Photoporation: a promising strategy for the generation of CAR-NK cells

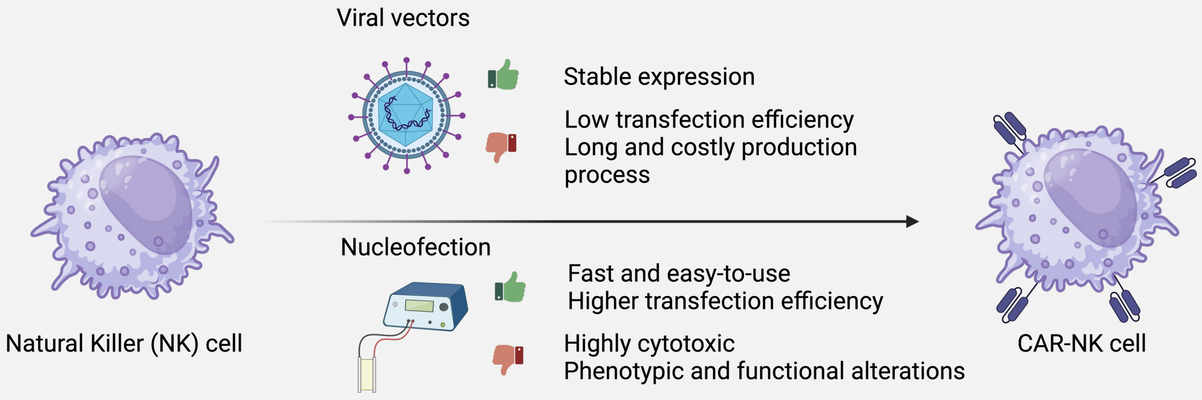
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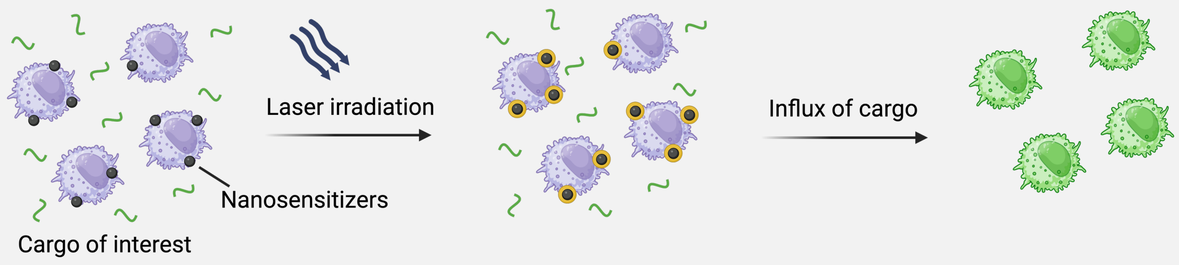
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INTRODUCTION

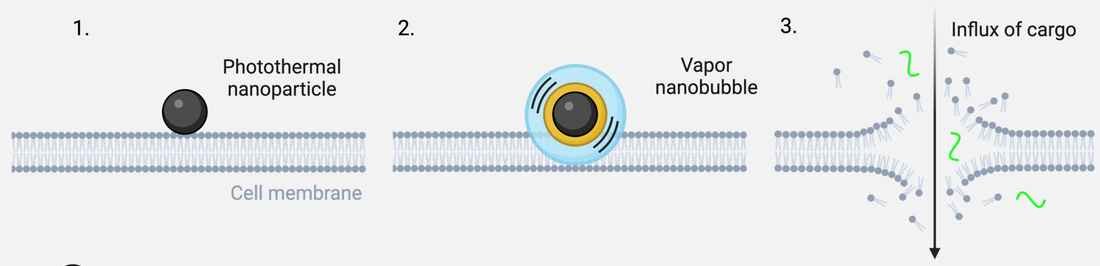


Nanoparticle - sensitized photoporation:

A physical transfection method based on the interaction of pulsed laser light and photothermal nanosensitizers



