light on iPSCs

PHOTOPORATION AS A GENTLE YET EFFECTIVE TRANSFECTION TECHNIQUE

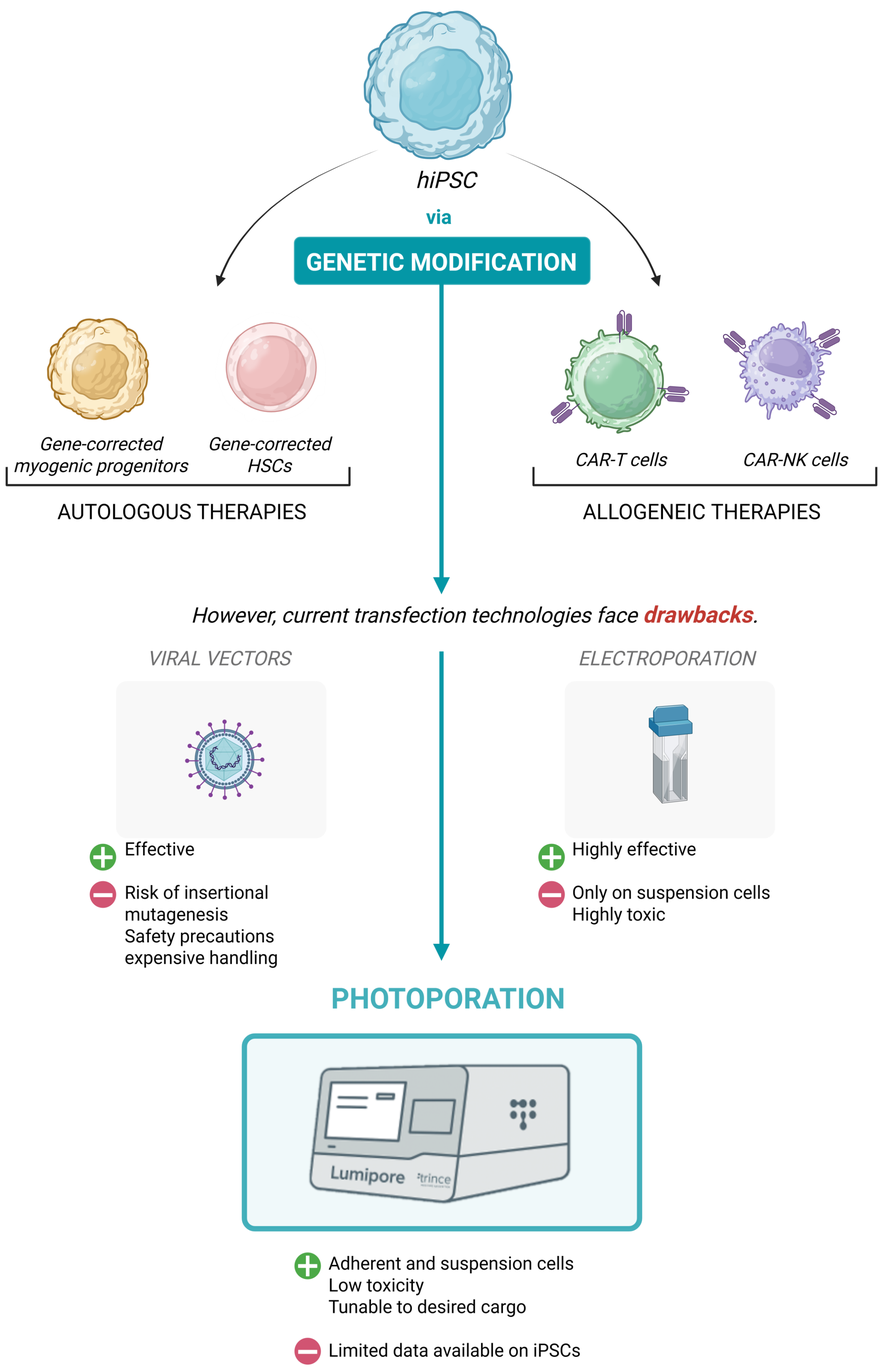
C. De Clercq [1], A. Ahmad [1], D. Sahin [1], A. Harizaj [2], B. Vanmeerhaeghe [1], B. Huang [1], K. Ling Liang [3], J. Ramon [1], F. Sauvage [1], S. De Smedt [1], T. Taghon [3], K. Braeckmans [1]

