Transfecting hiPSCs Robustly and Safely Using Photothermal Electrospun Nanofibers

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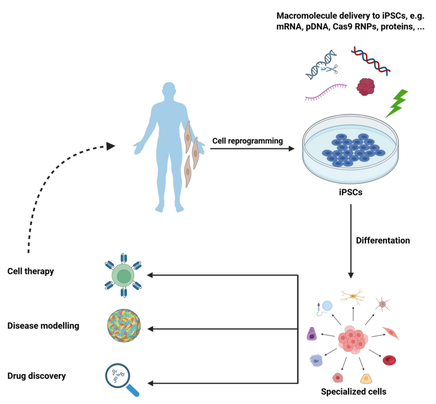
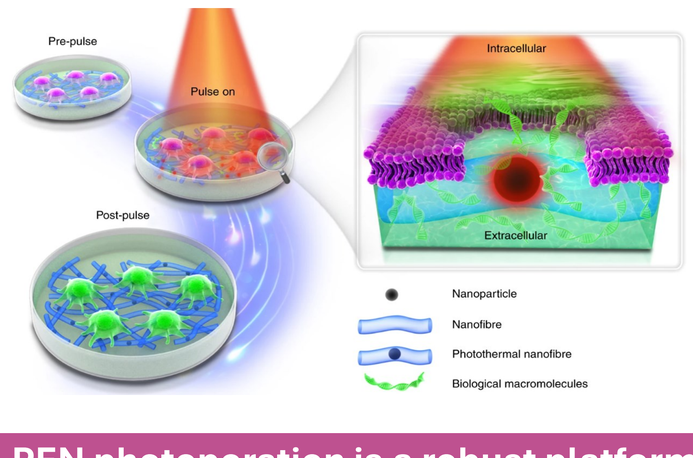
Introduction

- **iPSCs are uniquely defined by their pluripotent phenotype and their relative ease of production.**
- **Genetically engineered iPSCs hold promise for diverse applications** such as cell therapy, disease modelling, and drug discovery. **Gene-modified iPSCs could therefore be a game-changer for improving human health.**
- **Genetically engineering iPSCs requires delivering large macromolecules** inside their cytosol, e.g. mRNA, pDNA, proteins, …
- **iPSCs are hard-to-transfect:** current transfection technologies typically show low transfection efficiencies or high toxicity and phenotypic changes in iPSCs.



Methods – PEN photoporation

- Our approach relies on **iron oxide photothermal nanoparticles** (IONPs) **encapsulated in a biocompatible polycaprolactone**-based polymer. Cells are transiently cultured onto the photothermal substrate before being **irradiated with a 532 nm nanosecond-pulsed laser at low fluences (< 0.3 J/cm^2).**
- Laser irradiation causes photothermal effects to **locally permeabilize the cell membrane, enabling the entry of cargo molecules from the transfection medium into the cytosol.** The generated membrane pores are repaired within seconds to minutes, therefore minimally impacting cell viability.
- The encapsulation of the iron oxide photothermal sensitizer into polycaprolactone nanofibers ensures that **cells are not directly exposed to nanoparticles, alleviating concerns regarding potential (long term) toxicity.**
- Here, we show that the **PEN photoporation** platform enables to **effectively and gently deliver macromolecules into iPSCs.**



PEN photoporation is a robust platform

First, we investigated the effect of the nanofiber size, IONP content, and laser pulse fluence on the cell transfection performance. For this, we used a HeLa cell model in which we delivered fluorescently-labeled dextran molecules of 10 kDa (FD10) or 500 kDa (FD500). **Neither nanofiber diameter (400-600 nm) nor IONP content (0.2-5 wt%) had a significant effect on the transfection yield**, i.e. the percentage of succesfully transfected and alive cells.

