European Journal of Pharmaceutics and Biopharmaceutics 145 (2019) 1–6

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Bacterial membrane vesicles as promising vaccine candidates

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A B S T R A C T

Both Gram-positive and Gram-negative bacteria can release nano-sized lipid bilayered structures, known as membrane vesicles (MVs). These MVs play an important role in bacterial survival by orchestrating interactions between bacteria and between bacteria and host. The major constituents of MVs are proteins, lipids and nucleic acids. Due to the immunogenicity of the membrane lipids and/or proteins of the MVs, in combination with adjuvant danger signals and the repeating patterns on the nanosized surface, MVs can effectively stimulate the innate and adaptive immune system. Since they are non-replicating, they are safer than attenuated vaccines. In addition, by genetic engineering of the donor cells, further improvements to their safety profile, immunogenicity and yield can be achieved. To date, one MV-based vaccine against *Neisseria meningitidis* (*N. meningitidis*) serogroup B was approved. Other (engineered) MVs in the pipeline study are mostly in the preclinical phase.

1. Introduction

The first observation of membrane vesicles (MVs) produced by Gram-negative bacteria can be traced back to the 1960s [1]. Later on, MVs from Gram-positive bacteria were also confirmed to exist [2–4]. Both Gram-negative and Gram-positive bacteria release MVs under normal culture conditions. Release is often increased under stressed circumstances. MVs were regarded as cellular debris or microscopy artifacts for a long time [5]. Only recently, the important functional roles of MVs have been reported. These roles include: removal of stress products from the bacterial cells, convey antimicrobial properties against competitive micro-organisms, transfer of antibiotic resistance, contribution to horizontal gene transfer, nutrient acquisition, and nucleation point for the formation of bacterial biofilms [6–8].

The biogenesis of MVs in Gram-negative bacteria and Gram-positive bacteria are different. Understanding the unique bacterial architecture help understand those mechanisms involved. For Gram-negative bacteria, the envelope consists of two membranes, outer membrane (OM) and inner membrane (IM). There is also a thin highly cross-linked peptidoglycan layer (PG) bridging these two layers. One proposed MVs biogenesis mechanism for Gram-negative bacteria is that membrane budding occurs when the OM-PG, OM-PG-IM interaction within envelope temporarily decrease, thus allowing the dissociation of OM and PG [9]. It was also noted recently that explosive cell lysis mediated by cryptic prophage endolysin can serve as a new mechanism for the biogenesis of OMVs [10]. Gram-positive bacteria contains only one membrane and a thick PG layer, it is also proposed that endolysin can trigger the formation of MVs [11].

MVs are 20–250 nm in size and they exhibit certain similarities in composition to the membrane of the bacteria from which they are released. However, specific proteins such as virulence factors and small RNA are more abundant in MVs than parental bacteria *Porphyromonas gingivalis* and *Pseudomonas aeruginosa* respectively, which implies that a sorting process is involved in biogenesis [12–14].

MVs offer potential as a platform for vaccines. First of all, a range of antigens and danger signals are displayed either on the surface of MVs or encapsulated in the interior, which can stimulate both innate and adaptive immunity. Secondly, MVs are replication-defective and therefore safer compared to traditional attenuated bacterial vaccines. However, there are still several challenges to overcome in the application of MVs, such as unacceptable toxicity of the associated danger signals, poor yield of production and poor immunogenicity [8].

This review will first focus on current approaches to address the difficulties in the translation of MVs-based vaccines from concept to reality. Secondly, it discusses two examples the current state-of-the-art of MVs-based vaccine: Bexsero® and Bera-V.

2. Production and isolation of MVs

MVs are naturally produced by bacteria. Their production can also be induced by treating with detergents such as sodium deoxycholate, enzymatic degradation by lysozyme or mechanical shearing force [7]. The molecular composition of naturally produced MVs differs from induced MVs [15,16].

The workflow for harvesting of MVs consists of cultivation, removal of bacterial cells, concentration, isolation and purification steps. Usually, bacteria are inoculated in a suitable medium to reach their exponential /stationary phase of growth [17]. The compositions of MVs is different for the different growth phases. For example, Acm (a collagen binding adhesin) is detected in MVs isolated from *Enterococcus*

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https://doi.org/10.1016/j.ejpb.2019.09.021
Received 3 July 2019; Received in revised form 17 September 2019; Accepted 23 September 2019
Available online 24 September 2019
0939-6411/

Table 1
Summary of genes used in MV engineering.

Objective	Gene	Encoding protein	Ref
Detoxification	Δ*msbB (LpxM)*	Lipid A acyltransferase catalysing final myristoylation step	[25]
	Δ*LpxL*	Lipid A biosynthesis lauroyltransferase	[27,42]
	Δ*pagP*	Lipid A palmitoyltransferase PagP	[27]
	Δ*LpxP*	Lipid A biosynthesis palmitoleoyltransferase	[27]
	Δ*eptA*	Lipid A phosphoethanolamine transferase	[27]
	::*pagL*	Lipid A deacylase PagL which removes 3-hydroxydodecanoic acid moiety at the 3′ position	[21]
	::*lpxF*	Lipid A 4′-phosphatase which removes the 4′-phosphate moiety from lipid IV(A) and penta-acylated lipid A	[29,31]
Antigen display	OmpA	Outer membrane protein A	[25,36]
	ClyA	Cytolysin A	[34–35,43]
	Hbp	Hemoglobin protease	[37,44–45]
	Glycotopes	Gene cluster for synthesis of *F. tularensis* Schu S4 O-antigen polysaccharide	[39]
Increase yield	Δ*rmpM*	Protein links OM and peptidoglycan	[23]
	Δ*nlpl*	OM-anchored Lpp	[28]
	Δ*TolR*	Tol-pal complex	[41]
	Δ*TolA*	Tol-pal complex	[38]
	Δ*pbp4* (Gram-positive)	Penicillin-binding protein 4	[33]
	Δ*tagO* (Gram-positive)	N-acetylglucosamine-phosphate transferase enzyme	[33]

faecium E155 in their stationary-phase, but not in log-phase MVs. The opposite is true for PtsD (Enzyme IID subunit of the phosphotransferase system) [18]. Usually, a higher yield of MVs is obtained when bacteria are cultured up to the stationary phase, but it runs the risk of reducing sample purity due to contamination with membrane fragments as a result of bacterial lysis [18].

