

ENGINEERING OUTER MEMBRANE VESICLES FOR THERAPEUTIC APPLICATIONS: ADVANCES, CLINICAL TRANSLATION AND FUTURE PERSPECTIVES

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Abstract

Outer membrane vesicles (OMVs) are naturally released lipid-bilayer nanovesicles produced by Gram-negative bacteria that contain diverse bacterial components, including proteins, lipids, lipopolysaccharides (LPS), nucleic acids and enzymes. Their nanoscale architecture, biological activity and intrinsic capacity to interact with host cells make OMVs attractive platforms for therapeutic delivery and immunomodulation. However, naturally produced OMVs exhibit substantial heterogeneity and may possess undesirable inflammatory activity, particularly because of their LPS content. Engineering approaches have therefore been developed to control vesicle production, composition, cargo loading, surface properties and biological activity.

This review examines recent advances in the engineering of OMVs for therapeutic applications, including genetic engineering, hypervesiculation, LPS detoxification, cargo loading, surface functionalization, hybrid systems, stimuli-responsive approaches and programmable engineering. Particular emphasis is placed on emerging applications in cancer therapy, antimicrobial delivery, nucleic acid delivery and immunotherapy. The review further evaluates key barriers to clinical translation, including scalable manufacturing, purification, characterization, batch-to-batch consistency, pharmacokinetics, biodistribution, safety, immunogenicity and regulatory requirements. Finally, emerging opportunities involving synthetic biology, computational design and artificial intelligence are discussed in the context of rational OMV engineering.

Overall, engineered OMVs represent a promising biological nanoplatform, but their clinical development will require reproducible manufacturing, well-defined quality attributes, controlled immunogenicity, validated therapeutic efficacy and appropriate regulatory frameworks.

INTRODUCTION

Outer membrane vesicles (OMVs) are nanoscale extracellular vesicles naturally produced by Gram-negative bacteria. In recent years, increasing attention has been directed toward OMVs as biological nanocarriers because they possess a lipid bilayer structure, contain diverse biological molecules, and can interact with mammalian cells. These characteristics provide opportunities for their application in drug delivery, vaccination, immunotherapy and other biomedical applications (Huang et al., 2022).

Despite these advantages, naturally produced OMVs have several characteristics that limit their direct therapeutic use. Their molecular composition can vary according to bacterial strain, growth conditions and vesicle biogenesis, resulting in heterogeneous vesicle populations. In addition, the presence of lipopolysaccharide (LPS) and other pathogen-associated molecular patterns can induce strong inflammatory responses. Variability in vesicle composition, cargo loading, production yield, purification and biological activity also creates challenges for reproducible manufacturing and clinical development (Manno et al., 2025).

Engineering strategies have therefore been developed to modify OMVs according to specific therapeutic requirements. Genetic engineering can alter vesicle production, cargo incorporation and surface composition, whereas metabolic and chemical approaches can modify undesirable components or introduce additional functions.

Cargo-engineering strategies enable the incorporation of proteins, nucleic acids and small molecules, while surface functionalization can potentially improve cellular targeting. More recently, hybrid systems, stimuli-responsive designs, synthetic biology and computational approaches have been explored to increase the functional capabilities and controllability of OMVs (Bukke et al., 2026).

The therapeutic potential of engineered OMVs extends beyond their established applications as vaccine platforms. Preclinical studies have investigated their use in cancer immunotherapy, antimicrobial delivery, nucleic acid delivery and targeted therapeutic systems. However, translation from experimental models to clinical applications remains limited by challenges involving safety, manufacturing, characterization, pharmacokinetics, biodistribution and regulatory requirements (Luo et al., 2024).

This review critically examines current strategies for engineering OMVs for therapeutic applications, with particular emphasis on genetic engineering, LPS modification, cargo loading, surface functionalization, hybrid and stimuli-responsive systems and emerging programmable approaches. We further discuss therapeutic applications, manufacturing and quality-control requirements, clinical evidence, major knowledge gaps and future directions required to advance engineered OMVs toward clinically viable therapeutic platforms.

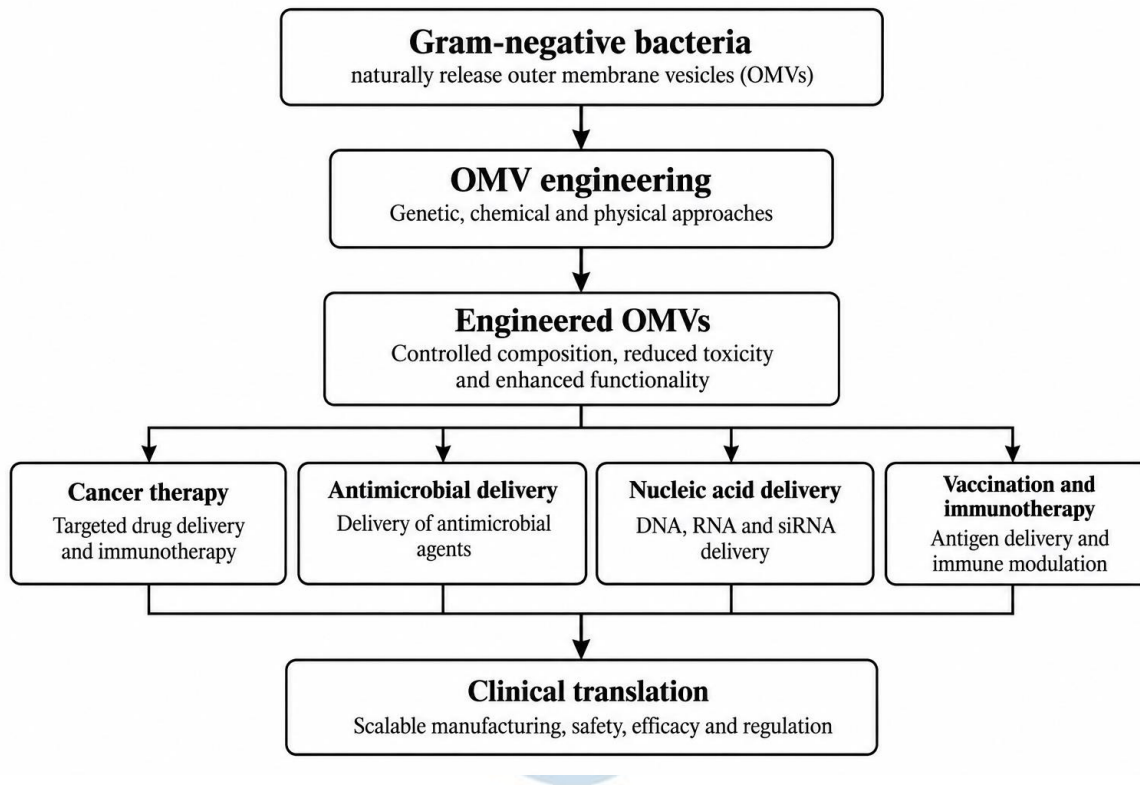


Figure 1. Gram-negative bacteria naturally release outer membrane vesicles (OMVs) which can be engineered to control their composition, safety and therapeutic functions. Engineered OMVs have potential applications in cancer therapy, antimicrobial and nucleic-acid delivery, vaccination and immunotherapy, while clinical translation requires scalable manufacturing, safety, efficacy and regulatory control.

