REVIEW

Prokaryotic microvesicles Ortholog of eukaryotic extracellular vesicles in biomedical fields

Halimeh Mobarak[1], Farzin Javid[1], Maryam Taghavi Narmi[1], Narges Mardi[2], Fatemeh Sadeghsoltani[3], Parisa Khanicheragh[4], Samaneh Narimani[5], Mahdi Mahdipour[5], Emel Sokullu[6], Ferzane Valioglu[7] and Reza Rahbarghazi[1,5*]

Abstract

Every single cell can communicate with other cells in a paracrine manner via the production of nano-sized extracellular vesicles. This phenomenon is conserved between prokaryotic and eukaryotic cells. In eukaryotic cells, exosomes (Exos) are the main inter-cellular bioshuttles with the potential to carry different signaling molecules. Likewise, bacteria can produce and release Exo-like particles, namely microvesicles (MVs) into the extracellular matrix. Bacterial MVs function with diverse biological properties and are at the center of attention due to their inherent therapeutic properties. Here, in this review article, the comparable biological properties between the eukaryotic Exos and bacterial MVs were highlighted in terms of biomedical application.

Keywords Exosomes, Bacterial microvesicles, Prokaryotic cells, Eukaryotic cells, Biological properties, Biomedical applications

Introduction

In the course of the evolution process, numerous biological mechanisms have been created to help prokaryotes and eukaryotes survive by using inter- and intra-species interaction [1]. During the last decades, the critical role of extracellular vesicles (EVs) has been proved in reciprocal cell–to–cell communication in a paracrine manner [2–4]. From the ultrastructural aspect, the term EVs encompasses heterogeneous bilayered nano-sized vesicles with the potential to carry numerous signaling molecules to promote synchronous multicellular dynamic growth and function [5, 6]. The production and release of EVs are thought to be a conserved biological phenomenon almost in all types of eukaryotic and prokaryotic (Gram-negative, and Gram-positive bacteria) cells [1, 7, 8].

Almost all types of unicellular structures such as archaea, bacteria, viruses, fungi, and parasites can produce MVs and release them to the microenvironment [9–22]. Membrane vesicles (MVs) are heterogenic in terms of morphology, size, and cargo type similar to eukaryotic EVs (Fig. 1, Table 1) [7, 23–25]. MVs structure in pathogenic and non-pathogenic Gram-negative

*Correspondence:
Reza Rahbarghazi
Rezarahbardvm@gmail.com; rahbarghazir@tbzmed.ac.ir
[1] Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran
[2] Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[3] Department of Clinical Biochemistry and Laboratory Medicine, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[4] Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
[5] Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[6] Biophysics Department, Koç University School of Medicine, Rumeli Feneri, 34450, Sariyer, Istanbul, Turkey
[7] Technology Development Zones Management CO, Sakarya University, Sakarya, Turkey

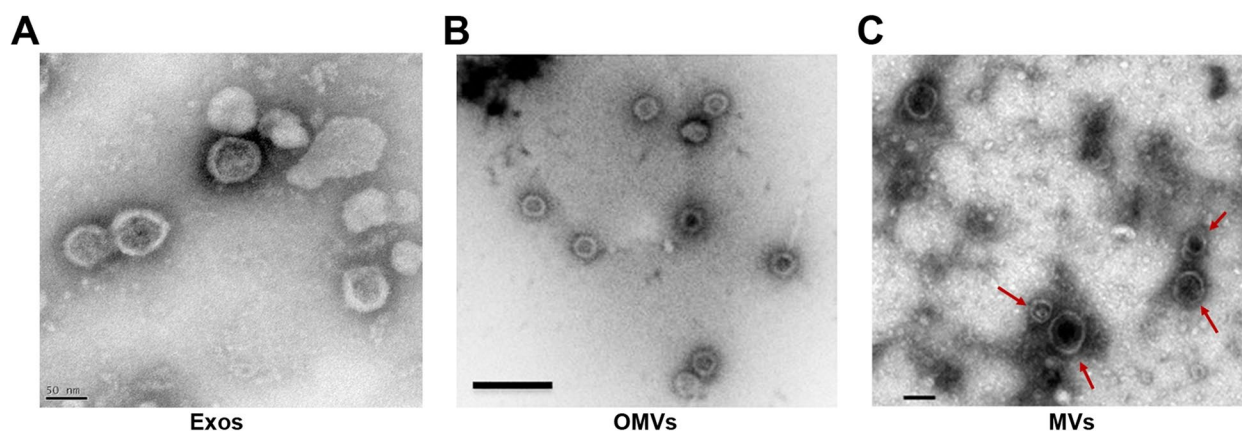Mobarak *et al. Cell Communication and Signaling* (2024) 22:80

Page 2 of 19

Fig. 1 Similarities between eukaryotic and prokaryotic EVs (**A**-**C**). Ultrastructural analysis of human bone marrow mesenchymal stem cell Exos (**A**). Images revealed cup-shaped and nearly round morphology in isolated Exos [30]. (Copyright 2021, Frontiers in Cell and Developmental Biology). TEM images of *Capnocytophaga ochracea* OMVs at stationary phase after exposure to pH value of 5.1 (**B**: Scale bar: 200 nm) [31]. (Copyright 2021, Frontiers in Microbiology). MVs (red arrows) were isolated by ultracentrifugation from *Staphylococcus aureus* cultured in Tryptic Soy Broth medium (**C**: Scale bar: 100 nm) [32]. (Copyright 2018, Nature Communications)

Table 1 Comparison between Eukaryotes, Prokaryotes, and Archaea EVs

Domains of life		Origin	Diameter (nm)	Terms
Eukaryotes	Mammalian cells	Apoptosis process: with origin cell membrane and components	1000–5000	Apoptotic bodies (ABs)
		The outward budding of the cell membrane	100–1000	Microvesicles, Extracellular membrane vesicles, Microparticles, Ectosomes, Exovesicles
		Endosomal origin: early and late endosome for intraluminal vesicles (ILVs) formation then Multivesicular Bodies (MVB) fusion with the cell membrane	30–150	Exosomes (Exos)
Prokaryotes	Gram-negative bacteria	Outer membrane	10–500	Outer membrane vesicles (OMVs)
	Gram-positive bacteria	Cytoplasmic membrane	20–400	Membrane vesicles (MVs)
Archaea	Archaea	Cytoplasmic membrane	50–230	Membrane vesicles (MVs)

bacteria, known also as outer membrane vesicles (OMVs), are nano-sized and originate from the cells' outer membrane (OM). Whereas, Gram-positive bacteria-derived MVs are released from the single cytoplasmic cell membrane surrounded by a peptidoglycan-rich cell wall [26]. It has been postulated that microorganism EVs contain virulent factors and pathogenic compounds that help in the progression of infection in the host cells. Besides, microorganism EVs are involved in the regulation of the immune system, and the neutralization of antibiotics and bacteriophages [27, 28]. To date, there is a gap in terms of microorganism EV impacts on multicellular systems and how and whether these EVs can regulate the function of eukaryotic cells under physiological and pathological conditions. The most of previously conducted studies have been limited to the evaluation of eukaryotic EVs, especially humans and other species. It is thought that focusing on microorganism EVs and their interaction with eukaryotic systems can give us an insightful vision of biological phenomena that occur following the activation of single-celled organisms inside the metazoan niche [7, 8, 29]. The existence of paracrine interaction between the cells in multicellular (metazoan) and unicellular creatures via EVs highlights the fact that the vesicular transmission of signaling molecules is a conserved biological phenomenon. It is recommended biologists evaluate ultrastructural and cytochemical features and biogenesis signaling pathways of eukaryotic and prokaryotic EVs. Here, in this review article, we tried to scrutinize the distinct properties of prokaryotic EVs and their physiological roles in comparison with eukaryotic EV counterparts.

Eukaryotic EVs

In eukaryotes, three types of EVs have been identified based on biogenesis, content, and size of vesicles. Among EVs, apoptotic bodies exhibit an average diameter of 1000 to 5000 nm and are produced during the activation of the apoptotic process via the disassembly of the cell membrane. Microvesicles, also known as microparticles, ectosomes, and exovesicles, range between 100 to 1000 nm and are generated by the evagination of cell membranes under physiological and pathological conditions in response to diverse stimuli. The last and smallest EV type is named exosomes (Exos) with an average diameter between 30 and 150 nm. Exos are produced by the activity of the endosomal system and released to the extracellular matrix (ECM) after the fusion of endosomes with the cell membrane (Fig. 2). Upon the invagination of the cell membrane, early endosomes are generated with numerous Exos entering the host cells. Molecular investigations have revealed that early endosomes can be directed toward the Golgi apparatus and/or lysosomal degradation. In an alternative pathway, early endosomes mature into late endosomes where numerous intraluminal vesicles (ILVs) are generated via the invagination of the endosomal membrane. The phenomenon is continued by the maturation of late endosomes toward multivesicular bodies (MVBs) where these vesicles can commit lysosomal degradation or fuse with the cell membrane to release luminal cargo of ILVs into ECM, hereafter called Exos. To date, the isolation and purification of EV types is one of the most challenging issues in biomedical fields. Practically, the isolation and purification of the EV subpopulation are not possible because of overlapping density, diameter size, cargo type, and even lack of definite markers [3, 33, 34].

Molecular investigations have revealed the existence of specific proteome [growth factors, cytokines, proteins, and enzymes], transcriptome [mRNAs, microRNAs], and lipid contents in the lumen of eukaryotic EVs [36, 37]. Besides their roles in mutual cell-to-cell interaction under different conditions, EVs are thought as valid diagnostic tools for the detection and monitoring of certain pathologies. Using engineering modalities, EVs can
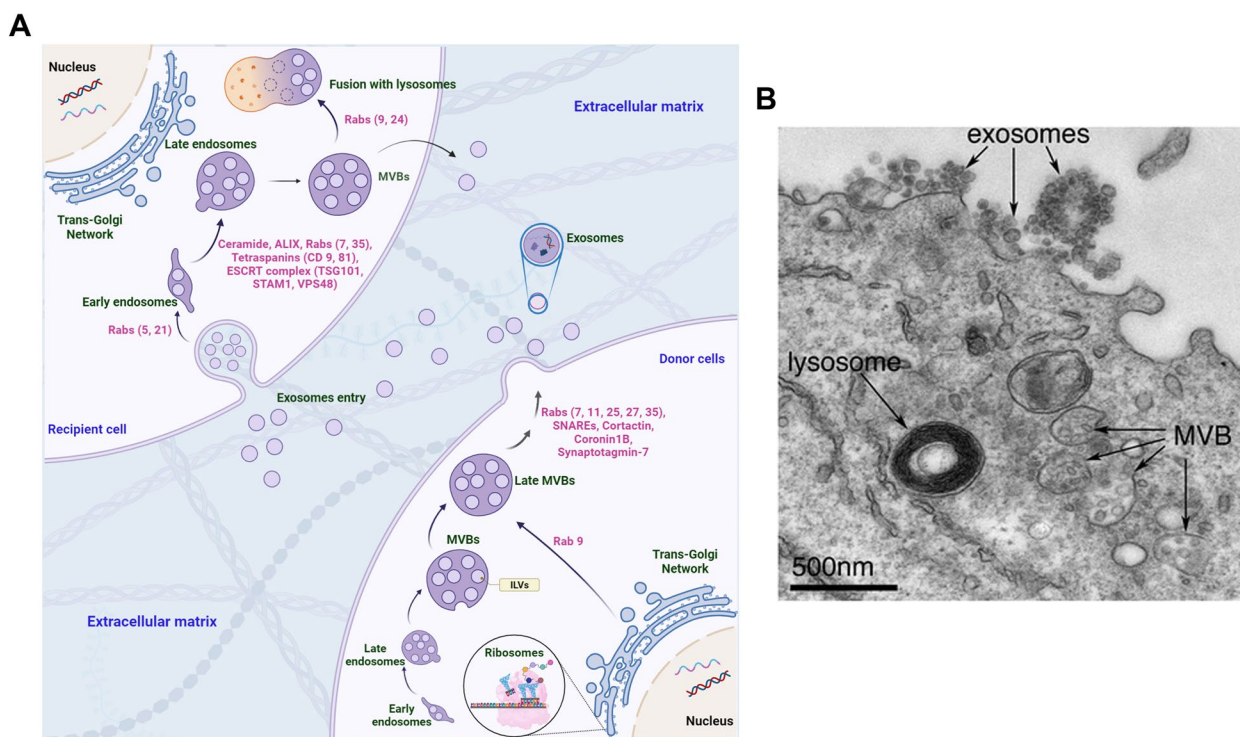
Fig. 2 Exosome (Exo) biogenesis pathway (**A**). Exos are generated and released using the endosomal system. In the recipient cells, internalized Exos are sequestrated inside the early endosomes followed by maturation into the mature endosomes. Inside the mature endosomes, numerous intraluminal vesicles (ILVs) are generated via the invagination of the endosomal membrane. In this step, several signaling molecules are sequestered into the ILV lumen. After that, late endosomes can mature into MVBs. In the following steps, MVBs can fuse with lysosomes for content degradation or make close connections with cell membranes for the release of ILVs into the extracellular matrix where they hereafter are known as Exos. Ultrastructural images of B lymphocyte with expelled Exos at the plasma membrane. MVBs can be directed toward lysosomal degradation or release their content into the extracellular matrix. Released ILVs (black arrows) are named Exos out of the parent cells. Reprinted adapted from [35]. (Copyright 2016, BMC Biology)

Mobarak *et al. Cell Communication and Signaling*   (2024) 22:80

Page 4 of 19

be used for the on-target delivery of therapeutics such as drugs, genes, and certain immunogens with promising therapeutic outcomes [38, 39]. It was suggested that EVs can be actively involved in the progression of distinct pathological conditions such as anaplastic changes, neurodegenerative, infectious cardiovascular diseases, and senile changes [7, 40, 41].

