REVIEW

Exploiting virus-like particles as innovative vaccines against emerging viral infections

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Emerging viruses pose a major threat to humans and livestock with global public health and economic burdens. Vaccination remains an effective tool to reduce this threat, and yet, the conventional cell culture often fails to produce sufficient vaccine dose. As an alternative to cell-culture based vaccine, virus-like particles (VLPs) are considered as a high-priority vaccine strategy against emerging viruses. VLPs represent highly ordered repetitive structures via macromolecular assemblies of viral proteins. The particulate nature allows efficient uptake into antigen presenting cells stimulating both innate and adaptive immune responses towards enhanced vaccine efficacy. Increasing research activity and translation opportunity necessitate the advances in the design of VLPs and new bioprocessing modalities for efficient and cost-effective production. Herein, we describe major achievements and challenges in this endeavor, with respect to designing strategies to harnessing the immunogenic potential, production platforms, downstream processes, and some exemplary cases in developing VLP-based vaccines.

Keywords: virus-like particles, emerging viruses, expression systems, vaccine design, downstream processes

Introduction

Infectious diseases are the leading cause of death among children and adolescents globally, and one of the primary causes of mortality in adults (Andre et al., 2008). Most of these deaths disproportionately burden low- and middle-income countries. For these countries, high-impact, low-cost public health interventions remain a key strategy for mitigating health and economic burden by infectious diseases. Immunization remains among the most cost-effective measures, second only to clean water. However, some vaccines are unavailable, inaccessible, and/or unaffordable for the populations most in need (Giersing et al., 2016a). Therefore, investments into the research, development, and deployment of vaccines and delivery technologies against emerging and re-emerging pathogens are likely to yield considerable dividends in global health.

Vaccination against infectious diseases is among the most effective of all global public health missions and saves the lives of 2.5 million people among children younger than age 5 every year worldwide (Giersing et al., 2016a, 2016b). However, vaccination has some challenges to solve. Most of all, the World Health Organization warned in its 2007 report that infectious diseases are emerging at an unprecedented rate (Andre et al., 2008). Since the 1970s, about 40 infectious diseases have been newly discovered, including SARS, MERS, Ebola, chikungunya, avian flu, swine flu, and, most recently, Zika. In the modern world, people travel much more than in the past, live in more densely populated areas, and come into closer contact with wild animals. As a consequence, the potential for emerging infectious diseases to spread rapidly and cause global epidemics becomes a major concern. The 2009 pandemic H1N1 influenza of swine origin is a prime example of such an emerging disease. Frequent changes in the influenza virus genome can cause epidemics and pandemics by successful immune evasion from prior infection or vaccination. Moreover, there has been potential zoonotic transmission from domestic or wild animals, some of which serves as mixing vessels for the generation of novel influenza. In fact, the 2009 pandemic H1N1 influenza turned out to be a genetic reassortment among four different viral species of avian, swine, and human origin (Shapshak et al., 2011; York and Donis, 2013). Although direct human-to-human transmission is not officially announced, the highly pathogenic avian influenza (HPAI) H5N1 virus remains on the watch list, especially when considering extremely high mortality among humans. To help meet this challenge, therapeutic and prophylactic interventions are dearly needed. This work encompasses both basic research and more directed research in developing and evaluating vaccines to prevent infection by these agents.

Challenges in the development of vaccines against an emerging virus

There are several challenges in developing a vaccine against
an emerging virus (Søborg et al., 2009; Bowick and McAuley, 2011). First, our understanding about the pathogenicity is limited and protective antigens are poorly characterized, especially with newly discovered viruses. In addition, for highly variable viruses, it is virtually impossible to predict the genetic variants that would cause the next outbreak or pandemic.

Second, it is hard to find appropriate animal challenge models for the evaluation of vaccine efficacy. Vaccine candidates need to be pre-clinically tested in animal models for safety, immunogenicity, and protective efficacy. Ideally, an animal model should have a well-known immune system and similar susceptibility, and immune responses to the pathogen as the natural host. Other important factors in choosing an ideal animal model are price, size, possibility to use in large numbers, and ethical considerations. However, due to the lack of knowledge on pathogenicity (e.g., route of infection and cellular receptors for viral entry, etc), an ideal animal model closely mimicking human infection is hard to establish.

