Illuminating corneal cell therapy: Photoporation as non-viral gateway for genetic enhancement

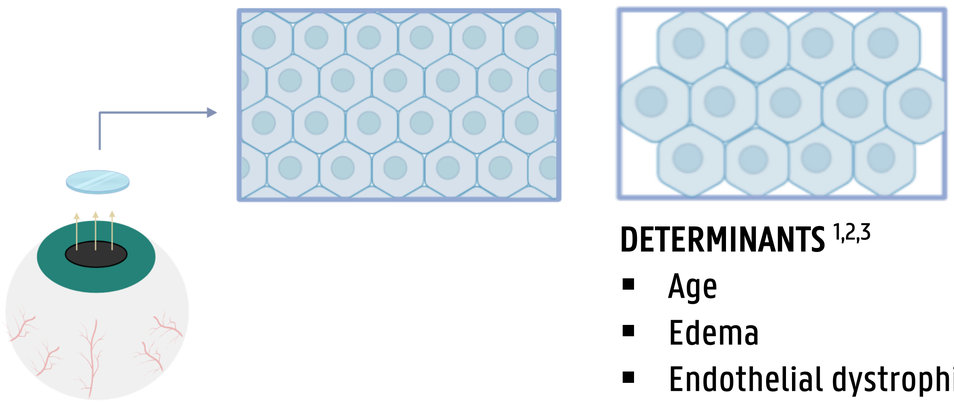
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INTRODUCTION

Corneal endothelium (CE) **Reduced endothelial cell density (ECD)**



DETERMINANTS [1,2,3]

- Age
- Edema
- Endothelial dystrophies (e.g. Fuchs endothelial dystrophy)

Corneal transplantation is the current treatment option for corneal blindness. [4,5,6]

~ 185.000 transplantations/year

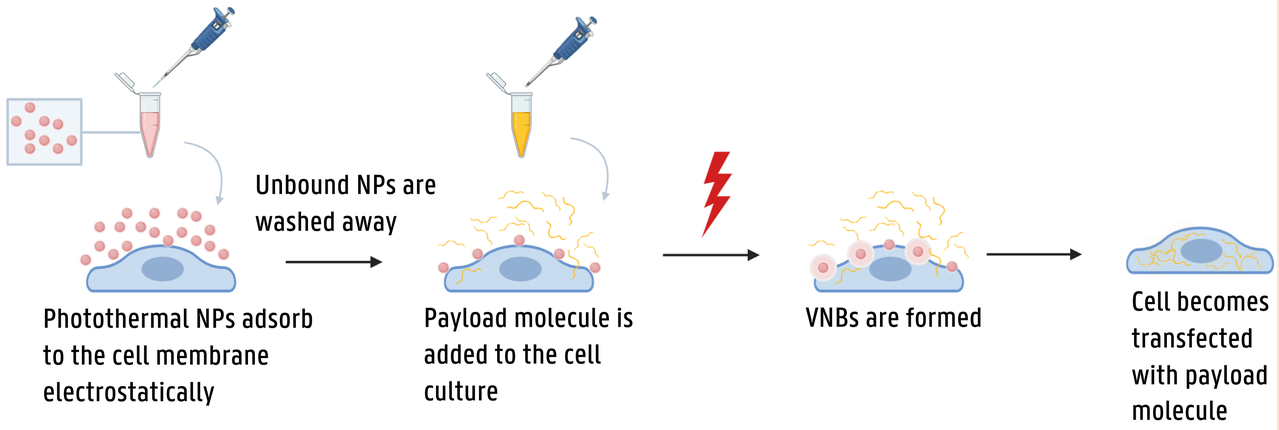
Donor shortage + low endothelial cell counts (ECD < 2000-2500 cells/mm^2) [4,5,6]

~ 30% of CE grafts worldwide is discarded each year[7]