Bacterial EVs

As abovementioned, bacteria can also secrete nano-sized EVs or MVs which are heterogeneous based on size, density, amount, and cargo component within the same species. It was suggested that the growth phase, niche condition, and several external stimuli can affect the quality of MV production in bacteria [28, 42, 43]. Noteworthy, the heterogeneity is related to engaging different biogenesis pathways, membrane structure of the parent bacteria, growth conditions, and genetic traits [44]. Gram-negative bacteria possess an OM harboring lipopolysaccharide (LPS), and a thin periplasmic peptidoglycan layer in the periplasmic space (Fig. 3). The peptidoglycan composites, known also mureins, are located between the outer and inner membranes [45]. These bacteria also contain a hard biopolymer peptidoglycan in the cell wall which is involved in the production of EVs. Along with these comments, Gram-negative bacteria OMVs have a large amount of LPS, proteins (cytoplasmic,

periplasmic, and membrane-bound), outer membrane lipids, virulence factors, immunomodulatory factors, ribonucleic acids, toxins, and other pathogen-associated molecular patterns (PAMPs) (Fig. 3 and Table 2) [1, 24, 28, 46–49]. It is speculated that the introduction of MV PAMP contents to host pattern recognition receptors (PRR) in immune/non-immune cells results in immune tolerance, pathological conditions, and protective immunity [44]. Some of the PRRs located in the intestinal epithelial cells contain the cytoplasmic nucleotide-binding oligomerization domain (NOD) and transmembrane Toll-like receptors (TLRs). Either TLR or NOD-like receptor (NLR) families are stimulated in response to bacterial EVs with crucial roles in OMV/MV-mediated pathologies [26].

The existence of OMVs was first indicated in the Gram-negative bacterium (*Escherichia coli*) in 1966. After that, OMVs were detected in patients' cerebrospinal fluid with meningococcal disease. Later, the Gram-negative pathogens OMVs originated from specific species such as *Neisseria meningitides, Helicobacter pylori*, and *Haemophilus influenza* were confirmed [26]. Microorganism EVs produced by other prokaryotes such as Gram-positive bacteria, and other archaea are called MVs [89].

In Gram-positive bacteria, a thick layer of peptidoglycan surrounds the cell membrane with no outer membrane [90]. Some phyla such as Mycobacteria, and
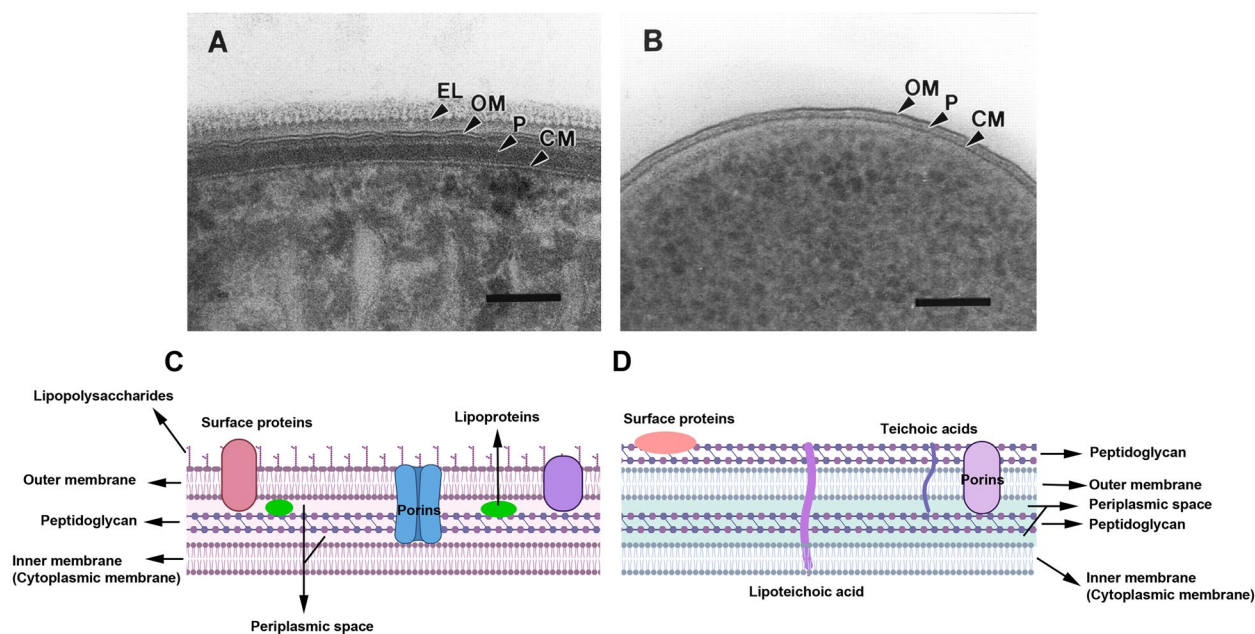
Fig. 3 Ultrastructural TEM images of cryo-sectioned Gram-negative cell envelopes. **A**: *Cyanobacterium Phormidium uncinatum* and **B**: *Escherichia coli. Cyanobacterium Phormidium uncinatum* has a combination of Gram-positive and –Negative structures. Note the thick peptidoglycan layer and outer membrane. The external layer (EL) consists of S- layer and oscillin fibrils with serrated surface morphologies (Scale bar: 100 nm). Reprinted adapted from [50]. (Copyright 2000, Journal of Bacteriology) [Cytoplasmic membrane: CM; outer membrane: OM; and Peptidoglycan: P]. Typical cell wall structure in Gram-negative (**C**) and Gram-positive bacteria (**D**)

Mobarak *et al. Cell Communication and Signaling*   (2024) 22:80

Page 5 of 19

Table 2 The function of MVs in Gram-negative/positive bacteria and archaea

Functions	Domain	Species	References
Delivery of toxic factors to eukaryotes/prokaryotes	Gram-negative bacteria	*Pseudomonas aeruginosa, Bordetella pertussis, Escherichia coli, Aggregatibacter actinomycetemcomitansm, Vibrio cholera, Pseudomonas fragi, Kingella kingae, Salmonella enterica pullorum, Pantoea agglomerans, Proteus vulgaris, Morganella morganii, Phyllobacterium trifolii*	[51–58]
Exchange of proteins (e.g., OM proteins (OmpA, OmpC, and OmpF), periplasmic proteins (alkaline phosphatase and AcrA), Hemin binding protein C and virulence factors (adhesins, invasins, and other enzymes) between cells	Gram-negative bacteria	*Neisseria meningitides, Escherichia coli, Gemmata obscuriglobus, Bartonella henselae*	[59–62]
Exchange of lipids (e.g., glycerophospholipids, phosphatidylethanolamine, phosphatidyl-glycerol, and cardiolipin) between cells	Gram-negative bacteria	*Escherichia coli, Pseudomonas syringae, Myxococcus xanthus*	[63–65]
	Gram-positive bacteria	*Bacillus anthracis, Staphylococcus aureus*	[66, 67]
Biofilm production	Gram-negative bacteria	*Pseudomonas aeruginosa, Helicobacter pylori*	[68–70]
Response to stress	Gram-negative bacteria	*Escherichia coli Salmonella typhimurium, Pseudomonas aeruginosa*	[71–73]
Transfer of genetic materials (DNA/RNA)	Gram-negative bacteria	*Pseudomonas aeruginosa, Escherichia coli, Neisseria gonorrhoeae, Borrelia burgdorferi, Haemophilus influenza, Moraxella osloensis, Salmonella typhimurium, Serratia marcescens,* Shigella spp. (*Shigella dysenteriae,* and *Shigella flexneri*), *Shewanella vesiculosa, Porphyromonas gingivalis, Yersinia pestis,* and *Vibrio cholerae*	[74–80]
	Gram-positive bacteria	*Ruminococcus* spp. *Mycobacteriaceae*	[81, 82]
	Archaea	Thermococcales (*Thermococcus kodakarensis*, and *Nautilia lithotrophica*)	[83, 84]
Promote antibiotics resistance	Gram-negative bacteria	*Porphyromonas gingivalis, Pseudomonas aeruginosa, Pseudomonas putida, Escherichia coli*	[85–88]

Actinobacteria, are completely different from Gram-positives and Gram-negatives. For instance, ultrastructural analyses have revealed that three main macromolecules – peptidoglycan, arabinogalactan (a highly branched polysaccharide), and mycolic acids (a specific lipid component in the cell wall), made the core structure of the mycobacterial cell wall. Because of the high density of lipids in their cell wall, they are also mentioned as acid-fast bacteria [91]. Gram-positive bacteria MVs originate from cell membranes with cytoplasm- and membrane-associated proteins and factors [89]. The existence of specific protein coating, namely the S layer, in Gram-positive and negative bacteria leads to physical interaction between the bacteria and the neighboring niche. Notably, it is suggested that the bacterial S layer can be eliminated after a prolonged culture period used for MV isolation [92].

As a common belief, most archaea species can constitutively produce MVs with an average size of 50–250 nm (Fig. 4). This phenomenon is initiated via the bulging of cell membranes using electron tomography and micrographs in *Sulfolobus* and *Thermococcales* species [83,

93]. It should not be neglected that MVs are not confined to extra-organism niches. These natural nanoparticles can also participate in intra-bacterial metabolism. For instance, numerous MVs have been identified in the periplasm space of Ignicoccus species. It is thought that these MVs play a key role in the transfer of several signaling molecules between the inner and OM. Of note, ultrastructural analyses have revealed the lack of an S layer in *Ignicoccus* species [94].

Like eukaryotic EVs, the production of MVs is constituent in different conditions. For example, the release of OMVs has been documented after in vitro microbiological cultures (biofilms, etc.) or in vivo expansion in biofluids (cerebrospinal fluid, and blood) and solid tissue samples (gastric tissue) [8, 95]. To resist the insulting conditions, most bacteria exhibit communities and attach to beneath a slim layer of biofilm composed of polysaccharides, proteins, and genetic elements. Emerging data have revealed the involvement of OMVs in the formation of biofilms [89]. *Pseudomonas aeruginosa* can prolong pulmonary infection after the production of biofilm [68]. Data confirmed that OMVs can promote the formation of
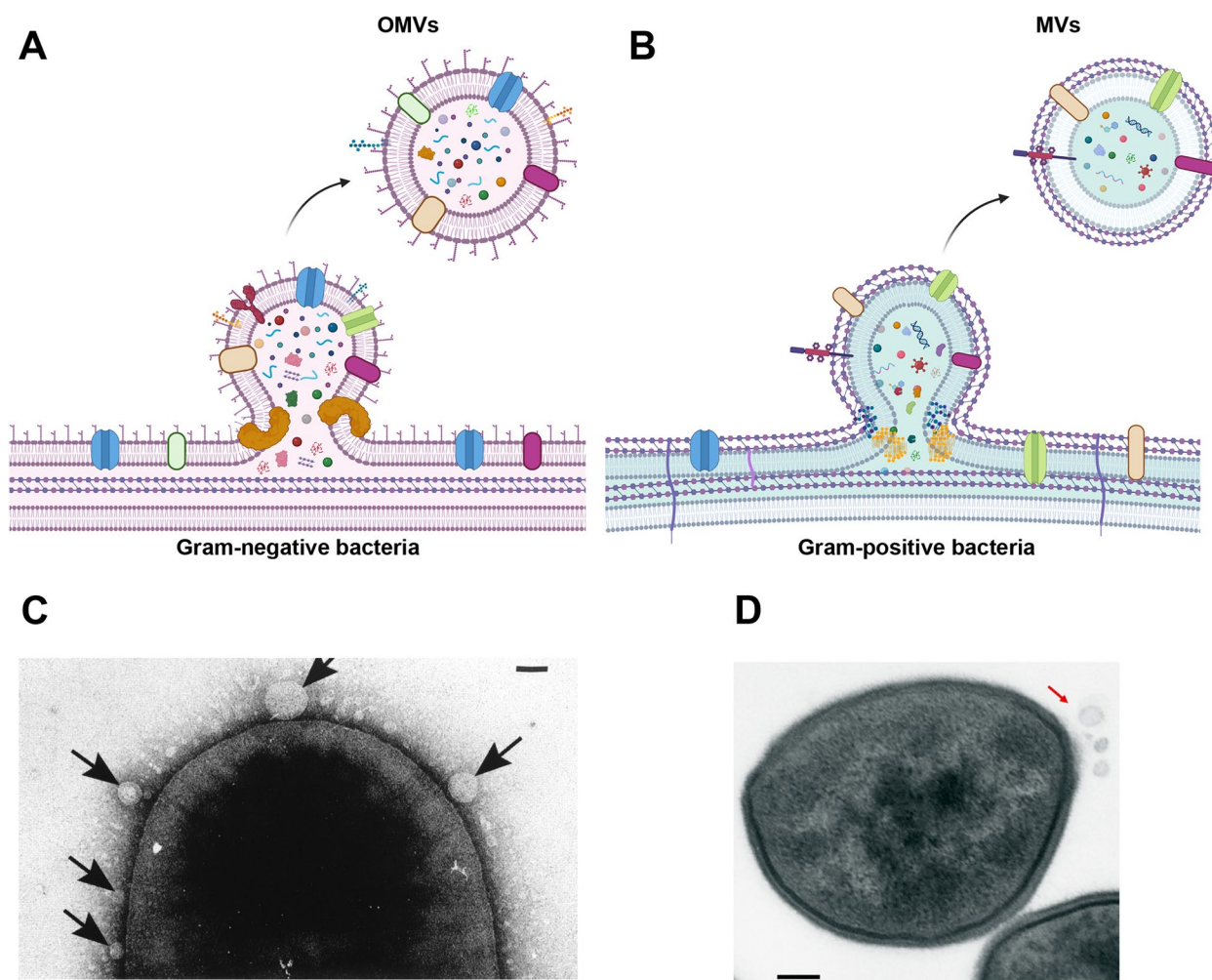
Fig. 4 Steps for the generation of OMVs and MVs in Gram-negative (**A**) and Gram-positive (**B**) bacteria, respectively. TEM image of production of MVs (black arrows) by Gram-negative bacterium *Serratia marcescens* (**C**). Samples were stained with 2% uranyl acetate solution and imaged at 60 kV (Scale bar: 100 nm). Reprinted adapted from [52]. (Copyright 1998, Journal of Bacteriology). Ultrastructural imaging revealed the formation of MVs (red arrows) on the cell wall of *Staphylococcus aureus* cultured in the Tryptic Soy Broth medium (Scale bar: 100 nm) [32]. (Copyright 2018, Nature Communications)