Third, some emerging viruses have high mortality rates, with no treatment or prophylaxis available, and must be manipulated under high bio-safety conditions, sometimes requiring BSL-4 facilities. Vaccine manufacturing in most cases requires the production of target viruses in large quantities by cell culture. Similar concerns apply to veterinary vaccines against highly contagious viruses, e.g., foot and mouth disease virus (FMDV), where BSL-3 level facilities is required during production.

Fourth, the time required to develop, validate, produce in a large scale, and deliver a new vaccine should ideally be as short as possible. This could be facilitated by the use of well-known vaccine platforms that have already been tested and validated against similar pathogens. Vaccines to be used to control an ongoing outbreak should be stockpiled in advance or produced in a timely manner, and provide protection preferably by a single administration. And yet, due to the lack of adaptation in cell substrates approved by regulatory authorities, newly isolated viruses are often difficult or impossible to produce in high titers enough to deliver the vaccine in a timely manner.

Various types of vaccines with wide spectrum of efficacy and safety have been developed in the last century (see Fig. 1). The conventional vaccines, inactivated or live attenuated vaccines require the culture of infectious viruses in cell culture. For newly discovered emerging viruses, the conventional cell culture often fails to produce sufficient vaccine dose for testing of immunogenicity, safety, antigenic variability, and cross-protection across the pathogens. Therefore, other strategies that would circumvent the cell culture method must be considered. First, a viral antigen can be produced by recombinant or synthetic peptides/proteins (Wang et al., 2010; Pallister et al., 2011; Turley et al., 2011). This strategy is safe in production processes and involves no viral replication. The prospective antigens could be easily modified for enhanced protection. However, recombinant soluble antigens are themselves poorly immunogenic and would require potent adjuvants or boosts to enhance immunogenicity. Second strategy involves the use of nucleic acids (DNAs or recently RNAs) as immunogen, where inoculation of cDNAs encoding viral antigens leads to uptake and expression of the cDNA by antigen-presenting cells to trigger protective immune responses (Powell, 2004; Liu et al., 2006) (Fig. 1). DNA vaccines have many advantages. Primarily, production from bacterial system in high purity is possible without high-level biosafety requirements. In addition, they can lead to humoral and T cell responses. Introduction of replicons in plasmids could increase immunogenicity by allowing limited level of replication after cellular uptake. And yet, the difficulties in delivery and consequently relatively low level of immunogenicity remains to be improved, preferably by combining with adjuvants or heterologous prime-boost strategies. Third strategy is viral vectored vaccines (Gunther, 2011; Sabchareon et al., 2012). For several decades, recombinant viruses have been used as vectors for protein expression and vaccination. For this purpose, viruses are manipulated to enhance their safety and immunogenicity by eliminating virulence factors; to increase coding capacity by eliminating non-essential genes; and to change tropism by changing envelope proteins. For this purpose, various type of vectored vaccine platforms are now available. A major concern is the pre-existing immunity against the viral vector that often compromises the vaccine efficacy. Fourth, recombinant bacteria can serve as vectors for the in vivo delivery of antigens or DNA, as well as production host for subunit vaccines (Pei et al., 2005). They elicit both humoral and T cell responses, and inherent replicon activity increase immunogenicity. For this purpose, several non-toxic or even probiotic bacteria are available.

**Fig. 1.** Contrasting features among various types of vaccines. Pros and cons in terms of safety, efficacy, productivity and stability are compared.
VLPs share common features of self-assembly into particulate form, which is necessary for immune stimulation. The lack of genetic materials is a common requirement for VLP-based vaccines. Alternatively, VLPs could also be used as delivery vehicles for nucleic acids or proteins.

Vaccine-like particles as a high-priority vaccine strategy against emerging viruses

