Self-Assembled Nanoparticles: Exciting Platforms for Vaccination

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Vaccination is successfully advanced to control several fatal diseases and improve human life expectancy. However, additional innovations are required in this field because there are no effective vaccines to prevent some infectious diseases. The shift from the attenuated or inactivated pathogens to safer but less immunogenic protein or peptide antigens has led to a search for effective antigen delivery carriers that can function as both antigen vehicles and intrinsic adjuvants. Among these carriers, self-assembled nanoparticles (SANPs) have shown great potential to be the best representative. For the nanoscale and multiple presentation of antigens, with accurate control over size, geometry, and functionality, these nanoparticles are assembled spontaneously and mimic pathogens, resulting in enhanced antigen presentation and increased cellular and humoral immunity responses. In addition, they may be applied through needle-free routes due to their adhesive ability, which gives them a great future in vaccination applications. This review provides an overview of various SANPs and their applications in prophylactic vaccines.

1. Introduction

In 1796, Edward Jenner first attempted to control fatal contagious smallpox scientifically by vaccination. Since then, vaccines have been under development for disease prevention, immunity improvement, and life quality enhancement. To date, vaccine production has been licensed to prevent more than 30 infectious agents, such as smallpox, polio, diphtheria, and tetanus, likely saving 6 million lives each year.\[^{1,2}\] Although recognized as the best milestone in human medical history, vaccination remains an interesting and vast field open to innovation.\[^{3}\]

The lack of effective vaccines against emerging and reemerging diseases is one challenge in the vaccination field caused by complex mechanisms that enable pathogens to escape the host immune system. The influenza virus is a typical example with a high likelihood of antigenic drift and shift, making infection a persistent threat to public health worldwide. Seasonal influenza epidemic and episodic outbreaks cause ≈3 to 5 million cases of severe illness and as many as 650 thousand deaths worldwide each year.\[^{4,5}\] To date, there is no universal vaccine to protect against different subtypes of the influenza virus. The development of universal anti-influenza vaccines would lead to vaccines for other similar infectious diseases. Existing vaccines have deficiencies, creating another difficulty in vaccination development. For example, the live attenuated vaccine is highly immunogenic but poses a risk of reverting to the virulent form.\[^{6}\] Inactivated, toxoid, and recombinant vaccines may elicit a weaker immuneresponsethatleadtorequirementsofadjuvantsormultipledosagesofadministration.\[^{6,7}\] Antibody-dependent enhancement (ADE) is another concern which needs to be taken into consideration for the live attenuated vaccine and inactivated vaccine.\[^{8,9}\] There are additional problems, such as the possible damage to antigens, in vivo instability, induced high toxicity, and the need for cold supply chains, also taking the vaccination filed intopromotion.\[^{10,11}\]

These limitations can be sufficiently addressed by nanoparticle systems, especially self-assembled nanoparticles (SANPs). SANPs are particles on a nanoscale with basic blocks autonomously organizing together.\[^{12}\] Through either encapsulation or conjugation with antigens, these minuscule particulates can function as both delivery platforms and adjuvants.\[^{13}\] Ability for antigen presentation to resident dendritic cells (DCs) have been improved on the account that SANP-based vaccines move through lymphatic vessels directly to lymph nodes by caveolae or receptor-mediated endocytosis, which enables cross-presentation processes and the induction of cellular and humoral responses.\[^{11,14,15}\] SANPs also have great capacities for increasing antigen stability, prolonging circulation time, amplifying the activity of antigens and adjuvants, overcoming biological barriers, and achieving targeted delivery.\[^{16–18}\] Thus, SANPs are new and promising platforms for antigen delivery.

In addition, with their high capacity for adsorption onto skin and mucosa, some SANPs can be supplied via needle-free administration, activating the immune response from the first line of defence in the human body and thus broaden the protective effects.\[^{17,19,20}\] This technique reduces pain and suffering, avoids
the potential dangers associated with incorrect or repeated use of injection devices, enables faster vaccine delivery, and decreases the demand for vaccination training, which is essential for realizing widespread coverage and lower overall costs, compared to traditional vaccination forms of administration.

In this review, we focus on the unique features of some SANPs and highlight the relevant examples for anti-influenza virus vaccines to generate a better understanding of their applications. The potential to use needle-free administration is also described.

