Biotechnology Advances 35 (2017) 565–574

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Bioengineering bacterial outer membrane vesicles as vaccine platform

Matthias J.H. Gerritzen[a,b], Dirk E. Martens[b], René H. Wijffels[b,c], Leo van der Pol[d], Michiel Stork[a,*]

[a] Institute for Translational Vaccinology (Intravacc), Process Development Bacterial Vaccines, P.O. Box 450, 3720 AL Bilthoven, The Netherlands
[b] Wageningen University, Bioprocess Engineering, P.O. Box 16, 6700 AA Wageningen, The Netherlands
[c] Nord University, Faculty of Biosciences and Aquaculture, P.O. Box 1409, 8049 Bodø, Norway
[d] Institute for Translational Vaccinology (Intravacc), Molecular Biology and Immunology, P.O. Box 450, 3720 AL Bilthoven, The Netherlands

ARTICLE INFO

Keywords:
Outer membrane vesicles
Extracellular vesicles
Vaccine platform
Bioengineering
Nanobiotechnology

ABSTRACT

Outer membrane vesicles (OMVs) are naturally non-replicating, highly immunogenic spherical nanoparticles derived from Gram-negative bacteria. OMVs from pathogenic bacteria have been successfully used as vaccines against bacterial meningitis and sepsis among others and the composition of the vesicles can easily be engineered. OMVs can be used as a vaccine platform by engineering heterologous antigens to the vesicles. The major advantages of adding heterologous proteins to the OMV are that the antigens retain their native conformation, the ability of targeting specific immune responses, and a single production process suffices for many vaccines. Several promising vaccine platform concepts have been engineered based on decorating OMVs with heterologous antigens. This review discusses these vaccine concepts and reviews design considerations as the antigen location, the adjuvant function, physiochemical properties, and the immune response.

1. Vaccine platforms

Many vaccines are developed based on Pasteur's principle: “isolate, inactivate and inject” or by the selection of attenuated strains (Rappuoli 2004). Although many very effective vaccines have been developed by these methods, they have often led to adverse effects. Nowadays, vaccines must combine the lowest possible adverse effects with a high efficacy. Vaccine platforms can standardize the provoked immune response with high safety and low adverse effects, while the design allows for easy switching of displayed antigens leading to high efficacy. Protection against different diseases can be addressed by presenting different antigens.

The development of a highly safe and effective vaccine requires a lot of resources and time. At the same time the upcoming post-antibiotic era may require the development of more vaccines that also need to be developed in a short period (WHO 2014). The development trajectory can be shortened by vaccine platforms (Kushnir et al. 2012), since these platforms provide a blueprint for development of many different vaccines instead of a specific development trajectory for each separate vaccine with all its uncertainties. The safety of a vaccine platform can be established by thorough development of the platform itself. Once the safety and efficacy of a platform has been established, development time can be reduced for new vaccines, since less testing will be required and unexpected failures will occur less. This advantage of vaccine platforms will reduce significantly the time to market, which is notoriously long for new vaccines.

A vaccine platform should provoke a strong specific immune response. This response is triggered by conformationally correct antigen presentation, **PAMPs** (see glossary) to activate antigen presenting cells, and a nanosized particulate nature. Furthermore, it should be possible to easily add antigens onto the vaccine platform. Platform nanovaccines can be based on many components, for example **VLPs**, **ISCOMs**, polymeric nanoparticles, inorganic nanoparticles, liposomes, and emulsions. A component that is often overlooked are OMVs (Zhao et al. 2014). While many nanoparticles are capable of transferring heterologous antigens to antigen presenting cells, the ability to properly stimulate the immune system is often not natively present (Singh et al. 2007). OMVs, however, combine antigen presentation with proper adjuvant properties, making them highly suitable as a vaccine platform.

OMVs are non-replicative vesicles that are naturally produced by Gram-negative bacteria and contain excellent intrinsic immunostimulatory properties based on their particulate nature and composition (Ellis and Kuehn 2010). The vesicles consist of phospholipids, LPS, outer membrane proteins and entrapped periplasmic components (Kulp and Kuehn 2010). OMVs are ascribed many biological functions such as cell to cell communication, surface modifications and the expulsion of components (Kulp and Kuehn 2010). Overall, OMVs have been shown to be highly stable even upon elevated temperatures and several chemical treatments (Arigita et al. 2004). This review addresses the latest state of research with respect to the development of an OMV

* Corresponding author.
E-mail address: michiel.stork@intravacc.nl (M. Stork).

http://dx.doi.org/10.1016/j.biotechadv.2017.05.003
Received 15 February 2017; Received in revised form 24 April 2017; Accepted 12 May 2017
Available online 15 May 2017

