


REVIEW ARTICLE

Interactions between extracellular vesicles and microbiome in human diseases: New therapeutic opportunities

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Abstract

In recent decades, accumulating research on the interactions between microbiome homeostasis and host health has broadened new frontiers in delineating the molecular mechanisms of disease pathogenesis and developing novel therapeutic strategies. By transporting proteins, nucleic acids, lipids, and metabolites in their versatile bioactive molecules, extracellular vesicles (EVs), natural bioactive cell-secreted nanoparticles, may be key mediators of microbiota–host communications. In addition to their positive and negative roles in diverse physiological and pathological processes, there is considerable evidence to implicate EVs secreted by bacteria (bacterial EVs [BEVs]) in the onset and progression of various diseases, including gastrointestinal, respiratory, dermatological, neurological, and musculoskeletal diseases, as well as in cancer. Moreover, an increasing number of studies have explored BEV-based platforms to design novel biomedical diagnostic and therapeutic strategies. Hence, in this review, we highlight the recent advances in BEV biogenesis, composition, biofunctions, and their potential involvement in disease pathologies. Furthermore, we introduce the current and emerging clinical applications of BEVs in diagnostic analytics, vaccine design, and novel therapeutic development.

KEYWORDS

biotherapy, extracellular vesicles, microbiome, microbiome–host interaction, vaccine

Highlights

- Bacterial extracellular vesicles (BEVs) are key intermediators of microbiota–host communications by transporting their versatile bioactive cargoes, including proteins, nucleic acids, lipids, and metabolites.

Rongjin Luo, Yanmin Chang, and Huaizhen Liang contributed equally to this work.

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- BEVs are proposed to exert both positive and negative roles in diverse physiological and pathological processes.
- BEVs secreted by pathogenic bacteria are strongly associated with the onset and progression of various human pathologies.
- BEV-based platforms show promising clinical potential, including application in diagnostic analytics, vaccine design, and novel therapeutic development.

INTRODUCTION

It is well established that the human body is a symbiotic ecosystem that contains a diverse range of commensal microorganisms, including eukarya, bacteria, and archaea, collectively termed microbiota [1, 2]. More than 100 trillion microbial cells, outnumbering human cells by 10 to 1, are estimated to inhabit almost every niche of the human body, especially the skin, oral cavity, respiratory, gastrointestinal, and urogenital tracts [3, 4]. Metagenomic sequencing data indicate that the human microbiome contains approximately 8 million unique protein-coding genes, which is 360 times higher than the total number of human genes (between 20,000 and 25,000) [5, 6]. The composition and quantity of the microbiota community are evolutionarily maintained in homeostasis and perform specific genetic and metabolic functions in human health. However, dysbiosis of the microbiome, caused by internal and external factors in certain circumstances, may result in the onset of various diseases, including inflammatory bowels, autoimmune, metabolic, psychological/neurological diseases, and cancer [7–10]. Moreover, with our rapidly advancing understanding of microbiota–host interactions and their regulatory networks, it is becoming increasingly clear that the microbiome is of great importance for the analysis of the host disease state and the development of novel therapeutic strategies.

Extracellular vesicles (EVs) are nano-sized, heterogeneous membrane-bound structures that are secreted by nearly all types of cells. They carry a diverse array of biologically active molecular cargos from parent cells, including lipids, proteins, nucleic acids, and small molecules, and serve as key intermediaries of intercellular communications [11–13]. Accumulating evidence has demonstrated that EVs are responsible for the complex and bidirectional relationship between microbiota and host cells, exerting an essential role in microbiota–host communications that are heavily involved in the regulation of various pathophysiological processes such as inflammation, angiogenesis, nutrient metabolism, and adaptive and innate immunity

responses [14–17]. Accordingly, diverse diagnostic and therapeutic approaches have been designed and clinically applied based on our increased understanding of the pivotal role of EVs in regulating microbiota–host connections [18–21].

Recently, EV-mediated interactions between the microbiome and the host have attracted much attention and have been intensively investigated. The biological properties of eukaryotic EVs have been well described. In this review, we mainly highlight the current state of knowledge of microbiota-secreted EVs, especially bacterial EVs (BEVs), focusing on their origin, biogenesis, composition, and, more specifically, their role in pathogenesis. Finally, we discuss the advances in BEV-based clinical utilization, including diagnostics, vaccines, and novel therapeutics (Figure 1).

ORIGIN, CLASSIFICATION, AND BIOGENESIS OF BEVs

Nowadays, the process of EV production has been clearly identified in nearly all cell types, including eukaryotic and prokaryotic cells. As early as 1967, researchers observed with electron microscopy, a process of spherical membrane-bound structures being released in the form of bulging out and pinching off from part of the cell wall of *Vibrio cholerae* (Gram-negative bacteria), and it was postulated to be relevant to the excretion product with cholera toxin [22]. Subsequently, in the early 1980s, EVs were found to be secreted from maturing sheep reticulocytes and function as a mechanism for iron transfer [23]. In 2007, the identification of abundant functional RNA present in EVs suggested a crucial role for EVs in cell-to-cell communication by RNA delivery [24]. Over the past two decades, several researchers have elucidated the characteristics of EV biogenesis, composition, biofunction, and clinical applications, especially EVs derived from eukaryotes. Researchers have reached a consensus that EV production is a highly regulated process, transporting diverse bioactive molecules into recipient cells either in their microenvironment or in

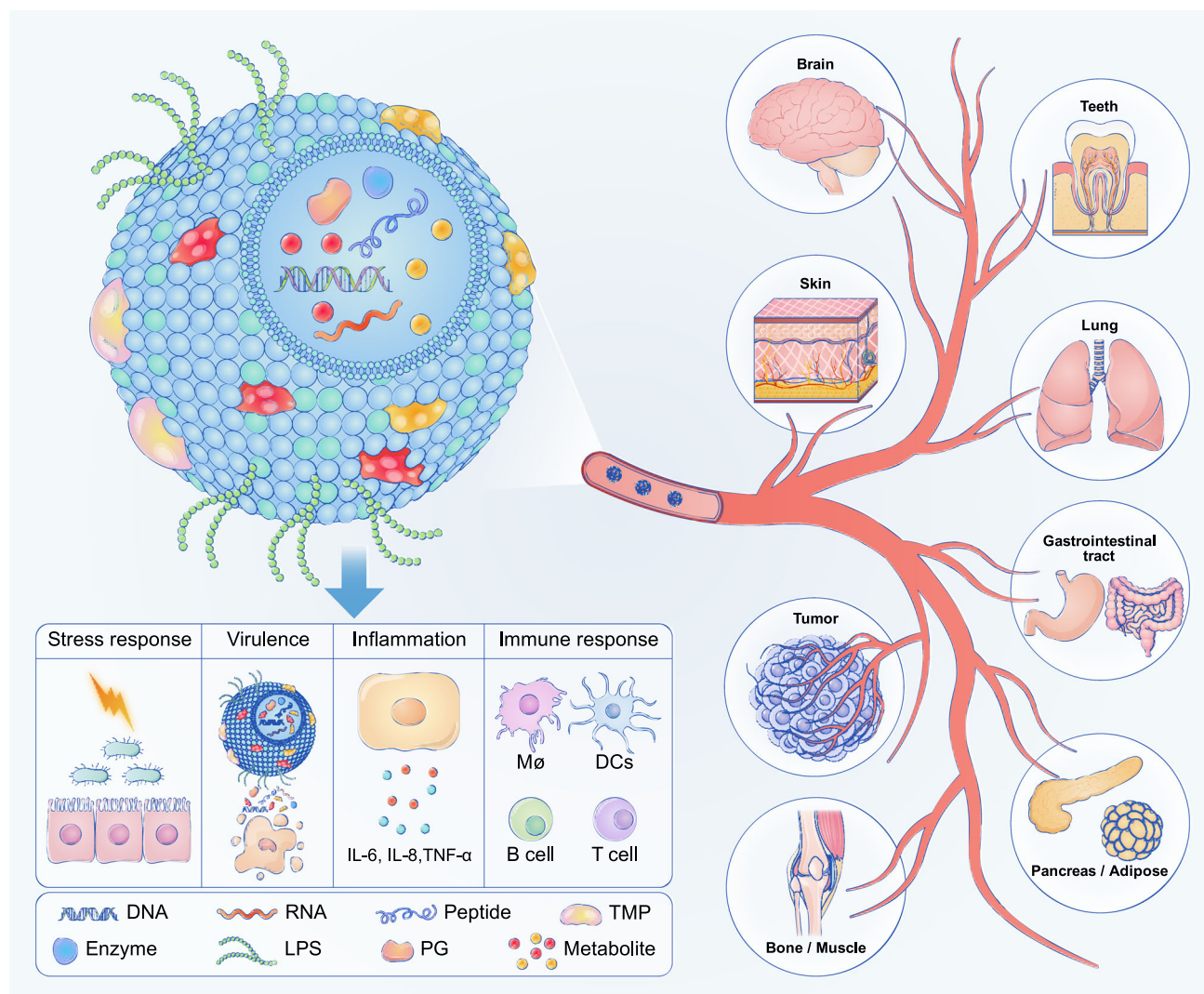


FIGURE 1 Schematic illustration of bacterial extracellular vesicles composition, biological functions, and their clinical correlations with various organs and tissues. DC, dendritic cell; IL-interleukin; LPS, lipopolysaccharide; PG, peptidoglycan; TMP, transmembrane protein; TNF, tumor necrosis factor.

distant tissues, initiating a variety of essential cellular signal transduction events [11, 25, 26].

As mentioned above, EVs are the generic name for cell-derived membrane vesicles that vary in origin, size, and composition. To date, diverse classification methods have been introduced to define and distinguish each subset of EVs, but an accurate and standard classification method is still lacking [27–29]. Currently, eukaryotic EVs are generally categorized into three main subgroups depending on their biogenesis, size, and secretion: exosomes (30–150 nm), ectosomes or microvesicles (100–1000 nm), and apoptotic bodies (1000–10,000 nm) [29, 30]. Among the subtypes of EVs, exosomes are the most intensively studied nanovesicles. They are formed by inward invagination of the endosomal membrane, resulting in the formation of intraluminal vesicles (ILVs) within multivesicular bodies (MVBs). MVBs can either

be directed to degradation by fusion with lysosomes, or directed to release their intraluminal vesicle cargo as exosomes. In contrast to exosomes, ectosomes are larger nanovesicles released directly from plasma membrane budding. Apoptotic bodies are large EVs released from apoptotic cells and eventually eliminated via phagocytosis [25, 31]. Eukaryotic EV generation and secretion is a complicated and finely tuned process and has been thoroughly summarized in the literature [25, 31–35]. Moreover, EVs are highly heterogeneous in nature, and the biophysical and biochemical properties of EVs often overlap between EV subtypes. It is important to understand the EV subtypes and their individual features before exploring their functional properties.

Since the importance of microbiota for human health has been revealed over the past decades, microbiota-derived vesicles (MEVs) are an emerging topic of study.

The human body harbors a variety of microorganisms including yeasts, archaea, parasites, viruses, and protozoa, of which bacterial taxa are currently the most intensively studied [36]. BEVs are nanovesicles naturally produced by all bacteria as part of their normal growth, ranging from approximately 20 to 300 nm. Specifically, BEVs released by Gram-negative bacteria and Gram-positive bacteria are referred to as outer membrane vesicles (OMVs) and membrane vesicles (MVs), respectively [14, 18] (Figure 2A,B). The mechanisms of BEV biogenesis in Gram-negative and Gram-positive bacteria follow two very different patterns based on their morphology, structure, and composition. OMVs were first found to originate from controlled blebbing of the outer membrane, which contains periplasmic contents, including outer membrane proteins, lipoproteins, and lipids [39, 40]. In addition, recent studies have revealed another type of OMV, called outer-inner membrane vesicles (OIMVs), which are formed by fission of a protrusion of the outer and plasma membranes or explosive cell lysis and entrap both periplasmic and cytoplasmic components, including DNA and ATP [41–44]. In contrast to Gram-negative bacteria, Gram-positive bacteria lack outer membranes but possess an extremely thick peptidoglycan (PG) cell wall, which is thought to prevent vesicular secretion. Emerging research has confirmed the existence of MVs derived

from abundant Gram-positive bacteria, which are postulated to originate from the blebbing of membrane material and encapsulate diverse membrane and cytoplasmic components [39, 45–47]. In addition, several membranous structures, referred to as nanotubes, nanowires, or nanopods protruding from the cytoplasmic membrane of Gram-positive bacteria or the outer membrane of Gram-negative bacteria, are also considered simple versions of BEVs [48–50]. Despite the different patterns of BEV production, the secretion of BEVs is an evolutionarily conserved characteristic among microbiota, suggesting the critical role of BEVs in the physical growth and survival of microbiota.

EV generation in eukaryotes is mainly mediated by endosomal sorting complexes required for transport pathway proteins and their homologues, which have been well documented in many excellent studies. However, the mechanisms of BEV biogenesis in bacteria remain poorly understood. To date, a series of models for OMV formation in Gram-negative bacteria have been proposed, based on genetic and biomedical studies (Figure 3A). The first model is a decreased interaction between the outer membrane and the PG layer, and PG-associated lipoproteins, such as Lpp and the Tol-Pal system, are a major class of surface proteins that are covalently anchored to the outer membrane and maintain membrane integrity and permeability. Lpp and Tol-Pal mutations or their

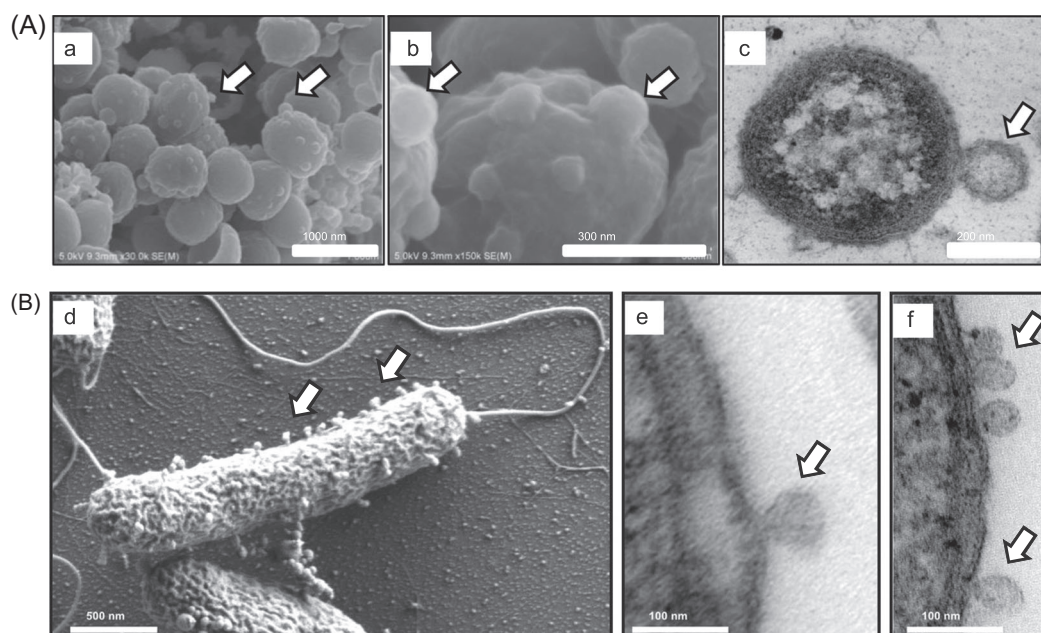


FIGURE 2 Visualization of BEVs release. (A) Cultured *Porphyromonas gingivalis* was observed with SEM (a, b) and TEM (c), representative graphs of whole cells with attached BEVs were illustrated. Reproduced with permission [37]. Copyright 2020, Elsevier. (B) BEVs detachment from *Vibrio harveyi* was observed with TEM (d) and SEM (e, f). Reproduced with permission [38]. Copyright 2018, American Society for Microbiology. The arrows indicate BEVs. BEV, bacterial extracellular vesicle; SEM, scanning electron microscope; TEM, transmission electron microscopy.

absence have previously been found to be associated with the increased release of periplasmic proteins and the formation of OMVs [51–53]. In the second model, local turgor pressure overload and imbalanced accumulation of multiple molecules, such as PG fragments, lipopolysaccharide (LPS), and misfolded proteins, into a defined area of the bacterial periplasm results in a strong pressure in the periplasmic space, deforming the upper outer membrane, leading to vesicle release [54, 55]. In the third model, changes in membrane physicochemical

properties and asymmetric distribution of phospholipids between the inner and outer leaflets of the outer membrane are proposed to initiate membrane curvature, leading to asymmetric membrane expansion, bulging, and subsequent release of OMVs [56, 57]. In the fourth model, LPS remodeling is linked to OMV production, which yields two different types of LPS: the A-band LPS (neutral charge) and the B-band LPS (anionic charge). Only B-band LPS was found packaged into OMVs, and mutants that only produce B-band LPS were detected with

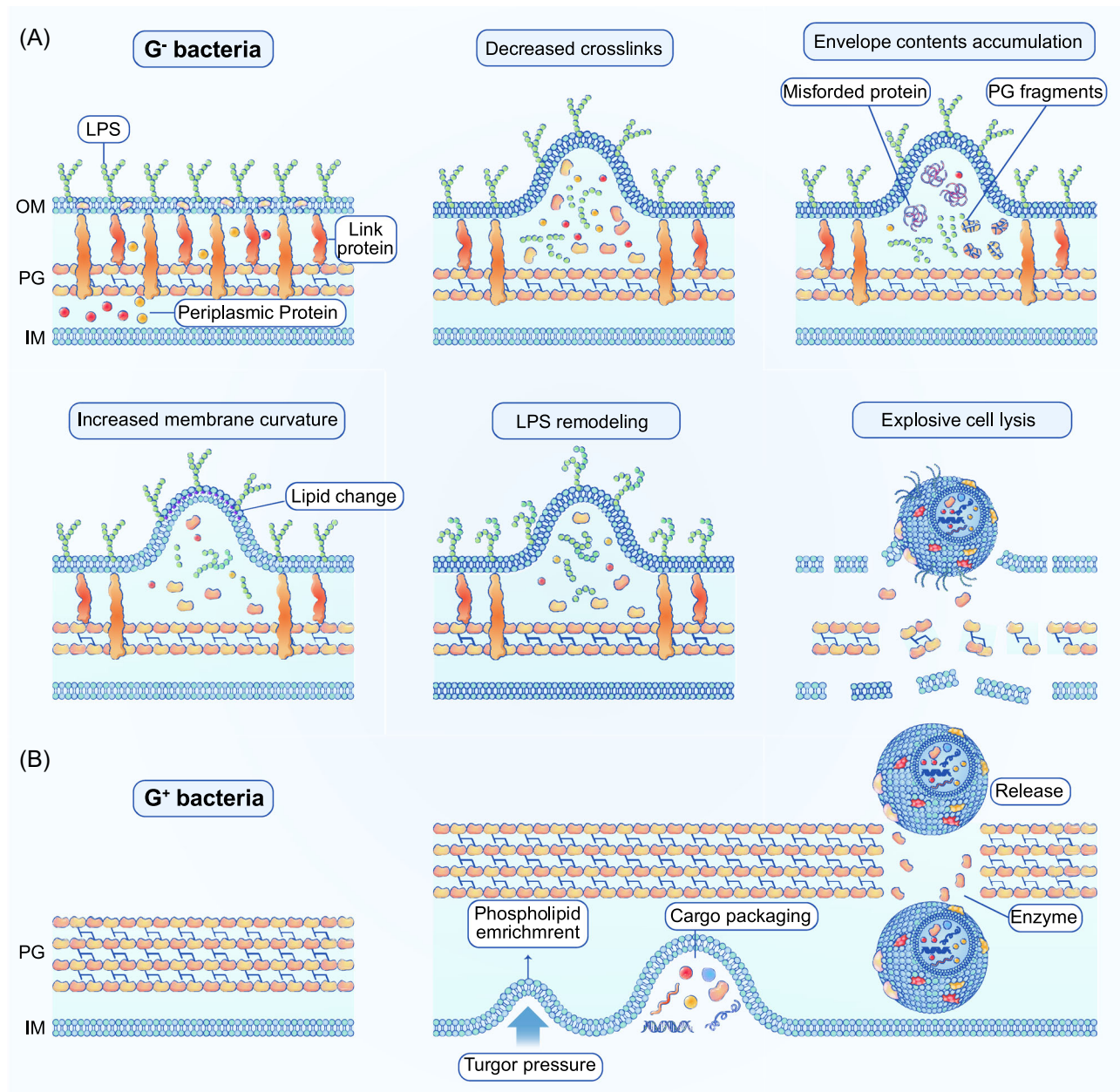


FIGURE 3 Potential mechanisms of BEVs biogenesis. (A) Gram-negative bacteria cell envelope structure and related BEVs generation models, including decreased cross-links between PG layer and outer membrane, local pressure overload, increased outer membrane curvature, LPS remodeling, and explosive cell lysis. (B) Gram-positive bacteria cell envelope structure and related BEVs generation machinery. BEV, bacterial extracellular vesicle; LPS, lipopolysaccharide; PG, peptidoglycan.

increased OMV production [58, 59]. Moreover, in *Porphyromonas gingivalis*, conformational alterations of LPS increase its membrane curvature and consequently augment OMV production [60, 61]. Explosive cell lysis was reported to be an active OMV secretion system, mediated by cryptic prophage endolysin, a key enzyme required for cytoplasmic content release; endolysin-deficient mutants are defective in MV production [44]. By contrast, Gram-positive bacteria possess thick PG cell walls compared with Gram-negative bacteria, suggesting the existence of a distinctly different mechanism of MV production. One hypothesis is that the accumulation of phospholipids in the outer leaflet of the membrane leads to rapid extension and outward bulging of the cytoplasmic membrane; turgor pressure elicited by the hypotonic environment may further promote the formation of MVs [62–65]. Mass spectrometry proteomic analyses of MVs revealed the presence of multiple PG-degrading enzymes, such as transpeptidases and autolysins, inside the MVs, suggesting that cell wall modification or loosening by enzymes may enable the trafficking of MVs [66–69] (Figure 3B). Moreover, protein channels or structural cables in the PG cell walls are postulated to be another mechanism of MV release [45]. Hence, research on MV biogenesis in Gram-positive bacteria is still in its infancy, and diverse internal and external factors affecting membrane fluidity and cell wall integrity appear to be critical determinants of MV generation.