To harvest MVs, one of the critical steps is to remove the intact bacteria via a series of centrifugation and filtration steps. Next, the supernatant/flow-through needs to be concentrated. The most extensively-used methods include ammonium sulphate precipitation methods, ultrafiltration, tangential flow filtration and hollow-fiber filtration. Subsequently, density gradient-ultracentrifugation (gUC) and size-exclusion chromatography (SEC) can be used to further purify MVs from co-isolated contaminants such as large molecular weight soluble proteins, and fragments of flagella, pili, and fimbriae. Density gUC is regarded as golden standard to obtain pure MVs samples, although it is less practical for large scale production. After ultracentrifugation, MVs migrate to the fractions with density similar to their buoyant density. SEC separates MVs based on size rather than density, as a result, both purification steps may yield different MV populations. After isolation and purification, absence of live bacteria/spores is confirmed by inoculation in a nutrient-rich liquid medium.

3. Characterization and quality control of MVs

Quality control of EVs has proven to be challenging. To assist the field, the International Society for Extracellular Vesicles published a guideline on the minimal characterization of EVs that should be reported to assert the presence of bona fide vesicles. These characterization criteria are known as the 'minimal information for studies of EVs (MISEV)'. It suggests that three or more MV protein markers should be detected by either western blot, flow cytometry or proteomic analysis. At least two different methods should be used to assess MV population heterogeneity. Transmission electron microscopy (TEM) or atomic force microscopy (AFM) which shows both close-up and wide-field images should be presented in a publication, and the size of EVs should be examined by nanoparticle tracking analyzer (NTA), dynamic light scattering (DLS), or resistive pulse sensing [19]. MV yield should be normalized to the number of bacteria by measuring OD value or calculating the Colony Forming Unit (CFU) value. In the MVs product development process, the batch-to-batch variation should also be taken into account. Several approaches can be used examine the variation in protein composition, such as Coomassie blue staining or silver staining of electrophoresis gels. Zeta-potential analysis which measures the surface charge of nanoparticle in solution can give information on the aggregation tendency. Lipidomics or lipid staining methods can offer extra information on the lipid compositions among different batches. Regarding the nucleic acid content, next generation sequencing techniques can provide profiles of DNA fragments or RNA species present.

4. Engineering of MVs

Since MVs are produced from bacteria, toxicity and undesirable immunogenicity remain a point of concern. In addition, their yield is often low and the desired immunogenicity can be insufficient. With genetic engineering, tailoring of the properties of MVs has become possible.

4.1. Detoxification

Lipopolysaccharide (LPS) is one of the major outer membrane constituents of Gram-negative bacteria. It can cause fever, inflammation and septic shock via activation of Toll-like receptor 4 (TLR 4) [20]. Therefore, lowering the LPS content of MVs can be important to limit the inflammatory response. Detergent extraction has been used to reduce the amount of LPS. In addition to LPS, the detergent may reduce/cleave other surface pathogen associated molecular pattern (PAMPs) such as surface-exposed lipoprotein factor H binding protein (fHbp), which may be unwanted [21]. It is also reported that detergent-extraction methods may increase OMVs' aggregation tendency, which could compromise product quality [22]. A detergent-free method, based on EDTA extraction, showed less tendency to aggregate, while maintaining yield and immunogenicity [23].

Genetic engineering is a more elegant method that may be preferred for MVs detoxification, various strategies are listed in Table 1 and discussed below. Since the toxicity of LPS is correlated to the degree of acylation [24], one of the strategies is based on reducing the number of LPS acyl chains. For example, a mutation in the *lpxL1* gene (which encodes lipid A biosynthesis lauroyl acyltransferase) in *Neisseria meningitides*, produces penta-acylated LPS. This has reduced toxicity compared to the original hexa-acylated LPS [23]. In a similar approach, inactivating the gene *msbB (*also known as *LpxM),* which encodes the lipid A acyltransferase in *Escherichia coli* strain O157:H7 and *Salmonella enterica* serovar Typhimurium, reduced the toxicity of OMVs [25–26]. In *E. coli* strain BL21, the lipid A structure was modified by deletion of a range of genes that encode enzymes which play important roles in its biosynthesis pathway such as *lpxL, lpxM, pagP, lpxP* and *eptA*. As a consequence, the lipid A precursor lipid IV_A was incorporated into MVs. This strongly attenuates toxicity, since lipid IV_A is a human Toll like receptor 4 (TLR4) antagonist [20,27–28]. In addition, a Δ*msbB/*Δ*pagP*

mutant was generated in *E. coli* K-12 strain W3110, leading to penta-acylated lipids and reducing pyrogenicity while maintaining their adjuvant activity on T cells [29,30].

Another approach to attenuate the toxicity induced by LPS is to modulate the phosphorylation of lipid A. By knocking in the *lpxF* gene, which encodes lipid A 4′-phosphatase, the 4′-phosphate moiety from lipid IV_A and penta-acylated lipid A, can be removed [31]. In the *E. coli* K-12 strain W3110 Δ*msbB*/Δ*pagP* mutant, the additional knock in of *lpxF*, TLR4 activation was further reduced in in-vitro and in-vivo tests [29].

Studies on attenuating the toxicity of MVs with other target molecules than LPS are few to date. One good example is the deletion of a global regulator (*agr*), which regulates the secretion of virulence factors and surface proteins [32]. After this deletion, the toxicity of MVs from *Staphylococcus aureus* was significantly reduced [33].

4.2. Antigen display

Genetic engineering offers an alternative to working with highly pathogenic microorganisms. Heterologous MV-vaccines, where non-pathogenic bacteria are genetically modified to express the antigens of pathogens either on the surface or in the lumen, form a safer alternative.

One of the strategies is to fuse antigens to membrane proteins. Cytolysin A (ClyA), a pore forming transmembrane protein which is enriched in MVs is often used for display. For example, β-lactamase, organophosphorous hydrolase, anti-digoxin single-chain Fv antibody fragment, and green fluorescent protein (GFP) were coupled to the C-terminus of ClyA. This resulted in the display of all of these antigens on the surface of MVs [34]. GFP fused to ClyA on MVs generated a strong anti-GFP antibody response in mice [35]. MVs can also be engineered using outer membrane protein (OMP). Kim et al. successfully localized a FLAG tag into the lumen of MVs from *E. coli* by fusing the protein to the C-terminus of the β-barrel domain of outer membrane protein A (OmpA) [25]. Also, antigens from *Staphylococcus* group A and group B fused to OmpA in *E. coli* resulted in MVs with staphylococcal antigens, which elicited high antibody titers [36].