### 2. Brief Introduction of SANP-Based Vaccine Development

SANPs are nanosized spherical tubular, or fibrillar particles generated from several oligomers spontaneously arranging themselves into organized structures. This self-assembly process happens when the various attractive/repulsive forces inter- and intra- molecules achieve an equilibrium with the expenditure of minimal free energy, resulting in the generation of different non-covalent interactions, such as hydrogen bonds, van der Waals, hydrophobic, electrostatic, and \( \pi-\pi \) interactions. Thus, the process is governed not only by the constituents themselves but also by environmental parameters such as temperature, water content, ionic strength, and pH. SANPs have a superiority of displaying the targeted epitopes or antigens in defined pattern at a high density on their surface, which resembles the pathogen-associated molecular patterns (PAMPs) in nature. So both the innate and adaptive immune systems can be induced to recognize such systems. The multivalent antigen presentation and structurally ordered antigenic array also enables the efficient cross-linking events between the antigens and the host cell BCRs, delivering strong activation signals to the B cell and a critical step to induce a potent immune reaction. Other processes can also account for the increased immune response like lower thresholds of B cell activation, promotion of long-lived plasma cell differentiation, and stimulation of DC-mediated priming of T helper cells.

An immunologic adjuvant is the substance that acts to improve, prolong, or boost antigen-specific immune responses, but is not immunogenic itself. Therefore, due to the ability to accelerate the innate and adaptive response, the SANPs are investigated as not only delivery platforms but also promising parts of immune adjuvants without the requirement of additional immune potentiators. Unlike the traditional chemical adjuvant like aluminum hydroxide or phosphates (alum) with toxicities and unwanted inflammatory side effects, SANPs are efficient, safe, and stable adjuvants, which can evoke the epitope-specific B- and T-cell immune responses, protect payloads from enzymatic degradation, and increase internal circulation time.

In addition, self-assembly technology is simple to operate at low cost because it usually depends on water as the solvent and no special devices are required. Besides the mentioned unique features, SANPs are excellent vehicles compared to the non-SANPs as summarized in Table 1. Therefore, SANPs have good prospects for industrial applications.

A conceptual workflow for SANP-guided vaccine development is shown in Figure 1A. To design a novel nanovaccine, an appropriate SANP must be chosen to deliver the screened pathogen epitopes. Then, it can be expressed and generated in proper systems in conjugation with characterization and animal immunity studies. Vaccines proven effective in animal models will be tested in clinical trials and applications. The design step is of vital importance as it determines the efficiency of the new vaccine in the following process. With the development of computational and structural biology, SANP-based vaccine can be designed by computational tools with expected immunodominant epitopes, molecular mass, diameter, geometry, and complementary packing arrangements. Bioinformatics with huge volume of data can further help researchers to predict the possible efficiency before performing lab experiments, which is convenient and time-saving. And the possibility of the ab initio design of new protein–protein interfaces catalyzes the development of SANP field, which enriches our molecular repertoire of nanoparticle scaffolds. SANPs are generated with several typical compositions borrowed from natural molecules such as liposomes, sugars, polymers, peptides and proteins, or synthesized from a novel computational sequence design. Based on differences in their smallest elements, SANPs can be divided into two classes: 1) non-protein-derived synthetic particles and 2) protein-based

### Table 1. The comparison between the non-SANPs, protein-based SANPs, and nonprotein SANPs.

| Characteristic                  | Non-SANPs                                      | Non-protein SANPs                       | Protein-based SANPs                     |
|--------------------------------|------------------------------------------------|------------------------------------------|------------------------------------------|
| Antigen formulation            | Admixing                                       | Encapsulation, adsorption, or chemical interactions | Covalent bonds or chemical interactions |
| Antigen presentation           | Single antigen                                  | Ordered repetitive antigens              | Highly ordered repetitive antigens, especially for display of large recombinant subunit antigens, such as trimeric viral surface proteins |
| Shapes                         | No fixed shape                                  | Linear, branched, or spherical           | Tubular, fibrillar, or spherical         |
| Safety concerns                | Toxics and unwanted inflammatory side effects (i.e., Alum) | Some being safe (i.e., chitosan) while some having safety concerns (i.e., AS03) | Biosafety |
| Controllability                | Not controllable                                | Controlled by the environment            | Designed at will                        |
| Manufacturability              | Easy for large-scale manufacture                | Easy for large-scale manufacture         | Stringent processes to remove endotoxin and microbial contamination |
| Cost                           | Lower cost                                      | Lower cost                               | Higher                                  |

A conceptual workflow for SANP-guided vaccine development is shown in Figure 1A.
biological entities (Figure 1B), both of which are discussed, with awe-inspiring anti-influenza vaccine examples highlighted. And the differences between them are also summarized in Table 1.

3. Non-Protein SANPs

Non-protein nanoparticles appear to be good platforms for antigen delivery, providing not only a potent intrinsic adjuvant but also antigen stabilizers. Some of non-protein nanoparticles, such as chitosan, are so effective that they can be applied in one-dose administration. Their structures depend on their own structural parameters such as ratio, molecular weight of hydrophilic/hydrophobic parts, and stimuli-like chemical composition of the solution and the surroundings, including the temperature and light.[32–34] As a consequence, they are smart chemical nanoparticles based on artificial designs with well-controlled assembled structures. They can be administered
by alternative non-invasive routes with dry powder formulations for long-term preservation. The interaction between the particles and the antigens is quite different, such as encapsulation within PLGA and liposomal nanoparticles, adsorption to isomatrix and emulsions while chemically connection with self-assembled peptides. Several non-protein SANPs, including emulsions, lipid-based structures, polymers, and peptide-assembled antigen delivery technologies, are introduced in this section.

