light on PSCs

PHOTOPORATION AS A GENTLE YET EFFECTIVE TRANSFECTION TECHNIQUE

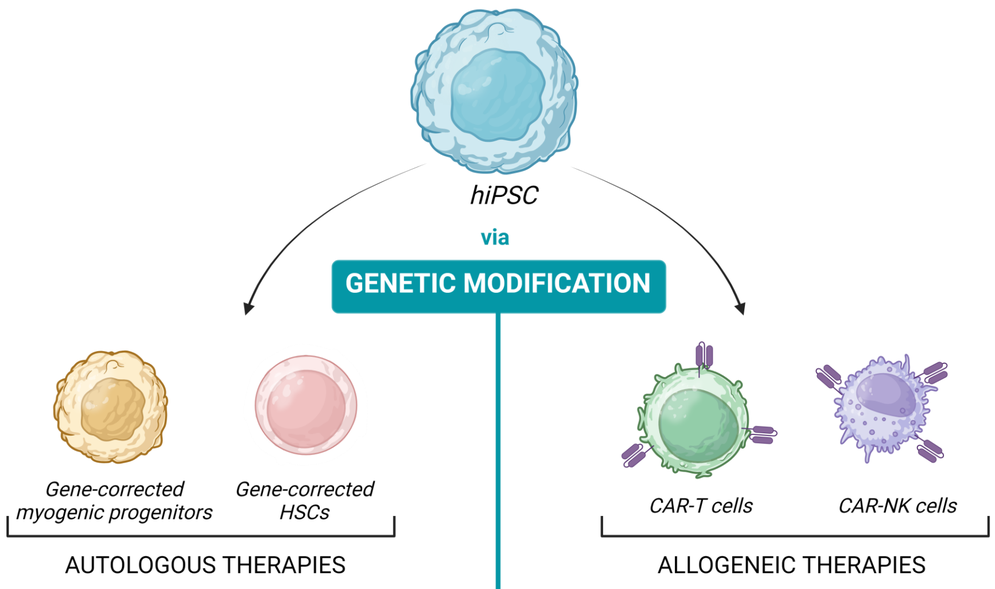
GHENT UNIVERSITY

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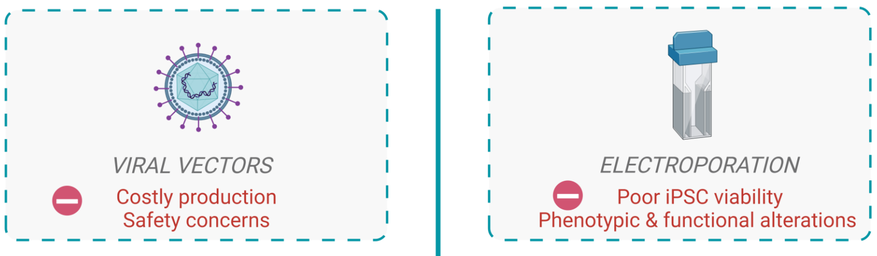
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Laboratory for General Biochemistry and Physical Pharmacy

INTRODUCTION



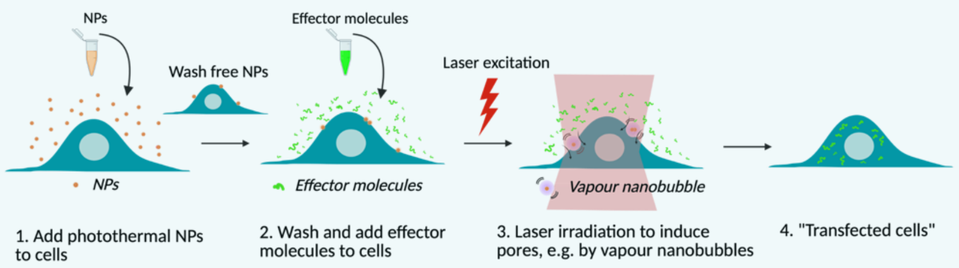
*However, current technologies face **drawbacks**.*



PHOTOPORATION

WHAT Photoporation is a novel and innovative **intracellular delivery** technique.

