International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 04 Issue: 10 | Oct-2020 ISSN: 2582-3930

# A Role of Bacterial Outer Membrane Vesicles in Bio Medicine: Mini Review

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**Abstract -**Nowadays, Bacterial Outer Membrane Vesicles (OMVs) are gaining considerable importance in drug industries due to their immuno-modulatory property. OMVs are nanostructures released by gram-negative bacteria in the log phase of the bacterial life cycle. They are rich in lipopolysaccharide (LPS), therefore they can induce an immune response. In recent years, scientists have discovered the significance of OMVs as a vaccine and adjuvant in many diseases. A vaccine boosts secondary immunity against the respective parent pathogen. An adjuvant assists vaccine work better and more efficiently. As conventional vaccines and adjuvants are less effective and show some side effects. Therefore, it is necessary to find out new potent vaccines and adjuvant which will have better efficacy and lesser side effects. OMVs can answer all these problems since they own natural adjuvant capability. In this review, we summarized the biomedical applications of OMVs in drug industries.

***Key Words***: Outer Membrane Vesicles, Lipopolysaccharides, Vaccines, Adjuvants, Immuno-modulators, drug delivery tools

## 1. INTRODUCTION

Outer Membrane Vesicles are heterogeneous, spherical, bioactive structures. Their size varies from 20nm-300nm [1,5]. OMVs are composed of Lipopolysaccharide (LPS), Pathogen Associated Molecular Patterns(PAMPs) phospholipids, nucleic acids (DNA and RNA), peptidoglycans, Lipids, outer membrane, and inner membrane proteins, hydrophobic quorum sensing molecules, virulence factors, toxins, and adhesins [2,3].

OMVs are first discovered in *Vibrio cholera*[3,4] by Indian scientists Prof. Smriti Narayan Chatterjee and J. Das in 1966-67 and their structure was determined by using Transmission Electron Microscopy (TEM) [6].

### 1.1 Production of OMVs

Outer Membrane Vesicles are secreted into surrounding through a process called blebbing [7]. Biogenesis explains the synthesis of outer membrane vesicles. When this spherical structure gets separated from the bacterial membrane, it carries all the parental bacterial membrane componentsalong withit. In many cases, OMVs fuse with the lysosomes and degrade their content [3,9]. The fate of OMVs depends upon the content and composition of the growth medium, temperature, oxidative stress, antibiotic addition etc. The production of OMVs is a stress-mediated phenomenon [7]. The protein number and composition of the extracellular vesicle is a strain-dependent phenomenon. Eg. *N. meningitides* and *E. coli* bear different numbers of proteins in OMVs [8].

### 1.2 Function of OMVs

OMVs play very important roles in bacterial cell life.They serve as a weapon in bacterial interspeciescompetition, attractbacteriophages, help in nutrient predation deliver the secreted content outside the cell, Mis-folded, or aggregated proteins are released through OMVs on the bacterial outer surface, act as an immuno-modulator due to the presence ofLPS and PAMPs [1,3].

### 1.3 Isolation Methods of OMVs

Mainly ultracentrifugation, Density Gradient Centrifugation, Filtration, and Ammonium Sulphate Precipitation are major techniques used in the isolation of OMVs [1]. There are other detergents based or detergent free techniques commonly used with sodium deoxycholate in conjunction with EDTA. But, detergent-based protocols do not always work properly so, Ultrafiltration and Ultracentrifugation are widely accepted methods. Sometimes ultracentrifugation can be combined with Density Gradient Centrifugation [9].

The supernatant is filtered through a 0.45µm pore size membrane filter followed by a 0.22µm sterile filter. Ultracentrifugation is used to separate the molecules based upon their density. The filtered cell-free supernatant is centrifuged at 150,000 g for 2 h at4℃. The morphological structure of OMVs is determined by Transmission Electron Microscopy and 3-D structure by Scanning Electron Microscopy [10,11].