The hemoglobin protease (Hbp) autotransporter-based fusion has also been explored in the MV field. Antigens from *Mycobacterium tuberculosis* including ESAT6 were fused to Hbp for surface modification of *E. coli* OMVs [37]. These antigens together with Hbp are subsequently released into the extracellular space [38]. It will be explained in more detail in the section on Heterologous vaccines.

Instead of fusing protein domains, another strategy is based on engineering glycotopes onto the lipid A moiety of LPS. With this method, the pathogen-mimetic glycotopes are genetically coupled to the O-antigen polysaccharide moiety of LPS. *E. coli* expressing the gene cluster encoding glycotopes from the highly virulent *Francisella tularensis*, resulted in MVs displaying the pathogens glycotope signature. The resulting OMVs elicited the IgG titers and protected mice from an *F. tularensis* challenge [39].

So far, it has not clearly been established what the preferred location of antigens associated with MVs is for vaccination: inside the lumen or on the surface. On the surface, it can be expected that exposure to immune cells is more direct to elicit an immune response. However, many studies report luminal loading to be able to induce antibody production [40]. For example, Fantappie‘ and co-worker showed that encapsulated Group A streptococcal (GAS) antigens Slo, spyCEP, Spy0269 inside *E. coli* MVs, are still able to raise protective antibodies in a mouse model of lethal GAS infection [36].

4.3. Higher MV production

The yield of MVs is one of the limiting factors for their application. Since, in principle, the outer membrane of Gram-negative bacteria remains stably connected to the inner layers, shedding of OMVs is necessarily restricted.

To increase yield, hypervesiculating bacteria can be engineered by creating mutations in genes encoding Lpp or Tol-pal proteins that connect membrane to peptidoglycan layer. When the *rmpM* gene, which belongs to peptidoglycan-associated Lpp family, was deleted in in *N. meningitidis*, MV production increased [23]. In a systematic screening of genes which are important in the vesiculation of an *E. coli* strain, higher MV production was achieved by knocking out *nlpI*, a gene encoding outer membrane anchored Lpp [28]. A study using *Shigella boydii* in which *tolA* was deleted showed a 60% higher vesiculation rate, and, surprisingly, also improved immunogenicity in adult Balb/c mice [41].

For Gram-positive bacteria, the thick outer layer of highly crosslinked peptidoglycan is the main barrier for the biogenesis of MVs. Therefore, higher yield of MVs usually requires degradation of the peptidoglycan layer or reduction of crosslinking. When *S. aureus* was treated with sublethal concentrations of Penicillin G (Pen G) or knocking out peptidoglycan proteins *pbp4* or *tagO*, MV yield increased significantly [33].

5. OMV vaccines against meningococci

Outbreaks of *N. meningitides* caused signficiant morbidity and mortality. There are 12 different *N. meningitidis* serogroups which are classified based on the type of capsular polysaccharide. Of these, six serogroups A, B, C, X, Y and W are the main subtypes contributing to global meningococcal disease [46]. For serogroup A, C, Y and W, a capsular polysaccharide vaccine has been developed. Since capsular polysaccharides from serogroup B are similar to components of human neural cells, there is a risk of an autoimmune response [46]. For this particular subtype OMV-based vaccine serves as an effective therapeutic alternative and a good example of the current state of the art.

The first monovalent OMV-based vaccines, each directed at one particular strain of meningococci type B include the MenBvac®, VA-Mengoc-BC® and MeNZB®. In these products, serosubtype outer membrane protein Porin A (Por A) is the dominant antigen. Other antigens include Porin B (Por B), reduction modifiable protein (Rmp), and Opc A, a protein involved in bacterial invasion [47].

MenBvac® is a first-generation OMV-based vaccine developed in Norway in 1984–1986 in a period when serogroup B meningococci were highly prevalent in Northern Europe with ~10% fatality in infected patients [48]. The OMVs were isolated from wild-type strain 44/76-SL by a deoxycholate extraction method. The adjuvant in this formulation is aluminum hydroxide. Other compositions include OM protein, thiomersalate and sucrose [48]. In 1991, a clinical study in healthy volunteers appeared promising [49]. However, in the subsequent large-scale randomised trial, the formulation failed to protect 40% of the vaccinated volunteers [48]. This product was also used in the control of an *N. meningitides* epidemic in France starting in 2006 [50], Studies showed that children of 1–5 year-old immunized with MenBvac®, showed serum bactericidal activity in 84.1% of children after 6 weeks, while this was 10.8% before immunization [51].

VA-Mengoc-BC® was developed in 1987–1989 in Brazil [52]. The formulation consists of OMVs isolated from wild-type strain CU-385, OMPs from serogroup B meningococci, polysaccharide from serogroup C meningcocci, LPS, aluminium hydroxide, and sodium thiomersalate [53]. It showed 83% efficacy in a clinical trial in Cuba, eliciting long-lasting and high antibody production [53]. VA-Mengoc-BC® is used in the public vaccination scheme [53].

MeNZB® was developed in New Zealand. It is based on OMVs isolated from the NZ98/554 strain which caused an epidemic in New Zealand around 2000. This OMV-based vaccine induced a 4-fold rise in MenB-antibody titers in 96% of adults, and ~75% of children, toddlers, and infants [54]. It appears a safe and efficient vaccine for the control of this specific serotype of meningococci [54].

Though these meningococcal OMV vaccines have shown promise to control MenB outbreaks in different regions, they do not offer broad

protection. Since the major antigen Por A is highly variable, these monovalent vaccines showed efficacy only for certain type of *N. meningitides*. It has proven to be a major challenge to find a product that has a broader coverage of the different strains of serogroup B meningococci [46].