HOW In its traditional form, photoporation uses a combination of **laser light** and **photothermal nanoparticles** to generate pores in the cell membrane (1,2).

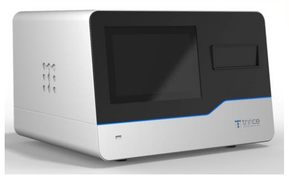


WHY Photoporation offers a **gentler** alternative, thus better preserving the quality of the final engineered cell product. This technology can be used **spatially-selective** and in **high throughput** (3).

METHODS

- 80 nm cationic **gold nanoparticles** (PDDAC AuNPs) and 500 nm Bovine Serum Albumin (BSA) coated **polydopamine nanoparticles** (PDNPs) were used as photothermal sensitizers. These nanosensitizers were custom made by Trince.
- **FITC-labelled dextran** of **150 kDa** (FD150) was used a model cargo molecule (2 mg/mL) whereas **eGFP mRNA** (0,025 mg/mL) served as a functional test.
- Laser irradiation was performed by the **Lumipore™** v1 device.
- Delivery efficiency, viability and relative mean fluorescence intensity (rMFI) were quantified using flow cytometry. Expression of eGFP and stemness markers was measured 24 hours post photoporation.
- Viability was measured by means of the metabolic activity assay CellTiter-Glo.
- hEMP (human Embryonic Mesodermal Progenitor) generation (4) was initialized 24 hours post photoporation. Cells were harvested after 3 days and analysed on the expression of markers via flow cytometry.

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RESULTS

Photoporation using 80 nm gold nanoparticles efficiently delivers FD150

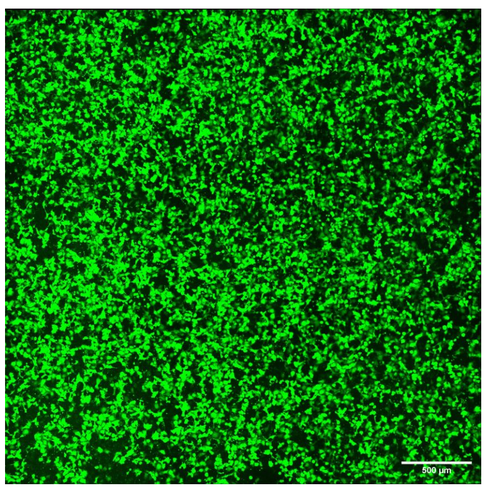
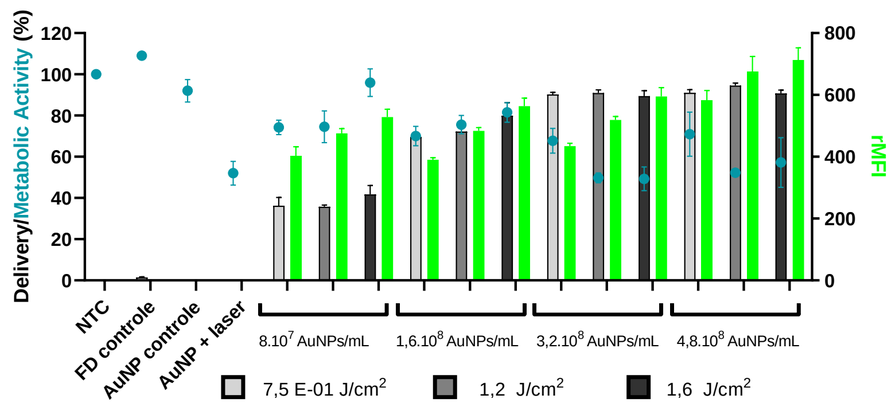


Figure 1. *Confocal microscopy overview of iPSCs after AuNP-photoporation (3,2.10⁷ AuNPs/mL and 2,1 J/cm²) with FD150 (green channel). Scale bar: 500 µm.*



