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COMMENTARY

Recent advancements in combination subunit vaccine development

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ABSTRACT

Viral structural proteins share a common nature of homotypic interactions that drive viral capsid formation. This natural process has been mimicked *in vitro* through recombinant technology to generate various virus-like particles (VLPs) and small subviral particles that exhibit similar structural and antigenic properties of their authentic viruses. Therefore, such self-assembled, polyvalent, and highly immunogenic VLPs and small subviral particles are excellent subunit vaccines against individual viruses, such as the VLP vaccines against the hepatitis B virus, human papilloma virus, and hepatitis E virus, which have already been in the markets. In addition, various antigens and epitopes can be fused with VLPs, small subviral particles, or protein polymers, forming chimeric mono-, bi-, or trivalent vaccines. Owing to their easy-production, non-infectiousness, and polyvalence, the recombinant, chimeric vaccines offer a new approach for development of safe, low-cost, and high efficient subunit vaccines against a single or more pathogens or diseases. While the first VLP-based combination vaccine against malaria has been approved for human use, many others are under development with promising future, which are summarized in this commentary.

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Introduction

Unlike the traditional live-attenuated or inactivated/killed virus vaccines that needs a cultivation of infectious virions, the recombinant protein-based, non-replicating subunit vaccines do not involve in an infectious agent in their production processes and therefore, are considered to be safer than the traditional vaccines. In fact, many viruses, such as human noroviruses (huNoVs), cannot be cultivated efficiently to date,[1] making vaccine development against huNoVs difficult and therefore, subunit vaccines the only choice. In other cases, highly virulent viruses, such as poliovirus and variola virus, are risky to cultivate in large scale, making the subunit vaccine approach a safer choice. Other scenarios, in which the subunit vaccine approach is helpful, include the development of vaccines against malaria caused by a large protozoan parasite and other non-infectious diseases caused by certain protein factors of humans, such as hypertension and cancer. In these circumstances, a self-assembled, polyvalent, and highly immunogenic viral particles or protein polymers are used as a platform to increase the immunogenicity of the specific antigens of pathogens or the protein factors that cause the diseases. These chimeric vaccines are combination subunit vaccines that can be designed and used as mono-, bi-, or even trivalent vaccines against one or more pathogens and/or diseases. Development and productions of subunit and combination subunit vaccines through a well-established expression system, including recombinant bacteria, yeast, baculovirus in insect cells, and/or various viral or plasmid vectors in mammalian, avian, or plant cells, are considered to be more cost-effective compared with those of traditional live-attenuated or inactivated/killed virus vaccines. Thus, the subunit vaccine approach would help to extend vaccine distribution more widely to developing countries and remote areas, where the vaccines are usually highly demanded.

Homotypic interactions of viral capsid proteins and subviral particle and polymer formation

Over the long-course of evolution, viruses have developed a common feature to assemble themselves efficiently during viral replication, in which the viral structural proteins are able to spontaneously assemble into spherical or rod-shaped capsids after the viral capsid proteins are produced in host cells. The basic driving force behind these self-assembled capsids is homotypic interactions of the viral capsid proteins. In many cases, heterotypic interactions are also required to assemble more complex capsids that are composed of more than one viral structural proteins. These unique features of viral structural proteins have been utilized to generate various virus-like particles (VLPs), small subviral particles, and/or protein polymers *in vitro* (Figs. 1 and 2) through one of the well-established recombinant protein expression systems. To date, at least 30 different VLPs, small subviral particles and/or polymers representing more than 20 viral families have been generated (for reviews see Refs. 2-4).