BEV production is a well-orchestrated and complex process that involves multiple regulatory factors. Genetic regulation of bacterial vesiculation has been widely introduced in a variety of studies, of which a genetic network composed of nearly 150 genes has been implicated in the regulation of vesiculation; loss of function mutation of specific genes could result in significant changes in the quantity and cargo content of BEV production [56, 70–72]. Furthermore, environmental stress factors, including exposure to antibiotics or unfavorable bacterial growth conditions, such as aberrant temperature or pH, have also been reported to modulate BEV production [73–76]. Nutrient availability and growth phases from which BEVs are isolated have been shown to influence BEV secretion and cargo packaging [77–79]. Hence, these observations led us to speculate that microbiota can dynamically optimize BEV production and selectively sort cargoes into BEVs based on their own circumstances.

CARGO PACKAGING INTO BEVs

Generally, BEVs incorporate different biological components, including proteins, lipids, and nucleic acids, according to their parental cells. The quantity and variety

of cargo selectively packaged into BEVs are influenced by their producer bacteria and environmental factors, which largely determine their distinct biofunctions under certain conditions. Thus, researchers have introduced various approaches and techniques, such as next-generation sequencing (NGS), electron microscopy, mass spectroscopy, and proteomic analysis, to decipher the structure and composition of BEVs and subsequently investigate their role in diverse pathophysiological processes. However, it is still a great challenge to thoroughly interpret the composition of BEVs and the specific mechanisms by which cargo is selectively enriched in BEVs. In this section, we briefly recapitulate the general information on BEV composition and associated mechanisms.

Proteins. The protein content of BEVs has been validated in nearly all strains of bacteria, which are mainly composed of outer membranes, periplasmic, and cytoplasmic proteins [69]. With the rapid advancement of proteomic techniques, more than 3000 BEV-associated proteins have been identified, which can be functionally classified as structural proteins, porins, ion channels, transporters, enzymes, and proteins related to stress responses [80–83]. Global proteomics highlights the unique protein profiles of BEVs, which endow them with distinct functional capabilities, such as signal transduction, pathogenicity, biofilm formation, metabolism, antibiotic resistance, and bacterial survival [38, 84–87]. The distinct features of dynamic changes in protein composition and BEV yield efficiency when bacteria are grown under different environmental conditions, suggests that cargos in BEVs may be selectively enriched compared to their parent bacteria, although few studies have determined the specific sorting mechanisms. For instance, the protein composition of BEVs released by *Mycobacterium avium* cultured in a metal mix medium mimicking the phagosome environment is distinct from that of BEVs produced in minimal medium [88].

Lipids. Lipids are the main structural constituents of BEVs' biological membranes and are mostly derived from the cytoplasmic membrane and endomembrane. Consistently, experimental evidence from *Escherichia coli* revealed a conserved lipid composition between the outer membranes and BEVs, including LPS, phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin, which are closely related to membrane curvature [89, 90]. Importantly, some specific lipid species were also found to be selectively enriched in BEVs, and lipid analysis data showed that the content of phosphatidylglycerol and stearic acid, which are involved in membrane fluidity and rigidity, present in BEVs was significantly higher than that in the cellular outer

membrane [56, 91]. Another study on *Listeria monocytogenes* showed that unsaturated fatty acids, including phosphatidylethanolamine and sphingolipids, were more abundant in BEVs, while glycolipids were relatively underexpressed, indicating that the lipid composition of BEVs is not uniform across bacterial species [92]. Furthermore, it has been revealed that *P. gingivalis* BEVs also possess unique properties in terms of LPS composition [60]. Therefore, the lipid composition of BEVs may vary depending on the bacterial species and environmental conditions that are adapted to their biological functions.

Nucleic acids. It has been well established that BEVs can incorporate bacterial genetic material, including DNA and, more recently, different types of RNAs [93]. BEVs allow the delivery of DNA into other bacterial cells. Both chromosomal and plasmid DNA have been detected in the lumen or surface of BEVs derived from bacteria such as *E. coli*, *Neisseria gonorrhea*, *P. gingivalis*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* [70, 94–96]. Although there is a limited understanding of its biological functions, evidence suggests that BEV-bound DNA may play a role in biofilm formation, immunoregulation, adhesion, and virulence [96–98]. Previous studies have well demonstrated that BEVs contain abundant RNAs, including messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), and small RNA (sRNA), of which a large proportion of these BEV sRNAs and noncoding RNAs are from uncharacterized intergenic regions and may be responsible for regulating gene expression in target cells [47, 96, 99]. Recent evidence has shown that mRNAs in BEVs may be translated to produce microbial proteins in target cells, and sRNAs in EVs may interact with specific target cell factors to attenuate or enhance gene expression via transcription, mRNA processing, and translation [100]. Vast numbers of BEV-associated RNAs have been validated by RNA profiling analysis, but their functions remain largely unclear [47]. Several studies have reported that the delivery of BEV RNAs to host cells may participate in the regulation of inflammation, immune response, infection, and chemotherapeutic resistance [101, 102]. Overall, abundant coding and regulatory nucleic acids have been well verified to be enriched in BEVs, yet their exact composition and function require further investigation.

As mentioned above, the BEV's cargo composition is highly heterogeneous and largely depends on its origin, release location, and physiological or pathological state. Although considerable research has been conducted to elucidate the potential mechanisms that confer selective enrichment of proteins, lipids, and nucleic acids in BEVs, the exact sorting mechanisms are still unclear [103–105].

One proposed mechanism for selective cargo delivery into BEVs involves outer membrane lipid chaperones. Both neutral (A-band LPS) and negatively charged (B-band LPS) O antigen residues are present on the outer membrane; only B-band LPS is found to be associated with BEVs. In BEVs released by the B-band LPS mutant strain of *P. gingivalis*, the enrichment process of virulence factors such as gingipains was apparently abolished, suggesting that outer membrane lipid chaperones with high A-LPS composition may play a critical role in selective cargo packaging due to their affinity for the overall charge [59, 60]. Consistently, a comprehensive analysis of BEV cargoes of group A *Streptococcus* identified that the lipid composition of the bacterial membrane was responsible for the selective enrichment of specific proteins and RNA species [47]. Besides, a recent study on *Staphylococcus aureus* characterized the BEVs' protein composition and revealed that the main protein cargoes were positively charged, constituting more small residues and fewer aromatic and aliphatic groups compared with the whole-cell proteome, which further implicated the role of physicochemical properties (and potential charge) in cargo sortation [104]. Furthermore, in contrast to the listed passive sorting mechanisms, researchers have speculated alternative active sorting mechanism that the membrane convex or concave topological domain shaped during BEV budding may selectively enrich specific complementary molecules and guide them into BEVs [106].

BEVs BIOLOGICAL FUNCTIONS

Since the discovery of BEVs, research in the field has predominantly focused on their contributions to a wide spectrum of physiological and pathophysiological conditions. After detachment from the parent cells, BEVs translocate and interact with the cell surface of recipient cells subsequently transferring cargoes to the cytoplasm, thereby triggering downstream signaling pathways. To date, it has been well demonstrated that BEVs play an important role in a broad spectrum of biological events, including biofilm formation, antibiotic resistance, delivery of virulence factors, immunomodulation, and inflammation [107–110]. In this section, we mainly focus on recent advances in the field of bacteria–host interactions.

Defensive stress response

BEV secretion serves as a conservative stress response strategy and has been implicated in diverse unfavorable environmental conditions, such as heat shock, nutrient

deficiency, pH changes, temperature extremes, and the presence of antibiotics [111]. Generally, multiple direct and indirect mechanisms regarding the protective role of BEV secretion against stressors have been introduced, including promoting biofilm formation, exporting misfolded aggregates, and decoying membrane-targeted attacks [112].

Biofilms are essential and complex structures that enable bacteria to thrive in a protected microenvironment and are deeply involved in bacterial growth, resistance, and survival [113]. The effects of BEVs on biofilm formation could be positive or negative, based on differences in bacterial species and physical state. Notably, BEV cargoes are suggested to be fundamental constituents of biofilms; for instance, environmental DNA forms a key biofilm substrate, and adhesion-related proteins confer adhesive properties. Moreover, many biofilm matrix proteins, such as leucine aminopeptidase PaAP, have been identified in BEVs derived from *P. aeruginosa*, *V. cholerae*, and *Helicobacter pylori* [114–116]. BEVs released in biofilms can also sequester antibiotics, thereby promoting bacterial survival and biofilm development. Evidence from *Aeromonas* strains and *Enterobacter cloacae* indicates that even the quantity and concentration of BEVs are positively correlated with the formation of biofilms [117, 118].

Given the fact that BEVs are spherical nanostructures containing bacterial membrane components, outer membrane proteins, lipids, and soluble materials bound to the external surface, which confer the potential for BEVs to act as decoys, enabling bacteria to evade external attacks [119]. For instance, phage treatment serves as an efficient alternative intervention to limit bacterial infection, which depends largely on the presence of phage receptors on the outer membrane surface. During phage treatment of *V. cholerae*, secreted BEVs can act as natural decoys to defend bacteria from phage predation and weaken the outcome of phage treatment [120]. T4 is a well-studied bacteriophage, and there is an obvious binding and reduction of infection when T4 is co-incubated with BEVs, allowing enterotoxigenic *E. coli* (ETEC) to escape many lethal environmental stresses [121]. Thus, BEV production may effectively enhance survivability by neutralizing environmental attacks that target parent bacteria.

Moreover, BEVs have been suggested to play an essential role in antimicrobial resistance by harboring neutralized enzymes or directly sequestering antimicrobials away from bacteria [122]. BEV production is positively linked to antibiotic treatment, and analysis of the proteome composition of BEVs revealed the presence of multiple antibiotic-degrading enzymes, including β -lactamase, indicating a strong relationship between BEV production and antibiotic resistance [123, 124]. BEV-mediated absorption can help bacteria fight

antibiotics. An obvious decline in the therapeutic efficiency of antimicrobial peptide (AMPs) polymyxin B and colistin in *E. coli* was observed with the supplementation of BEVs or in a hyper-vesiculating mutant of *E. coli* [121]. Blocking BEV release can increase bacterial susceptibility to antibiotics [125]. In addition, adaptive mechanisms involving intra- and inter-species transfer of functional antibiotic enzymes and transporters to remove antibiotics have also been reported to reduce the efficacy of antibiotics and contribute to bacterial virulence [126].

Delivery of virulence factors

As mentioned above, intensive research has recognized the critical role of BEVs as vehicles to deliver virulence effectors to host cells, allowing pathogenic bacteria to access distal sites within the body without directly translocating from their environmental niche [127]. Generally, virulence factors originate from the BEV lumen and membrane structure, including toxins and adhesins. Cargo inclusion into BEVs offers several distinct advantages over other delivery strategies, such as immune system evasion, protection from proteolytic degradation, increased concentration and stability, long-distance delivery, and host-cell targeting specificity [127, 128]. Proteomic analysis of BEV compositions has revealed the presence of diverse pathogenic virulence factors, including leukotoxin, Shiga toxin, hemolysin, genotoxins, β -lactamase, and active AmpC [127, 129, 130]. For example, *H. pylori* is the most important contributor to the development of chronic inflammation and peptic ulceration, and *H. pylori*-derived BEVs containing oncogenic virulence factor cytotoxin-associated gene A (cagA) result in increased epithelial permeability and disrupt the integrity of epithelial barriers [131]. Similarly, gingipain is the typical toxin released by *P. gingivalis* that causes severe damage to the supporting tissues of teeth and periodontitis, while its virulence was observed to be enhanced by the synergistic effect of BEVs [132]. The cytotoxicity of purified *Mycobacterium ulcerans* BEVs containing the toxin mycolactone is obviously amplified compared to that of purified toxin alone, indicating the pivotal role of toxin-associated BEVs in bacterial pathogenicity [133]. Furthermore, BEV-mediated long-distance delivery of bacterial proteins to host cells may represent a general pathogenic pattern observed for multiple pathogens [134, 135].

Pro- and anti-inflammatory responses

Inflammation is an adaptive response by the host to ensure removal of detrimental stimuli and promote

damage tissue repair, which is usually timely terminated upon restoration of tissue homeostasis, and excessive inflammation (termed a cytokine storm) can be harmful to the body. All structural and functional components of BEVs originate from bacteria, such as LPS, lipoproteins, and toxins, and it is not surprising that BEVs contribute to the onset and progression of inflammation in the host. Although BEV-associated inflammation is known to be present in diverse host tissues, the mechanisms involved have not been extensively researched [136]. Mounting evidence suggests that pattern recognition receptors (PRRs) expressed in host immune cells are responsible for sensing and recognizing BEVs via pathogen-associated molecular patterns (PAMPs). Different PRR family members have been identified based on their localization, including Toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), and NOD-like receptors (NLRs) [136]. PRR activation signals transduced by common adaptor proteins lead to upregulation of genes, transcription factors, and kinases involved in inflammatory responses. As the loading content and composition of BEVs vary among bacterial species, the extent of the proinflammatory response can be different. For example, THP-1 cells exposed to *Filifactor alocis* showed increased expression of various chemokines, such as C-C Motif chemokine ligand 1 (CCL1), CCL2, CCL5, C-X-C motif chemokine ligand 1 (CXCL1), CXCL10, intercellular adhesion molecule 1 (ICAM-1), interleukin-6 (IL-6), and IL-8 [137], whereas *Streptococcus pneumoniae*-secreted BEVs elicited enhanced production of IL-6, IL-8, IL-10, and tumor necrosis factor (TNF) in human monocyte-derived dendritic cells [138]. NOD-like receptor thermal protein domain associated protein 3 (NLRP3) inflammasome complex activation is responsible for the maturation of caspase-1 and subsequent secretion of mature IL-1 family cytokines, including IL-1 β and IL-18. Recent studies have reported that PG containing BEVs released by mucosal pathogens *H. pylori* and *P. aeruginosa* could be specifically detected by the NOD1 receptor, resulting in NF- κ B pathway-dependent activation of NLRP3 inflammasome and IL-1 β release [139–141]. Collectively, these studies highlight that there are a broad range of mechanisms by which BEVs mediate the induction of proinflammatory responses in the host.

Apart from the proinflammatory effects of pathogenic bacteria-derived BEVs mentioned above, the anti-inflammatory activity of BEVs has also been described in various nonpathogenic species, mostly commensal and probiotic bacteria. The decreased abundance of the *Lactobacillus* population is a typical probiotic that is tightly involved in the onset and progression of inflammatory gastrointestinal diseases; in LPS- and

dextran sulfate sodium (DSS)-mediated inflammation in vitro and in vivo, *Lactobacillus*-derived BEVs showed a marked anti-inflammatory effect by inhibiting cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS) and nuclear factor- κ B (NF- κ B) expression [142]. Similarly, *Lactobacillus*-derived BEVs can also reduce interferon gamma (IFN- γ) production and dampen proinflammatory cytokine release in a monocyte-dependent manner [143]. Another study utilized *E. coli* Nissle 1917 (EcN) purified BEVs to treat DSS-induced colitis in a mouse model, and the results showed that pretreatment with BEVs significantly attenuated DSS-induced weight loss, clinical symptoms, and histological scores [144]. Furthermore, nucleic acids packaged inside BEVs also regulate inflammatory responses. sRNA52320, a small sRNA detected in *P. aeruginosa*-secreted BEVs, was found to downregulate LPS-induced cytokine release via the mitogen-activated protein kinase (MAPK) signaling pathway [145]. Collectively, the contribution of BEV-mediated inflammation to host benefits and pathogenicity has been well illustrated in the literature, while emerging questions regarding the nature of these components and the underlying mechanisms in host–pathogen interactions have yet to be fully answered.

Immunomodulation

To date, there have been extensive studies aimed at exploring the role and underlying mechanisms of BEVs in the occurrence and development of various human pathologies, of which BEV-associated immunological balance has attracted much research interest. Generally, BEVs can confer immunosuppressive or immunostimulatory roles depending on the active components within the EVs and the distinct characteristics of diverse diseases. Mounting evidence has established that BEVs tightly interact with a range of innate and adaptive immune cells [18].

Monocyte-macrophages are the most widely investigated innate immune cells responsible for host tissue surveillance as well as rapid detection and elimination of invasive pathogens. In cases of infection caused by *P. gingivalis*, *Treponema denticola*, *Legionella pneumophila*, and *Salmonella typhimurium*, secreted BEVs can interact with and activate monocytes-macrophages, resulting in the release of cytokines, including nitric oxide (NO), TNF- α , IFN- α , IL-6, IL-8, and IL-1 β [140, 146]. *H. pylori*-derived BEVs have also been shown to reduce cytokine responses by promoting IL-10 production in human peripheral blood mononuclear cells [147]. In terms of the regulatory influence of BEVs on neutrophils, it is suggested that purified BEVs can directly interact with

and activate neutrophils, as demonstrated in *S. aureus* [148]. Neutrophil extracellular traps (NET) are specific neutrophil-related antimicrobial mechanisms, and *S. pneumoniae* BEVs packaged with extracellular DNase TatD may subvert immune responses by disrupting NET formation, thereby modulating the host's response to infection [149]. Dendritic cells (DCs) are antigen-presenting cells (APCs) that bridge innate and adaptive immune systems. DCs stimulated by *S. typhimurium*-derived BEVs displayed increased surface expression of MHC-II and CD86 and inflammatory mediators (NO, TNF- α , and IL-12), compared to levels induced by bacterial cells, indicating the potent proinflammatory and antigenic properties of BEVs [150]. Interestingly, in the case of the human commensal *Bacteroides fragilis* regulating the human immune response, purified BEVs delivered to intestinal dendritic cells exhibit anti-inflammatory effects by promoting IL-10 production and reducing IL-17A expression from CD4⁺ Foxp3⁺ regulatory T cells [151]. In summary, these studies demonstrated that the outcome of BEV and DC interactions is largely dependent on the microbial species and host physiological state.

In addition to the aforementioned immunomodulatory effects of BEVs on innate immune cells, BEVs have also been shown to modulate the adaptive immune response of the host. *Moraxella catarrhalis*, a respiratory pathogen residing in the tonsils, is readily endocytosed and eliminated by human tonsillar B cells. However, BEVs containing DNA have the potential to interact with and activate the B cell TLR9 signaling pathway; thus, BEVs serve as a decoy to ultimately favor bacterial survival [152]. In addition, combinations of BEVs from *Neisseria meningitidis* serogroup A and W₁₃₅ showed the ability to induce an IgG2a/IgG2b isotype profile and IFN- γ production, suggesting the production of long-term B memory cells and activation of cell-mediated immunity [153]. Nevertheless, more evidence regarding how BEVs directly interact with B cells and the underlying mechanisms is still needed. By contrast, T cell responses elicited by BEVs have been illustrated in a broad spectrum of pathogenic species, such as *S. aureus*, *Francisella tularensis*, and *S. typhimurium* [154, 155]. In adoptive transfer and gene-knockout research, *E. coli*-derived BEV immunization effectively stimulated T cell immunity and systemic inflammatory response syndrome primarily via Th1 and Th17 cell responses, ultimately preventing bacteria-induced lethality [156]. BEVs can also exhibit an immunosuppressive role in T-cell responses. The addition of *H. pylori*-derived BEVs to peripheral blood mononuclear cells increased the expression of COX-2, PGE2, and IL-10, accompanied by the subsequent

inhibition of T cell proliferation [157]. Thus, BEVs may possess versatile immunomodulatory roles in T cell responses in the host, based on their origin.

BEVs IN PATHOGENESIS

Over the past decades, with the rapid advancement of detection and analytical techniques, mounting evidence has demonstrated the pivotal role of BEVs in the initiation and progression of a wide spectrum of human pathologies, including gastrointestinal, respiratory, dermatological, neurological, periodontal, metabolic, and musculoskeletal diseases, and cancer.

Gastrointestinal disease. Compared to other organs, the gastrointestinal tract harbors the largest number and variety of microbes that play essential roles in energy acquisition, metabolism, and immune regulation. BEVs released into the gut lumen have the potential to cross the mucus layer and mediate cross-kingdom interactions with host cells, which are strongly implicated in host health. Several studies have confirmed the pathogenic role of BEVs in various gastrointestinal disorders such as peptic ulcers, enteric infections, and inflammatory bowel disease (IBD) [158]. *H. pylori* colonization of the human stomach has been well established as the principal etiologic agent of peptic ulcer disease. On the one hand, *H. pylori* BEVs containing several virulence factors (VacA, urease, and CagA) are readily internalized, leading to impaired bioactive function and disruption of tight junctions in gastric epithelial cells [86, 159]. On the other hand, *H. pylori* BEVs may contribute to proinflammatory effects on epithelial and immune cells by promoting IL-6, IL-8, TNF- α , and IFN- α production [160]. *V. cholerae* and pathogenic *E. coli* are representative etiologic agents implicated in gastroenteritis. BEVs secreted from *V. cholerae* and pathogenic *E. coli* serve as carriers for many virulence factors such as cholera toxin, heat-labile enterotoxin, hemolysin, and Shiga toxin, resulting in endothelial cytotoxicity, apoptosis, and inflammatory cytokine release [161, 162]. IBD, which comprises Crohn's disease and ulcerative colitis, is characterized by chronic relapsing-remitting inflammation of the gastrointestinal tract. Alterations in intestinal microbial homeostasis are closely associated with the pathogenesis and development of IBD [163]. BEVs derived from pathogenic *E. coli* are found to stimulate inflammatory production via the TLRs pathway in intestinal epithelial cells, and even induce endothelial and epithelial mitochondria-associated apoptosis via toxin delivery [164–166]. Despite the above facts, there are still many unanswered questions concerning the regulatory mechanisms of BEV-mediated microbe–host interactions.