5.1. Broadening coverage

Peter et al. designed OMV-based vaccines that should provide broader coverage. A bivalent vaccine was obtained by replacing less immunogenetic Class 3 OMPs in a H44/76 strain with high immunogenic Class 1 OMPs, from *N. meningitidis* strain 2996. Interestingly, the resulting OMVs can induce high antibody titers against both strains [55]. Later on, a hexavalent OMV was reported. This formulation consists of mixed OMVs in 1:1 ratio from two different strains of *N. meningitidis* PL16215 and PL10124, with each strain expressing three different class 1 OMP variants of Por A [56]. In order to further broaden the bacterial coverage, a nonavalent OMV was created on the basis of the hexavalent version. OMVs were added from strain HP1416 engineered to express an additional three Por A subtypes, this OMV-based product triggered high serum bactericidal activity (SBA) titers against certain *N. meningitidis* strain in mice model [57].

5.2. Reverse vaccinology

Reverse vaccinology is a genome-based method to identify potential antigens for vaccine purposes. It can help to shorten the time for vaccine development and is especially helpful when the pathogen can not be easily cultured in vitro [58]. When this technique emerged in 2000, it was used to design an efficient meningococci B vaccine. First, the sequence of the genome of virulent *N. meningitidis* strains was determined. Open reading frames, potentially encoding surface-exposed proteins were identified. A total of 350 candidates were identified, amplified and cloned in *E. coli* and tested in mice. [59] Five proteins which were able to induce protective immunity and selected for the final multicomponent vaccine. This '5CVMB' design included three antigens with broad coverage: *Neisseria* heparin binding antigen (Nhba), fHbp and *Neisseria* adhesin A (NadA), and two with less broad coverage the genomic *Neisseria* antigen (GNA) 2091 and 1030 [59,60]. This formulation appeared effective against 78% of a representive population of 85 pathogenic meningococcal strains, indicating much broader coverage compared with MenBvac® and MeNZB® [60]. To facilitate large-scale manufacture, four of the five antigens were ultimately expressed as fusion protein, and renamed as '4CMenB', tradename 'Bexsero' (Fig. 1) [61].

The first clinical study showed that 4CMenB induced a higher human serum bactericidal activity than 5CVMB immunization in adults with a good safety profile [62]. In Phase II and Phase III studies, infants and children were included, demonstrating a preferred schedule of two doses administered 1 to 6 months apart [63]. A follow-up study showed that 77–94% of the subjects still had protective antibody titers for up to 18–24 months after the last vaccination [64]. Based on these promising results, 4CMenB has been integrated into the vaccination program in different countries since 2013.

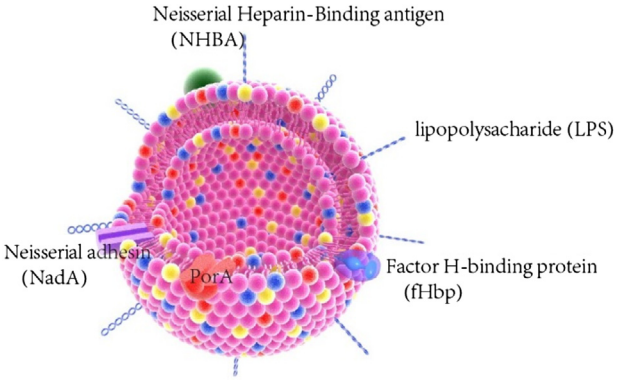

Fig. 1. Structure of the 4CMenB OMV vaccine.

5.3. Recent developments

In October 2014 a bivalent recombinant lipidated meningococcal complement factor H binding protein (fHbp) vaccine (trade name *Trumenba®*) was approved by the FDA for patients aged 10 to 25 years. Although fHbp has a high variability between strains, it can be broadly divided into two subgroups. The vaccine consists of fHbp of both subgroups. The first clinical studies showed that the vaccine induced bactericidal antibodies against various *N. meningitides* isolates [65].

Dowling et al. attenuated toxicity of *N. meningitides* OMVs by knocking down the gene *lpxL1* causing penta-acylated LPS to be formed. The OMVs caused a reduced inflammatory response in human leukocytes in vitro while still inducing antigen-specific antibodies in mice [66].

Zhang et al. tried to increase the fHbp content in the OMVs by inserting *fHbp* gene into the *porB* locus, resulting in OMVs without Por B. Interestingly, higher PorA expression was observed. Infant rhesus macaques vaccinated with these OMVs showed antigen-specific antibody responses against the genetically added fHbp [67].

6. Towards heterologous MV platforms

An example of a novel platform for vaccine development is based on OMVs from an engineered *Salmonella enterica* serovar Typhimurium strain. A particular advantage of this platform is the fact that the surface of OMVs can be decorated with multiple antigens of choice at a high density [68]. The OMVs are produced by *Salmonella enterica* serovar Typhimurium strain SL3261, which carries a mutated LPS through *msbB* gene knock-out. In addition, *ΔTolRA* was created leading to hypervesiculation of the bacteria.

Antigen loading of the OMVs is achieved using Hbp. As mentioned before, Hbp is a virulence factor, which is secreted by Gram-negative bacteria via the autotransporter pathway [69]. Autotransporters have three domains, one N-terminal signal peptide, a passenger domain, and a C-terminal β-helical domain. the N-terminal signal peptide facilitates crossing of the inner membrane. Then the C-terminal β-domain helps to target the passenger domain to the outer membrane [70].

For antigen display, a passenger subdomain was replaced by an ESATs antigen which is secreted by *Mycobacterium tuberculosis*. After fusing the antigen with Hbp, the cleavage site between the passenger domain and the β-domain was disrupted, which helps the antigen to remain attached to the surface of the bacteria or OMVs [71] Fig. 2.

