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Review

Outer Membrane Vesicles (OMVs) as Antibiotic Carriers: A Promising Approach

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Abstract

The misuse and overuse of antibiotics have driven the emergence of antibiotic-resistant bacteria in recent decades. With the increasing incidence of resistant strains and the significant slowdown in new antibiotic discoveries, treating bacterial infections has become more challenging. Therefore, there is an urgent need to explore alternative treatments, such as using bacterial outer membrane vesicles (OMVs) for targeted delivery. OMVs are nanoscale, spherical structures originating from Gram-negative bacteria's outer membrane. These vesicles are naturally released by almost all types of Gram-negative bacteria into their environment during growth and play crucial roles in pathogenesis by transporting specific biomolecules, such as toxins and other virulence factors, to host cells. Due to their unique ability to encapsulate and transport various bioactive molecules across the Gram negative cell membrane, nanosized OMVs hold significant potential as a novel platform for antibiotic delivery. This review discusses biogenesis, biofunctions, and antibacterial applications of OMVs.

Keywords: Antibacterial therapy, antibiotic resistance, drug delivery, Gram-negative bacteria, OMVs

1. Introduction

Antibiotics are commonly used for the treatment of bacterial infections. The frequent or improper use of antibiotics has resulted in the widespread development of bacterial resistance to these drugs. The increasing incidence of multidrug-resistant pathogenic bacteria has made conventional antimicrobial treatment ineffective (1). Particularly, Gram-negative bacteria pose a significant challenge to antibiotic treatment due to their intricate, double-membrane cell structure (2). In an effort to combat infections caused by Gram-negative bacteria, research has focused on developing new types of drugs and discovering new therapeutic approaches to overcome the limitations of current medications.

Gram-negative bacteria, like most other cells, release membrane vesicles, known as outer membrane vesicles (OMVs), to mediate a range of cellular functions. OMVs are small, spherical, bilayered particles released during normal growth and stress conditions (3). They range in size from 20 to 300 nm in diameter and consist of components from the outer membrane and periplasmic space of the bacterium (3). OMVs play crucial roles in many bacterial

activities including intercellular communication, biofilm formation and pathogenesis (1). In recent years, it has been established that OMVs are involved intracellular communication, known as quorum sensing, both within bacterial communities and between bacteria and host cells. This communication is facilitated by the capability of OMVs to transport a diverse array of biomolecules including proteins, lipids, nucleic acids, and small signaling molecules across the Gram negative cell envelope.

OMVs can encapsulate and transport these molecules, thereby enhancing their stability and concentration in the extracellular environment (4). The unique property of OMVs to transport molecules suggests that OMVs can serve as promising vehicles for antibiotic delivery, offering a novel approach to combat bacterial infections caused by Gram negative bacteria. OMVs as antibiotic carriers could enhance drug delivery efficiency, reduce side effects, and potentially overcome mechanisms of resistance. This paper aims to provide a comprehensive overview of the potential of using OMVs as antibiotic carriers, including their biogenesis, biofunctions, isolation, and purification. In addition, the limitations and concerns regarding the clinical use of OMVs are discussed.

2. Formation and Composition of OMVs

OMVs were first identified in the 1960s, but it is only in recent decades that research on their formation, functions, and potential applications has significantly expanded (5). The formation of OMVs is a complex and highly regulated process influenced by various factors. For instance, conditions such as temperature fluctuations, ultraviolet radiation, nutrient availability, environmental stress, antibiotic exposure, and changes in bacterial growth status (6). OMVs are essentially formed through the outward budding of the outer membrane (OM) of Gram-negative bacteria. The budding occurs at sites where the lipoprotein connections between the peptidoglycan layer and the outer membrane are absent or disrupted, encapsulating biological molecules such as proteins, genetic material, and virulence factors derived from both the outer membrane and the periplasm (Fig 1) (1, 7).

Although various models have been proposed to explain the complex biogenesis process, such as the enrichment or depletion of specific local proteins, alterations in membrane curvature, and the accumulation of periplasmic proteins and peptidoglycan fragments, the exact mechanism of OMV formation is not fully understood (8).