Biological Basis of Outer Membrane Vesicles

OMVs are formed from the outer membrane of Gram-negative bacteria whose envelope contains the inner membrane, periplasmic space, peptidoglycan layer and outer membrane. The outer membrane is composed mainly of LPS, phospholipids, outer-membrane proteins and lipoproteins and these components influence the composition and properties of the vesicles that bud from it. While an OMV is formed some of these compounds and structures get into the vesicle thus the composition of the bacterial outer membrane determines that of the OMVs (Xue et

al., 2026). OMVs appear when some part of the outer membrane of the bacteria protrudes and detaches from it and forms a membrane-enclosed vesicle. Thus, any changes in the composition or stability of the outer membrane can have an impact on the quantity of OMVs produced and their characteristics (Xue et al., 2022). This fact represents one of the basic principles in OMV engineering. Natural OMVs contain proteins, lipids, LPS, DNA, RNA and other bacterial components. Their intrinsic ability to transport biological material makes them attractive as delivery carriers (PAN et al., 2024). The fact is that a protein expressed by bacteria cannot be directly included into OMVs just because of its localization inside bacterial cells should be regulated in order to make it possible to associate the needed molecule with OMV either its surface or interior (Caruana & Walper, 2020). However, successful engineering requires control over the localization of desired molecules so that cargo is incorporated into the OMV surface or lumen. This point matters as there are different functions

for surface-displayed and internally delivered molecules in engineered OMVs. So OMV engineering starts from genetic modification of OMV-producing bacteria (Chen et al., 2022). It is possible to introduce or delete some genes in order to alter OMV production, composition and other parameters like inflammation caused by LPS. Finally, the interaction of engineered OMVs with a target host is required. OMVs are able to bind to host cells and enter them in order to deliver molecules that could act biologically. The overall engineering concept is therefore to modify the producer bacterium, control OMV production and composition, manipulate the vesicle surface or cargo and achieve a defined biological effect in target cells (Chen et al., 2022; Wang et al., 2026).

Biogenesis of OMVs

Outer membrane vesicles (OMVs) are spherical structures that are released from the outer membrane of Gram-negative bacteria (Kim et al., 2023). Their biogenesis is a multifactorial, regulated phenomenon and is not simply a consequence of cell lysis. Several coordinated mechanisms contribute to this process.

Classical blebbing: This principal pathway involves periplasmic stress, often associated with localized buildup of lipopolysaccharides (LPS) driving outward blebbing of the outer membrane. The resulting outward bulge then separates from the underlying peptidoglycan layer, ultimately leading to vesicle scission. The process begins with outward protrusion of the outer membrane, commonly occurring in regions that lack membrane-peptidoglycan bonds. Antibiotics that disrupt peptidoglycan integrity or reposition or destroy OM-peptidoglycan linkages can cause this disruption (Wang et al., 2025). Moreover, the accumulation of periplasmic constituents or specific curvature-inducing proteins can generate pressure that promotes this bulging (Li et al., 2024).

Explosive cell lysis: Under acute stress conditions such as antibiotic exposure, explosive cell lysis can act as an important complementary route to OMV formation. In this scenario, membrane fragments that are liberated during catastrophic cellular

rupture can self-seal into OMVs, markedly increasing the total vesicle output (Zhu et al., 2025).

Stress-induced vesiculation and regulation: OMV production is understood as a distinct bacterial stress response mechanism. Periplasm-driven stress is widely viewed as a key initiating factor: accumulation of misfolded proteins or peptidoglycan fragments within the periplasmic space produces turgor pressure and triggers envelope stress responses (Zhang et al., 2026). Vesiculation then occurs through a “pressure-release valve” mechanism. OMV generation is regulated and depends on growth conditions, stress responses and genetic factors. For example, elevated growth temperatures or envelope stress can raise OMV production (Wang et al., 2025). Likewise, mutations in genes that participate in the SigmaE stress response pathway or in OM-peptidoglycan linkages can also modify vesiculation levels (Gonçalves et al., 2025; Li et al., 2024; Woo et al., 2022).

The idea of increased OMV production is associated with stress responses and genetic factors. Mutations in genes involved in the SigmaE stress response pathway or OM-peptidoglycan linkages (such as OmpA) can influence vesiculation levels (Gonçalves et al., 2025; Wang et al., 2025; Zhang et al., 2026). In addition, during acute stress conditions like antibiotic treatment, explosive cell lysis can substantially enhance vesicle yield. Collectively, this suggests that particular conditions or genetic alterations can increase the rate of OMV production, which is consistent with the concept of hypervesiculation (Yu et al., 2026; Zhang, J. et al., 2026). Outer membrane vesicles (OMVs) house a broad range of bacterial constituents. These constituents support their functions in bacterial pathogenesis and communication and they also underlie their potential use as vaccine platforms (Magaña et al., 2023; Yu et al., 2026).

Outer membrane proteins, lipids and periplasmic contents: OMVs are composed mainly of bacterial proteins, lipids and periplasmic contents, thereby mirroring the composition of the bacterial outer membrane from which they

originate. They are characterized as small spherical lipid nanoparticles (Chen et al., 2025).

LPS is a highly immunogenic outer membrane structure present exclusively in Gram-negative bacteria. It comprises three principal components: the outermost O antigen (polysaccharides), the central core oligosaccharide and lipid A, the innermost segment that is inserted into the outer membrane. LPS functions as a key pattern-associated molecular pattern (PAMP) by activating Toll-like receptor 4 (Lieberman, 2022).

OMVs include phospholipids which can accumulate within the outer membrane when disruptions occur in ATP-binding cassette transport systems resulting in enhanced vesiculation (Zahid et al., 2025). Although the OMV is not described explicitly as containing “fragments,” the peptidoglycan layer is an essential element of the Gram-negative cell envelope and its disruption can cause hypervesiculation. Peptidoglycans are also recognized as PAMPs by the host immune system. OMVs may carry nucleic acids which they can deliver to nearby cells. Intracellular Toll-like receptor 9 recognizes unmethylated CpGs that are found in prokaryotic genomic DNA present in OMVs.

In addition to structural proteins OMVs transport a variety of proteins. These include immunogenic proteins and glycans displayed on their surface which contribute to their natural adjuvant properties. The quantity of outer membrane and periplasmic proteins incorporated into OMVs differs across organisms (Cheng et al., 2025; Cui et al., 2023).

OMVs produced by pathogenic bacteria can fuse with other bacterial membranes, thereby delivering virulence factors such as toxins and antibiotic resistance plasmids. These virulence factors promote bacterial pathogenesis (Xuan & Xuan, 2024).

Physiological functions

Outer membrane vesicles (OMVs) are naturally released lipid bilayer vesicles produced by Gram-negative bacteria. They carry a wide range of biological cargo including proteins, enzymes, lipopolysaccharides (LPS), peptidoglycan, DNA

and RNA. Because they can transport these molecules, OMVs support key physiological roles in bacterial communication, stress adaptation, interactions with the microbiome and host-microbe relationships (Magaña et al., 2023; Zahid et al., 2025).

