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# Subviral particle as vaccine and vaccine platform

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Recombinant subvirual particles retain similar antigenic features of their authentic viral capsids and thus have been applied as nonreplicating subunit vaccines against viral infection and illness. Additionally, the self-assembled, polyvalent subviral particles are excellent platforms to display foreign antigens for immune enhancement for vaccine development. These subviral particle-based vaccines are noninfectious and thus safer than the conventional live attenuated and inactivated vaccines. While several VLP vaccines are available in the markets, numerous others, including dual vaccines against more than one pathogen, are under clinical or preclinical development. This article provides an update of these efforts.

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## Introduction

Most viruses share common spherical or rod-shaped capsids built by multiple subunits of capsid proteins that encapsulate the viral genome. Through bioengineering technology viral capsid proteins can be produced *in vitro*, resulting in self-assembled, empty virus-like particles (VLPs) (reviewed in [1,2••]). In addition, smaller particles with less subunits can be produced for some viruses by expression of portions of the major viral capsid proteins [3–7]. These artificial subviral particles retain the structures and antigenic properties of their native viruses, including the virus-specific molecular patterns and high density of B-cell and T-cell epitopes to induce potent innate, humoral, and cellular immune responses, respectively, in animals and humans [1,2••]. Thus, these subviral particles are excellent source of materials for vaccine development against many viruses and their associated diseases. VLPs are usually made by an eukaryotic expression system, including the baculovirus/insect cells, yeast, and mammalian cells, while the smaller subviral particles and hepatitis B virus (HBV) VLPs can be produced through the *E. coli* expression system (Table 1), which is more cost-effective. Sevral subviral particle-based vaccines are currently available in the market, while many others are under clinical or preclinical development.

The self-assembled, polyvalent subviral particles are also excellent platforms for antigen presentation to enhance immunogenicity. Through genetic engineering or chemical conjugation heterologous antigens or peptide epitopes can be inserted or conjugated onto the surface of the subviral particles. The polyvalent presentation of the foreign antigens or epitopes on the subviral particles leads to enhanced immunogenicity, providing an effective approach for novel vaccine development. On the other hand, the immunogenicity of the subviral particle is generally maintained without disrupption by the foreign insertion, and thus the chimeric particles can be used as dual or even multivalant vaccines against two or more pathogens. A number of such chimeric particles have been under preclinical development, pointing to a new direction of highly efficient, low cost vaccines against major infectious diseases.

## Subviral particles as vaccines

Over 30 different subviral particles (Table 1), representing at least 21 viral families, have been generated so far through recombinant baculovirus, yeast, mammalian cells and *E. coli* expression systems. Most of them are VLPs comprising one or more full-length viral structural proteins, while others are smaller subviral particles formed by truncated capsid proteins [3–7]. The most complex subviral particles are VLPs of the rotavirus, influenza virus and coronavirus that contain up to four structure proteins. The smaller subviral particles include the E2 particles (~23 nm) of the hepatitis E virus (HEV) that are composed of the truncated protruding (P) P1 and P2 domains (~30 kDa) of HEV VP1 [2••,3,8] and the P particles (~20 nm) of norovirus (NoV) that are formed by 24 copies of the P domain (~34 kDa) of the NoV capsid protein VP1 [4,6,9•].

Most subviral particles can be easily produced in the laboratory (Table 1) and several of them have reached the markets as effective vaccines after successfully scaled-up production through Good Manufacturing Practices (GMP). These subviral particles are excellent immunogens inducing strong humoral and cellular immune responses as shown by numerous studies (Table 1). Immunization of subviral particle vaccines in different animal species and humans, through various routes, such as intranasal, intramuscular, and intraperitoneal administrations, stimulated high antibody as well as high $CD4^+$ proliferative and cytotoxic T lymphocyte (CTL) responses (Table 1).

