Improved in vitro in vivo correlation (IVIVC) in freeze-dried particle deposition patterns of antigen-containing liposomes

Matteo Aroffu[1,2], Fátima García-Villén[2], Maria Letizia Manca[1], Maria Manconi[1] & José Luis Pedraz[2]
[1] Department of Scienze della Vita e dell'Ambiente, University of Cagliari, Italy
[2] Laboratory of Pharmaceutics, School of Pharmacy, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, Spain

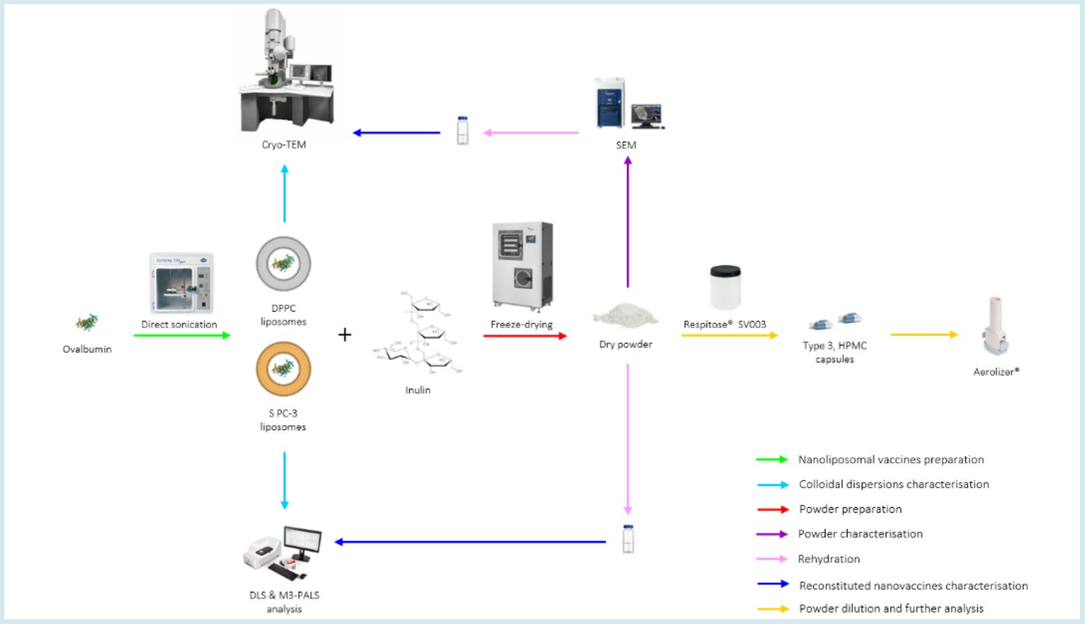
INTRODUCTION

- The major challenge in vaccine administration is related to the sensitivity of antigens to conditions such as aerosolisation [1] and metabolically active environments [2].
- Liposomes in form of dry powders are a valid strategy to improve stability while allowing administration to the lungs [3].
- However, since the mode of inhalation, as well as the impedance of the mucus, can negatively affect the therapeutic outcome, it is now crucial to perform analysis with improved in vitro-in vivo correlation (IVIVC) [4].

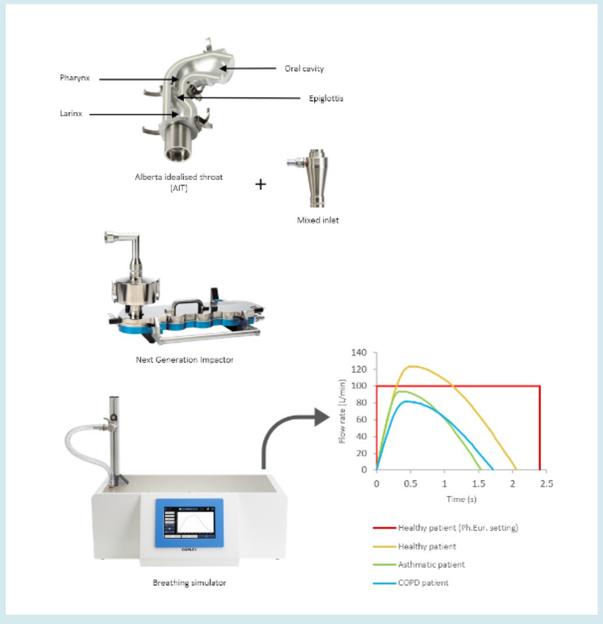
OBJECTIVE

1. Obtain liposomal nanovaccine powders suitable for pulmonary administration.
2. Explore antigen deposition under IVIVC conditions.
3. Assess mucus hindrance in vesicle crossing.