ECM by the transport of adherent substances and nutrients. In support of this notion, OMV-producing bacteria have robust biofilm generation capacity compared to OMV-free stains [85]. In an experiment, it was found that the relationship between distinct properties such as extracellular ATP, MV release and biofilm, and bacterial viability was investigated. Based on the obtained data, the existence of such conditions potentiates the *Shewanella vesiculosa* M7T strain to resist harsh niches such as Antarctica [49]. As described for eukaryotic EVs, MV cargo can differ based on the parent organism, strain, and mechanism of production [49]. Therefore, it is logical to postulate that these parameters can affect MV cargo such as the content of bacterial pro-inflammatory factors [LPS, OM proteins (OMPs), periplasmic compounds, polysaccharides, nucleic acids, and phospholipids] [47, 96, 97]. Based on previous data, phosphoglycerolipids (PLs), the most abundant lipids, exist at high levels inside the OMVs but not MVs [98]. For this purpose, Gram-negative bacteria OMVs have been used for the fabrication of vaccines [99]. The transfer of LPS by OMVs to the immune cells leads to the production of pro-inflammatory cytokines without the emergence of severe pathological responses [100]. Besides, MVs can be donated and received for the modulation of bacterial activity. Of course, the mutual transfer of MVs is not the sole pathway for the interchange of varied substances between bacteria. Recent data have indicated the formation of tubular structures namely nanotubes between bacteria from the same and different species for reciprocal

Mobarak *et al. Cell Communication and Signaling*   (2024) 22:80

Page 7 of 19

interchange of biomolecules [101]. It seems that the formation of nanotubes is done in both Gram-negative and Gram-positive bacteria. For example, genetic materials such as DNA, toxin-related tRNAase, and plasmids can be transferred via nanotubes in *Bacillus subtilis* which lacks an OM. Other species such as *Staphylococcus aureus* and *Escherichia coli* have the potential to maintain inter-bacterial interchange via nanotubes [101, 102].

Biogenesis of MVs in bacteria

It has been postulated that several molecular mechanisms are involved in the formation of bacterial EVs with different subtypes, specific cargoes, and biological functions [44]. In short, Gram-negative bacteria usually use two basic pathways for EV formation. In the first mechanism, bacterial OM is an envelope for the released OMVs while the second mechanism consists of explosive bacterial lysis leading to the generation of outer-inner membrane vesicles (OIMVs) and explosive outer-membrane vesicles (EOMVs) [44, 98]. Gram-positive bacteria, the cytoplasmic membrane vesicles (CMVs) are produced via endolysin-triggered bubbling cell death [98]. The membrane blebbing pathway causes the formation of OMVs via the disruption of cross-junctions between the OM and the underlying peptidoglycan cell wall [44].

Emerging data have indicated that the activity of certain enzymes in Gram-positive bacteria leads to the weakening of cell wall peptidoglycan structure and further release of MVs [103]. The phenomenon of *Staphylococcus aureus* MVs releasing was first approved using ultrastructural electron microscopy analyses. Data indicated the disruption of the peptidoglycan layer that potentiates cytoplasmic membrane protrusion via cell wall, and MVs release [90]. To be specific, local accumulation of lipids loosens the physical connection of OM with the beneath peptidoglycan. Along with these changes, the concentration of peptidoglycan components at the site of MV formation increases the sequestration of protein inside the MVs. The activity of integral membrane proteins and certain OM molecules leads to lipid remodeling and vesicle formation [74, 104–106]. The accumulation of phenol-soluble modulins (PSMs) in the cytoplasmic membrane results in MV formation by increasing membrane fluidity [26].

In Gram-negative bacteria, the OM-peptidoglycan connection is loosened in specific sites without proper attachment. Various misfolded proteins are also recruited and accumulated as nanodomains in the sites with less content of peptidoglycans. With the progression of these changes, molecular changes in LPS facilitate fluidity and bulging in OM, resulting in the release of OMVs based on certain phospholipid types [107]. Gram-positive bacteria MVs directly protrude from the cell membrane, thus

containing a large number of cytoplasmic compounds [98, 108]. It is possible these harbor several toxins [109], peptides [110], and genomic elements [74, 110, 111]. Most previously conducted experiments on MVs have been dine in certain species like *Staphylococcus aureus* [90], and *Bacillus* spp. [109, 112], *Streptococcus* spp. [113–115], *Listeria monocytogenes* [116, 117], and *Clostridium* spp. [111, 118]. Preliminary data on Gram-negative bacteria OMVs have been obtained from strains of *Escherichia coli*. It should not be forgotten that released OMVs carry some identical factors related to host bacteria. For instance, researchers claimed that the releasing MVs in *Escherichia coli* spp. encompass lipids and proteins from the mutant stains such as 12,408 [119], W3110 [120], and JC411 [121]. These features indicate that the content of release MVs can be used for immunophenotyping of parent bacteria. Avila-Calderón and co-workers highlighted the importance of lipoproteins, LPS, and peptidoglycan in OMVs biogenesis [122]. As abovementioned, they also claimed that weak lipoprotein linkages in the OM layer can increase the possibility of bulging and vesicle formation. Besides, free unbound peptidoglycan residues and the negative charge of LPS are also helpful in vesicle formation. It has been shown that specific conditions can affect the MV capacity in bacteria. For example, the phenomenon of bleb formation or bulging is increased on the external surface of *Escherichia coli* after being exposed to antibiotics such as polymyxin B [123]. Likewise, carbapenem antibiotic, imipenem, can promote high protein-content OMVs in *Acinetobacter baumannii* [124]. These data indicate a close relationship between the insulting conditions and the formation of MVs. It seems that the formation of MVs occurs in response to insulting conditions to increase bacterial resistance and circumvent the harmful factors besides their original paracrine activity in bacteria-to-bacteria communication. In support of this notion, data have indicated that the reduction and loss of antibiotics can diminish MV-related blebs in OM [122, 123]. Of course, it should not be neglected that the existence of antibiotic can also weaken the stability of OM and inner structures of bacterial membranes which per se increase MV formation. Due to existence of biomarkers inside the MVs, these nano-sized particles can be used for the diagnosis and follow-up of pathological conditions inside the body. MVs of various pulmonary tract pathogens transport specific cargoes containing nucleic acids, proteins, lipoproteins, fatty acids, glycolipids, and relevant virulence factors such as *Pseudomonas aeruginosa* CFTR inhibitory factor, *Streptococcus pneumoniae* pneumolysin, and *Legionella pneumophila* macrophage infectivity potentiator [108, 115, 125, 126]. It is possible that some factors are release by MVs and do not exist in ECM fluid. For example, macrophage infectivity

Mobarak *et al. Cell Communication and Signaling*   (2024) 22:80

Page 8 of 19

potentiator and flagellin are only exist inside the bacterial MVs and cannot be detected in ECM. In an experiment, it was suggested that in Gram-positive bacteria such as *Bacillus subtilis* and *Streptococcus pyogenes*, MVs biogenesis depends on prophage-encoded endolysin cell death with simultaneous cell wall peptidoglycan disruption. This biological process involved in Gram-negative bacteria OMV production such as *Pseudomonas aeruginosa,* is prophage-mediated explosive cell lysis. In both mechanisms, the integrity of the membrane is blunted and the production of MVs/OMVs associated with defective prophage activation through genotoxic stress [26, 127].

Certain envelopes and environmental stresses including OM curvature-inducing structures and misfolded protein accumulation, exposure to antibiotics, and undesirable bacterial growth under improper pH values and/or temperatures can affect OMV biogenesis. A bacterial membrane integrity regulator, namely the transmembrane Tol-Pal protein system, functions as a factor in the regulation of OMV biogenesis in pathogenic and non-pathogenic bacteria such as *Escherichia coli*, *Shigella boydii*, and *Helicobacter pylori*. These studies highlighted the modifications in cell membranes such as OM blebbing and its relationship with OMV biogenesis [26, 72].

Interactions of MVs with eukaryotes and prokaryotes

Interactions with eukaryotic hosts

The presence of compounds such as toxins, adhesion molecules, siderophores, immune evasion proteins, and antibiotic resistance proteins in MV lumen highlights their roles in bacterial virulence [1, 128]. For example, *Listeria monocytogenes* MVs pore-forming toxin namely listeriolysin O. This toxin helps the bacterium to circumvent the host cell vacuoles [128]. The existence of various compounds inside the *Staphylococcus aureus* MVs such as lipase, protein A and SbI, staphopain A, etc. contribute to ECM degradation and bacterial invasion [1, 90, 129–132]. Pneumolysin is a pore-forming toxin inside *Streptococcus pneumoniae* MVs and is released from the infected eukaryotic cells. These features indicate the critical role of this factor in the infectivity of this bacterium [133]. Likewise, *Acinetobacter baumannii* can transfer virulence factor, Omp33–36 porin by OMVs to human immune cells such as macrophages. Data indicated that the uptake of these factors can result in apoptotic changes via the activation of Caspases and excessive autophagy response [134]. *Acinetobacter baumannii* OMVs contain other protein factors with the potential to alter other eukaryotic cell behavior such as oxidative stress in macrophages [135]. The higher levels of proteins and toxins inside the bacterial MVs lead to distance function and efficient immunomodulatory properties. Due to

the existence of various delivery routes, bacterial MVs can easily enter the eukaryotic cells, resulting in the cell membrane integrity loss, and rupture of cells. Compared to free soluble factors, MVs can efficiently introduce large levels of bacterial toxins into the eukaryotic cytosol [1]. Four different mechanisms can be involved in Gram-positive bacteria MVs uptake by eukaryotic cells as follows; Direct fusion with the cell membrane, Dynamin-related endocytosis, Caveolin-mediated endocytosis, and Clathrin-related endocytosis [1, 51, 132, 136, 137]. Besides, the close interaction of bacterial MVs with cholesterol-rich domains has been also indicated. For instance, *Staphylococcus aureus* toxin A can enter the human HEp-2 laryngeal cancer cells via MVs following the application of the cholesterol-destroying agent methyl-β-cyclodextrin [136]. Wang and co-workers claimed that the uptake of *Staphylococcus aureus* MVs by THP-1 macrophages is done in a dynamin-dependent endocytosis manner in which the incubation of cells with dynasore inhibitor blunt these effects [132]. Of course, it should not be forgotten that the route of MV entry depends on the size and type of target cells [1, 138].