3.1. Water-In-Oil Emulsion

Aluminum compounds were the first adjuvants, invented approximately a century ago, and are widely used today. Water-in-oil emulsion technology, with oil as the main component, represents a second relatively new adjuvant. It was proven to be effective in various animal studies because of its ability to induce relatively high titers of antibodies, extend the time of antigen stimulation, and reduce the vaccination dose and frequency.

Freund’s adjuvant, composed of complete and incomplete Freund’s adjuvant (CFA and IFA, respectively), is among the classic oil adjuvants. IFA is a mixture of mineral oil (white oil of low viscosity) and emulsifier (lanolin or Tween-80), inducing only Th2 cytokine secretion. CFA, in addition of inactivated mycobacteria, is a very potent adjuvant that can stimulate strong humoral and cellular immune responses with higher toxicity. However, both CFA and IFA are potentially carcinogenic to mice and humans which are no longer utilized in vaccine production.

New oil-in-water emulsions have been successfully developed in recent years. MF-59 is a new squalene-based adjuvant that is easier to metabolize than mineral oil. Following alum, MF-59 is the second adjuvant applied in humans approved by the Food and Drug Administration (FDA). It can expand the protection range and enhance the immune response, which is 3–50-fold greater than that evoked by alum at the same antigen dose. With negligible toxicity and side effects, such as mild local pain and fever in adults, it has been successfully applied in marketed vaccines for influenza (FLUAD and Focetria). Another novel licensed vaccine adjuvant is AS03, based on functionalized doxorubicin with a hydrophobic lipid tail conjugated by a solubility-promoting poly(ethylene glycol) polymer (amph-DOX). The targeting ability to mitochondrial of the amph-DOX is pretty efficient, leading to a significant increase of reactive oxygen species levels in tumor cells. Thus, the antitumor efficacy has been markedly improved than the unmodified doxorubicin.

By integrating functional viral envelope glycoproteins, liposomes were developed into an interesting novel type of antigen adjuvant named virosomes, retaining the cell-binding and membrane fusion ability of the native virus. Mimicking natural infection, the vaccines manifest an improved ability to be captured by antigen presenting cells (APCs) and enhanced antigen processing in the context of the major histocompatibility complex (MHC) I pathway. Thus, they are highly efficacious and able to induce both B- and T-cell responses. A vaccine against influenza, licensed as Inflexal V (Figure 2A), has already reached the market. Generated with a mixture of three monovalent virosome pools, this vaccine was shown to be highly immunogenic and is the only adjuvanted influenza vaccine currently licensed for use in all age groups.

ISCOMATRIX particles are another useful lipid-based vehicle for delivery of vaccines against influenza viruses. They consist of phospholipids, saponins, and cholesterol components that assemble spontaneously into spherical cage-like nanoparticles. Associated with hydrophobic and hydrophilic antigens with good stability, ISCOMATRIX particles are able to induce both humoral and cellular immune responses. Chung et al. found that the ISCOMATRIX adjuvanted vaccine promotes a greater immune response against avian influenza virus in a clinical trial. Another group found that ISCOMATRIX might perform slightly better than other non-alum adjuvants in terms of releasing vaccine doses. However, its potential application in human vaccines has been impeded by toxicity concerns attributed to certain saponins, such as Quil A and QS-21.

Lipoproteins also have vast space for improvement as antigen delivery scaffolds. Rui Kuai et al. have developed a potent adjuvanted lipoprotein nanodiscs with MPLA, a TLR4 agonist, and CpG, a TLR9 agonist co-delivered, which can be readily combined with a variety of subunit antigens and significantly increase activation of dendritic cells to achieve the efficient vaccines. For example, when protein convertase subtilisin/kexin 9 (PCSK9) loaded on the system, strong humoral responses were generated and total plasma cholesterol levels had 17–30% reduction. While for addition of ovalbumin or E7 peptide antigen, the particles also enhanced the antigen specific CD8+ T cell responses and promoted regression of tumors in the treated animals. At the same time, they have generated another effective nanodiscs based on an ApoA1 mimetic 37-mer peptide and 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC). In addition of doxorubicin (DOX), a widely used chemotherapeutic agent, the particles were tested to be useful with immunogenic cell death of cancer cells and exerted antitumor efficacy without any overt off-target side effects in animal tumor models.
3.3. Polymeric Particles