As a result, OMVs from attenuated *S*. Typhimurium was successfully decorated with one, two or three antigens from *M. tuberculosis* (ESAT6, Ag85B and Rv2660c) and major OMP epitopes from *Chlamydia trachomatis* [38]. In vitro data showed that the antigen Ag85B delivered by OMVs is able to be recognized and processed by dendritic cells, and subsequently activate *M. tuberculosis*-specific T cells [44]. In addition, when OMVs from the *Salmonellae* were decorated with high-density *Streptococcus pneumoniae* antigens pneumococcal surface protein A variants (PspA) and pneumolysin (Ply) on the surface utilizing this Hbp platform, mice were protected from pneumococcal colonization after intranasal administration [45]. When compared with previously mentioned membrane/transmembrane fusion methods, the Hbp platform allows higher density display of antigens. An additional advantage is the fact that it is better suited for antigens with larger sizes and complicated structures [71]. This Hbp-based platform was further explored to developed OMVs against by decorating with fragments of protective protein [72]. One of the disadvantages of the Hbp platform is that the expression of the Hbp chimera might be lower when the number, size,

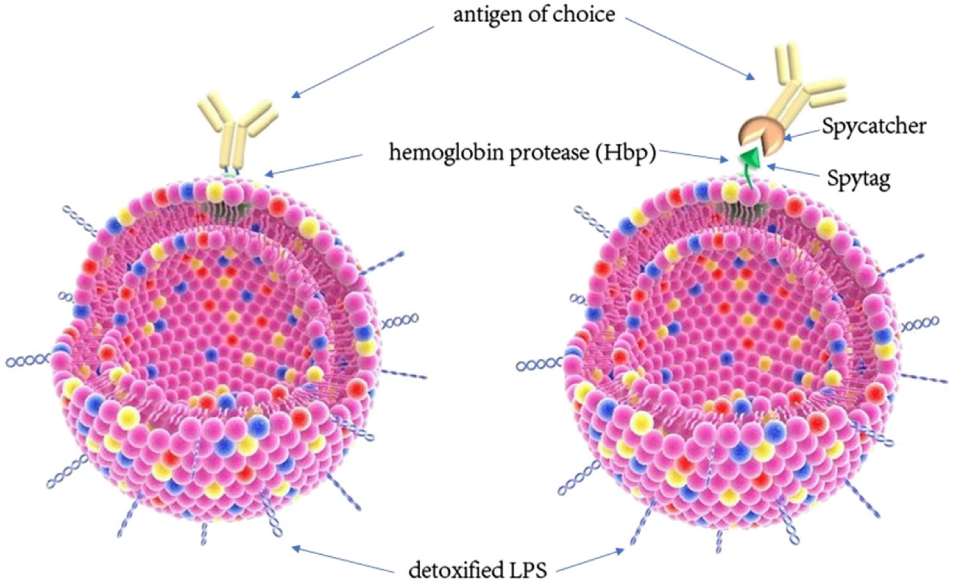

Fig. 2. Hbp based display of antigens on the surface of OMVs, via fusion with Hbp or equipping Hbp with a Spytag functionality with cognate Spycatcher coupling of the antigen of choice.

and complexity of the coupled antigens increases [73].

One of the solutions to circumvent these issues is to use the Hbp platform together with protein ligation technologies such as 'SpyTag-SpyCatcher' or 'SnoopTag-SnoopCatcher'. 'SpyTag' is a short peptide that can form a covalent bond when it encounters its protein partner 'SpyCatcher' [74]. Due to the strong binding, it can serve as 'superglue' to fuse antigens of interest to OMVs. Bart et al decorated the MV platform with pneumococcal antigens PspAα and SP1690 by firstly coupling the SpyTag to Hbps, and subsequently fusing the purified pneumococcal antigens to Spycatcher [73].

7. Final remarks

MVs from Gram-positive and Gram-negative bacteria are attractive vaccine platforms. Genetic engineering approaches enable precise tailoring of MVs to achieve safe, highly immunogenic and efficient vaccines. The first OMV-based engineered vaccine provides an example for the rational design of such platforms. Currently, new opportunities are emerging with heterologous engineered OMV vaccines that can have broad therapeutic activity by displaying various antigens on their surface.

Acknowledgement

LJ acknowledges financial support from the China Scholarship Council (CSC). Prof. Rob Willems (Department of Medical Microbiology, UMC Utrecht) is acknowledged for giving suggestions for this manuscript. Prof. Joen Luirink (Faculty of Science, Molecular Microbiology, Vrije Universiteit Amsterdam) is acknowledged for discussion on the work he has published.