VLPs, structurally resembling infectious virions, are non-infectious due to the lack of the viral genome required for replication (Kushnir et al., 2012; Chen and Lai, 2013; Zeltins, 2013). They still possess immune-stimulatory and self-adjuvanting properties of natural viruses, as they are comprised of capsid proteins that can initiate an immune response. VLPs have distinctive features of size and geometry, which are critical for their ability to potently activate B cells and elicit robust and long-lasting antibody responses. In turn, these two features of VLPs can be exploited as safe and effective vaccines (Fig. 2). First, VLPs are mostly in the range of 20 to 100 nm in diameter, a size that allows for free entry into the lymphatic vessels, passive drainage to the sub-capsular area within lymph nodes, and recognition and uptake by various antigen-presenting cells (Manolova et al., 2008). VLPs can traffic into the B cell follicles by the aid of specific interactions with complement components or natural IgM antibodies (Link et al., 2012). Second, VLPs also show a special geometry that enables them to highly activate B cells. Epitopes are presented on the multivalent and highly repetitive outer structures of VLPs and thus lead to cross-link B cell receptors. These interactions strongly stimulate B cells and induce potent and long-lasting antibody responses (Jennings and Bachmann, 2008; Bachmann and Jennings, 2010; Zabel et al., 2013). Furthermore, these responses can overcome B cell tolerance and allow robust antibody responses to be induced against self-antigens, providing technical platform for therapeutic vaccines against metabolic diseases (Bachmann et al., 1993; Bachmann and Zinkernagel, 1997; Chackerian et al., 2008). VLPs can stimulate both innate and adaptive immune responses, based on their particulate structure which can be easily recognized and absorbed by antigen-presenting cells (Keller et al., 2010). Since the development of the first approved recombinant vaccine against hepatitis B virus in 1986, VLPs have demonstrated success as a reliable vaccine platform (Table 1).

VLPs are diverse in structure and classified as capsid-based (non-enveloped) or enveloped (Fig. 2). Both capsid-based and enveloped VLPs can be single or multilayered and composed of single or multiple proteins. Among the simplest is a non-enveloped single-layered VLP, such as the human papillomavirus (HPV) VLP vaccines. These simple VLPs can be produced in both prokaryotic and eukaryotic expression systems. Sometimes, the capsid proteins of simple VLPs are recombinantly produced and assembled in a cell-free environment to form homogenous VLPs (Salunke et al., 1986; Chen et al., 2001; Bundy et al., 2008; Bundy and Swartz, 2011). Non-enveloped multiple-capsid protein VLPs are more complex and usually produced in higher eukaryotic hosts such as yeast (Rodriguez-Limas et al., 2011; Li et al., 2013), insect cells (Palomares et al., 2012; Fernandes et al., 2013) and plants (Scotti and Rybicki, 2013). These expression systems allow

| VLP-based vaccines | Development | Year | Approval | Method of production |
|--------------------|-------------|------|---------|---------------------|
| HBV (Hepatitis B virus) | 1982 | | 1986 | Yeast |
| HPV (Human papilloma virus) | 1991 | | 2006 | Yeast, Insect cells |
| HEV (Hepatitis E virus) | 1993 | | 2011 | E. coli |

Fifth, virus-like particles (VLPs), nanoparticles, or peptides in multimeric assembly provides an alternative vaccine platform (Akahata et al., 2010; Steel et al., 2010). Recombinant surface antigens constituting natural virions are assembled in a highly ordered conformation as an empty particle devoid of viral genetic materials. This feature can be explored as a way to increase the immunogenicity of viral antigens by delivering them in multimeric conformation, preferably as VLPs. Mimicking the morphology of natural infectious virions, but without virally derived genetic materials, the VLPs are highly immunogenic with proven protective immune response and safety profile. As the next wave of technical development as vaccine platform, we discuss VLPs in more details.
for co-expression of the different capsid proteins and complex assembly of the VLPs within a cell.

In enveloped VLPs, the assembly of matrix proteins provides a molecular scaffold, where viral antigens are embedded into lipid membrane. Different types of glycoproteins can be embedded in the lipid bilayer and then become the target immunological antigen for generating neutralizing antibodies. This enveloped VLPs are not structurally uniform and challenging to characterize. Influenza VLPs are a well-studied example of enveloped VLPs (Kang et al., 2012), in which matrix M1 proteins and glycoprotein hemagglutinin (and/or neuraminidase) are embedded in the lipid bilayer.