**Table 1**

**Some known subviral particles that have been studied as vaccines or immunogens**

| Virus family | Virus species | Subviral particle | Production system | Immune responses in lab animals (mice) | Neutralization/protection against virus and diseases (mice) | Clinical trial/ commercial use | Reference |
|---|---|---|---|---|---|---|---|
| Arteriviridae | PRRSV | VLP | Baculovirus | Ab, T cell | Neutralization | | [46] |
| Birnaviridae | IBDV | VLP | Baculovirus | Ab (chicken), | Neutralization, protection (chicken) | | [47,48] |
| Bunyaviridae | RVFV | VLP | Baculovirus | Ab, T cell (rat) | Neutralization/protection (rat) | | [49] |
| Caliciviridae | NoV | VLP | Baculovirus | Ab, T cell | Block NoV-receptor interaction, protection (human) | Phase I and II | [19••,50] |
| | RHDV | VLP | Baculovirus | Ab (rabbit) | Protection (rabbit) | | [51,52] |
| | NoV | P particle | *E. coli* | Ab, T cell | Block NoV-receptor interaction | | [9•,38•] |
| | NoV | Polyvalent complex | *E. coli* | Ab, T cell | Block NoV-receptor interaction | | [53] |
| Circoviridae | PCV | VLP | *E. coli* | Ab (pig) | Protection (pig) | Commercial use | [17,18] |
| Coronaviridae | SARS-CoV | VLP | Baculovirus | T cell | | | [54,55] |
| | IBV | VLP | Baculovirus | Ab, T cell (chicken) | Neutralization (chicken) | | [56] |
| Filoviridae | EBOV | VLP | Mammalian cells | Ab (guinea pig) | Protection (guinea pig) | | [57,58] |
| Flaviviridae | HCV | VLP | Baculovirus | Ab, T cell (primate) | Protection | | [59–62] |
| Hepadnaviridae | HBV | VLP | Yeast | Ab (monkey, chimpanzee) | Protection (chimpanzee, human) | Commercial use | [14,15] |
| | HBV | VLP | *E. coli* | Ab, T cell (human) | | Phase I | [26,63] |
| | HBV | VLP | *E. coli* | Ab | Protection against *B. burgdorferi* | | [64,65] |
| Hepeviridae | HEV | VLP | Baculovirus | Ab | Protection (monkey, human) | Phase I and II | [20,21,66,67] |
| | HEV | E2 particle | *E. coli* | Ab (monkey) | Protection (monkey, human) | Commercial use | [3,68] |
| Herpesviridae | EVB | VLP | HEK293 cell line | Ab, T cell | | | [69] |
| Nodaviridae | BV | VLP | Baculovirus | | Protection (European Sea Bass) | | [70,71] |
| | FHV | VLP | Baculovirus | Ab (rat) | | | [72] |
| Orthomyxoviridae | Flu virus | VLP | Baculovirus | Ab, T cell (ferret) | Protection (ferret) | | [73–75] |
| Paramyxoviridae | NDV | VLP | Baculovirus | Ab (chicken) | Protection (chicken) | | [76] |
| | RSV | VLP | Baculovirus | Ab | Neutralization/ protection | | [77] |
| Parvoviridae | PPV | VLP | Baculovirus | Ab (guinea pig, pig) | Protection (pig) | | [78] |
| | CPV | VLP | Baculovirus, *E. coli* | Ab (dog), T cell | Protection (dog) | | [79–81] |
| | GPV | VLP | Baculovirus | Ab (goose) | Neutralization | | [82] |
| | PV B19 | VLP | Baculovirus, yeast | Ab (human) | Neutralization (human) | Phase I | [83,84] |
| Papillomaviridae | HPV | VLP | Baculovirus, yeast | Ab (rabbit) | Neutralization, protection (human) | Commercial use | [10–13] |
| | HPV | Capsomere | *E. coli* | Ab (dog) | Protection (dog) | | [7,11,85,86] |