One of the most interesting properties of OMVs is associated with tumoricidal effects [23, 24, 139, 140]. In a study conducted by Aly and co-workers, they monitored the anticancer effects of *Salmonella typhimurium* strain ATCC14028 OMVs on human different carcinoma cell lines including colorectal, hepatocellular carcinoma, and breast cancer) in in vitro conditions and mouse model of breast adenocarcinoma (Ehrlich solid carcinoma). The incubation of tumor cells with *Salmonella typhimurium* OMVs led to the reduction of tumor mass, cancer cell dynamic growth, and induction of apoptosis (Caspase-3↑ and Bax↑), autophagy (Beclin-1↑), and activation of CD49b⁺ pan-NK cells. Along with these changes, the expression of genes associated with proliferation (Ki-67↓), and angiogenesis (VEGF↓) was also inhibited [140]. These data support the oncostatic effects of bacterial OMVs as a safe alternative therapeutic option along with conventional medications. Whether and how OMVs can exert anti-tumor effects has been not completely addressed. In an interesting experiment, single-dose administration of attenuated *Salmonella typhimurium* OMVs (0.11 mg/kg) and photothermal therapy in mice bearing colon (CT26 cells) and breast cancer cells (4T1 cells) led to blackening of tumor mass in the early hours after treatment. One reason for these effects would be that OMVs cause massive RBC extravasation in the tumor vascular system, resulting in the loss of blood support and effective therapeutic outcomes [24]. Despite therapeutic outcomes, it is postulated that OMVs should be used in in vivo conditions with some caution. For instance, underestimation of OMV doses can lead to immunotoxicity. To this end,

Mobarak *et al. Cell Communication and Signaling*   (2024) 22:80

Page 9 of 19

synthetic bacterial vesicles (SyBVs) have been developed by using chemical engineering of lysozyme, detergents at high pH values in in vitro conditions without any toxic effects on immune cells. To be specific, the levels of DNA, RNA, cytosolic factors, and other biological contaminants were trivial in SyBVs. To yield proper tumoricidal effects, co-administration of SyBVs and tumor cell EVs is suggested [47]. The entry and on-target uptake of MVs should not be neglected. Data have indicated that hydrophilicity and net electrostatic charge are key elements in the attachment of MVs to eukaryotic cells and other bacterial strains [97]. Interestingly, *Pseudomonas aeruginosa* OMVs can attach and fuse with the OM of Gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*, and other *Salmonella enterica* spp.), leading to the transfer of OMV cargo into periplasm space. While Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*, and *Enterococcus hirae*) cannot adsorb the OMVs. The reason would be that the net charge of *Pseudomonas aeruginosa* OMVs is negative which blunts their physical interaction with the surface of Gram-negative bacteria. At physiological pH values, the surface charge of Gram-negative bacteria is highly increased due to LPS, and numerous free carboxyl and amino terminus. Due to the suitable hydrophilic surfaces of *Bacillus subtilis*, OMVs can easily attach to bacterial surfaces and enter as compared to other gram-positive bacteria [45, 97, 141]. Generally, many pathways are involved in the uptake of OMVs/MVs into target cells, including direct fusion to the plasma membrane, dynamin-, caveolin-, and clathrin-mediated endocytosis, phagocytosis, lipid-raft-dependent and lipid-raft-independent endocytosis, and macropinocytosis (Fig. 5). It seems that MVs use several routes to enter the target cell especially depending on size heterogeneity. In clathrin-based endocytosis, the OMVs with a maximum size of 120 nm enter the host cells via cell surface ligands while the particles with an average size of around 1 µm cross the cell membrane via macropinocytosis [142]. Using fluorochrome-labeled bacterial EVs with several inhibitors, different internalization mechanisms have been identified [44, 143]. Notably, the composition of OMVs pre-determines the fate of these nanoparticles in the host cells. For instance, the existence of LPS and O-antigens at the periphery of OMVs activates lipid raft-dependent entry into the host eukaryotic cells [144]. In vitro analyses indicated that *Moraxella catarrhalis* OMVs harbor ubiquitous surface proteins A1/A2 and Moraxella IgD-binding factor which are involved in the activation of B cells. The exposure of human pulmonary epithelial cells (A549 cell line) with *Moraxella catarrhalis* OMVs led to close interaction with lipid raft domains and internalization after the activation of TLR2 (Fig. 5) [144]. It was suggested that treatment of

A549 cells with filipin promoted the disruption of cholesterol-containing lipid raft structures and prevented compartmentalization of TLR2 into the raft structure [144]. In the absence of O-antigens, OMVs can be internalized using clathrin-mediated endocytosis [145]. In an experiment conducted by O'Donoghue and co-workers, they claimed that the inhibition of raft-mediated endocytosis using methyl-β-cyclodextrin and/or filipin reduces the *Escherichia coli* serotype O157:H7 strain Sakai 813 OMVs in HeLa cells [145]. To be specific, OMVs are internalized into the target cells using macropinocytosis, and raft- and clathrin-dependent endocytosis. However, the lipid raft is the main entry route for the bacterial OMVs in eukaryotic cells. Therefore, one could hypothesize that the dynamic growth of bacteria, environmental stress, and culture medium composition can affect the OMV composites and thus the entry route [145].

In gram-positive bacteria, three main mechanisms dynamin-, and clathrin-dependent endocytosis, and membrane fusion are possible routes for the entry of MVs into the host eukaryotic cells [1]. The inhibition of dynamin with dynasore, and dynamin-dependent endocytosis, can abolish the entry of *Staphylococcus aureus* MVs into the monocyte-macrophage lineage [132]. Likewise, the inhibition of lipid raft cholesterol using MβCD can reduce the entry rate of *Staphylococcus aureus* MVs into HepG2 cells [136]. In terms of bacterial MVs with other microbial strains, the existence of certain cell-wall attacking enzymes, especially in OMVs can facilitate the entry into the cytosol of host bacteria [103].

Of course, it should not be forgotten that OMVs can exert various cytopathic effects using different underlying mechanisms based on the parent bacteria. For example, *Acinetobacter baumannii* OMVs can increase the possibility of mitochondrial dysfunction in alveolar macrophages and pulmonary epithelial cells. The cytopathic properties of other bacterial EVs in the host gastrointestinal epithelium and mucosal barrier were indicated after exposure to OMVs of *Helicobacter pylori* [146], *Campylobacter jejuni* [147], *Treponema denticola* [148], and *Porphyromonas gingivalis* [149]. Histological examinations revealed the destruction of epithelial layers in gastrointestinal tissue and periodontium via affecting the function of tight junction proteins (i.e. zonula occludens and E-cadherin), and entry of oncogenic virulence factors such as cytotoxin-associated gene A. *Streptococcus pneumonia* MVs inhibit ECM neutrophil function and complement system activity. The exposure of *Staphylococcus aureus* MVs bearing alpha toxin can result in massive keratinocyte necrosis, eosinophilic reaction, and atopic dermatitis. Likewise, *Mycobacterium tuberculosis* MVs increase excessive CD4 lymphocyte activity and anergy [108, 150]. Group B *Streptococci* spp. produce MVs with
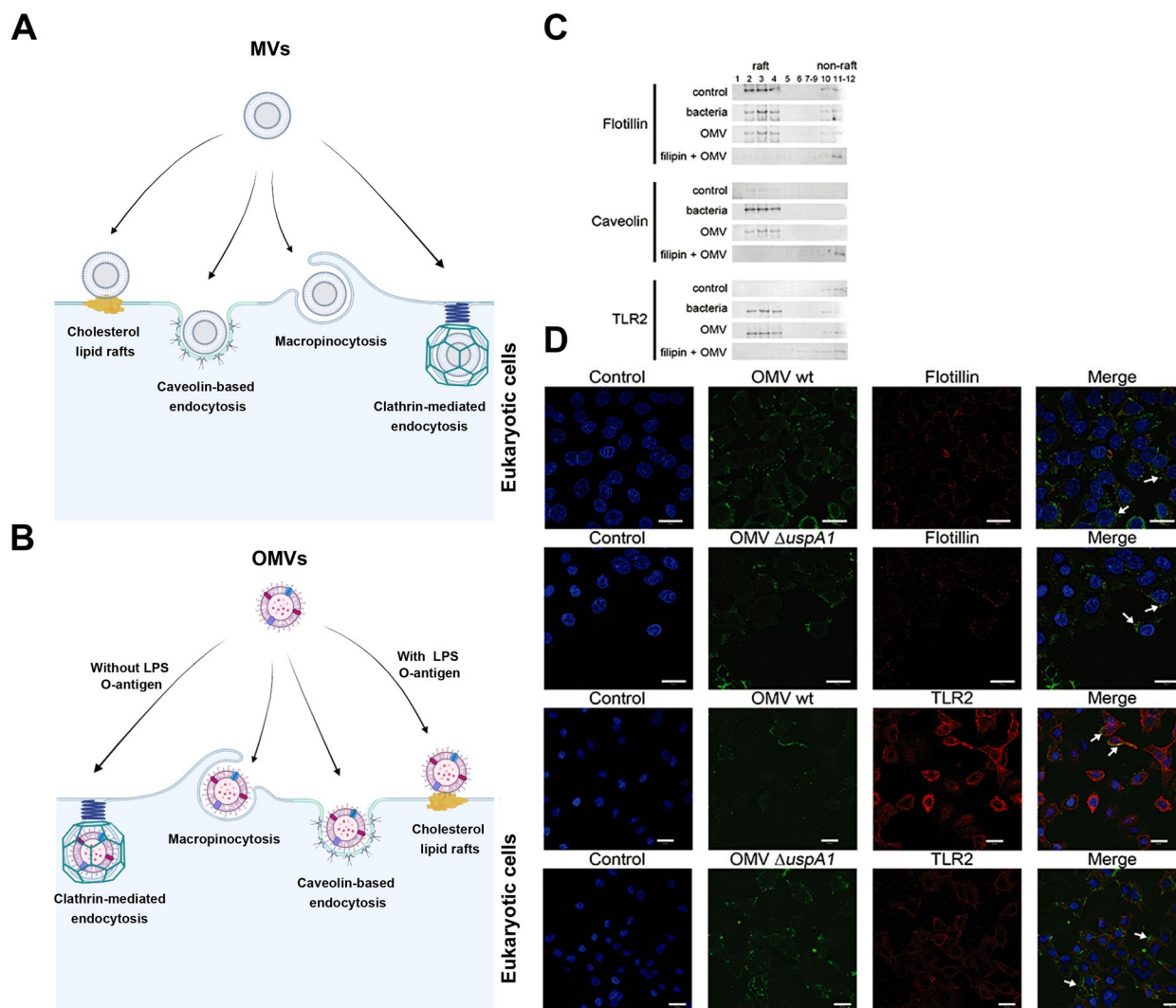
Fig. 5 Several mechanisms of bacterial MVs internalization into the eukaryotic cells (**A**-**B**). The attachment of OMVs with eukaryotic cells led to the compartmentalization of TLR2 into the raft. Western blotting indicated that treatment of A549 cells with filipin reduced the levels of Flotillin and Caveolin, as raft fraction. Along with these changes, TLR2 clustering also diminished lipid rafts (**C**). A549 cell lysates were obtained from non-treated control, formaldehyde-treated *Moraxella catarrhalis* (named bacteria), *Moraxella catarrhalis* OMVs, and filipin-treated cells + OMVs and electrophoresed in discontinuous sucrose gradients. Immunofluorescence staining of A549 cells for monitoring receptor clustering after 1-hour treatment with OMVs from wild type and/or UspA1-deficient (OMV ΔuspA1) *Moraxella catarrhalis* (50 μg/ml) (**D**). Co-localization of OMVs occurs with flotillin and TLR2. This reaction is not associated with the activity of surface OMVs UspA1. Blue nuclei were stained with DAPI (Scale bar: 20 μm) [144]. **Abbreviations**: ubiquitous surface proteins A1 (UspA1). (Copyright 2010, Cellular Microbiology)

the pontifical to loosen the integrity of natural cell barriers such as the blood-brain-barrier interface. Under such conditions, the recruitment of leukocytes, macrophages, and collagen degradation is increased [151]. Bacterial MVs can act as a two-edged sword in terms of infections inside the body. Alvarez-Jiménez et al. indicated that infection of human neutrophils with *Mycobacterium tuberculosis* (strain Mtb H37Rv) in in vitro conditions leads to the release of EVs which per se increases the intracellular elimination bacterial via macrophages

activation, ROS production, and autophagic response. Mtb-bearing EVs can activate Toll-like receptors (TLR2 and 6) and co-stimulatory factors CD80 and CD86, with simultaneous induction of LC3-II, TNF-α, and IL-6, and reduction of TGF-β in macrophages compared to the groups exposed to natural bacteria-free EVs (Fig. 6) [9]. In another study, it was indicated that *Mycobacterium smegmatis* and *Mycobacterium avium*) infected macrophages can release EVs with potent inflammatory capacities. The higher levels of heat shock protein 70 on the surface of
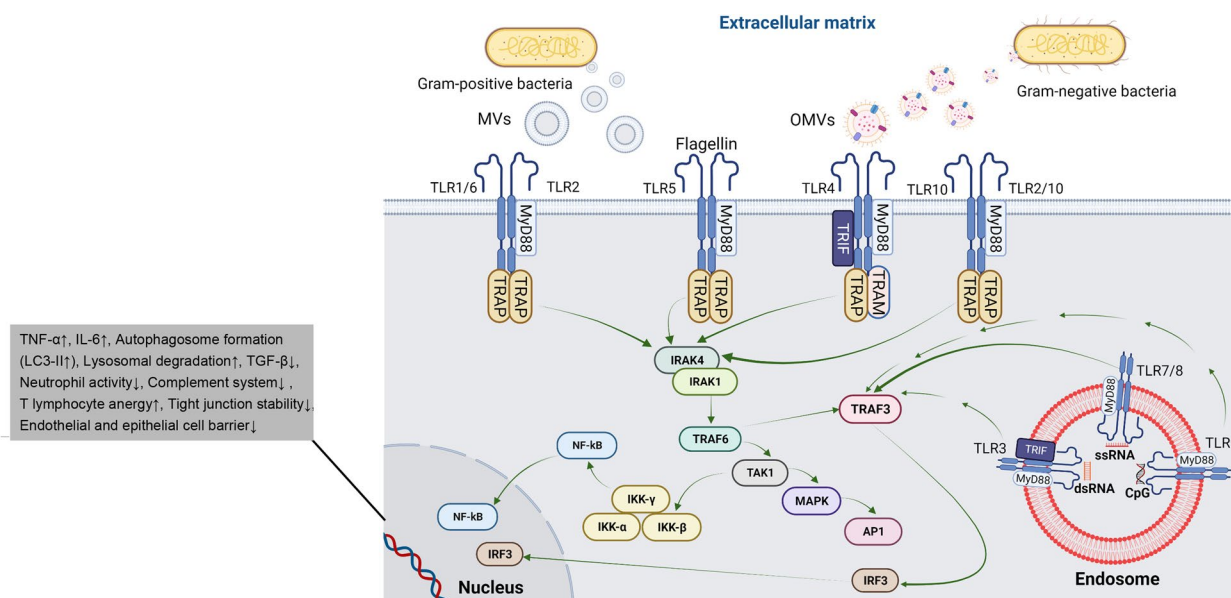
Fig. 6 The possible effect of bacterial MVs and OMVs via engaging Toll-like receptor signaling pathway on different cell lineage

bacterial-infected EVs lead to the activation of TNF-α and NF-κB signaling pathways. These features coincide with active lysosomal activity, autophagolysosome formation, and elimination of intracellular bacteria [152].