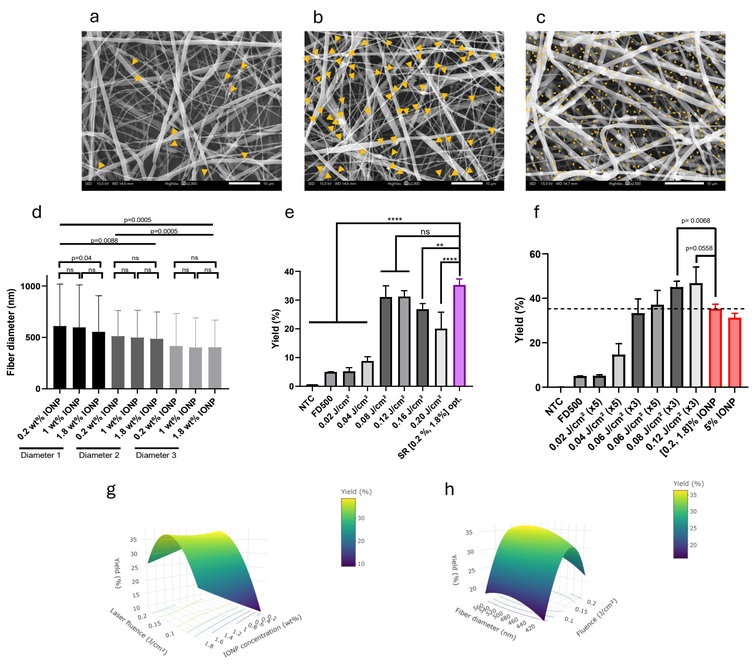


Figure 1: (a)-(c) Representative SEM pictures showing PEN nanofibers encapsulating 0.2%, 1%, and 5% IONPs (indicated with arrow heads). (d) PEN nanofibers with three distinct diameters were fabricated using electrospinning. (e) FD500 delivery yield was similar for fibers with 5% IONP or 1% IONP. Grey bars show the transfection yield achieved with 5% IONP PEN substrates whereas the purple one shows the optimum reached with 1% IONP substrates. (f) PEN substrates can be repetitively laser-activated. Grey bars show the transfection yield of 1% IONP PEN substrates when repetitively irradiated, whereas red bars show the optima reached with 1% IONP and 5% IONP substrates without repetitive irradiation. (g)-(h) Laser pulse fluence impacts the FD500 yield the most, but not the IONP content or nanofiber diameter.

hiPSCs maintain proliferation when cultured on PEN

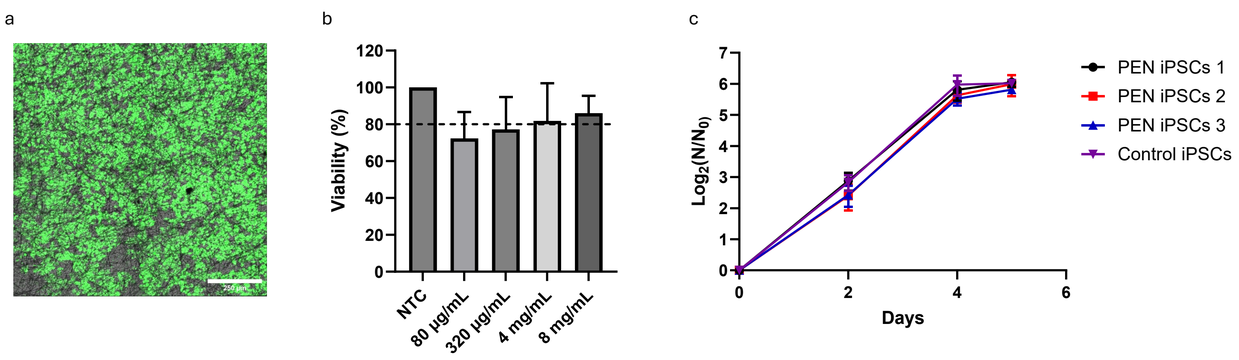


Figure 2: (a) Representative confocal microscopy image showing viable iPSCs stained with calcein AM (green) cultured on PEN nanofibers (transmission). (b) Matrigel coating of PEN nanofibers enables enhanced iPSC viability, as quantified by the Cell-Titer Glo assay. (c) iPSCs cultured on PEN nanofibers can be retrieved from the fibers and expanded similar to control iPSCs.

hiPSCs maintain pluripotency when cultured on PEN

iPSCs cultured onto PEN nanofibers express pluripotency markers similar as iPSCs cultured in a conventional plate.

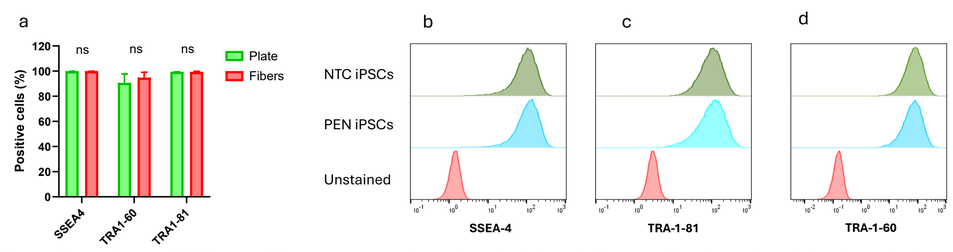


Figure 3: iPSCs cultured onto PEN nanofibers were retrieved and either (a) immediately stained for the pluripotency markers SSEA-4, TRA-1-60, and TRA-1-81 , or (b) expanded for > 2 weeks before being stained for the same markers.

iPSCs cultured on PEN nanofibers can be differentiated into e.g. embryonic mesodermal precursors (EMPs) for the generation of off-the-shelf T cells.