VLPs can function in various ways and thus have a wide range of applications (see Fig. 2). First, VLPs can function as immunogens: for vaccination against the cognate virus, VLPs can be generated from unmodified components as in the case for non-enveloped HPV vaccines Gardasil1 and Cervarix1 and enveloped influenza virus vaccine candidates (Galarza et al., 2005). Second, novel immunological antigens can be displayed on VLPs. Novel antigens are placed on the surface of VLPs either as fusion protein or chemical conjugation (Peacey et al., 2007; Jennings and Bachmann, 2008). Therefore, VLPs can be an effective platform that is exploited for generation of epitope-based VLPs to target various diseases (Plummer and Manchester, 2011). Heterologous antigen presentation on non-enveloped VLPs can be achieved through fusion of small epitopes or large antigens [e.g., hepatitis B virus core antigen (Whitacre et al., 2009)]. On enveloped VLPs, heterologous antigen presentation can be accomplished by insertion of glycoproteins [e.g., herpes simplex virus glycoprotein inserted into simian immunodeficiency virus VLPs (McGuigan et al., 1993)]; or by heterologous head domain fusion onto transmembrane region [e.g., vesicular stomatitis virus glycoprotein ectodomain fused to rabies virus glycoprotein (Kato et al., 2011)]. Third, VLPs are recently developed as delivery vectors for nucleic acids or proteins. Here, the properties of some viral capsid proteins allow engineered VLPs to encapsulate proteins, molecules, or nucleic acids. Examples of packaging nucleic acids include the non-enveloped Simian virus 40 (Kawano et al., 2013) and enveloped retrovirus VLPs (Keswani et al., 2013). Likewise, heterologous proteins can be encapsulated and delivered in both enveloped and non-enveloped VLPs. DNA-loaded VLPs are developed for gene therapy (Ramqvist et al., 2007; Seow and Wood, 2009) and therapeutic VLPs encapsulate proteins or drug molecules (Ashley et al., 2011; Kaczmarczyk et al., 2011).

**Design of VLP-based vaccine platforms**

Computational structure modeling and bioinformatics techniques have been increasingly applied on design of VLP-based vaccines (Ding et al., 2010; Pankavich and Ortoleva, 2012; Roldão et al., 2012). Currently, there are 540 completed icosahedral virus capsid structures available from VIPERdb database (http://viperdb.scripps.edu/), from which the designing principle of multi-molecular assembly could be deduced. The data have been generated from structural and computational analyses, as well as high-quality renderings for 3D information. Structural analyses, such as X-ray crystallography and cryo-electron microscopy (Carrillo-Tripp et al., 2009), provide a major tool for determining crystal structures of viral capsids (Grimes et al., 1998; Prasad et al., 1999; Wynne et al., 1999). Computational analyses can complement to these empirical structural analyses, and in combination provide a tool to rational design of monomers for expedited assembly into VLPs, followed by experimental verification (Kuroda et al., 2012; Zhang et al., 2013). Thus, design of VLP-based vaccines will increasingly benefit from protein engineering of monomers especially considering their ability for inter-molecular interactions. Enhanced computer modeling techniques are required for designing engineered VLP with novel epitopes transplanted on its surface.

To maximize the immunogenic potential of particle platform technologies, target antigens must be displayed at high density on the surface of VLPs. Different approaches have been tried to engineer and tailor VLPs as molecular scaffolds for antigen display. In order to maximize this critical feature, VLPs should be able to display foreign antigens of various sizes, structures, and compositions. One way is to generate recombinant fusions in which foreign antigens are inserted into specific sites within the viral structural protein so that the foreign antigen is displayed on the surface of the resulting VLP. However, peptide insertions with a view to introduce novel epitopes, often adversely affect the folding of monomers, which consequently fail to assemble into regular and compact structure. This often results in misfolding into amorphous aggregates, resulting in lowering of immunogenicity and/or vaccine efficacy. Such problems can be circumvented by initially preforming of VLPs as scaffolds to which foreign antigens or epitopes are subsequently conjugated. Post-production modifications of VLPs can take advantage of naturally occurring sites of conjugation, e.g., amino or sulfhydryl groups exposed on the VLP surface.

In HBeAg VLPs, like many other VLPs, foreign antigen insertion sites are identified by a combination of empirical approach and rational design based on structural categorizations. First, by a comprehensive combinatorial approach with multiple insertion sites, compensatory mutations, and robust screening methods, the Billaud group greatly enhanced the success rate for producing chimeric Woodchuck Hepatitis Virus core antigen VLPs (Billaud et al., 2005). Second, transforming the subunits of VLPs into a more thermodynamically stable conformation can dramatically enhance the success rate for engineered VLPs with novel peptide epitope insertions. Third approach is to circumvent potential disruption of folding and assembly by chemically cross-linking antigens to preformed VLPs. These approaches potentially increase the cost of production or decrease scalability along the downstream manufacturing processes, but allow VLPs to display various antigens, including peptides, whole proteins, carbohydrates, and other small molecules.

