exos can lead to inhibition of T cells function and development of Tregs by inducing an inhibitory effect on DCs (Aggarwal and Pittenger, 2005; Jiang et al., 2005). Moreover, MSCs lead to recruitment and stimulation of polymorphonuclear (PMN) cells such as neutrophils, by secreting

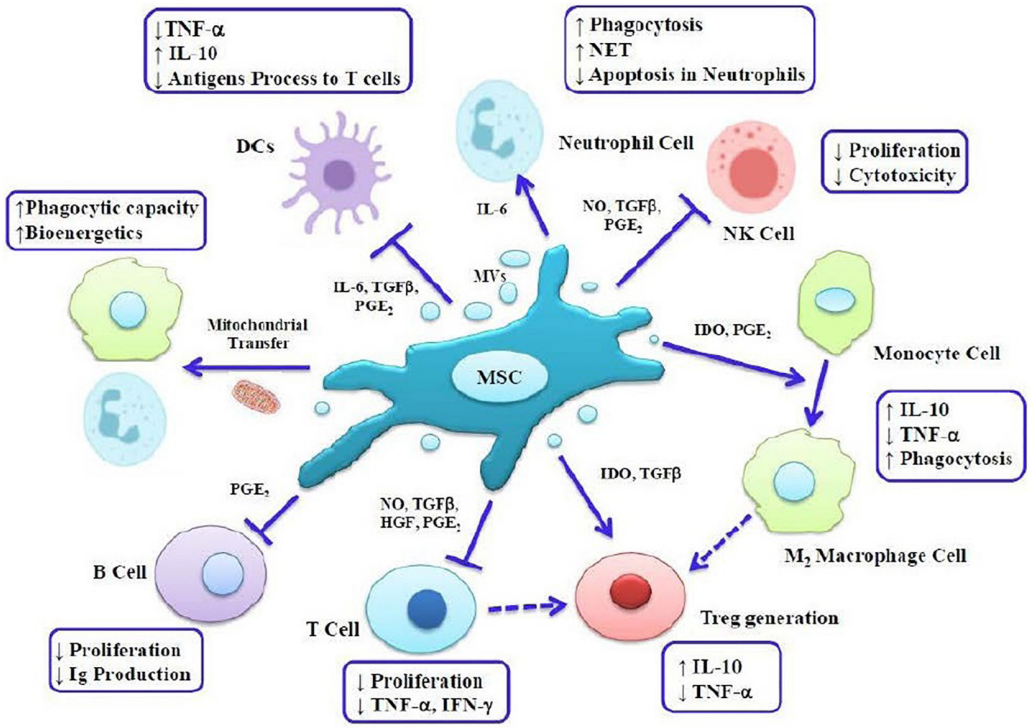

FIGURE 2 | The antibacterial effects of MSC can be indirectly mediated by increasing phagocytic activity of macrophages and neutrophils. These cells can also induce immunomodulatory effects by modulating immune responses and regulating cytokine homeostasis. MVs, microvesicles; DC, dendritic cell; NKC, natural killer cell; NET, neutrophil extracellular trap; IFN, interferon; TNF, tumor necrosis factor; NO, nitric oxide; PGE2, prostaglandin E2; TGF, transforming growth factor; IDO, indoleamine 2,3 dioxygenase; Treg, T regulatory cell; Ig, immunoglobulin.

IL-6 and IL-8 (Brandau et al., 2014). Neutrophils induce the killing of microorganisms by phagocytosis and internalization of them into the phagolysosome, and it has also been shown that these cells, through mechanism of neutrophil extracellular traps (NETs), immobilize microorganisms to prevent their spread in the environment (Hirschfeld, 2014; Jackson et al., 2016). Studies show that MSCs and MSC-Exos not only increase phagocytosis activity of neutrophils but also protect neutrophils from apoptosis (Harrell et al., 2019; Qian et al., 2021). In addition, research showed that direct co-culture of MSCs and their exosomes with macrophages induce mitochondrial transfer from MSCs to macrophages *via* formation of structures called tunneling nanotubes (TNT) that leads to increase in the phagocytic activity of macrophages and improvement in their bioenergetics (Hirschfeld, 2014; Qian et al., 2021).

PATHOGENESIS OF *N. GONORRHOEAE* AND *C. TRACHOMATIS* IN THE FEMALE REPRODUCTIVE TRACT

Neisseria gonorrhoeae and *Chlamydia trachomatis* are gram-negative bacteria that are both considered obligate human pathogens, and they are known as the most common cause of sexually transmitted diseases (STDs) (Dehghan Marvast et al., 2016, 2018; Chen et al., 2018; Lenz and Dillard, 2018). *N. gonorrhoeae* mainly affects the mucous membranes of female reproductive tracts. This infection starts from the lower reproductive tract including the vagina and ectocervix and can spread to the upper female genital tract (endometrium and fallopian tubes) (Lenz and Dillard, 2018). *Chlamydia* is also an intracellular pathogen that infects the epithelial cells of the endocervix in women and the urethra in men (O'Connell and Ferone, 2016). During its evolutionary cycles, *Chlamydia* forms structures called elementary bodies (EBs) and reticulate bodies (RBs). EBs are infective forms that are metabolically inactive, but after chlamydia enters the host cell, EBs convert to RBs that are metabolically active but non-infectious and are considered as the replicating form of the bacteria (Brunham and Rey-Ladino, 2005). *N. gonorrhoeae* and C. trachomatis infections can be symptomatic or asymptomatic and without treatment lead to complications such as pelvic inflammatory disease (PID), obstruction of FT, tubal scarring, and loss of ciliated cells function in these areas (Dehghan Marvast et al., 2017; Tsevat et al., 2017). Studies have reported that *N. gonorrhoeae* attach to non-ciliated cells through pili and Opa proteins in FT but lead to loss of ciliated cells function and eventually the death of these cells (Edwards and Apicella, 2004; Quillin and Seifert, 2018). Various studies have linked the death of these cells to the presence of toxic factors in bacteria, including lipopolysaccharide (LPS) and lipooligosaccharide (LOS), which induce the host immune system (Gregg et al., 1981; Christodoulides, 2019;

Gulati et al., 2019). In the gonococcal infections following exposure to pathogen associated molecular patterns (PAMPs), the secretion of cytokine TNF is one of the first responses of the host immune system (Patrone and Stein, 2007). One study reported that increase in the concentration of TNF was associated with decrease in function of ciliated cells (McGee et al., 1999). On the other hand, studies have reported that the reduction in ciliated cell activity and death of these cells during gonococcal infections has been attributed to the induction of apoptosis in FT epithelial cell by the TNF cytokine (Edwards and Apicella, 2004; Morales et al., 2006). Evidences also are showed that other factors, including IL-1, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), and granulocyte macrophage colony-stimulating factor (GM-CSF) secreted during gonococal infections (Maisey et al., 2003; Velasquez et al., 2012). Moreover, one study reported that the levels of cytokines IL-2 and IL-12 were rapidly upregulated during exposure to *N. gonorrhoeae* infection, such that IL-2 was associated with lymphocyte proliferation, while IL-12 increased IFNγ production by lymphocytes and NKC (Rarick et al., 2006). Although magnitude Th17 responses in gonococcal infections lead to the release of IL-17 and the recruitment of neutrophils, the relative resistance of *N. gonorrhoeae* to neutrophil function and the lack of an effective response to pathogen clearance have been reported (Witt et al., 1976; Liu et al., 2012). Moreover, immune responses in *C. trachomatis* infection include activation of Th1 and proinflammatory cytokines IL-2, IL-6, TNF, and INF-γ (Arno et al., 1990). One study reported that INF-γ levels in endocervical secretions of women with C. trachomatis infection were five times higher than uninfected women (Sellami et al., 2014). Also, studies show that TLR-2 and TLR-4, which are increased in *C. trachomatis* infection, play an important role in inducing innate and acquired immune responses (Agrawal et al., 2011; Lovett and Duncan, 2019).

