Gentle delivery of macromolecules in monocytes and macrophages by photoporation

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Introduction

Pivotal in inflammation and tissue homeostasis, monocytes and macrophages are an enticing therapeutic target. Harnessing their desirable properties often involves the intracellular delivery of functional macromolecules. Here, we explored photoporation as an up-and-coming gentle intracellular delivery method. Upon laser irradiation of photothermal nanoparticles, photothermal effects – *e.g.*, vapor nanobubbles (VNBs) – induce transient membrane permeabilization, allowing the influx of external molecules in the cell. In this study, we used polydopamine nanoparticle (PDNP) nanosensitizers for photoporation of monocyte/macrophage-like cell lines. The impact on cellular phenotype was investigated and compared to Nucleofection® for the intracellular delivery of model macromolecules (**Figure 1**).

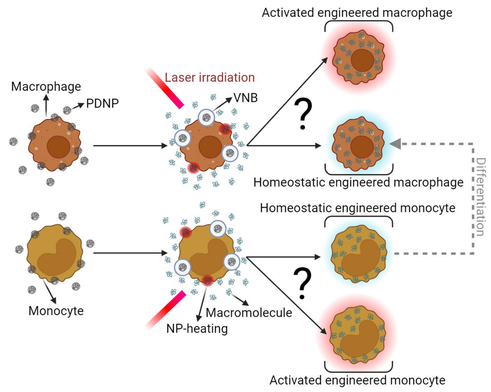


Figure 1 Schematic overview of polydopamine nanoparticle (PDNP)-sensitized photoporation.

Results

Identification of optimal photoporation parameters

- Increases in PDNP size/concentration & laser pulse fluence led to an enhancement in delivery efficiencies, but at the expense of cell viability, resulting in a maximum achievable delivery yield (= delivery efficiency x relative viability) (**Figure 2**).
- 500 nm PDNPs resulted in the highest delivery yield in all cell types, though optimal PDNP concentrations varied between cell types.
- THP-1 monocyte-like (THP-1Mo) and THP-1 macrophage-like cells (THP-1Ma) reached their highest yields at a laser pulse fluence of 1.13 J/cm² instead of 0.54 J/cm² for the RAW264.7 cells.

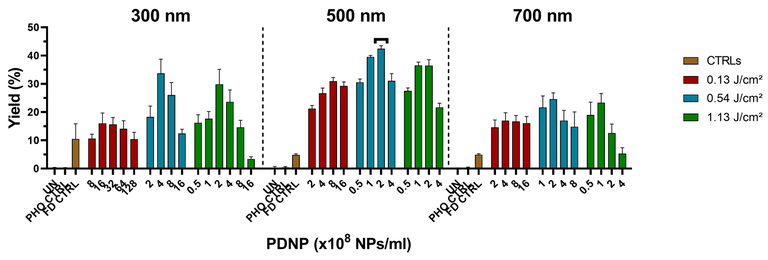


Figure 2 Optimization of FITC-Dextran 500 kDA (1 mg/ml) delivery yield in RAW264.7 murine macrophage-like cells by tailoring PDNP size, PDNP concentration and laser fluence. Black square bracket indicates optimal photoporation conditions.

Delivery of eGFP-mRNA

- Photoporation of monocyte-like cells (THP-1Mo) resulted in similar eGFP-mRNA transfection yields in comparison to Nucleofection®. Relative to Nucleofection® and the results for THP-1Mo cells, lower transfection yields were achieved in photoporated macrophage-like cells (RAW264.7 & THP-1Ma) (**Figure 3**).
- Significantly less cytotoxicity was inflicted by photoporation as compared to Nucleofection®.

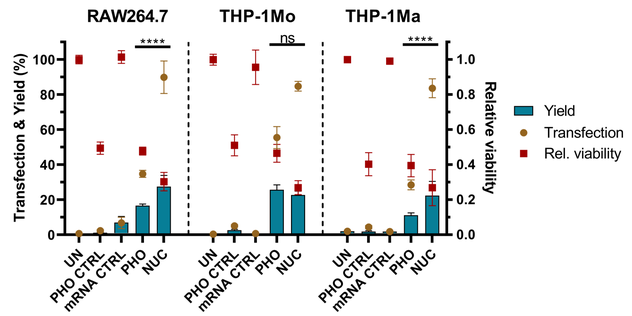


Figure 3 Comparison of eGFP-mRNA (0.1 mg/ml) transfection efficiency, cell viability and transfection yield by photoporation (PHO) and Nucleofection® (NUC) of RAW264.7, THP-1Mo and THP-1Ma. Incubation of cells with 0.1 mg/ml eGFP-mRNA (mRNA CTRL) did not induce transfection. Two-way ANOVAs with Dunnett's multiple comparisons test (PHO as reference) were performed to determine statistically different yields. Data are represented by the mean ± standard deviation of three biological replicates, each consisting of three technical replicates. ns = nonsignificant, * p < 0.05, ** p < 0.01, *** p < 0.001 & **** p < 0.0001.