References

[1] H.A. Bladen, J.F. Waters, Electron microscopic study of some strains of Bacteroides, J. Bacteriol. 86 (6) (1963) 1339–1344.
[2] D.W. Dorward, C.F. Garon, DNA is packaged within membrane-derived vesicles of Gram-negative but not Gram-positive bacteria, Appl. Environ. Microbiol. 56 (6) (1990) 1960–1962.
[3] W. Konings, A. Bisschop, M. Veenhuis, C. Vermeulen, New procedure for the isolation of membrane vesicles of Bacillus subtilis and an electron microscopy study of their ultrastructure, J. Bacteriol. 116 (3) (1973) 1456–1465.
[4] E.Y. Lee, D.Y. Choi, D.K. Kim, J.W. Kim, J.O. Park, S. Kim, S.H. Kim, D.M. Desiderio, Y.K. Kim, K.P. Kim, Gram-positive bacteria produce membrane vesicles: proteomics-based characterization of Staphylococcus aureus-derived membrane vesicles, Proteomics 9 (24) (2009) 5425–5436.
[5] T.J. Beveridge, Structures of gram-negative cell walls and their derived membrane vesicles, J. Bacteriol. 181 (16) (1999) 4725–4733.
[6] A.J. McBroom, M.J. Kuehn, Release of outer membrane vesicles by Gram-negative bacteria is a novel envelope stress response, Mol. Microbiol. 63 (2) (2007) 545–558.
[7] A. Kulp, M.J. Kuehn, Biological functions and biogenesis of secreted bacterial outer membrane vesicles, Annu. Rev. Microbiol. 64 (2010) 163–184.
[8] J.L. Baker, L. Chen, J.A. Rosenthal, D. Putnam, M.P. DeLisa, Microbial biosynthesis of designer outer membrane vesicles, Curr. Opin. Biotechnol. 29 (2014) 76–84.
[9] B.L. Deatherage, J.C. Lara, T. Bergsbaken, S.L.R. Barrett, S. Lara, B.T. Cookson, Biogenesis of bacterial membrane vesicles, Mol. Microbiol. 72 (6) (2009) 1395–1407.
[10] L. Turnbull, M. Toyofuku, A.L. Hynen, M. Kurosawa, G. Pessi, N.K. Petty, S.R. Osvath, G. Cárcamo-Oyarce, E.S. Gloag, R. Shimoni, Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms, Nat. Commun. 7 (2016) 11220.
[11] M. Toyofuku, N. Nomura, L. Eberl, Types and origins of bacterial membrane vesicles, Nat. Rev. Microbiol. 1 (2018).
[12] M.F. Haurat, J. Aduse-Opoku, M. Rangarajan, L. Dorobantu, M.R. Gray, M.A. Curtis, M.F. Feldman, Selective sorting of cargo proteins into bacterial membrane vesicles, J. Biol. Chem. 286 (2) (2011) 1269–1276.
[13] Q. Zhang, Y. Pan, R. Yan, B. Zeng, H. Wang, X. Zhang, W. Li, H. Wei, Z. Liu, Commensal bacteria direct selective cargo sorting to promote symbiosis, Nat. Immunol. 16 (9) (2015) 918.
[14] J.-W. Choi, J.-H. Um, J.-H. Cho, H.-J. Lee, Tiny RNAs and their voyage via extracellular vesicles: secretion of bacterial small RNA and eukaryotic microRNA, Exp. Biol. Med. 242 (15) (2017) 1475–1481.
[15] Y.M. Gnopo, H.C. Watkins, T.C. Stevenson, M.P. DeLisa, D. Putnam, Designer outer membrane vesicles as immunomodulatory systems–Reprogramming bacteria for vaccine delivery, Adv. Drug Deliv. Rev. 114 (2017) 132–142.
[16] L. van der Pol, M. Stork, P. van der Ley, Outer membrane vesicles as platform vaccine technology, Biotechnol. J. 10 (11) (2015) 1689–1706.
[17] J. Klimentová, J. Stulík, Methods of isolation and purification of outer membrane vesicles from gram-negative bacteria, Microbiol. Res. 170 (2015) 1–9.
[18] T. Wagner, B. Joshi, J. Janice, F. Askarian, N. Škalko-Basnet, O. Hagestad, A. Mekhlif, S. Wai, K. Hegstad, M. Johannessen, Enterococcus faecium produces membrane vesicles containing virulence factors and antimicrobial resistance related proteins, J. Proteomics 187 (2018) 28–38.
[19] J. Lötvall, A.F. Hill, F. Hochberg, E.I. Buzás, D. Di Vizio, C. Gardiner, Y.S. Gho, I.V. Kurochkin, S. Mathivanan, P. Quesenberry, Minimal Experimental Requirements for Definition of Extracellular Vesicles and Their Functions: a Position Statement from the International Society for Extracellular Vesicles, Taylor & Francis, 2014.
[20] H.C. Watkins, C.G. Rappazzo, J.S. Higgins, X. Sun, N. Brock, A. Chau, A. Misra, J.P. Cannizzo, M.R. King, T.R. Maines, Safe recombinant outer membrane vesicles that display M2e elicit heterologous influenza protection, Mol. Ther. 25 (4) (2017) 989–1002.
[21] A. Zariri, J. Beskers, B. van de Waterbeemd, H.J. Hamstra, T.H. Bindels, E. van Riet, J.P. van Putten, P.J.I. van der Ley, Meningococcal outer membrane vesicle composition-dependent activation of the innate immune response, Immunity 84 (10) (2016) 3024–3033.
[22] P. Kaaijk, I. van Straaten, B. van de Waterbeemd, E.P. Boot, L.M. Levels, H.H. van Dijken, G.P.J.V. van den Dobbelsteen, Preclinical safety and immunogenicity evaluation of a nonavalent PorA native outer membrane vesicle vaccine against serogroup B meningococcal disease, 2013, 31 (7), 1065–1071.
[23] B. Van de Waterbeemd, M. Streefland, P. Van der Ley, B. Zomer, H. Van Dijken, D. Martens, R. Wijffels, L. Van der Pol, Improved OMV vaccine against Neisseria