1. Laboratory of General Biochemistry and Physical Pharmacy, Ghent University 2.Trince 3. Department of Diagnostic Sciences, Ghent University

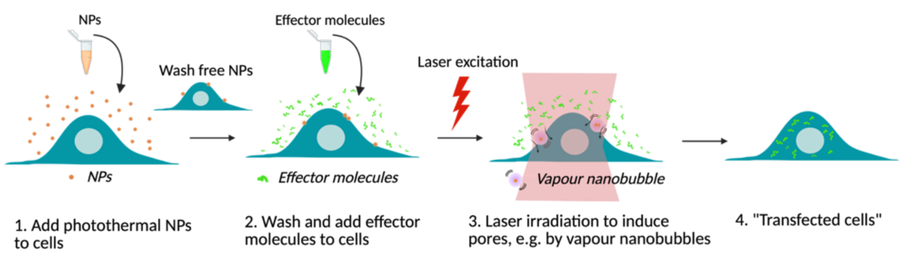
GHENT UNIVERSITY

Laboratory for General Biochemistry and Physical Pharmacy

INTRODUCTION



METHODS



- 80 nm cationic **gold nanoparticles** (PDDAC AuNPs) and 250 nm, 500 nm and 750 nm Bovine Serum Albumin (BSA) coated **polydopamine nanoparticles** (PDNPs) were used as photothermal sensitizers. These nanosensitizers were custom made by Trince.
- iPSCs are cultured using eTESR on Matrigel-coated plates. Cells are split twice a week, Y-27632 (10 uM) is added to promote cell survival.
- **FITC-labelled dextran** of **150 kDa** (FD150) was used a model cargo molecule (2 mg/mL) whereas **eGFP mRNA** served as a functional test.
- Laser irradiation was performed by the **Lumipore™** v1 device.
- Delivery/transfection efficiency, viability and relative mean fluorescence intensity (rMFI) were quantified using **flow cytometry**. Expression of eGFP was measured 24 hours post photoporation with flow cytometry as well. Metabolic activity was measured by means of the metabolic activity assay **CellTiter-Glo**.
- h**EMP** (human Embryonic Mesodermal Progenitor) generation (1) was initialized 24 hours post photoporation. Cells were harvested after 3 days and analysed on the expression of markers via flow cytometry. EMPs can be distinguished by a downregulation of CD326 (EpCAM) and an upregulation of CD56 (NCAM).
- To investigate the expression of **markers of the undifferentiated state**, iPSCs were reseeded 24 hour after photoporation and analysed after 1,5 days via antibody staining and flow cytometry.