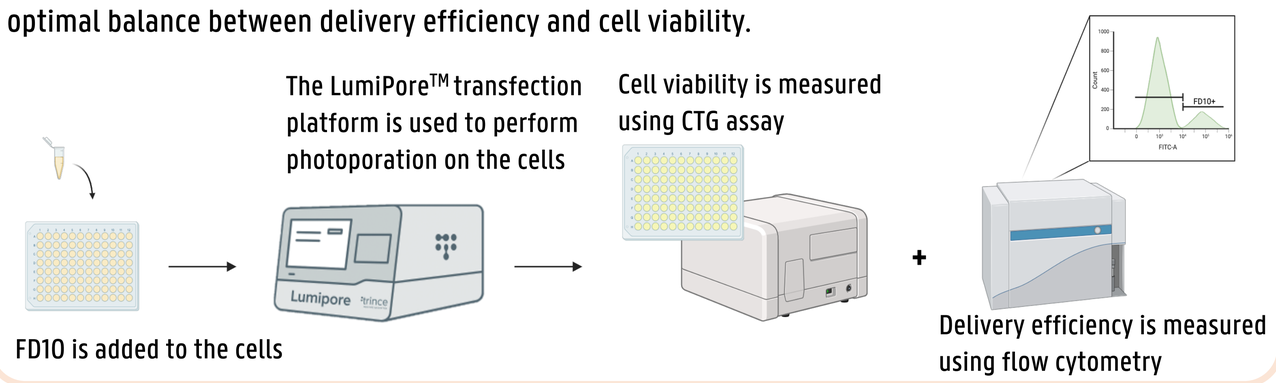
Increasing endothelial cell counts in corneal grafts to improve their quality and availability by **genetic engineering of corneal endothelial cells (CECs)**.

METHODS

Photoporation is a non-viral gentle transfection technology which employs a laser trigger in combination with photothermal nanoparticles (NPs) (e.g. gold (Au) or polydopamine). When high-intensity nanosecond laser pulses are applied, these NPs are rapidly heated. Above a critical temperature (~ 300°C), the surrounding water will start to evaporate, resulting in the formation of water vapor nanobubbles (VNBs) that initially expand and then implode. The resulting mechanical pressure waves induce transient pores in the plasma membrane, allowing exogenous cargo molecules (e.g. FD10, siRNA) to diffuse into the cytosol.[8]



Optimization of NP-mediated photoporation: FITC-dextran with molecular weight of 10 kDa (FD10) is used as model molecule for siRNA to optimize photoporation parameters such as **NP concentration** and **laser pulse fluence**. Using flow cytometry, both the percentage of FD10 positive cells and the fluorescence intensity per cell are quantified. Cell viability is measured using the CellTiter-Glo (CTG) luminescent assay. The aim is to determine the optimal intracellular delivery yield by determining the optimal balance between delivery efficiency and cell viability.