meningitidis using genetically engineered strains and a detergent-free purification process, Vaccine 28 (30) (2010) 4810–4816.
[24] R.T. Ranallo, R.W. Kaminski, T. George, A.A. Kordis, Q. Chen, K. Szabo, M.M.J.I. Venkatesan, Virulence, inflammatory potential, and adaptive immunity induced by Shigella flexneri msbB mutants, Immunity 78 (1) (2010) 400–412.
[25] S.-H. Kim, K.-S. Kim, S.-R. Lee, E. Kim, M.-S. Kim, E.-Y. Lee, Y.S. Gho, J.-W. Kim, R.E. Bishop, K.-T. Chang, Structural modifications of outer membrane vesicles to refine them as vaccine delivery vehicles, Biochimica et Biophysica Acta (BBA)-Biomembranes 1788 (10) (2009) 2150–2159.
[26] S.-R. Lee, S.-H. Kim, K.-J. Jeong, K.-S. Kim, Y.-H. Kim, S.-J. Kim, E. Kim, J.-W. Kim, K.-T. Chang, Multi-immunogenic outer membrane vesicles derived from an MsbB-deficient Salmonella enterica serovar typhimurium mutant, J. Microbiol. Biotechnol. 19 (10) (2009) 1271–1279.
[27] U. Mamat, K. Wilke, D. Bramhill, A.B. Schromm, B. Lindner, T.A. Kohl, J.L. Corchero, A. Villaverde, L. Schaffer, S.R. Head, Detoxifying Escherichia coli for endotoxin-free production of recombinant proteins, Microb. Cell Fact. 14 (1) (2015) 57.
[28] A.J. McBroom, A.P. Johnson, S. Vemulapalli, M.J. Kuehn, Outer membrane vesicle production by Escherichia coli is independent of membrane instability, J. Bacteriol. 188 (15) (2006) 5385–5392.
[29] T.-Y. Lee, C.-U. Kim, E.-H. Bae, S.-H. Seo, D.G. Jeong, S.-W. Yoon, K.-T. Chang, Y.S. Kim, S.-H. Kim, D.-J. Kim, Outer membrane vesicles harboring modified lipid A moiety augment the efficacy of an influenza vaccine exhibiting reduced endotoxicity in a mouse model, Vaccine 35 (4) (2017) 586–595.
[30] D.H. Lee, S.-H. Kim, W. Kang, Y.S. Choi, S.-H. Lee, S.-R. Lee, S. You, H.K. Lee, K.-T. Chang, E.-C. Shin, Adjuvant effect of bacterial outer membrane vesicles with penta-acylated lipopolysaccharide on antigen-specific T cell priming, Vaccine 29 (46) (2011) 8293–8301.
[31] https://www.uniprot.org/uniprot/A0Q4N6 (accessed 5-14).
[32] K.E. Traber, E. Lee, S. Benson, R. Corrigan M. Cantera B. Shopsin R.P.J.M. Novick, agr function in clinical Staphylococcus aureus isolates, 2008, 154 (Pt 8), 2265.
[33] X. Wang, C.D. Thompson, C. Weidenmaier, J.C. Lee, Release of Staphylococcus aureus extracellular vesicles and their application as a vaccine platform, Nat. Commun. 9 (1) (2018) 1379.
[34] J.-Y. Kim, A.M. Doody, D.J. Chen, G.H. Cremona, M.L. Shuler, D. Putnam, M.P. DeLisa, Engineered bacterial outer membrane vesicles with enhanced functionality, J. Mol. Biol. 380 (1) (2008) 51–66.
[35] D.J. Chen, N. Osterrieder, S.M. Metzger, E. Buckles, A.M. Doody, M.P. DeLisa, D. Putnam, Delivery of foreign antigens by engineered outer membrane vesicle vaccines, Proc. Natl. Acad. Sci. 107 (7) (2010) 3099–3104.
[36] L. Fantappie, M. de Santis, E. Chiarot, F. Carboni, G. Bensi, O. Jousson, I. Margarit, G. Grandi, Antibody-mediated immunity induced by engineered Escherichia coli OMVs carrying heterologous antigens in their lumen, J. Extracell. Vesicles 3 (1) (2014) 24015.
[37] W.S. Jong, Z. Soprova, K. de Punder, C.M. ten Hagen-Jongman, S. Wagner, D. Wickström, J.-W. de Gier, P. Andersen, N.N. van der Wel, J. Luirink, A structurally informed autotransporter platform for efficient heterologous protein secretion and display, Microb. Cell Fact. 11 (1) (2012) 85.
[38] M.H. Daleke-Schermerhorn, T. Felix, Z. Soprova, C.M. ten Hagen-Jongman, D. Vikström, L. Majlessi, J. Beskers, F. Follmann, K. De Punder, N.N. van der Wel, Decoration of outer membrane vesicles with multiple antigens by using an autotransporter approach 80 (18) (2014) 5854–5865.
[39] L. Chen, J.L. Valentine, C.-J. Huang, C.E. Endicott, T.D. Moeller, J.A. Rasmussen, J.R. Fletcher, J.M. Boll, J.A. Rosenthal, J. Dobruchowska, Outer membrane vesicles displaying engineered glycotopes elicit protective antibodies, Proc. Natl. Acad. Sci. 113 (26) (2016) E3609–E3618.
[40] M.J. Gerritzen, D.E. Martens, R.H. Wijffels, L. van der Pol, M.J.B.A. Stork, Bioengineering bacterial outer membrane vesicles as vaccine platform, 2017, 35 (5), 565–574.
[41] S. Mitra, R. Sinha, J. Mitobe, H. Koley, Development of a cost-effective vaccine candidate with outer membrane vesicles of a tolA-disrupted Shigella boydii strain, Vaccine 34 (15) (2016) 1839–1846.
[42] P. Van Der Ley, L. Steeghs, H.J. Hamstra, J. ten Hove, B. Zomer, L.J.I. van Alphen, Modification of lipid A biosynthesis inNeisseria meningitidis lpxL mutants: influence on lipopolysaccharide structure, toxicity, and adjuvant activity, Immunity 69 (10) (2001) 5981–5990.
[43] V. Gujrati, S. Kim, S.-H. Kim, J.J. Min, H.E. Choy, S.C. Kim, S.J.A.N. Jon, Bioengineered bacterial outer membrane vesicles as cell-specific drug-delivery vehicles for cancer therapy, 2014, 8 (2), 1525–1537.
[44] M.H. Daleke-Schermerhorn, T. Felix, Z. Soprova, M. Corinne, D. Vikström, L. Majlessi, J. Beskers, F. Follmann, K. De Punder, N.N. van der Wel, Decoration of outer membrane vesicles with multiple antigens by using an autotransporter approach, Appl. Environ. Microbiol. 80 (18) (2014) 5854–5865.
[45] K. Kuipers, M.H. Daleke-Schermerhorn, W.S. Jong, M. Corinne, F. van Opzeeland, E. Simonetti, J. Luirink, M.I. de Jonge, Salmonella outer membrane vesicles displaying high densities of pneumococcal antigen at the surface offer protection against colonization, Vaccine 33 (17) (2015) 2022–2029.
[46] X. Bai, J. Findlow, R. Borrow, Recombinant protein meningococcal serogroup B vaccine combined with outer membrane vesicles 11 (7) (2011) 969–985.
[47] J. Holst, B. Feiring, L.M. Næss, G. Norheim, P. Kristiansen, E.A. Høiby, K. Bryn, P. Oster, P. Costantino, M.-K.J.V. Taha, The concept of “tailor-made”, protein-based, outer membrane vesicle vaccines against meningococcal disease. 2005, 23 (17–18), 2202–2205.
[48] G. Bjune, E. Hoiby, J. Gronnesby, O. Arnesen, J.H. Fredriksen, A. Lindbak, H. Nokleby, E. Rosenqvist, L. Solberg, O. Closs, Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway, Lancet 338 (8775) (1991) 1093–1096.