trince PRECISION TRANSFECTIONS

nature awards the spinoff prize

THE WEBSITE

NOVISTEM

European Innovation Council

Funded by the European Union

RESULTS

AuNP-photoporation efficiently delivers FITC-labelled dextrans of 150 kDa

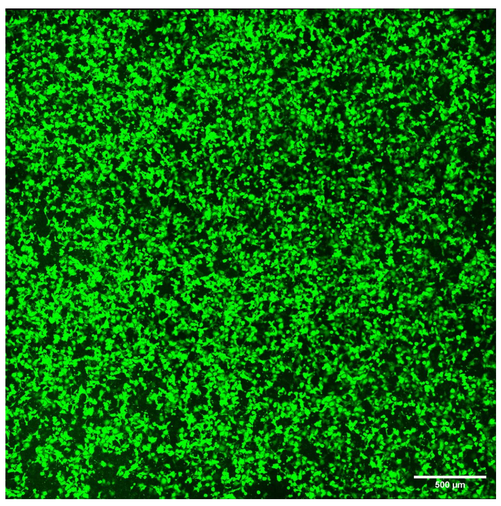
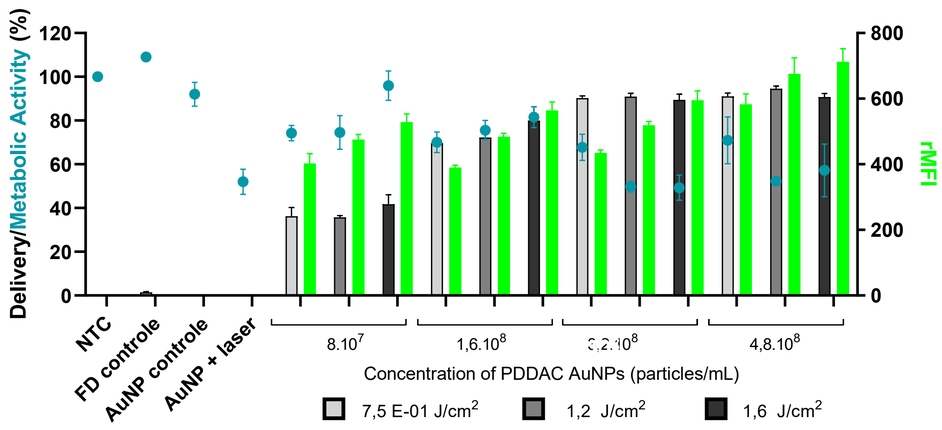


Figure 1. *Confocal microscopy overview of iPSCs after AuNP-photoporation (3,2.10⁸ AuNPs/mL and 2,1 J/cm²) with FD150 (green channel). Scale bar: 500 µm.*