## 2. OMVs AS VACCINES

Vaccines serve a very important part in the prevention and treatment of diseases. Vaccines often carry pathogen linked factors that havebeen obtained from a disease-causing agent.LPS in OMVs generatesan immune response by interacting with Toll-like Receptors (TLR) and hence Antigen Presenting Cells (APCs). The presence of PAMPs structures in OMVs makes them outstanding vaccines against many pathogens [12].

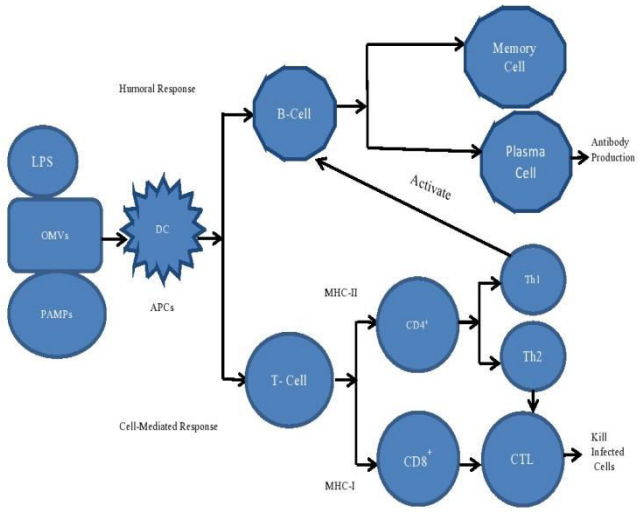


**Fig 1:** Flowchart displaying secondary immune response produced by OMVs.

LPS-Lipopolysaccharide, PAMPs-Pathogen Associated Molecular Patterns, DC- Dendritic Cell, APCs-Antigen Presenting Cells, CTL-Cytotoxic T Lymphocyte

Initially, OMVs were used as adjuvants in meningitidis, but due to their advanced functions scientist discovered their role as vaccines. The immune response developed by the OMVs vaccine in meningitides is strain-dependent. The PorA protein in *N. meningitides* is strain fixed. Using bioengineering tools scientists have developed a multivalent PorA vaccine which is four times more effective than the conventional vaccines [13].

D. Bottero and the group studied the role of the OMV vaccine in pertussis. They found that OMVs based vaccine obtained from *B. pertussis* can trigger Th1 and Th17 mediated immune response in pertussis infection [14].

Moreover, Oh Youn Kim *et. al* reported the antitumor activity of OMVs without any adverse effects by the production of antitumor cytokines CXCL10 and interferon-γ, but more research has to be carried out in this area[15].

Similarly, Binbin Zheng and co-workers found that synthetically obtained OMVs triggers many Pattern Recognition Receptors (PRRs) signaling pathways in antitumor therapy [16].

Allan and group demonstrated that *Psedomonas aeruginosa* produces gentamicin containing OMVs when treated with gentamicin antibiotic and shows bactericidal activities. As a result, gentamicin loaded OMVs performed delivery vehicle function to the selected *Burkholderia cepacia* bacterium [17].

Rafael Prados-Rosales et. al. showed that different strains of mycobacteria produce membrane vesicles in very conserved ways. When these vesicles are administered in mice, they produced TLR2 dependent immune response and stimulate cytokines secretion in wild type mice. The group has also revealed that only MVs from virulent strains of mycobacteria can produce TLR2 manner immune response [10, 19].

*V. cholerae* OMVs induce a long-term immune response with high-titer against the parent gastrointestinal pathogen[23].

Alves et. al. suggested that when OMVs are combined with enzymes, their functional activity gets increased, and simultaneously incorporation of phosphotriesterase (PTE) does not affect the enzyme kinetics [30].

