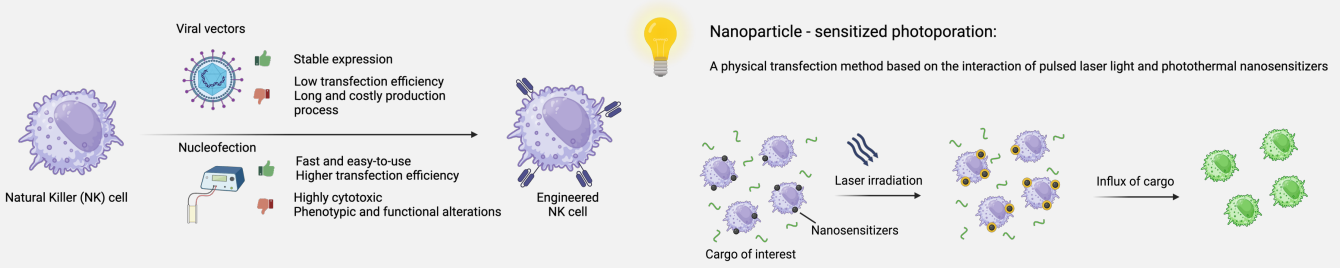
Photoporation: a promising strategy for the generation of engineered NK cell therapies

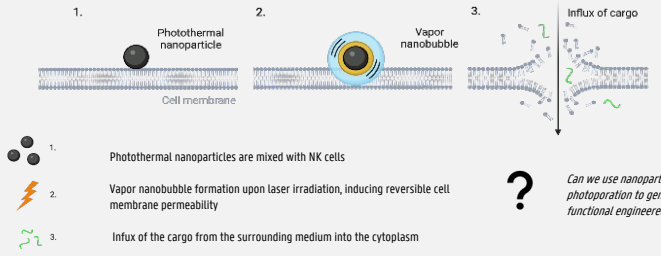
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Introduction

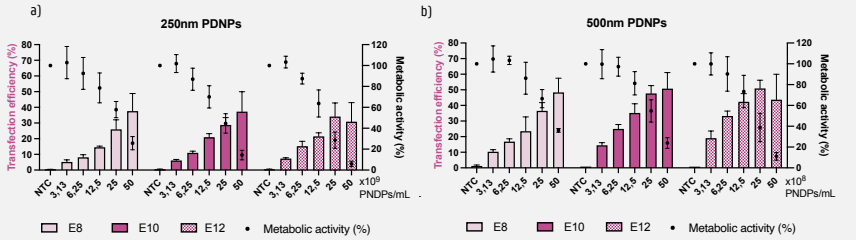


Materials and methods



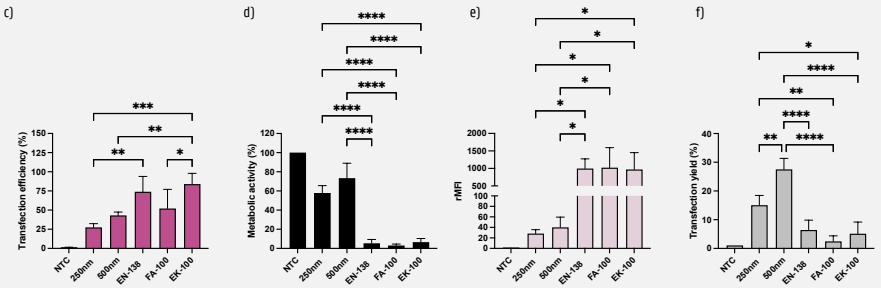
Results

Screening optimal photoporation parameters



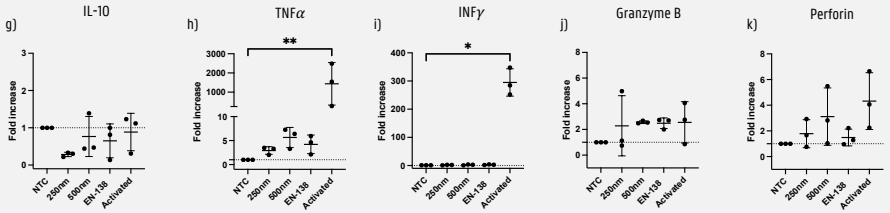
Graph a,b) Transfection of expanded NK cells with 0,25mg/mL eGFP mRNA. The transfection efficiency (left y-axis, bars) for increasing concentrations of 250nm (a) and 500nm (b) polydopamine nanoparticles (PDNPs) irradiated with laser fluence E8 (light pink), E10 (dark pink) and E12 (white and pink). The corresponding metabolic activity (right y-axis, black dots) was calculated using the CellTiter Glo assay.