Effect on homeostasis

Proliferative capacity

- No effect of photoporation on proliferation of RAW264.7 or THP-1Mo cells was found.
- Nucleofection® did not affect the proliferation of RAW264.7 cells, while it did stunt growth of THP-1Mo cells (**Figure 4A**). This was the result of a decreased proliferation rate in the first day post-treatment (**Figure 4B**)

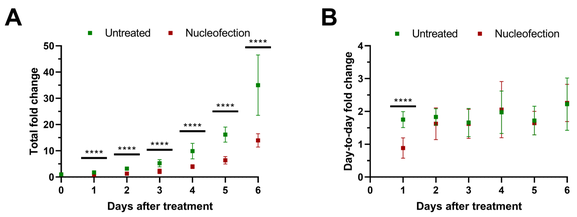


Figure 4 A) Total fold change in THP-1Mo cell number with respect to amount of cells at day 0, measured for 6 days after treatment by nucleofection. All cells were seeded at the same density, with the untreated cells serving as a baseline. B) Fold change of THP-1Mo on day *n* with respect to number of cells on day *n-1*. Data are represented by the mean ± standard deviation of three biological replicates, each consisting of three technical replicates. Multiple t-tests – Holm-Sidak-adjusted p values – were performed to determine statistically different results between treated cells and untreated cells (* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001).

Cell morphology

- Morphology was unaltered 24 hours post-treatment with photoporation of RAW264.7 & THP-1(Mo/Ma) (**Figure 5**).
- Multinuclear cells were present in Nucleofection®-treated RAW264.7 (**Figure 5 insets**), indicating a fusogenic altered phenotype – found in *e.g.*, osteoclasts –, while THP-1(Mo/Ma) were not affected morphologically.

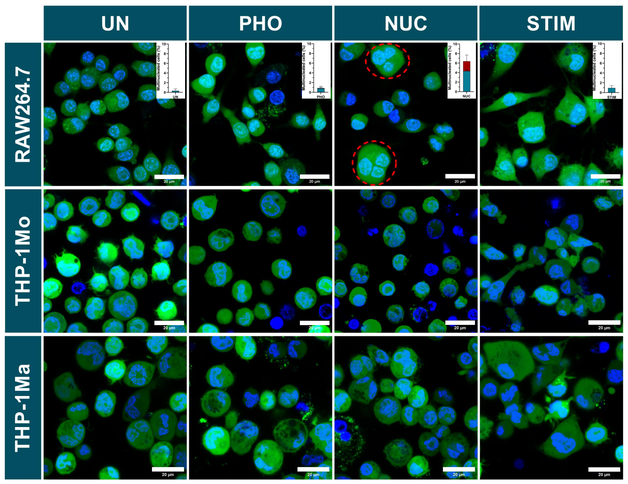


Figure 5 Representative images (24 hours post-treatment) of THP-1(Mo/Ma) & RAW264.7 cells subjected to photoporation (PHO), Nucleofection® (NUC) or stimulation (STIM) - IFN-γ (20 ng/ml) & LPS (100 ng/ml) for RAW264.7 & THP-1Ma, and 15 ng/ml PMA for THP-1Mo cells. Multinucleated RAW264.7 cells are indicated by brown circles, with the insets showing the percentage of double nucleated (blue) and triple (or more) nucleated cells (red). Cytoplasm and viable cells were visualized by Calcein AM (green); while the nucleus was stained by Hoechst 33342 (blue). Scale bars = 20 µm.

Cell surface markers

- A small decrease in CD80⁺, without change in expression levels, was observed in photoporated RAW264.7 cells, with Nucleofection® leading to an increase in % positive cells and expression levels of CD206 & MHCII (**Figure 6A**).
- Neither photoporation nor Nucleofection® influenced the expression of canonical activation/polarization markers in THP-1Mo (CD14, CD11 & CD36) or THP-1Ma (CD80, CD206 & HLA-DR) (**Figure 6A&B**).