Polymeric nanoparticles can be obtained either from the self-assembly of monomeric units or preformed polymers of varying forms, such as linear, slightly branched, or hyperbranched 3D network.[68] They are attractive in vaccine engineering because of their biocompatibility, biodegradability, and adjustable properties (size, composition, and surface properties), which reproduce the natural particulate form of pathogenic agents while allowing controlled release.[69] Their ability to be highly decorated promotes their roles as immune potentiators and extends their circulation time in vivo.[70]

Polysaccharide is a typical natural polymer. Its components can act as pathogen-associated molecular patterns (PAMPs) and enhance the immune response against relevant antigens.[71,72] Other features, such as high biocompatibility, low toxicity, and facile techniques for production, make them particularly applicable for vaccine development.[73] Chitosan, mainly obtained from the deacetylation of chitin,[74] was the first polysaccharide used for antigen delivery. With efficacious and sustainable immune responses by activation of the STING pathway, chitosan represents a single-dose formulation vaccine.[15,75] Its cationic character allows electrostatic interactions with negatively charged antigens under physiological conditions and makes interactions with mucosal tissues easier.[74] Thus, it was validated as an elicitor of strong cross-reactive protection when administered through the intranasal route.[76] Its derivative, termed N-trimethyl chitosan (TMC), is also an effective platform because of its higher water...

Figure 2. Application of non-protein-based SANPs. A) Cartoon model (left) and EM image (right) of virosomes reconstituted from influenza virus without an inner core and genome. Reproduced with permission.[59] Copyright 2009, Elsevier BV. B) Generation of self-assembled peptide nanoparticles. a) Schematic diagram of Uni4MC fabrication. Recombinant tetrameric M2e, referred to as 4MtG, was self-assembled into Uni4MC nanoparticles by desolvation. b) Schematic diagram of double-layered nanoparticle generation. Trimeric head-removed HAs (hrHAs), forming an additional layer, are bound to the Uni4MC surface via DTSSP crosslinking. Reproduced with permission.[87] Copyright 2018, Nature Publishing Group. C) Self-assembled peptide nanoparticle characterization. Scanning EM image of Uni4MC, Uni4C1 (hrH1-coated double-layer Uni4MC), and Uni4C3 (hrH3-coated double-layer Uni4MC). Scale bars, 500 nm. Reproduced under the terms of the Creative Commons CC-BY License.[86] Copyright 2018, The Authors, published by Springer Nature.
solubility and pH-independent cationic nature.\[73,77,78] Following the discovery of chitosan-based vehicles, other polysaccharides, such as dextran, mannan, and beta glucans, are also promising for use in nanovaccineengineering.\[79-81]

Poly(lactic-co-glycolic acid) (PLGA), another polymer extensively used in antigen delivery, has been approved by the FDA and European Medicines Agency (EMA) because it undergoes hydrolysis efficiently in vivo.\[77,82] When covalent attachment of antigens, an amphiphilic block co-polymer based on PLGA can spontaneously self-assemble into uniform nanoparticles. These particles can prevent antigen degradation within 4 weeks under physiological conditions. Thus, they are critical for long-term immune response and single-dose administration, but have yet to be evaluated in clinical trials. In addition, its PEGylated derivative has been broadly adopted for enhancing the delivery of hydrophobic drugs since their hydrophilic coating was proven to be essential for increasing stability in contact with mucosal fluids.\[83]

“Star” nanoparticles also appeal the attention of researchers with a poly(amidoamine) (PAMAM) dendrimer as core and biocompatible N-[2-hydroxypropyl] methacrylamide (HPMA)-based polymer as extending arms.\[84] When injected into mice, they can traffic to lymph nodes (LNs) by 4 h following vaccination, where they were taken up by subcapsular macrophages and then resident dendritic cells (DCs), playing as a powerful platform for efficient antigen delivery. Animal studies performed on mice and nonhuman primate has been proposed that high titers of antibody were elicited to the Env variable loop 3 (V3) of the human immunodeficiency virus (HIV) when these particles co-deliver the V3 and T-helper peptides.

3.4. Self-Assembled Peptides

Peptides are used as basic units in the formation of SANPs due to an easy modification process during and after biosynthesis, as well as effortless acquisition and resistance to non-native conditions.\[85] Alpha-helices are representative of coiled coil motifs that form a hydrophobic core and interhelical salt bridges through the arrangement of hydrophobic and charged amino acids.\[86] Thus, many scientists have used peptides with alpha-helices to build various self-assembling protein structures.