Interactions with bacteria

The reciprocal interaction between homogenous and heterogeneous bacteria via MVs has been reported [8, 52]. For instance, the fusion of *Lactobacillus acidophilus* MVs with other bacteria species, such as *Escherichia coli* and *Lactobacillus delbrueckii*. Upon the entry of *Lactobacillus acidophilus* MVs, the growth of bacteria is inhibited due to the delivery of an antimicrobial peptide bacteriocin [153]. Of note, the fusion of *Bacillus subtilis* lipophilic probe R18-labelled MVs with other *Bacillus subtilis* cells has been indicated [154]. The existence of degrading enzymes can facilitate the uptake of these particles by other bacteria [103]. Regarding the fact that MVs contain several quorum-sensing molecules such as N-acyl-homoserine lactones and Pseudomonas quinolone signal, it is logical to hypothesize that bacterial MVs are involved in interbacterial paracrine activity [1, 98, 155]. Factors like the blaOXA-24 gene that exists inside the MVs can induce resistance to carbapenem antibiotics in *Acinetobacter baumannii* cells [156]. Since EVs can fuse with bacterial membranes, likely, horizontal transfer of genes through EVs into bacteria is also possible [153, 154]. A similar process can occur in EV-embedded RNAs, in which recipient bacteria can utilize EVs-delivered RNAs [1].

Purification of eukaryotic and prokaryotic MVs

In eukaryotic EVs, differentiation of each subpopulation was performed based on diameter size and the existence of specific surface protein markers such as tetraspanins (CD9, CD63, and CD81), heat shock proteins (Hsp70, Hsp90), MHC molecules and proteins involved in the biogenesis of MVBs (i.e. TSG101 and ALIX), and other factors like GTPases, SNAREs, Annexins, flotillins). Besides common factors, EVs harbor certain markers of their parent cells. Despite these features, the purification of EV subpopulations is difficult due to the overlap between some of these factors [6, 157]. Even though, poor purification efficiency can affect the clinical applications of EVs with varied therapeutic outcomes. The lack of a suitable purification method can lead to low-rate EVs and a highly heterogeneous EV population. To date, various isolation techniques have been introduced to isolate the EVs while preserving their therapeutic properties [6, 158–164]. Ultracentrifugation is the most routine approach in EV extraction. It was suggested that the formation of EV aggregates, and loss of functionality at higher speeds are the main disadvantages [143, 165]. Other methods such as filtration, precipitation, microfluidics, size exclusion chromatography, and affinity-based approaches are also used in EV isolation.

Due to efficient production and reduced toxicity of LPS, the application of detergents has been commercialized for OMV purification [166]. The characterization of bacterial MVs is done based on their shape, size distribution, concentration, surface or internal contents using electron microscopy, light scattering-based

methods (e.g., nanoparticle tracking analysis and dynamic light scattering), and western blotting and/or flow cytometry [108]. Because none of these approaches can yield comprehensive data about MVs, a panel of these approaches is usually used [108]. It should not be forgotten that the bacterial source, culture conditions, and various parameters can affect the physicochemical properties of bacterial OMVs. For instance, in experiments conducted by Adriani et al. OMVs were isolated from bacteria grown in two different temperatures of 37 and 42 °C. Data confirmed that temperature can affect OMV protein content. Ultrastructural analysis has revealed a spherical electron-dense shape in both groups while the heterogeneity of isolated OMVs was high in groups exposed to the higher temperature. Likewise, the type of isolation can affect the structure of isolated OMVs. In the presence of deoxycholate detergent, the content of bacterial LPS is reduced in OMVs while the immunogenicity rate is not affected [166]. Recently, density gradient centrifugation has been performed as a common and most recommended method for MVs purification from samples. This approach is eligible to eliminate other cellular structures such as flagella and protein aggregates. Like eukaryotic EVs, size exclusion chromatography is also applicable for the isolation of bacterial MVs [27]. Despite both density gradient centrifugation, size exclusion chromatography can be used for the isolation of prokaryotic MVs, density gradient centrifugation is a time-consuming and labor-intensive method for MVs isolation and is not suitable for high-throughput applications while size exclusion chromatography is less time-consuming and can separate a generic mixed population of MVs [27, 167, 168].

Singorenko et al. claimed that a single purification method could be used to isolate the bacterial MVs from every strain within a bacteria species. Notably, data indicated that there is little difference in molecular patterns of OMVs isolated from UPEC536 and Nissle 1917 strains of *Escherichia coli* using density gradient centrifugation and size exclusion chromatography methods. Interestingly, *Escherichia coli* strain UPEC536 OMVs exhibited more homogeneity in size, density, and molecular contents relatively. On the contrary, *Mycobacterium smegmatis* MVs are heterogeneous and more problematic for the application of simple size exclusion isolation [27]. In an infection environment with stress such as lack of iron, certain bacteria types such as *Mycobacterium tuberculosis*, *Escherichia coli*, and *Haemophilus influenza* can release MVs with different cargo and size [27, 169]. *Acinetobacter baumannii* strain ATCC19606 can produce various sizes of OMVs at the growth phase. To be specific, the size of OMVs is small in the early phase of the growth cycle while the size of OMVs becomes medium, or large in early-mid, and stationary log phases, respectively [170]. These data show the need to use optimized fractionation protocols for the isolation of MVs depending on bacterial strain and growth phase [27] (Table 3).

Table 3 Effects of prokaryotic EVs on eukaryotic cells

Bacterial EVs	Recipient cells	Contents	Functions	References
Acinetobacter baumannii	Human macrophages cells	Virulence factor: Omp33–36 porin	Apoptosis↑, and autophagy modulation	[134]
Clostridioides difficile	Human colorectal epithelial Caco-2 cells	A total of 262 proteins	Pro-inflammatory response↑ and cytotoxicity of colonic epithelial cells↑	[118]
Vibrio cholerae	Human intestinal cell lines	Outer membrane porins, i.e., OmpU and OmpT bioactive cholera toxin		[51]
Helicobacter pylori	Macrophage RAW264.7 cells	Epimerase_2 domain-containing protein (Epi_2D), Probable malate: quinone oxidoreductase (Pro_mqo), and Probable cytosol aminopeptidase (Pro_ca)	Th2 immune response↑	[171]
Escherichia coli Nissle 1917 and Commensal ECOR63	Intestinal Epithelial Cells	The tcpC gene, such as ECOR63.	ZO-1↑ and claudin-14↑, and claudin-2↓, and protects epithelial barrier function	[172]
Salmonella typhimurium	Human colorectal carcinoma (HTC116), breast cancer (MCF-7), and hepatocellular carcinoma (HepG2) cell lines		Anti-neoplastic activity↑, tumor volume↓, tumor growth (Ki-67↓), Caspase-3↑, Bax↑, Beclin-1↑, and CD49b↑, down-regulated the Angiogenesis (VEGF↓)	[140]

OMVs/MVs as novel therapeutics

As above-mentioned, several studies have confirmed the existence of diverse biological roles for MVs/OMVs. Bacterial EVs are actively involved in gene transfer, biofilm formation, nutrient acquisition, pathogenesis, and defense promotion because of toxins and immunomodulatory products [microbe-associated molecular patterns (MAMPs)] [26]. Like eukaryotic EVs, emerging data have pointed to the fact that MVs are potential therapeutic targets and can be used as biological shuttles in the transfer of target compounds. Either eukaryotic EVs or bacterial OMVs/MVs can harbor biomaterials via circulation with suitable stability and on-target delivery efficiency (Table 4). To yield higher therapeutic outcomes, bacterial OMVs/MVs can be sophistically engineered [143]. Alves and co-workers used engineered *Escherichia coli* bacteria for the production of phosphotriesterase (PTE) enzyme. Data indicated that sequestrated PTE inside the OMVs exhibits better enzymatic activity compared to free soluble forms. These features demonstrate that OMVs are valid bioshuttles for drug stability and delivery purposes [173]. To this end, the elucidation of mechanisms associated with cellular uptake seems mandatory. Besides,

parameters such as administration route and parent bacterial source are critical issues in terms of bacterial MV/OMV bio-distribution [143, 174]. Recent decades have witnessed the advent of engineered OMV/MV, as tumoricidal agents, in cancer biology. Compared to conventional medications, the application of OMVs/MVs has superior on-target efficiencies [26]. For example, siRNA- and drug-loaded *Escherichia coli* OMVs were successfully used for targeting HER2, an EGF receptor, on the surface of tumor cells. Data confirmed that the size of the tumor mass decreased without prominent side effects. The treatment of hepatocellular carcinoma HepG2 cells with *Lactobacillus rhamnosus* MVs led to the regulation of tumor cells [139, 175]. Based on data from different studies, antibiotic-loaded OMVs can be used for the control of bacteria in the target tissues. For example, Huang and co-workers reported the reduction of intestinal bacterial load in a mouse model after administration of antibiotic-loaded OMVs [176]. Using certain bacterial EVs, it is possible to cross the epithelial and mucosal barrier [177].

The global application of antibiotics has led to the emergence of resistant bacterial species and thus biologists and clinicians are looking for different modalities

Table 4 Different approaches used in bacterial OMVs for different therapeutic purposes

Bacterial Species	Cargo Loaded	Loading Method	Application	References
Klebsiella pneumoniae	Doxorubicin-loaded OMVs	Incubation	Anti-tumor efficacy in non-small-cell lung cancer	[178]
Escherichia coli	Loading indocyanine green to modified OMVs with a synthe-sized αvβ3 integrin targeting ligand and arginyl-glycyl-aspartic acid	The fusion effect and electrostatic interaction	Melanoma	[179]
Escherichia coli	Spy ligation to factor hemoglobin protease on the surface of OMVs	Incubation	Vaccine design	[180]
Escherichia coli	Melanin	Genetic engineering of parent bacteria	Cancer	[181]
Escherichia coli	siRNA	Electroporation	Cancer therapy	[182]
Escherichia coli	NanoLuc Luciferase enzyme	Genetic engineering	Bioluminescence Imaging	[183]
Escherichia coli	Inhibitor of Indoleamine2,3-dioxygenase	Electroporation	Cancer Immunotherapy	[184]
Escherichia coli	NanoLuc Luciferase enzyme	Genetic engineering	As modular nanodevices for bio-sensing and bioimaging	[185]
Klebsiella pneumonia	Doxorubicin	Incubation	Anti-tumor in non-small-cell lung cancer	[178]
Pseudomonas aeruginosa	Gold NPs	Electroporation	Drug delivery	[186]
Escherichia coli	PD-1 Plasmid	Engineered to express the targeted polypeptide LyP1	Cancer Immunotherapy	[187]
Escherichia coli	Mesoporous silica nanoparticles loaded with 5-FU	Ultracentrifugation	Drug delivery system	[188]
Acinetobacter aumannii	Antibiotics	ND	Vesicle-based drug efflux mechanism	[176]
Salmonella typhimurium	*Ovalbumin*	Genetic manipulation	Maturation of human monocyte-derived dendritic cells	[189]
Escherichia coli	Shiga toxin	Ultracentrifugation	Cytotoxicity assays	[190]

Mobarak *et al. Cell Communication and Signaling*   (2024) 22:80

Page 14 of 19

to overcome drug-resistant microbes. Prokaryotic EVs are de novo vaccine candidates for the prevention of different bacterial infections [26]. Both OMVs and MVs exhibit inherent immunogenicity for the stimulation of immune cells against bacteria. Because of relatively simple and cost-effective production and ease of application of almost all kinds of modern technologies for modification of bacterial EV contents and surface, these natural nanoparticles are future vaccination agents. It is postulated that several antigens can be simultaneously loaded into the EVs from parent bacteria especially non-replicating microbes. By refining the bacterial EV contents, it is possible to make them more resistant to degrading enzymes to strengthen their immunogenic and on-target efficiencies. In support of this notion, previous data have indicated that the treatment of OMVs with vaccination protocols and formulation did not alter their stabilities over time [26, 173]. In an experiment conducted by Huang et al., immunogenic properties of *Acinetobacter baumannii* OMVs (AbOMVs) were examined in in vitro and in vivo settings. The injection of AbOMVs led to the production of specific IgG in a mouse model. The combination of anti-serum with quinolone antibiotics yielded proper bactericidal effects. Data indicated that simultaneous administration of levofloxacin with anti-AbOMVs anti-serum reduces the bacterial load in pulmonary tissues and spleen. The levels of recruited immune cells are also decreased within the lung parenchyma [191]. Kim and co-workers proved that *Escherichia coli* OMVs can stimulate the immune system response and thus reduce the lethal rate in infected mice via the production of certain cytokines such as IFN-γ and IL-17 [192]. Other experiments have shown the immunogenic properties of *Neisseria meningitidis*, *Vibrio cholera*, *Salmonella typhimurium*, and *Staphylococcus aureus* OMVs/MVs as novel vaccination tools [26, 191, 193, 194]. Furthermore, many studies have shown the potential of EVs derived from Gram-negative and Gram-positive bacteria in vaccine evolution. Bacterial EVs are being expanded as vaccines against some bacterial infections induced by *Klebsiella pneumoniae*, *Bordetella pertussis*, *Vibrio cholerae*, *Clostridium perfringens*, *Salmonella typhimurium*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*. Previous data have indicated the stimulation of cellular and humoral immune responses in vaccinated animals with bacterial EVs, leading to the reduction of bacterial load and infection. Indeed, EV-based vaccines have immune-stimulatory efficacy comparable to inactivated whole-cell vaccines [195, 196].

