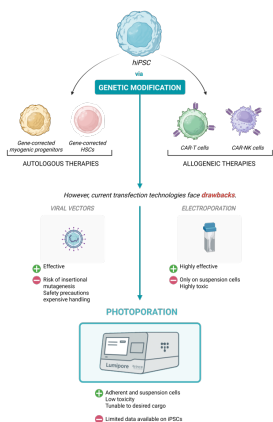


INTRODUCTION



METHODS

Figure 1. Traditional photoporation workflow

- 80 nm cationic gold nanoparticles (PDDAu NPs) 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 μ M) is added to promote cell survival.
- 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**.
- hEMP** (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.

RESULTS

Polydopamine photoporation can efficiently deliver large biomolecules

Delivery of eGFP mRNA

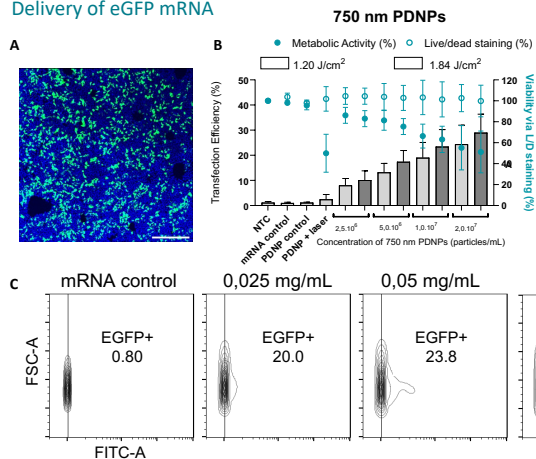


Figure 2. (A) Confocal microscopy image of eGFP expression (green channel) in iPSC29 24 hours after 750 nm PDNP-photoporation with 0,1 mg/mL eGFP mRNA. Nuclei were stained with Hoechst 33342 (blue channel). (B) Screening of particle concentrations for 750 nm using 0,025 mg/mL eGFP mRNA. (C) Flow cytometry plots for transfection of eGFP mRNA in increasing concentrations (FITC-A channel) using 750 nm PDNPs.

Delivery of fluorescently labelled CRISPR-Cas9 RNPs

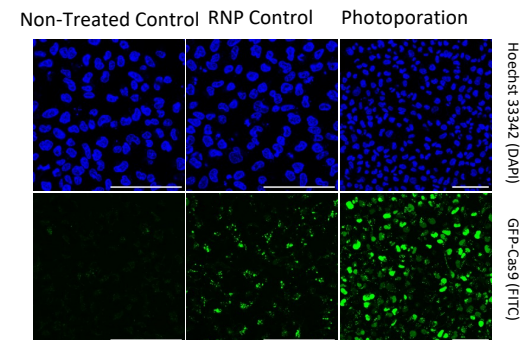


Figure 3. Confocal microscopy image of GFP-labelled Cas9 nuclease (green channel) in iPSC29. Images were taken 6 hours after photoporation with 250 nm PDNPs (1,84 J/cm² and 1,6.10⁹) and 2 μ M RNPs. Nuclei were stained with Hoechst 33342. Scale bar: 100 μ m.

Delivery of CRISPR-Cas9 RNPs for CD90 (Thy-1) knock-outs

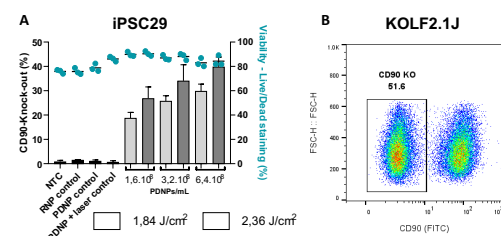


Figure 4. (A) Flow cytometry readout of CD90 knockout on iPSC29 72 hours after using 250 nm PDNP photoporation. Immunofluorescence and flow cytometry was used to stain the CD90 surface antigen, and incubation with TO-PRO-3 (APC) allowed to gate on viable cells. (B) Dot plot gating of CD90 negative KOLF2.1J cells created with 250 nm PDNPs (6,4.10⁸ PDNPs/mL and 2,36 J/cm²).

Delivery of CRISPR-Cas9 RNPs for Beta-2-microglobulin (B2M) knock-outs

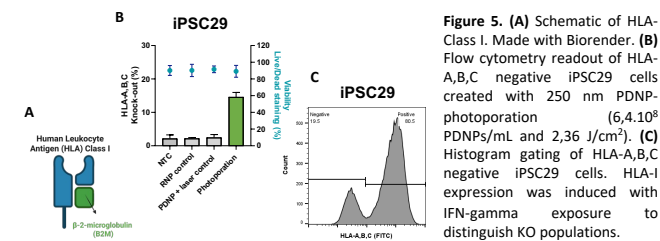


Figure 5. (A) Schematic of HLA-Class I. Made with Biorender. (B) Flow cytometry readout of HLA-A,B,C negative iPSC29 cells created with 250 nm PDNP-photoporation (6,4.10⁸ PDNPs/mL and 2,36 J/cm²). (C) Histogram gating of HLA-A,B,C negative iPSC29 cells. HLA-I expression was induced with IFN-gamma exposure to distinguish KO populations.

iPSC characteristics are perfectly preserved

Expression of surface markers of the undifferentiated state

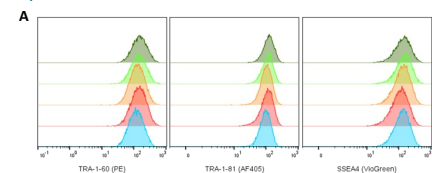
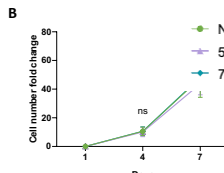
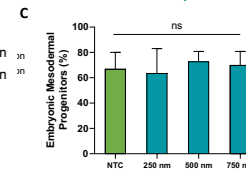


Figure 6. (A) Expression of stemness markers TRA-1-60, TRA-1-81, and SSEA4 on photoporated iPSCs. AuNP-photoporation used 3,2.10⁸ AuNPs/mL and 1,63 J/cm², whereas PDNP was executed with 1,84 J/cm² and 1,6.10⁹; 1,0.10⁷ and 2,0.10⁷ particles/mL for 250,500 and 750 nm TRA-1-81, respectively. (B) Proliferation of photoporated iPSCs compared to Non-Treated Control (NTC). (C) iPSCs underwent a 3,5 day guided differentiation towards Embryonic Mesodermal Progenitors (EMPs) 24 hours post-photoporation. (D) Long-term metabolic activity, on day 4 and day 7 after photoporation as measured with CellTiter-Glo. Fluences and particle concentrations for all graphs are the same as mentioned in part A. Statistical analysis was performed using Ordinary one-way ANOVA with Dunnett's multiple comparisons test and a single pooled variance (n=3).

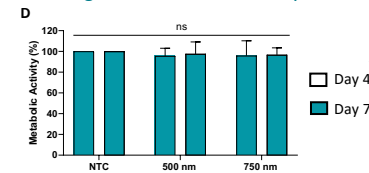
Proliferation



Differentiation potential



Long-term metabolic activity



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