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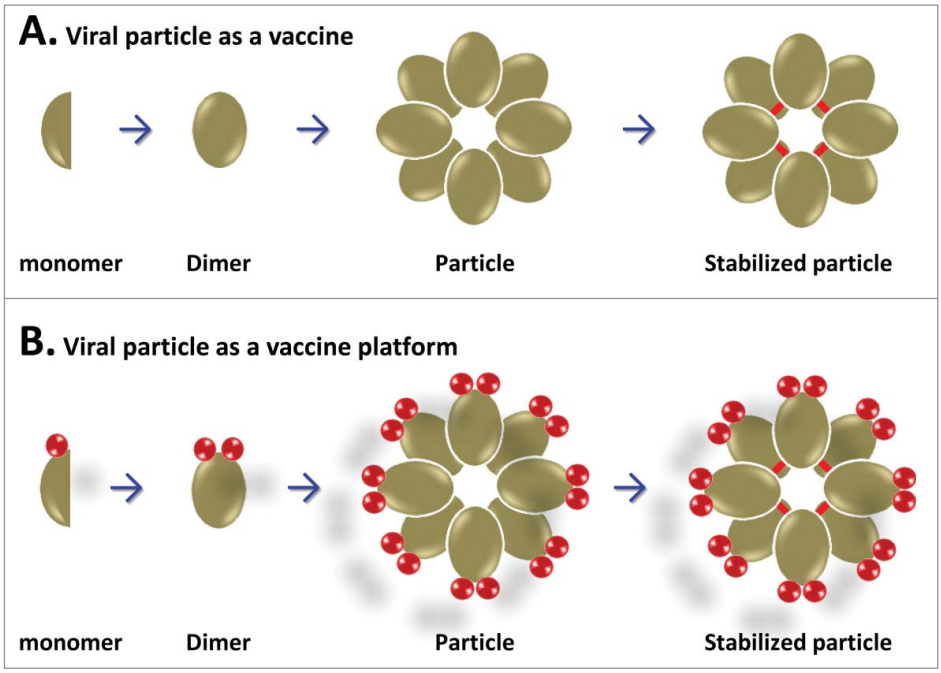

Figure 1. Schematic illustrations of a subviral particle formation (A) and its application as a polyvalent vaccine platform for combination subunit vaccine development (B). (A) Stepwise illustration of a subviral particle formation via homotypic interaction of the viral protein. The viral proteins are generated as monomers that can self-assemble into dimers and then a subviral particles via homotypic interaction of the viral proteins. Intermolecular disulfide bonds (red bars) may be introduced to stabilize the particle formation. (B) Application of the subviral particle as a polyvalent platform for a combination subunit vaccine development. A foreign antigen (red ball) is inserted to the top surface of the viral protein. Through dimerization and particle formation, multiple copies of the antigen are presented on the outermost surface of the subviral particle as a combination bivalent vaccine.

Generally, production of a single major viral capsid protein through an appropriate expression system leads to the formation of corresponding VLPs. For example, expression of the major huNoV capsid protein (VP1) via recombinant baculoviruses in inset cells formed huNoV VLPs.[5] Most of currently generated VLPs are made by a single capsid protein (for a review see[2-4]). However, in some cases, more than one capsid proteins are required to assemble more complex VLPs. For instance, double-layered VLPs of rotavirus (RV) can be made by co-expression of VP2 and VP6 proteins using the baculovirus expression system,[6] while triple-layered RV VLPs can be generated through constitutive co-expression of VP2, VP6, and VP7 in stably transfected high-5 insect cell lines.[7] The most complex recombinant VLPs are the severe acute respiratory syndrome coronavirus (SARS-CoV) VLPs that were made by co-expression of 4 structural proteins, including the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins, simultaneously using the baculovirus system.[8] These data suggest that VLPs of other viruses may also be generated based on the same self-assembly principle and appropriate expression approaches.

By contrast, small subviral particles can also be constructed by generating truncated viral structural proteins. For example, 3 different huNoV protruding (P) particles self-assembled, when modified P domains of huNoV capsid protein (VP1) were generated via the bacterial expression system, including the 24mer P particles,[9,10] the 12mer small P particles,[11] and the 36mer large P particles.[12] The stability of these P particles can be further enhanced by artificially introducing disulfide bonds into the core of the P particles via an end-linked cysteine-containing peptide to the P domain.[9-11] Similarly, expression of the truncated P1 and P2 domains of hepatitis E virus (HEV) VP1 forms 23 nm-particles, named E2 particles.[3,13] In addition, the homotypic interaction feature of viral structural and other proteins have also been employed to generate different protein polymers through DNA recombinant technology (Fig. 2). These include 1) lineage polymers through fusion of 2 dimeric proteins together;[14] 2) network polymers via fusion of 3 dimeric proteins covalently;[14] and 3) agglomerate polymers through fusion of an oligomeric protein with a dimeric protein (Fig. 2).[15] The driving forces behind these protein polymer formation are the intermolecular dimerization and/or oligomerization among the homologous proteins. These different VLPs, small subviral particles, and protein polymers have been further studied as vaccine candidates (see below).

