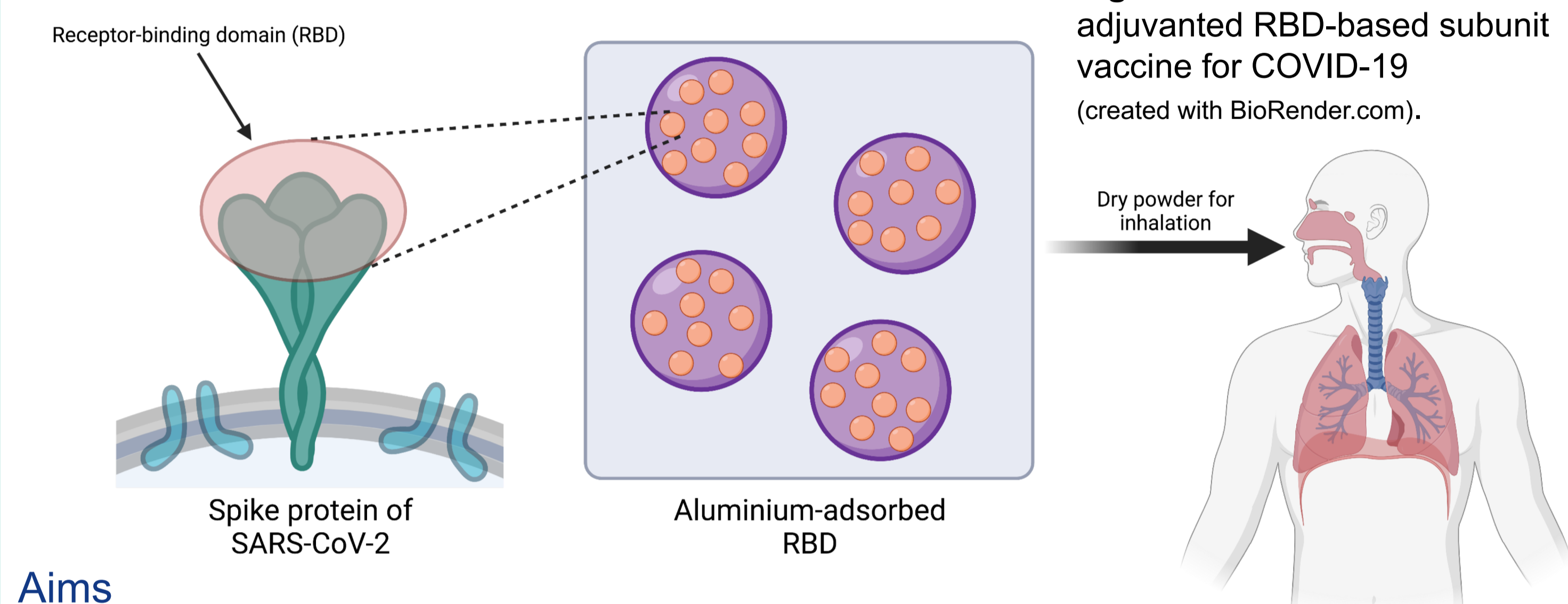


## Introduction

Mass vaccination against COVID-19 remains one of the mainstay approaches in combating the pandemic. The key target antigen of SARS-CoV-2 is the **receptor-binding domain (RBD)** of the surface spike protein. The RBD binds to angiotensin-converting enzyme 2 (ACE2) to gain entry into host cells.

Although parenteral COVID-19 vaccines are able to provide adequate systemic immunity, **mucosal vaccination** can induce **robust mucosal immune responses at the initial site of infection** while also eliciting systemic immune protection. Inhaled vaccines may be formulated into a dry powder that does not require the cold chain during transport and storage, representing a significant economic and logistical relief.



### Aims

- To develop an aluminium-adsorbed RBD-based subunit COVID-19 vaccine for inhalation by spray-freeze-drying
- To characterise the spray-freeze-dried (SFD) powder vaccine in terms of particle size, antigen-adsorbent binding efficiency, and *in vivo* efficacy