Extracellular communication and stress adaptation: OMVs function as delivery vehicles for transporting signaling molecules and functional cargo between bacterial cells, thereby enabling communication among bacteria and coordinated community-level behavior. OMV biogenesis and cargo composition are shaped by environmental stressors such as shifts in temperature, oxygen availability, nutrient availability and other unfavorable conditions. By exporting selected molecules and components that are associated with stress, OMVs help bacteria adapt and persist in changing microenvironments (Garling et al., 2025b).

Immunomodulation and host communication: The immune responses may be altered by bacterial components that are delivered by OMVs when they interact with host cells (Kaparakis-Liaskos & Ferrero, 2015). For instance, Caspase-11-dependent inflammatory signaling can be triggered by LPS delivered via OMVs. Their inherent capacity to engage host cells also positions OMVs as promising candidates for therapeutic delivery. Microbiome and biofilm interaction: OMVs can influence microbiome composition and function and help coordinate communication within microbial communities. Biofilm-associated community behavior may be further maintained by their contributions to signaling and stress adaptation (Huang et al., 2022).

Antimicrobial reactions and Virulence: OMVs have the ability to carry and discharge molecules linked to virulence to promote bacterial pathogenicity and host interactions (Magaña et al., 2023). Antimicrobial exposure can potentially alter OMV production and cargo including during aminoglycoside treatment (Liu et al., 2023). Nucleic acid transport: The presence of DNA and RNA inside OMVs indicates a potential role in the

extracellular delivery of genetic material. While this alone does not prove direct horizontal gene transfer, OMVs offer a protected pathway for nucleic acid transport and have been explored for uses such as CRISPR-based delivery (Marinacci et al., 2023).

Engineering Strategies for Therapeutic OMVs

OMV engineering focuses on modifying the bacterial strains that produce OMVs, the composition of the vesicles, their cargo or their surfaces to optimize their yield, reduce endotoxicity and enable programmable payload loading. These modifications can influence OMV biogenesis, composition, targeting and therapeutic function, making OMVs increasingly useful as biological nanocarriers. Genetic engineering primarily modifies the bacterial producer, whereas cargo and surface engineering can be performed either genetically during OMV biogenesis or post-production on isolated vesicles. These methods can also be used to produce customized OMVs with multiple engineered properties (Chaudhari et al., 2025; Nie et al., 2024; Zhao et al., 2025; Zhao, X. et al., 2025).

Genetic engineering: The main focus of Genetic engineering is to modify the bacterial strains that produce OMVs to optimize their yield, reduce endotoxicity and enable programmable payload loading. This involves changing the bacterial genome to influence OMV biogenesis and content making them safer and more effective carriers (Lieberman, 2022). Because OMVs originate from the bacterial outer membrane, modifications to genes controlling membrane stability, LPS biosynthesis, protein expression and intracellular trafficking can consequently alter the quantity and composition of the resulting vesicles (Zhang et al., 2026).

OMV production is strongly influenced by the physical relationship between the bacterial outer membrane and the underlying peptidoglycan layer. In Gram-negative bacteria proteins such as Lpp, OmpA and components of the Tol-Pal complex contribute to maintaining these connections and preserving outer-membrane stability (Juodeikis & Carding, 2022). The Tol-Pal

complex is a group of proteins involved in maintaining the organization and integrity of the bacterial envelope and disruption of this system can weaken the physical constraints that normally stabilize the outer membrane (Woo et al., 2022). Genetic disruptions or point mutations in these components such as knocking out tolR, weaken the connections between the outer membrane and the underlying cell-envelope structures (Abubakari et al., 2026; Juodeikis & Carding, 2022). When these constraints are reduced, localized regions of the outer membrane can become less tightly attached and deform outward. These regions form what can be described as a “structurally relaxed zone” from which the membrane can bleb and eventually bud off as an OMV (Magaña et al., 2023; Velimirov & Velimirov, 2024). Consequently, the bacterium releases a greater number of vesicles, producing what are commonly termed high-yield vesicle strains. For example, *Escherichia coli* Tol-Pal mutants are known to form increased outer membrane vesicles, demonstrating the role of Tol-Pal in controlling outer membrane stability and organization (Laotee & Arunmanee, 2024). The objective of hypervesiculation is therefore not simply to make the bacterium release vesicles randomly but to genetically alter the physical constraints governing membrane curvature and separation. However, increasing OMV production can also alter the composition of the vesicle and bacterial fitness, meaning that a high vesicle yield does not necessarily correspond to optimal vesicle quality (Kim et al., 2026).

One of the major limitations of OMVs for therapeutic applications is their natural association with lipopolysaccharide (LPS). LPS is an important component of the Gram-negative outer membrane and is therefore incorporated into OMVs during vesicle formation (Ayesha et al., 2023). Its lipid A component is particularly important because it is recognized by the innate immune system and can produce strong inflammatory responses. Excessive LPS activity can result in systemic toxicity and represents a major obstacle to the clinical application of OMVs (Li et al., 2025). Genetic engineering can address this

problem by modifying genes involved in lipid A biosynthesis, including *msbB*, *lpxL* and *lpxM*. These genes participate in determining the structure of lipid A and their modification can alter its acylation pattern, phosphate content or number of acyl chains. As a result, the lipid A incorporated into the resulting OMVs can possess substantially reduced immunogenicity and systemic toxicity (Zhang et al., 2026).

The objective is not necessarily to eliminate the immune-stimulating properties of LPS. Instead of

this the engineered OMVs can be designed to retain a degree of their beneficial adjuvant-like immunostimulatory activity while reducing excessive inflammatory responses (Li et al., 2023). This balance is important because some immune activation can be advantageous for vaccines, whereas uncontrolled inflammation can increase toxicity and sepsis-related risks. LPS-detoxified OMVs can therefore provide a potentially safer platform for human vaccine development and therapeutic applications (Zhou et al., 2025).

Table 1. Major strategies for engineering therapeutic OMVs.

Engineering strategy	Main purpose/approach	Representative features or applications	Reference
Genetic engineering	Modification of the bacterial producer to control OMV production, composition and function	Alters OMV biogenesis, cargo incorporation, surface composition and therapeutic properties	Chaudhari et al., 2025
Hypervesiculation	Increase OMV production by modifying membrane-stability and vesicle-biogenesis pathways	Modification of <i>Lpp</i> , <i>nlpI</i> , <i>Tol-Pal</i> and <i>VacJ/Yrb</i> pathways can enhance vesicle release	Juodeikis & Carding, 2022; Zahid et al., 2025
LPS detoxification	Reduce excessive inflammatory activity associated with OMV-associated LPS	Modification of <i>msbB</i> , <i>lpxL</i> and <i>lpxM</i> can reduce LPS-associated immunogenicity and toxicity	Zhang et al., 2026
Cargo loading	Control incorporation of therapeutic molecules into OMVs	Proteins, nucleic acids, small molecules and enzymes can be incorporated for targeted delivery	Kelwick et al., 2023; Yu et al., 2026
Surface engineering	Modify the OMV surface to improve interaction with target cells	Surface display and targeting ligands can improve cellular targeting and therapeutic activity	Zhao et al., 2025
Hybrid systems	Combine OMVs with synthetic biomaterials	Potential combinations include liposome-OMV hybrids, polymer-coated OMVs, hydrogels and nanocomposites	Nie et al., 2024; Chen et al., 2025
Stimuli-responsive systems	Enable biological activity or cargo release in response to specific stimuli	Potential responses include pH, enzymes, ROS, light, magnetic fields and ultrasound	Liu et al., 2022; Zhou et al., 2025