But although lymphocyte proliferative responses in gonococcal infections are increased compared to healthy individuals, these immune responses cannot provide strong protection against recurrence of the infection (Zhu et al., 2012). Moreover, *N. gonorrhoeae* are able to manipulate and effect the function of host immune cells, so that gonococcal infections have been shown to exert immunosuppressive signaling by inhibiting the proliferation of DCs, T cells, and B cell (Manicassamy et al., 2009; Escobar et al., 2018). On the other hand, *N. gonorrhoeae* induces the expression of immunosuppressive cytokines such as TGF-β and IL-10 so that it has been stated that *N. gonorrhoea* suppresses the activity of Th1 and Th2 by inducing the expression of TGF-β (Mascellino et al., 2011).

Evidence suggests that the immune responses generated during the pathogenesis of Neisseria and Chlamydia are polarized toward cytotoxic responses and provide the conditions for obstruction and scaring in FT (Menon et al., 2015; Jefferson et al., 2021). According to research, different mechanisms are involved in inducing infertility following *N. gonorrhoeae* and *C. trachomatis* infection. The first mechanism involves the ascension of the infection to the upper reproductive tract (Hafner, 2015). The second mechanism involves the persistence of the infection, which leads to long-term pathological immune responses and thus provides the conditions for damage to the epithelial cells of FT (Batteiger et al., 2010). It has also been suggested that treatment failures by antibiotics lead to recurrence of the infection and the development of infertility *via* persistence of infection (Menon et al., 2015). The third mechanism involves the secretion of cytokines from pathogen-infected epithelial cells, which induce proinflammatory immune responses that lead to severe epithelial cell damage and fibrosis or scarring following repair mechanisms by infiltrating fibroblasts (Darville, 2021). Since that salpingitis induced by *N. gonorrhoeae* and *C. trachomatis* infections leads to pathological immune responses and induces infertility, it is necessary to create a good balance between immune activation and immune suppression.

THERAPEUTIC POTENTIAL OF MESENCHYMAL STEM CELL-DERIVED EXOSOMES ON SALPINGITIS INDUCED BY *N. GONORRHOEAE* AND *C. TRACHOMATIS* INFECTIONS

Due to the unique life cycles of *N. gonorrhoeae* and *C. trachomatis*, the major PID caused by these infections are chronic, so antibiotic therapy is less effective, which often leads to persistence of the infection and reinfection (Chen et al., 2020). Various studies listed in **Table 1** have reported the positive effects of MSC-Exos in the treatment of gynecological diseases (Sun et al., 2019; Zhang et al., 2019; Liu C. et al., 2020; Xin et al., 2020; Zhao et al., 2020; Lee et al., 2021; Liao et al., 2021). Today, MSC-Exos are used in cell therapy, regenerative medicine, auto-immune, and microbial disease due to their unique properties such as high proliferative capacity, easy isolation, and secretion of bioactive factors, as well as having anti-apoptotic, antimicrobial, antiscarring, tissue repair, and wound healing effects (Ha et al., 2020; Raghav et al., 2021).

Exosomes Isolation of Mesenchymal Stem Cell

Various techniques are used to separate exosomes from MSCs, including ultracentrifugation, ultrafiltration, precipitation, immunological separation, chromatography, and nanoFACS (Théry et al., 2018; Rezaie et al., 2021). However, each of these methods has advantages and disadvantages, and studies have reported that the ultracentrifugation method is the most common standard method for isolating exosomes (Momen-Heravi et al., 2013). But Klymiuk et al. (2019) in their study stated that the ultrafiltration method had higher results and efficiencies in size-based isolation compared to the ultracentrifugation method, and a 50-fold increase in concentration and less time for isolation compared to the ultracentrifugation method was reported. On the other hand, due to several overlapping features between exosomes and viruses such as size, shape, density, and biogenesis, Rezaie et al. (2021) reported that nanoFACS and immunological methods are more suitable for isolating exosomes from viruses in infected samples.

Moreover, in most studies it has been stated that in order to achieve better specificity and recovery in the separation of EV or EV subtypes, the use of a combination of techniques or additional techniques is recommended (Nikfarjam et al., 2020; Liangsupree et al., 2021).

Advantages and Limitations of Mesenchymal Stem Cells-Derived Exosomes Application

Various studies have shown the superiority of using exosomes rather than MSCs. The risk of tumor formation has not been reported in exosome-based therapies, while the tumorigenic risk in MSC-based therapies has been observed in several studies (Mendt et al., 2019; Wei et al., 2021). In addition to the fact that lower side effects of exosomal therapy than mesenchymal transplantation have been reported in various studies, Sun et al. (2015) have reported increased expression of HLA and immunological rejection in MSCs transplantation. In addition, studies have shown that exosomes are not affected by apoptotic processes and cell death due to their non-cellular nature, and therefore their stability is greater in the damaged area (Lou et al., 2017; Wang et al., 2021). Exosomes are also less expensive to produce than MSCs and are more stable to store and easier for storage and, recently, it has received more attention than cell-based therapy due to the ability of exosomes to transport therapeutic biomolecules and facilitate repair of the damaged site (Babaei and Rezaie, 2021). Despite the advantages of exosome therapy and its therapeutic potential compared to their parent cells, several disadvantages have been reported, including the lack of renewal potential, the loss of some paracrine factors during the use of isolation methods, and the possibility of viral infections transmission and short half-life of exosomes (Takahashi et al., 2013; Babaei and Rezaie, 2021).

Application Studies of Mesenchymal Stem Cell-Derived Exosomes

Different evidences mentioned in **Table 1** have reported the antimicrobial effects of MSCs and MSC-Exos. However, these studies are more limited to animal studies and clinical dates are low. In the evaluation of antimicrobial activity of MSCs in chronic infections associated with biofilm formation, it has been reported that co-administration of MSCs with antibiotics affected both direct and indirect pathways of these cells, such that secretion factors of MSCs inhibited biofilm formation and disrupted the growth of stabilized biofilms (Johnson et al., 2017). It has also been suggested that administration of these cells with antibiotics can have a synergistic effect in reducing a variety of multi-drug resistance (MDR) in bacterial infections (Chow et al., 2020; Russell et al., 2020). Liao et al. reported that hUC-MSC reduced hydrosalpinx, macrophage infiltration, and the expression of IL-10 in the oviduct. Also, they observed that hUC-MSC induced anti-apoptotic effects by reducing the expression level of caspase-3. In addition, it was reported that pregnancy rate increased significantly, and these effects were attributed to the anti-inflammatory and anti-apoptotic properties of hUC-MSC (Liao et al., 2019). In addition, Li et al. (2017) observed that WJ-MSCs restored the epithelial structure of the FT and concentration of TNF was decreased significantly in the treatment group with WJ-MSCs, and they also reported that WJ-MSCs improved the secretion of oviduct glycoprotein and fertility partially in rabbits with chronic salpingitis. Furthermore, Ebrahim et al. (2018) revealed that hUC-MSC-EV alone or in combination with estrogen significantly reduced intrauterine adhesions in female rats due to decrease in inflammatory cytokines (TNF-α, IL-1, IL-6) and fibrotic markers (RUNX2, TGF-β, collagen-I). Also, Ding et al. reported that hUMSC-Exos due to microRNA-17-5P repaired the phenotype and function of the ovary, elevated ovarian cells proliferation, and decreased ROS accumulation in POI mouse model (Ding et al., 2020).