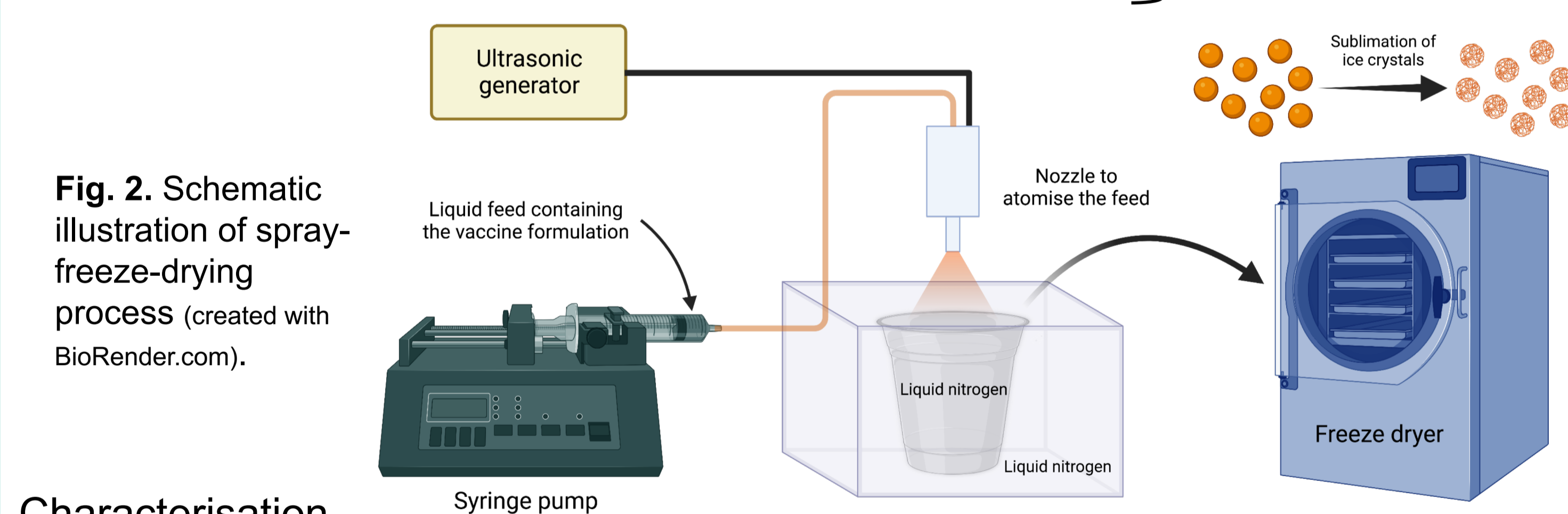
## Methods

### Spray-freeze-drying

Antigen: **RBD protein**, 0.15 mg/mL (developed by Microbiology Department, HKU)

Excipient 1: **2-hydroxypropyl-beta-cyclodextrin**, 2% w/w (protein stabiliser)

Excipient 2: **aluminium hydroxide**, 0.5 mg/mL aluminium (adjuvant to enhance antibody responses)



### Characterisation

#### Laser diffraction (LD) → particle size distribution

SFD powder was dispersed from a dry powder inhaler (breezhaler®, Novartis) at an airflow rate of 60 L/min into a laser diffractometer (HELOS/KR+INHALER, Sympatec). Measurements were done in triplicate.

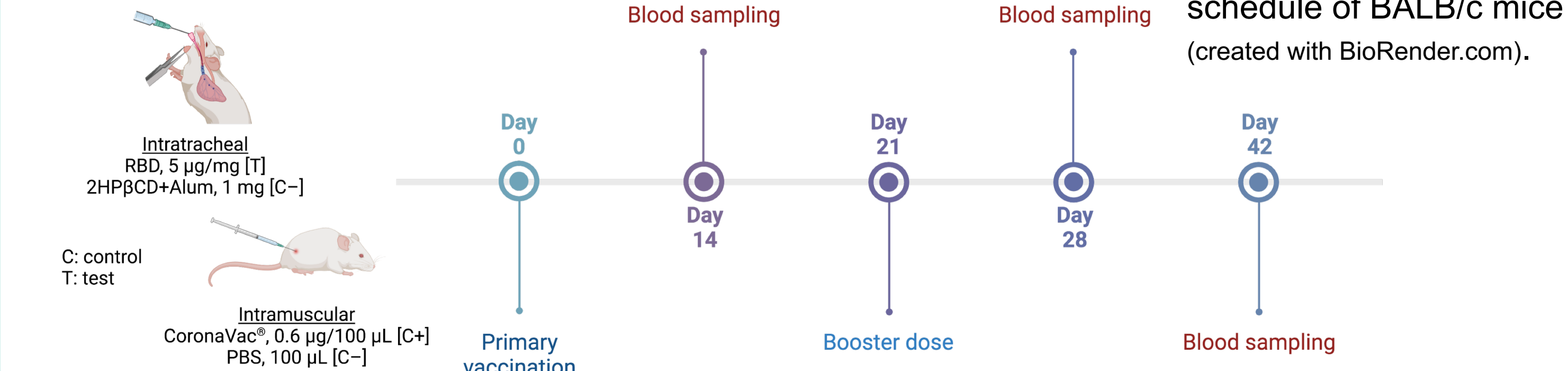
#### Scanning electron microscopy (SEM) → particle morphology

Powder sample was coated with gold-palladium (Q150R ES Plus, Quorum Technologies) and imaged at an accelerating voltage of 5 kV (Hitachi S-4800).

