Nanoparticle system for improved pulmonary delivery

Background

The lung offers various beneficial characteristics as a target organ for therapeutic approaches. Beside the large (hundreds of square meters) and very well perfused (5 l/min) surface area, extremely thin epithelium (0.1–0.2 µm) and high blood volume in pulmonary capillaries (0.25 l), it also captivates with a relatively low enzyme activity and slow surface clearance.

Owing to its location and function, the pulmonary region is susceptible to a number of specific diseases, being directly accessible to harmful substances (1). Unfortunately, treatment of lung-related pathologies poses a challenge for impermeable therapeutic agents. As these agents cannot cross the cellular membrane, they can greatly benefit from a targeted carrier.

Technology

We identified SP-B, an endogenous protein, as a key component in lung surfactant that strongly improves lung cell permeability for cytosolic delivery. SP-B, combined with our nanoparticle technology, can be applied to deliver various cargo (such as proteins, drug molecules and siRNA) to pulmonary cells. E.g., we integrated SP-B's functionality in a proteolipid nanoparticle by enveloping an siRNA loaded dextrane core with a specific composition of lipids and SP-B.

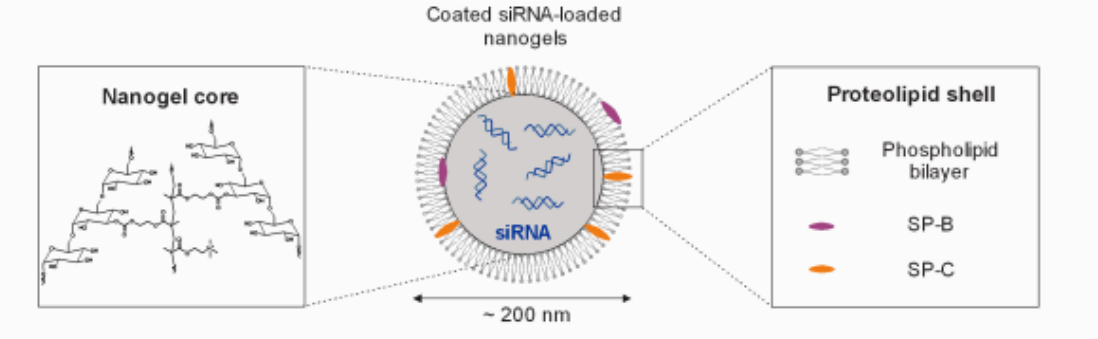

Figure 1. Schematic illustration of the (proteo)lipid coated siRNA-loaded nanogels (siNGs).

The developed nanocomposites have a low *in vivo* toxicity and show a high uptake by alveolar macrophages, a main target cell type for treatment of inflammatory pulmonary pathologies (2).

Unique selling points

- Reduced seed-mediated off-target effects (enabling low-dosage strategies)
- Improved release of therapeutic agent in the cytosol
- SP-B promotes prolonged lung distribution and retention
- Reduced non-specific interactions by the negative lipid coat
- Customizable membrane charge density (modular approach for intracellular delivery of membrane-impermeable agents)
- No interference of lung fluid components

Proof of concept

These results demonstrate the potential of the endogenous protein **SP-B** as an intracellular siRNA **delivery enhancer**. Indeed, SP-B's wide range for nanoparticles/cargo (high siRNA loading capacity, controlled and enhanced siRNA release (> 10-fold improvement of siRNA delivery efficiency)) is paving the way for future design of nanoformulations for siRNA and other inhalation therapies (2).

In figure 1, several delivery compositions were compared:

- siNGs: siRNA-loaded nanogels (siRNA against eGFP)
- coated siNGs (CS): siNGs coated with porcine pulmonary surfactant (CS)
- coated siNGs (LIP): siNGs coated with lipid mixture
- + SP-B: siNGs coated with lipid mixture and SP-B (0.4 wt%)
- + SP-C: siNGs coated with lipid mixture and SP-C (0.7 wt%)
- + SP-B, + SP-C: siNGs coated with lipid mixture, SP-B (0.4 wt%) and SP-C (0.7 wt%)

The siNGs and CS-coated siNGs act as reference for delivery efficiency. They are cationic and can therefore easily access negatively charged tissue, but cannot be applied *in vivo*. In contrast, the (proteo)lipid coating (LIP-coated siNGs, +SP-B, +SP-C, +SP-B and SP-C) allows *in vivo* targeting.

The absence of SPs (LIP-coated siNGs coated with the DPPC:DOPC:eggPG (LIP) mixture only) impaired cellular internalization and abrogated the gene silencing potential of the siNGs (Fig. 2 right). Interestingly, despite a similar reduction in cellular siRNA uptake, the addition of 0.4 wt% SP-B resulted in 40% eGFP gene suppression, an effect that could not be mimicked by SP-C. Although the extent of eGFP silencing obtained by the Curosurf® coating could not be reached, these pilot data clearly suggest that SP-B is a key molecular determinant for the enhanced siRNA delivery effects initially observed earlier for Curosurf®.