CONCLUSION

Experimental studies show that MSCs and MSC-Exos have a high potential for the treatment of inflammatory and microbial diseases. Furthermore, MSC-Exos have similar abilities to their parent cells, which have a high potential for modulating immune responses due to their therapeutic biomolecules. However, the priority of using MSC-Exos compared to cell-based therapy in terms of safety and stability has been reported in several studies. In addition, MSC-Exos induce the phagocytic activity of neutrophils and macrophages and improve the bioenergetics of them to provide the conditions for increasing the survival of these cells and the continuity of their function in bacterial phagocytes. On the other hand, MSC-Exos play an important role in preventing the pathological immune response by interacting with immune cells and reprogramming M1 macrophages to the M2 phenotype and converting Th to Tregs. Therefore, it can be said that MSC-Exos due to these properties can inhibit pathological immune responses during *N. gonorrhoeae* and *C. trachomatis* infections, and in this way MSC-Exos provide the conditions for tissue repair and prevent severe tissue damage during infection.

AUTHOR CONTRIBUTIONS

MZ wrote the draft of manuscript. LD, MI, and SM revised the parts of Infectious and infertility. BA read the manuscript and did the final revision and agreed with the final version of the manuscript.

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Stem Cell-Derived Exosome as Potential Therapeutics for Microbial Diseases

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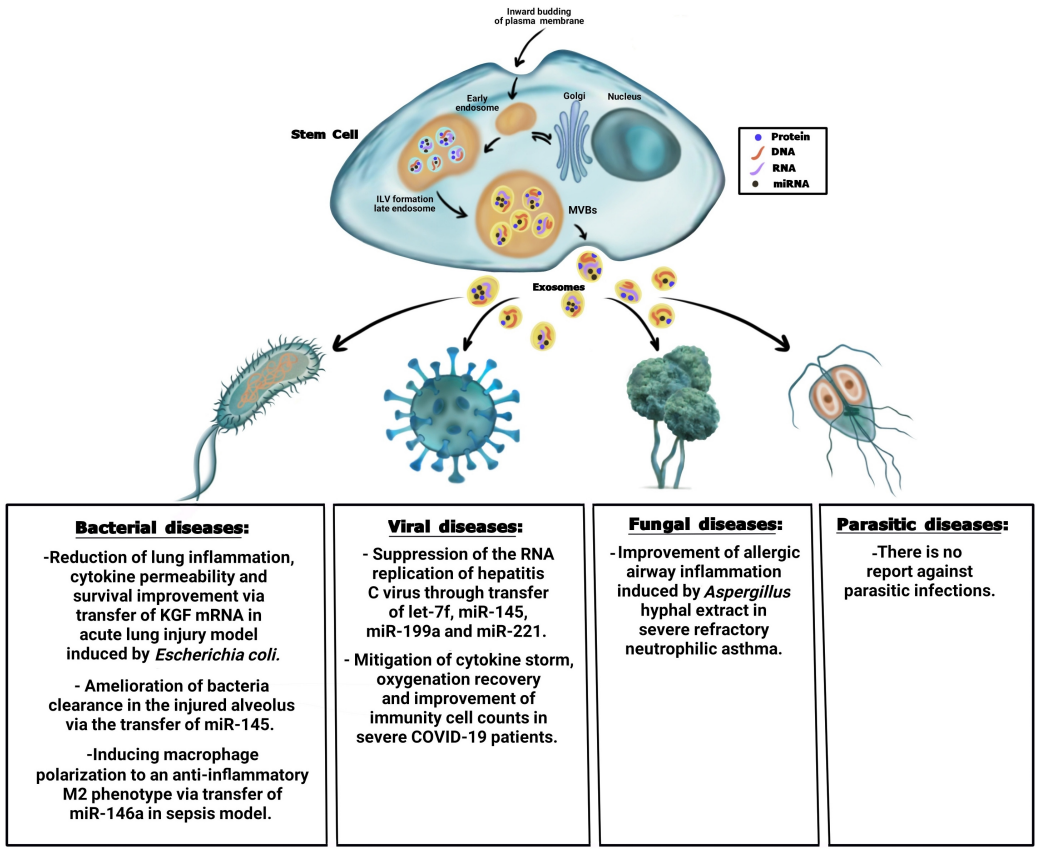
Exosomes, as the smallest extracellular vesicles that carry a cargo of nucleic acids, lipids, and proteins and mediate intercellular communication, have attracted much attention in diagnosis and treatment in the field of medicine. The contents of exosomes vary depending on the cell type and physiological conditions. Among exosomes derived from several cell types, stem cell-derived exosomes (stem cell-Exo) are increasingly being explored due to their immunomodulatory properties, regenerative capacity, anti-inflammatory and anti-microbial functions. Administration of stem cell-Exo, as a cell-free therapy for various diseases, has gained great promise. Indeed, the advantages of exosomes secreted from stem cells outweigh those of their parent cells owing to their small size, high stability, less immunogenicity, no risk of tumorigenesis, and easier condition for storage. Recently, the use of stem cell-Exo has been proposed in the field of microbial diseases. Pathogens including bacteria, viruses, fungi, and parasites can cause various diseases in humans with acute and chronic complications, sometimes resulting in mortality. On the other hand, treatments based on antibiotics and other chemical compounds have many side effects and the strains become resistant to drugs in some cases. Hence, this review aimed to highlight the effect of stem cell-derived extracellular vesicles including stem cell-Exo on microbial diseases. Although most published studies are preclinical, the avenue of clinical application of stem cell-Exo is under way to reach clinical applications. The challenges ahead of this cell-free treatment that might be applied as a therapeutic alternative to stem cells for translation from bench to bed were emphasized, as well.

Keywords: exosome, stem cell, anti-microbial, pathogen, therapy

INTRODUCTION

Almost all physiological and metabolic processes depend on cell-to-cell communication. Extracellular Vesicles (EVs) are one of the most important mediators of intercellular communication (Wang et al., 2018; Larabi et al., 2020), which include a collection of vesicles enclosed in a phospholipid bilayer membrane and released by various cells into the extracellular



GRAPHICAL ABSTRACT | Summary of the antimicrobial effect of stem cell-derived exosomes on bacteria, viral, fungal, and parasitic diseases.

space. The process of EVs release is evolutionary conserved and occurs in both prokaryotes and eukaryotic cells. EVs were first identified by Dr. Ross Johnstone in 1983 during reticulocyte maturation. Before that, EVs were considered "garbage bags," but they turned out to play an important role in intercellular communication by transferring different kinds of protein, nucleic acid, and lipid within an organism or between species (Keshtkar et al., 2018; Larabi et al., 2020). The International Society of Extra Cellular Vesicles (ISEV) has classified EVs into three groups of exosomes, microvesicles, and apoptotic bodies based on their size, biogenesis, release routs, cargos, and function (**Figure 1**). It has been shown that all types of cell are able to release EVs, which are observed in almost all body fluids such as blood, normal urine, breast milk, bronchial lavage fluid, saliva, cerebrospinal fluid, amniotic fluid, and synovial fluid (Keshtkar et al., 2018; Zhao et al., 2020).