Comparison to state-of-the-art Nucleofection

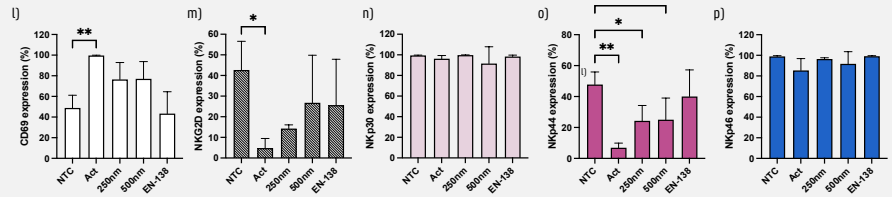


Graph c-f) Transfection of expanded NK cells with 0,25mg/mL eGFP mRNA using photoporation and Nucleofection. Comparison of the transfection efficiency (c), metabolic activity (d), relative mean fluorescence intensity (e) and transfection yield (f), 24 hours after photoporation/Nucleofection. Samples were photoporated with 25x10⁸ or 12,5x10⁸ PDNPs/mL for the 250nm and 500nm PDNPs, respectively. For Nucleofection, the pulse codes EN-138, FA-100 and EK-100 were used. Statistical analysis was performed using an ordinary one-way ANOVA with Tukey's multiple comparisons test, with a single pooled variance (n≥3, *p <0,05; **p < 0,01; ***p < 0,001; ****p < 0,0001).

Evaluation NK cell phenotype after photoporation/Nucleofection



Graph g-k) The presence of different cytokines in the cell supernatant measured using the LEGENDPlex assay 24h after treatment, expressed as fold change compared to NTC samples. Statistical analysis was performed with the Kruskal-Wallis test and Dunn's multiple comparisons test (n=3, *p < 0,05; **p < 0,01).



Graph l-p) Expression levels of five surface markers (CD69, NKG2D, NKp30, NKp44, NKp46) 24h after treatment. Statistical analysis was performed using an Ordinary one-way ANOVA and Dunnett's multiple comparisons test, with a single pooled variance (n=3, *p < 0,05; ** p < 0,01).

Proliferation capacity of NK cells after photoporation/Nucleofection

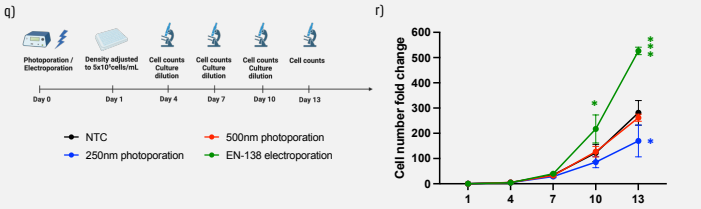
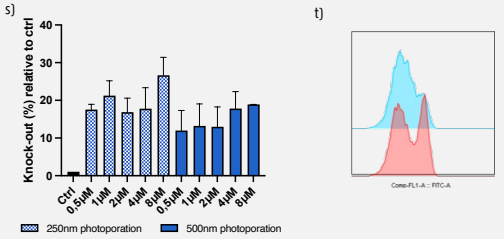


Illustration q) Schematic summary of the experimental procedure and corresponding timing. Cells were treated on day 0 and the cell density was adjusted to 5x10⁵ cells/mL the following day (day 1). From day 4-13 cell densities were determined and dilutions were performed. Graph r) The cell number fold change (relative to cell numbers on day 1) of expanded NK cells at different time points after photoporation with 250 nm (25x10⁸ PDNPs/mL) and 500 nm (12,5x10⁸ PDNPs/mL) PDNPs, and nucleofection with the EN-138 pulse code. Statistical analysis was performed using Ordinary one-way ANOVA with Dunnett's multiple comparisons test and a single pooled variance (**n=1**, *p < 0,05; ***p<0,001).

Cas9/RNPs mediated NKG2A KO in NK cells



Graph s,t) Knock-out of the NKG2A receptor in expanded NK cells with Cas9/RNPs. s) The knock-out efficiency 48h after photoporation using 250 nm (25x10⁸ PDNPs/mL) and 500 nm (12,5x10⁸ PDNPs/mL) PDNPs. The efficiency expressed as a percentage, relative to the corresponding control (using scrambled gRNA) for increasing concentration of the Cas9/RNPs. t) A flow cytometry histrogram showing the difference in distribution of fluorenscence intensity values after photoporation with 250nm PNDPs and 8uM of Cas9/RNPs (red for the control and blue for the actual KO).

Conclusions and future perspectives

- Photoporation can be used **to transfect expanded NK cells**
- Photoporation **outperforms Nucleofection** in terms of transfection yield
- **Phenotype** of NK cells does **not appear to be altered** after photoporation/Nucleofection
- Photoporation can be used for **NKG2A receptor KO** in expanded NK cells

Next steps; the **comparison of NKG2A KO efficiency** between photoporation and Nucleofection

Evaluate the cytotoxicity of NKG2A KO NK cells (e.g. co-culture with K562 cells)

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