The VLPs interact with various components of the immune system to provoke protective responses: receptors for the entry into antigen-presentation cells and molecular components in the trafficking pathway. Thus, VLP-based vaccines usually provoke strong immune responses, and by modifying VLP structures the relative degree of immune responses can be further tuned for a balanced induction of humoral and cell-mediated immunity. Overall impact on elicited im-
Table 2. Comparison of different expression platforms for producing VLPs

|              | Speed | Cost effectiveness | Scalability | Yield | VLP complexity |
|--------------|-------|--------------------|-------------|-------|----------------|
| Bacteria     | +++   | +++                | +++         | +++   | +              |
| Yeast        | +++   | +++                | +++         | ++    | ++             |
| Baculovirus-insect cells | ++ | +                 | ++         | ++    | +++            |
| Mammalian cells | +   | +                 | ++         | +     | ++             |
| Plants       | ++   | ++                 | ++         | ++    | ++             |

+, low; ++, medium; ++++, high

Fig. 3. Novel VLP production platform from bacterial host. Target antigen is expressed as fusion to RNA-interaction domain (RID). Using RNAs as molecular chaperones, the monomers are expressed as soluble, properly folded form, which, upon cleavage, assembles into VLPs.

Expression platforms for VLPs

In developing VLP-based vaccines, serious considerations should be given to a robust and faithful production platforms that enables the delivery of vaccines in a timely and reproducible manner. As the significance of VLPs has been appreciated for developing next-generation vaccines, several expression platforms for producing VLPs have been developed, including bacteria (Escherichia coli), yeast, insect cells, mammalian cells, and plants (Table 2). The E. coli expression system is easy to use and the least expensive. A major drawback for bacterial host, however, is that E. coli cytoplasm does not provide optimal milieu for the folding of human infecting viral antigens, and thus rarely successful for VLP assembly. Moreover, this system lacks a post-translational modification capacity and thus its use is limited to produce simple VLPs, such as non-enveloped VLPs. So far, commercial success is made for hepatitis E virus (HEV) vaccines based on chemical refolding of inclusion bodies (Hecolin, Xiamen Innovax Biotech Co. Ltd.). To be widely applicable for VLP vaccines, a robust expression platform is yet to be developed ensuring soluble expression of viral antigens amenable to folding and self-assembly.

A novel protein folding vehicle was recently developed that could be implemented for VLP assemblies in E. coli (Fig. 3). It has been shown that RNA binding could affect the overall kinetic network of protein folding pathway in favor of enhanced folding over off-pathway aggregation (Choi et al., 2008, 2009, 2013). In addition, the RNA binding-mediated solubility is greatly enhanced for increasing soluble yield of passenger proteins and this feature could be usefully implemented for high-throughput protein expression. The RNA-interaction mediated folding vehicle provides novel expre-
ssion platform for VLP vaccine, e.g., HPV as well as noro-virus (unpublished results). Fascinating possibility remains if the RNA-based molecular chaperones for protein folding could be extended to the production multi-component VLPs.

Yeast systems are popularly used in producing VLP-based vaccines. For example, the hepatitis B virus (HBV) vaccine Engerix-B® (GlaxoSmithKline) and the human papillomavirus (HPV) vaccine Gardasil® (Merck & Co., Inc) (Kim and Kim, 2017) were produced from recombinant yeasts. Yeast systems elicit high expression and easy scale-up and, unlike the E. coli system, provide post-translational modification of the expressed proteins. In addition, yeast has low risk of contamination by adventitious agents during scale-up of production. Yeast has been mostly applied to produce VLPs with simple structures, but currently its application has been extended to VLPs for enveloped viruses. Furthermore, it has been recently found that yeast systems enable VLPs to be produced either intracellularly or using secretion pathways. To enhance the quality and quantity of yeast-based VLPs, various factors, such as the type of plasmid and promoter and, especially the secretability and processing on exit out of the yeast cells, need to be carefully taken into account (Bae et al., 1998; Lee et al., 1999; Han et al., 2005).