Wang and co-workers desolvated tetrameric M2e (ectodomain of influenza matrix 2 protein) into nanoparticles and coated them with headless hemagglutinins (HAs) (Figure 2B,C).\[87] Mice vaccinated with them exhibited robust long-lasting immunity and were fully protected against lethal challenges by divergent influenza A viruses from both groups. After uptake into cells, these SANPs are disassembled and activated physiologically, implying their wide utilization for antigen delivery and controlled release. In addition, they can be developed with another layered nanoparticle format composed of nucleoprotein (NP) peptide cores and M2e peptide coatings. Via dissolvable microneedle patch-based skin vaccination, these nanoparticles increased antigen-presentation time and activated CD8+ T cells, correlating with stronger immune responses.\[88]

Lynn et al. have fabricated a novel vaccine vehicles derived from the charge-modified CD8 epitope which can autonomously assemble themselves into nanoparticles without the concerns about their composition.\[89] These particle have the uniform size (≈20 nm) and can provide the precise loading of diverse neoantigens, leading to the design of personalized vaccines for cancer therapy. Mice vaccinated with particles in combination with TLR-7/8a showed enhanced tumor clearance and ≈50% of high predicted binding affinity between the neoantigens and MHC-I. When tested in nonhuman primates, CD8 T cells are also activated.

Thus, nanoparticle cores derived from the conserved epitopes of pathogens and coated with other antigens represent a convenient solution to effective vaccine design without the addition of other encapsulation materials.

4. Protein-Based Nanoparticles

Protein-based SANPs are derived from the polymerization of protein monomers with fixed shapes. Therefore, their form and antigen concentration can be precisely controlled. In contrast to inorganic particles, they have various excellent characteristics, that is, tissue and cell targeting, biocompatibility, and biodegradability. New evidence has shown that they can activate innate immune responses without conventional adjuvants, acting as pure antigen carriers for delivery ad as innate stimulators.\[15] They are able to co-deliver additional immunostimulatory molecules, such as cytokines and TLR agonists\[90–93] with the purpose of increasing the overall immune response level. Generally, antigens are linked to protein-based particles by covalent bonds. But when a new virus, such as severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) emerges, high-throughput screening can be utilized by genetically fused platform/antigen with SpyCatcher/SpyTag system\[94,95] to form new isopeptide bonds and to find available epitopes rapidly on account that the system has been successfully implemented for antigen decoration of virus-like particles (VLPs) first.\[96] However, their dry formulation can be challenging and may require the addition of other materials.\[20] In this regard, three kinds of cage-like nanoparticles are introduced in this section.

4.1. Ferritin

Ferritin is probably the most ancient molecule presenting in a wide variety of biological species, such as eubacteria, archaea, plants, and animals.\[97–99] It is associated with iron stores and plays a key role in iron metabolism for iron detoxification and reservation,\[100,101] which protects the cell from oxidative damage. Its typical form is an almost spherical protein shell composed of 24 self-assembled subunits with remarkable thermal and chemical stability.\[85,102,103] Hence, ferritin nanoparticles are potentially well suited to carry and expose immunogens for vaccine design without the requirement for a cold chain. They can also be made into stable and spray-dried vaccines for needle-free administration.

Recently, Qi et al. constructed a novel nanovaccine named 3M2e-rHF by fusing the 3 sequential repeats of M2e (3M2e) with a recombinant human ferritin heavy chain (rHF), which self-assembled when expressed in Escherichia coli (Figure 3A).\[104] Mice generated robust immune responses when intranasally vaccinated with 3M2e-rHF nanoparticles in the absence of an
adjuvant, inducing complete protection against lethal dose challenges of homo-subtypic H1N1 and hetero-subtypic H9N2 virus. A mechanistic analysis suggested that M2e-specific mucosal secretory IgA and T-cell immune responses may play critical roles in preventing influenza virus infection when 3M2e-rHF nanoparticles are used as an immunization agent delivered through the intranasal route. The results indicate that self-assembled ferritin nanoparticles can be promising vehicles to deliver antigens with characteristics such as convenient administration, cost efficiency and suitability for large-scale production.

Ferritin can also be utilized as a scaffold for trimeric antigen presentation, that is, HA, the key antigenic component of influenza virus. Nabel and colleagues genetically fused the ectodomain of A/New Caledonia/20/1999 HA to Helicobacter pylori non-haem ferritin, generating a SANP with eight HAs in three-fold axis symmetry on its surface. Antibody inhibited HA was elicited in mice and ferrets at a level tenfold higher than that induced by the licensed inactivated vaccine. They neutralized H1N1 viruses from 1934 to 2007 and protected ferrets from an unmatched 2007 H1N1 virus. This ferritin-based nanoparticle was also used to display the H1 HA stem region only and triggered broadly cross-reactive antibodies against diverse group 1 influenza strains. Nevertheless, it could not provide cross-group protection against group 2 viruses, such as H3N2 and