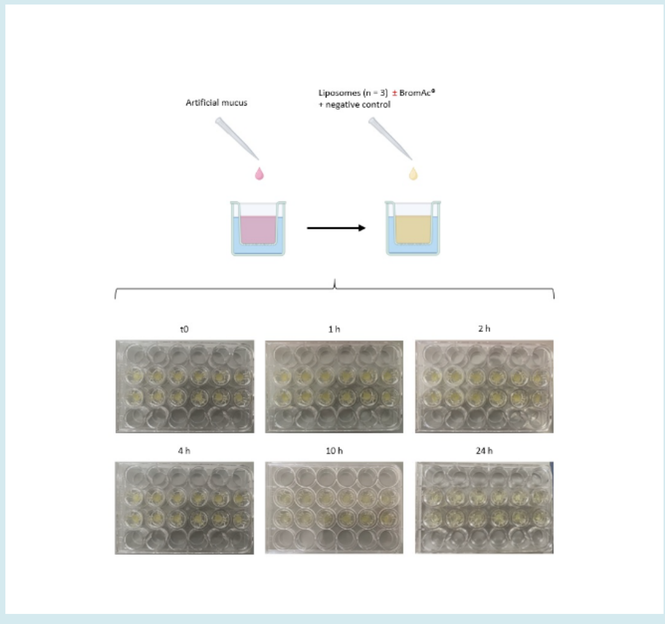
METHODS



Ovalbumin-loaded liposomal nanovaccines were prepared by direct sonication. Inulin was chosen as the cryoprotectant and lipid to cryoprotectant ratios from 1:1 to 1:3 were tested. Only formulations with a final to initial size ratio (Sf/Si) < 1.3 were further characterised for physicochemical and morphological properties. To perform the in vitro studies on aerodynamic performance, the Aerolyzer® was chosen as the dry powder inhaler (DPI) and freeze-dried powders were diluted 1:10 with Respitose® SV003.



The Next Generation Impactor (NGI) was paired with the adult Alberta Idealised Throat (AIT) and the BRS 3000 breath simulator to improve in vitro in vivo correlation (IVIVC); the former provides a more anatomically accurate throat and the latter allows for realistic inhalation profiles based on literature data.



A mucus penetration study was conducted using an artificial mucus model on Transwell® (6.5 mm well, 5.0 µm pore). The vesicles were tested either in the absence or presence of the patented mucolytic agent BromAc® at a concentration of N-acetyl cysteine of 2% (w/v) and bromelain of 100 µg/ml.

RESULTS

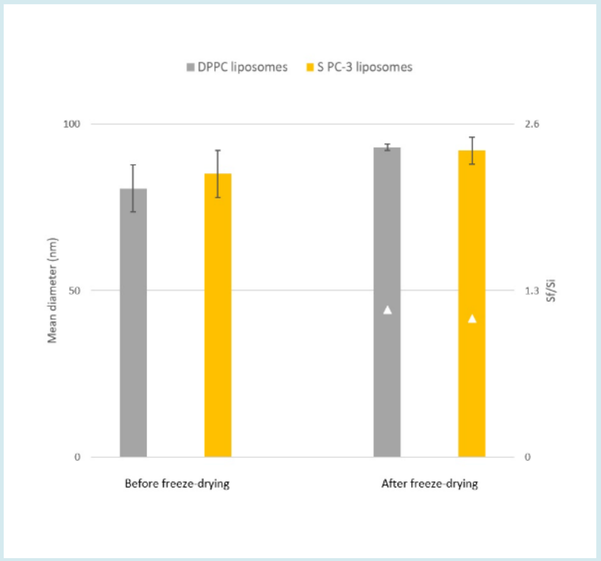


Fig.1 Mean diameter (nm) before and after freeze-drying and rehydration of DPPC liposomes (in grey) and S PC-3 liposomes (in yellow). Final size to initial size ratio (Sf/Si) is showed as well as parameter of freeze-drying effectiveness.