Respiratory disease. Numerous studies have characterized the respiratory microbiome in human health and disease. An imbalance in the respiratory microbiome is associated with a broad range of lung diseases, including chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, asthma, and acute lung injuries [167]. Chronic airway inflammation is a common feature in multiple chronic lung diseases, including asthma and COPD. *S. aureus* colonization of the nasopharynx may be a risk factor for lung disease. Kim et al. have provided new evidence that the addition of *S. aureus* BEVs to macrophages and airway epithelial cells results in the production of IL-6 and TNF- α , and repeated airway exposure with *S. aureus* BEVs can induce Th1 and Th17 neutrophilic pulmonary inflammation in a TLR2-dependent manner [168]. Similar outcomes were observed in BEVs that were mainly derived from indoor dust Gram-negative bacteria such as *E. coli* [80, 169]. Furthermore, under conditions of microbe-associated acute lung injury, BEVs containing PAMPs and damage-associated molecular patterns (DAMPs) are known to trigger inflammatory cell (macrophage and neutrophil) infiltration and cytokine (IL-6, TNF- α , and IL-1 β) expression in lung tissues [170].

Dermatological disease. Acne vulgaris (acne) is a highly prevalent inflammatory skin condition that involves the microbiome and its interactions with the innate immune system. A microbial imbalance of skin bacteria including *Cutibacterium acnes*, *Staphylococcus epidermidis*, and *S. aureus* has been implicated in the pathophysiology of inflammatory acne [171]. Keratinocytes are the main cell type of the epidermis and participate in the activation of inflammation and immune responses when exposed to DAMPs and PAMPs. Proteomic analysis identified multiple virulence factors (such as NlpC/P60, CAMP factor, and Hta domain protein) in *C. acnes*-derived BEVs [172]. Meanwhile, *C. acnes*-derived BEVs significantly induced IL-8 and GM-CSF production and dysregulated epidermal differentiation in epidermal keratinocytes, leading to acne-like phenotypes [173]. Atopic dermatitis, characterized by epidermal thickening and infiltration by eosinophils and mast cells, is intrinsically associated with abnormal microbiome homeostasis in the skin, especially *S. aureus*. A previous metagenomic analysis together with serum detection of pathogen-specific EVs in atopic dermatitis patients showed that *S. aureus* is the most dominant microbe detected in atopic dermatitis lesions, and its EV-specific IgG and IgE were twofold higher in atopic dermatitis patients than in normal controls [174]. Histological analyses observed abundant staphylococcal protein A (SPA) in the epidermis of atopic dermatitis lesions, and proteomic analysis revealed that *S. aureus* BEVs contain various pathogenic

molecules (such as a-hemolysin and cysteine protease), indicating its potential links with epidermal barrier dysfunction [175, 176]. Moreover, intact *S. aureus* BEVs stimulated the release of proinflammatory cytokines (including IL-1b, IL-6, IL-8, and MIP-1a) in keratinocytes in a TLR2- and NOD2-dependent manner. Topical application of intact *S. aureus* BEVs specifically induced infiltration of inflammatory cells and inflammatory responses in atopic dermatitis-like skin lesions in an in vivo model [176]. Thus, *S. aureus* BEVs may be potential therapeutic targets for the management of atopic dermatitis aggravation.

Neurological disease. Emerging evidence has demonstrated the critical role of microbiota in bidirectional communication between the gut and the brain, and it is not surprising that BEVs may participate in the pathogenesis and progression of many neurological conditions [177]. The blood-brain barrier (BBB) is a highly selective barrier that controls molecular and cellular trafficking between the bloodstream and neural tissue. Several studies have provided evidence for the presence of bacterial components, such as rRNA and rDNA, in brain tissues, and it has been speculated that a fraction of these microbial nucleic acids may cross the BBB through BEVs delivery [178, 179]. Moreover, BEVs can disrupt BBB integrity and result in increased access of other BEVs or bacterial products to the brain [180, 181]. Neuroinflammation is a common pathological feature that contributes to abnormal protein aggregation, neuronal dysfunction, and even cell death in multiple neuropsychiatric disorders including Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), and depression/anxiety. On the one hand, once translocated into the brain, BEVs directly alter neurologic function and induce pathology through releasing their biological cargoes, such as enzymes and regulatory sRNAs [181, 182]. *Aggregatibacter actinomycetemcomitans* BEVs ferry sRNA across the BBB to increase brain TNF- α levels and subsequent neurologic effects via TLR8 and NF- κ B signaling pathways [182]. By contrast, BEVs containing various DAMPs/PAMPs (such as LPS, lipoprotein, and nucleic acids) readily stimulate host immune and nonimmune cells via TLR and NOD signaling pathways to produce systemic inflammation, ultimately resulting in neuroinflammation [183]. Thus, BEVs may represent an important alternative pathway in the pathogenesis of neurological disorders.

Periodontal disease. Periodontal disease is a common infectious disease that involves a range of bacterial species and is characterized by a surrounding inflammatory microenvironment and destruction of periodontal structures. BEVs derived from *P. gingivalis* have been implicated in the formation of oral inflammatory

microenvironments. As early as 1985, researchers observed the phenomenon of BEVs produced by *P. gingivalis*, but their pathogenic role was not defined [184]. Proteomics and RNA-seq analyses have revealed that *P. gingivalis* BEVs are enriched in diverse virulence factors or modulators of virulence factors, including gingipains, heme-binding lipoproteins, and noncoding RNAs, enabling bacteria to penetrate deep tissues and activate an inflammatory host response [185]. Compared with the parent bacteria, BEVs showed amplified production of cytokines and proapoptotic factors (including TNF- α , IL-6, IL-8, IFN- γ , LDH, and 7-AAD) in macrophages and endothelial cells, leading to cellular impairment, connective tissue destruction, and alveolar bone resorption [128, 186, 187]. In addition, recent understanding of the constituents of BEVs released by *A. actinomycetemcomitans*, *Filifactor alocis*, and *Fusobacterium nucleatum* also provides clues for deciphering the pathogenic role of BEVs in periodontal disease [84, 137, 188, 189].

Metabolic disease. Mounting evidence has established the central role of the microbiome in host energy homeostasis and its alterations have strongly been linked to the onset and progression of multiple metabolic disorders, especially obesity and diabetes [190–192], although the exact mechanisms remain unclear. In this regard, BEVs are postulated to be key mediators of microbiota–host communication by encapsulating a wide range of bioactive molecules. *Akkermansia muciniphila* is a beneficial bacterium that modulates intestinal barrier integrity. *A. muciniphila* EV administration to a high-fat diet (HFD)-induced diabetic mice resulted in decreased gut barrier permeability, reduced body weight gain, and improved glucose tolerance. Moreover, there are fewer *A. muciniphila* EVs in the fecal samples of patients with type 2 diabetes (T2D), indicating a positive relationship with energy metabolism [193]. In addition, obesity and insulin resistance are often associated with low-grade inflammation, with adipocytes and macrophages being master players. TLR-4 dependent cytokine production is an important pathway for BEV-mediated proinflammatory tissue; TLR-4 overexpression has been detected in adipose tissue, skeletal muscle, and liver [194, 195]. Under HFD conditions, TLR4 depletion-mice showed decreased blood glucose and glucose tolerance and improved insulin sensitivity. Furthermore, *Pseudomonas panacis* BEVs were reported to block the insulin-signaling pathway in both skeletal muscle and adipose tissue and induce a typical diabetic phenotype in mice [196]. Moreover, BEVs derived from *P. gingivalis* were also introduced to participate in the progression of T2DM, and BEVs containing the active protease gingipains were translocated to the liver in mice, which resulted in changes in glucose metabolism and

reduced insulin sensitivity. *P. gingivalis* BEVs also attenuated insulin-induced Akt/GSK-3 β signaling in hepatic HepG2 cells [37, 197]. These results revealed a complex and multifaceted relationship between BEVs and host metabolism.

Musculoskeletal disease. Bone homeostasis is dynamically maintained by the balance of bone formation and bone absorption, and alterations in intestinal microbiota composition and metabolites are implicated in impaired bone metabolism by affecting mineral absorption and stimulating inflammation [198, 199]. Abnormal bone loss can be caused by aging, hormonal imbalance, and dietary factors, all of which have been documented to be accompanied by microbiome changes [41, 200–203]. In addition, a recent study by Liu et al. reported that gut microbiota from healthy children alleviated bone loss and altered bone metabolism in ovariectomy-mice by reversing the ovariectomy-induced reduction of *A. muciniphila*. Notably, the protective effects of *A. muciniphila* treatment against osteoporosis are largely attributed to the secretion of BEVs, which translocate and accumulate in bone tissues to promote osteogenic activity and inhibit osteoclast formation [204]. Similarly, Chen et al. also revealed that the composition of *Lactobacillus animalis*, a well-studied probiotic strain, was reduced in mice with glucocorticoid-induced osteonecrosis of the femoral head (ONFH), and oral supplementation with *L. animalis* significantly mitigated the extent of ONFH by promoting vascularization, osteogenesis, and suppressing cell apoptosis. Mechanistically, *L. animalis*-BEVs can selectively ferry various functional proteins and deliver them to the femoral head, whereby BEVs prevent trabecular bone damage and bone loss by improving the activity and function of endothelial and bone cells [205]. Additionally, it has been demonstrated that a shift in microbiota is identified as a key risk factor influencing the pathogenesis of rheumatoid arthritis (RA), which is a chronic inflammatory disease characterized by synovial inflammation, leading to progressive cartilage and bone destruction [201]. *P. gingivalis* is an important pathogenic bacterium linked to RA, and DNA derived from *P. gingivalis* has been detected in the synovial fluid of patients with RA [206]. Peptidylarginine deiminase (PAD), an enzyme in *P. gingivalis*, is reported to mediate the citrullination of human proteins and potentially contribute to the loss of tolerance to citrullinated proteins in RA. Gabarrini et al. found that PPAD can be modified by A-LPS and is associated with BEVs escaping proteolytic degradation [207]. Thus, BEV-dependent regulatory mechanisms may represent alternative pathways for maintaining bone homeostasis.

Cancer. Alterations in the diversity and function of microbiome homeostasis are associated with the

pathogenesis of diverse tumors. BEVs detached from the outer membrane can enter the circulatory system to disseminate to distant organs and tissues, possibly contributing to tumorigenesis. There are some reasonable tumor-promoting mechanisms of BEVs, including direct impact on genomic stability or cell cycle, generation of a proinflammatory tumor microenvironment, and production of certain carcinogens [208]. As mentioned above, TLRs are typical PRR-sensing BEV components, including nucleic acids, LPS, and peptidoglycans, and trigger temporal inflammation to ensure pathogenetic clearance from the body, while uncontrolled chronic inflammation also results in disease, including cancer [209]. For instance, metagenomic analyses have shown enrichment of *F. nucleatum* in colorectal carcinoma tissue, and BEVs released by *F. nucleatum* may drive oncogenesis by suppressing the immune response and eliciting inflammation [210, 211]. Similarly, *H. pylori*-derived BEVs were found to be upregulated in the gastric juice of patients with gastric cancer. *H. pylori*-derived BEVs containing CagA and VacA are suggested to hold capabilities of alteration of cell growth, damage to DNA, and aneuploidy, implying its tight association with gastric cancer [102]. Additionally, *P. gingivalis*, the major pathogen in periodontal disease, was recently shown to promote the progression of oral squamous cell carcinoma (OSCC). *P. gingivalis* BEVs are packaged with diverse sRNAs with the potential to target host mRNA function

and/or stability, of which sRNA23392 was found to promote invasion and migration of OSCC cells [212]. To date, various lines of evidence have shown that the impact of BEVs on oncogenesis is largely context-dependent and the potential role of BEVs in tumorigenesis are well investigated in multiple studies [102, 213–215]; however, the underlying mechanisms are still not fully elucidated.

BEVs IN CLINICAL APPLICATION

Based on the distinct features of BEVs for cost-effectiveness in production, stability to transport and storage, convenience for modification, and proven immunomodulatory properties, BEVs have emerged as candidates with strong clinical potential, including application in disease diagnoses, vaccine development, and novel therapeutics (Figure 4).

BEVs in diagnostic analytics

As discussed above, an imbalance in microbiome homeostasis has been strongly associated with the onset and progression of a wide range of human pathologies, and disease-associated microbiome changes may be reflected in the abundance and composition of BEVs in

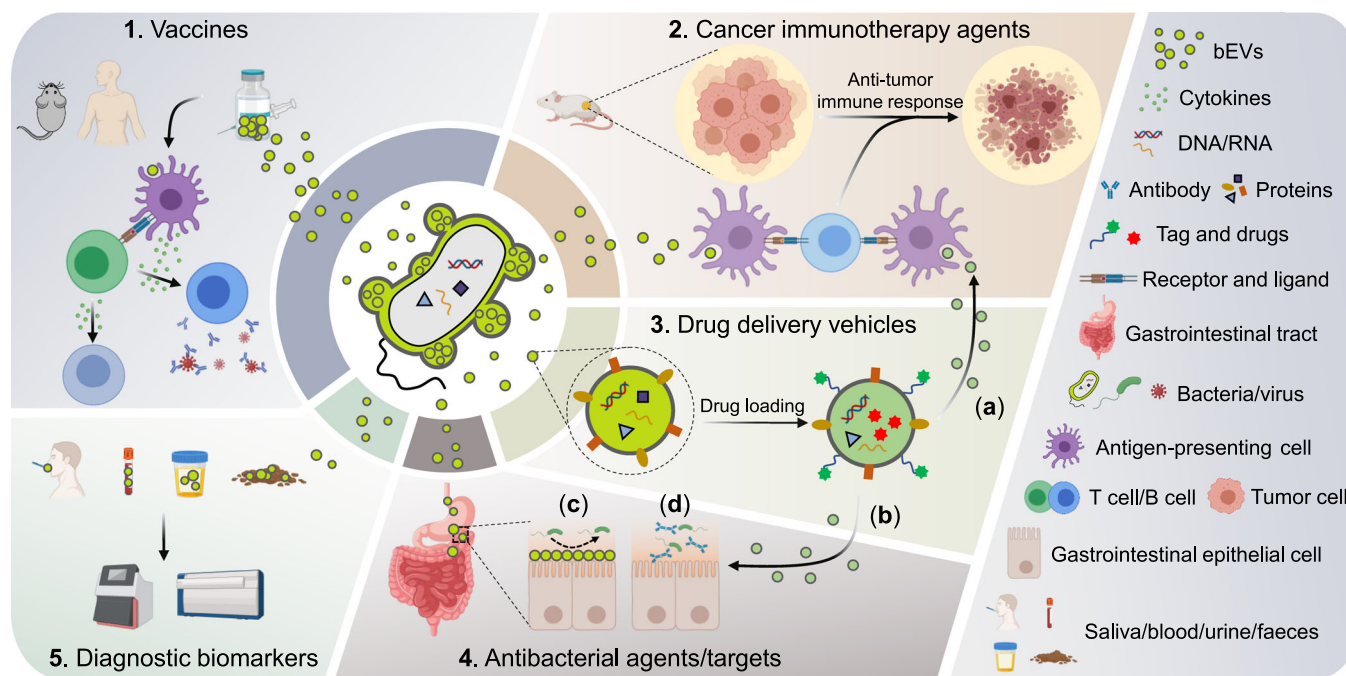


FIGURE 4 BEVs clinical applications at a glance. BEVs have emerged as candidates with strong clinical potential, including utilization in vaccine development, disease diagnoses, and novel therapeutics. Reprinted with permission [21]. Copyright 2020, Elsevier. BEV, bacterial extracellular vesicle.

biofluids [216]. BEVs released by microbiota can directly penetrate the mucosa and adjacent epithelium, reach the bloodstream, and subsequently travel throughout the body [217]. BEVs packaged with diverse parental genomic and metabolic information indicate their origin and reflect the composition and metabolic status of the microbiota. In this context, it is desirable to detect BEVs profiles in the urine, blood, and other bodily fluids to assist in the diagnosis of physiological and disease states.

In recent years, advanced and accurate meta-omics technologies (metagenomics and metabolomics) combined with bioinformatics analytic technologies have provided a detailed landscape of the phylogeny, interactions, and functions of BEVs in health and disease conditions. In contrast to human cell-derived EVs, the exploration of BEVs as biomarkers in disease is still in its infancy. To date, several BEV-based disease diagnosis models have been developed for Alzheimer's [218], atopic dermatitis [219], allergies [220], respiratory diseases [221], and cancer [222]. Despite these encouraging results, a variety of challenges still need to be addressed to facilitate clinical adoption. One of the issues is the requirement for a special technological platform to collect, screen, and interpret large datasets. In addition, the actual presence of BEV extracts is highly complex and depends on their source and isolation approaches, which largely weakens the sensitivity and specificity of BEVs' diagnostic efficiency.

Notably, BEV-based diagnostic models integrated with other diagnostic modalities can provide improved diagnostic performance. A combination of metagenomic and metabolomic analyses of BEVs isolated from the feces of patients with colorectal cancer and healthy participants demonstrated an association between microbial changes and metabolic alterations, indicating that BEVs carry a dynamic range of metabolic information reflecting the host's nutritional state, metabolism, and immune responses in the presence of disease [223]. Furthermore, BEV-metagenomics and immunoassays can be used to develop a novel diagnostic methodology. For example, exposure to BEVs in indoor dust is involved in the pathogenesis of respiratory diseases, and the combination of BEVs metagenomic characteristics assessment and ELISA-based analysis of serum IgGs against core pathogenic indoor dust BEVs was found to be an effective diagnostic tool for lung disease [224]. Therefore, BEV-based diagnostic technologies have the potential to be widely applied in the clinical setting.

BEVs in vaccine design

Rapid spread of antibiotic resistance has provoked an urgent need for novel antimicrobial therapeutics,

including vaccine formulations. BEVs are nonreplicating entities, with a size of 20–250 nm, and incorporate multiple PAMPs mimicking the immunogenic properties of the producing bacteria, which has increased the appeal of BEVs as attractive vaccine candidates [225]. In recent years, the potential of BEVs as vaccines against bacterial pathogens has been extensively investigated [226, 227].

As discussed before, BEVs are primarily composed of bacterial outer membrane constituents and thus contain key antigenic components, such as LPS, lipoproteins, and nucleic acids, to stimulate humoral and cellular immune responses. The intrinsic features of BEVs have been exploited in the field of vaccinology for decades. The most representative and successful BEV vaccine against *Neisseria meningitidis* was first licensed for use in Cuba as early as 1987 and subsequently employed as a vaccine to combat the outbreak of *meningococcal* group B disease in Norway, New Zealand [227]. Similarly, BEVs derived from many other Gram-negative and Gram-positive bacteria are being developed as candidate vaccines against pathogenic infections caused by, *V. cholerae*, *Klebsiella pneumoniae*, *Bordetella pertussis*, *S. typhimurium*, *S. aureus*, *S. pneumoniae*, and *Clostridium perfringens*. BEVs vaccines have immunostimulatory efficiency comparable to that of the whole inactivated cell vaccine, and animals vaccinated with BEVs displayed cell-mediated and humoral immune responses, resulting in decreased bacterial burden and lethal challenge [228–231]. These studies highlight the potential of BEVs in vaccine development.

In addition, BEVs can be developed as adjuvants to enhance and shape the quality and quantity of immune response. BEVs contain multiple PAMPs (such as LPS and lipoproteins) in their native conformations that interact with the corresponding PPRs (such as TLRs and NLRs), promoting APCs maturation and antigen-specific immune response activation [216]. For instance, recent studies have reported that coimmunization of *E. coli* BEVs with malaria transmission-blocking antigens in mice elicited robust humoral and cellular immune responses, and antibody titers were found to be comparable to conventional adjuvants, such as cholera toxin and MF59C.1, accompanied by no significant side effects or weight loss [232]. Similarly, BEVs also display strong adjuvant activity against influenza vaccine antigens and hepatitis B virus surface vaccine antigens [233, 234]. Intranasal injection of the influenza vaccine with BEVs acquired from a combination of ammonium sulfate precipitation and DGC, significantly stimulated antigen-specific humoral and cellular immune responses in mice, leading to a significantly higher protection rate against challenge with a lethal dose of homologous or heterologous influenza viruses without adverse effects [233].

These results demonstrate the potential of BEVs as safe and effective adjuvants for vaccine utilization.

Despite these promising data, several challenges must be addressed before BEVs can safely be used for vaccination, such as their high reactogenicity, heterogeneity, strain variation, and low expression of relevant protective antigens [227]. Genetic engineering techniques are under investigation to address these challenges by improving BEVs immunogenicity, safety, and production while removing BEVs toxins and deleterious compounds. Genetic manipulation of the parent bacteria can refine the functionality of BEVs; for instance, heterologous antigens can be anchored to native proteins located on the surface of BEVs, including the cytolysin A protein of *E. coli* or factor H binding protein (fHbp) of *N. meningitidis*, resulting in their association with OMVs at high concentration [235–237]. These engineered BEVs based vaccines have been successfully validated against pathogens in models of infection with H1N1 influenza, *A. baumannii*, and *S. pneumoniae* [236–238]. Furthermore, the issue of low yield efficiency that restricts the use of BEVs as potential vaccines is largely resolved by genetically engineered approaches to alter the structure and integrity of the bacterial outer membrane in hypervesiculating bacteria [235, 238]. However, it remains unclear whether these modifications to the bacterial outer membrane result in distinct characteristics of BEVs compared with their wild-type counterparts.

BEVs in therapeutics development

In recent years, precision medicine, also known as personalized medicine, has attracted increasing research interest, which enables the individualization of patient care from diagnosis to therapy. Given the inherent structural and functional characteristics of BEVs, such as nanoscale size, cell-free system, immunogenicity, and ease of genetic engineering editing, BEVs have shown great therapeutic potential in precision medicine, and their clinical applications have been widely explored.