**Table 1** (*Continued*)

| Virus family | Virus species | Subviral particle | Production system | Immune responses in lab animals (mice) | Neutralization/protection against virus and diseases (mice) | Clinical trial/ commercial use | Reference |
|---|---|---|---|---|---|---|---|
| Picornaviridae | EMCV | VLP | Baculovirus | Ab (pig) | Neutralization | | [87] |
| | CVB3 | VLP | Baculovirus | Ab | Protection | | [88] |
| | CVA16 | VLP | Baculovirus | Ab | Protection | | [89] |
| | EV71 | VLP | Baculovirus, yeast | Ab, T-cell (monkey) | Neutralization (monkey), protection | | [90–92] |
| | FMDV | VLP | Baculovirus, *E. coli* | Ab, T cell (dog, cattle) | Protection (guinea pig, dog, cattle) | | [93,94] |
| | PyV | VLP | Yeast | Ab, T cell | | | [95–97] |
| | PyV | VLP | Yeast | T cell | Protection | | [98] |
| | PyV | VLP | *E. coli* | Ab | Protection | | [99] |
| | PyV | VLP | Baculovirus | Ab, T cell | Against tumor growth, protection | | [100,101] |
| | PyV | Pentamer/ capsoid | *E. coli* | Ab (piglet) | | | [102] |
| Polyomaviridae | SV40 | VLP | Baculovirus | Ab, T cell | | | [103] |
| Reoviridae | RV | VLP | Baculovirus, *E. coli* | Ab, T cell | Protection (mouse, pig) | | [104–106] |
| | BTV | VLP | Baculovirus | Ab | Neutralization, protection (sheep) | | [107–109] |
| Retroviridae | HIV | VLP | Baculovirus | Ab, CTL | Neutralization | | [110] |
| Togaviridae | CHIKV | VLP | Baculovirus | Ab (monkey) | Protection (monkey) | | [111] |

Ab, antibody; *B. burgdorferi*, *Borrelia burgdorferi*; BV, betanodavirus; BTV, bluetongue virus; CHIKV, chikungunya virus; CoV, coronavirus; CPV, conine parvovirus; CTL, cytotoxic-T-lymphocyte; CVA16, coxsackievirus A-16; CVB3, coxsackievirus B3; *E. coli*, *Escherichia coli*; EBOV, ebolavirus; EMCV, encephalomyocarditis virus; EV71, Enterovirus 71; EBV, epstein–barr virus; FHV, flock house virus; Flu virus, influenza virus; FMDV, foot-and-mouth disease virus; GPV, Goose parvovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; IBDV, Infectious bursal disease virus; IBV, infectious bronchitis virus; NDV, Newcastle disease virus; NoV, norovirus; PCV, porcine circovirus; PPV, porcine parvovirus; PRRSV, porcine reproductive and respiratory syndrome virus; PV, parvovirus; PyV, polyomavirus; RV, rotavirus; RHDV, Rabbit haemorrhagic disease virus; RSV, respiratory syncytial virus; RVFV, Rift Valley fever virus; SARS, severe acute respiratory syndrome; SV40, simian vacuolating virus 40 or simian virus 40; VLP, virus-like particle.

These features support the subviral partciels to be highly efficient vaccines against many infectous diseases.

To date five subviral particle-based vaccines are commercially available for human use. The two VLP vaccines against human papillomavirus (HPV) are made by L1, the major capsid protein of HPV16 [10], through recombinant yeasts (Gardasil®, Merck & Co., NJ, USA) or baculoviruses in insect cells (Cervarix®, GlaxoSmithKline, London, UK) [10–13]. Both vaccines have been proven for the prevention of cervical and anogenital infection and diseases associated with HPVs. The other two commercial VLP vaccines against hepatitis B viruses (HBVs), Recombivax HB® (Merck & Co., NJ, USA) and Engerix-B® (GlaxoSmithKline, London, UK), are made by the small surface antigen of HBV (HBsAg) through recombinant yeasts (*Saccharomyces cerevisiae*) [14,15]. These vaccines have been proven effective worldwide against HBV infection. Most recently, a further subviral particle vaccine against HEVs, the HEV 239/Hecolin® (Xiamen Innovax Biotech, Xiamen, China) that is made through the *E. coli* system, has been proven by the Chinese health authorities for human use in China [16]. In addition, there are two other subviral particle vaccines, the Ingelvac CircoFLEX® (Boehringer Ingelheim, Germany) and Porcilis PCV® (Intervet International, The Netherlands), that are commcercially available for use in domestic pigs against porcine circovirus infection and diseases [17,18]. Furthermore, the NoV VLP vaccine has shown significant protection against NoV diarrhea in phase II clinical trials [19••,20,21], while many other subviral particle vaccines are under intensive preclinical development (Table 1).