Figure 3. Application of protein-based SANPs. A) Derivation of ferritin nanoparticles presenting influenza virus 3M2e peptide. a) Construction of a 3M2e-rHF monomer. Three sequential repeats of M2e peptides, referred to as 3M2e, were fused to the N-terminus of recombinant human ferritin heavy chain (rHF) (purple: 3M2e; blue: rHF). b) Formation of a 3M2e-rHF nanoparticle. c) Transmission (left) and immunogold (right) EM images of 3M2e-rHF cages. Scale bars, 20 nm. Reproduced with permission. Copyright 2018, Wiley-VCH. B) Generation of influenza A/Indonesia/05/2005 (H5N1) virus-like particles. a) Baculovirus construct composed of Tn7 regions, gentamicin resistance gene (Gm), polyhedron (PoH) promoters, influenza genes (HA, M1, and NA), and polyadenylation signals. b) Immunogold EM image of purified H5N1 VLPs probed with anti-H5 primary antibody. Scale bars, 100 nm. Reproduced under the terms of the Creative Commons Public Domain. Copyright 2008, The Authors, published by PLOS. C) Construction of flock house virus-like particles with multivalent presentation of the influenza virus HA2 alpha-helix. a) Positions on the FHV capsid chosen for antigen insertion or replacement (blue: 206 loop; red: 264 loop). A subunit is shown in the inset, and three of each are present at 60 sites in one FHV-like particle (PDB: 4FSJ). b) Ribbon diagram of B2/alpha-helix design for FHV capsid attachment. B2 (yellow and orange), a small helix-turn-helix scaffold, maintains the secondary structure of several helices. An equivalent length of helical residues in the B2 protein (orange) is replaced by the HA2 alpha-helix (magenta). Then, the C terminal is slightly truncated. c) The B2/A-helix construct. Arrows indicate the insertion points into the 206/264 loops. d) Negative-stain images of the assembled icosahedral nanoparticles with the insertion at site 264. Scale bars, 50 nm. Reproduced under the terms of the Creative Commons Attribution License. Copyright 2012, The Authors, published by PLOS.
H7N9. Recent studies have shown that H7N9 can trigger multiple HA stem-directed antibody lineages in humans that recognize HA molecules from both group 1 and group 2.\textsuperscript{[107]} Thus, with an iterative structure-based design, Corbett et al. displayed stable stem trimers from H3N2 and H7N9 viruses on ferritin, which activated cross-group reactive antibodies in mice.\textsuperscript{[108]} Further development of a mosaic ferritin nanoparticle were able to evoke antibody responses neutralizing H1N1 viruses spanning the 90 years with diverse hypervariable receptor-binding domain (RBD) of influenza virus hemagglutinins shown on the surface in well-ordered pattern. The reason can be attributed to the heterotypic RBDs in the adjacent supplying an avidity advantage (RBD) of influenza virus hemagglutinins shown on the surface.

Furthermore, based on precise protein engineering, a ferritin nanoparticle that shows two or more antigens simultaneously is under development. Georgiev et al designed a two-component ferritin with four trimers for each antigen derived from the HIV-1 envelope and influenza HA in a defined geometric pattern, evoking neutralizing antibodies against the respective viruses in guinea pigs.\textsuperscript{[110]} At the same time, another group implemented M2e on the ferritin surface while packaging fluorescent reporter protein (GFP) into the internal cavity.\textsuperscript{[111]} Antibody responses against both the surface antigen and the loaded cargo protein were elicited. These results serve as proofs-of-concept that multimeric conserved epitopes can be placed on a single ferritin particle.

4.2. Lumazine Synthase

Lumazine synthase (LS), an enzyme involved in riboflavin biosynthesis, is distributed extensively in organisms, including archaea, eubacteria, bacteria, fungi, and plants.\textsuperscript{[112–114]} With as many as 12 pentamers or more than 300 subunits, a number of porous shell-like structures have been reported, all of which were able to generate strong humoral and cellular immunity.\textsuperscript{[115,116]} Foreign peptides or proteins inserted at its N-terminus do not disrupt general peptide folding, representing another example of a particular base for the repetitive display of vaccine candidates.

Using LS from \textit{Brucella abortus} (BLS) as a carrier for one or four copies of the M2e peptide (BLS-M2e or BLS-4M2e, respectively), Alvarez et al. efficiently expressed chimeric proteins in \textit{E. coli}. Mice immunized with BLS-M2e induced a remarkable humoral immune response with different adjuvants, while BLS-4M2e alone elicited similar levels and protected 60% of the animals from influenza virus challenge.\textsuperscript{[117]} Thus, with a simple purification process, LS is an excellent platform applicable in the absence of strong adjuvants. There remains undeniable room for LS development because of the positive attempts made to enhance the immunoreactivity of HIV epitopes.\textsuperscript{[118]}

4.3. Virus-Like Particle

VLPs, which are non-replicating non-infectious particles, arise from the self-assembly of viral capsid proteins without genetic material.\textsuperscript{[119,120]} They are spherical, rod-like, or icosahedral supramolecules with diameters varying from 10 nm to 1 µm.\textsuperscript{[121,122]} They show similarity with the perfect structure of a viral capsid, which has been proven to be immunogenic. The process for their purification is based on capsid engineering in high amounts and requires only standard biological facilities.\textsuperscript{[123]} VLPs are thus attractive delivery nanocarriers for which there are notable examples (Figure 3B,C).