Therefore, native or natural BEVs derived from commensal bacteria and probiotics may serve as potential therapeutic candidates. For example, *L. animalis* reduction has been associated with glucocorticoid-induced osteonecrosis of the femoral head. Administration of *L. animalis* BEVs greatly improved bone microarchitecture and mitigated the extent of osteonecrosis by enhancing blood vessel abundance, increasing osteogenic activity, and reducing apoptosis [205]. Direct replenishment of *A. muciniphila* BEVs has also been shown to restore multiple pathological events, including rebalancing bone

metabolism to prevent osteoporosis, improving glucose tolerance and obesity, and enhancing epithelial function to relieve IBD [193, 204, 239]. In addition, treatment with *Lactobacillus plantarum* BEVs showed therapeutic effects on stress-induced depression-like behaviors by altering the expression of neurotropic factors in the hippocampus of mice [240]. Therefore, BEV-based therapeutic strategies have emerged as novel and promising treatment modalities for many refractory diseases.

BEVs can also serve as potent drug delivery vehicles based on their intrinsic properties, such as loading capacity, specific targeting, and temperature stability. BEV-based delivery platforms present distinct advantages compared with conventional nanomaterials, such as liposomes, polymers, and metal-based nanoparticles, especially for intercell communication [241]. Researchers have developed various strategies to ensure successful loading of bioactive substances into BEVs; compounds or biomolecules of interest can be either actively encapsulated by parent cell engineering approaches or by directly modifying BEVs using exogenous incubation, permeabilization, electroporation, among others [242–245]. BEVs can be loaded with various effective proteins, RNA, and small-molecule drugs, thereby preventing enzymatic degradation, enabling controlled release, achieving distant delivery, and synergistically enhancing therapeutic effects [246–249]. In a dextran sodium sulfate-induced colitis animal model, *Bacteroides thetaiotaomicron* BEVs loaded with the human therapeutic protein keratinocyte growth factor-2 (KGF-2) in a stable form, are found to alleviate disease severity and promote intestinal epithelial repair and recovery [250]. In particular, the specificity of engineering BEVs to target tumor cells by conjugating them with ligands for tumor cell receptors, and their ability to protect their contents, such as chemotherapeutic agents, from protease or nuclease degradation may confer BEV-based anticancer therapies with greater specificity and fewer adverse side effects compared to traditional indiscriminate chemotherapies [242]. A previous study developed engineered *E. coli* BEVs decorated with HER2 ligands and loaded with specific antitumor siRNA, which showed high specificity to cancer cells and impressive tumor cell-killing effects [251]. Similarly, epidermal growth factor (EGF) receptors are commonly expressed on the surface of numerous tumor cells of epithelial origin; thus, engineered BEVs expressing EGF on the surface show high specificity to tumor cells and significantly hinder tumor growth and reduce tumor burden without notable adverse effects when loaded with chemotherapeutics [252]. Furthermore, BEV-based anticancer therapies may also be combined with other novel techniques, such as immunotherapeutics and nanotechnologies, to amplify the

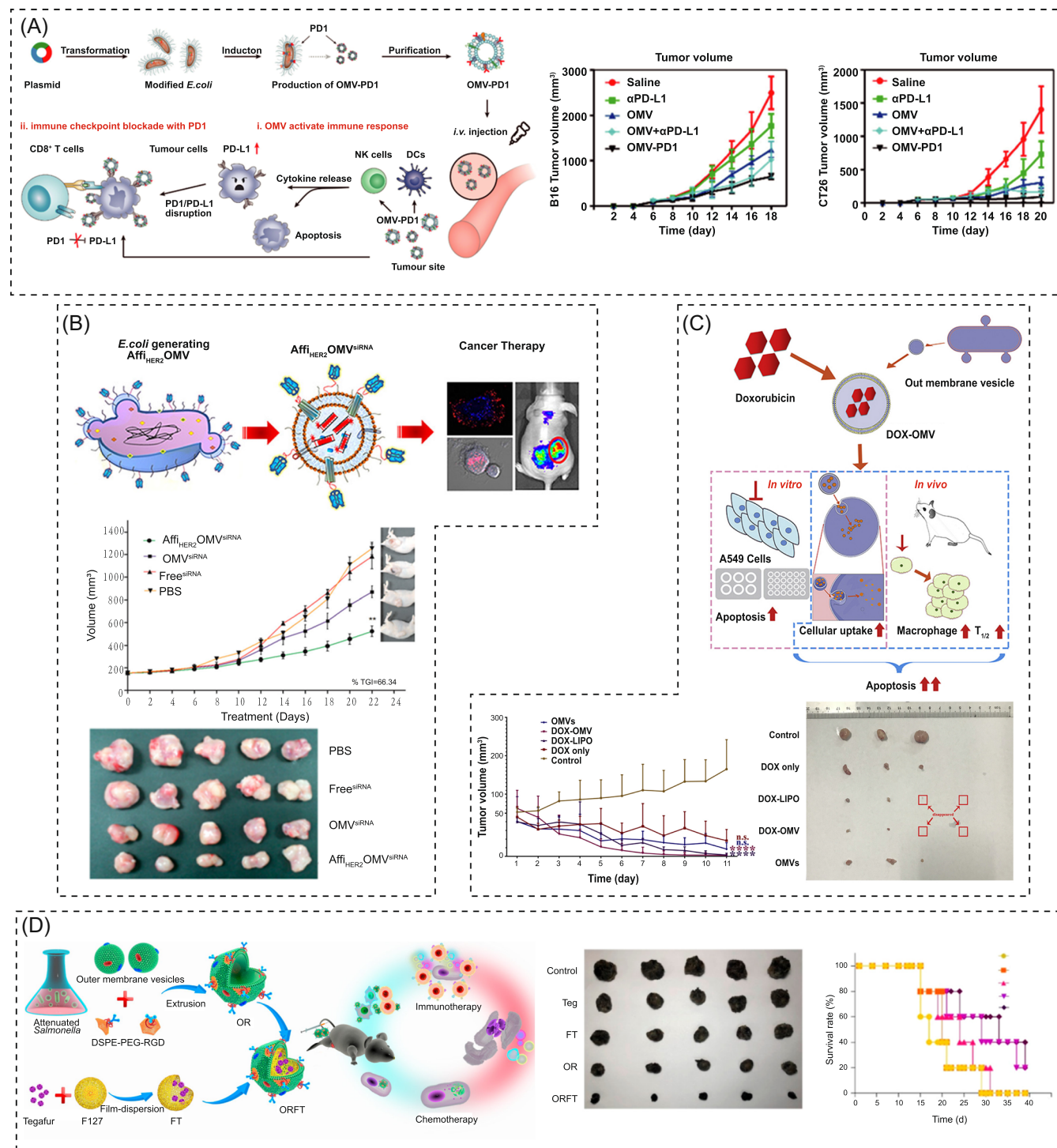


FIGURE 5 BEVs-based therapeutic application in cancer treatment. (A) schematic illustration of the development of genetically engineered BEVs that modified with PD1 overexpression, leading to enhanced immune activation and subsequent increased antitumor efficacy. Reproduced with permission [257]. Copyright 2020, American Chemical Society. (B) Schematic representation of the BEVs-based small interfering RNA delivery system, by which si-HER2 are translocated to tumor cells and contribute to targeted gene silencing and significant tumor growth regression. Reproduced with permission [251]. Copyright 2014, American Chemical Society. (C) schematic illustration of BEVs-based drug delivery system, doxorubicin-loaded BEVs exerted improved efficacy of tumor chemoimmunotherapy. Reproduced with permission [255]. Copyright 2020, Elsevier. (D) schematic illustration of combination of BEVs and nanomedicine approaches, BEVs coated on drug-loaded polymeric micelles were highly effective cancer immunotherapy and chemotherapy. Reproduced with permission [256]. Copyright 2020, American Chemical Society. BEV, bacterial extracellular vesicle.

therapeutic efficacy [253–257] (Figure 5A–D). To further enhance the functionality and expandability of BEVs, researchers have designed and constructed a hybrid eukaryotic–prokaryotic vesicle (EPV) nanoplatfrom by fusing attenuated *Salmonella* BEVs with tumor cell membranes and vesicles, which evoke potent tumor-specific immune responses. In addition, the EPV platform possesses high expandability to load the complementary photothermal modality and protects mice from melanoma challenge [258]. Thus, the combination of BEVs and other conventional treatments may shed light on novel therapeutics for cancer.

CONCLUDING REMARKS

Over the past decades, potent associations between microbiota homeostasis and human health, especially gut microbiota, have been intensively investigated. BEVs derived from various commensal and pathogenic bacteria have been shown to profoundly participate in a wide range of physiological and pathological processes and show great potential in biomedical applications. Within this context, our knowledge regarding the origin, biogenesis, composition, biofunctions, and clinical applications are rapidly expanding. Accumulating evidence has demonstrated that BEVs generation and release present a conserved and active secretory mechanism in which molecules of definite composition are destined to be entrapped in BEVs, thereby conferring their versatile biofunctions, including biofilm formation, antibiotic resistance, virulence factor delivery, inflammation, and immunomodulation. The biological properties of BEVs, including their size, content, and yield, are significantly influenced by multiple genetic and environmental factors. Moreover, BEVs shuttled bioactive molecules throughout the body may provide a novel observation window for the pathogenesis of diverse systemic diseases.

Furthermore, owing to their distinct characteristics of high biocompatibility, low toxicity, immunogenicity, ease of manipulation, and potential for cell targeting, BEVs are of great interest for broad utilization in clinical settings, including disease diagnoses, vaccine formulation, and novel therapeutics.

Despite the recent remarkable achievements in our knowledge of BEVs' generation and preliminary application, several puzzles remain regarding the underlying mechanism of BEVs' biogenesis and their exact functional role in disease. For example, BEVs can be released by cells in distinct forms, depending on their growth conditions, extent of stress, and species. Is there a universal pathway through which cells sense stimuli to drive BEV generation? How are cargo sorted and selectively packaged in BEVs? How do external factors influence the BEVs' content and release? How do BEVs distribute throughout the body and interact with host cells? Are they destined for specific target cells? Are BEVs' contents that have not been identified endowed with specific biological functions? In the future, deeper and broader cooperation in areas of high-throughput sequencing, multiomics approaches, artificial intelligence analytics, and nanobiotechnology may provide new lights on resolving these questions and facilitating translational applications. In addition, there are several ongoing challenges and technical issues in the biomedical utilization of BEVs. For example, techniques for BEV preparation, purification, and identification are still in their infancy, and more reliable, reproducible, efficient, and standardized BEV production procedures are needed. BEV-associated immunogens, such as LPS, are critical for vaccine development but can also elicit immunotoxicity and raise safety concerns. Thus, balancing the high efficacy of immunogenicity and eliminating immunotoxicity will be an intriguing topic in the future. Furthermore, BEV-based cell- or tissue-targeted therapeutics have shown great promise in precision medicine,

TABLE 1 Characteristics of the main types of extracellular vesicles (EVs).

	Type	Size (nm)	Biogenesis	Reference
Eukaryotic EVs	Exosomes	30–150	Released by multivesicular bodies fusing with plasma membrane	[30, 31, 71]
	Microvesicles	100–1000	Outward budding of the plasma membrane	[30, 31, 71]
	Apoptotic bodies	>1000	Programmed cell death	[30, 31, 71]
Bacterial EVs	Outer membrane vesicles	20–300	Blebbing of the outer membrane of Gram-negative bacteria	[39, 71, 110]
	Outer-inner membrane vesicle	60–160	Explosive cell lysis and cell budding of Gram-negative bacteria	[39, 43, 44]
	Cytoplasmic membrane vesicles	20–400	Extrusion of the cell membrane and release through cell wall pores or holes of Gram-positive bacteria	[39, 71, 110]

especially for cancer treatment, while their accuracy, specificity, and efficiency need to be further improved.

Collectively, research in the field of BEVs is rapidly evolving and will continue to revolutionize our understanding of BEVs' biology, particularly BEVs' biogenesis, biofunctions, and biomedical applications. The BEV-based platform is expected to offer an innovative way to think about pathogenesis and treatment modalities for various conditions (Table 1).

AUTHOR CONTRIBUTIONS

Rongjin Luo, Yanmin Chang, and Huaizhen Liang: conceptualization, literature review, and writing - original draft; **Weifeng Zhang and Yu Song:** literature review, visualization; **Gaocai Li and Cao Yang:** supervision, writing - review & editing, and funding acquisition. All authors have read the final manuscript and approved it for publication.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

No new data and script were used in this paper. Supporting Information materials (figures, tables, scripts, graphical abstract, slides, videos, Chinese translated version and updated materials) may be found in the online DOI or iMeta Science <http://www.imeta.science/>.

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REFERENCES

- Belkaid, Yasmine, and Timothy W. Hand. 2014. "Role of the Microbiota in Immunity and Inflammation." *Cell* 157: 121–41. <https://doi.org/10.1016/j.cell.2014.03.011>
- Jenkins, Samir V., Kieng B. Vang, Allen Gies, Robert J. Griffin, Se-Ran Jun, Intawat Nookaew, and Ruud P. M. Dings. 2018. "Sample Storage Conditions Induce Post-Collection Biases in Microbiome Profiles." *BMC Microbiology* 18: 227. <https://doi.org/10.1186/s12866-018-1359-5>
- Ferreira, Miguel R., Ann Muls, David P. Dearnaley, and H. Jervoise N. Andreyev. 2014. "Microbiota and Radiation-Induced Bowel Toxicity: Lessons From Inflammatory Bowel Disease for the Radiation Oncologist." *The Lancet Oncology* 15: e139–47. [https://doi.org/10.1016/s1470-2045\(13\)70504-7](https://doi.org/10.1016/s1470-2045(13)70504-7)
- Willing, Benjamin P., Shannon L. Russell, and B. Brett Finlay. 2011. "Shifting the Balance: Antibiotic Effects on Host-Microbiota Mutualism." *Nature Reviews Microbiology* 9: 233–43. <https://doi.org/10.1038/nrmicro2536>
- Gill, Steven R., Mihai Pop, Robert T. Deboy, Paul B. Eckburg, Peter J. Turnbaugh, Buck S. Samuel, Jeffrey I. Gordon, et al. 2006. "Metagenomic Analysis of the Human Distal Gut Microbiome." *Science* 312: 1355–59. <https://doi.org/10.1126/science.1124234>
- Lozupone, Catherine A., Jesse Stombaugh, Antonio Gonzalez, Gail Ackermann, Doug Wendel, Yoshiki Vázquez-Baeza, Janet K. Jansson, Jeffrey I. Gordon, and Rob Knight. 2013. "Meta-Analyses of Studies of the Human Microbiota." *Genome Research* 23: 1704–14. <https://doi.org/10.1101/gr.151803.112>
- Moloney, Rachel D., Lieve Desbonnet, Gerard Clarke, Timothy G. Dinan, and John F. Cryan. 2014. "The Microbiome: Stress, Health and Disease." *Mammalian Genome* 25: 49–74. <https://doi.org/10.1007/s00335-013-9488-5>
- Cryan, John F., and Timothy G. Dinan. 2012. "Mind-Altering Microorganisms: The Impact of the Gut Microbiota on Brain and Behaviour." *Nature Reviews Neuroscience* 13: 701–12. <https://doi.org/10.1038/nrn3346>
- Köhling, Hedda L., Sue F. Plummer, Julian R. Marchesi, Kelly S. Davidge, and Marian Ludgate. 2017. "The Microbiota and Autoimmunity: Their Role in Thyroid Autoimmune Diseases." *Clinical Immunology* 183: 63–74. <https://doi.org/10.1016/j.clim.2017.07.001>
- Fröhlich, Eleonore, and Richard Wahl. 2019. "Microbiota and Thyroid Interaction in Health and Disease." *Trends in Endocrinology & Metabolism* 30: 479–90. <https://doi.org/10.1016/j.tem.2019.05.008>
- van Niel, Guillaume, David R. F. Carter, Aled Clayton, Daniel W. Lambert, Graça Raposo, and Pieter Vader. 2022. "Challenges and Directions in Studying Cell-Cell Communication by Extracellular Vesicles." *Nature Reviews Molecular Cell Biology* 23: 369–82. <https://doi.org/10.1038/s41580-022-00460-3>
- Martínez, M. Carmen, and Ramarosan Andriantsitohaina. 2017. "Extracellular Vesicles in Metabolic Syndrome." *Circulation Research* 120: 1674–86. <https://doi.org/10.1161/CIRCRESAHA.117.309419>
- Buzas, Edit I. 2022. "The Roles of Extracellular Vesicles in the Immune System." *Nature Reviews in Immunology* 4: 1–15. <https://doi.org/10.1038/s41577-022-00763-8>
- Ñahui Palomino, Rogers A., Christophe Vanpouille, Paolo E. Costantini, and Leonid Margolis. 2021. "Microbiota-Host Communications: Bacterial Extracellular Vesicles As a Common Language." *PLoS Pathogens* 17: e1009508. <https://doi.org/10.1371/journal.ppat.1009508>
- Raposo, Graça, and Philip D. Stahl. 2019. "Extracellular Vesicles: A New Communication Paradigm?" *Nature Reviews Molecular Cell Biology* 20: 509–10. <https://doi.org/10.1038/s41580-019-0158-7>
- Lee, Kyung-Eon, Jeon-Kyung Kim, Sang-Kap Han, Dong Yun Lee, Hae-Ji Lee, Sung-Vin Yim, and Dong-Hyun Kim. 2020. "The Extracellular Vesicle of Gut Microbial *Paenalcigenes Hominis* is a Risk Factor for Vagus Nerve-Mediated Cognitive Impairment." *Microbiome* 8: 107. <https://doi.org/10.1186/s40168-020-00881-2>
- Erttmann, Saskia F., Patrycja Swacha, Kyaw Min Aung, Björn Brindefalk, Hui Jiang, Anetta Härtlova, Bernt Eric Uhlin, Sun N. Wai, and Nelson O. Gekara. 2022. "The Gut Microbiota

- Prime Systemic Antiviral Immunity Via the cGAS-STING-IFN-I Axis." *Immunity* 55: 847–861. <https://doi.org/10.1016/j.immuni.2022.04.006>
18. Gilmore, William J., Ella L. Johnston, Lauren Zavan, Natalie J. Bitto, and Maria Kaparakis-Liaskos. 2021. "Immunomodulatory Roles and Novel Applications of Bacterial Membrane Vesicles." *Molecular Immunology* 134: 72–85. <https://doi.org/10.1016/j.molimm.2021.02.027>
19. Jung, Anna Lena, Bernd Schmeck, Marie Wiegand, Katrin Bedenbender, and Birke J. Benedikter. 2021. "The Clinical Role of Host and Bacterial-Derived Extracellular Vesicles in Pneumonia." *Advanced Drug Delivery Reviews* 176: 113811. <https://doi.org/10.1016/j.addr.2021.05.021>
20. Wang, Zhenzhen, Kristen D. Popowski, Dashuai Zhu, Blanca López de Juan Abad, Xianyun Wang, Mengrui Liu, Halle Lutz, et al. 2022. "Exosomes Decorated With a Recombinant SARS-CoV-2 Receptor-Binding Domain as an Inhalable COVID-19 Vaccine." *Nature Biomedical Engineering* 6: 791–805. <https://doi.org/10.1038/s41551-022-00902-5>
21. Xie, Junhua, Qiqiong Li, Freddy Haesebrouck, Lien Van Hoecke, and Roosmarijn E. Vandenbroucke. 2022. "The Tremendous Biomedical Potential of Bacterial Extracellular Vesicles." *Trends in Biotechnology* 40: 1173–94. <https://doi.org/10.1016/j.tibtech.2022.03.005>
22. Chatterjee, S. N., and J. Das. 1967. "Electron Microscopic Observations on the Excretion of Cell-Wall Material by *Vibrio Cholerae*." *Journal of General Microbiology* 49: 1–11. <https://doi.org/10.1099/00221287-49-1-1>
23. Pan, Bin-Tao, and Rose M. Johnstone. 1983. "Fate of the Transferrin Receptor During Maturation of Sheep Reticulocytes In Vitro: Selective Externalization of the Receptor." *Cell* 33: 967–78. [https://doi.org/10.1016/0092-8674\(83\)90040-5](https://doi.org/10.1016/0092-8674(83)90040-5)
24. Valadi, Hadi, Karin Ekström, Apostolos Bossios, Margareta Sjöstrand, James J. Lee, and Jan O. Lötval. 2007. "Exosome-Mediated Transfer of mRNAs and microRNAs is a Novel Mechanism of Genetic Exchange Between Cells." *Nature Cell Biology* 9: 654–59. <https://doi.org/10.1038/ncb1596>
25. Colombo, Marina, Graça Raposo, and Clotilde Théry. 2014. "Biogenesis, Secretion, and Intercellular Interactions of Exosomes and Other Extracellular Vesicles." *Annual Review of Cell and Developmental Biology* 30: 255–89. <https://doi.org/10.1146/annurev-cellbio-101512-122326>
26. Ahmadi Badi, Sara, Arfa Moshiri, Abolfazl Fateh, Fatemeh Rahimi Jamnani, Meysam Sarshar, Farzam Vaziri, and Seyed Davar Siadat. 2017. "Microbiota-Derived Extracellular Vesicles as New Systemic Regulators." *Frontiers in Microbiology* 8: 1610. <https://doi.org/10.3389/fmicb.2017.01610>
27. Gould, Stephen J., and Graça Raposo. 2013. "As we Wait: Coping With an Imperfect Nomenclature for Extracellular Vesicles." *Journal of Extracellular Vesicles* 2: 20389. <https://doi.org/10.3402/jev.v2i0.20389>
28. Witwer, Kenneth W., Deborah Ci Goberdhan, Lorraine O'Driscoll, Clotilde Théry, Joshua A. Welsh, Cherie Blenkinsop, Edit I. Buzás, et al. 2021. "Updating MISEV: Evolving the Minimal Requirements for Studies of Extracellular Vesicles." *Journal of Extracellular Vesicles* 10: e12182. <https://doi.org/10.1002/jev2.12182>
29. Lötval, Jan, Andrew F. Hill, Fred Hochberg, Edit I. Buzás, Dolores Di Vizio, Christopher Gardiner, Yong Song Gho, et al. 2014. "Minimal Experimental Requirements for Definition Of Extracellular Vesicles and Their Functions: A Position Statement From the International Society for Extracellular Vesicles." *Journal of Extracellular Vesicles* 3: 26913. <https://doi.org/10.3402/jev.v3.26913>
30. Gandham, Srujan, Xianyi Su, Jacqueline Wood, Angela L. Nocera, Sarath Chandra Alli, Lara Milane, Alan Zimmerman, Mansoor Amiji, and Alexander R. Ivanov. 2020. "Technologies and Standardization in Research on Extracellular Vesicles." *Trends in Biotechnology* 38: 1066–98. <https://doi.org/10.1016/j.tibtech.2020.05.012>
31. van Niel, Guillaume, Gisela D'Angelo, and Graça Raposo. 2018. "Shedding Light on the Cell Biology of Extracellular Vesicles." *Nature Reviews Molecular Cell Biology* 19: 213–28. <https://doi.org/10.1038/nrm.2017.125>
32. Bebelman, Maarten P., Martine J. Smit, D. Michiel Pegtel, and S. Rubina Baglio. 2018. "Biogenesis and Function of Extracellular Vesicles in Cancer." *Pharmacology & Therapeutics* 188: 1–11. <https://doi.org/10.1016/j.pharmthera.2018.02.013>
33. Abels, Erik R., and Xandra O. Breakefield. 2016. "Introduction to Extracellular Vesicles: Biogenesis, RNA Cargo Selection, Content, Release, and Uptake." *Cellular and Molecular Neurobiology* 36: 301–12. <https://doi.org/10.1007/s10571-016-0366-z>
34. Maas, Sybren L. N., Xandra O. Breakefield, and Alissa M. Weaver. 2017. "Extracellular Vesicles: Unique Intercellular Delivery Vehicles." *Trends in Cell Biology* 27: 172–88. <https://doi.org/10.1016/j.tcb.2016.11.003>
35. Mathieu, Mathilde, Lorena Martin-Jaular, Grégory Lavie, and Clotilde Théry. 2019. "Specificities of Secretion and Uptake of Exosomes and Other Extracellular Vesicles for Cell-to-Cell Communication." *Nature Cell Biology* 21: 9–17. <https://doi.org/10.1038/s41556-018-0250-9>
36. Cryan, John F., Kenneth J. O'Riordan, Caitlin S. M. Cowan, Kiran V. Sandhu, Thomaz F. S. Bastiaanssen, Marcus Boehme, Martin G. Codagnone, et al. 2019. "The Microbiota-Gut-Brain Axis." *Physiological Reviews* 99: 1877–2013. <https://doi.org/10.1152/physrev.00018.2018>
37. Seyama, Mariko, Kaya Yoshida, Kayo Yoshida, Natsumi Fujiwara, Kisho Ono, Takanori Eguchi, Hotaka Kawai, et al. 2020. "Outer Membrane Vesicles of *Porphyromonas gingivalis* Attenuate Insulin Sensitivity by Delivering Gingipains to the Liver." *Biochimica et Biophysica Acta (BBA)—Molecular Basis of Disease* 1866: 165731. <https://doi.org/10.1016/j.bbadis.2020.165731>
38. Brameyer, Sophie, Plener Laure, Muller Axel, Klingl Andreas, Wanner Gerhard, and Jung Kirsten. 2018. "Outer Membrane Vesicles Facilitate Trafficking of the Hydrophobic Signaling Molecule CAI-1 Between *Vibrio harveyi* Cells." *Journal of Bacteriology* 200: e00740-17. <https://doi.org/10.1128/JB.00740-17>
39. Toyofuku, Masanori, Nobuhiko Nomura, and Leo Eberl. 2019. "Types and Origins of Bacterial Membrane Vesicles." *Nature Review Microbiology* 17: 13–24. <https://doi.org/10.1038/s41579-018-0112-2>
40. Kaparakis-Liaskos, Maria, and Richard L. Ferrero. 2015. "Immune Modulation by Bacterial Outer Membrane