#### Polyacrylamide gel electrophoresis (SDS-PAGE) → antigen-adsorbent binding

Reconstituted SFD powder was loaded into each well (Mini-PROTEAN® Tetra system, Bio-Rad) and subjected to electrophoresis. The gel was then stained in Coomassie blue dye. The intensity of the protein band reflects the amount of free, unbound antigen.

### Immunogenicity assessment



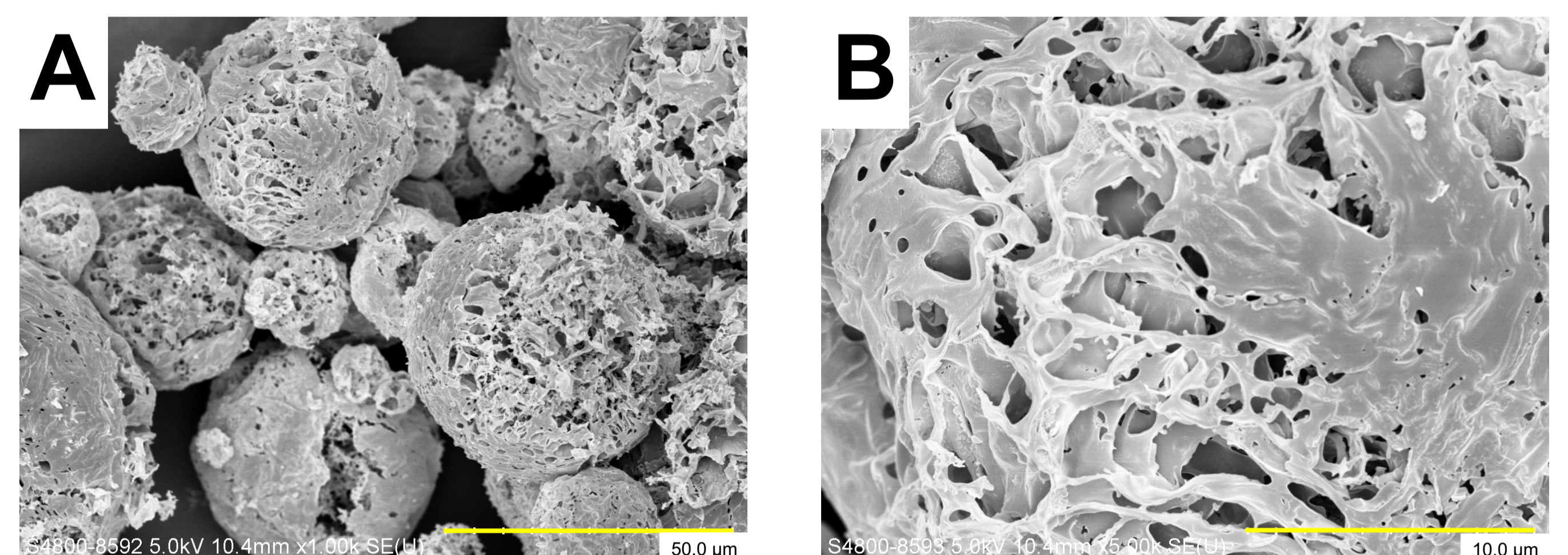
## Results & Discussion

The volume diameters (Table 1) suggest that the particles were probably too coarse for deep lung penetration, but ideal for deposition in the human nasal cavity. Particles of diameters > 10 µm would be preferentially deposited along the nasal passage. However, considering the particle size range, the majority of the SFD particles probably would deposit in the anterior vestibular region in humans due to significant inertial impaction. To maximise deposition the central nasal regions, the particle sizes should fall between 10 and 11 µm.

**Table 1.** Processing yield and volumetric size distribution (n=3) of the SFD particles. Data are presented as mean ± standard deviation.