VLPs, small subviral particles, and viral protein polymers as vaccines

The artificially made VLPs, small subviral particles, and viral protein polymers maintained the basic molecular patterns and the major B- and T-cell epitopes of their parental viruses which are highly immunogenic because of their polyvalence nature, and thus are able to elicit potent innate, humoral, and cellular immune responses.[3,16] Among these different particles and polymers, VLPs are the first to be developed and characterized into subunit vaccines (reviewed in[2-4]). So far several VLP-based vaccines have been commercially available for human use globally. These include 2 human papillomavirus (HPV) VLP vaccines consisting of L1 protein, the major capsid protein of HPV16,[17] for prevention of cervical and anogenital infection and diseases

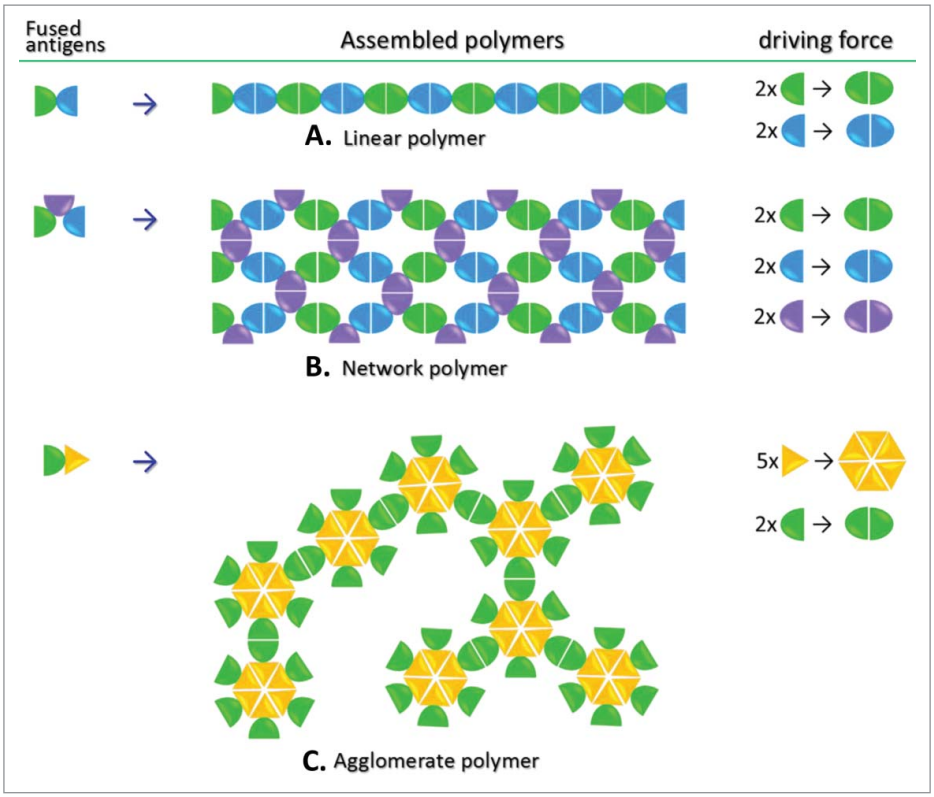

Figure 2. Schematic illustration of the formations of 3 viral protein polymers. (A) Lineage polymer formation. Fusion of 2 dimeric viral proteins (in green and blue, respectively) forms a lineage polymer via intermolecular dimerization of the homologous proteins. This lineage polymer can be used as a bivalent vaccine. (B) Network polymer formation. Fusions of 3 dimeric viral proteins (in green, blue, and purple, respectively) assembles into a network polymer via intermolecular dimerization of the homologous proteins. This network viral protein polymer can be used as a trivalent vaccine. (C) Agglomerate polymer formation. Fusion of a dimeric and an oligomeric viral proteins together assembles into an agglomerate polymer via intermolecular dimerization and oligomerization of the homologous proteins. This agglomerate polymer can be used as a bivalent vaccine.

associated with HPVs (Gardasil1, Merck & Co., New Jersey, USA; Cervarix1, GlaxoSmithKline, London, UK)[17-20] and 2 hepatitis B viruses (HBVs) VLP vaccines that are composed of the small surface antigen of HBV (HBsAg) against HBV infection (Recombivax HB1, Merck & Co., New Jersey, USA; Engerix-B1, GlaxoSmithKline, London, UK).[21,22] In addition, a small subviral particle vaccine that is formed by truncated P1 and P2 domains of the HEV capsid protein has recently been developed as an HEV vaccine for humans in China (HEV 239/Hecolin1, Xiamen Innovax Biotech, Xiamen, China).[23] These successful VLP and small subviral particle vaccines have endowed the feasibility and usefulness of the subunit vaccine approach. In addition to these 5 subunit vaccines that have been approved for commercial use, many others are under development, among which some, such as huNoV VLP vaccine[24] and parvovirus VLP vaccine,[25,26] have reached the stage of clinical trials.