3. Functions of OMVs

OMVs have the ability to naturally package and deliver a diverse array of molecular cargoes including proteins, lipids, nucleic acids, and small signaling molecules. Through their cargoes, OMVs carry out various physiological and pathological biofunctions**.** They have been shown to play crucial roles in bacterial communication (quorum sensing), biofilm formation, genetic exchange and pathogenesis.

3.1. Bacterial Communication and Biofilm Formation

OMVs are involved in quorum sensing, a bacterial communication process that relies on the production and detection of signaling molecules known as autoinducers. OMVs can encapsulate and transport these signaling molecules, thereby enhancing their stability and concentration in the extracellular environment. By facilitating the transfer of quorum-sensing signals, OMVs help bacteria monitor their population density and coordinate collective behaviors such as virulence factor production, antibiotic production, motility and biofilm formation (9).

Bacterial biofilms play a significant role in the development of chronic infections in humans. Biofilms are clusters of microorganisms that mainly consist of polysaccharides, secreted proteins, and extracellular DNA. Bacteria in biofilms can display antibiotic resistance up to 1,000 times higher than their planktonic counterparts. OMVs have been identified as crucial elements within the biofilm matrix (10). This discovery highlight that OMVs participate to biofilm formation

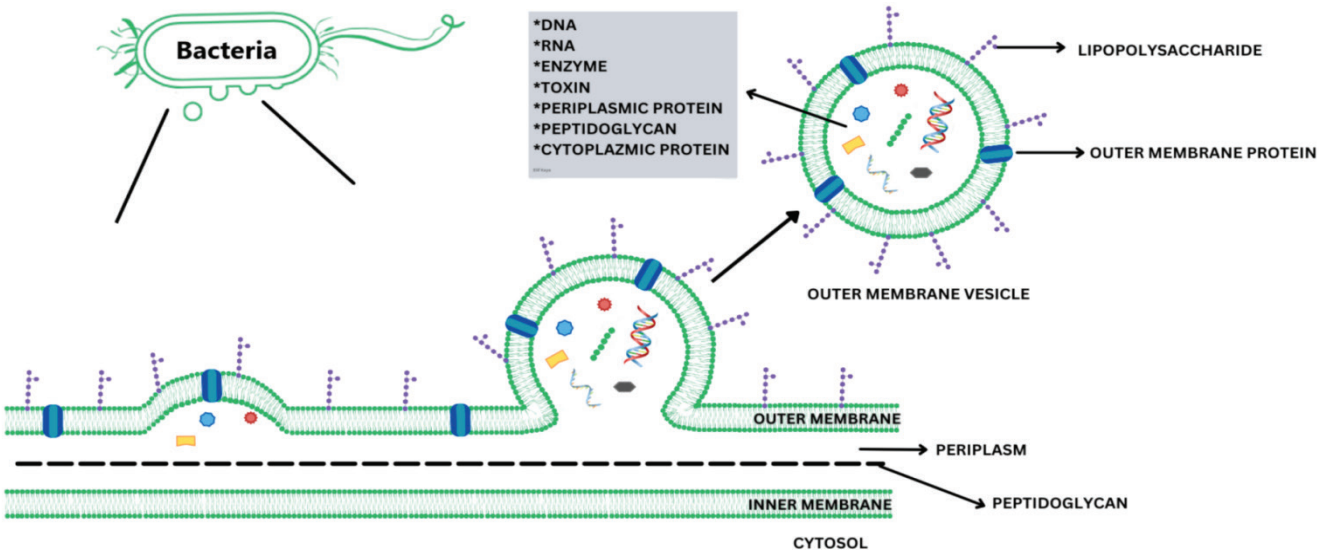

Figure 1. Representation of the formation, release and composition of OMVs. OMVs bud from outer membrane containing proteins and lipids from the outer membrane and material from the periplasm.

3.2. Horizontal Gene Transfer

OMVs can carry genetic material, such as plasmids or fragments of chromosomal DNA, facilitating horizontal gene transfer between bacterial cells. This property is especially important in the spread of antibiotic resistance genes, which can be delivered to neighboring cells through OMVs, contributing to the emergence of multidrug-resistant bacteria (1,11).