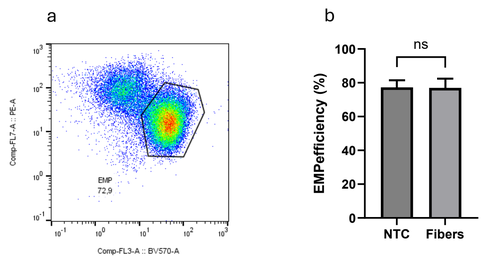


Figure 4: iPSCs cultured onto PEN nanofibers were retrieved, expanded, and differentiated into EMPs. (a) Representative flow plot showing more than 70% EMP induction efficiency for iPSCs cultured onto PEN nanofibers. (b) iPSCs cultured onto PEN nanofibers differentiate into EMPs similar to control iPSCs.

PEN photoporation enables delivering macromolecules and mRNA

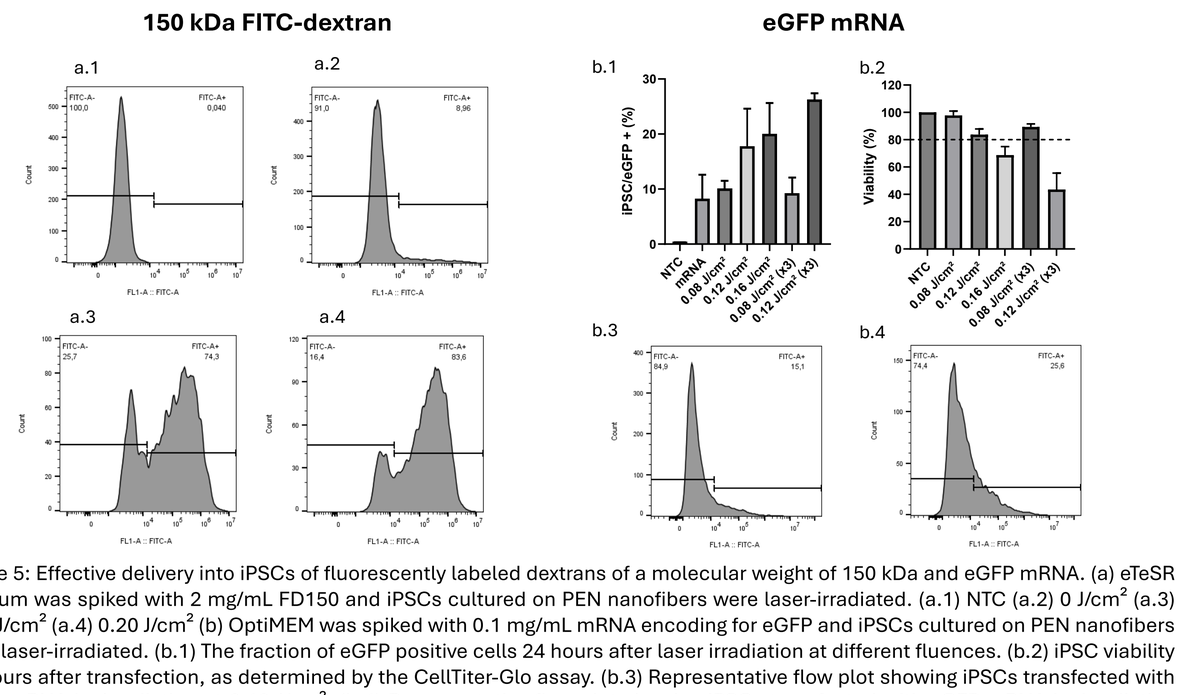


Figure 5: Effective delivery into iPSCs of fluorescently labeled dextrans of a molecular weight of 150 kDa and eGFP mRNA. (a) eTeSR medium was spiked with 2 mg/mL FD150 and iPSCs cultured on PEN nanofibers were laser-irradiated. (a.1) NTC (a.2) 0 J/cm^2 (a.3) 0.12 J/cm^2 (a.4) 0.20 J/cm^2 (b) OptiMEM was spiked with 0.1 mg/mL mRNA encoding for eGFP and iPSCs cultured on PEN nanofibers were laser-irradiated. (b.1) The fraction of eGFP positive cells 24 hours after laser irradiation at different fluences. (b.2) iPSC viability 24 hours after transfection, as determined by the CellTiter-Glo assay. (b.3) Representative flow plot showing iPSCs transfected with eGFP mRNA by irradiation at 0.12 J/cm^2. (b.4) Representative flow plot showing iPSCs transfected with eGFP mRNA by irradiating thrice at 0.12 J/cm^2.