Yield	D <sub>10</sub>	D <sub>50</sub>	D <sub>90</sub>	Span
86.1%	10.75 ± 1.10 µm	37.62 ± 3.35 µm	70.93 ± 5.65 µm	1.60 ± 0.02

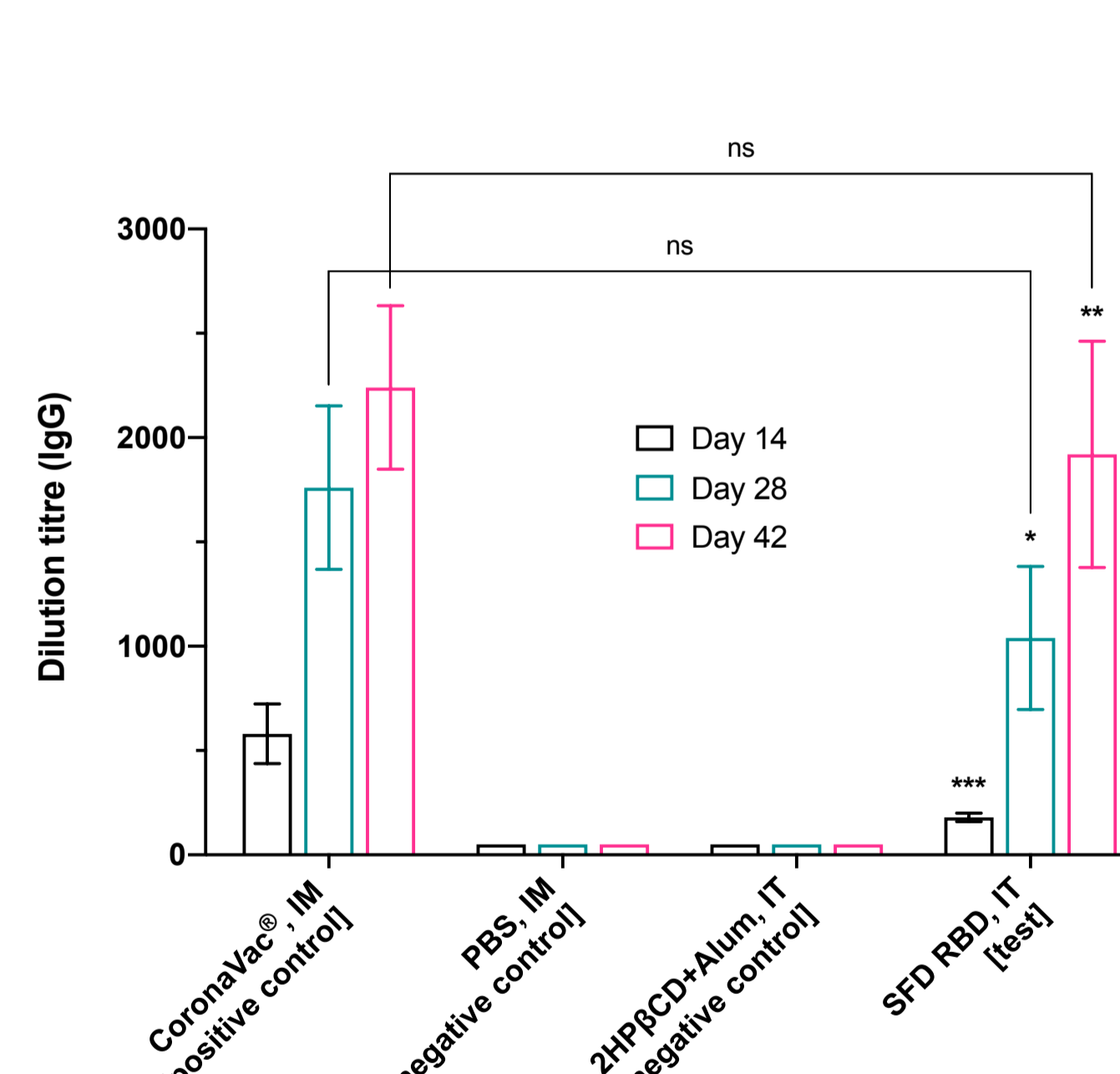
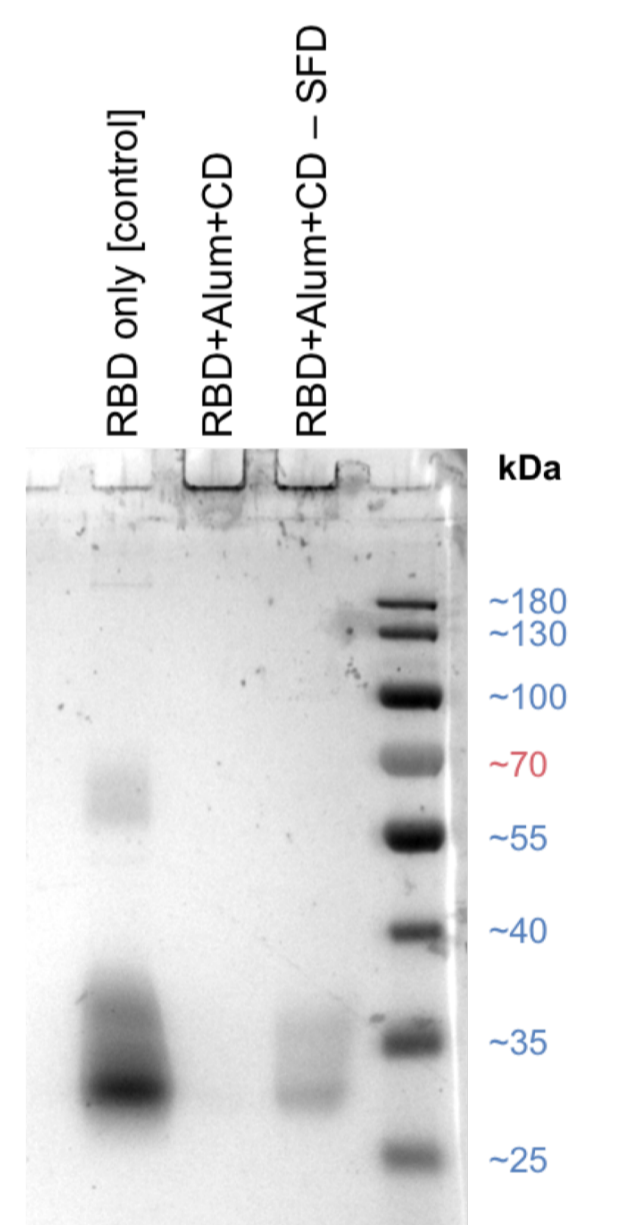
Yield is defined as the percentage of the powder mass in the product collection vessel to the mass of the total solid loading in the feed suspension. D<sub>10</sub>/D<sub>50</sub>/D<sub>90</sub> represent the diameters of volume-equivalent spheres at 10/50/90% cumulative volumes, respectively. Span = (D<sub>90</sub> - D<sub>10</sub>) / D<sub>50</sub>