The baculovirus-insect cell and mammalian-cell systems have an advantage of eliciting more complete post-translational modification including glycosylation and expressing multiple component VLPs (Rodriguez-Limas et al., 2011). VLP-base vaccines produced using these systems include the HBV vaccine GenHevac B® (Pasteur-Mérieux Aventis) and the HPV vaccine Cervarix® (GlaxoSmithKline). These systems are more expensive than bacterial and systems, and downstream processes are usually more complex. The bacterial system, if optimized in the downstream recovery process (Fig. 3), would afford VLP vaccines (HPV vaccine, for instance) in developing countries.

Recently, plants become newly appreciated as bioreactors, as it has been realized that plants, as molecular pharming, can work as a factory for recombinant expression of proteins of interest (Ma et al., 2015; Marsian and Lomonossoff, 2016). Innovative methods for rapid production of useful proteins in plants rely on transient expression to bypass the requirement for tedious transformation and regeneration steps. This strategy can be applied to produce viral coat proteins to be assembled into VLPS. As a protein production system, plants are cost-effective and highly scalable in manufacturing requiring only water, carbon dioxide, inorganic nutrients, and sunlight for growth (Twyman et al., 2003; Lico et al., 2008; Paul et al., 2011; Marsian and Lomonossoff, 2016). Plant expression systems provide complex but distinctive protein assembly and post-translational modifications as in mammalian-cell systems, and show high-level immunogenicity. Glycosylation in yeast and insect cells is limited to very simple and inconsistent glycoforms, whereas plants can generate a wide variety of glycosylation types (Chen and Lai, 2013). Recent success on genetically engineered plants that support humanized glycosylation plant lines holds a great potential for their applications (Marsian and Lomonossoff, 2016). Furthermore, unlike mammalian cell cultures, they bring a low risk of contamination derived from endotoxins and mammalian pathogens.

**Downstream processes of VLP-based vaccines**

VLP production in recombinant host is invariably associated with numerous impurities from cell substrates and the manufacturing process. US the Food and Drug Administration (FDA) summarized these impurities into two subgroups, process-related and product-related contaminants (US FDA, 1999). Process-related contaminants are predominantly attributed to host cell impurities, such as cells, cell debris, host cell-derived proteins, DNAs, proteases, endotoxins, polysaccharides, and lipids. Impurities intrinsic to the production process are media components, anti-foam reagents, and other potential hazards supplemented during upstream or downstream processing, such as stabilizers, excipients, proteases, and nucleases. A list of inactive ingredients allowed in final drug formulations is provided by FDA (http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm). The World Health Organization (WHO) states that quality monitoring of final VLP-based vaccine bulks should include, among others (WHO, 2006): testing for sterility (bacteria, fungi); testing for virus clearance; summary of vaccine composition (protein, lipid, polysaccharides); control of residual DNA, protein and endotoxin content; determination of protein purity (by SDS-PAGE or similar); and description of residual chemicals. Several factors - expression host, the cellular environment, the type of target viral proteins, and the virus subtype - exert influence on the quality of in vivo-assembled VLPs in recombinant systems (Mach et al., 2006). It is practically very difficult to sufficiently control all of these factors during VLP assembly in vivo. As an alternative, therefore, disassembly and reassembly in vitro were developed and optimized for several VLPS (McCarthy et al., 1998; Ren et al., 2006; Xing et al., 2010; Link et al., 2012; Roldão et al., 2012). The disassembly and reassembly in vitro allows easy control over encapsulated host cell impurities and lead to increase the homogeneity, stability, and reactivity of VLPS (Zhang et al., 2012a, 2012b). Molecular dynamic simulations enable a better understanding of the assembly mechanism and prediction of the quality of newly designed VLP constructs (Zhang et al., 2014). Other product-related contaminants include VLP aggregates formed during both upstream and downstream processing. Modeling the competition between aggregation and self-assembly into ordered structure has been developed (Shi et al., 2005; Ding et al., 2010; Sanchez-Rodriguez et al., 2012), which, if properly implemented, may help enhance the quality and the purification yields.

The final bulk products need to meet the desired purity criteria for recombinant VLPS (Effio and Hubbuch, 2015). The downstream purification process ranges from the initial product isolation out of cells to concentration (clarification and capturing), intermediate purification, and polishing of the molecule of interest (Effio and Hubbuch, 2016). The methods of choice for these processes include chromatography, filtration, and integrating purification techniques. Process-related contaminants need be separated particularly at the beginning of the process, whereas product-related contaminants and residual process-related impurities are removed preferably in the final polishing step.