MATERIALS and METHODS



1. Photothermal nanoparticles are mixed with NK cells

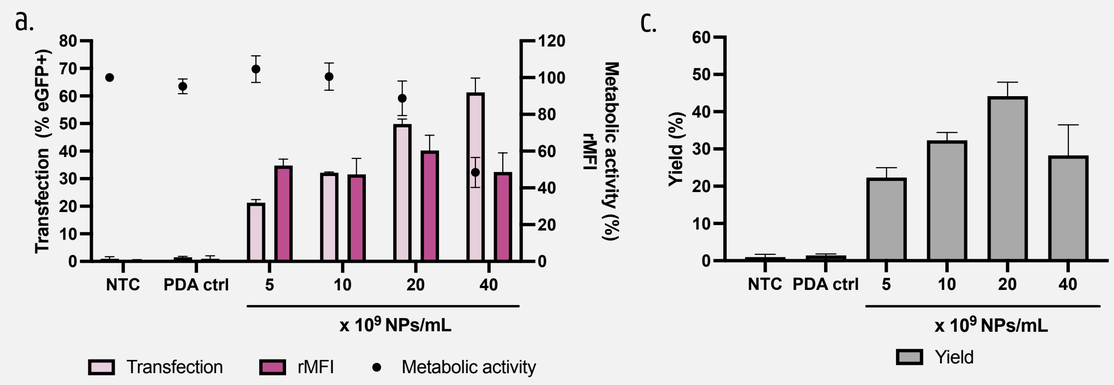
2. Vapor nanobubble formation upon laser irradiation, inducing reversible cell membrane permeability

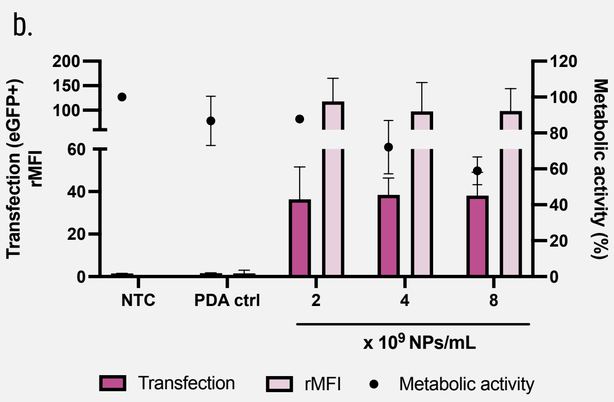
3. Influx of the cargo from the surrounding medium into the cytoplasm

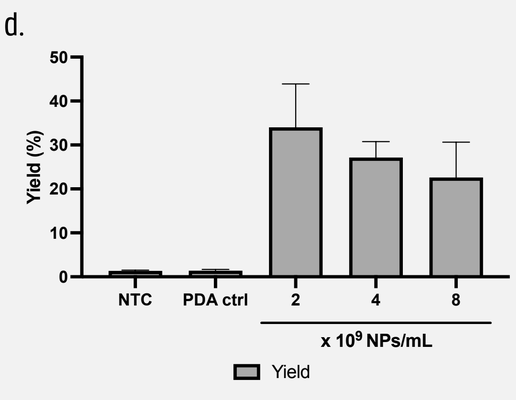
? *Can we use nanoparticle-sensitized photoporation to generate highly functional CAR-NK cells?*

RESULTS

Screening for the optimal PDA-BSA particle size and concentration



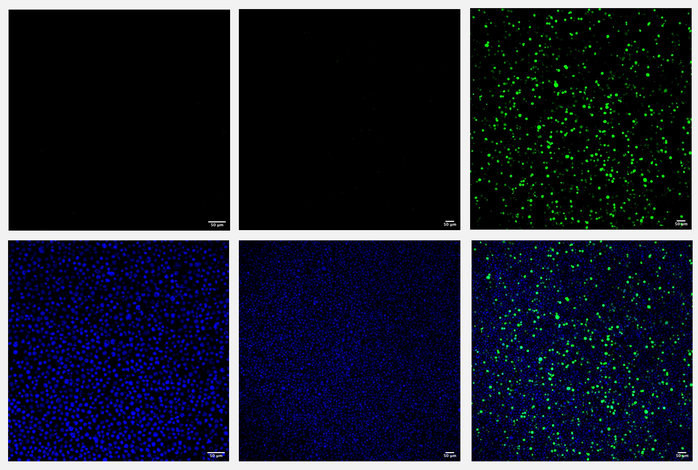




Graph a,b) Transfection of NK-92MI cells with 0,3μM eGPF-mRNA for increasing concentrations of PDA-BSA nanoparticles. Transfection efficiency (left y-axis, dark pink) and relative mean fluorensce intensity (right (a)/ left (b) y-axis, light pink) 24 hours after photoporation with 250nm (a) and 500nm (b) PDA-BSA nanoparticles. The corresponding metabolic activity (right y-axis, black dots) was calculated using the CellTiter-Glo assay.