The earliest VLP-derived vaccines were “plain,” obtained simply by the self-assembly of virus capsid proteins. Some have been successfully marketed, such as hepatitis B virus (HBV)-like particles and human papilloma virus (HPV)-like particles. In 2001, Latham et al. generated influenza VLPs composed of HA, neuraminidase (NA), matrix 1 protein (M1), and matrix 2 protein (M2) from the Sf9 cell surface, bearing surface projections that closely resemble those of the wild-type influenza virus.\textsuperscript{[124]} A study revealed that HA on VLPs can elicit high levels of virus-specific antibody responses in vivo and antibody-secreting cell (ASC) responses in vitro.\textsuperscript{[125]} Based on these results, several significant anti-influenza VLP vaccines have been developed in the past few years, such as the H5N1 VLP vaccine\textsuperscript{[126,127]} and the H7N9 VLP vaccine,\textsuperscript{[128]} both of which have been entered into clinical trials. Recently, VLPs have been developed in combination with other techniques, that is, computationally optimized broadly reactive antigen (COBRA) methodology led to a consensus HA gene based on H5 HA sequences from 2005 to 2006.\textsuperscript{[129]} Therefore, a vaccine with a cocktail of VLPs displaying consensus H1, H3, H5 and H7 HA may have cross-protective ability.

The VLPs derived from other viruses can also serve as a “chimeric” delivery platform due to their efficiency in promoting phagocytosis by APCs and subsequent activation.\textsuperscript{[110,111]} Ward and co-workers produced a VLP vaccine based on H5N1 HA in \textit{Nicotiana benthamiana}, triggering strong immune responses and complete protection levels in mice.\textsuperscript{[132]} This is the first vaccine to leverage the ability of plants to produce enveloped influenza VLPs budding from the plasma membrane. This VLP vaccine was found to be effective in a phase 1–2 clinical trial, generating T cell activation and a cross-reactive response.\textsuperscript{[133]} Currently, a phase 3 trial is underway to test its efficacy in adults and the elderly and lot-to-lot consistency in healthy adults. Such VLPs represent very promising vaccines owing to their potential to address several limitations of currently licensed products, such as response time and scalability during a pandemic. Other virus-like particles can also be utilized as promising carriers, such as bacteriophages\textsuperscript{[134–136]} and flock house viruses.\textsuperscript{[137]}

4.4. De Novo Designed Protein-Based SANP

At present, natural components are generally chosen as vaccine carriers, which has been limited by their restricted available number and fixed physical structure and chemical properties.\textsuperscript{[138]} And their interaction with the special host cell and immune system is another factor limiting their application. Thus, engineering de novo protein assemblies has been pushed as a growing field, in which can make the vaccine scaffolds more smart, powerful and adaptive to the chosen epitopes.\textsuperscript{[135]}

Babapoor et al. have built a self-assembling icosahedral nanoparticle composed of de novo designed trimeric coiled coils and pentameric coiled coils derived from Cartilage Oligomeric
Matrix Protein (COMP). The two coiled coils were connected by a short linker region and self-assembly occurs when their coiled-coil domains associate with each other. A novel vaccine representing M2e in tetrameric forms on this particle have been constructed, for which induced a significantly higher antibody responses in chickens after each inoculation. The results propose that the self-assembling polypeptide nanoparticle shows promise as a potential platform for development of an anti-avian influenza vaccine.

Another computational designed protein named I53-50, comprising 20 trimeric “A” components and 12 pentameric “B” components for a total of 120 subunits, has also been applied in the nanovaccine field. For instance, respiratory syncytial virus (RSV) is a worldwide threat to public health, especially infants and older adults, with no available vaccines. Most current candidates are based on the prefusion conformation of the fusion protein (Pre F). Due to its unstability and rapid conformation changes, the engineered antigen defined as DS-Cav1 has been generated based on the locked structure of the PreF. However, it may not be fully stable and also requires adjuvants to be effective. The 3-fold symmetry axes of the I53-50A provides a unique platform for the DS-Cav1 display at controllable density in highly ordered pattern, which resembles its natural conformation on the viral surface while retain its immunogenicity. Animal studies in mice and nonhuman primates have proven this system to be effective as about 10-fold higher neutralizing antibody responses were induced than trimeric DS-Cav1 and a total increase to be effective as about 10-fold higher neutralizing antibody responses were induced than trimeric DS-Cav1 and a total increase of 100-fold compared to the native PostF soluble molecule. This two-component protein nanoparticle has also been applied to the manufacture of a novel HIV vaccines displaying 20 stabilized native-like HIV-1 envelope trimer antigens.