Programmable / AI-assisted engineering	Enable rational control and computational optimization of OMV properties	Synthetic biology, machine learning and computational design can support programmable OMV production	Feng et al., 2022; Abubakari et al., 2026
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Programmable payload loading: Genetic engineering can also be used to control which molecules are incorporated into OMVs. This involves removing unwanted or virulence-associated genes while introducing genes encoding specific antigens or therapeutic proteins, frequently by linking them to outer membrane or periplasmic proteins that favor their incorporation into vesicles (Gonçalves et al., 2025; Kelwick et al., 2023). This produces a process of “selective streamlining” in which endogenous components that are unnecessary or undesirable can be removed without disrupting the fundamental process of vesicle biogenesis. The resulting bacterial strain can consequently act as a producer of customized OMVs, carrying predetermined combinations of antigens or therapeutic molecules. Such customized vesicles have potential applications in vaccines and tumor immunotherapy (Yu et al., 2026).

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) is a programmable genetic engineering system originally derived from a bacterial defense mechanism against foreign genetic material. In the commonly used CRISPR-Cas9 system, a guide RNA is designed to recognize a specific DNA sequence, while the Cas9 protein acts as the DNA targeting and cutting enzyme (Wen et al., 2026). Once the guide RNA directs Cas9 to the selected sequence, the DNA can be disrupted or modified through cellular repair mechanisms (Cheng et al., 2025). In OMV engineering, CRISPR can therefore be used to selectively modify genes involved in OMV production, envelope stability, virulence, LPS synthesis or cargo composition. It can be used to create gene knockouts, insert desired sequences or modify multiple genes simultaneously. This provides considerably greater control over the bacterial producer than nonspecific genetic modification. Related approaches such as CRISPR

interference (CRISPRi) use a modified Cas protein that binds DNA without cutting it, allowing researchers to reduce the expression of selected genes rather than permanently deleting them (Karimbakhsh et al., 2026).

Synthetic biology involves designing biological systems using defined genetic components to give cells predictable and programmable functions (Feng et al., 2022; Yue et al., 2022). In OMV engineering, synthetic biology expression systems can be used to introduce specific antigen or therapeutic protein sequences and control when and how strongly they are expressed (Karimbakhsh et al., 2026). A desired protein can be placed under the control of a selected promoter which regulates its expression and can additionally be linked to sequences or proteins that direct it toward the outer membrane or periplasm. The resulting system can therefore coordinate protein expression → intracellular localization → incorporation during OMV biogenesis (Alexander & van Pijkeren, 2023). Future strategies using synthetic biology aim toward the de novo creation of synthetic OMVs or OMV-like structures with controllable features, potentially increasing yield and reducing batch-to-batch variability.

Cargo engineering: This type of engineering focuses on efficiently loading therapeutic payloads into OMVs and exploiting their nanoscale vesicular structure as a drug-delivery system. The engineering objective is to introduce the desired molecule while maintaining both OMV structural integrity and cargo functionality. Cargo can be introduced either during OMV formation through genetic engineering or after OMV isolation through physical loading techniques. Genetically encoded cargo can be directed toward the bacterial membrane, periplasm or an OMV-associated protein allowing it to become incorporated naturally during vesicle formation.

Alternatively, preformed OMVs can be physically manipulated to introduce cargo.

Passive loading relies on the natural movement or diffusion of molecules into or association with OMVs when the vesicles are incubated with the desired cargo. This approach is generally most suitable for small lipophilic molecules which can interact readily with the lipid membrane and partition into the vesicle structure (Ban et al., 2023). Because passive loading does not require substantial physical disruption of the membrane it can be relatively simple and gentle. However, the amount of cargo incorporated depends strongly on the physicochemical properties of the cargo and its interactions with the OMV membrane.

Active loading deliberately alters the OMV membrane or physically manipulates the vesicle to facilitate cargo incorporation (Ban et al., 2023). Electroporation uses high-voltage electrical pulses to temporarily disrupt the OMV membrane and generate transient and reversible pores. Molecules are present in the OMV lumen. This approach is particularly relevant to charged macromolecules such as siRNA, mRNA and plasmid DNA (Zhou et al., 2025). Sonication uses ultrasonic energy to generate mechanical disturbances in the vesicle membrane. These transient membrane perturbations can facilitate the incorporation of both small molecules and larger macromolecular cargo. The degree of physical disruption must be controlled because excessive membrane disturbance can compromise vesicle integrity (Liu et al., 2025). Freeze-thaw loading uses repeated freezing and thawing to alter the physical state of the OMV membrane. Temporary membrane disruption allows cargo to enter after which the membrane can reseal when the vesicle returns to a warmer state (Ban et al., 2023).

Co-extrusion and microfluidic approaches provide more controlled physical methods of incorporating cargo or nanomaterials. In co-extrusion, drug or nanomaterial-containing vesicles are passed through membranes containing defined pores. Mechanical shear and controlled mixing can promote the formation of more uniform composite vesicle structures and improve cargo incorporation. Microfluidic systems

similarly allow OMVs and cargo to be manipulated under highly controlled flow and mixing conditions (Canalejo-Marco et al., 2025).

OMVs can carry a broad range of molecules. Small molecular drugs that include chemotherapeutic agents can be incorporated into OMVs allowing the vesicles to function as nanoscale drug carriers. Their lipid membrane and internal aqueous environment provide opportunities for association with molecules possessing different physicochemical properties (Liu et al., 2025).

Proteins and peptides can either be encapsulated within the OMV lumen or displayed on its surface. Surface display is particularly useful when the biological activity of the protein or peptide requires direct interaction with a target cell whereas internal encapsulation can protect the cargo from the extracellular environment (Garling et al., 2025b). OMVs can also deliver nucleic acids including siRNA, miRNA, mRNA and DNA. Encapsulated DNA is protected from extracellular nucleases increasing its persistence and potentially facilitating horizontal gene transfer (Marinacci et al., 2023). OMVs naturally contain multiple RNA species including rRNA, tRNA, mRNA and sRNAs and some of these molecules appear to undergo active sorting into vesicles. Once delivered, these RNAs to recipient cells can influence gene expression (Juodeikis & Carding, 2022).

CRISPR/Cas systems can also be considered potential OMV cargo. A CRISPR system requires the appropriate combination of components such as a guide RNA and a Cas protein to recognize and modify a selected genetic sequence. Although specific loading methods are not detailed here, incorporating such gene editing systems would require advanced strategies capable of delivering the required components while maintaining their functionality (Cheng et al., 2025). OMVs can additionally encapsulate periplasmic enzymes including alkaline phosphatases, proteases, glycosidases and lipases as well as enzymes involved in peptidoglycan modification. Encapsulation can protect these enzymes from extracellular proteolysis while enabling their activity to occur in a localized environment (Dean et al., 2023).