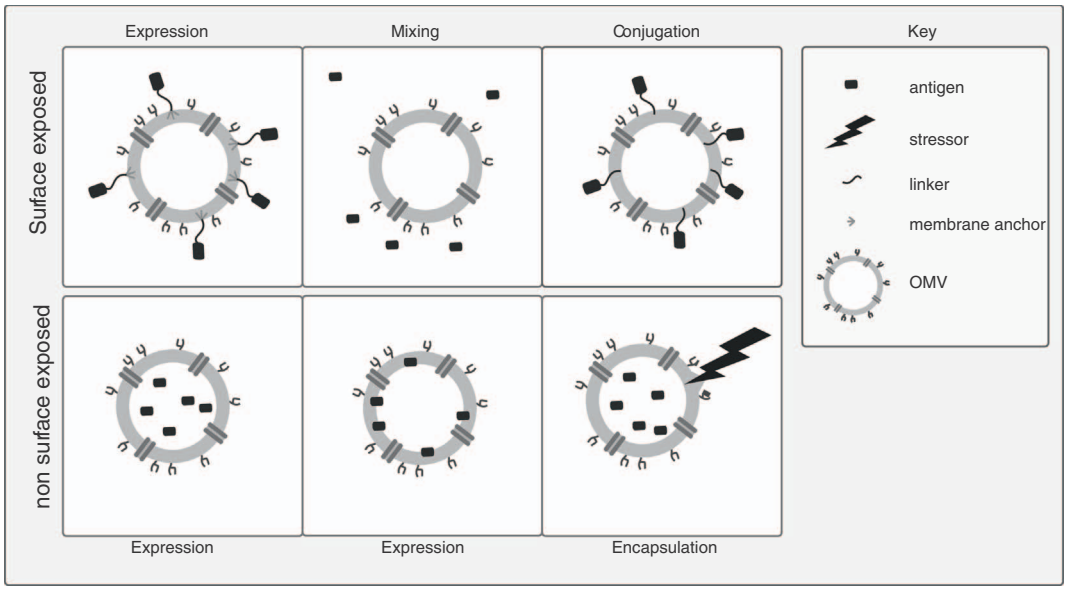

Fig. 1. Methods of antigen decoration on OMVs. Top row shows surface exposed antigens on the vesicles, bottom row shows the antigens as luminal cargo of OMVs. Antigens can be produced by the OMV production bacterium (left), while antigen addition to purified vesicles can be divided in mixing, conjugation and encapsulation (middle and right).

based vaccine platform. First we discuss the location of the antigen, which is either inside the OMV or displayed on the OMV surface. Location is important for the provoked type of immune response. Two approaches of location specific antigen addition are discussed, namely the endogenous addition based on antigen production by the bacterium itself and the exogenous methods that introduce the antigen in a separate process step. The bioengineering of the provoked immune response and the endotoxicity is discussed. Additionally, we discuss the bioengineering of the physiochemical properties of the OMV and the potential of outer membrane vesicles as vaccine platforms. Lastly we propose a uniform naming of different vesicles based on the origin of the OMVs.

2. Designing the OMV: antigen location

Heterologous antigens on OMVs can be presented with or without surface exposure, attached to the vesicle or non-attached and directly produced by the bacterium or combined in a later production stage. Various possibilities of antigen locations and their production method are schematically shown in Fig. 1. At this moment it is unclear what the most preferred setup for an OMV based vaccine platform is. This section describes the impact of the heterologous antigen location, the endogenous loading of antigens to the vesicle lumen and the vesicle surface, and the exogenous loading of antigens to the vesicle lumen and the vesicle surface.

Surface exposed antigens are accessible for antigen-specific B cell binding, while the inside of the vesicle is shielded from these cells. Luminal antigens may be skewed towards cytotoxic T-cell responses (Galen and Curtiss, 2014), hence the desired immune response determines the design of the OMV. Many groups have expressed antigens in the lumen of OMVs to develop OMV vaccines (Table 1) (Bartolini et al. 2013; Fantappie et al. 2014; Kesty and Kuehn 2004; Kim et al. 2009; Muralinath et al. 2011; Schild et al. 2009). Surprisingly, these studies also find antibody-mediated immune responses against the luminal heterologous antigen. Muralinath et al. studied the luminal expression of Pneumococcal PspA in *Salmonella enterica* serovar Typhimurium OMVs (Muralinath et al. 2011). These vesicles triggered minor antibody responses against OMVs and PspA in immunized mice and provided protection in a challenge experiment. OMVs without PspA or purified PspA alone did neither evoke an antibody response nor provide protection. Schild et al. showed minor specific antibody responses against periplasmic alkaline phosphatase (PhoA) from *Escherichia coli* expressed in *Vibrio cholerae* OMVs (Schild et al. 2009). In addition to the minor antibody titers found in the previous two studies, it was shown by Fantappie et al. that also high functional antibody titers can be obtained by expressing heterologous antigens in their native conformation in the lumen of *E. coli* OMVs (Fantappie et al. 2014). This systematic study characterized the vesicles by showing incorporation of the antigens and antigen localization. Because of the findings in a previous study that the Chlamydial HtrA protein expressed in OMVs was partially surface exposed, the authors checked the antigen localization (Box 1) by proteinase K treatment (Bartolini et al. 2013). After all, contamination with surface exposed antigen could be a cause for the observed response. The heterologous antigens in OMVs were found not to be surface exposed. Further analysis of these proteins showed their native conformation in the vesicle lumen that remarkably appears to be sufficient to trigger antibody mediated responses.

Antigens can be presented on the surface of the OMV with exposure to the exterior side of the vesicle. We recently studied the expression of the Borrelial surface-exposed lipoprotein **OspA** in *Neisseria meningitidis* OMVs (Salverda et al. 2016). Expression of the protein in meningococci did not result in surface exposure on OMVs. To obtain surface exposure, OspA was fused to a Neisserial lipoprotein. The immunogenicity of this surface exposed fusion construct was compared to that of a luminal expressed OspA in mice. Results showed that only the surface-exposed OspA was able to elicit an OspA-specific antibody response. In a study on Salmonella OMVs by Muralinath et al., higher immune responses against outer membrane proteins and LPS were found than against the heterologous expressed antigen present in the vesicle lumen (Muralinath et al. 2011).

It remains unclear whether antigens in the lumen of OMVs provide sufficient antibody responses. The observed antibody responses of some studies may be biased by extracellular antigen or surface attached antigen. On the contrary the lack of an antigen specific antibody response against non-surface exposed OspA may not be predictive for other antigens (Salverda et al. 2016). Antibody responses have been observed for all surface exposed antigens, while for luminal antigens the provoked responses remain ambiguous. Altogether more research is required on the exact effect of the antigen location on efficacy of an OMV vaccine platform.