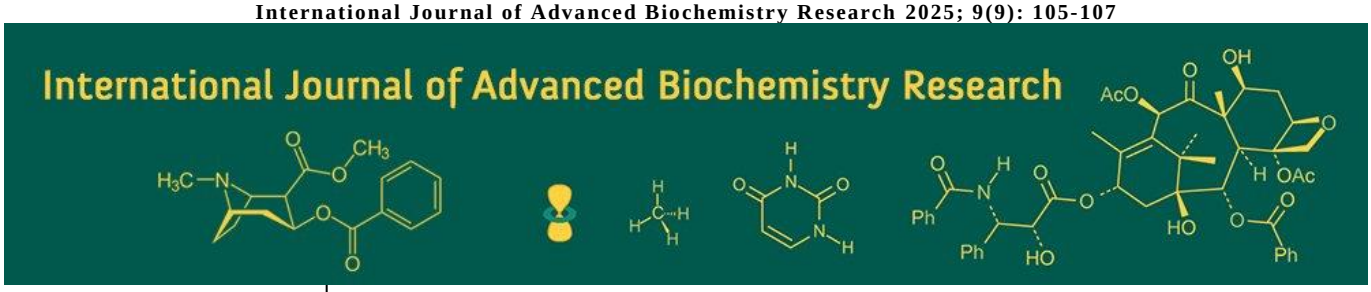

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Outer membrane vesicles (OMVs) as a versatile platform for vaccines and adjuvants: Strategies, advances, and perspectives

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Abstract
Outer membrane vesicles (OMVs) are nano-sized, spherical particles naturally released by Gram-negative bacteria through outer membrane blebbing. Composed of lipopolysaccharides (LPS), outer membrane proteins, phospholipids, and periplasmic components, OMVs play critical roles in bacterial communication, virulence, and host-pathogen interactions. Their intrinsic immunostimulatory properties and ability to deliver bioactive molecules have positioned OMVs as a powerful platform for vaccine development and drug delivery. This review comprehensively examines the bioengineering of OMVs for vaccine applications, focusing on their self-adjuvanting nature, antigen delivery capabilities, and potential in combating infectious diseases and cancer. We discuss strategies for modifying OMVs—including detergent extraction, genetic detoxification of LPS, and surface display of heterologous antigens—to enhance safety, immunogenicity, and targeting. The clinical success of OMV-based vaccines such as Bexsero® and PedvaxHIB highlights their translational potential. Furthermore, we explore the emerging role of OMVs in cancer immunotherapy, where they induce immunogenic cell death, promote trained immunity, and can be engineered to deliver chemotherapeutics or siRNA. Advances in scalable production and purification methods are also addressed, paving the way for broader clinical adoption. Collectively, OMVs represent a versatile and multifunctional platform that integrates antigen presentation, immune activation, and targeted delivery, making them a cornerstone of next-generation therapeutic and prophylactic strategies.

Keywords: Outer membrane vesicles (OMVs), vaccine delivery, self-adjuvanticity, bacterial vesicles, cancer immunotherapy, antigen presentation, trained immunity, drug delivery, nanovaccines, bioengineering

Introduction
Outer membrane vesicles (OMVs) are 20-300 nm spherical nanoparticles naturally shed by Gram-negative bacteria during growth. These vesicles are composed of a lipid bilayer enriched with outer membrane proteins, lipopolysaccharides (LPS), phospholipids, and periplasmic components, encapsulating a diverse cargo of proteins, nucleic acids, and metabolites [1]. Initially considered byproducts of bacterial stress or growth, OMVs are now recognized as key mediators of intercellular communication, host-pathogen interactions, and immune modulation [2]. Their ability to transfer virulence factors, modulate host immune responses, and deliver functional biomolecules has sparked significant interest in harnessing OMVs for biomedical applications, particularly in vaccinology and targeted therapy.

One of the most compelling attributes of OMVs is their inherent immunogenicity. They contain pathogen-associated molecular patterns (PAMPs) such as LPS, lipoproteins, and bacterial DNA, which activate Toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) on antigen-presenting cells (APCs), triggering robust innate and adaptive immune responses [3]. This self-adjuvanting property eliminates the need for external adjuvants, simplifying vaccine formulation and enhancing immunogenicity. Licensed OMV-based vaccines, such as Bexsero® for meningococcal disease and PedvaxHIB for *Haemophilus influenzae* type b, exemplify the successful translation of this platform into clinical practice [4]. Beyond prophylactic vaccines, OMVs are being engineered to serve as as delivery vehicles for heterologous antigens, anticancer drugs, and nucleic acids, enabling

targeted therapy for infectious diseases and malignancies.

This review explores the bioengineering strategies used to optimize OMVs for vaccine development and therapeutic delivery. We discuss their structural and functional characteristics, methods for enhancing safety and immunogenicity, and their expanding applications in infectious disease prevention and cancer immunotherapy. Furthermore, we highlight recent advances in scalable production and purification technologies that are critical for the clinical translation of engineered OMVs.