- Vesicles." *Nature Review Immunology* 15: 375–87. <https://doi.org/10.1038/nri3837>
41. Wang, Zhongxiang, Kai Chen, Congcong Wu, Junhao Chen, Hao Pan, Yangbo Liu, Peng Wu, et al. 2021. "An Emerging Role of Prevotella Histicola on Estrogen Deficiency-Induced Bone Loss Through the Gut Microbiota-Bone Axis in Postmenopausal Women and in Ovariectomized Mice." *American Journal of Clinical Nutrition* 114: 1304–13. <https://doi.org/10.1093/ajcn/nqab194>
 42. Li, Jie, Farooq Azam, and Si Zhang. 2016. "Outer Membrane Vesicles Containing Signalling Molecules and Active Hydrolytic Enzymes Released by a Coral Pathogen *Vibrio shilonii* AK1." *Environmental Microbiology* 18: 3850–66. <https://doi.org/10.1111/1462-2920.13344>
 43. Pérez-Cruz, Carla, Lidia Delgado, Carmen López-Iglesias, and Elena Mercade. 2015. "Outer-Inner Membrane Vesicles Naturally Secreted by Gram-Negative Pathogenic Bacteria." *PLoS One* 10: e0116896. <https://doi.org/10.1371/journal.pone.0116896>
 44. Turnbull, Lynne, Masanori Toyofuku, Amelia L. Hynen, Masaharu Kurosawa, Gabriella Pessi, Nicola K. Petty, Sarah R. Osvath, et al. 2016. "Explosive Cell Lysis as a Mechanism for the Biogenesis of Bacterial Membrane Vesicles and Biofilms." *Nature Communications* 7: 11220. <https://doi.org/10.1038/ncomms11220>
 45. Brown, Lisa, Julie M. Wolf, Rafael Prados-Rosales, and Arturo Casadevall. 2015. "Through the Wall: Extracellular Vesicles in Gram-Positive Bacteria, Mycobacteria and Fungi." *Nature Review Microbiology* 13: 620–30. <https://doi.org/10.1038/nrmicro3480>
 46. Briaud, Paul, and Ronan K. Carroll. 2020. "Extracellular Vesicle Biogenesis and Functions in Gram-Positive Bacteria." *Infection and Immunity* 88: e00433-20. <https://doi.org/10.1128/IAI.00433-20>
 47. Resch, Ulrike, James A. Tsatsaronis, Anaïs Le Rhun, Gerald Stübiger, Manfred Rohde, Sergo Kasvandik, Susanne Holzmeister, et al. 2016. "A Two-Component Regulatory System Impacts Extracellular Membrane-Derived Vesicle Production in Group A Streptococcus." *mBio* 7: e0020716. <https://doi.org/10.1128/mBio.00207-16>
 48. Remis, Jonathan P., Dongguang Wei, Amita Gorur, Marcin Zemla, Jessica Haraga, Simon Allen, H. Ewa Witkowska, et al. 2014. "Bacterial Social Networks: Structure and Composition of *Myxococcus xanthus* Outer Membrane Vesicle Chains." *Environmental Microbiology* 16: 598–610. <https://doi.org/10.1111/1462-2920.12187>
 49. Wei, Xueming, Christopher N. Vassallo, Darshankumar T. Pathak, and Daniel Wall. 2014. "Myxobacteria Produce Outer Membrane-Enclosed Tubes in Unstructured Environments." *Journal of Bacteriology* 196: 1807–14. <https://doi.org/10.1128/JB.00850-13>
 50. Baidya, Amit K., Saurabh Bhattacharya, Gyanendra P. Dubey, Gideon Mamou, and Sigal Ben-Yehuda. 2018. "Bacterial Nanotubes: A Conduit for Intercellular Molecular Trade." *Current Opinion in Microbiology* 42: 1–6. <https://doi.org/10.1016/j.mib.2017.08.006>
 51. Fung, J., T. J. MacAlister, and L. I. Rothfield. 1978. "Role of Murein Lipoprotein in Morphogenesis of the Bacterial Division Septum: Phenotypic Similarity of Lkyd and Lpo Mutants." *Journal of Bacteriology* 133: 1467–71. <https://doi.org/10.1128/jb.133.3.1467-1471.1978>
 52. Cascales, Eric, Alain Bernadac, Marthe Gavioli, Jean-Claude Lazzaroni, and Roland Llobes. 2002. "Pal Lipoprotein of *Escherichia coli* Plays a major Role in Outer Membrane Integrity." *Journal of Bacteriology* 184: 754–59. <https://doi.org/10.1128/JB.184.3.754-759.2002>
 53. Pantua, Homer, Elizabeth Skippington, Marie-Gabrielle Braun, Cameron L. Noland, Jingyu Diao, Yutian Peng, Susan L. Gloor, et al. 2020. "Unstable Mechanisms of Resistance to Inhibitors of *Escherichia coli* Lipoprotein Signal Peptidase." *mBio* 11: e0201820. <https://doi.org/10.1128/mBio.02018-20>
 54. Roy, Rakesh, Ren-In You, Chan-Hua Chang, Chiou-Ying Yang, and Nien-Tsung Lin. 2021. "Carboxy-Terminal Processing Protease Controls Production of Outer Membrane Vesicles and Biofilm in *Acinetobacter baumannii*." *Microorganisms* 9: 1336. <https://doi.org/10.3390/microorganisms9061336>
 55. Marchant, Pedro, Alexander Carreño, Eduardo Vivanco, Andrés Silva, Jan Nevermann, Carolina Otero, Eyleen Araya, et al. 2021. "One for All": Functional Transfer of OMV-Mediated Polymyxin B Resistance From *Salmonella Enterica* Sv. Typhi ΔtolR and ΔdegS to Susceptible Bacteria." *Frontiers in Microbiology* 12: 672467. <https://doi.org/10.3389/fmicb.2021.672467>
 56. Roier, Sandro, Franz G. Zingl, Fatih Cakar, Sanel Durakovic, Paul Kohl, Thomas O. Eichmann, Lisa Klug, et al. 2016. "A Novel Mechanism for the Biogenesis of Outer Membrane Vesicles in Gram-Negative Bacteria." *Nature Communications* 7: 10515. <https://doi.org/10.1038/ncomms10515>
 57. Henderson, Jeremy C., Shawn M. Zimmerman, Alexander A. Crofts, Joseph M. Boll, Lisa G. Kuhns, Carmen M. Herrera, and M. Stephen Trent. 2016. "The Power of Asymmetry: Architecture and Assembly of the Gram-Negative Outer Membrane Lipid Bilayer." *Annual Reviews of Microbiology* 70: 255–78. <https://doi.org/10.1146/annurev-micro-102215-095308>
 58. Kadurugamuwa, J. L., and T. J. Beveridge. 1995. "Virulence Factors Are Released From *Pseudomonas Aeruginosa* in Association With Membrane Vesicles During Normal Growth and Exposure to Gentamicin: A Novel Mechanism of Enzyme Secretion." *Journal of Bacteriology* 177: 3998–4008. <https://doi.org/10.1128/jb.177.14.3998-4008.1995>
 59. Nguyen, T. T. 2003. "Effect of Surface Lipopolysaccharide on the Nature of Membrane Vesicles Liberated From the Gram-Negative Bacterium *Pseudomonas Aeruginosa*." *Journal of Electron Microscopy* 52: 465–69. <https://doi.org/10.1093/jmicro/52.5.465>
 60. Haurat, M. Florencia, Joseph Aduse-Opoku, Minnie Rangarajan, Loredana Dorobantu, Murray R. Gray, Michael A. Curtis, and Mario F. Feldman. 2011. "Selective Sorting of Cargo Proteins into Bacterial Membrane Vesicles." *Journal of Biological Chemistry* 286: 1269–76. <https://doi.org/10.1074/jbc.M110.185744>
 61. Gui, M. J., S. G. Dashper, N. Slakeski, Y. Y. Chen, and E. C. Reynolds. 2016. "Spheres of Influence: Porphyromonas Gingivalis Outer Membrane Vesicles." *Molecular Oral Microbiology* 31: 365–78. <https://doi.org/10.1111/omi.12134>
 62. Schlatterer, Katja, Christian Beck, Dennis Hanzelmann, Marco Lebtig, Birgit Fehrenbacher, Martin Schaller,

- Patrick Ebner, et al. 2018. "The Mechanism Behind Bacterial Lipoprotein Release: Phenol-Soluble Modulins Mediate Toll-Like Receptor 2 Activation via Extracellular Vesicle Release From *Staphylococcus aureus*." *mBio* 9: e0185118. <https://doi.org/10.1128/mBio.01851-18>
63. Surve, Manalee V., Anjali Anil, Kshama G. Kamath, Smita Bhutda, Lakshmi Kavitha Sthanam, Arpan Pradhan, Rohit Srivastava, et al. 2016. "Membrane Vesicles of Group B Streptococcus Disrupt Feto-Maternal Barrier Leading to Preterm Birth." *PLoS Pathogens* 12: e1005816. <https://doi.org/10.1371/journal.ppat.1005816>
 64. Roier, Sandro, Franz G. Zingl, Fatih Cakar, Sanel Durakovic, Paul Kohl, Thomas O. Eichmann, Lisa Klug, et al. 2016. "A Novel Mechanism for the Biogenesis of Outer Membrane Vesicles in Gram-Negative Bacteria." *Nature Communications* 7: 10515. <https://doi.org/10.1038/ncomms10515>
 65. Wang, Xiaogang, Christopher D. Thompson, Christopher Weidenmaier, and Jean C. Lee. 2018. "Release of *Staphylococcus aureus* Extracellular Vesicles and Their Application as a Vaccine Platform." *Nature Communications* 9: 1379. <https://doi.org/10.1038/s41467-018-03847-z>
 66. Typas, Athanasios, Manuel Banzhaf, Carol A. Gross, and Waldemar Vollmer. 2011. "From the Regulation of Peptidoglycan Synthesis to Bacterial Growth and Morphology." *Nature Reviews. Microbiology* 10: 123–36. <https://doi.org/10.1038/nrmicro2677>
 67. Abrahams, Katherine A., and Gurdyal S. Besra. 2018. "Mycobacterial Cell Wall Biosynthesis: A Multifaceted Antibiotic Target." *Parasitology* 145: 116–33. <https://doi.org/10.1017/S0031182016002377>
 68. Jeon, Jinseong, Hyuck J. Mok, Youngwoo Choi, Seung C. Park, Hunho Jo, Jin Her, Jin-Kwan Han, et al. 2017. "Proteomic Analysis of Extracellular Vesicles Derived From *Propionibacterium Acnes*." *Proteomics—Clinical Applications* 11: 1600040. <https://doi.org/10.1002/prca.201600040>
 69. Lee, Eun-Young, Do-Young Choi, Dae-Kyum Kim, Jung-Wook Kim, Jung Ok Park, Sungjee Kim, Sang-Hyun Kim, et al. 2009. "Gram-Positive Bacteria Produce Membrane Vesicles: Proteomics-Based Characterization of *Staphylococcus aureus*-derived Membrane Vesicles." *Proteomics* 9: 5425–36. <https://doi.org/10.1002/pmic.200900338>
 70. Kulp, Adam J., Bo Sun, Teresa Ai, Andrew J. Manning, Nichole Orench-Rivera, Amy K Schmid, and Meta J Kuehn. 2015. "Genome-Wide Assessment of Outer Membrane Vesicle Production in *Escherichia coli*." *PLoS One* 10: e0139200. <https://doi.org/10.1371/journal.pone.0139200>
 71. Gill, Sukhvinder, Ryan Catchpole, and Patrick Forterre. 2019. "Extracellular Membrane Vesicles in the Three Domains of Life and Beyond." *FEMS Microbiology Reviews* 43: 273–303. <https://doi.org/10.1093/femsre/fuy042>
 72. Nevermann, Jan, Andrés Silva, Carolina Otero, Diego P. Oyarzún, Boris Barrera, Fernando Gil, Iván L. Calderón, and Juan A. Fuentes. 2019. "Identification of Genes Involved in Biogenesis of Outer Membrane Vesicles (OMVs) in *Salmonella enterica* Serovar Typhi." *Frontiers In Microbiology* 10: 104. <https://doi.org/10.3389/fmicb.2019.00104>
 73. Bonnington, Katherine E., and Meta J. Kuehn. 2016. "Outer Membrane Vesicle Production Facilitates LPS Remodeling and Outer Membrane Maintenance in *Salmonella* During Environmental Transitions." *mBio* 7: e01532-16. <https://doi.org/10.1128/mBio.01532-16>
 74. Cao, Yina, Yan Zhou, Dongru Chen, Ruixue Wu, Lihong Guo, and Huancai Lin. 2020. "Proteomic and Metabolic Characterization of Membrane Vesicles Derived From *Streptococcus Mutans* at Different pH Values." *Applied Microbiology and Biotechnology* 104: 9733–48. <https://doi.org/10.1007/s00253-020-10563-6>
 75. Klimentova, Jana, Ivona Pavkova, Lenka Horcickova, Jan Bavlovic, Olga Kofronova, Oldrich Benada, and Jiri Stulik. 2019. "Francisella Tularensis Subsp. Holarctica Releases Differentially Loaded Outer Membrane Vesicles Under Various Stress Conditions." *Frontiers in Microbiology* 10: 2304. <https://doi.org/10.3389/fmicb.2019.02304>
 76. Obana, Nozomu, Ryoma Nakao, Kyoko Nagayama, Kouji Nakamura, Hidenobu Senpuku, and Nobuhiko Nomura. 2017. "Immunoactive Clostridial Membrane Vesicle Production is Regulated by a Sporulation Factor." *Infection and Immunity* 85: 00096-17. <https://doi.org/10.1128/IAI.00096-17>
 77. Malabirade, Antoine, Janine Habier, Anna Heintz-Buschart, Patrick May, Julien Godet, Rashi Halder, Alton Etheridge, et al. 2018. "The RNA Complement of Outer Membrane Vesicles From *Salmonella enterica* Serovar Typhimurium Under Distinct Culture Conditions." *Frontiers in Microbiology* 9: 2015. <https://doi.org/10.3389/fmicb.2018.02015>
 78. Konečná, Klára, Jana Klimentová, Oldřich Benada, Ivana Němečková, Ondřej Jandourek, Petr Jílek, and Marcela Vejsová. 2019. "A Comparative Analysis of Protein Virulence Factors Released Via Extracellular Vesicles in Two *Candida albicans* Strains Cultivated in a Nutrient-Limited Medium." *Microbial Pathogenesis* 136: 103666. <https://doi.org/10.1016/j.micpath.2019.103666>
 79. Turner, Lorinda, Natalie J. Bitto, David L. Steer, Camden Lo, Kimberley D'Costa, Georg Ramm, Mitch Shambrook, et al. 2018. "*Helicobacter pylori* Outer Membrane Vesicle Size Determines Their Mechanisms of Host Cell Entry and Protein Content." *Frontiers in Immunology* 9: 1466. <https://doi.org/10.3389/fimmu.2018.01466>
 80. Kim, Y.-S., E.-J. Choi, W.-H. Lee, S.-J. Choi, T.-Y. Roh, J. Park, Y.-K. Jee, et al. 2013. "Extracellular Vesicles, Especially Derived From Gram-Negative Bacteria, in Indoor Dust Induce Neutrophilic Pulmonary Inflammation Associated With Both Th1 and Th17 Cell Responses." *Clinical and Experimental Allergy* 43: 443–54. <https://doi.org/10.1111/cea.12085>
 81. Stentz, Régis, Emily Jones, Rokas Juodeikis, Udo Wegmann, Maria Guirro, Andrew J. Goldson, Arlaine Brion, et al. 2022. "The Proteome of Extracellular Vesicles Produced by the Human Gut Bacteria *Bacteroides Thetaiotaomicron* In Vivo Is Influenced by Environmental and Host-Derived Factors." *Applied and Environmental Microbiology* 88: e0053322. <https://doi.org/10.1128/aem.00533-22>
 82. Zaborowski, Mikołaj P., Leonora Balaj, Xandra O. Breakefield, and Charles P. Lai. 2015. "Extracellular Vesicles: Composition, Biological Relevance, and Methods of Study." *Bioscience* 65: 783–97. <https://doi.org/10.1093/biosci/biv084>
 83. Lee, Eun-Young, Dong-Sic Choi, Kwang-Pyo Kim, and Yong Song Gho. 2008. "Proteomics of Gram-Negative