## Subviral particles as vaccine platforms

In addition to being vaccines, the subviral particles can also be used as vaccine platforms to present foreign antigens and small peptide epitopes of heterologous pathogens for novel vaccine development. The highly stable structures of most subviral particles tolerate an exogenous insertion, which can be achieved through either recombinant DNA technology or chemical conjugation. The native antigenic properties of the inserted antigens or epitopes usually are preserved on the surface of the chimeric particles, while the immunogenicity of the antigen/epitope is significantly enhanced by the polyvalent nature of the subviral particles functioning as an adjuvant. In addition, the major antigenic determinants of the subviral particle carriers are generally preserved, and thus the resulting chimeric particles can be used as a dual vaccine against the pathogens of the insertion and the carrier.

**Table 2**

**Some subviral particle platforms for display of heterologous antigens and epitopes for vaccine development**

| Virus species | Subviral particle | Displayed epitope or antigen | Production system | Immune response in animal (mouse) | Neutralization/ protection against pathogens and diseases (mouse) | Clinical trial | Reference |
|---|---|---|---|---|---|---|---|
| Vaccine candidates that are in clinical trials | | | | | | | |
| HBV | VLP | CSP antigen of *P. falciparum* | Yeast | Ab, T cell (human) | Protection against malaria (human) | Phase I, II, and III | [23•,24••, 112,113] |
| | VLP | CSP epitopes of *P. falciparum* | *E. coli* | Ab (monkey, human) | Protection against malaria (monkey) | Phase I | [25,26] |
| | VLP | M2e epitope (influenza virus) | *E. coli* | Ab | Protection | Phase I | [27,29,33] |
| Bacteriophage | VLP | Nicotine | *E. coli* | Ab (human) | Increase smoking cessation (human) | Phase I, II | [34,35] |
| Qβ | VLP | Angiotensin II epitopes | *E. coli* | Ab (rat, human) | Reduces blood pressure (rat) | Phase I | [36] |
| | VLP | allergen Der p 1 epitope | *E. coli* | Ab (human) | | Phase I | [114] |
| Some vaccine candidates that are in preclinical development | | | | | | | |
| CPMV | Virion | VP2 epitope of MEV | Cowpea leaf | Ab | Protection (minks) | | [115] |
| | Virion | Protein F epitope of *P. aeruginosa* | Cowpea leaf | Ab | protection | | [116] |
| | Virion | FnBP epitope of *T. aureus* | Cowpea leaf | Ab | protection against endocarditis (rat) | | [117] |
| FHV | VLP | Toxin of *Bacillus anthracis* | Baculovirus | Ab | Neutralization, protection (rat) | | [72] |
| Influenza virus | VLP | IBV S1 protein | Baculovirus | Ab, T cell (chicken) | Neutralization, protection (chicken) | | [118] |
| | VLP | HA/NA Epitope of NDV | Baculovirus | Ab (chicken) | Protection (chicken) | | [119] |
| | VLP | F or G antigen of RSV | Baculovirus | Ab | Neutralization/ protection | | [77] |
| HAV | VLP | Angiotensin II epitopes | Baculovirus | Ab (rat) | Reduced blood pressure (rat) | | [120] |
| HBV | VLP | SP55/SP70 epitopes of EV71 | *E. coli* | Ab | Neutralization/ protection | | [121] |
| | VLP | epitopes of HCV | *E. coli* | Ab, T cell, CTL | | | [122] |
| | VLP | HVR1 epitope of E2 of HCV | *E. coli* | Ab | Neutralization | | [123] |
| | VLP | EDIII antigen of DENV-2 | Yeast | Ab | Neutralization | | [124,125] |
| | VLP | E1 epitope of rubella virus | *E. coli* | Ab | | | [126] |
| | VLP | CSP epitopes of *P. falciparum* | *E. coli* | Ab, T cell (human) | | Phase I | [26,63] |
| | VLP | OspA antigen of *B. burgdorferi* | *E. coli* | Ab | Protection | | [64,65] |
| | VLP | VP2 five-mimotope of IBDV | *E. coli* | Ab (chicken) | Protection (chicken) | | [127] |
| | VLP | CFP-10 antigen of MTB | *E. coli* | Ab, T cell | | | [128] |
| HIV | VLP | Domain III of DENV1 or WNV | Baculovirus | Ab | Neutralization | | [129] |
| | VLP | F/G surface antigens of HMPV | Baculovirus | Ab | Neutralization/ protection | | [130] |
| NoV | P particle | VP8* antigen of RV | *E. coli* | Ab | Neutralization/ protection | | [9•] |
| | P particle | M2e epitope of influenza virus | *E. coli* | Ab | Protection | | [41] |
| | Polyvalent complex | VP8* antigen of RV | *E. coli* | Ab, T cell | Neutralization/ protection | | [53] |
| | Polyvalent complex | M2e epitope of influenza virus | *E. coli* | Ab | protection | | [53] |
| | Polyvalent complex | P domain antigen of HEV | *E. coli* | Ab | Neutralization | | [131] |
| PyV | VLP | Pre-S1 epitope of HBV | Yeast | Ab | | | [95] |
| | VLP | N-termini of NP of PUUV | Yeast | Ab | | | [96] |
| | VLP | CTL epitope of mucin 1 | Yeast | Ab, T cell | | | [97] |
| | VLP | GP33 CTL epitope of LCMV | Yeast | T cell | Protection | | [98] |
| | VLP | J8i antigen of GAS | *E. coli* | Ab | Protection | | [99] |
| | VLP | Her2 antigens of tumors | Baculovirus | T cell | Protection against tumor growth | | [100] |
| | VLP | PSA antigens of D2F2 tumors | Baculovirus | Ab, T cell | Protection against tumor growth | | [101] |
| | VLP | H190 epitope of influenza virus | *E. coli* | Ab | | | [132] |
| | Pentamer capsoid | B cell epitopes | *E. coli* | Ab (pig) | | | [102] |