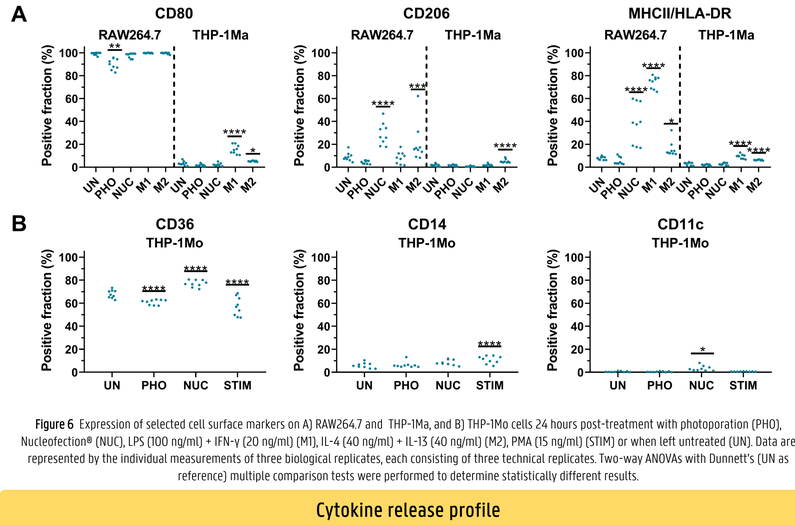


Figure 6 Expression of selected cell surface markers on A) RAW264.7 and THP-1Ma, and B) THP-1Mo cells 24 hours post-treatment with photoporation (PHO), Nucleofection® (NUC), LPS (100 ng/ml) + IFN-γ (20 ng/ml) (M1), IL-4 (40 ng/ml) + IL-13 (40 ng/ml) (M2), PMA (15 ng/ml) (STIM) or when left untreated (UN). Data are represented by the individual measurements of three biological replicates, each consisting of three technical replicates. Two-way ANOVAs with Dunnett's (UN as reference) multiple comparison tests were performed to determine statistically different results.

Cytokine release profile

- MDC and TNF-α secretion were slightly, yet significantly downregulated after photoporation of RAW264.7 cells (**Figure 7A**).
- Elevated levels of MCP-1 and IL-8 were detected following photoporation or Nucleofection® of THP-1Mo cells (**Figure 7B**).
- In THP-1Ma, photoporation and Nucleofection® increased secretion of IL-8, IL-10, IL-1β & TNF-α (**Figure 7C**).

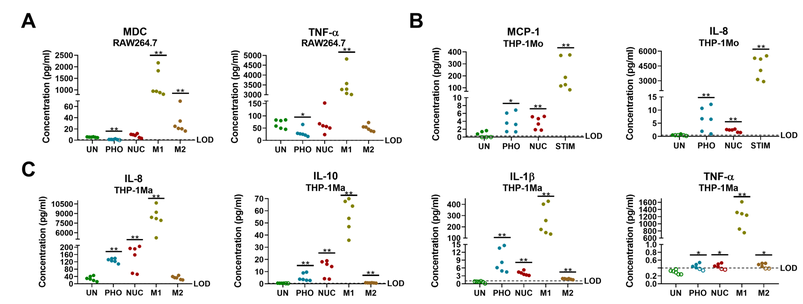


Figure 7 Cytokine levels that were significantly altered 24 hours post-treatment of A) RAW264.7 B) THP-1Mo & C) THP-1Ma. Treatments: photoporation (PHO), Nucleofection® (NUC), LPS (100 ng/ml) + IFN-γ (20 ng/ml) (M1), IL-4 + IL-13 (both 40 ng/ml) (M2), PMA (15 ng/ml) (STIM) and untreated (UN). ** p-value < 0.01 and * p-value < 0.05. Mann-Whitney U test performed with respect to UN, not corrected for mutiple testing due to low statistical power and low sample number (N = 12).

Conclusions

Overall, these findings show that PDNP photoporation-based intracellular delivery is competitive with the state-of-the-art Nucleofection® delivery method, while allowing for higher cell viability, spatiotemporal selectivity, flexibility in execution (one NP type vs. cell specific buffers/kits) and no risk of inducing (multinucleated) phenotypically divergent monocytes/macrophages in established cell lines.

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Research Foundation Flanders
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