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.*

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

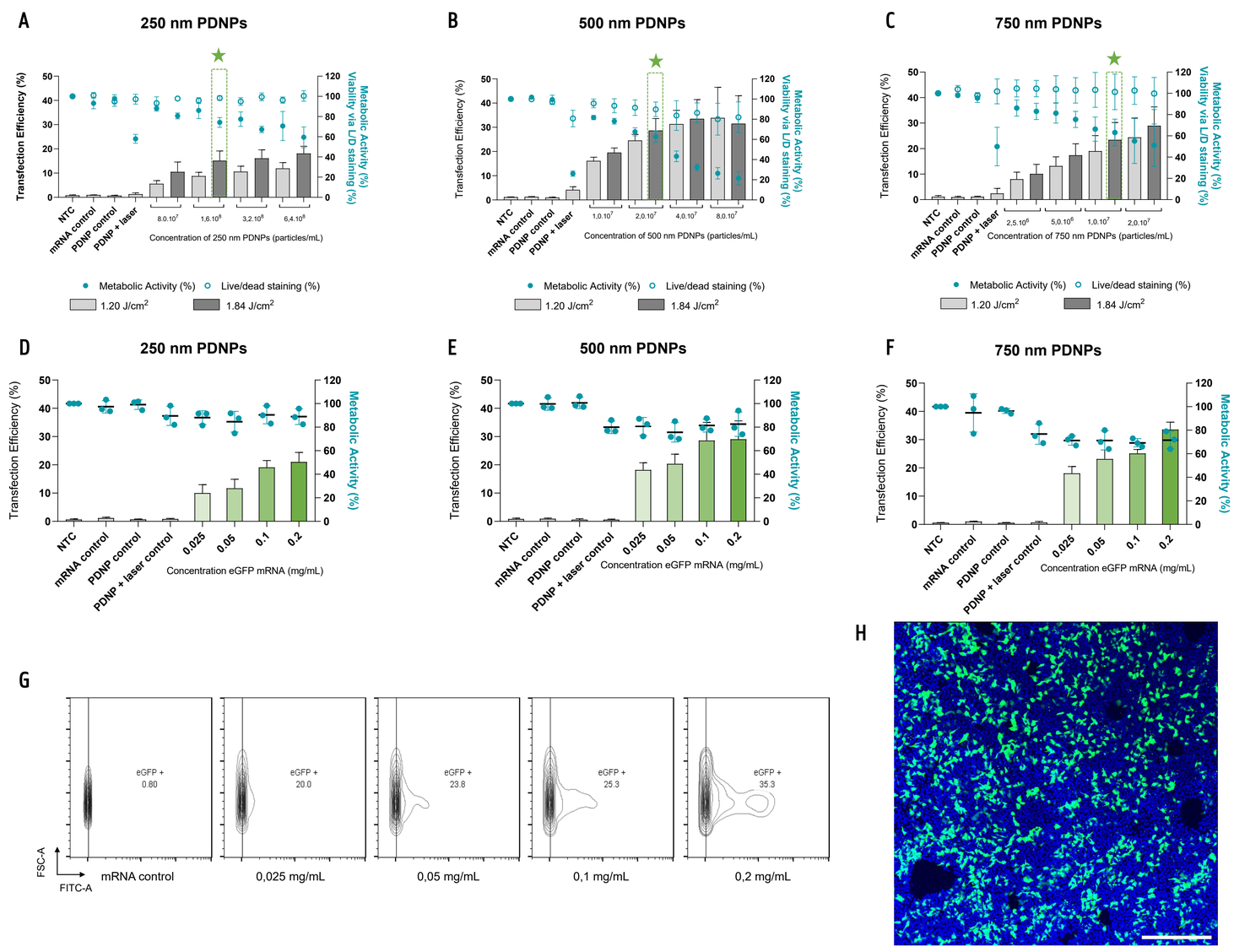


Figure 2. *eGFP expression in iPSCs 24 hours post photoporation **(A-C)** screening of particle concentrations for 250 nm **(A)**, 500 nm **(B)** and 750 nm **(C)** using 0,025 mg/mL eGFP mRNA. Optimal conditions, those that yielded the most amount of metabolically active yet transfected cells, are highlighted. Delivery efficiency (left y-axis, grey bars) was obtained by flow cytometry, similar to viability (right y-axis, blue circles), relative to NTC, which was obtained by live/dead staining. The corresponding metabolic activity (right y-axis, blue dots) was determined with the CellTiter-Glo assay. **(D-F)** Increasing concentrations of eGFP mRNA were used together with the optimal concentrations shown above for 250 nm **(D)**, 500 nm **(E)** and 750 nm **(F)**. **(G)** Flow cytometry plots for transfection of eGFP mRNA (FITC-A channel) using 750 nm PDNPs. **(H)** Confocal microscopy image of eGFP expression (green channel) in iPSCs 24 hours after 750 nm PDNP-photoporation with 0,1 mg/mL eGFP mRNA. Nuclei were stained with Hoechst 33342 (blue channel).*

The undifferentiated state and high differentiation potential of iPSCs is preserved