On the other hand, bacterial EVs can be used as effective and safe adjuvants for the development of certain vaccine types to elevate and regulate the immune response. For example, *Neisseria lactamica* EVs exhibited the powerful adjuvant activity against hepatitis B virus surface vaccine antigens [197]. Also, it has been reported that intranasal vaccination with *Escherichia coli* EVs can provoke immune system responses against the malaria without any side effects or weight loss in mice model, and antibody titers were comparable with other common adjuvants (e.g., MF59C.1 and cholera toxin) [198].

Despite these advantages, the application of BEVs should be done under special consideration. For instance, it was suggested that *Bacteroides fragilis* OMVs can stimulate the proliferation of cancer cells more than normal cells [199]. Besides, another side effect related to microbial MVs application is an excessive inflammatory response. Prolonged inspiration of BEVs promotes the recruitment of immune cells such as neutrophils and Th17 T lymphocytes into the pulmonary tissue, resulting in chronic pathological conditions and anaplastic changes [200]. The direct exposure of gastric epithelial cells to *Helicobacter pylori* OMVs can increase in situ levels of different cytokines such as IL-1β, −6, and TNF-α by macrophages and IL-17 and INF-γ by T lymphocytes. Inflamed epithelial cells also release IL-8 because of virulent factors vacuolating cytotoxin A and cytotoxin-associated gene A [201]. Bacterial lipids, mainly LPS, are endotoxic and can facilitate bacterial virulence [202]. The mutual interaction between the bacterial via EVs can lead to changes in growth rate and invasion [203]. *Staphylococcus aureus* MVs can facilitate interbacterial communication, resulting in antibiotic resistance and changes in EV release. The prominent heterogeneity, significant variation between batches, and standard production protocols make the application of bacterial EVs problematic in in vivo conditions.

Conclusions

Recent data have shown the similarity between eukaryotic EVs and prokaryotic MVs in terms of size, luminal contents, structure, etc. Compared to eukaryotic EVs, bacterial MVs possess relatively homogenous particle sizes [27]. Like Exos and microvesicles, bacterial MVs, and OMVs are alternates to synthetic polymeric micelles and liposomes in the delivery of therapeutics and specific signaling molecules to the targeted sites. Due to inherent immunogenic properties, both naïve and engineered bacterial MVs/OMVs can be used for vaccination and alleviation of pathological conditions. Because of specific features such as ease of production on large scales, it seems that these particles will be used as magic bullets for therapeutic purposes to reduce the possibility of antibiotic resistance and cancer patients.

Mobarak *et al. Cell Communication and Signaling*   (2024) 22:80

Page 15 of 19

Abbreviations

AbOMVs	*Acinetobacter baumannii* OMVs
CMVs	Cytoplasmic membrane vesicles
Exos	Exosomes
EOMVs	Explosive outer-membrane vesicles
ECM	Extracellular matrix
EVs	Extracellular vesicles
ILVs	Intraluminal vesicles
LPS	Lipopolysaccharide
MVs	Membrane vesicles
MVBs	Multivesicular bodies
NLR	NOD-like receptor
NOD	Nucleotide-binding oligomerization domain
OMVs	Outer membrane vesicles
OM	Outer membrane
OIMVs	Outer-inner membrane vesicles
PAMPs	Pathogen-associated molecular patterns
PLs	Phosphoglycerolipids
PSMs	Phenol-soluble modulins
PTE	Phosphotriesterase
SyBVs	Synthetic bacterial vesicles
TLRs	Toll-like receptors

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

References

1. Briaud P, Carroll RK. Extracellular vesicle biogenesis and functions in gram-positive Bacteria. Infect Immun. 2020;88(12)
2. Han C, et al. Exosome-mediated communication between tumor cells and tumor-associated macrophages: implications for tumor microenvironment. Oncoimmunology. 2021;10(1):1887552.
3. Yáñez-Mó M, et al. Biological properties of extracellular vesicles and their physiological functions. J Extracell Vesicle. 2015;4(1):27066.
4. Neven KY, Nawrot TS, Bollati V. Extracellular vesicles: how the external and internal environment can shape cell-to-cell communication. Curr Environ Health Rep. 2017;4(1):30–7.
5. Mobarak H, et al. Physiological impact of extracellular vesicles on female reproductive system; highlights to possible restorative effects on female age-related fertility. Biofactors. 2019;45(3):293–303.
6. Ayala-Mar S, et al. Recent advances and challenges in the recovery and purification of cellular exosomes. Electrophoresis. 2019;40(23–24):3036–49.
7. Gill S, Catchpole R, Forterre P. Extracellular membrane vesicles in the three domains of life and beyond. FEMS Microbiol Rev. 2019;43(3):273–303.
8. Kim JH, et al. Gram-negative and gram-positive bacterial extracellular vesicles. Semin Cell Dev Biol. 2015;40:97–104.
9. Alvarez-Jiménez VD, et al. Extracellular vesicles released from myco-bacterium tuberculosis-infected neutrophils promote macrophage autophagy and decrease intracellular mycobacterial survival. Front Immunol. 2018;9:272.
10. Kamran-Sarkandi M, et al. Mycobacterium avium complex extracel-lular vesicles attenuate inflammation via inducing IL-10. Int J Mol Cell Med. 2018;7(4):241–50.
11. Layre E. Trafficking of mycobacterium tuberculosis envelope com-ponents and release within extracellular vesicles: host-pathogen interactions beyond the wall. Front Immunol. 2020;11:1230.
12. Carrière J, et al. Exosomes released from cells infected with Crohn's disease-associated adherent-invasive Escherichia coli activate host innate immune responses and enhance bacterial intracellular replica-tion. Inflamm Bowel Dis. 2016;22(3):516–28.
13. Teow SY, et al. Pathogenic role of exosomes in Epstein-Barr virus (EBV)-associated cancers. Int J Biol Sci. 2017;13(10):1276–86.
14. Cai C, et al. Macrophage-derived extracellular vesicles induce long-lasting immunity against hepatitis C virus which is blunted by polyunsaturated fatty acids. Front Immunol. 2018;9:723.
15. Zicari S, et al. Human cytomegalovirus-infected cells release extracel-lular vesicles that carry viral surface proteins. Virology. 2018;524:97–105.
16. Zhu Y, et al. Exosomes: another arena for the game between viruses and hosts. Sheng Wu Gong Cheng Xue Bao. 2020;36(9):1732–40.
17. Karkowska-Kuleta J, et al. Characteristics of extracellular vesicles released by the pathogenic yeast-like Fungi Candida glabrata, Can-dida parapsilosis and Candida tropicalis. Cells. 2020;9(7)
18. Vallhov H, et al. Extracellular vesicles released from the skin commen-sal yeast Malassezia sympodialis activate human primary keratino-cytes. Front Cell Infect Microbiol. 2020;10:6.
19. Bielska E, May RC. Extracellular vesicles of human pathogenic fungi. Curr Opin Microbiol. 2019;52:90–9.
20. Atayde VD, et al. Leishmania exosomes and other virulence factors: impact on innate immune response and macrophage functions. Cell Immunol. 2016;309:7–18.
21. Mardahl M, Borup A, Nejsum P. A new level of complexity in parasite-host interaction: the role of extracellular vesicles. Adv Parasitol. 2019;104:39–112.
22. Castelli G, et al. Exosome secretion by Leishmania infantum modulate the chemotactic behavior and cytokinic expression creating an envi-ronment permissive for early infection. Exp Parasitol. 2019;198:39–45.
23. Schwechheimer C, Kuehn MJ. Outer-membrane vesicles from gram-negative bacteria: biogenesis and functions. Nat Rev Microbiol. 2015;13(10):605–19.
24. Zhuang Q, et al. Bacteria-derived membrane vesicles to advance tar-geted photothermal tumor ablation. Biomaterials. 2021;268:120550.
25. McCaig WD, Koller A, Thanassi DG. Production of outer membrane vesicles and outer membrane tubes by Francisella novicida. J Bacte-riol. 2013;195(6):1120–32.
26. Gilmore WJ, Bitto NJ, Kaparakis-Liaskos M. Pathogenesis mediated by bacterial membrane vesicles. Subcell Biochem. 2021;97:101–50.
27. Dauros Singorenko P, et al. Isolation of membrane vesicles from prokaryotes: a technical and biological comparison reveals heteroge-neity. J Extracell Vesicles. 2017;6(1):1324731.
28. Tashiro Y, Takaki K, Futamata H. Targeted delivery using membrane vesicles in prokaryotes. Biophys Physicobiol. 2019;16:114–20.
29. Gould S, Garg S, Martin W. Bacterial vesicle secretion and the evolution-ary origin of the eukaryotic endomembrane system. Trends Microbiol. 2016;24:525–34.
30. Wang X, et al. Exosomes isolated from bone marrow mesenchymal stem cells exert a protective effect on osteoarthritis via lncRNA LYRM4-AS1-GRPR-miR-6515-5p. Front Cell Dev Biol. 2021;9:644380.
31. Naradasu D, et al. Biogenesis of outer membrane vesicles concentrates the unsaturated fatty acid of phosphatidylinositol in Capnocytophaga ochracea. Front Microbiol. 2021;12:682685.
32. Wang X, et al. Release of Staphylococcus aureus extracellular vesicles and their application as a vaccine platform. Nat Commun. 2018;9(1):1379.

33. Stahl PD, Raposo G. Exosomes and extracellular vesicles: the path forward. Essays Biochem. 2018;62(2):119–24.

34. Van Niel G, d'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol. 2018;19(4):213–28.

35. Edgar JR. Q&a: what are exosomes, exactly? BMC Biol. 2016;14(1):46.

36. Zidan AA, et al. Characterization of urine stem cell-derived extracellular vesicles reveals B cell stimulating cargo. Int J Mol Sci. 2021;22(1)

37. Tsatsaronis JA, et al. Extracellular vesicle RNA: a universal mediator of microbial communication? Trends Microbiol. 2018;26(5):401–10.

38. Chen S, Zhu X, Huang S. Clinical applications of extracellular vesicle long RNAs. Crit Rev Clin Lab Sci. 2020;57(8):508–21.

39. Abbasian N, et al. Vesicles bearing gifts: the functional importance of micro-RNA transfer in extracellular vesicles in chronic kidney disease. Am J Physiol Renal Physiol. 2018;315(5):F1430–f1443.

40. Takasugi M. Emerging roles of extracellular vesicles in cellular senescence and aging. Aging Cell. 2018;17(2):e12734.

41. Liu S, et al. Prions on the run: how extracellular vesicles serve as delivery vehicles for self-templating protein aggregates. Prion. 2017;11(2):98–112.

42. Orench-Rivera N, Kuehn MJ. Environmentally controlled bacterial vesicle-mediated export. Cell Microbiol. 2016;18(11):1525–36.

43. Domingues S, Nielsen KM. Membrane vesicles and horizontal gene transfer in prokaryotes. Curr Opin Microbiol. 2017;38:16–21.

44. Chronopoulos A, Kalluri R. Emerging role of bacterial extracellular vesicles in cancer. Oncogene. 2020;39(46):6951–60.

45. Mashburn-Warren L, Mclean R, Whiteley M. Gram-negative outer membrane vesicles: beyond the cell surface. Geobiology. 2008;6(3):214–9.

46. Go G, et al. Extracellular Vesicle–Mimetic Ghost Nanovesicles for Delivering Anti-Inflammatory Drugs to Mitigate Gram-Negative Bacterial Outer Membrane Vesicle–Induced Systemic Inflammatory Response Syndrome. Adv Healthcare Mat. 2019;8(4):1801082.

47. Park KS, et al. Synthetic bacterial vesicles combined with tumour extracellular vesicles as cancer immunotherapy. J Extracell Vesicles. 2021;10(9):e12120.

48. Soult MC, et al. Outer membrane vesicles alter inflammation and coagulation mediators. J Surg Res. 2014;192(1):134–42.

49. Baeza N, Mercade E. Relationship between membrane vesicles, extracellular ATP and biofilm formation in Antarctic gram-negative Bacteria. Microb Ecol. 2021;81(3):645–56.

50. Hoiczyk E, Hansel A. Cyanobacterial cell walls: news from an unusual prokaryotic envelope. J Bacteriol. 2000;182(5):1191–9.

51. Zingl FG, et al. Outer membrane vesicles of Vibrio cholerae protect and deliver active cholera toxin to host cells via Porin-dependent uptake. mBio. 2021;12(3):e0053421.

52. Li Z, Clarke AJ, Beveridge TJ. Gram-negative bacteria produce membrane vesicles which are capable of killing other bacteria. J Bacteriol. 1998;180(20):5478–83.

53. Donato GM, et al. Delivery of Bordetella pertussis adenylate cyclase toxin to target cells via outer membrane vesicles. FEBS Lett. 2012;586(4):459–65.

54. Choi DS, et al. Proteomic analysis of outer membrane vesicles derived from Pseudomonas aeruginosa. Proteomics. 2011;11(16):3424–9.

55. Kesty NC, et al. Enterotoxigenic Escherichia coli vesicles target toxin delivery into mammalian cells. EMBO J. 2004;23(23):4538–49.

56. Rompikuntal PK, et al. Perinuclear localization of internalized outer membrane vesicles carrying active cytolethal distending toxin from Aggregatibacter actinomycetemcomitans. Infect Immun. 2012;80(1):31–42.

57. Thompson SS, Naidu Y, Pestka JJ. Ultrastructural localization of an extracellular protease in Pseudomonas fragi by using the peroxidase-antiperoxidase reaction. Appl Environ Microbiol. 1985;50(4):1038–42.