Surface engineering: Surface engineering involves modifying the molecules exposed on the OMV exterior to improve pharmacokinetics, reduce immune clearance and enable targeted delivery to particular cells or tissues. Because the surface is the first part of the OMV encountered by a target cell, engineering it can substantially change how the vesicle interacts with its biological environment. Outer membrane proteins such as ClyA and OmpA can be genetically fused to a desired functional molecule. The scaffold directs the fusion protein toward the bacterial outer membrane and when OMVs subsequently bud from that membrane, the attached molecule becomes exposed on the vesicle surface (Laotee & Arunmanee, 2024). This approach can be used to display single-chain variable fragments (scFv), peptides, targeting ligands and immunomodulatory molecules. For example, scFvs can be engineered to recognize antigens that are associated with tumor. Other surface molecules such as mannose can enhance interaction with dendritic cells and potentially promote antigen presentation. PD-1-related domains and adaptors can also be incorporated to modify immune interactions (Liu et al., 2025). Consequently, surface engineering can convert OMVs into targeted carriers with defined receptor recognition (Canalejo-Marco et al., 2025).

PEGylation involves the covalent or non-covalent attachment of polyethylene glycol (PEG) chains to the OMV surface (Sun et al., 2024). PEG creates a hydrated, sterically shielding layer around the vesicle and modifies its hydrophobic-hydrophilic balance. This shielding can reduce adsorption of plasma proteins and opsonization, the process through which particles become marked for recognition and removal by immune and clearance systems (Zhao et al., 2025). By reducing nonspecific interactions, PEGylation can potentially prolong circulation and improve pharmacokinetic behavior. However, excessive surface shielding can also interfere with interactions required for cellular uptake means that PEG modification must be balanced against the need for biological recognition.

Click chemistry refers to a class of highly efficient, specific and robust chemical reactions that allow two appropriately functionalized molecules to be selectively connected. In OMV engineering, one chemical group can be introduced onto the vesicle while a complementary group is placed on a targeting ligand, drug or other functional molecule. The two can then be joined after OMV production, providing precise control over surface functionalization without requiring the targeting molecule to be genetically expressed by the bacterial producer (Huang et al., 2022; Sun et al., 2024).

Membrane decoration refers broadly to incorporating additional molecules into or onto the OMV membrane to change its properties or introduce new functions (Kelwick et al., 2023). These modifications can include lipids, polymers and targeting ligands which can alter membrane characteristics, cellular interactions and stability. Additional polymer coatings or co-assemblies can also provide structural support and influence OMV release properties (Chapagain et al., 2024).

Hybrid engineering: In Hybrid engineering, OMVs are combined with other materials to create platforms with properties that are not achievable using OMVs alone. The basic concept is to combine the biological functionality of OMVs with the structural, chemical or physical properties of synthetic biomaterials. The provided information specifically indicates that future approaches may combine synthetic biology and nanotechnology to support the de novo creation of synthetic OMVs or OMV-like structures with controllable features. However, the provided document does not explicitly detail specific hybrid constructs such as liposome-OMV hybrids, polymer-coated OMVs, cell-membrane-coated OMVs, hydrogels or nanocomposites. Therefore, these should be presented as broader or emerging hybrid-engineering concepts rather than as mechanisms directly established by the provided information. Liposome-OMV hybrids would conceptually combine OMV-derived biological components with liposome-derived synthetic lipid components. Polymer-coated OMVs would surround the vesicle with a polymeric layer capable

of altering stability, protection or release. Cell-membrane-coated OMVs would introduce membrane components derived from host cells to provide additional biological recognition. Hydrogels could function as three-dimensional reservoirs that retain OMVs and influence their local release. Nanocomposites could combine OMVs with nanoparticles possessing additional properties such as magnetic, optical or structural functionality. These approaches reflect the broader goal of combining OMVs with other biomaterials to overcome limitations associated with either system alone (Liu et al., 2022).

Stimuli-responsive OMVs: The provided information does not clearly explain specific pH-responsive, enzyme-responsive, ROS-responsive, light-responsive, magnetic or ultrasound-responsive OMVs. However, the combination of gene editing, synthetic biology and advanced formulation methods suggests the potential for developing such systems. In principle, these strategies could allow OMVs to release cargo or exert their biological effects after encountering a particular physiological condition or external stimulus.

pH-responsive OMV: These OMVs use a component whose structure or charge changes at a particular pH, potentially allowing cargo release under acidic conditions. An enzyme-responsive OMV contains a component that is cleaved or modified by a particular enzyme, using that enzyme as the activation signal. A ROS-responsive system would exploit chemical groups that change in response to reactive oxygen species. Light-responsive systems would incorporate components capable of undergoing controlled photochemical changes following exposure to an appropriate wavelength of light (Chen et al., 2024). Magnetic systems could incorporate magnetic materials whose movement or behavior is influenced by an external magnetic field, while ultrasound-responsive systems could use acoustic energy as an external physical trigger (Liu et al., 2022; Zhou et al., 2025). These concepts therefore represent a progression from passive delivery toward condition-dependent activation, although

their specific implementation and effectiveness must be established experimentally.

AI-designed OMVs would use artificial intelligence and machine learning to predict and optimize combinations of OMV characteristics. Instead of experimentally testing every possible combination of bacterial genotype, cargo, surface ligand and formulation, computational models could potentially analyze existing datasets and predict designs with desirable characteristics such as improved cargo loading, targeting efficiency, stability or stimulus responsiveness (Cheng et al., 2025; Li et al., 2026). The predicted design could then be experimentally produced and tested with the resulting data feed back into the computational model. This creates a continuous process in which AI can help the design and optimization of increasingly complex OMV systems.

Programmable engineering: Programmable OMVs represent the integration of synthetic biology with OMV engineering. The provided information highlights the potential for the de novo creation of synthetic OMVs or OMV-like structures with controllable features which could increase yield and reduce batch-to-batch variability. At the genetic level this concept involves controlling the pathways responsible for vesicle formation and cargo incorporation so that the properties of the resulting OMVs are more predictable (Kim et al., 2026; Zhao, X. et al., 2025). Rather than producing a vesicle whose composition is largely determined by natural bacterial physiology a programmable system would aim to deliberately control biogenesis, cargo and potentially surface properties. Understanding protein and sRNA sorting during OMV biogenesis is particularly important because this could allow researchers to design which biological molecules are preferentially incorporated into the vesicles (Juodeikis & Carding, 2022).

Living therapeutics: The concept of living therapeutics extends OMV engineering beyond isolated vesicles. The provided information highlights the broader potential of OMVs in drug delivery, vaccination and immune modulation, including personalized OMV-based vaccines and

their ability to interact with the host immune system (Charpentier et al., 2023). In this context, OMVs can be considered active biological agents capable of delivering therapeutic molecules and modulating host responses. A more advanced concept is to integrate OMV production with engineered living systems in which a living bacterial producer could potentially generate therapeutic vesicles continuously or within a

desired biological environment. Instead of relying entirely on externally manufactured OMVs, the living system could function as a biological source of therapeutic vesicles. Such approaches would combine OMV engineering with control over bacterial behavior and therefore introduce additional considerations concerning biological containment, host interactions and regulation of therapeutic activity.

Table 2. Therapeutic applications of engineered outer membrane vesicles (OMVs).

