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Synthetic virus-like particles prepared via protein corona formation enable effective vaccination in an avian model of coronavirus infection

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ABSTRACT

The ongoing battle against current and rising viral infectious threats has prompted increasing effort in the development of vaccine technology. A major thrust in vaccine research focuses on developing formulations with virus-like features towards enhancing antigen presentation and immune processing. Herein, a facile approach to formulate synthetic virus-like particles (sVLPs) is demonstrated by exploiting the phenomenon of protein corona formation induced by the high-energy surfaces of synthetic nanoparticles. Using an avian coronavirus spike protein as a model antigen, sVLPs were prepared by incubating 100 nm gold nanoparticles in a solution containing an optimized concentration of viral proteins. Following removal of free proteins, antigen-laden particles were recovered and showed morphological semblance to natural viral particles under nanoparticle tracking analysis and transmission electron microscopy. As compared to inoculation with free proteins, vaccination with the sVLPs showed enhanced lymphatic antigen delivery, stronger antibody titers, increased splenic T-cell response, and reduced infection-associated symptoms in an avian model of coronavirus infection. Comparison to a commercial whole inactivated virus vaccine also showed evidence of superior antiviral protection by the sVLPs. The study demonstrates a simple yet robust method in bridging viral antigens with synthetic nanoparticles for improved vaccine application; it has practical implications in the management of human viral infections as well as in animal agriculture.

1. Introduction

Vaccine is historically the most effective countermeasure against infectious threats, as agents resembling pathogens are administered to mount an immune response against specific targets. Amidst continuing and emerging viral threats, vaccine technology continues to advance with the aim of effectively promoting antiviral immune responses, and a major development effort lies in retaining or integrating virus-like features in vaccine formulations for improved immune processing. Several morphological and antigenic characteristics of viral particles have been demonstrated to promote immune potentiation. For example, particles at the nanoscale have been shown to have better lymphatic transport as compared to smaller subunit antigens [1,2]. In addition, the display of multiple antigens on a single particle facilitates more effective antigen presentation to immune cells [1]. As compared to traditional vaccine formulations, vaccines preserving virus-like features have shown superior capability in eliciting immune responses [3–5]. These results and observations have also prompted material scientists to apply synthetic nanomaterials towards mimicking viral features for vaccine development [6–9].

Given their high radii of curvature, synthetic nanoparticles frequently possess high surface energies that induce adsorption of biomolecules in a phenomenon known as protein corona formation. In protein-rich media, strong nanoparticle/protein association occurs spontaneously as a means to passivate surface energies, and the resulting particles are encased in a protein layer that dictates

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the particles' interactions with the environment [10,11]. While protein corona formation is gaining increasing scientific interest owing to its implications in biomedical applications [10,12,13], we herein demonstrate harnessing this phenomenon can be beneficial towards mimicking viral features for vaccine applications. We show that synthetic virus-like particles (sVLPs) with close semblance to native virions in physicochemical properties and antigen display can be facilely prepared through spontaneous antigen-particle association in optimized incubation conditions. Using 100 nm gold nanoparticles (AuNP), a biologically inert material commonly used for biomedical research [14–16], and a spike glycoprotein derived from an avian infectious bronchitis virus (IBV), a single-stranded positive-sense RNA virus that belongs to the family *Coronaviridae* [17], we controlled the incubation condition to prepare spike glycoprotein-laden sVLPs (Fig. 1). The morphological features and antigen display by the sVLPs were compared to native IBV viral particles using nanoparticle tracking analysis and immunogold staining. In addition, vaccination potency between the sVLPs and free spike glycoproteins was compared in an avian model of coronavirus infection. A commercial whole inactivated virus (WIV) formulation that is the current standard vaccine for IBV management was examined in parallel.

Coronaviruses are a major viral family of which the most publicized examples include the pathogens behind severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) [18]. In animals, IBV is a prime example of coronavirus that infects the respiratory and urogenital tracts of chickens, posing a serious economic threat as one of the most important pathogens in the poultry industry. The IBV spike glycoprotein, which forms the large, pental-shaped spikes on the surface of the virion, is chosen as the antigen candidate as it is implicated as a determinant of virus pathogenicity. Among coronaviruses, spike glycoproteins possess a variety of biological functions, including triggering cell attachment, inducing cell-cell fusion, and binding to cellular receptors [19,20]. As spike glycoproteins are the primary targets in ongoing vaccine development efforts for coronavirus vaccinations, the present study has broad implications across both human and animal disease management [21,22].