58. Maldonado R, et al. Cytotoxic effects of Kingella kingae outer membrane vesicles on human cells. Microb Pathog. 2011;51(1–2):22–30.

59. Ferrari G, et al. Outer membrane vesicles from group B Neisseria meningitidis Δgna33 mutant: proteomic and immunological comparison with detergent-derived outer membrane vesicles. Proteomics. 2006;6(6):1856–66.

60. Kesty NC, Kuehn MJ. Incorporation of heterologous outer membrane and periplasmic proteins into Escherichia coli outer membrane vesicles. J Biol Chem. 2004;279(3):2069–76.

61. Fuerst JA, Sagulenko E. Protein uptake by bacteria. Commun Integ Biol. 2010;3(6):572–5.

62. Roden JA, et al. Hemin binding protein C is found in outer membrane vesicles and protects Bartonella henselae against toxic concentrations of hemin. Infect Immun. 2012;80(3):929–42.

63. Horstman AL, Kuehn MJ. Enterotoxigenic Escherichia coli secretes active heat-labile enterotoxin via outer membrane vesicles. J Biol Chem. 2000;275(17):12489–96.

64. Chowdhury C, Jagannadham MV. Virulence factors are released in association with outer membrane vesicles of Pseudomonas syringae pv. Tomato T1 during normal growth. Biochimica et Biophysica Acta (BBA)-Proteins Proteomics. 2013;1834(1):231–9.

65. Pathak DT, et al. Cell contact-dependent outer membrane exchange in myxobacteria: genetic determinants and mechanism. PLoS Genet. 2012;8(4):e1002626.

66. Abrami L, et al. Hijacking multivesicular bodies enables long-term and exosome-mediated long-distance action of anthrax toxin. Cell Rep. 2013;5(4):986–96.

67. Wang X, et al. Staphylococcus aureus extracellular vesicles: a story of toxicity and the stress of 2020. Toxins (Basel). 2021;13(2)

68. Yu Q, et al. In vitro evaluation of tobramycin and aztreonam versus Pseudomonas aeruginosa biofilms on cystic fibrosis-derived human airway epithelial cells. J Antimicrob Chemother. 2012;67(11):2673–81.

69. Yonezawa H, Osaki T, Kamiya S. Biofilm formation by helicobacter pylori and its involvement for antibiotic resistance. Biomed Res Int. 2015, 2015; https://doi.org/10.1155/2015/914791.

70. Jarzab M, et al. Helicobacter pylori-derived outer membrane vesicles (OMVs): role in bacterial pathogenesis? Microorganisms. 2020;8(9):1328.

71. McBroom AJ, et al. Outer membrane vesicle production by Escherichia coli is independent of membrane instability. J Bacteriol. 2006;188(15):5385–92.

72. McBroom AJ, Kuehn MJ. Release of outer membrane vesicles by gram-negative bacteria is a novel envelope stress response. Mol Microbiol. 2007;63(2):545–58.

73. Tashiro Y, et al. Outer membrane machinery and alginate synthesis regulators control membrane vesicle production in Pseudomonas aeruginosa. J Bacteriol. 2009;191(24):7509–19.

74. Bitto NJ, et al. Bacterial membrane vesicles transport their DNA cargo into host cells. Sci Rep. 2017;7(1):7072.

75. Blenkiron C, et al. Uropathogenic Escherichia coli releases extracellular vesicles that are associated with RNA. PLoS One. 2016;11(8):e0160440.

76. Gaudin M, et al. Extracellular membrane vesicles harbouring viral genomes. Environ Microbiol. 2014;16(4):1167–75.

77. Dorward DW, Garon CF, Judd RC. Export and intercellular transfer of DNA via membrane blebs of Neisseria gonorrhoeae. J Bacteriol. 1989;171(5):2499–505.

78. Sjöström AE, et al. Membrane vesicle-mediated release of bacterial RNA. Sci Rep. 2015;5(1):1–10.

79. Pérez-Cruz C, et al. New type of outer membrane vesicle produced by the gram-negative bacterium Shewanella vesiculosa M7T: implications for DNA content. Appl Environ Microbiol. 2013;79(6):1874–81.

80. Ho MH, et al. Functional advantages of Porphyromonas gingivalis vesicles. PLoS One. 2015;10(4):e0123448.

81. Biton M, Abou Karam P, Regev-Rudzki N. Tuberculosis's cargoman: bacteria load RNA into host extracellular vesicles. EMBO Rep. 2019;20(3):e47719.

82. Klieve AV, et al. Naturally occurring DNA transfer system associated with membrane vesicles in cellulolytic Ruminococcus spp. of ruminal origin. Appl Environ Microbiol. 2005;71(8):4248–53.

83. Soler N, et al. Virus-like vesicles and extracellular DNA produced by hyperthermophilic archaea of the order Thermococcales. Res Microbiol. 2008;159(5):390–9.

84. Blesa A, Berenguer J. Contribution of vesicle-protected extracellular DNA to horizontal gene transfer in Thermus spp. Int Microbiol. 2015;18(3):177–87.

85. Baumgarten T, et al. Membrane vesicle formation as a multiple-stress response mechanism enhances Pseudomonas putida DOT-T1E cell surface hydrophobicity and biofilm formation. Appl Environ Microbiol. 2012;78(17):6217–24.

86. Grenier D, Bertrand J, Mayrand D. Porphyromonas gingivalis outer membrane vesicles promote bacterial resistance to chlorhexidine. Oral Microbiol Immunol. 1995;10(5):319–20.

Mobarak *et al. Cell Communication and Signaling*   (2024) 22:80

Page 17 of 19

87. Kadurugamuwa JL, Clarke AJ, Beveridge TJ. Surface action of gentamicin on Pseudomonas aeruginosa. J Bacteriol. 1993;175(18):5798–805.

88. Manning AJ, Kuehn MJ. Contribution of bacterial outer membrane vesicles to innate bacterial defense. BMC Microbiol. 2011;11(1):1–15.

89. Manning AJ, Kuehn MJ. Functional advantages conferred by extracellular prokaryotic membrane vesicles. J Mol Microbiol Biotechnol. 2013;23(1–2):131–41.

90. Lee EY, et al. Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of Staphylococcus aureus-derived membrane vesicles. Proteomics. 2009;9(24):5425–36.

91. Rohde M. The gram-positive bacterial Cell Wall. Microbiol Spectr. 2019;7(3):10–128.

92. Fagan RP, Fairweather NF. Biogenesis and functions of bacterial S-layers. Nat Rev Microbiol. 2014;12(3):211–22.

93. Ellen AF, et al. Proteomic analysis of secreted membrane vesicles of archaeal Sulfolobus species reveals the presence of endosome sorting complex components. Extremophiles. 2009;13(1):67–79.

94. Rachel R, et al. The ultrastructure of Ignicoccus: evidence for a novel outer membrane and for intracellular vesicle budding in an archaeon. Archaea. 2002;1(1):9–18.

95. Fiocca R, et al. Release of helicobacter pylori vacuolating cytotoxin by both a specific secretion pathway and budding of outer membrane vesicles. Uptake of released toxin and vesicles by gastric epithelium. J Pathol. 1999;188(2):220–6.

96. Schorey JS, Cheng Y, McManus WR. Bacteria- and host-derived extracellular vesicles - two sides of the same coin? J Cell Sci. 2021;134(11)

97. Tashiro Y, Uchiyama H, Nomura N. Multifunctional membrane vesicles in Pseudomonas aeruginosa. Environ Microbiol. 2012;14(6):1349–62.

98. Toyofuku M, Nomura N, Eberl L. Types and origins of bacterial membrane vesicles. Nat Rev Microbiol. 2019;17(1):13–24.

99. Brown L, et al. Through the wall: extracellular vesicles in gram-positive bacteria, mycobacteria and fungi. Nat Rev Microbiol. 2015;13(10):620–30.

100. Broz P. Inflammasomes: intracellular detection of extracellular bacteria. Cell Res. 2016;26(8):859–60.

101. Bron PA, et al. Renaissance of traditional DNA transfer strategies for improvement of industrial lactic acid bacteria. Curr Opin Biotechnol. 2019;56:61–8.

102. Dubey GP, Ben-Yehuda S. Intercellular nanotubes mediate bacterial communication. Cell. 2011;144(4):590–600.

103. Liu Y, et al. Gram-positive bacterial extracellular vesicles and their impact on health and disease. Front Microbiol. 2018;9:1502.

104. Berleman J, Auer M. The role of bacterial outer membrane vesicles for intra- and interspecies delivery. Environ Microbiol. 2013;15(2):347–54.

105. Kulp A, Kuehn MJ. Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annu Rev Microbiol. 2010;64:163–84.

106. Elhenawy W, et al. LPS remodeling triggers formation of outer membrane vesicles in Salmonella. mBio. 2016;7(4)

107. Mozaheb N, Mingeot-Leclercq M-P. Membrane vesicle production as a bacterial defense against stress. Front Microbiol. 2020;11:600221.

108. Behrens F, et al. Bacterial membrane vesicles in pneumonia: from mediators of virulence to innovative vaccine candidates. Int J Mol Sci. 2021;22(8)

109. Rivera J, et al. Bacillus anthracis produces membrane-derived vesicles containing biologically active toxins. Proc Natl Acad Sci U S A. 2010;107(44):19002–7.

110. Resch U, et al. A two-component regulatory system impacts extracellular membrane-derived vesicle production in group a Streptococcus. mBio. 2016;7(6):10–128.

111. Jiang Y, et al. Membrane vesicles of Clostridium perfringens type a strains induce innate and adaptive immunity. Int J Med Microbiol. 2014;304(3–4):431–43.

112. Brown L, et al. Extracellular vesicles produced by the gram-positive bacterium Bacillus subtilis are disrupted by the lipopeptide surfactin. Mol Microbiol. 2014;93(1):183–98.

113. Lee H, et al. Streptococcus equi-derived extracellular vesicles as a vaccine candidate against Streptococcus equi infection. Vet Microbiol. 2021;259:109165.

114. Wen ZT, et al. Multiple factors are involved in regulation of extracellular membrane vesicle biogenesis in Streptococcus mutans. Mol Oral Microbiol. 2021;36(1):12–24.

115. Olaya-Abril A, et al. Characterization of protective extracellular membrane-derived vesicles produced by Streptococcus pneumoniae. J Proteome. 2014;106:46–60.

116. Karthikeyan R, et al. Comprehensive proteomic analysis and pathogenic role of membrane vesicles of listeria monocytogenes serotype 4b reveals proteins associated with virulence and their possible interaction with host. Int J Med Microbiol. 2019;309(3–4):199–212.

117. Lee JH, et al. Transcription factor σB plays an important role in the production of extracellular membrane-derived vesicles in listeria monocytogenes. PLoS One. 2013;8(8):e73196.

118. Nicholas A, et al. Clostridium difficile-derived membrane vesicles induce the expression of pro-inflammatory cytokine genes and cytotoxicity in colonic epithelial cells in vitro. Microb Pathog. 2017;107:6–11.

119. Knox KW, Vesk M, Work E. Relation between excreted lipopolysaccharide complexes and surface structures of a lysine-limited culture of Escherichia coli. J Bacteriol. 1966;92(4):1206–17.

120. Katsui N, et al. Heat-induced blebbing and vesiculation of the outer membrane of Escherichia coli. J Bacteriol. 1982;151(3):1523–31.

121. Hoekstra D, et al. Release of outer membrane fragments from normally growing Escherichia coli. Biochim Biophys Acta. 1976;455(3):889–99.

122. Avila-Calderón ED, et al. Outer membrane vesicles of gram-negative Bacteria: an outlook on biogenesis. Front Microbiol. 2021;12:557902.

123. Koike M, Iida K, Matsuo T. Electron microscopic studies on mode of action of polymyxin. J Bacteriol. 1969;97(1):448–52.

124. Yun SH, et al. Antibiotic treatment modulates protein components of cytotoxic outer membrane vesicles of multidrug-resistant clinical strain, Acinetobacter baumannii DU202. Clin Proteomics. 2018;15(1):28.

125. Galka F, et al. Proteomic characterization of the whole Secretome of *legionella pneumophila* and functional analysis of outer membrane vesicles. Infect Immun. 2008;76(5):1825–36.

126. Jhelum H, Sori H, Sehgal D. A novel extracellular vesicle-associated endodeoxyribonuclease helps Streptococcus pneumoniae evade neutrophil extracellular traps and is required for full virulence. Sci Rep. 2018;8(1):7985.

127. Toyofuku M, et al. Prophage-triggered membrane vesicle formation through peptidoglycan damage in Bacillus subtilis. Nat Commun. 2017;8(1):481.

128. Coelho C, et al. Listeria monocytogenes virulence factors, including listeriolysin O, are secreted in biologically active extracellular vesicles. J Biol Chem. 2019;294(4):1202–17.

129. Lee J, et al. Staphylococcus aureus extracellular vesicles carry biologically active β-lactamase. Antimicrob Agents Chemother. 2013;57(6):2589–95.

130. Tartaglia NR, et al. Staphylococcus aureus extracellular vesicles elicit an Immunostimulatory response in vivo on the murine mammary gland. Front Cell Infect Microbiol. 2018;8:277.

131. Tartaglia NR, et al. Extracellular vesicles produced by human and animal Staphylococcus aureus strains share a highly conserved core proteome. Sci Rep. 2020;10(1):8467.