To summarize, ab initio design of new protein platforms by computational tools has showed a vast prospect in the engineering field. Based on it, novel structures with atomic-level accuracy can be fabricated at will, for which takes the influence factors into consideration and adjusts their surface properties, such as polarity, core hydrophobicity and backbone regularity. Although in its early stages, de novo design-based nanoparticles will have an increasing impact on next generation vaccines.

5. Needle-Free Administration

Currently, vaccines are administered mainly by needle- and spring-based injection, which causes pain and suffering and requires healthcare training for vaccination. Every year, as many as 0.3 billion injections are thought to be unsafe due to needle-stick injuries, which not only put the public and health workers at risk of blood transmitted virus infections such as HIV but also increase the economic burden on healthcare systems. Because of these problems, needle-free vaccination alternatives are being sought as the most feasible approaches.

Mucosal delivery mimics the natural infection process of many respiratory viruses, such as influenza virus, Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome-coronavirus (SARS-CoV). The mucosa layer, a dense and dynamic mucus layer, the intact and active antigens displayed on nanostructures can interact and cross barriers and be internalized by APCs upon translocation from the mucosa-associated lymphoid tissue (MALT) to lymph nodes or other secondary lymphoid organs. Thus, systemic immune responses and mucosal responses are induced during this process, providing more effective and broad protection against pathogens, even at the site of entry. To achieve successful immunization through the nasal cavity, SANPs appear to be a promising approach because of their capacity to increase residence time and to improve interaction with the mucosal epithelium. In addition to some relevant examples of polymers, protein-based nanoparticles can also be applied for nasal delivery without requiring conventional adjuvants. A ferritin nanoparticle displaying 3M2e had much greater protective efficacy through intranasal delivery to immunize mice than that delivered in an intramuscular manner. The digestive tract is also part of the mucosal system, with gut-associated lymphoid tissue (GALT) mainly composed of Peyer’s patches and M-cells. However, its harsh gastric environment requires specialized material to improve antigen stability and bioavailability.

For DNA-based vaccines, the skin is a suitable site for inoculation. The underlying viable epidermis is absent of blood vessels and sensory nerve endings but is abundant in APCs, such as keratinocytes, melanocytes, and Langerhans cells (LCs). The evenly distributed LCs are critical for the uptake and processing of foreign materials for both naïve and antigen-specific T-cells with CD4+ and CD8+ phenotypes. In addition, keratinocytes are important sources of pro-inflammatory cytokines that may enhance immune responses. Thus, the skin is not only a physical barrier but also an easily accessible and practical target site for vaccine administration. A novel DNA vaccine encoding HA bivalently targeted to MHC II molecules on APCs has been shown to induce rapid, enhanced, and long-lasting HA-specific antibody titer in mice, ferrets, pigs, and rhesus macaques and it will be tested in humans. Another vaccine involving a trivalent HA DNA prime-trivalent inactivated influenza vaccine (IIV3) booster regimen was tested to be safe and well tolerated in children. In addition to the jet injector used in the above examples, other methods, such as microneedle injection, epidermal powder immunization, and particle-mediated gene-gun DNA immunization, can be advantageously used for antigen delivery. In addition, the solid-state and dry formulations of these vaccines allow them to be effective with room temperature stability.

As needle-free vaccination prevents the risk of incorrect or repeated use of injection devices, it is more likely to be accepted by patients and reduces worries over cross contamination. Free of specific administration materials (needles and syringes) and specialized techniques, adults can easily vaccinate themselves and their families at home. Altogether, these technologies result in improved vaccine coverage and eventual cost reduction. Therefore, needle-free administration potentially attains the final aim of universal accessibility to vaccines that are simultaneously effective, affordable, and safe.

6. Conclusion

As stated above, SANPs are proposed to be efficient, safe, and uniform delivery platforms. Some SANP-based vaccines can be administered effectively by a pain-free oral, nasal, or skin route. Some can also be made into solid-state or dry formulations and
remain effective at room temperature for months. Thus, they can be developed independent of a cold chain and are more convenient for long-distance transportation, leading to more widespread implementation of vaccination program.

However, SANP-based vaccines are still in their infancy, with few having precisely established including antigen release properties, internal distribution, or prophylactic mechanism in vivo thus far. Animal models, such as mice, ferrets, and thesus monkeys, do not mimic the natural process of human disease development, thus the fates of these vaccines are difficult to predict in humans and it takes a considerable long time for completion of clinical trials and licensing by the FDA. Therefore, the current majority of vaccines are merely the products of experimental study.

Currently, continued interdisciplinary progress related to immunology, toxicology, material science, and computational biology is catalyzing the rapid development of new SANPs against different viruses and a clearer understanding of nanoparticle pharmacodynamics and biodistribution in animal models and humans. In the near future, we can design SANP-based vaccines at will in an efficient, optimized, and suitable manner to make the most infectious diseases preventable.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

ferritin, influenza virus, self-assembled nanoparticle, vaccine, virus-like particle

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