**Fig. 4.** Scanning electron micrographs of spray-freeze-dried vaccine at (A) 1000x and (B) 5000x magnifications. Large particles with spherical morphology and high porosity such as these are associated with improved dispersibility and aerosol delivery.

**Fig. 5.** SDS-PAGE gel image (one of three replicates). The binding efficiency of the RBD to the aluminium hydroxide particles in the feed suspension (middle lane) was 86.5 ± 3.7%, whereas it was 67.9 ± 7.8% in the reconstituted SFD vaccine (right lane). The binding was lower after spray-freeze-drying, implying that the technique could be a contributing factor, although most of the interactions were strong enough to withstand the process.

Alum: aluminium hydroxide; CD: 2-hydroxypropyl-beta-cyclodextrin; RBD: receptor-binding domain (antigen); SFD: spray-freeze-dried



**Fig. 6.** Humoral (IgG) immune response in BALB/c mice (n=5 per group). The *in vivo* potency of IT RBD was comparable to that of CoronaVac® (an inactivated COVID-19 vaccine) at days 28 and 42. The antigen-specific IgG titres induced by IT RBD were also significantly higher than those induced by the negative control groups (IT 2HPβCD+alum & IM PBS) as early as day 14.

Alum: aluminium hydroxide; CD: 2-hydroxypropyl-beta-cyclodextrin; IM: intramuscular; IT: intratracheal; ns: not significant; SFD: spray-freeze-dried.

\* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001 (cf. negative controls at the same time point)

## Conclusions

This work demonstrates the promising use of spray-freeze-drying to generate a powder subunit vaccine for COVID-19. The aluminium-adsorbed RBD antigen delivered via intratracheal administration stimulated satisfactory systemic immune response in mice.

There is room for optimisation of the particle size to better target mucosa-associated lymphoid tissues in humans and the aluminium concentration to maximise the efficiency of antigen-adsorbent binding. Future work also warrants exploration of the prophylactic efficacy when administered intranasally.

### References

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