Graph 1. *Intracellular delivery of FD150 in iPSCs for increasing concentrations AuNPs. Delivery efficiency (left y-axis, grey bars) and relative mean fluorescence intensity (right y-axis, green). The corresponding metabolic activity (left y-axis, blue dots) was determined with the CellTiter-Glo assay.*

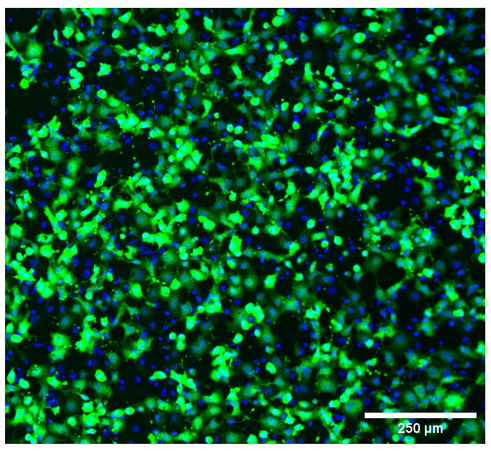
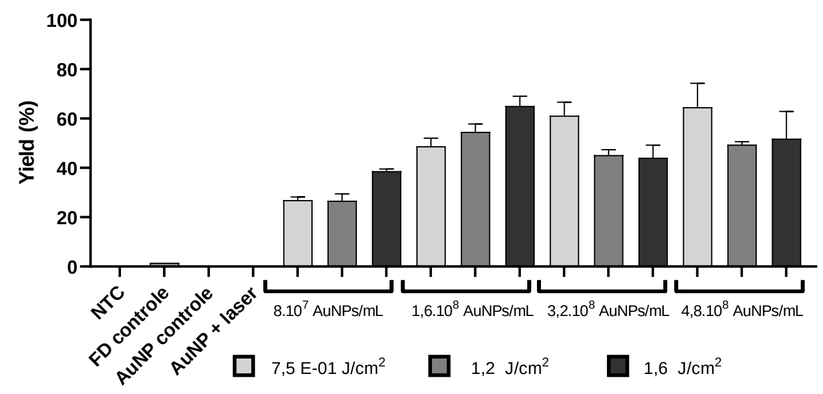
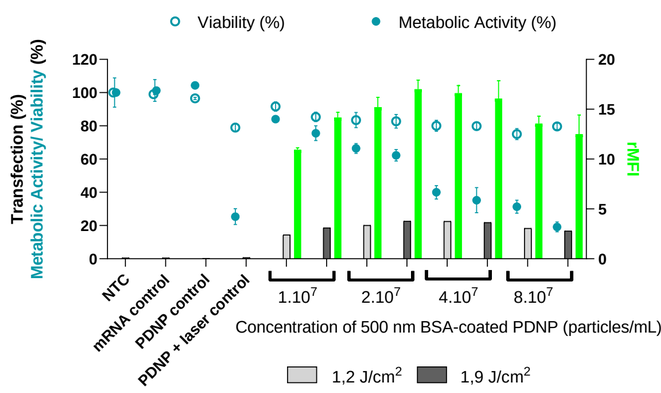


Figure 2. *Confocal microscopy image of iPSCs after AuNP-photoporation (concentration 3,2.10⁷ and 2,1 J/cm²) with FD150 (green channel). Nuclei were stained with Hoechst 33342 (blue channel). Scale bar: 250 µm.*