[49] E. Høiby, E. Rosenqvist, G. Bjune, O. Closs, L.J.N. Frøholm, Serological responses in Norwegian adult volunteers to a meningococcal 15: P1. 16 outer membrane vesicle vaccine (phase II studies), 1990, 241–246.
[50] F. Caron, I.P. Du Chatelet, J.-P. Leroy, C. Ruckly, M. Blanchard, N. Bohic, N. Massy, I. Morer, D. Floret, V. Delbos, From tailor-made to ready-to-wear meningococcal B vaccines: longitudinal study of a clonal meningococcal B outbreak 11 (6) (2011) 455–463.
[51] F. Caron, V. Delbos, E. Houivet, A.-E. Deghmane, J.-P. Leroy, E. Hong, J. Bénichou, M.-K.J.V. Taha, Evolution of immune response against Neisseria meningitidis B: 14: P1. 7, 16 before and after the outer membrane vesicle vaccine MenBvac, 2012, 30 (34), 5059–5062.
[52] R.A. Pérez, F.O. Meneses, VA-Mengoc-BC vaccine: its impact on meningococcal disease in children 1-4 years old 51 (3) (1999) 189–193.
[53] G. Sierra, H. Campa, N. Varcacel, I. Garcia, P. Izquierdo, P. Sotolongo, G. Casanueva, C. Rico, C. Rodriguez, M. Terry, Vaccine against group B Neisseria meningitidis: protection trial and mass vaccination results in Cuba, NIPH Ann. 14 (2) (1991) 195–207 discussion 208–10.
[54] P. Oster, D. Lennon, J. O'Hallahan, K. Mulholland, S. Reid, D. Martin, MeNZB™: a safe and highly immunogenic tailor-made vaccine against the New Zealand Neisseria meningitidis serogroup B disease epidemic strain, Vaccine 23 (17–18) (2005) 2191–2196.
[55] P. Van Der Ley, J.J.I. Poolman, Construction of a multivalent meningococcal vaccine strain based on the class 1 outer membrane protein, Immunity 60 (8) (1992) 3156–3161.
[56] I. Claassen, J. Meylis, P. van der Ley, C. Peeters, H. Brons, J. Robert, D. Borsboom, A. van der Ark, I. van Straaten, P.J.V. Roholl, Production, characterization and control of a Neisseria meningitidis hexavalent class 1 outer membrane protein containing vesicle vaccine, 1996, 14 (10), 1001–1008.
[57] G. Van Den Dobbelsteen, H. Van Dijken, H. Hamstra, R. Ummels, L. Van Alphen, P. Van Der Ley, From HexaMen to NonaMen: expanding a multivalent PorA-based meningococcal outer membrane vesicle vaccine, 14th International Pathogenic Neisseria Conference. Milwaukee WI, 2004, pp. 5–10.
[58] R. Rappuoli, Reverse vaccinology, Curr. Opin. Microbiol. 3 (5) (2000) 445–450.
[59] M. Pizza, V. Scarlato, V. Masignani, M.M. Giuliani, B. Arico, M. Comanducci, G.T. Jennings, L. Baldi, E. Bartolini, B. Capecchi, Identification of vaccine candidates against serogroup B meningococcus by whole-genome sequencing, Science 287 (5459) (2000) 1816–1820.
[60] M.M. Giuliani, J. Adu-Bobie, M. Comanducci, B. Aricò, S. Savino, L. Santini, B. Brunelli, S. Bambini, A. Biolchi, B. Capecchi, A universal vaccine for serogroup B meningococcus, Proc. Natl. Acad. Sci. 103 (29) (2006) 10834–10839.
[61] C. Mameli, E. Galli, C. Mantegazza, V. Fabiano, G.V.J.F.M. Zuccotti, The multicomponent meningococcal serogroup B vaccine (4CMenB): origin, composition, health impact and unknown aspects, 2015, 10 (10), 1579–1598.
[62] C. Mameli, E. Galli, C. Mantegazza, V. Fabiano, G.V. Zuccotti, The multicomponent meningococcal serogroup B vaccine (4CMenB): origin, composition, health impact and unknown aspects, Future Microbiol. 10 (10) (2015) 1579–1598.
[63] M.E. Santolaya, M.L. O'Ryan, M.T. Valenzuela, V. Prado, R. Vergara, A. Muñoz, D. Toneatto, G. Graña, H. Wang, R.J.T.L. Clemens, Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents in Chile: a phase 2b/3 randomised, observer-blind, placebo-controlled study, 2012, 379 (9816), 617–624.
[64] M.E. Santolaya, M. O'Ryan, M.T. Valenzuela, V. Prado, R.F. Vergara, A. Muñoz, D. Toneatto, G. Graña, H. Wang, P.M.J.H.V. Dull, Persistence of antibodies in adolescents 18–24 months after immunization with one, two, or three doses of 4CMenB meningococcal serogroup B vaccine, Immunotherapeutics 9 (11) (2013) 2304–2310.
[65] I.M. Feavers, M.C.J. Maiden, Recent progress in the prevention of serogroup B meningococcal disease 24 (5) (2017) e00566-16.
[66] D.J. Dowling, H. Sanders, W.K. Cheng, S. Joshi, S. Brightman, I. Bergelson, C. Pietrasanta, S.D. van Haren, S. van Amsterdam, J.J.F.I.I. Fernandez, A meningococcal outer membrane vesicle vaccine incorporating genetically attenuated endotoxin dissociates inflammation from immunogenicity, 2016, 7, 562.
[67] L. Zhang, Z. Wen, J. Lin, H. Xu, P. Herbert, X.-M. Wang, J.T. Mehl, P.L. Ahl, L. Dieter, R.J.V. Russell, Improving the immunogenicity of a trivalent Neisseria meningitidis native outer membrane vesicle vaccine by genetic modification, 2016, 34 (35), 4250–4256.
[68] http://aberabio.com/technology/.
[69] W.S. Jong, C.M. Ten Hagen-Jongman, T. Den Blaauwen, D. Jan Slotboom, J.R. Tame, D. Wickström, J.W. De Gier, B.R. Otto, J.J.M.M. Luirink, Limited tolerance towards folded elements during secretion of the autotransporter Hbp. 2007, 63 (5), 1524–1536.
[70] J.C. Leo, I. Grin, D. Linke, Type V secretion: mechanism (s) of autotransport through the bacterial outer membrane 367 (1592) (2012) 1088–1101.
[71] W.S. Jong, Z. Soprova, K. de Punder, C.M. ten Hagen-Jongman, S. Wagner, D. Wickström, J.-W. de Gier, P. Andersen, N.N. van der Wel, J.J.M.C.F. Luirink, A structurally informed autotransporter platform for efficient heterologous protein secretion and display, 2012, 11 (1), 85.
[72] K. Kuipers, M.H. Daleke-Schermerhorn, W.S. Jong, M. Corinne, F. van Opzeeland, E. Simonetti, J. Luirink, M.I.J.V. de Jonge, Salmonella outer membrane vesicles displaying high densities of pneumococcal antigen at the surface offer protection against colonization, 2015, 33 (17), 2022–2029.
[73] D. Houben, M.I. de Jonge, W.S. Jong, J. Luirink, Display of recombinant proteins on bacterial outer membrane vesicles by using protein ligation, Appl. Environ. Microbiol. 84 (8) (2018) e02567–e2617.
[74] S.C. Reddington, M. Howarth, Secrets of a covalent interaction for biomaterials and biotechnology: SpyTag and SpyCatcher 29 (2015) 94–99.