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

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RESULTS

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Screening for the optimal PDA-BSA particle size and concentration



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

x 10⁹ NPs/mL

NTC PDA ctrl

лU

x 10⁹ NPs/mL

PDA ctrl x 10⁹ NPs/mL

Tield

Graph c,d) Transfection yield of NK-92MI cells 24 hours after photoporation with increasing concentrations of 250mm (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) 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 20x10^o 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.

NPs/mL 500nm mRNA.

photoporation. Nuclei were stained with Hoechst. NTC = non-treated control cells, Control = cells photoporated with 2x10° 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-

f) Confocal images of eGFP-mRNA transfection of NK-92MI cells 24h after

NTC Control 2x10^o NPs/mL 500nm PDA-BSA

Comparing the results to state-of-the art Nucleofection



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, transference) 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) Transfection of NK-92MI cells with 0,3 µM eGPF-mRNA. Comparison of the transfection yield (fraction viable and transfected cells, calculated as the product of transfection efficiency 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 vield 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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