Structural and Functional Characteristics of OMVs

OMVs are derived from the outer membrane of Gram-negative bacteria and maintain a spherical, bilayered structure that encapsulates periplasmic content. The asymmetric lipid composition, with LPS predominantly in the outer leaflet and phospholipids in the inner leaflet, contributes to their stability and interaction with host cells [5]. Key components include porins, adhesins, and transporters, which not only support bacterial physiology but also serve as immunodominant antigens when presented on OMVs [6]. The presence of bioactive molecules such as cardiolipin and lipid A further enhances their immunostimulatory capacity by activating TLR4 and inflammasome pathways.

The biogenesis of OMVs is influenced by various environmental factors, including antibiotic stress, nutrient limitation, and oxidative conditions. Sub-inhibitory concentrations of antibiotics such as ceftazidime and ciprofloxacin have been shown to significantly increase OMV production, suggesting that stress responses can be leveraged to boost yield in biomanufacturing processes [4]. Additionally, mechanisms such as the twin-arginine translocation (Tat) pathway and local disruptions in lipoprotein-outer membrane interactions contribute to membrane curvature and vesicle formation [7]. The selective packaging of proteins into OMVs—such as PorB from *Neisseria gonorrhoeae*—is mediated by lipid rafts and protein-lipid interactions, allowing for the enrichment of specific antigens and functional molecules [6].

Engineering OMVs for Enhanced Safety and Immunogenicity

While native OMVs are highly immunogenic, their endotoxic LPS content can cause reactogenicity. To improve safety, several strategies have been developed. Detergent extraction (dOMVs) reduces LPS levels and has been used in licensed vaccines like Bexsero® and VA-MENGOC-BC® [3]. Genetic modifications, such as deletion of *msbB* or *lpxL1* genes, result in penta-acylated LPS with reduced TLR4 activation, thereby lowering pyrogenicity while preserving immunogenicity [8]. These modified OMVs (mdOMVs) offer a safer profile for repeated dosing and use in vulnerable populations.

Antigen loading and surface display are critical for vaccine efficacy. OMVs can be engineered to display heterologous antigens via genetic fusion with outer membrane proteins (e.g., ClyA, OmpA, or autotransporters), chemical conjugation, or membrane insertion [9]. This enables high-density antigen presentation and improved immune recognition. For example, the Hib polysaccharide in PedvaxHIB is conjugated to an outer membrane protein complex from *N. meningitidis*, resulting in 93-100% efficacy in high-risk infant populations [10]. Proteome-minimized OMVs, which lack non-essential proteins, offer a "clean" platform for precise antigen display and reduced off-target immune activation [11].

OMVs in Infectious Disease Vaccines

OMVs have been successfully deployed in vaccines against several pathogens. The most notable example is Bexsero®, a multicomponent vaccine against *Neisseria meningitidis* serogroup B, which combines dOMVs with recombinant proteins (NHBA, NadA, fHbp) and is recommended for infants as young as two months. Clinical studies have confirmed its immunogenicity, although co-administration with routine vaccines is associated with increased reactogenicity, particularly fever [12].

OMVs are also being explored for vaccines against *Helicobacter pylori*, *Klebsiella pneumoniae*, and enterotoxigenic *E. coli* (ETEC). For instance, a combined OMV-based vaccine targeting both *Vibrio cholerae* and ETEC has shown promise in preclinical models [13]. The ability of OMVs to stimulate both mucosal and systemic immunity makes them ideal candidates for oral or intranasal vaccines, especially when combined with mucosal adjuvants like retinoic acid [14].

OMVs in Cancer Immunotherapy and Drug Delivery

Beyond infectious diseases, OMVs are emerging as powerful tools in oncology. They can be loaded with chemotherapeutic agents, siRNA, or tumor-associated antigens (TAAs) to target cancer cells directly while simultaneously activating dendritic cells and promoting cross-presentation [15]. Engineered OMVs expressing SIRPα-Fc enhance phagocytosis of tumor cells by reprogramming tumor-associated macrophages (TAMs), demonstrating their potential in modulating the tumor microenvironment [4].

OMVs also induce immunogenic cell death (ICD), characterized by the release of damage-associated molecular patterns (DAMPs) such as calreticulin, ATP, and HMGB1, which act as danger signals to recruit and activate APCs [16]. This transforms the tumor into an "in situ vaccine" site, amplifying anti-tumor immunity. When combined with checkpoint inhibitors or oncolytic viruses, OMVs can synergistically enhance therapeutic efficacy [17].

Moreover, OMVs can stimulate trained immunity—long-term functional reprogramming of innate immune cells—offering durable protection against tumor recurrence [18]. Their natural tropism for immune cells and ability to accumulate in tumors via the enhanced permeability and retention (EPR) effect further enhance their therapeutic potential [19].

Scalable Production and Future Perspectives

The clinical translation of OMVs requires scalable, reproducible, and GMP-compliant manufacturing processes. Recent advances in continuous bioprocessing, combined bind-elute and size exclusion chromatography, and non-chromatographic purification methods are addressing these challenges [20, 21]. The integration of synthetic biology tools—such as CRISPR-based genome editing and biosensors for real-time monitoring—further enables precise control over OMV composition and function.

Future directions include the development of hybrid OMV-liposome systems, OMV-based mRNA delivery, and personalized cancer vaccines tailored to individual

mutanomes. As our understanding of OMV biogenesis and host interactions deepens, engineered OMVs are poised to become a cornerstone of next-generation vaccines and targeted therapies.

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