Combination subunit vaccines against different pathogens or diseases

In addition to being used as vaccines against individual viral pathogens, the self-assembled, highly stable, and highly immunogenic VLPs, small subviral particles, and protein polymers are also excellent vaccine platforms for combination vaccine development against one or more pathogens or diseases. Foreign antigens or epitopes can be incorporated onto these VLPs, small subviral particles, or protein polymers for immunogenicity enhancement,[2,27-31] resulting in chimeric combination subunit vaccines against both pathogens that provide the platform and the inserted antigens or epitopes (Fig. 1B).[27,30,32] The vaccine RTS,S/AS01 or Mosquirix (GlaxoSmithKline, London, UK) against malaria is one of such combination vaccines that has reached the market. It consists of the HBV HBsAg VLPs[21] containing a portion of *Plasmodium falciparum*-derived circumsporozoite protein (CSP) with a liposome-based adjuvant.[30,33-35] It should be noted that, although the Mosquirix has been approved by the European Medicines Agency (EMA) for active immunization of children aged 6 weeks to 17 months against malaria, the World Health Organization (WHO) did not recommend inclusion of this vaccine in the Expanded Programme of Immunisations (EPI) due to the rapidly decline of the vaccine protection, particularly in infants and the potential risk of meningitis as adverse effects.[33-36]

Other combination vaccines based on the same principle have also been developed and reached the stage of clinical trials. For example, the vaccine CYT006-AngQb (Cytos Biotechnology AG, Schlieren, Switzerland) that consists of VLPs covalently coupled with angiotensin II epitope for therapeutic treatment of hypertension has reached phase II clinic trial.[37] Another example is the vaccine NicVax (Nabi Biopharmacetuicals, Rockville, Maryland) that is composed of nicotine in form of hapten 3′-aminomethylnicotine conjugating to the exoprotein A complex of Pseudomonas

aeruginosa[38,39] to reduce or eliminate physical dependence to nicotine has reached the phase III clinical trial (http://phx.corporate-ir.net/phoenix.zhtml?c=100445&p=irol-newsArticle&ID=1586001&highlight). A further example is the vaccine VAX102Q (VaxInnate, Cranbury, New Jersey), a recombinant flagellin protein (a TLR5 ligand) with 4 tandem copies of M2e epitope of influenza virus fused at the C-terminus.[40-42] A phase II clinical trial is ongoing to test the efficacy of the vaccine as a universal vaccine against influenza virus (http://www.biocentury.com/companies/vaxinnate_corp).

Based on the same principle, small subviral particles can also be used as platforms for combination vaccine development. For example, NoV P particle contains 3 surface loops that has been shown to be able to hold a foreign antigen or epitopes without compromising the stability of the chimeric P particles.[27,32] The RV neutralizing antigen VP8*,[27] the M2e epitope[30] and the HA2 antigen[43] of influenza virus, the 4E10 and 10E8 epitopes of human immunodeficiency virus (HIV),[44] and the VP3 epitope of enterovirus 71 (EV71)[45] were inserted onto the surface loops of the P particles as chimeric P particle vaccines. Preclinical animal trials of these vaccine candidates revealed high antibody titers specific to the inserted antigen/epitopes and the P particle platform, respectively, and protected the vaccinated mice against infection of RV,[27] influenza virus,[30,43] and EV71.[45] These data indicated that the huNoV P particle is an excellent platform for combination vaccine development.