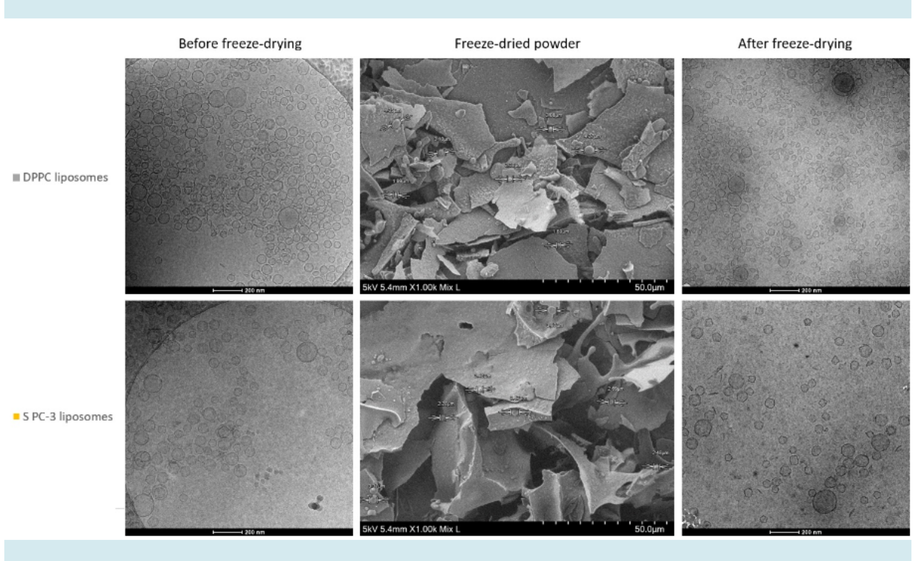


Fig.2 Cryo-TEM of DPPC liposomes (in the upper panel) and S PC-3 liposomes (in the lower panel), before freeze-drying (on the left column) and after freeze-drying and rehydration (on the right column). SEM of the respective powders are shown in the central column.

CONCLUSION

- Inulin was effective in protecting vesicularl vaccines with an inulin:lipid ratio of 3:1, as Sf/Si was <1.3 (**Fig 1**).
- The actual formation of liposomes and powders was confirmed by electron microscopy (**Fig. 2**).
- Reconstitution in water took place after a few seconds.
- The fine particle dose (FPD), as well as the fine particle fraction (FPF) and the stage of deposition (data not shown), were influenced by the condition (**Fig. 3**).
- The mucolytic agent BromAc® significantly improved mucus penetration of the nanovaccines (**Fig. 4**).

FUTURE WORK

- The reconstitution will be evaluated in a simulated interstitial lung fluid (SILF) as it is better representative of the environment in the lungs.
- Uptake studies will be carried out on alveolar macrophages, since they are the most relevant immune cell in the lungs.
- Confocal microscopy will be used to assess cellular uptake.

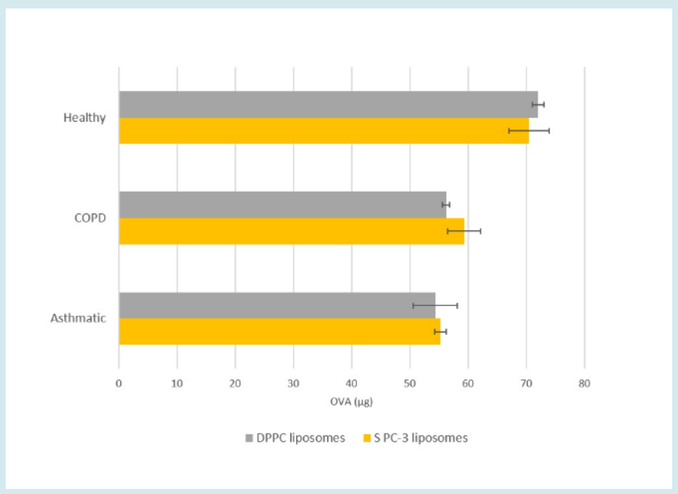


Fig. 3 Fine particle dose (FPD) for DPPC liposomes (in grey) and S PC-3 liposomes (in yellow) under simulation of healthy, asthmatic and COPD profiles.

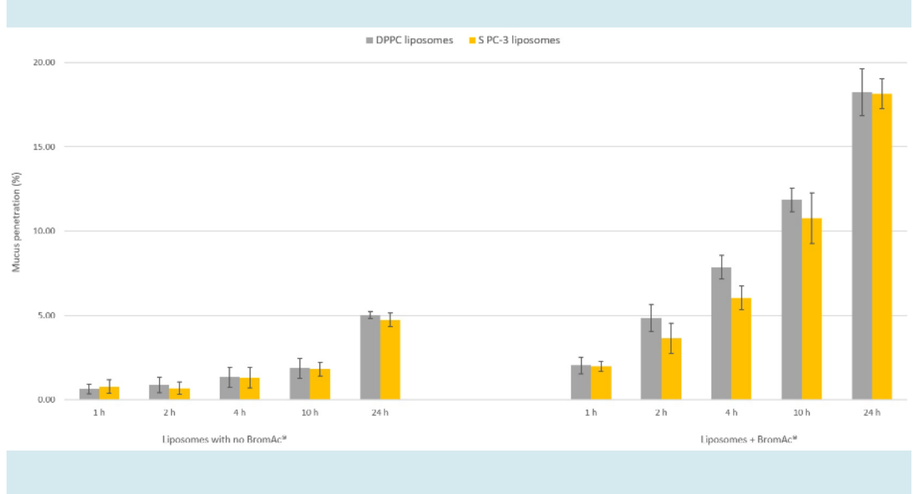


Fig. 4 Mucus penetration (%) of DPPC liposomes (in grey) and S PC-3 liposomes (in yellow) either in absence (on the left) or in presence (on the right) of BromAc® (2% w/v N-acetyl cysteine and 100 µg/ml Bromelain).

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