132. Wang X, Eagen WJ, Lee JC. Orchestration of human macrophage NLRP3 inflammasome activation by Staphylococcus aureus extracellular vesicles. Proc Natl Acad Sci U S A. 2020;117(6):3174–84.

133. Codemo M, et al. Immunomodulatory effects of pneumococcal extracellular vesicles on cellular and humoral host defenses. mBio. 2018;9(2):e00559.

134. Rumbo C, et al. The Acinetobacter baumannii Omp33-36 porin is a virulence factor that induces apoptosis and modulates autophagy in human cells. Infect Immun. 2014;82(11):4666–80.

135. Mendez JA, et al. Extracellular proteome of a highly invasive multidrug-resistant clinical strain of Acinetobacter baumannii. J Proteome Res. 2012;11(12):5678–94.

136. Gurung M, et al. Staphylococcus aureus produces membrane-derived vesicles that induce host cell death. PLoS One. 2011;6(11):e27958.

137. Choi EJ, et al. Propionibacterium acnes-derived extracellular vesicles promote acne-like phenotypes in human epidermis. J Invest Dermatol. 2018;138(6):1371–9.

138. Kaparakis-Liaskos M, Ferrero RL. Immune modulation by bacterial outer membrane vesicles. Nat Rev Immunol. 2015;15(6):375–87.

139. Kim OY, et al. Bacterial outer membrane vesicles suppress tumor by interferon-γ-mediated antitumor response. Nat Commun. 2017;8(1):1–9.

Mobarak *et al. Cell Communication and Signaling*   (2024) 22:80

Page 18 of 19

140. Aly RG, et al. Antineoplastic activity of Salmonella Typhimurium outer membrane nanovesicles. Exp Cell Res. 2021;399(1):112423.

141. Tashiro Y, et al. Variation of physiochemical properties and cell association activity of membrane vesicles with growth phase in Pseudomonas aeruginosa. Appl Environ Microbiol. 2010;76(11):3732–9.

142. Jan AT. Outer membrane vesicles (OMVs) of gram-negative Bacteria: a perspective update. Front Microbiol. 2017;8:1053.

143. De Sousa KP, et al. Isolation and characterization of extracellular vesicles and future directions in diagnosis and therapy. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2023;15(1):e1835.

144. Schaar V, et al. Multicomponent Moraxella catarrhalis outer membrane vesicles induce an inflammatory response and are internalized by human epithelial cells. Cell Microbiol. 2011;13(3):432–49.

145. O'Donoghue EJ, et al. Lipopolysaccharide structure impacts the entry kinetics of bacterial outer membrane vesicles into host cells. PLoS Pathog. 2017;13(11):e1006760.

146. Turkina MV, et al. Helicobacter pylori vesicles carrying CagA localize in the vicinity of cell–cell contacts and induce histone H1 binding to ATP in epithelial cells. FEMS Microbiol Lett. 2015;362(11)

147. Elmi A, et al. Campylobacter jejuni outer membrane vesicle-associated proteolytic activity promotes bacterial invasion by mediating cleavage of intestinal epithelial cell E-cadherin and occludin. Cell Microbiol. 2016;18(4):561–72.

148. Chi B, Qi M, Kuramitsu HK. Role of dentilisin in Treponema denticola epithelial cell layer penetration. Res Microbiol. 2003;154(9):637–43.

149. Nakao R, et al. Effect of Porphyromonas gingivalis outer membrane vesicles on gingipain-mediated detachment of cultured oral epithelial cells and immune responses. Microbes Infect. 2014;16(1):6–16.

150. Hong SW, et al. An important role of α-hemolysin in extracellular vesicles on the development of atopic dermatitis induced by Staphylococcus aureus. PLoS One. 2014;9(7):e100499.

151. Surve MV, et al. Membrane vesicles of group B Streptococcus disrupt Feto-maternal barrier leading to preterm birth. PLoS Pathog. 2016;12(9):e1005816.

152. Anand PK, et al. Exosomal Hsp70 induces a pro-inflammatory response to foreign particles including mycobacteria. PLoS One. 2010;5(4):e10136.

153. Dean SN, et al. Lactobacillus acidophilus membrane vesicles as a vehicle of Bacteriocin delivery. Front Microbiol. 2020;11:710.

154. Kim Y, Edwards N, Fenselau C. Extracellular vesicle proteomes reflect developmental phases of Bacillus subtilis. Clin Proteomics. 2016;13:6.

155. Mashburn LM, Whiteley M. Membrane vesicles traffic signals and facilitate group activities in a prokaryote. Nature. 2005;437(7057):422–5.

156. Rumbo C, et al. Horizontal transfer of the OXA-24 Carbapenemase Gene via outer membrane vesicles: a new mechanism of dissemination of Carbapenem resistance genes in Acinetobacter baumannii. Antimicrob Agents Chemother. 2011;55(7):3084–90.

157. Akbar N, et al. Isolation and characterization of human adipocyte-derived extracellular vesicles using filtration and ultracentrifugation. J Vis Exp. 2021;170:e61979.

158. Zhuo Z, et al. Targeted extracellular vesicle delivery systems employing superparamagnetic iron oxide nanoparticles. Acta Biomater. 2021;134:13–31.

159. Fuhrmann G, et al. Engineering extracellular vesicles with the tools of enzyme prodrug therapy. Adv Mater. 2018;30(15):1706616.

160. Zhu N, et al. A light-up fluorescence resonance energy transfer magnetic aptamer-sensor for ultra-sensitive lung cancer exosome detection. J Mater Chem B. 2021;9(10):2483–93.

161. Lane RE, et al. Purification protocols for extracellular vesicles. Methods Mol Biol. 2017;1660:111–30.

162. Balaj L, et al. Heparin affinity purification of extracellular vesicles. Sci Rep. 2015;5:10266.

163. Bruce TF, et al. Exosome isolation and purification via hydrophobic interaction chromatography using a polyester, capillary-channeled polymer fiber phase. Electrophoresis. 2019;40(4):571–81.

164. Huang S, et al. Evaluation of exosome loading characteristics in their purification via a glycerol-assisted hydrophobic interaction chromatography method on a polyester, capillary-channeled polymer fiber phase. Biotechnol Prog. 2020;36(5):e2998.

165. Mol EA, et al. Higher functionality of extracellular vesicles isolated using size-exclusion chromatography compared to ultracentrifugation. Nanomedicine. 2017;13(6):2061–5.

166. Adriani R, et al. Immunogenicity of Vibrio cholerae outer membrane vesicles secreted at various environmental conditions. Vaccine. 2018;36(2):322–30.

167. Bobrie A, et al. Diverse subpopulations of vesicles secreted by different intracellular mechanisms are present in exosome preparations obtained by differential ultracentrifugation. J Extracel Vesicles. 2012;1(1):18397.

168. Clark DJ, et al. Redefining the breast Cancer exosome proteome by tandem mass tag quantitative proteomics and multivariate cluster analysis. Anal Chem. 2015;87(20):10462–9.

169. Roier S, et al. A novel mechanism for the biogenesis of outer membrane vesicles in gram-negative bacteria. Nat Commun. 2016;7(1):1–13.

170. Koning RI, et al. Cryo-electron tomography analysis of membrane vesicles from Acinetobacter baumannii ATCC19606T. Res Microbiol. 2013;164(5):397–405.

171. Ahmed AAQ, et al. The impact of ExHp-CD (outer membrane vesicles) released from helicobacter pylori SS1 on macrophage RAW 264.7 cells and their immunogenic potential. Life Sci. 2021;279:119644.

172. Alvarez CS, et al. Outer membrane vesicles and soluble factors released by probiotic Escherichia coli Nissle 1917 and commensal ECOR63 enhance barrier function by regulating expression of tight junction proteins in intestinal epithelial cells. Front Microbiol. 2016;7:1981.

173. Alves NJ, et al. Protecting enzymatic function through directed packaging into bacterial outer membrane vesicles. Sci Rep. 2016;6(1):24866.

174. Nishida-Aoki N, et al. Altered biodistribution of deglycosylated extracellular vesicles through enhanced cellular uptake. J Extracell Vesicles. 2020;9(1):1713527.

175. Kim OY, et al. Bacterial protoplast-derived nanovesicles for tumor targeted delivery of chemotherapeutics. Biomaterials. 2017;113:68–79.

176. Huang W, et al. Development of novel nanoantibiotics using an outer membrane vesicle-based drug efflux mechanism. J Control Release. 2020;317:1–22.

177. Jahromi LP, Fuhrmann G. Bacterial extracellular vesicles: understanding biology promotes applications as nanopharmaceuticals. Adv Drug Deliv Rev. 2021;173:125–40.

178. Kuerban K, et al. Doxorubicin-loaded bacterial outer-membrane vesicles exert enhanced anti-tumor efficacy in non-small-cell lung cancer. Acta Pharm Sin B. 2020;10(8):1534–48.

179. Peng L-H, et al. Engineering bacterial outer membrane vesicles as transdermal nanoplatforms for photo-TRAIL–programmed therapy against melanoma. Sci Adv. 2020;6(27):eaba2735.

180. van den Berg Saparoea HB, et al. Display of recombinant proteins on bacterial outer membrane vesicles by using protein ligation. Appl Environ Microbiol. 2018;84(8):e02567–17.

181. Gujrati V, et al. Bioengineered bacterial vesicles as biological nano-heaters for optoacoustic imaging. Nat Commun. 2019;10(1):1114.

182. Gujrati V, et al. Bioengineered bacterial outer membrane vesicles as cell-specific drug-delivery vehicles for cancer therapy. ACS Nano. 2014;8(2):1525–37.

183. Huang Y, et al. Genetically engineered bacterial outer membrane vesicles with expressed nanoluciferase reporter for in vivo bioluminescence kinetic modeling through noninvasive imaging. ACS Appl Bio Mater. 2019;2(12):5608–15.

184. Li Y, et al. Antigen capture and immune modulation by bacterial outer membrane vesicles as in situ vaccine for Cancer immunotherapy post-Photothermal therapy. Small. 2022;18(14):2107461.

185. Chen Q, Rozovsky S, Chen W. Engineering multi-functional bacterial outer membrane vesicles as modular nanodevices for biosensing and bioimaging. Chem Commun. 2017;53(54):7569–72.

186. Ayed Z, et al. Electroporation of outer membrane vesicles derived from Pseudomonas aeruginosa with gold nanoparticles. SN Appl Sci. 2019;1:1–9.

187. Pan J, et al. Self-blockade of PD-L1 with Bacteria-derived outer-membrane vesicle for enhanced Cancer immunotherapy. Adv Mater. 2022;34(7):2106307.

188. Huang J, Wu Z, Xu J. Effects of biofilm nano-composite drugs OMVs-MSN-5-FU on cervical lymph node metastases from oral squamous cell carcinoma. Front Oncol. 2022;12:881910.

189. Schetters ST, et al. Outer membrane vesicles engineered to express membrane-bound antigen program dendritic cells for cross-presentation to CD8+ T cells. Acta Biomater. 2019;91:248–57.

Mobarak *et al. Cell Communication and Signaling*   (2024) 22:80

Page 19 of 19

190. Kehl A, et al. Modeling native EHEC outer membrane vesicles by creating synthetic surrogates. Microorganisms. 2020;8(5):673.

191. Huang W, et al. Anti-outer membrane vesicle antibodies increase antibiotic sensitivity of Pan-drug-resistant Acinetobacter baumannii. Front Microbiol. 2019;10:1379.

192. Kim OY, et al. Immunization with Escherichia coli outer membrane vesicles protects Bacteria-induced lethality via Th1 and Th17 cell responses. J Immunol. 2013;190(8):4092–102.

193. Oster P, et al. MeNZB™: a safe and highly immunogenic tailor-made vaccine against the New Zealand Neisseria meningitidis serogroup B disease epidemic strain. Vaccine. 2005;23(17):2191–6.

194. Choi SJ, et al. Active immunization with extracellular vesicles derived from Staphylococcus aureus effectively protects against staphylococcal lung infections, mainly via Th1 cell-mediated immunity. PLoS One. 2015;10(9):e0136021.

195. Luo R, et al. Interactions between extracellular vesicles and microbiome in human diseases: new therapeutic opportunities. iMeta. 2023;2(2):e86.

196. Micoli F, MacLennan CA. Outer membrane vesicle vaccines. In: Seminars in immunology. Elsevier; 2020.

197. Sardiñas G, et al. Outer membrane vesicles of Neisseria lactamica as a potential mucosal adjuvant. Vaccine. 2006;24(2):206–14.

198. Pritsch M, et al. Comparison of intranasal outer membrane vesicles with cholera toxin and injected MF59C. 1 as adjuvants for malaria transmission blocking antigens AnAPN1 and Pfs48/45. J Immunol Res. 2016;2016 https://doi.org/10.1155/2016/3576028.

199. Barteneva NS, et al. Extracellular vesicles in gastrointestinal cancer in conjunction with microbiota: on the border of kingdoms. Biochimica et Biophysica Acta (BBA)-reviews on. Cancer. 2017;1868(2):372–93.

200. Yang J, et al. The impact of bacteria-derived ultrafine dust particles on pulmonary diseases. Exp Mol Med. 2020;52(3):338–47.

201. Choi J-W, et al. Secretable small RNAs via outer membrane vesicles in periodontal pathogens. J Dent Res. 2017;96(4):458–66.

202. Yang J, et al. Microbe-derived extracellular vesicles as a smart drug delivery system. Transl Clin Pharmacol. 2018;26(3):103.

203. Lee EY, et al. Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of *Staphylococcus aureus*-derived membrane vesicles. Proteomics. 2009;9(24):5425–36.

Publisher's Note