Extracellular Vesicles, particularly exosomes, are released by cells during normal physiological and pathological conditions (Yuana et al., 2013). Production of EVs can be induced by various processes such as oxidative stress, hypoxia, senescence, inflammation, and infection (Keshtkar et al., 2018; Zhao et al., 2020). The number of released EVs depends on the physiological state of cell production and its microenvironment. The unique properties of EVs in delivering their active cargos to neighbor or distant cells have attracted much attention for the therapeutic application of these particles (Abreu et al., 2016; Zhang et al., 2019). In the following sections, three groups of EVs are reviewed, focusing on exosomes and exosomes derived from stem cells.

APOPTOTIC BODIES

Apoptotic bodies are the biggest vesicles whose diameters range from 50 to 5,000 nm and are released from dying cells (Doyle and Wang, 2019). The process of apoptotic body formation includes cell contracts and enhancement of hydrostatic pressure, leading to outward budding or fragmentation of plasma membrane from cytoskeleton in dying cells (**Figure 1**). It has been reported that apoptotic bodies carry chromatin, intact organelles, and glycosylated proteins such as histones and Heat Shock Protein (HSP)-60. The markers of apoptotic bodies include Annexin V, DNA fragments, and histones. However, their contents can be different depending on the cell type from which they are released (Doyle and Wang, 2019; Mohan et al., 2020; Rezaie et al., 2021).

MICROVESICLES

Microvesicles (MVs) are 100–1,000 nm EVs released from a verity of living cells into the extracellular space (**Figure 1**). MVs biogenesis is driven *via* direct outward budding of plasma membrane in the presence of cytoskeleton agents such as

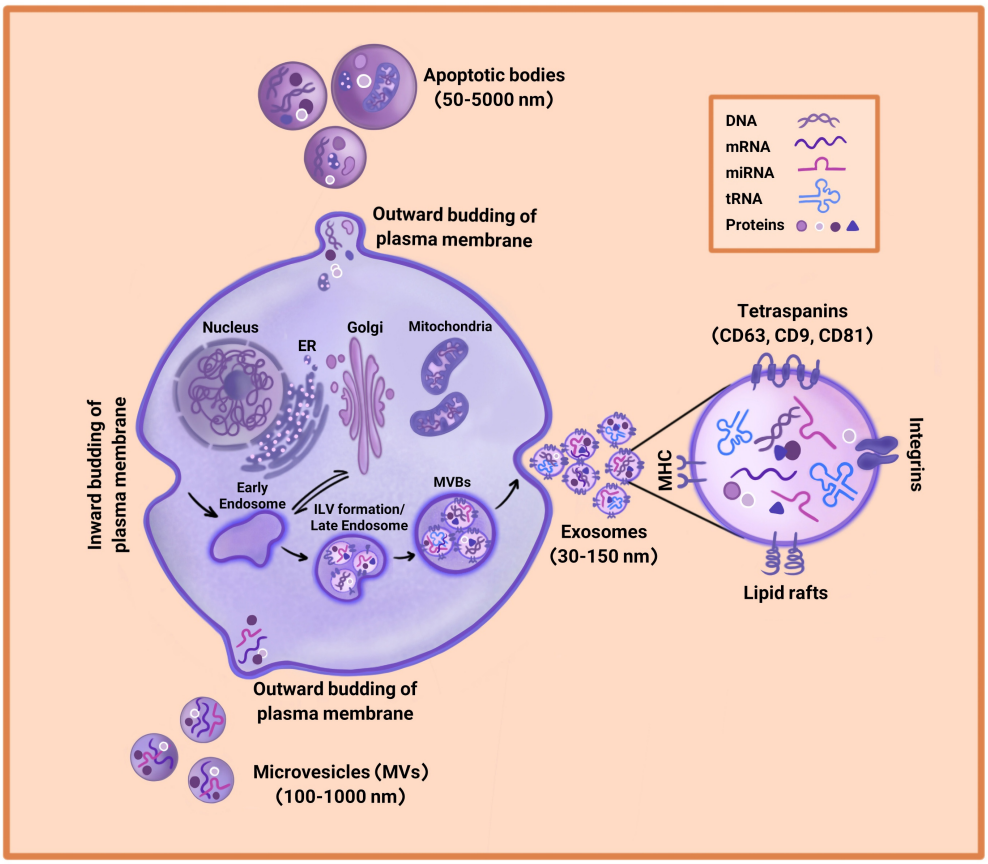


FIGURE 1 | Extracellular Vesicles (EVs) biogenesis. EVs consist of exosomes, microvesicles, and apoptotic bodies. Exosomes arise through the inward budding of plasma membrane and are the smallest in diameter (30–150 nm). Microvesicles are larger in diameter (100–1,000 nm) and are driven through an outward budding of the plasma membrane. Apoptotic bodies range from 50 to 5,000 nm in diameter and are generated by outward budding of the plasma membrane from dying cells. Due to different biogenesis mechanisms, the compositions of exosomes, microvesicles, and apoptotic bodies are varied.

microtubules, actin, kinesins, myosins, and tethering factors (Doyle and Wang, 2019; Rezaie et al., 2021). These vesicles isolate by ultracentrifugation at 10,000–20,000 × *g*. Since MVs are separated by the budding of plasma membrane, their composition mainly includes plasma membrane-associated proteins such as tetraspanins along with cytosolic proteins, cytoskeletal proteins, and HSPs. However, its compounds are not limited to proteins and contain lipids, mRNAs, and microRNAs. Moreover, MVs are one of the mediators of cell-to-cell communication between neighbor and distant cells, which interact through a specific ligand-receptor. MVs are able to transfer their contents to recipient cells and change the functionality of target cells based on physiological and pathological environmental conditions (Keshtkar et al., 2018; Doyle and Wang, 2019).

EXOSOMES

Exosomes are nano-vesicles with sizes ranging from 30 to 150 nm in diameter that sediment between 70,000 and 200,000 × *g*. Exosomes arise through a specific biogenesis pathway including the inward budding of plasma membrane that forms the early endosome (**Figure 1**). Then, the early endosome matures into late endosome with the accumulation of Intraluminal Vesicles (ILVs) in their lumen. The process of ILVs formation is mediated by the Endosomal Sorting Complex Required for Transport (ESCRT) or by ESCRT-independent mechanisms including tetraspanins or lipids such as ceramides. Since the late endosome contains ILVs, it is called Multivesicular Bodies (MVBs) (Yuana et al., 2013; Salimi et al., 2020). Finally, MVBs fuse with plasma membrane and generate exosomes (Larabi et al., 2020). After releasing into the extracellular space, exosomes are recognized by recipient cells through adhesion factors such as integrin followed by endocytic uptake. However, some exosomes directly fuse with the plasma membrane or interact with the lipid-ligand receptor and transmit their cargos (Keshtkar et al., 2018; Shi et al., 2021). Depending on the distance of the target cell, exosomes for distant cells may be absorbed through the paracrine or the endocrine pathway (Keshtkar et al., 2018).