**Table 2** (*Continued*)

| Virus species | Subviral particle | Displayed epitope or antigen | Production system | Immune response in animal (mouse) | Neutralization/ protection against pathogens and diseases (mouse) | Clinical trial | Reference |
|---|---|---|---|---|---|---|---|
| RHDV | VLP | 3A protein epitope of FMDV | Baculovirus | Ab, T cell (pig) | | | [51] |
| SV40 | VLP | HLA-CTL epitope of flu virus | Baculovirus | Ab, T cell | Protection | | [133] |

Ab, antibody; *B. burgdorferi*, *Borrelia burgdorferi*; CFP-10, antigen of culture filtrate protein 10; CPMV, cowpea mosaic virus; CSP, circumsporozoite protein; CTL, cytotoxic-T-lymphocyte; DENV-1/2, dengue virus type-1/2; EDIII, envelope domain III; EV71, Enterovirus 71; FHV, Flock House virus; Flu virus, influenza virus; FMDV, foot-and-mouth disease virus; FnBP, fibronectin-binding protein; GAS, Group A streptococcus; GP, glycoprotein; HA/NA, hemagglutinin/neuraminidase; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HMPV, human metapneumovirus; HVR1, hypervariable region 1; IBDV, infectious bursal disease virus; IBV, infectious bronchitis virus; INF, interferon; LCMV, lymphocytic choriomeningitis virus; M2e, ectodomain of influenza A virus M2 protein; MEV, Mink enteritis virus; MTB, mycobacterium tuberculosis; NDV, Newcastle disease virus; NP, nucleocapsid protein; NoV, norovirus; *P. falciparum*, *Plasmodium falciparum*; *P. aeruginosa*, *Pseudomonas aeruginosa*; PyV, polyomavirus; PUUV, Puumala hantavirus; RV, rotavirus; RHDV, rabbit haemorrhagic disease virus; RSV, respiratory syncytial virus; SV40, simian vacuolating virus 40 or simian virus 40; *T. aureus*, *Taphylococcus aureus*; VLP, virus-like particle; WNV, West Nile virus.