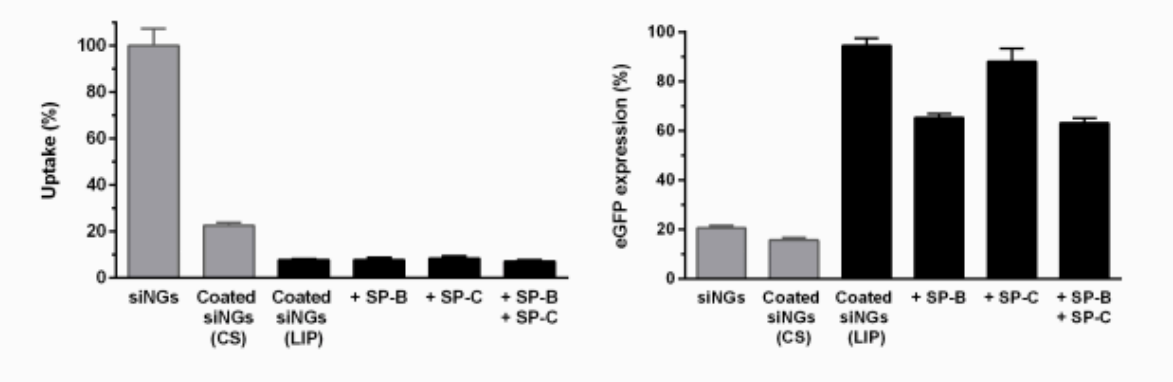

Figure 2. In vitro / lung cell lines. Biological activity of siNGs coated with surfactant protein containing proteolipid mixtures. Evaluation of (left) cellular uptake and (right) gene silencing potential of siNGs in H1299_eGFP cells determined via flow cytometry.

In vivo SP-B is critical for the developed formulation to obtain a significant silencing of TNFa in a murine LPS-induced acute lung injury model (2).

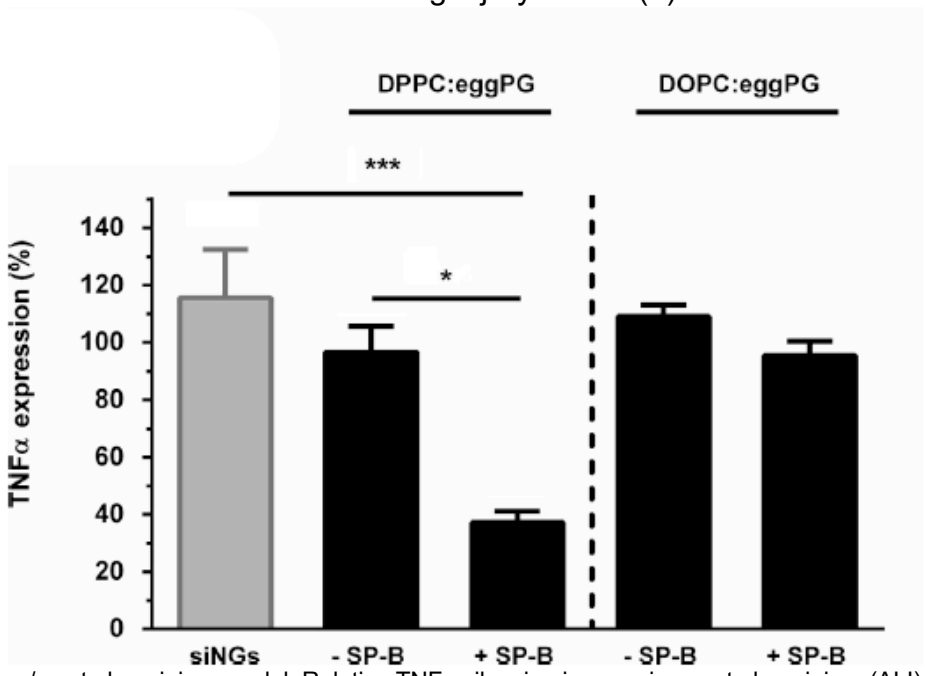

Figure 3. In vivo / acute lung injury model. Relative TNFa silencing in a murine acute lung injury (ALI) model. TNFa levels were quantified in BAL fluid extracted 24 h after LPS stimulation and thus 48 h after instillation of different siCTRL and siTNFa loaded NPs. The TNFa levels obtained with siTNFa loaded siNGs was normalized to the levels in BAL of mice that had received control siRNA (siCTRL). Mice were treated with a fixed NG dose (100 mg) loaded with 1 pmol siTNFa or siCTRL per mg NG (n = 4; *p ≤0.05, ***p ≤0.001).

Validation status:

- *In vitro* PoC in human lung cell assays
- *In vivo* PoC in CD45-KD healthy mice / TNFa-KD LPS-lung injury model

IP-position

International patent application WO2018/096057 A1 filed on 31/05/2018.

Partnering

We seek partnerships

- to explore SP-B technology for delivery of macromolecules in disease of interest
- for benchmarking/validation studies for local pulmonary applications

References

1. Kandil, R.; Merkel O.M. Pulmonary delivery of siRNA as a novel treatment for lung diseases. *Ther. Deliv.* **2019**, 10(4), 203–206.
2. Merckx, P.J.; De Backer, L.; Van Hoecke, L.; Guagliardo; R.; Echaide, M.; Baatsen, P.; Olmeda, B.; Saelens, X.; Perez-Gil, J.; De Smedt, S.C. Surfactant protein B (SP-B) enhances the cellular siRNA delivery of proteolipid coated nanogels for inhalation therapy Acta Biomaterialia 2018, 78: 236-246.
3. De Backer, L.; Naessens, T.; De Koker, S.; Zagato, E.; Demeester, J.; Grooten, J.; De Smedt, S.C.; Raemdonck, K. Hybrid pulmonary surfactant-coated nanogels mediate efficient in vivo delivery of siRNA to murine alveolar macrophages. *J Control Release*. **2015**, 217:53-63.