The importance of exosomes goes back to their contents since they contain a valuable shipment of proteins, lipids, and metabolites as well as a set of nucleic acids consisting of

microRNA, tRNA fragments, mRNAs, small RNA transcripts, and RNA-protein complexes (Wang et al., 2018; Larabi et al., 2020). Exosomes also carry chromosomal and mitochondrial DNA. It has turned out that these nucleic acids are essential in cell signaling transduction and regulation of biological function. Nucleic acids are functionally active when entering recipient cells. Although the composition of exosomes depends on the origin of the donor cell, there are multiple conserved proteins that are considered the specific markers of exosomes. The tetraspanin family of proteins (including CD9, CD63, and CD81) are the most important conserved proteins, which assist the connection of inside the cell to the outside environment. Apart from tetraspanin, other important cell adhesion molecules include integrins and antigen presentation molecules (MHC) (Nikfarjam et al., 2020). HSP70 and HSP90 are also known as the top exosomal markers involved in membrane remodeling *via* protein folding regulation and transformation (Shi et al., 2021). Alix, TSG101, and GTPases are other specific markers of exosomes. Exosomes are surrounded by a lipid bilayer membrane that preserves them from degradation by the immune system and separation from body fluids. Exosomes enclosed in the phospholipid membrane include high levels of cholesterol, sphingomyelin, ceramide, and lipid-rafts (Doyle and Wang, 2019; Larabi et al., 2020). These lipids are in fact the characteristic of the cellular source releasing exosomes. After isolation, exosomes are stable and can be stored at −80°C for a long time without losing their functionality, because of their biolayer lipid membrane.

Studies have shown that exosomes possess immunomodulatory potentials, one of which being communication between antigen-presenting and recipient cells. Exosomes also contain cytokines with antimicrobial properties and innate response signaling molecules that are important in response to viral and bacterial infections. The exosomal composition depends mainly on the source of donor cells, epigenetic changes, and physiological and pathological microenvironment conditions. Hence, exosomes have vital roles in intracellular communication and immune modulation in different physiological and pathological conditions (Doyle and Wang, 2019). Due to the broad biological functions of exosomes including maintaining homeostasis and transferring molecules between cells, these vesicles have attracted much attention in medical research, with a focus on the therapeutic application of exosomes in the last two decades.

STEM CELL-DERIVED EXOSOMES

It has been reported that exosomes are released by all kinds of cell including stem cells and immune cells that can enter body fluids including blood, saliva, amniotic fluid, urine, milk, cerebrospinal fluid, ascites, and semen (Wang et al., 2018) and move toward target cells. Among exosomes derived from several stem cell types, Mesenchymal Stem Cell-derived Exosomes (MSC-Exo) have received much attention due to their immunomodulatory, regenerative, and anti-inflammatory functions. MSCs exert immunoregulatory and tissue repair functions due to secreting paracrine factors including exosomes and MVs (Keshtkar et al., 2018; Zhao et al., 2020). MSC-Exo are involved in cellular processes including proliferation, transcription, migration, and differentiation. MSC-Exo also help the stimulation of angiogenesis, suppression of fibrosis, increase of neuronal survival and differentiation, induction of extracellular matrix remodeling, inhibition of local inflammation response, and adjustment of immune cells' activities (Zhao et al., 2020).

Extensive body of evidence has demonstrated that MSC-Exo mimic the beneficial effects of parent MSCs in animal models of various human diseases including cardiovascular, kidney, liver, lung, and neurodegenerative diseases, and other diseases (Keshtkar et al., 2018, 2020; Zhao et al., 2020). MSC-Exo were first separated in 2010 and decreased the infarct size in a mouse model of myocardial ischemia/reperfusion injury. The results of microarray analysis indicated that about 98% of miRNAs in stem cells were in exosomes and MVs (Hassanzadeh et al., 2021; Rezaie et al., 2021).

In addition to the aforementioned beneficial effects, stem cell-Exo present anti-microbial properties like parent cells. pathogens including bacteria, viruses, fungi, and parasites can cause various diseases in humans with acute and chronic complications, sometimes resulting in mortality. Moreover, the rising incidence of emerging infectious agents is alarming. On the other hand, treatments based on antibiotics and other chemical compounds have many side effects and the strains become resistant to drugs in some cases. Hence, exploring novel treatment approaches is always a necessity. Recently, various studies have presented the anti-microbial effects of stem cell-Exo in preclinical and few clinical trials. This review highlights the recent studies exploring the therapeutic potential of all kinds of stem cell-Exo along with immune-derived exosomes to combat with microbial infections and complications.

THE APPLICATION OF STEM CELLS THERAPY IN MICROBIAL DISEASES

To date, stem cell therapy has been promising in tissue and immune disorders. Successful attempts have been made mainly MSCs in the treatment of infectious diseases and controlling their complications. This part aims to summarize the advances in this field.

The anti-bacterial effect of MSCs has been investigated in various studies (Krasnodembskaya et al., 2010; Sung et al., 2016; Liu et al., 2017; Chow et al., 2020). Accordingly, these cells exert their effect through direct bacterial killing or indirectly by modulating the acute phase of the immune response (Pierce and Kurata, 2021). MSCs express various kinds of anti-microbial peptide and protein (AMPs), four of which are well known due to anti-bacterial properties including cathelicidin LL-37 (Krasnodembskaya et al., 2010), β-defensin-2 (BD-2) (Sutton et al., 2016), hepcidin (Alcayaga-Miranda et al., 2015), and Lipocalin-2 (Lcn2) (Gupta et al., 2012). Recent studies have suggested that MSCs improve bacterial clearance in preclinical models through the AMPs. Therefore, MSCs can augment the innate immune response against bacteria

(Alcayaga-Miranda et al., 2017). Yagi et al. (2020) assessed the anti-microbial activity of human Adipose-Derived MSCs (AD-MSCs) on *Staphylococcus aureus*. The findings indicated that human AD-MSCs conditioned medium significantly prevented the growth of *S. aureus*. The results also demonstrated that cathelicidin LL-37 played an important role in the anti-microbial activity of AD-MSCs (Yagi et al., 2020). A previous study also showed that the anti-microbial activity of BM-MSCs against the growth of Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*S. aureus*) bacteria was mediated by LL-37 (Krasnodembskaya et al., 2010). On the other hand, human umbilical cord blood-derived MSCs attenuated acute lung injury due to *E. coli* infection in mice. The results demonstrated that MSCs secreted BD-2 through the TLR-4 signaling pathway and mediated the anti-microbial effects (Rezaie et al., 2021). Moreover, menstrual-derived MSCs were responsible for bacterial clearance by the secretion of hepcidin in synergy with antibiotics in sepsis (Alcayaga-Miranda et al., 2015). MSCs also exerted anti-bacterial activity through the secretion of growth factors, especially Keratinocyte Growth Factor (KGF) (Lee et al., 2013). In the research performed by Lee et al. (2013) BM-MSCs improved alveolar fluid bacterial clearance and mitigated inflammation in an *E. coli* infection model in an *ex vivo* perfused human lung (Lee et al., 2013).

The application of stem cells for the treatment of viral infections has started recently. Studies on the use of stem cells in the treatment of viral diseases are mainly related to MSCs. There are several clinical trials on the use of MSCs in the treatment of viral infections (Sleem and Saleh, 2020). These trials have been mainly focused on COVID-19 (Leng et al., 2020; Zhang et al., 2020), Human Immunodeficiency Virus (HIV) (Zhang et al., 2013), and hepatitis B virus (Lin et al., 2017; Chen B et al., 2018a). During the COVID-19 crisis, stem cells were introduced as the most promising treatment option (Chrzanowski et al., 2020). A recent clinical trial also indicated that MSCs improved COVID-19 patients' outcomes. This improvement was dependent on the inhibition of the over-activation of the immune system. In fact, MSCs therapy decreased C-reactive protein and increased peripheral lymphocytes and IL-10 (Leng et al., 2020). Furthermore, Zhang et al. designed a pilot study to evaluate the responses of difficult-to-treat HIV-1-infected patients to human umbilical cord MSCs therapy. This treatment resulted in an increase in circulating naive and central memory CD4 T-cell counts and a decrease in systemic immune activation and inflammation. Furthermore, HIV-1-specific IFN-γ and IL-2 production was restored in immune non-responders (Zhang et al., 2013). Moreover, peripheral infusion of Bone Marrow–derived MSCs (BM-MSCs) significantly improved the survival rate in patients with hepatitis B virus–related acute-on-chronic liver failure because of recovering the liver function and reducing the incidence of severe infections (Lin et al., 2017).