Numerous chimeric particles with antigen or epitope insertions on the surface have been produced (Table 2), in which the foreign antigen is usually inserted into a surface loop of the subviral particles. The capacity of a foreign insertion is subviral particle-dependent, with a maximal insertion of 238 residues (green fluorescence protein, GFP) for the HBV VLP [22] and 159 residues (VP8* antigen of rotavirus) for the P particle of NoV [9•] being reported. A selection of proper sites of a subviral particle for insertion of exogenous antigens and/or epitopes is important for the generation of stable chimeric particles, the distal end of a flexible surface loops is generally a good choice.

The HBV VLP has been extensively studied as a vaccine platform for presentation of heterologous antigens and epitopes, with a chimeric VLP vaccines reaching to phase III and other two to phase I human trials. One is the RTS,S/AS01 malaria vaccine (GlaxoSmithKline) that comprises of the C-terminal half (189 residues) of the circumsporozoite protein (CSP) of *Plasmodium falciparum* on the surface of the HBV VLP (HBsAg) with adjuvant AS01 [23•]. This chimeric vaccine is currently under phase III evaluations with high protective efficacy [24••] and thus will most likely be the first malaria vaccine ever licensed and the first vaccine with a VLP-displayed antigen. Another VLP-based malaria vaccine is ICC-1132 (Malarivax) that is composed of a HBV VLP (HBcAg) displaying multiple epitopes of the *P. falciparum* CSP [25,26]. After testing in rodents and nonhuman primates [25], this vaccine candidate was assessed for safety and immunogenicity by a phase I human trial, which showed malaria- and HBV-specific immune responses [26], supportingg ICC-1132 as a potential dual vaccine. The other HBV VLP-based dual vaccine is the M2e-HBcAg chimera, in which the conserved M2e epitope of influenza A virus M2 protein is linked to the HBV VLP through either recombinant DNA technology [27] or chemical conjugation [28]. After a number of animal experiments showing specific immune reponses against the M2e epitope and HBV, as well as protective immunity against influenza virus infection [28–31], the the first phase I trial was performed in 2008, demonstrating its safety and immunogenicity in humans [32,33]. These data prove the concept that subviral particle can be a practical strategy of novel vaccine development.

Another well studied subviral partcile platform is the bacteriaphage Qβ VLPs that have been used to develop vaccines to control smoking addiction, hypertention and allergy. Nicotine was cross-linked to Qβ VLPs, forming nicotine-Qβ chimeric particle vaccine. Both phase I and II human trials of smokers showed high nicotine-specific immune responses in vaccinated subjects and revealed significantly increased abstinence rates of smoking [34,35]. The Qβ VLP was also used to display the epitopes of angiotensin II (Ang-Qβ) and the chimeric vaccine induced high level of angiotensin II-specific IgG and reduced systolic blood pressure in vaccinated rats [36]. A phase I human trial confirmed the high immunogenicity and safety of the chimeric vaccine [36]. In a separate study, an epitope of allergen Der p1 was covalently coupled to the Qβ VLPs (Der-P1-Qβ). This vaccine induced high immune response and has been shown to be safe in humans [37].

There are many other chimeric subviral particle-based vaccines that are in the preclinical evaluation, including those derived from VLPs of polyomaviruses, cowpea mosaic viruses, flock house virus, and NoVs (Table 2). The P particle of NoV that is formed by 24 copies or 12 dimers of the protruding (P) domain of NoV capsid protein (VP1) is highly stable and immunogenic [38•,39]. Three surface loops are identified on each of the P monomer that tolerate a heterologous insertion of at least 159 residues [9•,40]. Two chimeric P particles, each with the rotavirus surface spike protein VP8* [9•] and the conserved M2e epitope of influenza A viruses [41], have been successfully constructed. Both chimeric vaccines revealed strong humoral and cellular immune responses,

neutralization and protective efficacies against these viruses in mouse models [9•,38•,41], supporting the two chimeric particles as dual vaccines against rotavirus and NoV, and influenza virus and NoV, respectively.

