Photoporation for enhanced mRNA delivery to the ocular surface

Félix Sauvage[1]
Mike Wels[1]
Van Phuc Nguyen[2]
Aranit Harizaj[1]
Dimitri Roels[4]
Ilia Goemaere[1]
Katrien Remaut[1]
Yannis Paulus[2,3]
Koen Raemdonck[1]
Kevin Braeckmans[1]
Stefaan C. De Smedt[1]

[1] Laboratory of General Biochemistry and Physical Pharmacy, Faculty of Pharmaceutical Sciences, Ghent University, 9000 Ghent, Belgium
[2] Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, United States
[3] Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI, United States
Felix.sauvage@ugent.be

Introduction
Diseases of the ocular surface such as dry eye disease can severely impact vision. Current therapies typically rely on traditional approaches such as the use of artificial tears, antibiotics, or anti-inflammatory drugs. Recently, nucleic acid (NA) research have grown interest in ophthalmology by investigating the potential of siRNA and mRNA as for the treatment of ocular surface diseases. However, a major pitfall for using NAs lies in limited delivery efficiency to the ocular surface, as extra- and intracellular barriers impede their cell uptake and function[1]. To overcome this challenge, we introduce the use of photoporation to enhance mRNA delivery into cells at the surface of the eye. Photoporation makes use of pulsed-laser light to create transient pores in cell membranes through mechanical forces which arise by implosion of vapor nanobubbles (VNBs; **Figure 1**)[2]. Making use of polydopamine nanoparticles (PD NPs) as photosensitizers, we show improved delivery of mRNA in the corneal epithelium by using the mechanical forces induced by the implosion of VNBs to disrupt the mucus layer covering the ocular surface and form pores in the membrane of ocular surface cells. Our research demonstrates that photoporation using low fluences allows for safe and efficient mRNA delivery in rabbit corneas, offering a promising new approach for laser-based engineering of the ocular surface and potential treatment of various ophthalmological diseases.

Methods
Bovine eyes were used for the purpose of isolating and culturing primary bovine epithelial corneal cells (pBCECs). Additionally, intact bovine eyes were used as an *ex vivo* model to assess the impact of different NP sizes (90 nm versus 250 nm) and concentrations on photoporation efficiency. PD NPs were exposed to a pulsed laser (<7 ns; 561 nm) to produce VNBs, and the effectiveness of photoporation in delivering FITC-dextrans (FD; 10 kDa, 150 kDa, and 500 kDa) and mRNA to pBCECs and bovine corneas was investigated. To evaluate the transfection efficiency *in vivo*, rabbits were administered with PD NPs and eGFP-mRNA and their corneas were exposed to the pulsed laser (532 nm; <7 ns; 1.8 J/cm^2). The extent of transfection was measured by assessing fluorescence at the corneal level. Safety evaluations were performed through optical coherence tomography (OCT), H&E staining, TUNEL assay and electroretinography (ERG) to assess any potential corneal and retinal damage.

Results
The delivery of FD of different molecular weights was achieved with high efficiency in primary epithelial corneal cells. There was no significant difference in delivery yield between 90 nm and 250 nm NPs, but the latter demonstrated increased toxicity (**Figure 2a-d**). When applied to the surface of intact bovine eyes with FD10, optimal concentrations determined *in vitro* did not result in increased uptake when compared to FD alone. However, increasing the concentration of PD NPs clearly improved the uptake. Single particle tracking (SPT) measurements in corneal mucus reveals that photoporation induced mucus liquefaction suggesting facilitated diffusion of the cargo through the mucus, a major barrier to delivery to the ocular surface. In rabbits, photoporation using 250 nm NPs was found to be an efficient method for mRNA transfection (**Figure 3**) and to be safe, as there were no morphological changes in the cornea (determined via OCT, H&E staining and TUNEL assay) and no impact on retinal functions (determined via ERG; **Figure 4**).

Conclusions/impact
Our findings indicate that PD NPs were able to generate VNB, which effectively disrupted the corneal mucus and facilitated photoporation of corneal epithelial cells, resulting in a significant increase in delivery efficiency observed *ex vivo*. Furthermore, in an *ex vivo* setting, we were able to efficiently deliver FDs and mRNA to the corneal epithelial layer. In rabbits, we were able to safely and efficiently deliver mRNA to the cornea at low laser fluences, which is a promising development for the treatment of ocular surface diseases.

References

1. Wels, M., Roels, D., Raemdonck, K., De Smedt, S. C. & Sauvage, F. Challenges and strategies for the delivery of biologics to the cornea. *J. Controlled Release* **333**, 560–578 (2021).