The efficiency of stem cells in the treatment of parasitic infections has been reported in animal models (Zhang et al., 2014). However, there are limited therapeutic methods in parasitic infections, and drug resistance is a challenging issue in long-term drug administration (Ouellette, 2001; Montazeri et al., 2018; Ertabaklar et al., 2020). Previous studies indicated that stem cells played an important role in the treatment or control of schistosomiasis (Miranda et al., 2020), malaria (Souza et al., 2015), Chagas disease (Silva et al., 2014), and hydatid cyst (Abo-Aziza et al., 2019). Recently, Miranda et al. (2020) reported that AD-MSCs could decrease liver damage in schistosomiasis through controlling the granulomatous reaction. On the other hand, stem cells have been introduced as a new therapeutic option for malaria (Wang et al., 2015a). In this regard, BM-MSCs reduced mortality in infected mice. The results also revealed the reduction of parasitemia and morphological and functional improvement in vital organs (Souza et al., 2015). Furthermore, the effect of MSCs on protective immune responses was proposed in malaria-infected mice. Based on the results, these cells increased the production of IL-12, suppressed IL-10 production, and reduced regulatory T cells (Thakur et al., 2013). Moreover, the potentiality of stem cells was confirmed against myocarditis in Chagas disease. The findings showed that receiving cardiac MSCs attenuated myocarditis in a model of chronic Chagasic cardiomyopathy, but did not decrease fibrosis (Silva et al., 2014). Additionally, the combination of BM-MSCs transplantation with albendazole was effective in the modulation of humeral and cell-mediated immune responses against hydatid cyst antigens in experimentally infected rats (Abo-Aziza et al., 2019).

There are limited reports on the anti-fungal activity of stem cells. In a mouse model of severe refractory neutrophilic asthma, administration of BM-MSCs mitigated inflammation and improved the diseases induced by *Aspergillus via* Th17 inhibition. Moreover, a recent study indicated the anti-fungal activity of human uterine cervical stem cells conditioned medium against different species of Candida (Schneider et al., 2018).

THE APPLICATION OF STEM CELL-DERIVED EXOSOMES IN MICROBIAL DISEASES

An extensive body of evidence has indicated that a variety of cells including stem cells release exosomes and exert therapeutic properties in viral, bacterial, parasitic, and fungal infections, which will be discussed below.

THE APPLICATION OF STEM CELL-DERIVED EXOSOMES IN BACTERIAL DISEASES

Bacterial infections are a major public health problem, and the enhanced antibiotic resistance of bacteria requires finding new therapeutic options (Monsarrat et al., 2019). The anti-bacterial effects of stem cell-Exo and MVs have been investigated in different bacterial diseases, especially respiratory failure (Zhu et al., 2014). One of the main causes of respiratory failure is Acute Lung Injury (ALI) that is mainly induced by bacterial pneumonia. Studies have demonstrated that stem cell-Exo have the potential to reduce the severity of bacterial pneumonia. However, little is known regarding the underlying mechanisms of their anti-microbial activity. Zhu et al. (2014) disclosed that

BM-MSC-derived MVs were as effective as their parent cells in improving survival, restoring lung protein permeability, and reducing inflammation in an *E. coli* endotoxin-induced ALI mouse model. In fact, the administration of MVs decreased extravascular lung water, total protein level, and influx of neutrophils in Bronchoalveolar Lavage Fluid (BALF), indicating mitigation in pulmonary edema, lung protein permeability, and inflammation. Moreover, the anti-bacterial effect of MVs was in part through the transfer of KGF mRNA into the injured alveolus, which was eliminated after the administration of MVs derived from KGF siRNA-pre-treatment of BM-MSCs. KGF was known as a paracrine factor secreted by human MSCs and was previously revealed to restore alveolar fluid clearance (Abreu et al., 2016). This suggested the direct anti-bacterial activity of vesicles inherited from parent cells. In the same line, Monsel et al. (2015) reported that the administration of human MSC-derived MVs in an *E. coli* pneumonia mouse model resulted in a higher bacterial clearance, which was in part due to the increased monocyte phagocytosis. Moreover, survival improved partly through KGF secretion. The results also showed that the pre-stimulation of MSCs with a Toll like Receptor-3 (TLR-3) agonist could lead to the release of more effective MVs and further enhancement of bacteria's monocyte phagocytosis. It has been revealed that the binding and uptake of MSC-MVs into human monocytes and injured alveolar epithelial cells were mediated *via* the CD44 receptor on the mentioned target cells, which was necessary for their therapeutic effects. In addition, MVs enhanced intracellular ATP levels in injured alveolar epithelial cells and reduced the secretion of inflammatory cytokines including Tumor Necrosis Factor-alpha (TNF-α) in human monocytes, suggesting the metabolomics and immunomodulatory effects of MVs derived from MSCs. Interestingly, MSC-MVs expressed Cyclooxygenase2 (COX2) mRNA. COX2 is the key enzyme in Prostaglandin E2 (PGE2) synthesis that is an essential factor for transforming the polarization of monocyte-macrophage M1 into M2 phenotype. It was suggested that the increment in PGE2 secretion by monocytes following the transfer of COX2 mRNA from MSC-MVs to these cells caused the phenotype switch toward an anti-inflammatory state. Thus, it could be suggested that MSC-MVs mitigated lung inflammation, cytokine permeability, and bacterial growth and improved survival directly through KGF transfer or indirectly *via* activating monocytes. This therapeutic effect of MVs was abrogated by KFG neutralizing antibody, proposing a possible mechanism for the anti-bacterial effect of MSC-MVs (Monsel et al., 2015; Abreu et al., 2016). Since the anti-bacterial effect of KGF was previously reported in MSCs (Lee et al., 2013), these studies supported the hypothesis that MVs conserve the anti-microbial effects of parent cells partly through their growth factors content including KGF.

In addition to the beneficial effects of stem cell-Exo in a mouse model of ALI, Park et al. (2019) recently evaluated the therapeutic effects of BM-MSC-MVs on *ex vivo* perfused human lungs with severe pneumonia induced by *Escherichia coli* that resulted in the significant enhancement of alveolar fluid clearance, reduction of lung protein permeability leading to lower bacterial load, and decrement of the median pulmonary artery pressure. The anti-microbial activity of human BM-MSC-MVs could be further increased by the pre-treatment of MSCs with a TLR-3 agonist, Poly (I:C), before the isolation of MVs, which led to lower neutrophils infiltration in the injured lung. Additionally, isolated human alveolar macrophages increased anti-microbial activity with MSC-MVs treatment *in vitro*, which resulted in the enhancement of bacterial clearance in the injured lung (Park et al., 2019; Al-Khawaga and Abdelalim, 2020). A noteworthy point in the studies carried out by Monsel et al. (2015) and Park et al. (2019) was the preconditioning of MSCs with a TLR-3 agonist, Poly (I:C). Further studies showed that the pre-stimulation of parent MSCs with poly (I:C) could increase the anti-microbial and immunomodulatory proteomic profile of EVs (Mayo et al., 2019; Pierce and Kurata, 2021). They also indicated various AMPs in MSC-EVs including dermcidin, lactoferrin, lipocalin 1, lysozyme C, neutrophil defensin 1, S100A7 (psoriasin), S100A8/A9 (calprotectin), and histone H4. Several AMPs helped fight against various bacteria, fungi, and viruses (Pierce and Kurata, 2021). However, these AMPs remained unaltered by poly (I:C) pre-stimulation. Up to now, no study has been performed on the exact effect of AMPs through transfer with stem cell-Exo, which requires special attention in future. Furthermore, it should be noted that although many studies have dealt with MVs based on the separation method (Zhu et al., 2014; Monsel et al., 2015; Park et al., 2019), they have actually isolated a combination of MVs and exosomes.

