Femtosecond and nanoparticle-mediated gene therapy in the murine model for corneal dystrophy treatment

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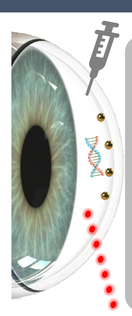


ABSTRACT: Endothelial diseases impact nearly 250 million people worldwide. Corneal transplantation, the sole approved treatment, is invasive and may lead to tissue rejection and subsequent vision loss. We propose a technique using a femtosecond laser and gold nanoparticles (AuNPs) for gene delivery into corneal endothelial cells (CECs). *Ex vivo* experiments on mice demonstrated effective optoporation with reduced side effects, enabling highly localized treatment with elevated efficacy (>50%) and low cellular death (<15%). Our technique holds promise for gene and drug delivery to CECs, with future work involving knock-out mice as a disease model for mRNA internalization.

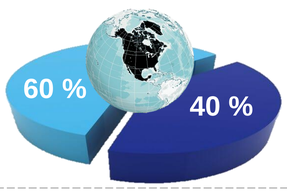
Introduction

Corneal endothelial dystrophies

Fuchs: hereditary dystrophy caused by genetical mutations (SLC4A11) [1].

One of the major causes of blindness

Responsible for 40% of corneal transplantations



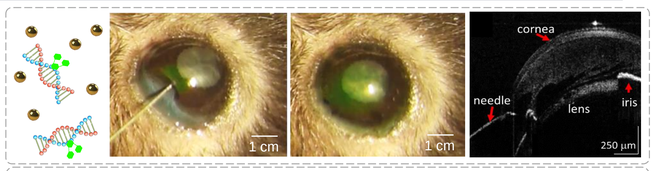
The hypothesis and the principle

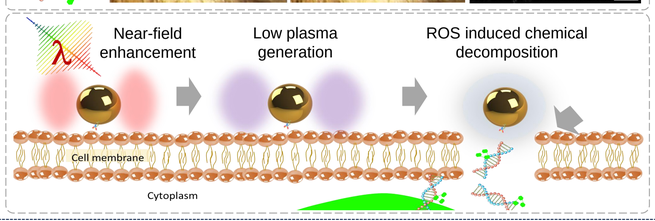
A combination of laser treatment enhanced by AuNPs can lead to a less invasive gene therapy in the CECs [2-5]

Intracameral injection

+

Femtosecond laser therapy λ= 800nm





Methods

Intracameral injection *

n=12

1ul of AuNPs (2E9NPs/ml)

+

Membrane impermeable fluorophores (2mM)

PI - Optoporation

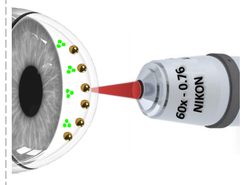
CALCEIN AM - CECs viability

DAPI - CECs

Adapted technique for mice (wild-type C57Bl/6).

*All experiments were conducted under ethical protocols approved by the Association for Research in Vision Science and Ophthalmology: ARVO.

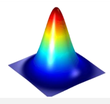
Transcorneal irradiation



fs laser 800nm @ 80MHz

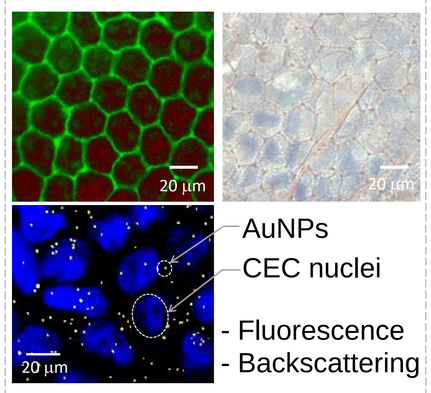
Central cornea scanning

Laser profile



Scanning speed	0.8-80 µm/s
Dwell time	0.5-500 ms
Optimized fluence	5-30 mJ/cm^2

Corneal tissue imaging

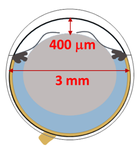


Efficacy quantification

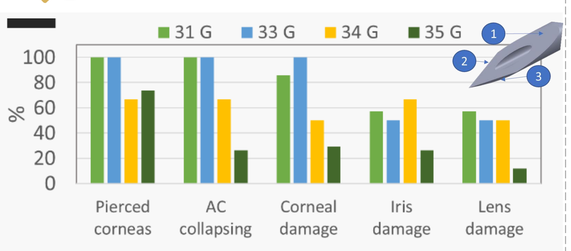
 # Irradiated cells Vs # cells expressing PI (red fluorescence)

