Lipid Polymer Hybrid Nanoparticles for Pulmonary mRNA Delivery

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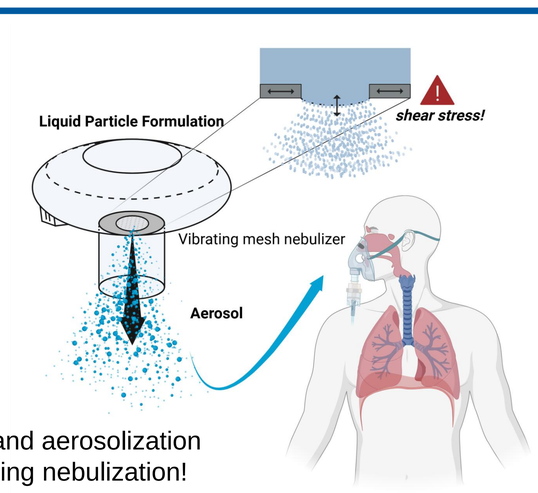
Why deliver mRNA into the lungs?

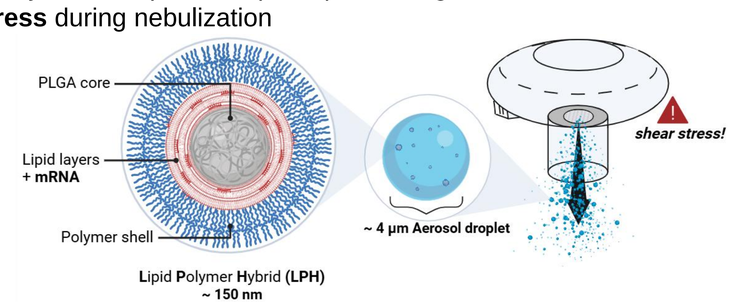
- Possible therapeutic areas:
  - Cystic fibrosis (CF)
  - Asthma
  - Primary ciliary dyskinesia (PCD)
  - α-1 antitrypsin deficiency
  - **Mucosal vaccination**
  - **Antiviral therapy**
  - …



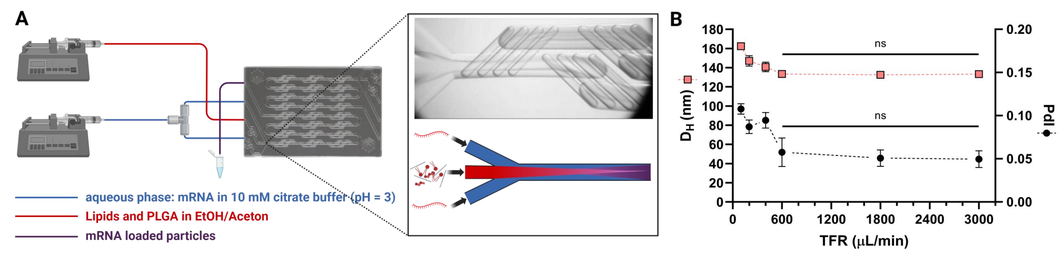
- Challenges:
  - **Technological Barriers:**
    Stable formulations required for storage and aerosolization
    ➢ Conventional LNPs tend to rupture during nebulization!
  - **Biological Barriers:**
    Penetration of extracellular barriers (mucus), cell-uptake and endosomal escape

Lipid Polymer Hybrids (LPHs)

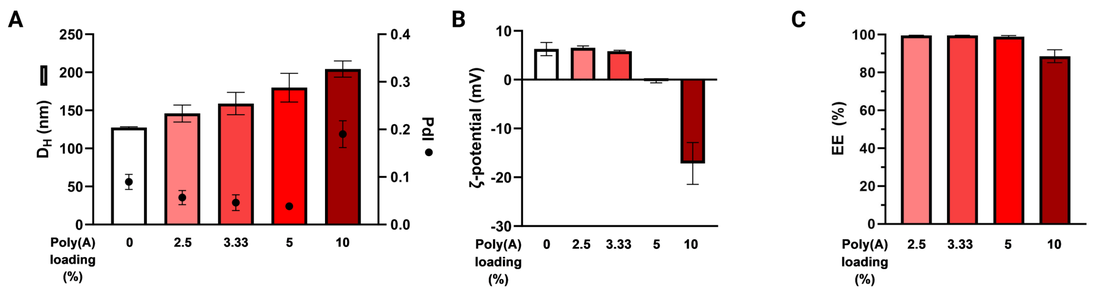
- **L**ipid **P**olymer **H**ybrid nanoparticles (LPHs) are designed to endure harsh mechanical forces and **shear stress** during nebulization