- Bacterial Outer Membrane Vesicles." *Mass Spectrometry Reviews* 27: 535–55. <https://doi.org/10.1002/mas.20175>
84. Liu, Jinjing, Ching-Lin Hsieh, Ozkan Gelincik, Bryan Devolder, Shizuko Sei, Sheng Zhang, Steven M. Lipkin, and Yung-Fu Chang. 2019. "Proteomic Characterization of Outer Membrane Vesicles From Gut Mucosa-Derived *Fusobacterium Nucleatum*." *Journal of Proteomics* 195: 125–37. <https://doi.org/10.1016/j.jprot.2018.12.029>
 85. Wagner, T., B. Joshi, J. Janice, F. Askarian, N. Škalko-Basnet, O. C. Hagestad, A. Mekhlif, et al. 2018. "Enterococcus Faecium Produces Membrane Vesicles Containing Virulence Factors and Antimicrobial Resistance Related Proteins." *Journal of Proteomics* 187: 28–38. <https://doi.org/10.1016/j.jprot.2018.05.017>
 86. Zavan, Lauren, Natalie J. Bitto, Ella L. Johnston, David W. Greening, and Maria Kaparakis-Liaskos. 2019. "*Helicobacter pylori* Growth Stage Determines the Size, Protein Composition, and Preferential Cargo Packaging of Outer Membrane Vesicles." *Proteomics* 19: e1800209. <https://doi.org/10.1002/pmic.201800209>
 87. Li, Ying, Lezhen Dong, Zhishen Mu, Lingyi Liu, Junsu Yang, Zufang Wu, Daodong Pan, and Lianliang Liu. 2022. "Research Advances of Lactoferrin in Electrostatic Spinning, Nano Self-Assembly, and Immune and Gut Microbiota Regulation." *Journal of Agricultural Food Chemistry* 70: 10075–89. <https://doi.org/10.1021/acs.jafc.2c04241>
 88. Chiplunkar, Sanket S., Carlos A. Silva, Luiz E. Bermudez, and Lia Danelishvili. 2019. "Characterization of Membrane Vesicles Released by *Mycobacterium Avium* in Response to Environment Mimicking the Macrophage Phagosome." *Future Microbiology* 14: 293–313. <https://doi.org/10.2217/fmb-2018-0249>
 89. Horstman, Amanda L., and Meta J. Kuehn. 2000. "Enterotoxigenic *Escherichia coli* Secretes Active Heat-Labile Enterotoxin Via Outer Membrane Vesicles." *Journal of Biological Chemistry* 275: 12489–96. <https://doi.org/10.1074/jbc.275.17.12489>
 90. Barák, Imrich, and Katarína Muchová. 2013. "The Role of Lipid Domains in Bacterial Cell Processes." *International Journal of Molecular Sciences* 14: 4050–65. <https://doi.org/10.3390/ijms14024050>
 91. Tashiro, Yosuke, Aya Inagaki, Motoyuki Shimizu, Sosaku Ichikawa, Naoki Takaya, Toshiaki Nakajima-Kambe, Hiroo Uchiyama, and Nobuhiko Nomura. 2011. "Characterization of Phospholipids in Membrane Vesicles Derived From *Pseudomonas Aeruginosa*." *Bioscience, Biotechnology, and Biochemistry* 75: 605–7. <https://doi.org/10.1271/bbb.100754>
 92. Coelho, Carolina, Lisa Brown, Maria Maryam, Raghav Vij, Daniel F. Q. Smith, Meagan C. Burnet, Jennifer E. Kyle, et al. 2019. "Listeria Monocytogenes Virulence Factors, Including Listeriolysin O, Are Secreted in Biologically Active Extracellular Vesicles." *Journal of Biological Chemistry* 294: 1202–17. <https://doi.org/10.1074/jbc.RA118.006472>
 93. O'Brien, Killian, Koen Breyne, Stefano Ughetto, Louise C. Laurent, and Xandra O. Breakefield. 2020. "RNA Delivery by Extracellular Vesicles in Mammalian Cells and its Applications." *Nature Reviews Molecular Cell Biology* 21: 585–606. <https://doi.org/10.1038/s41580-020-0251-y>
 94. Jiang, Yanlong, Qingke Kong, Kenneth L. Roland, and Roy, 3rd Curtiss. 2014. "Membrane Vesicles of *Clostridium perfringens* Type A Strains Induce Innate and Adaptive Immunity." *International Journal of Medical Microbiology* 304: 431–43. <https://doi.org/10.1016/j.ijmm.2014.02.006>
 95. Kim, Sarah, Franz Bauernfeind, Andrea Ablasser, Gunther Hartmann, Katherine A. Fitzgerald, Eicke Latz, and Veit Hornung. 2010. "Listeria Monocytogenes is Sensed by the NLRP3 and AIM2 Inflammasome." *European Journal of Immunology* 40: 1545–51. <https://doi.org/10.1002/eji.201040425>
 96. Domingues, Sara, and Kaare M. Nielsen. 2017. "Membrane Vesicles and Horizontal Gene Transfer in Prokaryotes." *Current Opinion In Microbiology* 38: 16–21. <https://doi.org/10.1016/j.mib.2017.03.012>
 97. Liao, Sumei, Marlise I. Klein, Kyle P. Heim, Yuwei Fan, Jacob P. Bitoun, San-Joon Ahn, Robert A. Burne, et al. 2014. "Streptococcus Mutans Extracellular DNA is Upregulated During Growth in Biofilms, Actively Released Via Membrane Vesicles, and Influenced by Components of the Protein Secretion Machinery." *Journal of Bacteriology* 196: 2355–66. <https://doi.org/10.1128/JB.01493-14>
 98. Senpuku, Hidenobu, Tomoyo Nakamura, Yusuke Iwabuchi, Satoru Hirayama, Ryoma Nakao, and Makoto Ohnishi. 2019. "Effects of Complex DNA and MVs with GTF Extracted From *Streptococcus mutans* on the Oral Biofilm." *Molecules* 24: 3131. <https://doi.org/10.3390/molecules24173131>
 99. Blenkiron, Cherie, Denis Simonov, Anita Muthukaruppan, Peter Tsai, Priscila Dauros, Sasha Green, Jiwon Hong, et al. 2016. "Uropathogenic *Escherichia coli* Releases Extracellular Vesicles That are Associated With RNA." *PLoS One* 11: e0160440. <https://doi.org/10.1371/journal.pone.0160440>
 100. Tsatsaronis, James A., Sandra Franch-Arroyo, Ulrike Resch, and Emmanuelle Charpentier. 2018. "Extracellular Vesicle RNA: A Universal Mediator of Microbial Communication?" *Trends in Microbiology* 26: 401–10. <https://doi.org/10.1016/j.tim.2018.02.009>
 101. Lee, Heon-Jin. 2019. "Microbe-Host Communication by Small RNAs in Extracellular Vesicles: Vehicles for Transkingdom RNA Transportation." *International Journal of Molecular Sciences* 20: 1487. <https://doi.org/10.3390/ijms20061487>
 102. Amatya, Sajeen Bahadur, Sonja Salmi, Veera Kainulainen, Peeter Karihtala, and Justus Reunanen. 2021. "Bacterial Extracellular Vesicles in Gastrointestinal Tract Cancer: An Unexplored Territory." *Cancers* 13: 5450. <https://doi.org/10.3390/cancers13215450>
 103. Wang, Xiaogang, William J. Eagen, and Jean C. Lee. 2020. "Orchestration of Human Macrophage NLRP3 Inflammasome Activation Bystaphylococcus Aureusextracellular Vesicles." *Proceedings of the National Academy of Sciences of the United States of America* 117: 3174–84. <https://doi.org/10.1073/pnas.1915829117>
 104. Tartaglia, Natayme Rocha, Aurélie Nicolas, Vinicius de Rezende Rodvalho, Brenda Silva Rosa da Luz, Valérie Briard-Bion, Zuzana Krupova, Anne Thierry, et al. 2020. "Extracellular Vesicles Produced by Human and Animal *Staphylococcus aureus* Strains Share a Highly Conserved Core Proteome." *Scientific Reports* 10: 8467. <https://doi.org/10.1038/s41598-020-64952-y>

105. Lee, Jaewook, Si-Hyun Kim, Dong-Sic Choi, Jong Seok Lee, Dae-Kyum Kim, Gyeongyun Go, Seon-Min Park, et al. 2015. "Proteomic Analysis of Extracellular Vesicles Derived From *Mycobacterium tuberculosis*." *Proteomics* 15: 3331–37. <https://doi.org/10.1002/pmic.201500037>
106. Barák, Imrich, and Katarína Muchová. 2013. "The Role of Lipid Domains in Bacterial Cell Processes." *International Journal of Molecular Sciences* 14: 4050–65. <https://doi.org/10.3390/ijms14024050>
107. Caruana, Julie C, and Scott A. Walper. 2020. "Bacterial Membrane Vesicles as Mediators of Microbe—Microbe and Microbe—Host Community Interactions." *Frontiers in Microbiology* 11: 432. <https://doi.org/10.3389/fmicb.2020.00432>
108. Carmen, Carmen, and Meta J. Kuehn. 2015. "Outer-Membrane Vesicles From Gram-Negative Bacteria: Biogenesis and Functions." *Nature Reviews Microbiology* 13: 605–19. <https://doi.org/10.1038/nrmicro3525>
109. Jan, Arif Tasleem. 2017. "Outer Membrane Vesicles (OMVs) of Gram-Negative Bacteria: A Perspective Update." *Frontiers in Microbiology* 8: 1053. <https://doi.org/10.3389/fmicb.2017.01053>
110. Fang, Yuan, Zhiwen Wang, Xili Liu, and Brett M. Tyler. 2022. "Biogenesis and Biological Functions of Extracellular Vesicles in Cellular and Organismal Communication With Microbes." *Frontiers in Microbiology* 13: 817844. <https://doi.org/10.3389/fmicb.2022.817844>
111. Ultee, Eveline, Karina Ramijan, Remus T. Dame, Ariane Briegel, and Dennis Claessen. 2019. "Stress-Induced Adaptive Morphogenesis in bacteria" *Advances in Microbial Physiology* 74: 97–141. <https://doi.org/10.1016/bs.ampbs.2019.02.001>
112. Mozaheeb, Negar, and Marie-Paule Mingeot-Leclercq. 2020. "Membrane Vesicle Production as a Bacterial Defense Against Stress." *Frontiers in Microbiology* 11: 600221. <https://doi.org/10.3389/fmicb.2020.600221>
113. Kumar, Ashutosh, Anwar Alam, Mamta Rani, Nasreen Z. Ehtesham, and Seyed E. Hasnain. 2017. "Biofilms: Survival and Defense Strategy for Pathogens." *International Journal of Medical Microbiology* 307: 481–89. <https://doi.org/10.1016/j.ijmm.2017.09.016>
114. Altindis, Emrah, Yang Fu, and John J. Mekalanos. 2014. "Proteomic Analysis of *Vibrio Cholerae* Outer Membrane Vesicles." *Proceedings of National Academy of Science of the United States of America* 111: E1548–1556. <https://doi.org/10.1073/pnas.1403683111>
115. Yonezawa, Hideo, Takako Osaki, Toshiyuki Fukutomi, Tomoko Hanawa, Satoshi Kurata, Cynthia Zaman, Fuhito Hojo, and Shigeru Kamiya. 2017. "Diversification of the AlpB Outer Membrane Protein of *Helicobacter pylori* Affects Biofilm Formation and Cellular Adhesion." *Journal of Bacteriology* 199: e00729–16. <https://doi.org/10.1128/JB.00729-16>
116. Esoda, Caitlin N., and Meta J. Kuehn. 2019. "*Pseudomonas aeruginosa* Leucine Aminopeptidase Influences Early Biofilm Composition and Structure Via Vesicle-Associated Antibiofilm Activity." *mBio* 10: e02548–19. <https://doi.org/10.1128/mBio.02548-19>
117. Seike, Soshi, Hidetomo Kobayashi, Mitsunobu Ueda, Eizo Takahashi, Keinosuke Okamoto, and Hiroyasu Yamanaka. 2021. "Outer Membrane Vesicles Released From *Aeromonas* Strains are Involved in the Biofilm Formation." *Frontiers In Microbiology* 11: 613650. <https://doi.org/10.3389/fmicb.2020.613650>
118. Bhar, Sutonuka, Mariola J. Edelmann, and Melissa K. Jones. 2021. "Characterization and Proteomic Analysis of Outer Membrane Vesicles From a Commensal Microbe, *Enterobacter cloacae*." *Journal of Proteomics* 231: 103994. <https://doi.org/10.1016/j.jprot.2020.103994>
119. Schwechheimer, Carmen, and Meta J. Kuehn. 2015. "Outer-Membrane Vesicles From Gram-Negative Bacteria: Biogenesis and Functions." *Nature Reviews. Microbiology* 13: 605–19. <https://doi.org/10.1038/nrmicro3525>
120. Reyes-Robles, Tamara, Rebecca S. Dillard, Lynne S. Cairns, Cecilia A. Silva-Valenzuela, Max Housman, Afsar Ali, Elizabeth R. Wright, and Andrew Camilli. 2018. "*Vibrio cholerae* Outer Membrane Vesicles Inhibit Bacteriophage Infection." *Journal of Bacteriology* 200: e00792–17. <https://doi.org/10.1128/JB.00792-17>
121. Manning, Andrew J., and Meta J. Kuehn. 2011. "Contribution of Bacterial Outer Membrane Vesicles to Innate Bacterial Defense." *BMC Microbiology* 11: 258. <https://doi.org/10.1186/1471-2180-11-258>
122. Roszkowiak, Justyna, Paweł Jajor, Grzegorz Guła, Jerzy Gubernator, Andrzej Żak, Zuzanna Drulis-Kawa, and Daria Augustyniak. 2019. "Interspecies Outer Membrane Vesicles (OMVs) Modulate the Sensitivity of Pathogenic Bacteria and Pathogenic Yeasts to Cationic Peptides and Serum Complement." *International Journal of Molecular Sciences* 20: 5577. <https://doi.org/10.3390/ijms20225577>
123. Yun, Sung Ho, Edmond Changkyun Park, Sang-Yeop Lee, Hayoung Lee, Chi-Won Choi, Yoon-Sun Yi, Hyun-Joo Ro, et al. 2018. "Antibiotic Treatment Modulates Protein Components of Cytotoxic Outer Membrane Vesicles of Multidrug-Resistant Clinical Strain, *Acinetobacter baumannii* DU202." *Clinical Proteomics* 15: 28. <https://doi.org/10.1186/s12014-018-9204-2>
124. Stentz, Régis, Nikki Horn, Kathryn Cross, Louise Salt, Charles Brearley, David M. Livermore, and Simon R. Carding. 2015. "Cephalosporinases Associated With Outer Membrane Vesicles Released by *Bacteroides* spp. Protect Gut Pathogens and Commensals Against β -lactam Antibiotics." *The Journal of Antimicrobial Chemotherapy* 70: 701–9. <https://doi.org/10.1093/jac/dku466>
125. Kosgodage, Uchini S., Paul Matewele, Giulia Mastroianni, Igor Kraev, Dominik Brotherton, Brigitte Awamaria, Anthony P. Nicholas, Sigrun Lange, and Jameel M. Inal. 2019. "Peptidylarginine Deiminase Inhibitors Reduce Bacterial Membrane Vesicle Release and Sensitize Bacteria to Antibiotic Treatment." *Frontiers in Cellular and Infection Microbiology* 9: 227. <https://doi.org/10.3389/fcimb.2019.00227>
126. Devos, Simon, Wouter Van Putte, Jolien Vitse, Gonzalez Van Driessche, Stephan Stremersch, Wim Van Den Broek, Koen Raemdonck, et al. 2017. "Membrane Vesicle Secretion and Prophage Induction in Multidrug-Resistant *Stenotrophomonas maltophilia* in Response to Ciprofloxacin Stress." *Environmental Microbiology* 19: 3930–37. <https://doi.org/10.1111/1462-2920.13793>

127. Bonnington, K. E., and M. J. Kuehn. 2014. "Protein Selection and Export Via Outer Membrane Vesicles." *Biochimica et Biophysica Acta (BBA)—Molecular Cell Research* 1843: 1612–19. <https://doi.org/10.1016/j.bbamcr.2013.12.011>
128. Cecil, Jessica D., Natalie Sirisaengtaksin, Neil M. O'Brien-Simpson, and Anne Marie Krachler. 2019. "Outer Membrane Vesicle-Host Cell Interactions." *Microbiology Spectrum* 7: 10.1128. <https://doi.org/10.1128/microbiolspec.PSIB-0001-2018>
129. Yokoyama, Keiko, Toshinobu Horii, Takafumi Yamashino, Shinnosuke Hashikawa, Soumitra Barua, Tadao Hasegawa, Haruo Watanabe, and Michio Ohta. 2000. "Production of Shiga Toxin by *Escherichia coli* Measured With Reference to the Membrane Vesicle-Associated Toxins." *FEMS Microbiology Letters* 192: 139–44. <https://doi.org/10.1111/j.1574-6968.2000.tb09372.x>
130. Kato, Satsuki, Yusuke Kowashi, and Donald R. Demuth. 2002. "Outer Membrane-Like Vesicles Secreted by *Actinobacillus Actinomycetemcomitans* are Enriched in Leukotoxin." *Microbial Pathogenesis* 32: 1–13. <https://doi.org/10.1006/mpat.2001.0474>
131. Turkina, Maria V., Annelie Olofsson, Karl-Eric Magnusson, Anna Arnqvist, and Elena Vikström. 2015. "*Helicobacter pylori* Vesicles Carrying CagA Localize in the Vicinity of Cell-Cell Contacts and Induce Histone H1 Binding To ATP in Epithelial Cells." *FEMS Microbiology Letters* 362: fnv076. <https://doi.org/10.1093/femsle/fnv076>
132. He, Yuhuan, Noriko Shiotsu, Yoko Uchida-Fukuhara, Jiajie Guo, Yao Weng, Mika Ikegame, Ziyi Wang, et al. 2020. "Outer Membrane Vesicles Derived from *Porphyromonas gingivalis* Induced Cell Death with Disruption of Tight Junctions in Human Lung Epithelial Cells." *Archives of Oral Biology* 118: 104841. <https://doi.org/10.1016/j.archoralbio.2020.104841>
133. Marsollier, Laurent, Priscille Brodin, Mary Jackson, Jana Korduláková, Petra Tafelmeyer, Etienne Carbonnelle, Jacques Aubry, et al. 2007. "Impact of *Mycobacterium Ulcerans* Biofilm on Transmissibility to Ecological Niches and Buruli Ulcer Pathogenesis." *PLoS Pathogens* 3: e62. <https://doi.org/10.1371/journal.ppat.0030062>
134. Bomberger, Jennifer M., Daniel P. Maceachran, Bonita A. Coutermarsh, Siying Ye, George A. O'Toole, and Bruce A. Stanton. 2009. "Long-Distance Delivery of Bacterial Virulence Factors by *Pseudomonas aeruginosa* Outer Membrane Vesicles." *PLoS Pathogens* 5: e1000382. <https://doi.org/10.1371/journal.ppat.1000382>
135. Bomberger, Jennifer M., Daniel P. Maceachran, Bonita A. Coutermarsh, Siying Ye, George A. O'Toole, and Bruce A. Stanton. 2009. "Long-Distance Delivery of Bacterial Virulence Factors by *Pseudomonas aeruginosa* Outer Membrane Vesicles." *PLoS Pathogens* 5: e1000382. <https://doi.org/10.1371/journal.ppat.1000382>
136. Takeuchi, Osamu, and Shizuo Akira. 2010. "+Pattern Recognition Receptors and Inflammation." *Cell* 140: 805–20. <https://doi.org/10.1016/j.cell.2010.01.022>
137. Kim, Hyun Young, Younggap Lim, Sun-Jin An, and Bong-Kyu Choi. 2020. "Characterization and Immunostimulatory Activity of Extracellular Vesicles From Filifactor Alocis." *Molecular Oral Microbiology* 35: 1–9. <https://doi.org/10.1111/omi.12272>
138. Mehanny, Mina, Marcus Koch, Claus-Michael Lehr, and Gregor Fuhrmann. 2020. "Streptococcal Extracellular Membrane Vesicles are Rapidly Internalized by Immune Cells and Alter Their Cytokine Release." *Frontiers in Immunology* 11: 80. <https://doi.org/10.3389/fimmu.2020.00080>
139. Kaparakis, Maria, Lynne Turnbull, Leticia Carneiro, Stephen Firth, Harold A. Coleman, Helena C. Parkinson, Lionel Le Bourhis, et al. 2010. "Bacterial Membrane Vesicles Deliver Peptidoglycan to NOD1 in Epithelial Cells." *Cellular Microbiology* 12: 372–85. <https://doi.org/10.1111/j.1462-5822.2009.01404.x>
140. Cecil, Jessica D., Neil M. O'Brien-Simpson, Jason C. Lenzo, James A. Holden, William Singleton, Alexis Perez-Gonzalez, Ashley Mansell, and Eric C. Reynolds. 2017. "Outer Membrane Vesicles Prime and Activate Macrophage Inflammation and Cytokine Secretion In Vitro and In Vivo." *Frontiers in Immunology* 8: 1017. <https://doi.org/10.3389/fimmu.2017.01017>
141. Cecil, Jessica D., Neil M. O'Brien-Simpson, Jason C. Lenzo, James A. Holden, Yu-Yen Chen, William Singleton, Katelyn T. Gause, et al. 2016. "Differential Responses of Pattern Recognition Receptors to Outer Membrane Vesicles of Three Periodontal Pathogens." *PLoS One* 11: e0151967. <https://doi.org/10.1371/journal.pone.0151967>
142. Choi, Ji Hyun, Chang Mo Moon, Tae-Seop Shin, Eun Kyoung Kim, Andrea McDowell, Min-Kyung Jo, Yang Hee Joo, et al. 2020. "Lactobacillus Paracasei-Derived Extracellular Vesicles Attenuate the Intestinal Inflammatory Response by Augmenting the Endoplasmic Reticulum Stress Pathway." *Experimental and Molecular Medicine* 52: 423–37. <https://doi.org/10.1038/s12276-019-0359-3>
143. Mata Forsberg, Manuel, Sophia Björkander, Yanhong Pang, Ludwig Lundqvist, Mama Ndi, Martin Ott, Irene Buesa Escibá, et al. 2019. "Extracellular Membrane Vesicles From *Lactobacilli* Dampen IFN- γ Responses in a Monocyte-Dependent Manner." *Science Reports* 9: 17109. <https://doi.org/10.1038/s41598-019-53576-6>
144. Fábrega, María-José, Alba Rodríguez-Nogales, José Garrido-Mesa, Francesca Algeri, Josefa Badía, Rosa Giménez, Julio Gálvez, and Laura Baldomà. 2017. "Intestinal Anti-Inflammatory Effects of Outer Membrane Vesicles From *Escherichia coli* Nissle 1917 in DSS-Experimental Colitis in Mice." *Frontiers in Microbiology* 8: 1274. <https://doi.org/10.3389/fmicb.2017.01274>
145. Koeppen, Katja, Thomas H. Hampton, Michael Jarek, Maren Scharfe, Scott A. Gerber, Daniel W. Mielcarz, Elora G. Demers, et al. 2016. "A Novel Mechanism of Host-Pathogen Interaction Through sRNA in Bacterial Outer Membrane Vesicles." *PLoS pathogens* 12: e1005672. <https://doi.org/10.1371/journal.ppat.1005672>
146. Galka, Frank, Sun Nyunt Wai, Harald Kusch, Susanne Engelmann, Michael Hecker, Bernd Schmeck, Stefan Hippenstiel, Bernt Eric Uhlin, and Michael Steinert. 2008. "Proteomic Characterization of the Whole Secretome of *Legionella pneumophila* and Functional Analysis of Outer Membrane Vesicles." *Infection and Immunity* 76: 1825–36. <https://doi.org/10.1128/IAI.01396-07>
147. Winter, Jody, Darren Letley, Joanne Rhead, John Atherton, and Karen Robinson. 2014. "*Helicobacter pylori* Membrane