Results

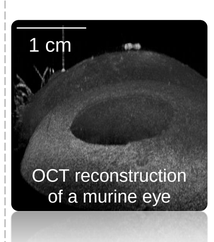
Intracameral injection *



Cell viability: evaluation of different needle gauge:



Minimized damaged was achieved with 35G needles



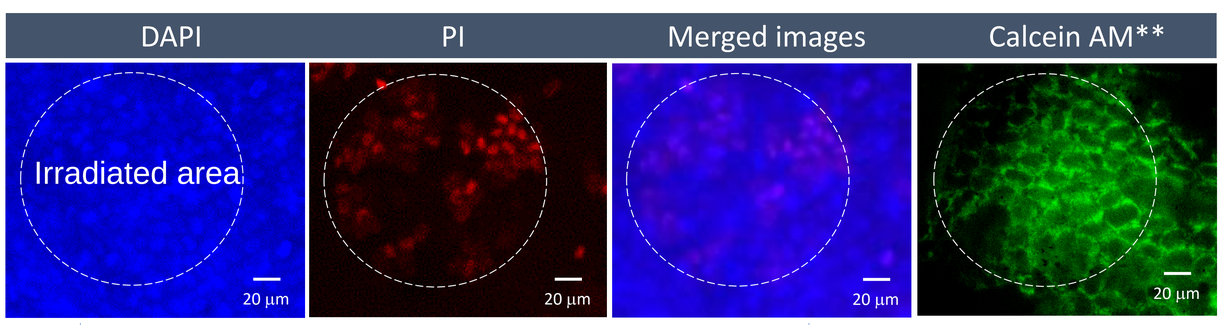
From OCT measurements:

- Precision
- Reproducibility
- CECs damage

- Submitted paper-

Transcorneal irradiation

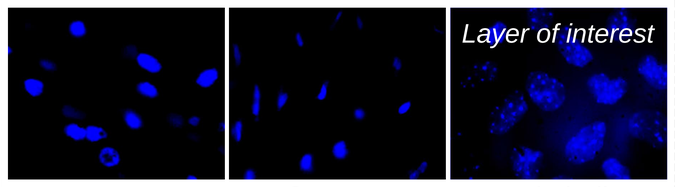
Optimized outcome parameters	Quantification
1) Scanning speed	➢ 80 µm/s
2) Dwell time	➢ 500 ms
3) Fluence	➢ 20 mJ/cm^2
4) NPs attachment (quantification of NPs/cell)	➢ 20±5 NPs/cell
5) Efficacy	➢ 50 % internalization
6) Cell viability after irradiation	➢ 76 % (at the irradiated area)



Transcorneal imaging

CECs direct imaging

Minimized side effects



Epithelium *Stroma* *Endothelium*

No optoporation effect was visible in the rest of the corneal layers

Negative controls

- **Irradiation**
  - Without NPs = No PI signal
- **No irradiation**
  - PI Incubation = No PI signal
  - NPs incubation = No effect in cell viability

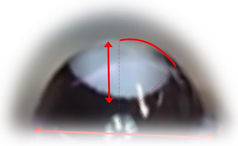
Conclusions

Our novel femtosecond laser and nanoparticle-assisted technique shows:

- Precise molecule delivery (CECs only)
- Selectivity (Focalized laser + AuNPs)
- Phototoxicity

To improve:

Reproducibility



(Limitation: murine cornea curvature radius)

High cell viability rate

=

Potential implementation for *in vivo* transfection

Working on:

- *Ex vivo* : Transfection (mRNA)
- *In vivo:* In preparation

KO mouse model SLC4A11

Potential clinical translatability:

- Less invasive alternative
- Injection translatable to other species
- Biocompatibility: AuNPs

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References

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