3.3. Pathogenesis

The role of OMVs in bacterial pathogenesis has been increasingly recognized. Many pathogenic bacteria release OMVs that help them establish infections by facilitating tissue invasion, immune evasion, and host cell interactions. OMVs contribute to bacterial pathogenesis by delivering microbial toxins and virulence factors directly into host cells during infection and weakening the immune response (12). For instance, *P. aeruginosa* produces OMVs that carry virulence factors, including toxins, that damage host cells and tissues (13). Similarly, *Porphyromonas gingivalis* OMVs carrying gingipain toxin can damage the supporting tissues of teeth, leading to severe periodontitis (1).

After mature OMVs formed, they gradually adhere to the host membrane, enter the cell, and release their cargo at a specific location. They can aid in immune evasion by modulating host immune responses or acting as decoys for antibodies (14). When OMVs interact with host cells, they can trigger immune responses such as the activation of macrophages, dendritic cells, or epithelial cells, either by inducing inflammation or immune tolerance. This immune modulation can be beneficial for bacteria, as it allows them to evade host defenses or establish chronic infections. In *Neisseria gonorrhoeae*, OMVs can trigger apoptosis of macrophages, affecting innate immun response and worsening the sexually transmitted disease gonorrhoea (15). OMVs from *Helicobacter pylori* (*H. pylori*) can reduce the secretion of interleukin 8 (IL-8) or lipopolysaccharide (LPS) and allow *H. pylori* to evade the immune response and establish a persistent infection (16).

4. Production, Isolation and Purification of OMVs

The process of isolating and purifying OMVs typically involves a series of steps, including

cultivation, low-speed centrifugation, sterile filtration, ultrafiltration, ultracentrifugation, density gradient separation, and gel filtration (17). The production of OMVs begins with culturing bacteria in an appropriate growth medium. Typically, OMVs are harvested from the culture after a sufficient cultivation period. After culturing, bacteria in the culture are removed by low-speed centrifugation (usually 2000×*g* ~ 10,000×*g*) (3). The supernatant is then passed through a sterile filter (0.22 μm or 0.45 μm) to eliminate any remaining bacteria. 100–500 kDa ultrafiltration membranes are used to to concentrate OMVs and remove non-OMV-associated proteins. Finally, the cell-free supernatant is subjected to ultracentrifugation at a speed range between 100,000 and 200,000 g at 4 ◦C for at least 2 h to collect OMVs (18). OMVs can be further purified by density gradient centrifugation with iodixanol, and ultracentrifuged at 100, 000 g for 18 h. After ultracentrifugation, the obtained OMV pellet is resuspended in PBS and stored at -20 or -80 ºC (17).

5. Characterization of OMVs

The determination of quantification and quality of purified OMVs is crucial for subsequent applications. The morphology and structure of OMVs are characterized by various optical microscopes such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), cryogenic-transmission electron microscopy (cryo-TEM), and atomic force microscopy (AFM) (19, 20). TEM is the most commonly employed technique to visualize OMVs morphology and structure. SEM shows the three-dimensional structure of OMVs, but its resolution is lower than TEM. Cryo-TEM has also been utilized to visualize the morphology and structure of OMVs (21).

Cryo-TEM examines a frozen sample, effectively preventing morphological changes caused by dehydration and chemical fixatives. Atomic Force Microscopy (AFM) is another method used to analyze the structure of OMVs with high resolution. It enables real-time visualization of OMVs in air or liquid without the need for extensive sample preparation, allowing for detailed observation of surface properties. (22). Dynamic Light Scattering (DLS) is usually employed to measure the OMVs hydrodynamic diameter, size distribution and zeta potential (23).

Nanoparticle Tracking Analysis (NTA) is an alternative light scattering technique for determining the size distribution and concentration of nanoparticles. NTA works on principles similar to DLS but offers enhanced accuracy in assessing the size distribution of heterogeneous particles. Furthermore, NTA not only measures the size of OMVs but also counts single particles, allowing for simultaneous measurement of OMV yield (21, 24).

6. OMVs as antibiotic delivery vehicles

The rising prevalence of multidrug-resistant pathogenic bacteria has rendered conventional antimicrobial treatments ineffective, underscoring the critical need for novel strategies to combat bacterial infections. The capacity of OMVs to carry diverse biomolecules across the Gram-negative bacterial cell membrane highlights their potential as natural carriers for delivering antibiotics (25). This approach could offer a significant advantage in overcoming the challenges associated with effectively treating infections caused by these difficult-to-treat bacteria.