Therapeutic application	Therapeutic role	Examples/cargo	Reference
Cancer therapy	Targeted drug delivery and cancer immunotherapy	5-Fluorouracil (5-FU), chlorin e6 (Ce6), Doxorubicin (DOX), KGF-2, melanin	Li et al., 2025; Zhang et al., 2026
Antimicrobial delivery	Delivery of antimicrobial agents and potential treatment of infectious diseases	Gentamicin and other antimicrobial cargoes	Li et al., 2025
Nucleic acid delivery	Transport and delivery of genetic material for gene regulation or therapy	Therapeutic siRNAs, DNA, RNA and CRISPR-related cargo	Marinacci et al., 2023; Li et al., 2025
Vaccination and immunotherapy	Antigen delivery, immune stimulation and immune modulation	SARS-CoV-2 spike protein receptor-binding domain; engineered antigens	Micoli et al., 2024; Yan et al., 2026

Clinical Translation of OMV-Based Vaccines

Outer membrane vesicles (OMVs) are gaining recognition as a versatile platform for next-generation vaccines due to their intrinsic immunogenicity, adjuvant properties and scalability. Their ability to simultaneously deliver antigens and pathogen-associated molecular patterns (PAMPs) makes them particularly attractive as vaccine platforms. However, translating OMVs from experimental systems into clinically approved products requires control over the entire development pathway from bacterial fermentation and vesicle isolation to characterization, quality control, pharmacokinetics, regulatory assessment and clinical evaluation. The central translational challenge is to convert a biologically heterogeneous vesicle population into a reproducible, well-characterized, safe, potent and scalable pharmaceutical product.

Manufacturing, Purification, Fermentation, Isolation and Scale-up: OMVs are naturally secreted by Gram-negative bacteria, but their low yield requires optimization of production. Clinical manufacturing therefore depends on suitable bacterial strains and controlled fermentation conditions, including growth, nutrients, temperature, pH, oxygenation, agitation, and culture duration. Detergent-extracted OMVs (dOMVs), produced using agents such as deoxycholate, increase yield and reduce LPS and lipoproteins but may remove protective antigens or disrupt vesicle structure. Modified OMVs (mdOMVs), including GMMAs, use genetic engineering to enhance vesicle release or introduce foreign antigens while preserving native vesicle structure and offering greater manufacturing control. OMV production is often highest during the late exponential phase, making harvest timing important. Chemical, genetic, and

physical approaches, including nutrient limitation, temperature changes, phages, antibiotics and high-pressure homogenization, can increase yield (Lu et al., 2025).

Genetic modification of OMV biogenesis-related genes such as *Lpp*, *nlpI*, *Tol-Pal*, and *VacJ/Yrb* can further enhance vesicle release (Zahid et al., 2025). However, increased yield must be balanced with consistent composition, antigen preservation, potency, structural integrity and safety.

Traditional methods like ultracentrifugation and density gradient centrifugation are laborious, time-consuming and require costly equipment. These limitations become particularly significant when production is moved from laboratory-scale experiments to manufacturing quantities. In addition, purification must remove bacterial cells, cellular debris, soluble proteins, nucleic acids and other contaminants without substantially reducing OMV recovery or damaging vesicle structure (Li et al., 2024). Emerging techniques include gradient filtration and size-exclusion chromatography (SEC) using zeolite columns which offer more efficient and scalable isolation while preserving OMV integrity (Cui et al., 2023). Magnetic extraction methods using iron oxide nanoparticles have also been developed for efficient recovery. The development of these methods is important because an ideal clinical purification process must simultaneously achieve high recovery, high purity, preservation of vesicle structure and reproducibility (Li et al., 2024).

For large-scale OMV production continuous bioprocessing platforms offer advantages over batch-based production and improve yield, reduce cost and maintain consistent quality. Continuous bioreactor systems have demonstrated stable and high-yield OMV production for *N. meningitidis*. Scale-up is not simply a matter of increasing the volume of a laboratory culture. Changes in oxygen transfer, mixing, nutrient gradients, shear conditions and bacterial growth dynamics can influence OMV production and composition. Therefore, a clinically viable manufacturing process must demonstrate that the characteristics of the vesicles remain consistent when production is increased from laboratory to industrial scale.

Characterization: Particle size, Purity, Cargo, potency and Stability

OMVs typically range from 20 to 250 nm but can expand up to 500 nm exhibiting diverse irregular shapes. Transmission electron microscopy and nanoparticle tracking analysis are employed to examine morphology and particle size. Particle characterization is important because vesicle dimensions and morphology can influence how OMVs interact with biological systems. The size, shape and rigidity of particles impact their cellular uptake, antigen presentation and immune cell activation. Consequently, particle size is not merely a physical measurement but can represent an important quality attribute associated with biological performance (Sun et al., 2024; Wo et al., 2023). For clinical manufacturing, characterization should therefore determine whether particle populations remain within a defined and reproducible range between production batches. Changes in size distribution may indicate alterations in bacterial growth, vesicle biogenesis, purification, aggregation or storage.

OMVs are heterogeneous in size, protein composition and content influenced by bacterial strain, biogenesis route, growth conditions and extraction methods. This heterogeneity represents one of the major challenges in translating OMVs into standardized pharmaceutical products because the biological activity of a preparation depends on its molecular composition (Dean et al., 2023). Proteomic analyses are used to assess the protein composition with 40-80% of detected proteins typically being outer membrane proteins. OMVs can contain fundamental outer membrane proteins specialized cargo proteins (e.g., toxins, enzymes) and sometimes contaminating proteins. OMVs also encapsulate small RNA and transfer RNA fragments as well as DNA and peptidoglycan. For vaccine development, this means that characterization must extend beyond simply measuring the number of vesicles produced. The identity and abundance of relevant antigens, membrane proteins, nucleic acids and other biologically active components must also be

evaluated. Improved isolation techniques are expected to diminish the presence of contaminating proteins thereby increasing the purity and consistency of the final product (Liu et al., 2023).

OMVs possess inherent immunogenicity and adjuvant characteristics, significantly enhancing innate and adaptive immune responses. Their small size and particle shape promote systemic distribution and induce long-term memory responses. These properties make OMVs particularly attractive because the vesicle itself can contribute to immune stimulation while simultaneously presenting vaccine antigens. OMVs exhibit remarkable stability under elevated

temperatures and various chemical treatments. This stability could provide practical advantages for vaccine storage and distribution particularly where maintaining strict cold-chain conditions is difficult (Wo et al., 2023; Yue et al., 2022). Concerns remain related to potential changings in OMV morphology, aggregation or antigen degradation during prolonged circulation in biological fluids requiring formulation refinement. Stability therefore needs to be evaluated not only during storage but also after administration. A clinically useful formulation must maintain the structural and functional characteristics necessary for effective antigen delivery (Chen et al., 2024; Liu et al., 2024).

Table 3. Key requirements and challenges for clinical translation of engineered OMVs.