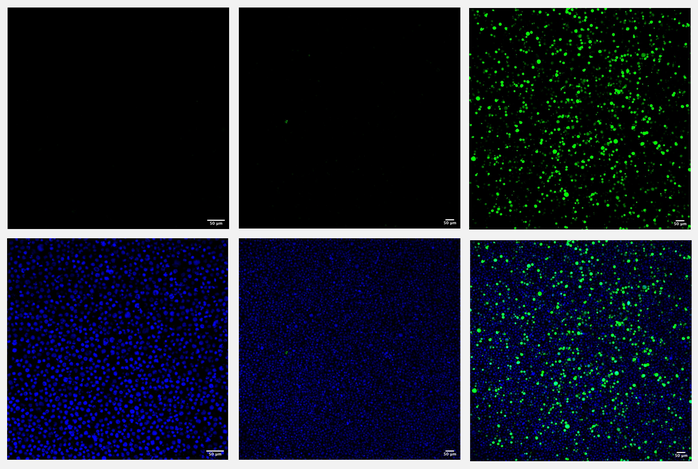
Graph c,d) Transfection yield of NK-92MI cells 24 hours after photoporation with increasing concentrations of 250nm (c) and 500nm (d) PDA-BSA nanoparticles. Transfection yield is the fraction viable and transfected cells, relative to the starting population. It was calculated as the product of transfection efficiency (%) and metabolic activity (%) determined by Cell TiterGlo assay).

e.



e) Confocal images of eGFP-mRNA transfection of NK-92MI cells 24h after photoporation. Nuclei were stained with Hoechst. NTC = non-treated control cells, Control = cells photoporated with 20x10⁹ NPs/mL 250nm PDA-BSA without the presence of mRNA. Last column represents cells photoporated with 20x10⁹ NPs/mL 250nm PDA-BSA in the presence of 0,3 μ M eGFP-mRNA.

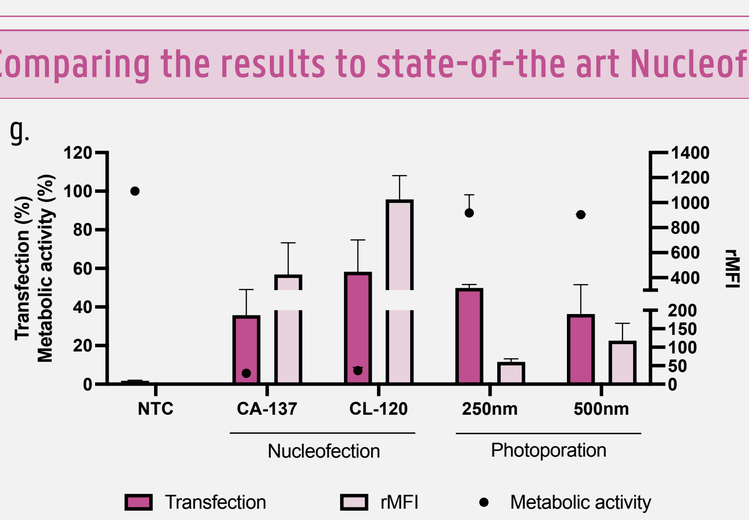
f.



f) Confocal images of eGFP-mRNA transfection of NK-92MI cells 24h after photoporation. Nuclei were stained with Hoechst. NTC = non-treated control cells, Control = cells photoporated with 2x10⁹ NPs/mL 500nm PDA-BSA without the presence of mRNA. Last column represents cells photoporated with 2x10⁹ NPs/mL 500nm PDA-BSA in the presence of 0,3 μ M eGFP-mRNA.

Comparing the results to state-of-the art Nucleofection

g.



g) Transfection of NK-92MI cells with 0,3μM eGPF-mRNA. Comparison of the transfection efficiency (dark pink, as a percentage), metabolic activity (black dots, as a percentage) and relative MFI (light pink, relative to the NTC) for photoporation with 20x10⁹ PDA-BSA nanoparticles/mL (250nm), 2x10⁹ PDA-BSA nanoparticles/mL (500nm) and two different Nucleofection protocols (CL-120 and CA-137).

h.

h) Transfection of NK-92MI cells with 0,3μM eGPF-mRNA. Comparison of the transfection yield (fraction viable and transfected cells, calculated as the product of transfection effciciency and metabolic activity) of photoporation with 20x10⁹ PDA-BSA nanoparticles/mL (250nm), 2x10⁹ PDA-BSA nanoparticles/mL (500nm) and two different Nucleofection protocols (CL-120 and CA-137). A one-way ANOVA with Tukey's multiple comparison test was used to compare the yield values.

CONCLUSION and FUTURE PERSPECTIVES

Photoporation can be used **to deliver large marcomolecules** in NK-92MI cells

Photoporation **outperforms Nucleofection** in terms of transfection yield

Next steps include the transfection with **mRNA encoding for a chimeric antigen receptor**

Study the cytotoxicity and functionality of CAR NK-92 cells created by photoporation

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Other posters on photoporation: n°744 and 799 by Chloë De Clercq