- Vesicles Stimulate Innate Pro- and Anti-Inflammatory Responses and Induce Apoptosis in Jurkat T Cells.” *Infection and Immunity* 82: 1372–81. <https://doi.org/10.1128/IAI.01443-13>
148. Askarian, Fatemeh, John D. Lapek, Mitesh Dongre, Chih-Ming Tsai, Monika Kumaraswamy, Armin Kousha, J. Andrés Valderrama, et al. 2018. “*Staphylococcus aureus* Membrane-Derived Vesicles Promote Bacterial Virulence and Confer Protective Immunity in Murine Infection Models.” *Frontiers In Microbiology* 9: 262. <https://doi.org/10.3389/fmicb.2018.00262>
 149. Jhelum, Hina, Hema Sori, and Devinder Sehgal. 2018. “A Novel Extracellular Vesicle-Associated Endodeoxyribonuclease Helps *Streptococcus Pneumoniae* Evade Neutrophil Extracellular Traps and is Required for Full Virulence.” *Scientific Reports* 8: 7985. <https://doi.org/10.1038/s41598-018-25865-z>
 150. Alaniz, Robert C, Brooke L. Deatherage, Jimmie C. Lara, and Brad T. Cookson. 2007. “Membrane Vesicles Are Immunogenic Facsimiles of *Salmonella Typhimurium* That Potently Activate Dendritic Cells, Prime B and T Cell Responses, and Stimulate Protective Immunity in Vivo.” *Journal of Immunology* 179: 7692–701. <https://doi.org/10.4049/jimmunol.179.11.7692>
 151. Chu, Hiutung, Arya Khosravi, Indah P. Kusumawardhani, Alice H. K. Kwon, Anilton C. Vasconcelos, Larissa D. Cunha, Anne E. Mayer, et al. 2016. “Gene-Microbiota Interactions Contribute to the Pathogenesis of Inflammatory Bowel Disease.” *Science* 352: 1116–20. <https://doi.org/10.1126/science.aad9948>
 152. Perez Vidakovics, Maria Laura A., Johan Jendholm, Matthias Mörgelin, Anne Månsson, Christer Larsson, Lars-Olaf Cardell, and Kristian Riesbeck. 2010. “B Cell Activation by Outer Membrane Vesicles—A Novel Virulence Mechanism.” *PLoS Pathogens* 6: e1000724. <https://doi.org/10.1371/journal.ppat.1000724>
 153. Romeu, Belkis, Miriam Lastre, Luis García, Bárbara Cedré, Aleida Mandariote, Mildrey Fariñas, Reynaldo Oliva, and Oliver Pérez. 2014. “Combined Meningococcal Serogroup A and W135 Outer-Membrane Vesicles Activate Cell-Mediated Immunity and Long-Term Memory Responses Against Non-Covalent Capsular Polysaccharide A.” *Immunologic Research* 58: 75–85. <https://doi.org/10.1007/s12026-013-8427-6>
 154. Schetters, Sjoerd T. T., Wouter S. P. Jong, Sophie K. Horrevorts, Laura J. W. Kruijsen, Steef Engels, Dorian Stolk, Maria H. Daleke-Schermerhorn, et al. 2019. “Outer Membrane Vesicles Engineered To Express Membrane-Bound Antigen Program Dendritic Cells for Cross-Presentation to CD8 T Cells.” *Acta Biomaterialia* 91: 248–57. <https://doi.org/10.1016/j.actbio.2019.04.033>
 155. Stevenson, Taylor C., Colette Cywes-Bentley, Tyler D. Moeller, Kevin B. Weyant, David Putnam, Yung-Fu Chang, Bradley D. Jones, Gerald B. Pier, and Matthew P. DeLisa. 2018. “Immunization With Outer Membrane Vesicles Displaying Conserved Surface Polysaccharide Antigen Elicits Broadly Antimicrobial Antibodies.” *Proceedings of the National Academy of Sciences of the United States of America* 115: E3106–115. <https://doi.org/10.1073/pnas.1718341115>
 156. Kim, Oh Youn, Bok Sil Hong, Kyong-Su Park, Yae Jin Yoon, Seng Jin Choi, Won Hee Lee, Tae-Young Roh, et al. 2013. “Immunization With *Escherichia coli* Outer Membrane Vesicles Protects Bacteria-Induced Lethality Via Th1 and Th17 Cell Responses.” *Journal of Immunology* 190: 4092–102. <https://doi.org/10.4049/jimmunol.1200742>
 157. Hock, Barry D., Judith L. McKenzie McKenzie, and Jacqueline I. Keenan. 2017. “*Helicobacter Pylori* Outer Membrane Vesicles Inhibit Human T Cell Responses Via Induction Of Monocyte COX-2 Expression.” *Pathogens and Disease* 75: ftx034. <https://doi.org/10.1093/femspd/ftx034>
 158. Shen, Qichen, Zhuizui Huang, Jiachen Yao, and Yuanxiang Jin. 2022. “Extracellular Vesicles-Mediated Interaction Within Intestinal Microenvironment in Inflammatory Bowel Disease.” *Journal of Advanced Research* 37: 221–33. <https://doi.org/10.1016/j.jare.2021.07.002>
 159. Olofsson, Annelie, Anna Vallström, Katja Petzold, Nicole Tegtmeier, Jürgen Schleucher, Sven Carlsson, Rainer Haas, et al. 2010. “Biochemical and Functional Characterization of *Helicobacter Pylori* Vesicles.” *Molecular Microbiology* 77: 1539–55. <https://doi.org/10.1111/j.1365-2958.2010.07307.x>
 160. González, María Fernanda, Paula Díaz, Alejandra Sandoval-Bórquez, Daniela Herrera, and Andrew F. G. Quest. 2021. “*Helicobacter pylori* Outer Membrane Vesicles and Extracellular Vesicles From *Helicobacter pylori*-Infected Cells in Gastric Disease Development.” *International Journal of Molecular Sciences* 22: 4823. <https://doi.org/10.3390/ijms22094823>
 161. Zingl, Franz G., Himadri B. Thapa, Martina Scharf, Paul Kohl, Anna M. Müller, and Stefan Schild. 2021. “Outer Membrane Vesicles of *Vibrio Cholerae* Protect and Deliver Active Cholera Toxin to Host Cells Via Porin-Dependent Uptake.” *mBio* 12: e0053421. <https://doi.org/10.1128/mBio.00534-21>
 162. Rueter, Christian, and Martina Bielaszewska. 2020. “Secretion and Delivery of Intestinal Pathogenic *Escherichia coli* Virulence Factors Via Outer Membrane Vesicles.” *Frontiers In Cellular and Infection Microbiology* 10: 91. <https://doi.org/10.3389/fcimb.2020.00091>
 163. Chang, Xin, Shu-Ling Wang, Sheng-Bing Zhao, Yi-Hai Shi, Peng Pan, Lun Gu, Jun Yao, Zhao-Shen Li, and Yu Bai. 2020. “Extracellular Vesicles With Possible Roles in Gut Intestinal Tract Homeostasis and IBD.” *Mediators of Inflammation* 2020: 1–14. <https://doi.org/10.1155/2020/1945832>
 164. Bielaszewska, Martina, Monika Marejková, Andreas Bauwens, Lisa Kunsmann-Prokscha, Alexander Mellmann, and Helge Karch. 2018. “Enterohemorrhagic *Escherichia coli* O157 Outer Membrane Vesicles Induce Interleukin 8 Production in Human Intestinal Epithelial Cells by Signaling Via Toll-Like Receptors TLR4 and TLR5 and Activation of the Nuclear Factor NF- κ B.” *International Journal of Medical Microbiology* 308: 882–89. <https://doi.org/10.1016/j.ijmm.2018.06.004>
 165. Bielaszewska, Martina, Christian Rüter, Lisa Kunsmann, Lilo Greune, Andreas Bauwens, Wenlan Zhang, Thorsten Kuczius, et al. 2013. “Enterohemorrhagic *Escherichia coli* Hemolysin Employs Outer Membrane Vesicles to Target Mitochondria and Cause Endothelial and Epithelial

- Apoptosis." *PLoS pathogens* 9: e1003797. <https://doi.org/10.1371/journal.ppat.1003797>
166. Bielaszewska, Martina, Christian Rüter, Andreas Bauwens, Lilo Greune, Kevin-André Jarosch, Daniel Steil, Wenlan Zhang, et al. 2017. "Host Cell Interactions of Outer Membrane Vesicle-Associated Virulence Factors of Enterohemorrhagic *Escherichia coli* O157: Intracellular Delivery, Trafficking and Mechanisms Of Cell Injury." *PLoS Pathogens* 13: e1006159. <https://doi.org/10.1371/journal.ppat.1006159>
 167. Yi, Xinzhu, Jingyuan Gao, and Zhang Wang. 2022. "The Human Lung Microbiome—A Hidden Link Between Microbes and Human Health and Diseases." *iMeta* 1: e33. <https://doi.org/10.1002/imt2.33>
 168. Kim, M.-R., S.-W. Hong, E.-B. Choi, W.-H. Lee, Y.-S. Kim, S. G. Jeon, M. H. Jang, Y. S. Gho, and Y.-K. Kim. 2012. "Staphylococcus aureus-derived extracellular vesicles induce neutrophilic pulmonary inflammation via both Th1 and Th17 cell responses." *Allergy* 67: 1271–81. <https://doi.org/10.1111/all.12001>
 169. Kim, You-Sun, Won-Hee Lee, Eun-Jeong Choi, Jun-Pyo Choi, Young Joo Heo, Yong Song Gho, Young-Koo Jee, Yeon-Mok Oh, and Yoon-Keun Kim. 2015. "Extracellular Vesicles Derived From Gram-Negative Bacteria, Such as *Escherichia coli*, Induce Emphysema Mainly Via IL-17A-mediated Neutrophilic Inflammation." *Journal of Immunology (Baltimore, Md.: 1950)* 194: 3361–68. <https://doi.org/10.4049/jimmunol.1402268>
 170. Li, Yue, Huan Li, Husheng Tong, Marc Maegelev, and Zhengtao Gu. 2022. "Outer Membrane Vesicles Derived From Heatstroke-Associated Intestinal Microbiota Promote Multiple Organ Injury in Mice." *Microbial Pathogenesis* 170: 105653. <https://doi.org/10.1016/j.micpath.2022.105653>
 171. Dréno, Brigitte, Marie Ange Dagnelie, Amir Khammari, and Stéphane Corvec. 2020. "The Skin Microbiome: A New Actor in Inflammatory Acne." *Am J Clin Dermatol* 21: 18–24. <https://doi.org/10.1007/s40257-020-00531-1>
 172. Jiang, Min, Xiaoyao Fan, Ziqi Jiang, Huyan Chen, Ye Liu, Tianze Yu, Qiong Huang, and Ying Ma. 2022. "Comparative Proteomic Analysis of Membrane Vesicles from Clinical C. Acnes Isolates with Differential Antibiotic Resistance." *Clinical, Cosmetic and Investigational Dermatology* 15: 703–12. <https://doi.org/10.2147/CCID.S363537>
 173. Choi, Eun-Jeong, Hyun Gee Lee, Il-Hong Bae, Wanil Kim, Jungwon Park, Tae Ryong Lee, and Eun-Gyung Cho. 2018. "Propionibacterium Acnes-Derived Extracellular Vesicles Promote Acne-Like Phenotypes in Human Epidermis." *Journal of Investigative Dermatology* 138: 1371–79. <https://doi.org/10.1016/j.jid.2018.01.007>
 174. Kim, Min Hye, Mina Rho, Jun Pyo Choi, Hyun Il Choi, Han Ki Park, Woo Jung Song, Taek Ki Min, et al. 2017. "A Metagenomic Analysis Provides a Culture-Independent Pathogen Detection for Atopic Dermatitis." *Allergy, Asthma & Immunology Research* 9: 453–61. <https://doi.org/10.4168/aaair.2017.9.5.453>
 175. Hirasawa, Yusuke, Toshiro Takai, Toshinobu Nakamura, Kouichi Mitsuishi, Hendra Gunawan, Hajime Suto, Takasuke Ogawa, et al. 2010. "Staphylococcus aureus extracellular protease causes epidermal barrier dysfunction." *Journal of Investigative Dermatology* 130: 614–17. <https://doi.org/10.1038/jid.2009.257>
 176. Jun, S. H., J. H. Lee, S. I. Kim, C. W. Choi, T. I. Park, H. R. Jung, J. W. Cho, S. H. Kim, and J. C. Lee. 2017. "Staphylococcus aureus-derived membrane vesicles exacerbate skin inflammation in atopic dermatitis." *Clinical & Experimental Allergy* 47: 85–96. <https://doi.org/10.1111/cea.12851>
 177. Sittipo, Panida, Jaeyoon Choi, Soojin Lee, and Yun Kyung Lee. 2022. "The Function of Gut Microbiota in Immune-Related Neurological Disorders: A Review." *Journal of Neuroinflammation* 19: 154. <https://doi.org/10.1186/s12974-022-02510-1>
 178. Zhan, Xinhua, Boryana Stamova, Lee-Way Jin, Charles DeCarli, Brett Phinney, and Frank R. Sharp. 2016. "Gram-Negative Bacterial Molecules Associate With Alzheimer Disease Pathology." *Neurology* 87: 2324–32. <https://doi.org/10.1212/WNL.0000000000003391>
 179. Emery, David C., Deborah K. Shoemark, Tom E. Batstone, Christy M. Waterfall, Jane A. Coghill, Tanya L. Cerajewska, Maria Davies, Nicola X. West, and Shelley J. Allen. 2017. "16S rRNA Next Generation Sequencing Analysis Shows Bacteria in Alzheimer's Post-Mortem Brain." *Frontiers In Aging Neuroscience* 9: 195. <https://doi.org/10.3389/fnagi.2017.00195>
 180. Wei, Shouchao, Wanjuan Peng, Yingren Mai, Kanglan Li, Wei Wei, Li Hu, Shaoping Zhu, et al. 2020. "Outer Membrane Vesicles Enhance Tau Phosphorylation and Contribute to Cognitive Impairment." *Journal of Cellular Physiology* 235: 4843–55. <https://doi.org/10.1002/jcp.29362>
 181. Aldick, Thomas, Martina Bielaszewska, Bernt Eric Uhlin, Hans-Ulrich Humpf, Sun Nyunt Wai, and Helge Karch. 2009. "Vesicular Stabilization and Activity Augmentation of Enterohaemorrhagic *Escherichia coli* Haemolysin." *Molecular Microbiology* 71: 1496–508. <https://doi.org/10.1111/j.1365-2958.2009.06618.x>
 182. Han, Eun-Chong, Song-Yi Choi, Youngkyun Lee, Jin-Woo Park, Su-Hyung Hong, and Heon-Jin Lee. 2019. "Extracellular RNAs in Periodontopathogenic Outer Membrane Vesicles Promote TNF- α Production in Human Macrophages and Cross the Blood-Brain Barrier in Mice." *The FASEB Journal* 33: 13412–22. <https://doi.org/10.1096/fj.201901575R>
 183. Fung, Thomas C. 2020. "The Microbiota-Immune Axis as a Central Mediator of Gut-Brain Communication." *Neurobiology of Disease* 136: 104714. <https://doi.org/10.1016/j.nbd.2019.104714>
 184. Williams, G. D., and S. C. Holt. 1985. "Characteristics of the Outer Membrane of Selected Oral Bacteroides Species." *Canadian Journal of Microbiology* 31: 238–50. <https://doi.org/10.1139/m85-046>
 185. Zhang, Zhiying, Dongjuan Liu, Sai Liu, Shuwei Zhang, and Yaping Pan. 2021. "The Role of Porphyromonas Gingivalis Outer Membrane Vesicles in Periodontal Disease and Related Systemic Diseases." *Frontiers In Cellular and Infection Microbiology* 10: 585917. <https://doi.org/10.3389/fcimb.2020.585917>
 186. Cecil, Jessica D., Neil M. O'brien-Simpson, Jason C. Lenzo, James A. Holden, William Singleton, Alexis Perez-Gonzalez, Ashley Mansell, and Eric C. Reynolds. 2017. "Outer Membrane Vesicles Prime and Activate Macrophage Inflammasomes and Cytokine Secretion In Vitro and In Vivo." *Frontiers In Immunology* 8: 1017. <https://doi.org/10.3389/fimmu.2017.01017>

187. Fleetwood, Andrew J., Man K. S. Lee, William Singleton, Adrian Achuthan, Ming-Chin Lee, Neil M. O'Brien-Simpson, Andrew D. Cook, et al. 2017. "Metabolic Remodeling, Inflammasome Activation, and Pyroptosis in Macrophages Stimulated by Porphyromonas Gingivalis and its Outer Membrane Vesicles." *Frontiers In Cellular and Infection Microbiology* 7: 351. <https://doi.org/10.3389/fcimb.2017.00351>
188. Kieselbach, Thomas, Vincent Zijng, Elisabeth Granström, and Jan Oscarsson. 2015. "Proteomics of Aggregatibacter Actinomycetemcomitans Outer Membrane Vesicles." *PLoS One* 10: e0138591. <https://doi.org/10.1371/journal.pone.0138591>
189. Kim, Hyun Young, Min-Kyoung Song, Younggap Lim, Ji Sun Jang, Sun-Jin An, Hong-Hee Kim, and Bong-Kyu Choi. 2022. "Effects of Extracellular Vesicles Derived From Oral Bacteria on Osteoclast Differentiation and Activation." *Sci Rep* 12: 14239. <https://doi.org/10.1038/s41598-022-18412-4>
190. Villard, Alexandre, Jérôme Boursier, and Ramarosan Andriantsitohaina. 2021. "Microbiota-Derived Extracellular Vesicles and Metabolic Syndrome." *Acta Physiologica* 231: e13600. <https://doi.org/10.1111/apha.13600>
191. Zhang, Yunzhen, Lezhen Dong, Lingyi Liu, Zufang Wu, Daodong Pan, and Lianliang Liu. 2022. "Recent Advances of Stimuli-Responsive Polysaccharide Hydrogels in Delivery Systems: A Review." *Journal of Agricultural and Food Chemistry* 70: 6300–6316. <https://doi.org/10.1021/acs.jafc.2c01080>
192. Liu, Yahui, Peifang Weng, Yanan Liu, Zufang Wu, Lei Wang, and Lianliang Liu. 2022. "Citrus Pectin Research Advances: Derived as a Biomaterial in the Construction and Applications of Micro/Nano-Delivery Systems." *Food Hydrocolloids* 133: 107910. <https://doi.org/10.1016/j.foodhyd.2022.107910>
193. Chelakkot, Chaithanya, Youngwoo Choi, Dae-Kyum Kim, Hyun T. Park, Jaewang Ghim, Yonghoon Kwon, Jinseong Jeon, et al. 2018. "Akkermansia Muciniphila-Derived Extracellular Vesicles Influence Gut Permeability Through the Regulation of Tight Junctions." *Experimental & Molecular Medicine* 50: e450. <https://doi.org/10.1038/emmm.2017.282>
194. Shi, Hang, Maia V. Kokoeva, Karen Inouye, Iphigenia Tzamelis, Huali Yin, and Jeffrey S. Flier. 2006. "TLR4 Links Innate Immunity and Fatty Acid-Induced Insulin Resistance." *Journal of Clinical Investigation* 116: 3015–25. <https://doi.org/10.1172/JCI28898>
195. Lu, Min, David Patsouris, Pingping Li, Jaime Flores-Riveros, James M. Frincke, Steve Watkins, Simon Schenk, and Jerrold M. Olefsky. 2010. "A New Antidiabetic Compound Attenuates Inflammation and Insulin Resistance in Zucker Diabetic Fatty Rats." *American Journal of Physiology-Endocrinology and Metabolism* 298: E1036–E1048. <https://doi.org/10.1152/ajpendo.00668.2009>
196. Michail, Sonia, Malinda Lin, Mark R. Frey, Rob Fanter, Oleg Paliy, Brian Hilbush, and Nicholas V. Reo. 2015. "Altered Gut Microbial Energy and Metabolism in Children with Non-Alcoholic Fatty Liver Disease." *FEMS Microbiology Ecology* 91: 1–9. <https://doi.org/10.1093/femsec/fiu002>
197. Choi, Youngwoo, Yonghoon Kwon, Dae-Kyum Kim, Jinseong Jeon, Su Chul Jang, Taejun Wang, Minjee Ban, et al. 2015. "Gut Microbe-Derived Extracellular Vesicles Induce Insulin Resistance, Thereby Impairing Glucose Metabolism in Skeletal Muscle." *Scientific Reports* 5: 15878. <https://doi.org/10.1038/srep15878>
198. Quach, Darin, and A Britton Robert. 2017. "Gut Microbiota and Bone Health." *Advances in Experimental Medicine and Biology* 1033: 47–58. https://doi.org/10.1007/978-3-319-66653-2_4
199. Sjögren, Klara, Cecilia Engdahl, Petra Henning, Ulf H. Lerner, Valentina Tremaroli, Marie K. Lagerquist, Fredrik Bäckhed, and Claes Ohlsson. 2012. "The Gut Microbiota Regulates Bone Mass In Mice." *Journal of Bone and Mineral Research* 27: 1357–67. <https://doi.org/10.1002/jbmr.1588>
200. Li, Jau-Yi, Benoit Chassaing, Abdul Malik Tyagi, Chiara Vaccaro, Tao Luo, Jonathan Adams, Trevor M Darby, et al. 2016. "Sex Steroid Deficiency-Associated Bone Loss Is Microbiota Dependent and Prevented By Probiotics." *Journal of Clinical Investigation* 126: 2049–63. <https://doi.org/10.1172/JCI86062>
201. Durado, Eduardo, Margarida Ferro, Catarina Sousa Guerreiro, and João Eurico Fonseca. 2020. "Diet as a Modulator of Intestinal Microbiota in Rheumatoid Arthritis." *Nutrients* 12: 3504. <https://doi.org/10.3390/nu12113504>
202. Dai, Hanhao, Wu Zheng, Jun Luo, Guoyu Yu, Chao Song, Yijing Wu, and Jie Xu. 2022. "Inhibiting Uptake of Extracellular Vesicles Derived From Senescent Bone Marrow Mesenchymal Stem Cells by Muscle Satellite Cells Attenuates Sarcopenia." *Journal of Orthopaedic Translation* 35: 23–36. <https://doi.org/10.1016/j.jot.2022.06.002>
203. Wu, Rongjie, Haotao Li, Chuanwei Sun, Jialin Liu, Duanrong Chen, Haiyang Yu, Zena Huang, et al. 2022. "Exosome-Based Strategy for Degenerative Disease in Orthopedics: Recent Progress and Perspectives." *Journal of Orthopaedic Translation* 36: 8–17. <https://doi.org/10.1016/j.jot.2022.05.009>
204. Liu, Jiang-Hua, Chun-Yuan Chen, Zheng-Zhao Liu, Zhong-Wei Luo, Shan-Shan Rao, Ling Jin, Teng-Fei Wan, et al. 2021. "Extracellular Vesicles From Child Gut Microbiota Enter Into Bone to Preserve Bone Mass and Strength." *Advanced Science* 8: 2004831. <https://doi.org/10.1002/adv.202004831>
205. Chen, Chun-Yuan, Shan-Shan Rao, Tao Yue, Yi-Juan Tan, Hao Yin, Ling-Jiao Chen, Ming-Jie Luo, et al. 2022. "Glucocorticoid-Induced Loss of Beneficial Gut Bacterial Extracellular Vesicles is Associated With the Pathogenesis of Osteonecrosis." *Science Advances* 8: eabg8335. <https://doi.org/10.1126/sciadv.abg8335>
206. Reichert, Stefan, Maximilian Haffner, Gernot Keyßer, Christoph Schäfer, Jamal M. Stein, Hans-Günter Schaller, Andreas Wienke, et al. 2013. "Detection of Oral Bacterial DNA in Synovial Fluid." *Journal of Clinical Periodontology* 40: 591–98. <https://doi.org/10.1111/jcpe.12102>
207. Gabarrini, Giorgio, Rick Heida, Nienke van Ieperen, Mike A Curtis, Arie Jan van Winkelhoff, and Jan Maarten van Dijk. 2018. "Dropping Anchor: Attachment of Peptidylarginine Deiminase Via A-LPS to Secreted Outer Membrane Vesicles Of Porphyromonas Gingivalis." *Scientific Reports* 8: 8949. <https://doi.org/10.1038/s41598-018-27223-5>
208. Chronopoulos, Antonios, and Raghu Kalluri. 2020. "Emerging Role of Bacterial Extracellular Vesicles in Cancer."