Immunomodulatory and immunostimulatory properties of stem cell-Exo partly depend on functional miRNAs by exosomes. Hao et al. (2019) investigated the effects of human MSC-Exo on *Escherichia coli* pneumonia-induced acute lung injury in C57BL/6 mice. They found that exosomes administration was associated with high levels of Leukotriene (LT) B4 and improvement of bacteria clearance in the injured alveolus. It has been found that LTB4 augmented phagocytosis and the release of anti-microbial agents and increased host defense against pneumonia and sepsis. Production of LTB4 was suppressed by an ATP-binding cassette transporter called Multidrug Resistance–Associated Protein 1 (MRP1). The underlying mechanism of the anti-microbial activity of MSC-Exo was through the inhibition of MRP1 expression partly *via* the transfer of miR-145, which resulted in increased LTB4 production that led to the enhancement of bacterial phagocytosis through LTB4/BLT1 signaling. Previous studies indicated that miR-145 was one of the top 10 most abundant miRNAs detected in MSCs and MSC-Exo, which could directly inhibit MRP1 expression in breast and gallbladder cancers (Hao et al., 2019).

The use of stem cell-Exo for reducing the complications caused by bacteria seems to be attractive. Sepsis is known as a serious and life-threatening condition with high morbidity and mortality, which increases when the host body's response to infections including bacterial infections causes injury to its own organs (Cheng et al., 2020). Interleukin-1b (IL-1b), as a serious pro-inflammatory cytokine, increases in the early stage of sepsis and is involved in the severity and evolution of organ dysfunction. In the study conducted by Song et al. (2017) BM-MSCs were pre-stimulated by IL-1b prior to the isolation of exosomes. Then, the effect of these exosomes was investigated in a cecal ligation and puncture-induced mouse model of

sepsis. The results showed that IL-1b enhanced the therapeutic effect of MSCs-Exo against sepsis by inducing macrophage polarization to an anti-inflammatory M2 phenotype (Song et al., 2017). The results also revealed that exosomes derived from MSCs contained high levels of miR-146a, which is a well-known anti-inflammatory microRNA. Transfer of miR-146a by exosomes to recipient macrophages regulated M1-M2 transition, reduced inflammation, and enhanced survival in septic mice. In addition, transfection of miR-146a inhibitors partially abrogated the immunomodulatory properties of exosomes. Overall, IL-1b pre-stimulation effectively increased the immuno-modulatory properties of MSCs partially through the exosome-mediated transfer of miR-146a (Song et al., 2017; Cheng et al., 2020). All in all, exosomes derived from stem cells had the anti-bacterial capacity against intracellular bacterial infections. It could be a proof-of-principle that therapeutic approaches based on exosomes derived from MSCs offer a promising path forward.

Research on the administration of exosomes for drug delivery, particularly antibiotics, is still in its initial steps. In the study carried out by Yang et al. (2018) exosomes were isolated form RAW 264.7 mice macrophages and were incubated with an antibiotic agent called linezolid. They evaluated the effect of linezolid-exosomes on Methicillin-Resistant *Staphylococcus aureus* (MRSA)-infected macrophages in a mouse model of MRSA. Briefly, *Staphylococcus aureus* lives inside phagocytes and is a strain with antibiotic resistance, which can lead to sepsis, infective endocarditis, osteomyelitis, and necrotizing pneumonia. The results showed that the delivery of exosome-encapsulated antibiotics was more effective against intracellular MRSA infections compared to free linezolid antibiotics (Yang et al., 2018). In this regard, exosomes derived from stem cells and immune cells had the capacity for delivery of anti-microbial agents against intracellular pathogen infections. Yet, further studies are required for clinical applications.

THE APPLICATION OF STEM CELL-DERIVED EXOSOMES IN VIRAL DISEASES

Some contents in exosomes derived from stem cells can play substantial anti-viral roles by inhibiting viral replication and inducing immune responses (Longatti, 2015). Clinical trials have shown that the exosomes released from different cells can be novel therapeutic strategies against viruses including hepatitis, HIV, and COVID-19. To the best of our knowledge, there are limited studies regarding the application of stem cell-Exo in viral diseases. Qian et al. (2016) investigated the effects of the secreted exosomes from umbilical-MSCs on hepatitis C virus infection *in vitro*. The results indicated that a profile of miRNAs in the exosomes including let-7f, miR-145, miR-199a, and miR-221 was involved in the direct suppression of the RNA replication of hepatitis C virus (Qian et al., 2016).

Exosomes also play important roles in the interplay between the virus and various immune cells in hepatitis viruses. In particular, virus-infected cells release exosomes that affect the host immune system. An *in vitro* study on hepatitis C virus infection showed that macrophages' exosomes contained miR-29 family members that exerted anti-viral effects on Huh7 cells (Zhou et al., 2016). Kouwaki et al. (2016) also reported that hepatitis B virus-infected hepatocytes released exosomes containing viral nucleic acid, which activated the innate immune response. They found that the microRNA levels of miR-21 and miR-29a increased in the exosomes of the infected hepatocytes that stimulated macrophages (Kouwaki et al., 2016). Furthermore, the previous studies emphasized that miR-21 was enriched in exosomes derived from BM-MSCs (Shi et al., 2018) and human umbilical cord MSCs (Chen et al., 2020). The presence of miR-29a was detected in BM-MSCs, as well (Lu et al., 2020; Tan et al., 2020). Therefore, the exosomes derived from such sources may be effective against hepatitis viruses.

Sims et al. (2014) described the role of neural stem cell-Exo in cellular viral entry. The findings showed that the exosomes contained T-cell immunoglobulin mucin protein 4, which acted as a phosphatidylserine receptor and mediated adenovirus type 5 entries. Clarifying the virus/exosome pathways and exosome trafficking may provide a potentially therapeutic option (Sims et al., 2014).

Recent studies have proposed the anti-HIV activity of exosomes (Yong et al., 2018). It has been conducted on the application of exosome-containing miRNAs in the treatment of HIV (Yong et al., 2018). In this context, a variety of miRNAs including miR-28, miR-150, miR-223, miR-382 (Wang et al., 2009), miR-29a, miR-29b, miR-149, miR-324, miR-378 (Hariharan et al., 2005), miR-125b (Mantri et al., 2012), and miR-198 (Sung and Rice, 2009) have been explored in the host exosomes involved in HIV therapy (Madison and Okeoma, 2015). Several studies proposed the presence of the mentioned miRANs in the exosomes derived from different sources of stem cells. Accordingly, the exosomes derived from BM-MSCs contained miR-29a (Lu et al., 2020; Tan et al., 2020), miR-150 (Qiu et al., 2021; Wu et al., 2021), miR-223 (Chen L et al., 2018b), miR-29a (Su et al., 2019), and miR-125b (Wang et al., 2019). miR-223 was also identified in the exosomes derived from umbilical cord MSCs (Wei et al., 2020; Liu et al., 2021). These findings indicated that MSCs could be considered in anti-HIV strategies. Recent studies on the treatment of viruses also indicated the potentiality of exosomes for encapsulating bioactive molecules in drug delivery systems. Therefore, transferring anti-HIV RNAs through artificial exosomes or exosomes derived from stem cells may be promising in the HIV treatment.