Kadurugamuwa and Beveridge (1996) were the first to discover that bacteria exposed to antibiotics release OMVs containing some of the drug. They cultured the *Pseudomonas aeruginosa* (*P. aeruginosa)* strain PAO1 in the presence of gentamicin and isolated gentamicin loaded OMVs (g-MVs) (26). Gentamicin containing OMVs (g-MVs) exhibited a significantly stronger inhibitory capacity against gentamicin-impermeable *P. aeruginosa* strains when compared to the effects of free antibiotics [10]. In another study, it was observed that g-MVs isolated from *Shigella flexneri* (*S. flexneri*) were able to attach to and penetrate human epithelial cells, effectively delivering the antibiotic to intracellular

S. flexneri, resulting in significant inhibition of bacterial growth (27). Similarly, gentamicin-containing *P. aeruginosa* OMVs were also reported to effectively kill some Gram-positive organisms such as *Bacillus subtilis* and *Staphylococcus aureus (*28). Study carried out by Huang et al demonstrated that quinolone antibiotics—such as levofloxacin, ciprofloxacin, and norfloxacin loaded OMVs isolated from *Acinetobacter baumannii* can effectively penetrate and kill pathogenic bacteria, including *Klebsiella pneumoniae* and *P. aeruginosa*, in vitro (20). Moreover, in a mouse model of intestinal bacterial infection, these antibiotic-loaded OMVs significantly reduced the bacterial load in the small intestine and feces of infected mice [20]. Tashiro et al reported that gentamicin-loaded OMVs from *Buttiauxella agrestis* (*B. agrestis*) demonstrated a potent bactericidal effect against *B. agrestis* as well as against *E. coli* and *P. aeruginosa (11)*.

The studies demonstrated that as nanoscale materials, OMVs can improve the efficiency of drug uptake and delivery. Additionally, OMVs offer excellent biostability, ensuring efficient drug transport. They protect bioactive molecules from degradation, enhancing cargo stability and enabling the efficient delivery of functional cargo to target cells. OMVs readily fuse with target cell membranes, exhibit excellent membrane stability and biocompatibility, and demonstrate prolonged circulation times within the bloodstream. These properties make them ideal for a variety of biomedical applications. Moreover, they can be genetically modified by molecular techniques. Compared to synthetic nanoparticles, OMVs generally exhibit lower toxicity, making them safer for therapeutic applications. Although OMVs have numerous advantages as antibiotic delivery platforms, several limitations hinder their widespread use as drug carriers such as pathogenicity, immunogenicity and controlled drug loading and release. One of the most significant drawbacks of OMV use in human is their potential to cause pathogenicity and immunogenicity in the host (1, 29). Derived from bacterial outer membranes, OMVs often contain LPS, which can provoke strong immune responses. High LPS levels may lead to systemic inflammation. In addition, OMVs derived from pathogenic bacteria can deliver virulence factors, leading to toxicity in host cells. Strategies such as LPS modification or genetic engineering of bacterial strains can help reduce this risk (30).

7. Conclusion

In today's of increasing antibiotic resistance, OMVs are attracting increasing attention as an alternative approach to the treatment of infections caused by Gram-negative bacteria due to their wide range of applications, especially in drug delivery. Compared to other drug delivery platforms, their lower toxicity, excellent biostability, and especially their ability to transport and protect the drugs they contain into targeted cells make OMVs an alternative to conventional antimicrobial therapies. This makes them a promising antibiotic delivery vehicle. However, the safety and efficacy of OMVs in clinical applications requires further study. Technical challenges such as the production, purity, stabilization and large-scale production of these structures should also be considered. Furthermore, further research on the effects of the biological materials carried by OMVs and their effects on the immune system is important. In conclusion, OMVs should be considered as a potential tool in the fight against antibiotic resistance and research in this field should be supported. Future studies will be critical for the safe and effective use of OMVs in clinical practice.

Conflicts of interest: The authors declare no conflicts of interest related to this work.

Ethics approval: Not applicable

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