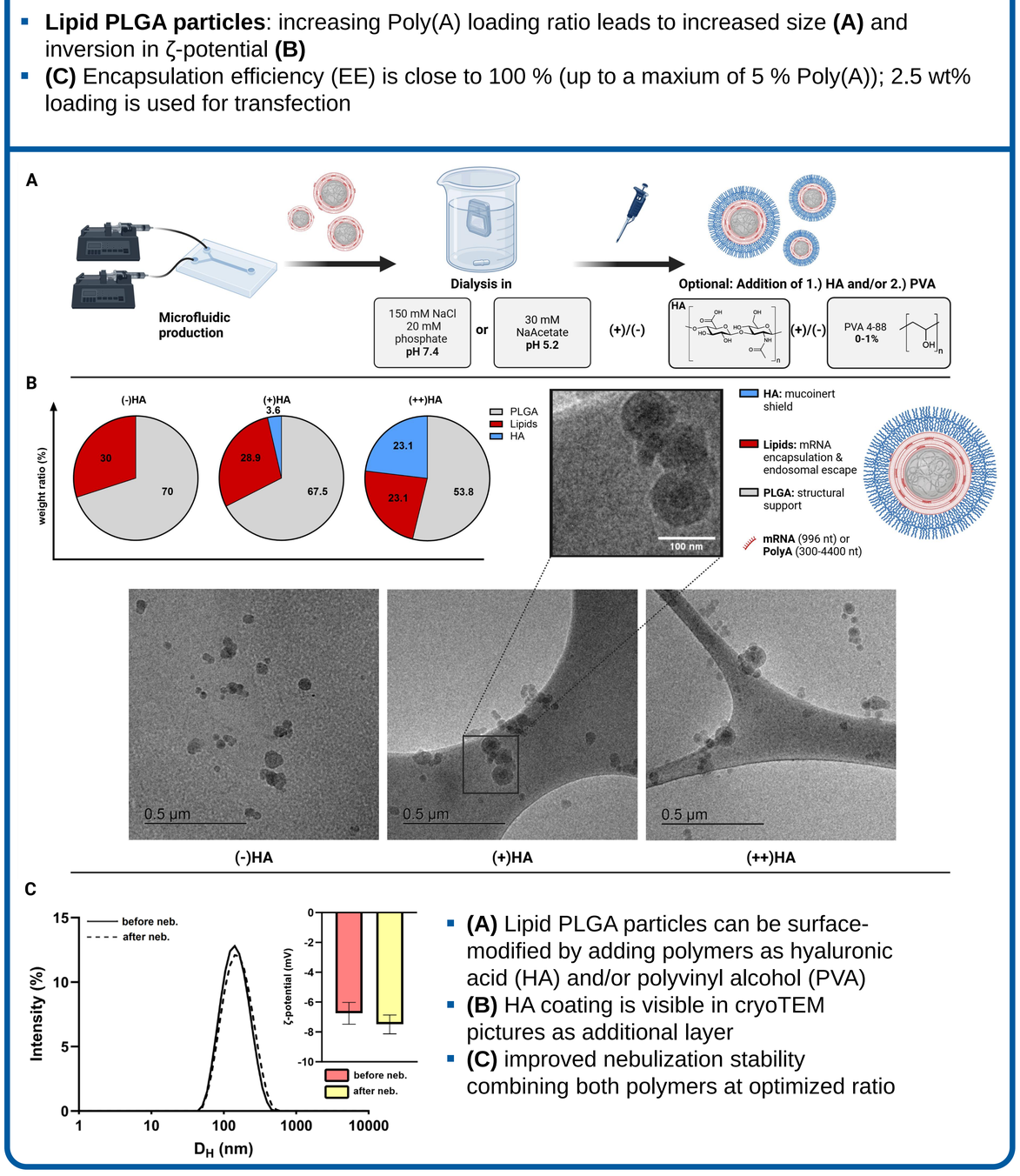
- Single step microfluidic production with a micromixer glass chip **(A).** Increasing total flow rate (TFR) reduces particle size and PdI **(B)**