## Challenges and future directions

The non-replicating subunit vaccine is an important option against many viral pathogens, particularly those that an *in vitro* cultivation system remains lacking such as human NoV, and that are too dangerous to culture, such as variola virus and Ebola virus. It is also a choice for future vaccines to avoid the safety concerns of conventional live attenuated or inactivated vaccines, such as a safe vaccine for eradication of poliovirus. The recent reports on the increased risk of intussusception of the two live attenuated rotavirus vaccines to vaccinated children [42•,43,44] is a new example of such concerns that could be prevented by a non-replicating subunit vaccine. However, based on current technology, it seems not possible to produce subviral particles of all known viral pathogens. Thus, the technology of subviral particle-based antigen presentation provides an important strategy for vaccine development against those viral pathogens. As shown in the two tables, many subviral particles are capable antigen carriers. Since the major antigenic determinants of many viral pathogens are known (Table 2), it would be straightforward for design and producing a new vaccine by taking advantage of this technology.

The past experience suggests that success of a chimeric vaccine may rely on certain levels of structural and/or chemical compatibility between the carriers and the inserted antigens. There is no simple solution to this technical challenge. If such a problem occurs, attempts of other carrier-antigen combinations are encouraged. In addition, a modification of the carrier vectors by including short flexible peptide adaptors to the two arms of the surface loops is an option. Furthermore, the maximal size of an inserted antigen may vary among different carriers, and therefore, selection of proper carriers for larger antigens is also recommended. Finally, selection of appropriate carrier–antigen combinations should be considered based on the target pathogens and host populations. For example, both NoVs and rotaviruses cause acute gastroenteritis in children, the selection of NoV P particle as carrier to present the rotavirus surface antigens is an ideal combination for a highly effective dual vaccine against the two most important causes of acute gastroenteritis in children.

Subviral particle-based vaccines may not be as immunogenic as those replicating viruses following a natural infection. Thus, development of strategies for a maximal efficacy of the subviral vaccines is important, for which optimization of the vaccine formulations and vaccination regimes may be the key, including increase of vaccine doses and dosages, identification of the best administration routes, and use of appropriate adjuvants. In the case that the antigen-presentation approach is used, rational designs of the vaccines by increasing the copy numbers of the inserted antigens/epitopes on each subviral particle carrier should be considered. In addition, insertion of a universal immunue stimulate elements, such as the T cell epitope, may be considered.

Currently, production of most subviral particles relies on a eukaryotic expression system, such as baculovirus/insect cells, yeasts or mammalian cells. Since bacteria can produce subviral particles at lower cost, attempt to improve the prokaryotic expression system for production of more subviral particles would help to reduce the cost of vaccine delivery in the developing countries. It is worth to point out that several smaller or simpler subviral particles, including the VLP of HBV (HBcAg) [22], the P particles of NoV [4,6], the E2 particles of HEV [2,3,8], and the small VLP [7] and the L1 capsomeres [45] of HPV, can be readily produced in *E. coli* with excellent quality and yields. Since all these viral pathogens are prevalent in the developing countries, further study to develop their subviral particles into cost-effective vaccines and vaccine platform for broad application in the developing world is highly significant. Finally, new concept of vaccine delivery, such edible vaccines produced by transgenic vegetbles containing related subviral particles should be explored.

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This review article summarizes the long journey of the three commercial subviral particle vaccines from bench to patients. The physical properties and structural features of the three VLP vaccines are analyzed. The authors also summarize how the crucial quality attributes of VLP-based human vaccines against diseases were assessed, controlled, and improved during bioprocessing through an array of structural and functional analyses.

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