RESULTS

Experiments were conducted on B4G12 – human CECs

(1) Effect of different incubationmedia on FD10 delivery using 60nm PDDAC-AuNPs

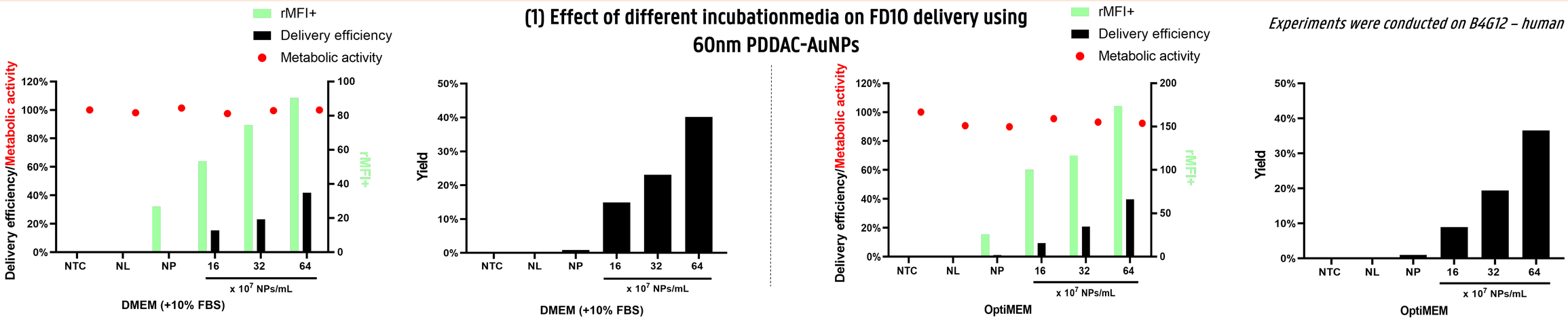


Figure 1: Intracellular delivery of FD10 to CECs via AuNP-mediated photoporation using FBS-supplemented Dulbecco's Modified Eagle Medium (DMEM) or OptiMEM as incubationmedia. CECs were incubated with three different concentrations of 60nm poly(diallyldimethylammonium chloride) (PDDAC) - coated AuNPs ranging from 16 x 10^7 to 64 x 10^7 nanoparticles (NPs)/mL. A fluence of 1.20 J/cm^2 was used to create VNBs to deliver 2 mg/mL FD10 into CECs. Non treated cells (NTC), cells treated without laser (NL), and cells treated without AuNPs (NP) are shown as controls. The percentage of FD10 positive cells (expressed as delivery efficiency) and the relative mean fluorescence intensity (rMFI) were determined with flow cytometry. The corresponding metabolic activity was determined with the CTG assay. The delivery yield is the product of the percentage of FD10 positive cells and viable cells. ***Figure 1*** represents results from one independent experiment (n=1).

(2) FD10 delivery via NP-mediated photoporation using…

Experiments were conducted on B4G12 – human CECs

a) … 60nm PDDAC-AuNPs **b) … 250nm PDNPs** **c) … 500nm PDNPs**

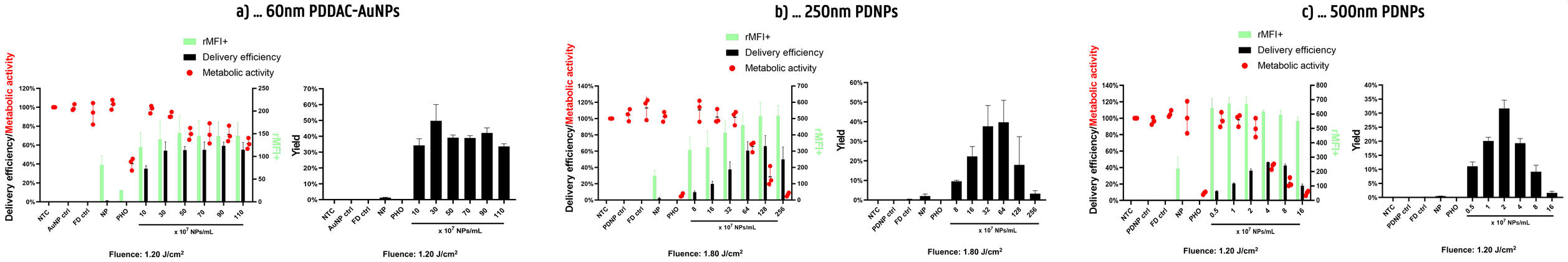


Figure 2: Illustration of the delivery efficiency, the metabolic activity, the rMFI, and the delivery yield for the intracellular delivery of FD10 to CECs. CECs were incubated with increasing concentrations of (a) 60nm PDDAC-AuNPs, (b,c) 250 and 500nm polydopamine nanoparticles (PDNPs). The percentage of FD10 positive cells (expressed as delivery efficiency) and the rMFI were determined with flow cytometry. The corresponding metabolic activity was determined with the CTG assay. The delivery yield is the product of the percentage of FD10 positive cells and viable cells. ***Figure 2*** represents results from three independent experiments (n=3).

CONCLUSIONS & FUTURE PERSPECTIVES

- No significant difference in delivery yield was observed between DMEM and OptiMEM as incubationmedia for AuNP-mediated photoporation.
- AuNP-mediated photoporation effectively delivers FD10 into CECs.
- PDNPs show comparable delivery performance as AuNPs, where smaller-sized PDNPs seem to increase the delivery yield.
- Future research will explore the transfection of CECs using fluorescently labeled siRNA.

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https://www.drugdelivery.be/

https://trincebio.com/

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