PEN photoporation enables gene editing in hiPSCs

PEN photoporation enables efficiently generating biallelic CD90 knock-outs in hiPSCs.

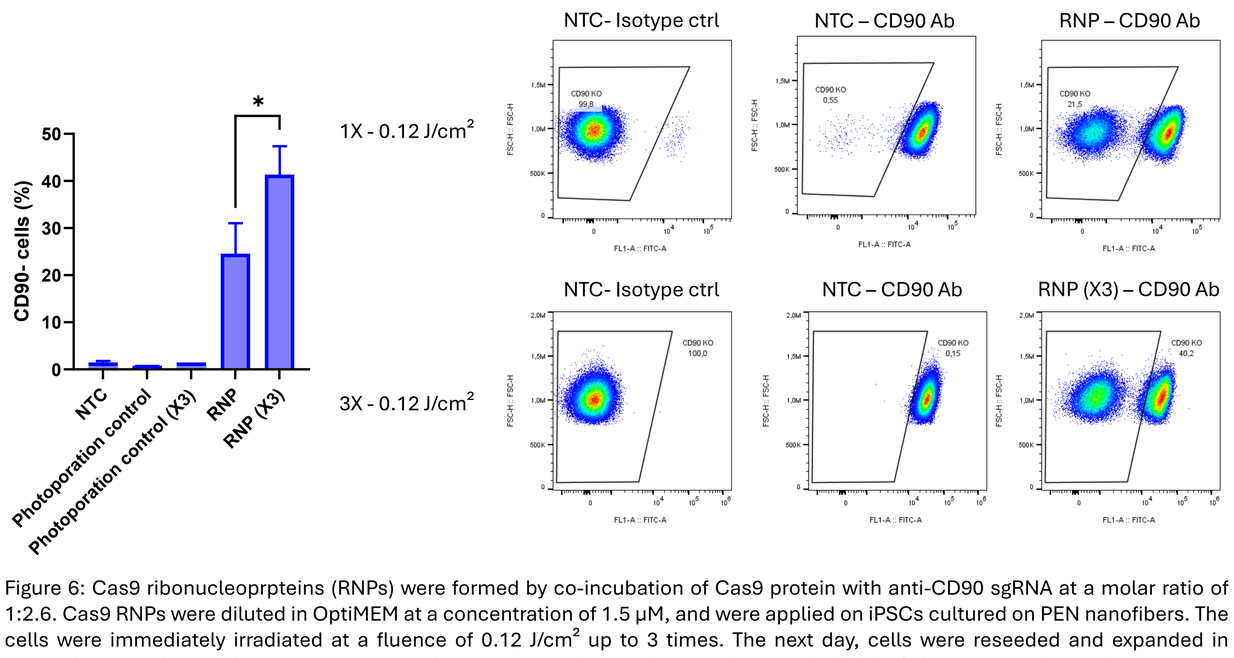


Figure 6: Cas9 ribonucleoprpteins (RNPs) were formed by co-incubation of Cas9 protein with anti-CD90 sgRNA at a molar ratio of 1:2.6. Cas9 RNPs were diluted in OptiMEM at a concentration of 1.5 µM, and were applied on iPSCs cultured on PEN nanofibers. The cells were immediately irradiated at a fluence of 0.12 J/cm^2 up to 3 times. The next day, cells were reseeded and expanded in conventional culture plates for 4 more days. Cells were eventually stained with an anti-CD90 FITC-labeled antibody to assess the rate of non-homologous end joining.

Conclusion

- **PEN photoporation is a robust yet gentle** method enabling the delivery of effector molecules up to 500 kDa **in iPSCs, including mRNA and Cas9 RNPs.**
- **PEN photoporation enables performing gene editing in iPSCs.**
- **iPSCs can be cultured** and transfected **on nanofiber substrates while maintaining (1) their viability and proliferative capacity, (2) the expression of pluripotency** markers and, **(3) their ability to undergo directed differentiation.**
- **PEN photoporation is a promising transfection technology for iPSC-based cell therapies.** While very gentle to the cells, further work is needed to increase the transfection efficiency of large constructs like mRNA and pDNA.

References: Xiong et al., Nature Nanotechnology, 2021. Berdecka et al., Applied Materials Today, 2024. Goemaere et al., International Journal of Molecular Sciences, 2023.

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