In the past, process development and optimization in downstream processes mostly depended on empirical approaches.
An exemplary case: norovirus VLP-based vaccines

Noroviruses are the most common cause of nonbacterial acute gastroenteritis in humans worldwide (Patel et al., 2009; Hall et al., 2011). These highly infectious viruses have recently been referred to as 'The Perfect Human Pathogen' since the virus is highly stable in the environment, infectious at low doses, and moderately virulent yet rapidly shed in large quantities by the host following natural infection. In addition, the virus is antigenically diverse and evolving, and induces limited immunity after natural infection (Hall, 2012). The lack of proper cell culture methods and animal infection models pose challenges to the development of the vaccine. As an alternative to cell culture method, the VLPs has enabled significant progress toward effective vaccine candidates designed to protect against multiple circulating norovirus strains. Vaccination with norovirus VLP vaccines has been shown to both induce antibodies that block virus-derived VLP carbohydrate binding and protect against homologous viral challenge in a clinical study. The first finding that recombinant norovirus VP1 capsid sequences can be expressed and self-assembled into VLPs that are morphologically similar to the infective virion (Jiang et al., 1992) led to extensive use of VLPs for understanding norovirus structure, immunogenicity, and receptor-binding activity.

In case of noroviruses, the inability to propagate viruses in cell culture precludes the development of live-attenuated or inactivated vaccine candidates. Thus, several expression systems or middle-income countries.

Other cases: HBV and papillomavirus VLP-based vaccines

After the successful launch in 1986 of the first recombinant vaccine produced from yeast, the production platform for the VLP-based hepatitis B virus vaccines has been extended to plants (Mechtcheriakova et al., 2006). It is demonstrated that a monomeric form of the core antigen HBCAg can be expressed and spontaneously form core-like particles in different heterologous expression systems including bacteria and plants. HBC particles with a spiky surface and repetitive presentation of antigenic determinants show a strong inherent immunogenicity and can be used for the display of foreign peptides (Peyret et al., 2015). Using HBCAg dimers produced by the tandem fusion of two HBCAg open reading frames, a carrier system for antigens is developed. These ‘tandem core’ system can still self-assemble into core-like particles and can be used to display correctly folded GFP or nanoparticles on their surface. These, in turn, can be used to bind their cognate antigens. Therefore, it is possible to display whole proteins on the particle surface.

Papillomaviruses are non-enveloped tumor-inducing DNA viruses, with over 100 serotypes mostly infecting humans, accounting for over 5% of all human cancers (Münger et al., 2004). Two types of HPV VLP vaccines, based on the self-assembly of L1 (the major capsid protein) expressed in yeast or insect cells, are currently on the market (Rybicki, 2014). As an alternative to yeast- or insect cell-based production, a low-cost plant-based vaccines are being developed, where HPV-8 L1 is successfully expressed as VLPs (Matić et al., 2012).

Challenges and perspectives

Over the last few decades, significant advances are made in
the design and manufacture of VLP vaccines, as vital alternative to traditional cell-culture vaccines. Capitalizing on strong immune stimulation intrinsic to particulate form of immunogen, continued research has focused on engineering the VLP assembly as to enhance the particle stability, and the diversity of antigens to be displayed on the surface of VLPs. Although only a few VLP-based vaccine have reached the market and no VLP-based vaccine against emerging viruses is currently available yet, there are great expectations for several VLP vaccines based on the positive results in clinical trials. Technical challenges still remains, however. As compared with VLPs made of single component, e.g., norovirus or HPV, the assemblies of viral capsids requiring multicomponents, e.g., FMDV, HFMV, is more complex. Further understanding on the folding of individual monomers and the complexities involved in multimeric macromolecular assemblies, as well as the enabling protein folding technologies, are required. On the downstream process especially for enveloped VLPs, the purification of VLPs from baculo-viral particles of similar size and biophysical characters still poses daunting challenge. To meet the regulatory requirements on the safety issue, proper inactivation of contaminating baculo-virions without sacrificing the immunogenicity of VLPs should also be combined. Despite such huddles, the recombinant VLP vaccine platform, as alternative to cell-culture methods, would emerge in the foreseeable future as a major manufacturing platform for vaccines combating emerging and re-emerging infectious diseases.

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