Clinical translation stage	Key requirement	Major challenge/consideration	Reference
Production	Controlled bacterial strain and fermentation	Low natural OMV yield; control of growth, nutrients, temperature, pH, oxygenation and harvesting	Lu et al., 2025; Zahid et al., 2025
Purification and isolation	High recovery and purity while preserving OMV structure	Removal of bacterial cells, debris, proteins and nucleic acids; scalable purification	Li et al., 2024
Scale-up	Reproducible large-scale production	Changes in oxygen transfer, mixing, nutrient gradients, shear conditions and bacterial growth can affect OMV properties	Kelwick et al., 2023
Characterization	Defined size, morphology and molecular composition	OMV heterogeneity and variation between production batches	Dean et al., 2023; Sun et al., 2024
Quality control	Consistent purity, cargo, LPS characteristics, stability and potency	Maintaining batch-to-batch consistency	Dean et al., 2023
Pharmacokinetics and biodistribution	Understanding tissue distribution, uptake and clearance	Biological distribution and persistence may vary with OMV properties and administration	Zhao et al., 2022; Li et al., 2026
Safety	Control of inflammatory activity and residual bacterial components	Excessive immune activation, endotoxin-associated effects, off-target interactions and long-term exposure	Liu et al., 2024;
Regulation and GMP	Defined manufacturing, quality and safety controls	Limited OMV-specific regulatory frameworks and challenges in GMP-compatible production	Park et al., 2023; Zhou et al., 2025

Sterility and Endotoxins: The presence of endotoxins such as LPS which are intrinsic to OMVs can trigger unwanted inflammatory responses. This represents one of the defining challenges of OMV-based vaccine development. LPS contributes strongly to OMV immunogenicity but excessive innate immune activation can result in unacceptable inflammatory or toxic responses (Park et al., 2023). Genetic detoxification strategies (e.g., lipid A mutations like lpxL1 and lpxL2 deletions, msbB or pagL deletions) are employed to reduce endotoxicity and improve tolerability. Comparative studies show that OMVs engineered with lipid A mutations exhibit significantly reduced immunotoxicity and pyrogenicity (Park et al., 2023).

Batch consistency and storage: Establishing robust manufacturing pipelines with reproducible and tightly controlled parameters is critical due to the heterogeneity of OMVs caused by bacterial strain variations, cultivation conditions and purification protocols. Advanced characterization tools for OMV profiling are necessary to ensure uniformity across production lots. Particularly, Batch-to-batch consistency is important because changes in OMV composition could alter vaccine potency or safety even when the manufacturing process appears superficially unchanged. Quality control must therefore assess multiple characteristics simultaneously including particle concentration, size distribution, morphology, antigen composition, LPS activity, purity and biological potency. OMVs can retain their integrity and immunogenicity after lyophilization or exposure to physiological temperatures. However, formulation optimization is needed to enhance durability and bioavailability for mucosal or systemic delivery. Storage conditions must therefore preserve both physical integrity and biological activity. Formulation development may ultimately determine whether OMV vaccines can be transported and stored efficiently without substantial loss of potency (Dean et al., 2023).

Pharmacokinetics, Immune Response, Safety and Regulation: OMVs due to their small size can access lymph nodes via lymphatic drainage or through phagocytosis subsequently being

transported by antigen-presenting cells (APCs). This behavior is particularly relevant for vaccines because delivery to lymphoid tissues and interaction with antigen-presenting cells can influence the magnitude and quality of the resulting immune response (Zhao et al., 2022). The in vivo kinetic biodistribution of OMVs shows peak concentration at 3 hours post-administration with subsequent decline in most organs within 24 hours. These kinetics demonstrate that OMVs can undergo relatively rapid distribution and clearance, although their behavior can vary according to their composition, administration route, surface properties and biological environment (Sun et al., 2024). In solid tumors OMVs can accumulate at tumor sites for extended periods by evading lymphatic clearance due to the enhanced permeability and retention (EPR) effect (Li et al., 2026). Although this phenomenon is particularly relevant to therapeutic OMVs, it also demonstrates how vesicle size and tissue physiology can influence biodistribution.

Immune response: OMVs activate both innate and adaptive immunity, stimulating a strong and enduring pathogen-specific immune response. Their immunological activity results from the combination of antigenic molecules and intrinsic PAMPs contained within the vesicles (Luo et al., 2024). They possess various PAMPs (e.g., LPS, lipoproteins, flagellin, CpG DNA) that activate Toll-like receptors (TLRs) and pattern recognition receptors (PRRs), leading to the secretion of proinflammatory cytokines and activation of dendritic cells. This initial innate response contributes to the development of an adaptive immune response (Park et al., 2023). Mature dendritic cells present bacterial antigens, stimulating the activation and proliferation of antigen-specific CD4⁺ and CD8⁺ T lymphocytes, leading to the generation of Th1 and Th2 cells, antibodies and cytotoxic T lymphocytes (CTLs). Consequently, OMVs can simultaneously provide antigenic stimulation and the innate immune signals required to enhance antigen presentation (Garling et al., 2025a). This dual functionality represents one of the major advantages of OMVs

as vaccine platforms because the vesicle can function as both an antigen carrier and an intrinsic adjuvant.

Safety: OMVs are non-replicative particles so they cannot induce disease. Nevertheless, non-replicative status does not eliminate all safety concerns. Concerns about unintended immune activation, autoimmunity and long-term immunological consequences require careful consideration. Preclinical studies must demonstrate an acceptable safety profile, particularly concerning endotoxins (Liu et al., 2024).

Safety evaluation must therefore consider both acute inflammatory effects and potential consequences of repeated or prolonged exposure. The balance between immunogenicity and inflammatory toxicity remains particularly important because many of the molecular components responsible for OMV immunogenicity are also capable of producing inflammatory responses.

Regulatory considerations: FDA, EMA, CMC and GMP

A major challenge is the lack of clear OMV-specific regulatory frameworks from agencies like the FDA and EMA. Developers often interpret general biologics or vaccine guidelines which can delay progress and introduce uncertainty (Park et al., 2023). This challenge becomes increasingly important as OMVs move from relatively conventional vaccine preparations toward genetically modified, detoxified, cargo-engineered or hybrid vesicles (Chen, M. Y. et al., 2025; Garling et al., 2025b). In the United States a comprehensive Investigational New Drug (IND) application including robust preclinical data, quality control procedures and detailed manufacturing protocols is required before clinical trials. In Europe the European Medicines Agency (EMA) mandates a full dossier of non-clinical and clinical data, safety assessments and manufacturing details (Zhou et al., 2025). The complexity of OMVs means that regulators must evaluate not only the final vaccine but also the biological source, manufacturing process, genetic

modifications, purification methods, composition, potency and safety profile of the vesicles.

GMP compliance is essential for clinical-grade OMV production. Current OMV production techniques are often labor-intensive, low-yield and challenging to scale for GMP compatibility. Emerging alternatives like tangential flow filtration and size-exclusion chromatography are being explored. Continuous manufacturing platforms are promising for improving yield, reducing costs and maintaining consistent quality. Continuous systems may also allow closer monitoring of production parameters and potentially reduce variability that is associated with repeated batch processing. Facility design must support sterile processing environments, biocontainment for pathogenic strains and rigorous cleaning and documentation procedures including HEPA-filtered cleanrooms and automated sterilization systems. These requirements demonstrate that clinical OMV manufacturing involves not only biological engineering but also substantial infrastructure and process-control requirements. The CMC framework is therefore central to clinical translation because it establishes how the product is manufactured, what characteristics define its quality, how those characteristics are measured, and how consistency between batches is demonstrated (Zhou et al., 2025).

