Inhalable SARS-CoV-2 Mimetic Particles Induce Pleiotropic Antigen Presentation

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Statement of Purpose: Covid-19 has caused over 4.5 million deaths worldwide and continues to ravage communities with limited access to injectable vaccines, or high rates of vaccine hesitancy. Inhalable vaccines have the potential to address these distribution and compliance issues as they are less likely to require cold storage, avoid the use of needles, and can elicit potent localized immune responses with only a single dose. Alveolar macrophages represent attractive targets for inhalable vaccines as they are abundant within lung mucosa (90-95% of all immune cells), are important mediators of mucosal immunity, and evidence suggests may be key cellular players in early Covid-19 pathogenesis. Here, we report inhalable coronavirus mimetic particles (CoMiP) designed to rapidly bind to, and be internalized by, alveolar macrophages to deliver nucleic acid encoded viral antigens, inducing expression of SARs-CoV-2 antigens at the local antigen presenting cells to kickstart immunogenic cascade.

Method: CoMiP synthesis began via electrostatic complexation of plasmid DNA encoding viral proteins with cationic poly-L-lysine. The resulting polyplex was then coated with anionic hyaluronic acid carbohydrates. Particle morphology size and surface charge were characterized by scanning electron microscopy, dynamic light scattering, and zeta potential measurements, respectively. Time dependent uptake and transgene expression in THP-1 monocyte cultures was confirmed via fluorescent confocal microscopy and flow cytometry using a model GFP-encoding reporter plasmid ($CoMiP_{GFP}$). Similarly, in *vitro* expression of SARs-CoV-2 antigens were quantified by flow cytometry and confocal microscopy in murine macrophage cells (RAW264.7), and results benchmarked against lipofectamine. In *vivo* immunization studies were conducted in C57BL/6 mice following intranasal administration of CoMiP loaded with Spike protein-encoding plasmid ($CoMiP_S$). Humoral (IgG) and mucosal (IgA) antibody production at different times after vaccination were quantified by ELISA. Biocompatibility of CoMiP was additionally assessed in *vivo* and in *vitro* by lung histology and MTT cytotoxicity assay, respectively.

Results: Structural characterization of CoMiP indicated the formation of spiky nanoparticles that resemble the size (~150nm) and surface topography of the SARS-CoV-2 virion (Figure 1A). Further surface charge characterization confirmed the display of hyaluronic acid at the surface of the particle, which is essential for recognition and rapid uptake by alveolar macrophages. Accordingly, robust particle internalization into human macrophages was observed after a 4 –hour incubation, and endosomal escape observed after 24 hours. Treatment of macrophages with CoMiP particles loaded with SARs-CoV-2 antigen-encoding plasmids exhibited robust transgene expression, particularly for spike, envelope and nucleocapsid proteins. Intranasal immunization of mice with CoMiP particles containing spike protein-encoding plasmids ($CoMiP_S$) showed a significant increase in total mucosal IgA in the lung, while differences in spike protein-specific IgA was not observed relative to controls. In conclusion, CoMiP carriers represent a versatile aerosolizable material that, with continued optimization, could lead to the development of safe and effective inhalable vaccines for SARS-CoV-2, and potentially other respiratory viruses.

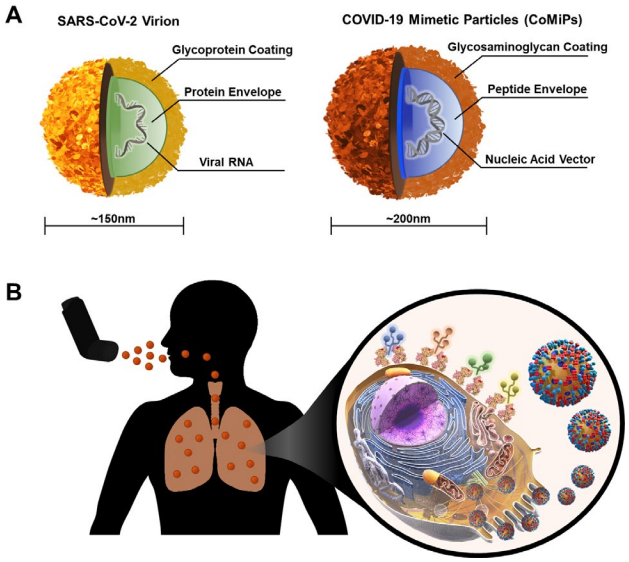

Figure 1: (A) Schematic representation of the structural and compositional similarities between SARS-CoV-2 virions (left) and rationally designed Covid-19 Mimetic Particles (CoMiP; right). (B) CoMiP aerosols engage CD44 receptors on the surfaces of alveolar macrophages in lung tissue to gain intracellular entry. Cytoplasmic delivery and expression of nucleic acid cargo leads to presentation of various encoded SARS-CoV-2 antigens to stimulate local mucosal immunity.