Another important concern about OMVs adjuvant is safety. And in order to achieve this safety, a reduction in the LPS layer is an important factor. LPS secretes some pro-inflammatory cytokines in the host cell. Thus, investigators are trying to alter the composition of OMVs by using modern Genetic engineering techniques. Eg. Kim et al. (2009) made a technology by inactivating the MsbB (LpxM) lipid Aacyl transferase; thereby, producing low toxicity OMVs in *E. coli* [20].

Maria Kaparakiset. al. demonstrated the exact mechanism of OMVs that is responsible for inflammation in the host epithelial cells through PRRs. Epithelial cells are known to be the first physical barriers of cells in the immune system. OMVs can invade this immune response by the production of cytokines and chemokines in different ways [21].

## 3. OMVs AS ADJUVANTS

Adjuvants help in decreasing the dose of antigen needed to show the same effect. Adjuvants can alter the defense mechanism of a specific type of immune cell Eg. Activation of T cells rather than B cells based on the cause of the requirement.Adjuvants cannot induce immunity on their own without forming a complex with the antigen or vaccine.Dendritic Cell (DCs) activation supports the hypothesis that OMVs have the potential to act as a vaccine and provide a platform for self-adjuvant function due to the presence of immunogenic proteins and toxic molecules.To prove the biomedical applications of OMVs many studies have carried out, and these findings can contribute to the development of OMV-based adjuvants against many pathogens [12,22].

Chandan Kanta Das et. al. concluded thatpositively charged hydrophobic molecules interact with negatively charged membranes through passive diffusion. This interaction makes dendritic derived OMVs able to act as a siRNA delivery tool to the brain [24].

Tae-Young Lee and colleagues carried out a study that showed that the influenza vaccine with OMVs adjuvant enhanced T-cell specific response in obese mice [25].

In hepatitis B infection OMVs show another evidence of its adjuvant activity. When OMVs are co-immunized with the

hepatitis B virus surface antigen (HbsAg), they produce antigen-specific IgG and IgA humoral responses [26].

Another experiment carried out by Jens et.al. Showed that OMVs derived from *Legionella pneumophila* can fuse with eukaryotic membranes, and activate TLR2-dependent signaling pathways of the host cell [27].

Furthermore, Kim et al. studied the first experiment demonstrating that OMVs produce both Th1 and Th17 cell responses in neutrophilic pulmonary [28].

Further study revealed that OMVs isolated from meningococci are strain-specific, bind with TLR-2 and TLR-4 receptors and recruit other immune cells. Thus, the conventional adjuvants used in meningococcal vaccines can be replaced by newly discoveredOMVs adjuvants [22].

Chen et. al. demonstrated that fusion of green fluorescent protein (GFP) with *E. coli* Cytolysin A protein can produce a strong protective humoral response without any adjuvant. This study proves that OMVs possess their own adjusting capacity [29].

Due to their extraordinary target-specific drug delivery mechanism, many conventional drug delivery tools are replaced by OMVs. Eg. Recent studies showed that OMVs carry anti-tumor siRNA to the target cell in cancer therapy, and help in increased half-life of the drug [18].

## 3. CONCLUSION

Bacterial OMVs have shown wide applications in drug industries as they are rich in PAMPs and LPS. We have discussed various functions of OMVs in drug industries. They can act as vaccines, adjuvants, immune-modulator, drug delivery tools, tumor suppressors etc. As OMVs fate is decided by their biological content, so their products can be enhanced by providing appropriate nutrients and monitoring the growth phase of culture.Recent techniques in Genetic Engineering increase the ease of artificial OMVs production.

However, many problems need to be addressed for the mass production of OMVs in the future. Detoxification of OMVs, Reduction in LPS content, Safety and efficacy, Route of administration, loading efficiency and cost aresome of the challenges that occur while working with OMVs. The use of novel strategies, nanotechnology-based approaches and high purification techniques are some of the solutions to the respective problems.

## ACKNOWLEDGEMENT

We would like to thank Mr. Mayur Desai, Mr. Prathmesh, Suryawanshi and Ms. Poonam Patel for their constant support and dedicated help.

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