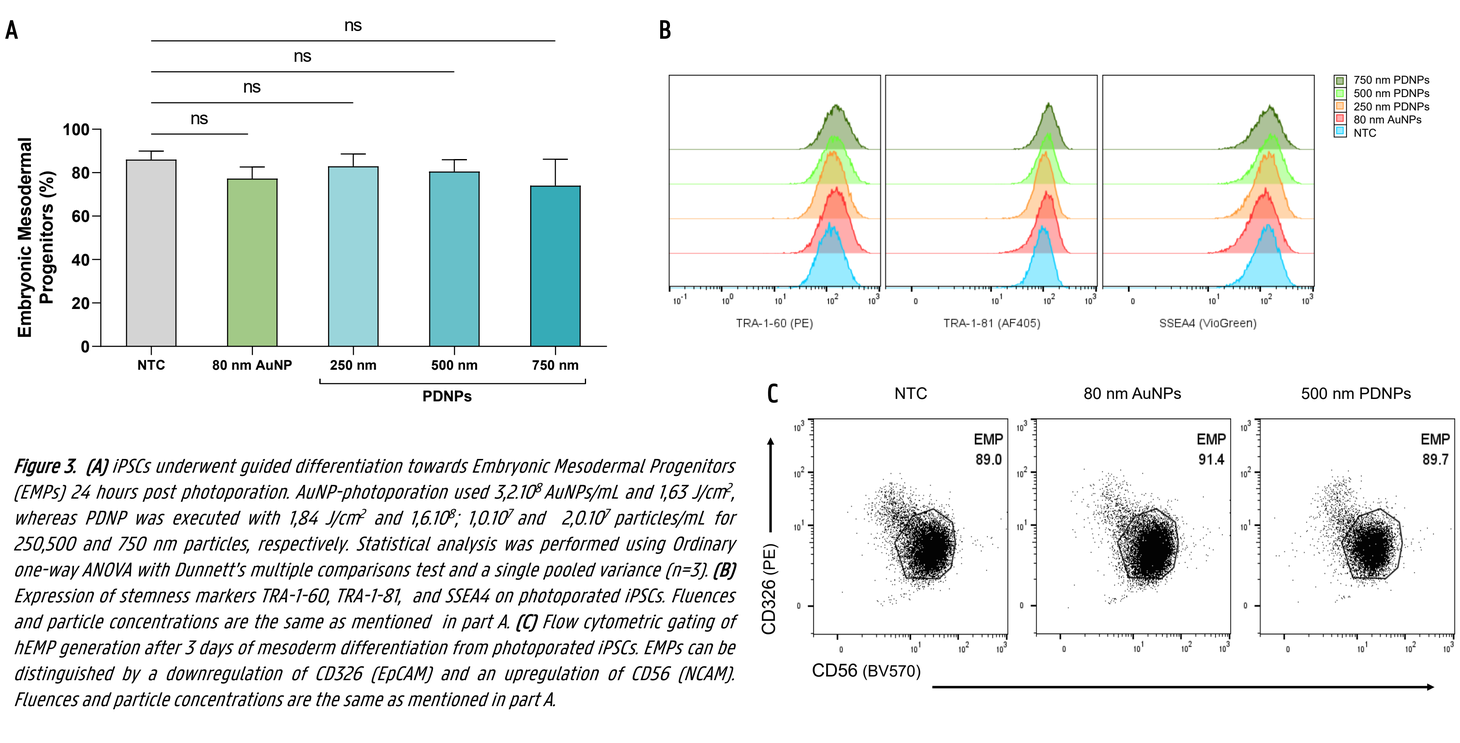


Figure 3. ***(A)*** *iPSCs underwent guided differentiation towards Embryonic Mesodermal Progenitors (EMPs) 24 hours post photoporation. AuNP-photoporation used 3,2.10⁸ AuNPs/mL and 1,63 J/cm², whereas PDNP was executed with 1,84 J/cm² and 1,6.10⁸; 1,0.10⁷ and 2,0.10⁷ particles/mL for 250,500 and 750 nm particles, respectively. Statistical analysis was performed using Ordinary one-way ANOVA with Dunnett's multiple comparisons test and a single pooled variance (n=3).* ***(B)*** *Expression of stemness markers TRA-1-60, TRA-1-81, and SSEA4 on photoporated iPSCs. Fluences and particle concentrations are the same as mentioned in part A.* ***(C)*** *Flow cytometric gating of hEMP generation after 3 days of mesoderm differentiation from photoporated iPSCs. EMPs can be distinguished by a downregulation of CD326 (EpCAM) and an upregulation of CD56 (NCAM). Fluences and particle concentrations are the same as mentioned in part A.*

CONCLUSION

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.

References.

1. Montel-Hagen A, S. Seet C., Li S., Chick B., Zhu Y., Chang P., Tsai S., Sun V., Lopez S., Chen H., He C., Jia Chin C., Casero D., M. Crooks G., Organoid-Induced Differentiation of Conventional T Cells from Human Pluripotent Stem Cells. Cell Stem Cell, Volume 24, Issue 3, 376-389(2019)

Contact

Chloe.DeClercq@UGent.be

https://www.drugdelivery.be/