2. Materials and methods

2.1. Cells and gold nanoparticles

S. frugiperda Sf9 (ATCC CRL-1711) insect cells were cultured in Grace's insect cell medium (Invitrogen, Carlsbad, CA) and supplemented with 10% FBS (Thermo Fisher, Rockford, IL) and 1% P/S/A antibiotics (Biological Industries, Beit-Haemek, Israel) at 27 °C. 100 nm gold nanoparticle (AuNP) solution was purchased from Sigma-Aldrich (St. Louis, MO).

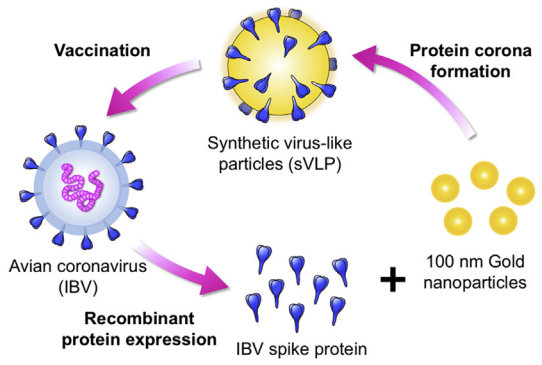

Fig. 1. Schematics illustrating the preparation of an avian coronavirus sVLPs. sVLPs are prepared in optimized mixtures containing viral proteins and 100 nm gold nanoparticles via spontaneous protein corona formation.

2.2. Propagation of IBV

Avian coronavirus IBV strain 2575/98 was propagated in 10-day-old specific-pathogen-free (SPF) chicken embryos via the allantoic route as previously described [23]. The virus titers of IBVs were determined with the method of Reed and Muench [24] in SPF chicken embryos and expressed as 50% embryo infectious dose (EID_{50}) [25]. The virus-containing allantoic fluid was concentrated and purified using sucrose gradient solution as previously described to derive the native virions [23].

2.3. Preparation of recombinant IBV spike proteins

Full spike (S) protein of avian coronavirus IBV was cloned and expressed using the Bac-to-Bac baculovirus expression system (Invitrogen). Briefly, a recombinant plasmid was constructed by inserting full spike protein gene of IBV strain 2575/98 (accession no. DQ646405) [26] into the pFastBac-1 vector using the following primer set: IBV-S-BamHI-f: 5′- TTGGG ATCCG ATGTT GGTGA AGTCA C-3′; IBV-S-SalI-f: 5′-CTTGT CGACA TTAAA CAGAC TTTTT AGGT-3′. The recombinant pFastBac-1 shuttle vector was then transposed to the bacmid in *E. coli* strain DH10Bac, and recombinant bacmid was purified using the HiPure Plasmid Midiprep kit (Invitrogen). Sf9 cells were used for transfection with the recombinant bacmid, and recombinant baculoviruses were then harvested in the supernatant and designated rBac-2575S. Recombinant spike proteins (r2575S) were harvested from Sf9 cells infected with rBac-2575S (multiplicity of infection = 1). Sf9 cells were washed and lysed with the I-PER insect cell protein extraction reagent (Thermo Fisher). Recombinant proteins were purified using the Glycoprotein Isolation Kit, ConA (Thermo Fisher) according to the manufacturer's instructions. After purification, r2575S protein was stored in 10% sucrose at −20 °C.

2.4. Preparation of synthetic virus-like particles

Citrate-buffered 100 nm gold nanoparticles were washed repeatedly in water to remove the citrate stabilizer, and the resulting pellet was resuspended in 10% sucrose. Protein solutions ranging in concentrations between 100 μg/mL to 3 mg/mL of purified spike proteins were then mixed with 1×10^{11}/mL of gold nanoparticles (determined by nanoparticle tracking analysis) in 10% sucrose. The mixtures were bath sonicated for 1 min followed by incubation in an ice bath for 30 min. The nanoparticles were then removed from unbound spike proteins via centrifugation at 1500*g* for 3 min. Following 3 centrifugal washes with 10% sucrose, pelleted nanoparticles were mixed with 1X PBS and sonicated in a bath sonicator for 30 s. Dispersible, stabilized sVLPs were retrieved and their protein content was quantified using a BCA protein assay (Thermo Fisher) with 25 μL of 1×10^{11} particles/mL following the manufacturer's protocol. Visualization of unstable nanoparticles and colloidally stable sVLPs was performed using a 200 kV high resolution transmission electron microscope (FEI Tecnai TF20). Particle stability was assessed by monitoring the size of sVLPs for 7 days. Particle size, polydispersity index (PDI), and concentrations were measured by nanoparticle tracking analysis using Nanosight NS-500 (Malvern, UK) at a concentration of 1×10^{8} particles/mL based on the manufacturer's instructions. Particle size and zeta potential were also measured by dynamic light scattering using Zetasizer Nano ZS at a concentration of