Physicochemical characterization of LPHs

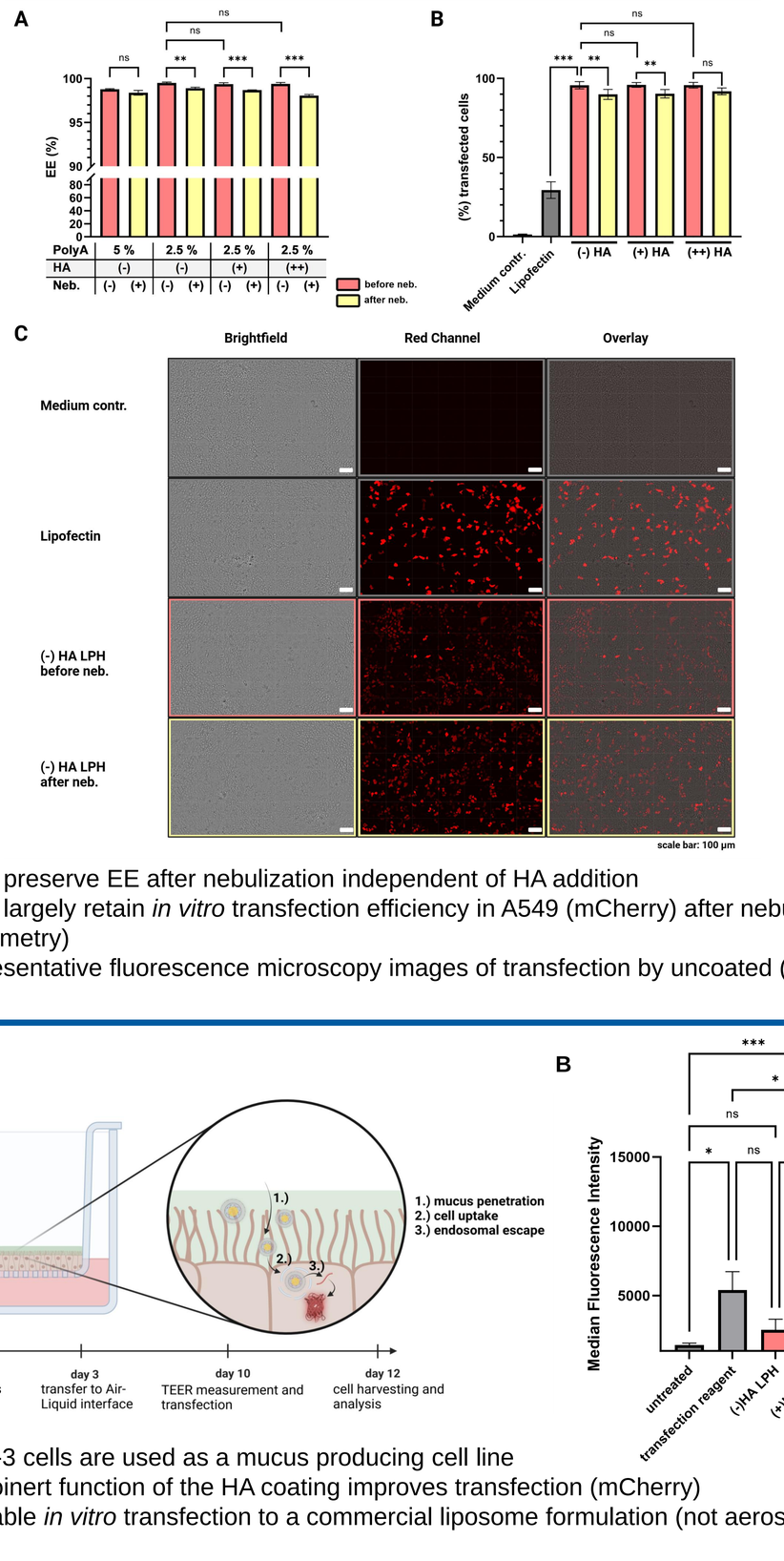


- **Lipid PLGA particles**: increasing Poly(A) loading ratio leads to increased size **(A)** and inversion in ζ-potential **(B)**
- **(C)** Encapsulation efficiency (EE) is close to 100 % (up to a maxium of 5 % Poly(A)); 2.5 wt% loading is used for transfection



- **(A)** Lipid PLGA particles can be surface-modified by adding polymers as hyaluronic acid (HA) and/or polyvinyl alcohol (PVA)
- **(B)** HA coating is visible in cryoTEM pictures as additional layer
- **(C)** improved nebulization stability combining both polymers at optimized ratio

Biological characterization of LPHs



- **(A)** LPHs preserve EE after nebulization independent of HA addition
- **(B)** LPHs largely retain *in vitro* transfection efficiency in A549 (mCherry) after nebulization (flow cytometry)
- **(C)** Representative fluorescence microscopy images of transfection by uncoated ((-)HA) LPHs

- **(A)** Calu-3 cells are used as a mucus producing cell line
- **(B)** Mucoinert function of the HA coating improves transfection (mCherry)
- ➢ Comparable *in vitro* transfection to a commercial liposome formulation (not aerosolizable!)

Conclusion and Outlook

- **LPHs** were successfully designed as platform for pulmonary mRNA delivery, overcoming the associated main barriers
  - ➢ Stability during nebulization, mucus penetration and endosomal escape
- Experiments with human lung organoids as well as *in vivo* experiments in mice (intratracheal and intranasal administration) are planned

References

[1] Lokugamage et al. (2021) *Nat Biomed Eng*
[2] Kliesch et al. (2022) *Pharmaceutics*
[3] Meyer et al. (2022) *Int J Pharm*
[4] Colombo et al. (2015) *J Control Release*

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