- Oncogene* 39: 6951–60. <https://doi.org/10.1038/s41388-020-01509-3>
209. Sheikh, Aadil, Joseph Taube, and K. Leigh Greathouse. 2021. "Contribution of the Microbiota and Their Secretory Products to Inflammation and Colorectal Cancer Pathogenesis: The Role of Toll-Like Receptors." *Carcinogenesis* 42: 1133–42. <https://doi.org/10.1093/carcin/bgab060>
 210. Shang, Fu-Mei, and Hong-Li Liu. 2018. "Fusobacterium nucleatum and Colorectal Cancer: A Review." *World Journal of Gastrointestinal Oncology* 10: 71–81. <https://doi.org/10.4251/wjgo.v10.i3.71>
 211. Nosh, Katsuhiko, et al. 2016. "Association of Fusobacterium Nucleatum With Immunity and Molecular Alterations in Colorectal Cancer." *World Journal of Gastroenterology* 22: 557–66. <https://doi.org/10.3748/wjg.v22.i2.557>
 212. Liu, Dongjuan, Sai Liu, Junchao Liu, Lei Miao, Shuwei Zhang, and Yaping Pan. 2021. "sRNA23392 Packaged By *Porphyromonas gingivalis* Outer Membrane Vesicles Promotes Oral Squamous Cell Carcinomas Migration and Invasion by Targeting desmocollin-2." *Molecular Oral Microbiology* 36: 182–91. <https://doi.org/10.1111/omi.12334>
 213. Tarashi, Samira, Mohammad Saber Zamani, Mir Davood Omrani, Abolfazl Fateh, Arfa Moshiri, Ahmad Saedisomeolia, Seyed Davar Siadat, and Stan Kubow. 2022. "Commensal and Pathogenic Bacterial-Derived Extracellular Vesicles in Host-Bacterial and Inter-bacterial Dialogues: Two Sides of the Same Coin." *J Immunol Res* 2022: 8092170. <https://doi.org/10.1155/2022/8092170>
 214. Hosseini-Giv, Niloufar, Alyza Basas, Chloe Hicks, Emad El-Omar, Fatima El-Assaad, and Elham Hosseini-Beheshti. 2022. "Bacterial Extracellular Vesicles and Their Novel Therapeutic Applications in Health and Cancer." *Frontiers in Cellular and Infection Microbiology* 12: 962216. <https://doi.org/10.3389/fcimb.2022.962216>
 215. Barteneva, Natasha S, Yeldar Baiken, Elizaveta Fasler-Kan, Kenneth Alibek, Sheng Wang, Natalia Maltsev, Eugene D Ponomarev, et al. 2017. "Extracellular Vesicles in Gastrointestinal Cancer in Conjunction With Microbiota: On the Border Of Kingdoms." *Biochimica et Biophysica Acta (BBA)—Reviews on Cancer* 1868: 372–93. <https://doi.org/10.1016/j.bbcan.2017.06.005>
 216. Li, Min, Han Zhou, Chen Yang, Yi Wu, Xuechang Zhou, Hang Liu, and Yucai Wang. 2020. "Bacterial Outer Membrane Vesicles as a Platform for Biomedical Applications: An Update." *Journal of Controlled Release* 323: 253–68. <https://doi.org/10.1016/j.jconrel.2020.04.031>
 217. Jang, Su Chul, Sae Rom Kim, Yae Jin Yoon, Kyong-Su Park, Ji Hyun Kim, Jaewook Lee, Oh Youn Kim, et al. 2015. "In Vivo Kinetic Biodistribution of Nano-Sized Outer Membrane Vesicles Derived From Bacteria." *Small* 11: 456–61. <https://doi.org/10.1002/smll.201401803>
 218. Wei, Shou-Chao, Wei Wei, Wan-Juan Peng, Zhou Liu, Zhi-You Cai, and Bin Zhao. 2020. "Metabolic Alterations in the Outer Membrane Vesicles of Patients with Alzheimer's Disease: An LC-MS/MS-based Metabolomics Analysis." *Current Alzheimer Research* 16: 1183–95. <https://doi.org/10.2174/1567205016666191121141352>
 219. Yang, Jinho, Andrea McDowell, Hochan Seo, Sungwon Kim, Taek Ki Min, Young Koo Jee, Youngwoo Choi, et al. 2020. "Diagnostic Models for Atopic Dermatitis Based on Serum Microbial Extracellular Vesicle Metagenomic Analysis: A Pilot Study." *Allergy, Asthma & Immunology Research* 12: 792–805. <https://doi.org/10.4168/aaair.2020.12.5.792>
 220. Lee, Yeong Seok, Jeong Hee Kim, and Dae Hyun Lim. 2021. "Urine Microbe-Derived Extracellular Vesicles in Children With Asthma." *Allergy, Asthma & Immunology Research* 13: 75–87. <https://doi.org/10.4168/aaair.2021.13.1.75>
 221. Samra, Mona Salem, Dae Hyun Lim, Man Yong Han, Hye Mi Jee, Yoon Keun Kim, and Jeong Hee Kim. 2021. "Bacterial Microbiota-Derived Extracellular Vesicles in Children With Allergic Airway Diseases: Compositional and Functional Features." *Allergy, Asthma & Immunology Research* 13: 56–74. <https://doi.org/10.4168/aaair.2021.13.1.56>
 222. Kim, Se Ik, Nayeon Kang, Sangseob Leem, Jinho Yang, HyunA Jo, Maria Lee, Hee Seung Kim, et al. 2020. "Metagenomic Analysis of Serum Microbe-Derived Extracellular Vesicles and Diagnostic Models to Differentiate Ovarian Cancer and Benign Ovarian Tumor." *Cancers (Basel)* 12: 1309. <https://doi.org/10.3390/cancers12051309>
 223. Kim, Da Jung, Jinho Yang, Hochan Seo, Won Hee Lee, Dong Ho Lee, Sungmin Kym, Young Soo Park, et al. 2020. "Colorectal Cancer Diagnostic Model Utilizing Metagenomic and Metabolomic Data of Stool Microbial Extracellular Vesicles." *Science Reports* 10: 2860. <https://doi.org/10.1038/s41598-020-59529-8>
 224. Yang, Jinho, Goohyeon Hong, Youn Seup Kim, Hochan Seo, Sungwon Kim, Andrea McDowell, Won Hee Lee, et al. 2020. "Lung Disease Diagnostic Model Through IgG Sensitization to Microbial Extracellular Vesicles." *Allergy, Asthma & Immunology Research* 12: 669–83. <https://doi.org/10.4168/aaair.2020.12.4.669>
 225. van der Pol, Leo, Michiel Stork, and Peter van der Ley. 2015. "Outer Membrane Vesicles as Platform Vaccine Technology." *Biotechnology Journal* 10: 1689–706. <https://doi.org/10.1002/biot.201400395>
 226. Sartorio, Mariana G, Evan J Pardue, Mario F Feldman, and M. Florencia Haurat. 2021. "Bacterial Outer Membrane Vesicles: From Discovery to Applications." *Annual Reviews of Microbiology* 75: 609–30. <https://doi.org/10.1146/annurev-micro-052821-031444>
 227. Micoli, Francesca, and Calman A MacLennan. 2020. "Outer Membrane Vesicle Vaccines." *Seminars in Immunology* 50: 101433. <https://doi.org/10.1016/j.smim.2020.101433>
 228. Huang, Weiwei, Yufeng Yao, Qiong Long, Xu Yang, Wenjia Sun, Cunbao Liu, Xiaomei Jin, et al. 2014. "Immunization Against Multidrug-Resistant *Acinetobacter baumannii* Effectively Protects Mice in Both Pneumonia and Sepsis Models." *PLoS One* 9: e100727. <https://doi.org/10.1371/journal.pone.0100727>
 229. Gaillard, María Emilia, Daniela Bottero, Agustina Errea, Maximiliano Ormazábal, M. Eugenia Zurita, Griselda Moreno, Martin Rumbo, et al. 2014. "Acellular Pertussis Vaccine Based on Outer Membrane Vesicles Capable of Conferring Both Long-Lasting Immunity and Protection Against Different Strain Genotypes." *Vaccine* 32: 931–37. <https://doi.org/10.1016/j.vaccine.2013.12.048>
 230. Raeven, René H. M., Jolanda Brummelman, Jeroen L. A. Pennings, Larissa van der Maas,

- Wichard Tilstra, Kina Helm, Elly van Riet, et al. 2016. "Bordetella Pertussis Outer Membrane Vesicle Vaccine Confers Equal Efficacy in Mice With Milder Inflammatory Responses Compared to a Whole-Cell Vaccine." *Scientific Reports* 6: 38240. <https://doi.org/10.1038/srep38240>
231. Choi, Seng Jin, Min-Hye Kim, Jinseong Jeon, Oh Youn Kim, Youngwoo Choi, Jihye Seo, Sung-Wook Hong, et al. 2015. "Active Immunization With Extracellular Vesicles Derived From *Staphylococcus aureus* Effectively Protects Against Staphylococcal Lung Infections, Mainly Via Th1 Cell-Mediated Immunity." *PLoS One* 10: e0136021. <https://doi.org/10.1371/journal.pone.0136021>
 232. Pritsch, Michael, Najib Ben-Khaled, Michael Chaloupka, Sebastian Kobold, Nicole Berens-Riha, Annabell Peter, Gabriele Liegl, et al. 2016. "Comparison of Intranasal Outer Membrane Vesicles With Cholera Toxin and Injected MF59C.1 as Adjuvants for Malaria Transmission Blocking Antigens AnAPN1 and Pfs48/45." *Journal of Immunology Research* 2016: 1–11. <https://doi.org/10.1155/2016/3576028>
 233. Lee, Tae-Young, Chang-Ung Kim, Eun-Hye Bae, Sang-Hwan Seo, Dae Gwin Jeong, Sun-Woo Yoon, Kyu-Tae Chang, et al. 2017. "Outer Membrane Vesicles Harboring Modified Lipid A Moiety Augment the Efficacy of an Influenza Vaccine Exhibiting Reduced Endotoxicity in a Mouse Model." *Vaccine* 35: 586–95. <https://doi.org/10.1016/j.vaccine.2016.12.025>
 234. Sardiñas, Gretel, Karen Reddin, Rolando Pajon, and Andrew Gorringer. 2006. "Outer Membrane Vesicles of *Neisseria lactamica* as a Potential Mucosal Adjuvant." *Vaccine* 24: 206–14. <https://doi.org/10.1016/j.vaccine.2005.07.064>
 235. Daleke-Schermerhorn, Maria H., Tristan Felix, Zora Soprova, Corinne M. Ten hagen-Jongman, David Vikström, Laleh Majlessi, Joep Beskers, et al. 2014. "Decoration of Outer Membrane Vesicles With Multiple Antigens by Using an Autotransporter Approach." *Applied and Environmental Microbiology* 80: 5854–65. <https://doi.org/10.1128/AEM.01941-14>
 236. Huang, Weiwei, Shijie Wang, Yufeng Yao, Ye Xia, Xu Yang, Kui Li, Pengyan Sun, et al. 2016. "Employing *Escherichia coli*-derived Outer Membrane Vesicles as an Antigen Delivery Platform Elicits Protective Immunity Against *Acinetobacter baumannii* Infection." *Scientific Reports* 6: 37242. <https://doi.org/10.1038/srep37242>
 237. Rappazzo, C. Garrett, Hannah C. Watkins, Cassandra M. Guarino, Annie Chau, Jody L. Lopez, Matthew P. DeLisa, et al. 2016. "Recombinant M2e Outer Membrane Vesicle Vaccines Protect Against Lethal Influenza A Challenge in BALB/c Mice." *Vaccine* 34: 1252–58. <https://doi.org/10.1016/j.vaccine.2016.01.028>
 238. Price, Nancy L., Guillaume Goyette-Desjardins, Harald Nothaft, Ezequiel Valguarnera, Christine M. Szymanski, Mariela Segura, and Mario F. Feldman. 2016. "Glycoengineered Outer Membrane Vesicles: A Novel Platform for Bacterial Vaccines." *Scientific Reports* 6: 24931. <https://doi.org/10.1038/srep24931>
 239. Díez-Sainz, Ester, Fermín I. Milagro, José I. Riezu-Boj, and Silvia Lorente-Cebrián. 2022. "Effects of Gut Microbiota-Derived Extracellular Vesicles on Obesity and Diabetes and Their Potential Modulation Through Diet." *Journal of Physiology and Biochemistry* 78: 485–99. <https://doi.org/10.1007/s13105-021-00837-6>
 240. Choi, Juli, Yoon-Keun Kim, and Pyung-Lim Han. 2019. "Extracellular Vesicles Derived From *Lactobacillus plantarum* Increase BDNF Expression in Cultured Hippocampal Neurons and Produce Antidepressant-Like Effects in Mice." *Experimental Neurobiology* 28: 158–71. <https://doi.org/10.5607/en.2019.28.2.158>
 241. Yoo, Jin-Wook, Darrell J. Irvine, Dennis E. Discher, and Samir Mitragotri. 2011. "Bio-Inspired, Bioengineered and Biomimetic Drug Delivery Carriers." *Nature Reviews Drug Discovery* 10: 521–35. <https://doi.org/10.1038/nrd3499>
 242. Gujrati, Vipul B., and Sangyong Jon. 2014. "Bioengineered Bacterial Outer Membrane Vesicles: What is Their Potential in Cancer Therapy?" *Nanomedicine* 9: 933–35. <https://doi.org/10.2217/nnm.14.56>
 243. Vader, Pieter, Emma A. Mol, Gerard Pasterkamp, and Raymond M. Schiffelers. 2016. "Extracellular Vesicles for Drug Delivery." *Advanced Drug Delivery Reviews* 106: 148–56. <https://doi.org/10.1016/j.addr.2016.02.006>
 244. Liu, Han, Hao Zhang, Yafei Han, Yan Hu, Zhen Geng, and Jiacan Su. 2022. "Bacterial Extracellular Vesicles-Based Therapeutic Strategies for Bone and Soft Tissue Tumors Therapy." *Theranostics* 12: 6576–94. <https://doi.org/10.7150/thno.78034>
 245. Li, Tong, Xian-Mao Lu, Ming-Rong Zhang, Kuan Hu, and Zhou Li. 2022. "Peptide-Based Nanomaterials: Self-Assembly, Properties and Applications." *Bioactive Materials* 11: 268–82. <https://doi.org/10.1016/j.bioactmat.2021.09.029>
 246. Allan, Nick D., and Terry J. Beveridge. 2003. "Gentamicin Delivery to Burkholderia Cepacia Group IIIa Strains Via Membrane Vesicles From *Pseudomonas aeruginosa* PAO1." *Antimicrobial Agents and Chemotherapy* 47: 2962–65. <https://doi.org/10.1128/AAC.47.9.2962-2965.2003>
 247. Alves, Nathan J., Kendrick B. Turner, Igor L. Medintz, and Scott A. Walper. 2016. "Protecting Enzymatic Function Through Directed Packaging Into Bacterial Outer Membrane Vesicles." *Scientific Reports* 6: 24866. <https://doi.org/10.1038/srep24866>
 248. Daniele, Lorenzo, and Anna Sapino. 2009. "Anti-HER2 treatment and breast cancer: state of the art, recent patents, and new strategies." *Recent Patents On Anti-cancer Drug Discovery* 4. <https://pubmed.ncbi.nlm.nih.gov/19149684>
 249. Gu, Ting-Wei, Mao-Ze Wang, Jie Niu, Yang Chu, Ke-Ran Guo, and Li-Hua Peng. 2020. "Outer Membrane Vesicles Derived From *E. coli* as Novel Vehicles for Transdermal and Tumor Targeting Delivery." *Nanoscale* 12: 18965–77. <https://doi.org/10.1039/d0nr03698f>
 250. Carvalho, Ana L., Sonia Fonseca, Ariadna Miquel-Clopés, Kathryn Cross, Khoon- S. Kok, Udo Wegmann, Katherine Gil-Cardoso, et al. 2019. "Bioengineering Commensal Bacteria-Derived Outer Membrane Vesicles for Delivery of Biologics to the Gastrointestinal and Respiratory Tract." *Journal of Extracellular Vesicles* 8: 1632100. <https://doi.org/10.1080/20013078.2019.1632100>
 251. Gujrati, Vipul, Sunghyun Kim, Sang-Hyun Kim, Jung Joon Min, Hyon E. Choy, Sun Chang Kim, and Sangyong Jon. 2014. "Bioengineered Bacterial Outer Membrane Vesicles as Cell-Specific Drug-Delivery Vehicles for Cancer Therapy." *ACS Nano* 8: 1525–37. <https://doi.org/10.1021/nn405724x>

252. Kim, Oh Youn, Nhung Thi Hong Dinh, Hyun Taek Park, Seng Jin Choi, Kahye Hong, and Yong Song Gho. 2017. "Bacterial Protoplast-Derived Nanovesicles for Tumor Targeted Delivery of Chemotherapeutics." *Biomaterials* 113: 68–79. <https://doi.org/10.1016/j.biomaterials.2016.10.037>
253. Wang, Dongdong, Conghui Liu, Shiquan You, Kai Zhang, Meng Li, Yu Cao, Changtao Wang, Haifeng Dong, and Xueji Zhang. 2020. "Bacterial Vesicle-Cancer Cell Hybrid Membrane-Coated Nanoparticles for Tumor Specific Immune Activation and Photothermal Therapy." *ACS Applied Materials & Interfaces* 12: 41138–47. <https://doi.org/10.1021/acsami.0c13169>
254. Zhuang, Qi, Jun Xu, Dashi Deng, Ting Chao, Junyan Li, Rui Zhang, Rui Peng, and Zhuang Liu. 2021. "Bacteria-Derived Membrane Vesicles to Advance Targeted Photothermal Tumor Ablation." *Biomaterials* 268: 120550. <https://doi.org/10.1016/j.biomaterials.2020.120550>
255. Kuerban, Kudelaidi, Xiwen Gao, Hui Zhang, Jiayang Liu, Mengxue Dong, Lina Wu, Ruihong Ye, et al. 2020. "Doxorubicin-Loaded Bacterial Outer-Membrane Vesicles Exert Enhanced Anti-Tumor Efficacy in Non-Small-Cell Lung Cancer." *Acta Pharmaceutica Sinica B* 10: 1534–48. <https://doi.org/10.1016/j.apsb.2020.02.002>
256. Chen, Qi, Hongzhen Bai, Wangteng Wu, Guojun Huang, Yang Li, Min Wu, Guping Tang, and Yuan Ping. 2020. "Bioengineering Bacterial Vesicle-Coated Polymeric Nanomedicine for Enhanced Cancer Immunotherapy and Metastasis Prevention." *Nano Lett* 20: 11–21. <https://doi.org/10.1021/acs.nanolett.9b02182>
257. Li, Yao, Ruifang Zhao, Keman Cheng, Kaiyue Zhang, Yazhou Wang, Yinlong Zhang, Yujing Li, et al. 2020. "Bacterial Outer Membrane Vesicles Presenting Programmed Death 1 for Improved Cancer Immunotherapy via Immune Activation and Checkpoint Inhibition." *ACS Nano* 14: 16698–711. <https://doi.org/10.1021/acs.nano.0c03776>
258. Chen, Qi, Guojun Huang, Wangteng Wu, Jianwei Wang, Jiawei Hu, Jianming Mao, Paul K. Chu, Hongzhen Bai, and Guping Tang. 2020. "A Hybrid Eukaryotic-Prokaryotic Nanoplatform with Photothermal Modality for Enhanced Antitumor Vaccination." *Advanced Materials* 32: e1908185. <https://doi.org/10.1002/adma.201908185>

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