2. Xiong, R. *et al.* Comparison of Gold Nanoparticle Mediated Photoporation: Vapor Nanobubbles Outperform Direct Heating for Delivering Macromolecules in Live Cells. *ACS Nano* **8**, 6288–6296 (2014).

Figures

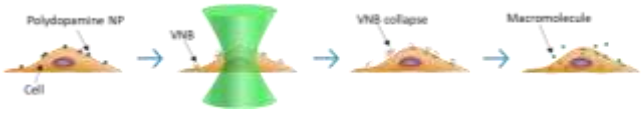

Figure 1. Principle of photoporation.

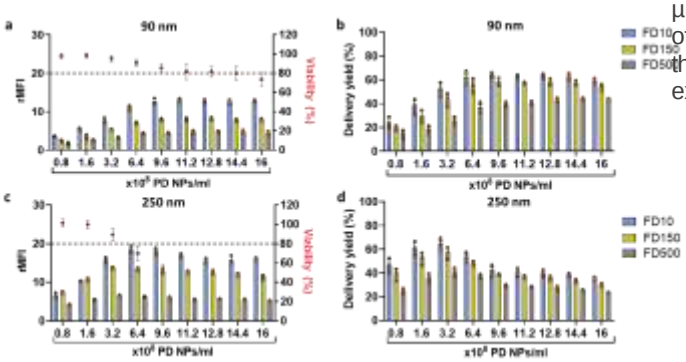

Figure 2. Photopoation of primary epithelial corneal cells. Cells were incubated with 90 nm (a and b) or 250 nm (c and d) PD NPs before being irradiated with a pulsed laser (1.8 J/cm2; 532 nm; pulse duration < 7 ns).

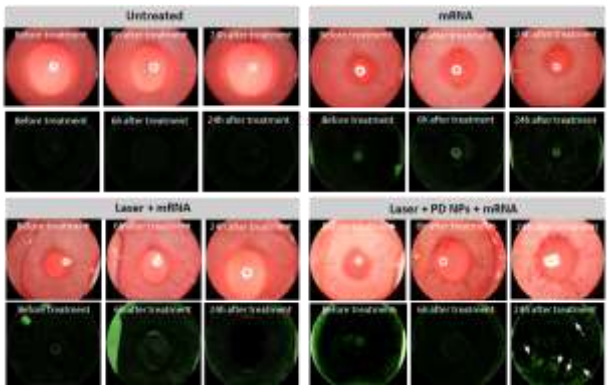

Figure 3. Fundus imaging (top row) and fluorescence imaging (bottom row) of corneas respectively untreated, treated with mRNA (control), treated with mRNA and laser irradiated (control) and treated with PD NPs (4.8x1011 NPs/ml) and mRNA (0.1 µg/µl) and laser irradiated (1.8 J/cm²; 532 nm; <7 ns). The images were taken before, 6 hours after, and 24 hours after the treatment, and are representative of the images obtained from three rabbits for each condition. The white arrows indicate eGFP-expression. Fundus imaging did not reveal any damage of the corneas.

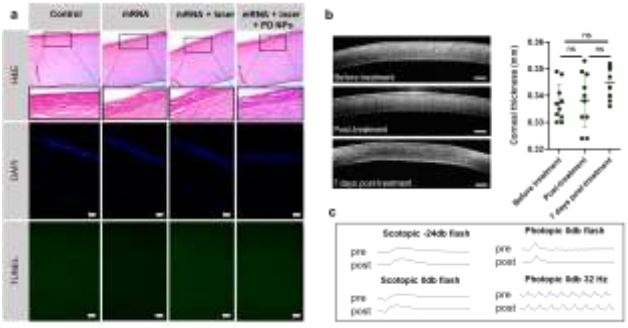

Figure 4. mRNA delivery in the corneal epithelium of rabbits in vivo by photoporation. (a) Fundus imaging (top row) and fluorescence imaging (bottom row) of corneas respectively untreated, treated with mRNA (control), treated with mRNA and laser irradiated (control) and treated with PD NPs (4.8x10^{11} NPs/ml) and mRNA (0.1 µg/µl) and laser irradiated (1.8 J/cm²; 532 nm; <7 ns). The images were taken before, 6 hours after, and 24 hours after the treatment, and are representative of the images obtained from three rabbits for each condition. The white arrows indicate eGFP-expression. Fundus imaging did not reveal any damage of the corneas. (b) Cryo-sections of the corneal epithelium 24 hours after treatment. Nuclei were stained with Hoechst; scale bar = 20 µm. eGFP-fluorescence could be detected in the epithelium of rabbits treated with PD NPs and mRNA and irradiated with the laser (1.8 J/cm²; 532 nm; <7 ns). In all control experiments, eGFP-expression did not occur.