Other combination vaccines under development include the 3 types of viral protein polymers (Fig. 2, see above).[14,15] For example, fusion of the dimeric P domains of huNoV and HEV, the major neutralizing antigens of the 2 viruses (Fig. 3), formed lineage polymers (Fig. 2), resulting in a bivalent vaccine against the 2 viruses.[46] Similarly, fusion of the P domains of huNoV, HEV, and astrovirus (AstV), the major neutralizing antigens of the 3 viruses (Fig. 3), formed network polymers (Fig. 2), leading to a trivalent vaccine against the 3 viruses.[47] Furthermore, the huNoV P domain can be modified into a oligomeric protein via an end-linked cysteine-containing peptide[9,10,48] and fused with a dimeric proteins forming agglomerate polymers, leading to a combination vaccine against huNoV and a selected pathogen.[15] In fact, a monomeric antigens, such as the RV VP8* antigen or the M2e epitope of influenza virus, can also be incorporated into these 3 polymers for enhanced immunogenicity for combination vaccine development.[14,15,49] Preclinical animal trials of these viral polymer-based combination vaccines showed good immune responses and neutralization activity against huNoVs, HEVs, and RVs.,[14,15,46,47,49] as well as protection of vaccinated mice against infection of influenza virus.[14,15]

Challenge and future direction

Development of effective, non-replicating subunit vaccines based on many known neutralizing antigens or epitope faces a common issue of low immunogenicity due to relative small sizes and low valences of these antigens/epitope. This issue can be solved by fusing these antigens or epitopes onto a large, polyvalent, and highly immunogenic VLP, small subviral particle, or protein polymer platform for improved immunogenicity as combination vaccines. While successful combination vaccines have been reported, challenges have also been encountered. Generally, while larger antigens contain more authentic antigenic features and are more immunogenic, they are less compatible with viral particle-based platforms compared with smaller epitopes that are easier to be incorporated onto the viral particles without compromising the stability of resulting chimeric vaccines. Therefore, a balance between the 2 factors need to be considered for the best vaccine outcomes.

Among different vaccine platforms, VLPs and small subviral particles usually form more unified particles after incorporation of the foreign antigens or epitopes[27,30] for better quality control compared with the protein polymer platform.[14,15] However, these viral particle-based platforms generally have limited surface space for foreign antigen/epitope insertions (Fig. 1B), which may lead to instability of the chimeric vaccines. On the other hand, the protein polymers themselves are made by

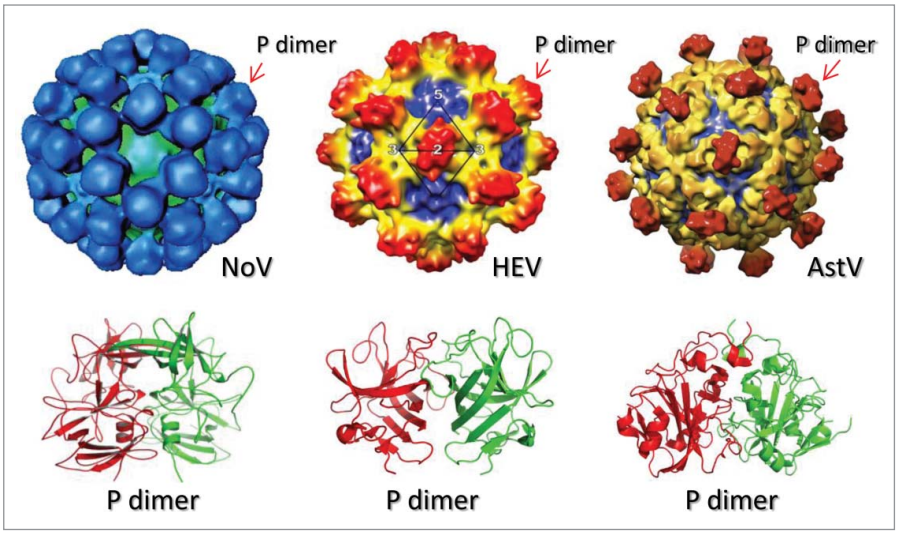

Figure 3. Schematic illustration of the common availability of viral dimeric surface proteins. The structures of norovirus (NoV), hepatitis E virus (HEV), and astrovirus (AstV) with indication of protruding (P) dimers on the capsid are shown in the top panel. The crystal structures of the P dimers are shown in the bottom panel. These dimeric viral proteins are ideal components for the viral protein polymer production.

different antigens (Fig. 2) and thus exhibit a much larger capacity and flexibility to incorporate larger antigens, however, the resulted polymer sizes may be dispersed, which may need better quality control for unified vaccine outcomes. In summary, further development of different vaccine platforms with favorable features and continual identification of new effective antigens and epitopes will facilitate the advancement of combination subunit vaccines against different human diseases.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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