Clinical Evidence and Development:

Several OMV-based vaccines have been licensed primarily for protection against *Neisseria meningitidis* serogroup B (MenB) and *Haemophilus influenzae* type b (Hib) infections (Lieberman, 2022). Examples include Bexsero (GSK), VA-MENGOC-BC (Cuba) and Hib-OMPC (e.g. PedvaxHIB, Comvax, VAXELIS) (Garling et al., 2025b). Bexsero, formulated with dOMVs from *N. meningitidis* strain NZ98/254 showed acceptable safety and immunogenicity leading to a 75% reduction in MenB cases in vaccine eligible infants in the UK (Velimirov & Velimirov, 2024). Its development demonstrates the feasibility of using OMVs as clinically effective

vaccine components while also illustrating the importance of controlling reactogenicity associated with meningococcal LPS. VAMENGOC-BC, a dOMV-based vaccine led to a dramatic 93-98% reduction in MenB disease incidence in Cuba (Zahid et al., 2025). This provides further evidence that OMV-based vaccination can produce substantial population level protection when appropriately developed and deployed. Hib-OMPC the first licensed OMV-based conjugate vaccine, uses a capsular polysaccharide from *H. influenzae* type b conjugated to OMPC from *N. meningitidis*, with dOMVs showing high efficacy in trials. This demonstrates that OMV-derived components can also function as carriers within conjugate vaccine designs rather than solely serving as complete vaccine particles.

Many OMV-based vaccines are in clinical development against various pathogens (e.g., Salmonella, Shigella, *N. gonorrhoeae*, SARS-CoV-2) (Micoli et al., 2024). GMMAs are in clinical trials for Salmonella and Shigella infections with some undergoing phase I/II trials (Zahid et al., 2025). Intranasal OMV-based COVID-19 vaccines (e.g., Avacc 10) and iNTS-GMMA vaccines for invasive non-typhoidal Salmonella infections are in early-stage clinical trials. These approaches demonstrate the increasing diversification of OMV technology beyond meningococcal vaccines and toward pathogens for which conventional vaccine development remains challenging. The movement into different administration routes, including intranasal delivery, is particularly significant because OMVs can potentially exploit their mucosal interactions and intrinsic immunostimulatory properties to generate immune responses at sites of pathogen entry.

Future Perspectives in OMV Engineering

Outer membrane vesicles (OMVs) are emerging as versatile platforms for therapeutics, vaccines, diagnostics, and pharmaceutical applications. The convergence of synthetic biology, artificial intelligence (AI) and OMV research is expected to accelerate their development and translation into applications for human and animal health

(Kelwick et al., 2023). Future research is shifting from naturally produced OMVs toward rationally engineered vesicles with controlled production, composition, surface properties, cargo loading and biological activity.

Synthetic biology provides a design-build-test-learn (DBTL) framework for engineering bacterial strains with defined OMV characteristics. Genes involved in OMV biogenesis, cargo incorporation, surface display and LPS production can be modified, followed by evaluation of vesicle yield, size, composition, stability, cargo loading and biological activity. Standardized protocols and biological metrology, supported by approaches such as MISEV2018, will be important because OMV preparations vary in size, composition, cargo, LPS content and activity (Hendrix et al., 2023). Automation using acoustic and liquid-handling robotics can increase experimental throughput and reduce manual errors while design-of-experiment and AI-guided approaches can identify combinations that optimize OMV production and function (Sahoo et al., 2025). Machine learning may further predict relationships between bacterial genotype and OMV properties, improving the DBTL cycle and reducing experimental workload (Li et al., 2026). Advances in protein design, including AlphaFold and protein large language models such as ESM-2, may support the development of novel OMV cargo and membrane proteins (Liu et al., 2022). Digital twins could eventually model OMV-producing systems and predict the effects of genetic or environmental changes, although this remains a prospective concept (Kim et al., 2026; Zhou et al., 2025).

Engineered OMVs can carry diverse therapeutic agents, including Gentamicin, SARS-CoV-2 spike protein receptor-binding domain, 5-Fluorouracil, chlorin e6, Doxorubicin, KGF-2, melanin and therapeutic siRNAs, supporting their potential in infectious disease and cancer therapy (Li et al., 2025). Surface engineering can improve targeting and control LPS-associated toxicity, while combining OMVs from different strains has demonstrated potential for improving vaccine efficacy (Yan et al., 2026). Future systems may

integrate therapeutic, targeting, imaging and sensing functions within individual vesicles. Precision medicine could further enable OMV composition to be adapted to individual disease characteristics through selection of specific cargoes, targeting ligands, and immunomodulatory components (Li et al., 2026). Hybrid systems combining OMVs with liposomes, polymers, hydrogels, cell membranes, or other nanomaterials may additionally provide improved stability, controlled release, imaging or responsiveness (Chen et al., 2025). Programmable OMVs could ultimately allow deliberate control of vesicle biogenesis, cargo incorporation, surface composition, targeting and biological responses (Wo et al., 2023).

OMVs may also contribute to microbiome engineering by delivering defined molecular signals to microbial populations and modifying microbial communication and function, although precise targeting within complex microbiomes remains challenging (Abubakari et al., 2026). OMV technology has already reached clinical application through FDA-approved vaccines against *Neisseria meningitidis*, while additional human and animal OMV-based vaccines are under development (Garling et al., 2025b; Zahid et al., 2025). However, further translation requires improved understanding of OMV heterogeneity and biogenesis, together with reproducible and scalable production, purification, characterization, formulation and storage processes (Kelwick et al., 2023). Key quality attributes include vesicle size, concentration, morphology, molecular composition, LPS characteristics, cargo identity, purity, stability and biological potency. Safety assessment must also address immune activation, residual bacterial components, off-target effects, biodistribution, persistence and interactions arising from multiple genetic or synthetic modifications.

Conclusion

OMVs have developed from a biological feature of Gram-negative bacteria into a versatile platform for vaccines, targeted drug delivery, cancer immunotherapy, diagnostics and other biomedical

applications. Their key advantages arise from their nanoscale architecture, natural cellular interactions, diverse molecular cargo and intrinsic immunostimulatory activity which can be further modified through genetic, cargo, surface and hybrid engineering (Chen et al., 2022; Wang et al., 2026; Yu et al., 2026). At the same time the same biological complexity that makes OMVs useful creates major translational challenges. LPS-associated inflammatory activity, heterogeneous composition, variable production, purification difficulties, batch-to-batch variability and the need for rigorous safety and potency testing must be addressed before increasingly sophisticated engineered OMVs can be widely adopted clinically (Park et al., 2023). The most promising path forward is therefore not simply to increase OMV yield but to achieve controlled and reproducible production in which vesicle composition, cargo, surface properties, stability, biodistribution and biological activity are defined and measurable. Integration of synthetic biology, high-throughput experimentation, computational design and artificial intelligence may accelerate this transition but emerging concepts such as fully programmable or personalized OMVs still require substantial experimental validation. Clinical translation will ultimately depend on a balance between engineering sophistication, manufacturing quality, regulatory clarity and demonstrated patient benefit.

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