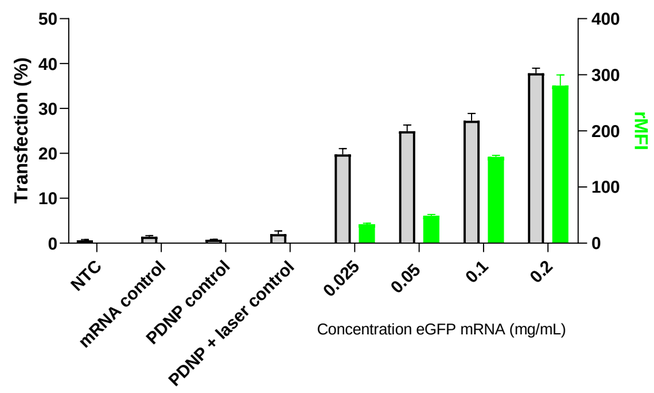


Graph 2. *Delivery yield of FD150 in iPSCs, defined as the fraction of cells that are both viable and FD150 positive.*

Polydopamine nanoparticles pose as a biocompatible alternative for the delivery of large biomolecules

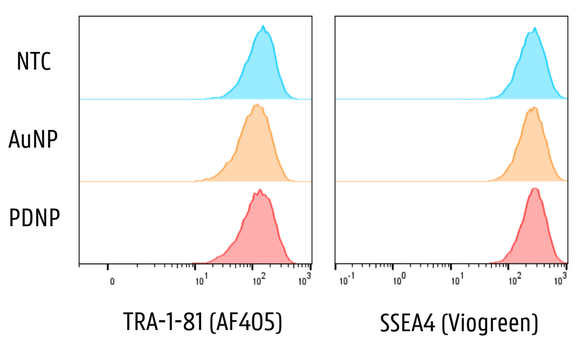


Graph 3. *eGFP expression in iPSCs for increasing concentrations PDNPs. Delivery efficiency (left y-axis, grey bars) and relative mean fluorescence intensity (right y-axis, green bars). Viability (left y-axis, blue circles), relative to NTC, was obtained by live/dead staining. The corresponding metabolic activity (left y-axis, blue dots) was determined with the CellTiter-Glo assay.*

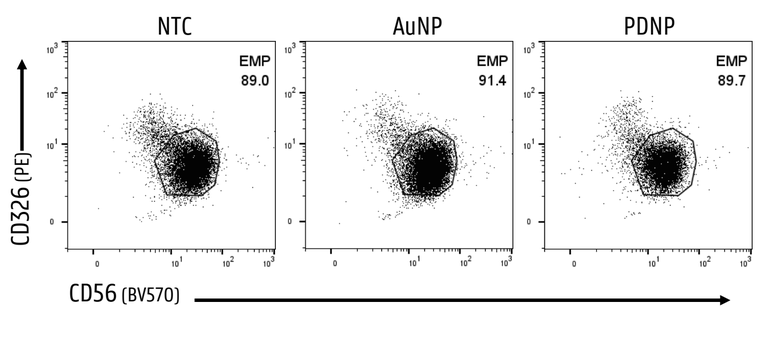


Graph 4. *eGFP expression in iPSCs after PDNP- photoporation for increasing concentrations eGFP mRNA. Delivery efficiency (left y-axis, grey bars) and relative mean fluorescence intensity (right y-axis, green bars).*

Photoporation preserves the undifferentiated state and differentiation potential



Graph 5. *Expression of stemness markers TRA-1-81 and SSEA4 on photoporated iPSCs. AuNP-photoporation was conducted with 3,2.10⁸ AuNPs/mL and 1,6 J/cm², whereas PDNP-photoporation was performed using 2.10⁷ PDNPs/mL and 1,8 J/cm².*



Graph 6. *Flow cytometric gating of hEMP generation after 3 days of mesoderm differentiation from photoporate iPSCs. AuNP-photoporation was conducted with 3,2.10⁸ AuNPs/mL and 1,6 J/cm², whereas PDNP-photoporation was performed using 2.10⁷ PDNPs/mL and 1,8 J/cm².*

CONCLUSION and FUTURE PERSPECTIVES

Photoporation can reach **high delivery percentages while maintaining high viability in iPSCs**. Pluripotency remains unaltered and cells still exhibit excellent differentiation potential. Other cargo and applications will be tested.

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