Several studies have demonstrated the beneficial effects of exosomes from stem cells on the treatment of respiratory viruses (Popowski et al., 2021). During the COVID-19 pandemic, researchers focused on the application of stem cell-Exo, as a treatment option. Considering the beneficial effects of stem cell-Exo on the management of cytokine storm, tissue repair, and viral suppression, exosomes may be considered a promising therapeutic option. A prospective non-randomized open-label cohort study proposed the safety and efficacy of exosomes derived from allogeneic BM-MSCs in severe COVID-19. This study revealed that these exosomes attenuated cytokine storm, recovered oxygenation, and improved immunity cell counts (Sengupta et al., 2020). Generally, the Receptor-Binding Domain (RBD) of the SARS-CoV-2 spike protein recognizes the Angiotensin-Converting Enzyme 2 (ACE2) receptor to enter

host cells. The application of exosomes that effectively bind to SARS-CoV-2 may prevent the virus from entering the cells. Interestingly, a previous study revealed that the exosomes expressing ACE2 dose-dependently prevented the binding of the RBD of the SARS-CoV-2 spike protein to ACE2 + cells (El-Shennawy et al., 2020). Hence, engineering of stem cell-Exo to overexpress ACE2 may competitively block the binding of SARS-CoV-2 to ACE2-expressing cells (Inal, 2020). In addition, stem cell-Exo contributed to organ regeneration and repair (Basu and Ludlow, 2016; Maqsood et al., 2020). Therefore, the tissue and organ destruction occurring in COVID-19 may be improved by using exosomes. Suppression of cytokine storm is yet another issue in COVID-19 management. The immunomodulatory function of MSC-Exo has made them a potential therapeutic option for cytokine storm. These exosomes decrease proinflammatory cytokines (Liang et al., 2020), inhibit $CD4^+$ and $CD8^+$ T cells (Taechangam et al., 2019), reduce the proliferation and activation of NK cells (Moloudizargari et al., 2021), and improve the release of IL-4, IL-10, and TGF-β (Jayaramayya et al., 2020). Based on these pieces of evidence, the anti-viral properties of the exosomes released from stem cells are related to their key molecules. These molecules may disturb the virus survival or inhibit the side effects caused by them.

THE APPLICATION OF STEM CELL-DERIVED EXOSOMES IN FUNGAL AND PARASITIC DISEASES

Similar to the studies performed on viruses, limited data are available for identifying the effects of stem cell-Exo on human fungal infections. Only one study conducted by Cruz et al. (2015) demonstrated that the systemic administration of exosomes derived from human BM-MSCs improved allergic airway inflammation induced by *Aspergillus* hyphal extract in an immunocompetent mouse model of severe refractory neutrophilic asthma (Cruz et al., 2015). Hence, research on the impact of stem cell-Exo on fungal diseases is still in its infancy, and further investigations are necessary.

Despite of distinct properties of stem cell-Exo, no evidence is available regarding the therapeutic application of stem cell-Exo in the context of parasite infections. There are more than 1 billion cases of parasitic diseases in the world including malaria (Murray et al., 2014) and neglected tropical diseases such as helminthiases, Chagas disease, and leishmaniosis (Coakley et al., 2015), with an increasing prevalence in developing regions such as Eastern Asia, Sub-Saharan Africa, and the Americas (Lustigman et al., 2012). Hence, attention has to be paid to the potential of exosomes as a biomarker and therapeutic agent in parasite diseases.

ADVANTAGES AND CHALLENGES IN THE USE OF STEM CELL-DERIVED EXOSOMES

Application of stem cells in the treatment of different human diseases, especially microbial infections, has shown effective outcomes. Nevertheless, there are still safety concerns like lower survival after transplantation as well as the possibility of pulmonary embolism, tumorginicity, and uncontrolled differentiation. Yet, stem cell-Exo are highly stable due to biolayer lipid membrane, small size, low immunogenicity, easy storage at −80°C for a long time without toxic agents, and easier procedure for delivery and management (Keshtkar et al., 2018). Like parent cells, exosomes have immunomodulatory and immunosuppressive properties that enable them to participate in various disease models. Thus, stem cell-Exo represent an alternative to stem cell therapies, with no safety issues regarding regenerative medicine.

Mesenchymal Stem Cell-derived Exosomes have been reported to decrease the side effects of cell therapy (Malekpour et al., 2021). Therefore, they can be a good platform for various applications in the treatment of various diseases. For instance, manipulation of exosomes by loading therapeutic compounds as well as transferring interfering RNA, miRNA, and oligonucleotides enhances their efficiency (Zhang et al., 2021). In the study by Melzer et al. (2019) evaluated the loading of compound paclitaxel into MSC-Exo. They showed that manipulated MSC-Exo notably decreased the breast tumor volume and suppressed the metastasis compared to MSC-Exo alone (Melzer et al., 2019). The biocompatibility potential of exosomes also makes them an ideal candidate for drug delivery like antibiotics (Huang and Lai, 2019). In the study carried out by Yang et al. (2018) exosomes were isolated form RAW 264.7 mice macrophages and were incubated with an antibiotic agent called linezolid. They evaluated the effect of linezolid-exosomes on Methicillin-Resistant *Staphylococcus aureus* (MRSA)-infected macrophages in a mouse model of MRSA. Briefly, *S. aureus* lives inside phagocytes and is a strain with antibiotic resistance, which can lead to sepsis, infective endocarditis, osteomyelitis, and necrotizing pneumonia. The results showed that the delivery of exosome-encapsulated antibiotics was more effective against intracellular MRSA infections compared to free linezolid antibiotics (Yang et al., 2018). Hence, they have been nominated as ideal vehicles for therapeutic applications. Despite the aforementioned advantages, research on development and treatment based on stem cell-Exo is still in its infancy. There are also some hurdles that must be overcome prior to translation from bench to bed. These include the lack of standard isolation and purification methods for exosomes, lack of complete information about the exact cargos of these vesicles, and existence of heterogeneity in released vesicles as a result of physiological changes in the cells' extracellular space (Huang and Lai, 2019; Brakhage et al., 2021).

CONCLUSION

Extracellular Vesicles, especially exosomes, secreted by stem cells have the same anti-microbial potential and immunomodulatory ability as their parent cells. Hence, clinical applications of stem cell-Exo can possibly overcome the shortage of stem cells for the treatment of microbial and other infectious diseases and, at the same time, affect the field of novel medicine from cellular to acellular therapy. Both intact and engineered exosomes have been applied and their therapeutic effects on various infectious diseases

have been demonstrated in preclinical studies and limited clinical trials. Exosomes perform a part of their antimicrobial activity through the direct transfer of mRNA, miRNA, and protein cargos, while their beneficial effects are mostly applied indirectly through the reprogramming of immune cells and the activation of innate and adaptive immune responses.

Although the underlying mechanism of stem cell-Exo has not been specified exactly and completely, the anti-microbial activity of exosomes appears to be more indirect than direct.

Moreover, many barriers are still needed to be eliminated prior to the application of stem cell-Exo as anti-microbial agents in clinical settings.

AUTHOR CONTRIBUTIONS

All authors took part in literature search, manuscript preparation, and editing.

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