Investigation of Nuvec Particles for Effective Gene Delivery

N. Ponnappan[1], J. Szczerkowski[1], M. Bachman[1], M. Main[1], D. Templeton[2], R. Harris[2], M. Janssen[2], N. Theobald[2], D. Yilmaz[1]

1. Medicines Discovery Catapult, Alderley Park, Cheshire, SK10 4ZF, UK.

2. N4 Pharma, Weston house Bradgate Park View, Chellaston, Derbyshire, DE73 5UJ

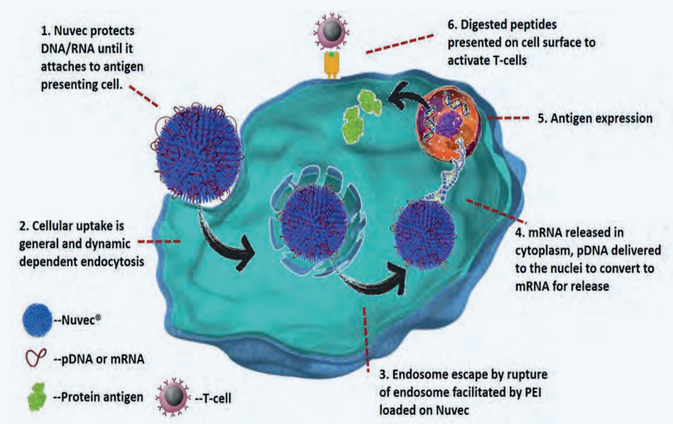
md.catapult.org.uk

Abstract

Effective delivery of nucleic acids into the cells holds great potential for vaccine development and cancer treatments. N4 Pharma has been developing Nuvec® – a unique non-viral adjuvant delivery system for the effective delivery of nucleic acids into the cells. Nuvec silica nanoparticles have a unique irregular (spiky) surface structure, coupled with polyethyleneimine (PEI), that simply and effectively traps and protects nucleic acids (such as mRNA / pDNA) as it travels into the cells.

Here we carried out biological characterisation of Nuvec particles using a range of methods. We determined the size, zeta potential and DNA/RNA loading capacity of different size Nuvec particles using DLS, zeta sizer and mass spectrometry respectively. Next, we determined the transfection efficiency of Nuvec at different Nuvec:nucleic acid ratios using both live cell imaging and flow cytometry methods. Furthermore, we investigated the stability of mRNA upon lyophilisation of Nuvec:mRNA complex and demonstrated that Nuvec particles protect the RNA from degradation even after lyophilisation. Additionally, we studied the cellular internalisation of Nuvec using high resolution microscopy. We analysed 3D reconstructed microscopy images and quantified the number of Nuvec particles inside the cells. Finally, we studied the mechanism of cellular internalisation in HEK293T cells upon inhibition of different endocytosis pathways.

Our data demonstrates that Nuvec particles provide protection to RNA/DNA, efficiently deliver DNA/RNA into the cells and result in protein expression. Furthermore, our data provides insights into the mechanisms involved in the cellular uptake of Nuvec particles. Altogether our findings provide guidance for the rational design of Nuvec for efficient gene delivery applications.



Method

Loading of Nuvec with different amounts of plasmid DNA/mRNA → Incubation at room temperature for 20 min → Size distribution and zeta potential analysis using DLS & zetasizer; Mass spectrometry analysis to assess the binding of DNA/mRNA molecule to Nuvec particles

Lyophilisation of complex → Transfection of HEK293T cells seeded in 96-well plate with nucleic acid: Nuvec complex → Incubation at 37°C, 5% CO_2 for different time-periods → Microscopy and flow cytometry analysis

Results

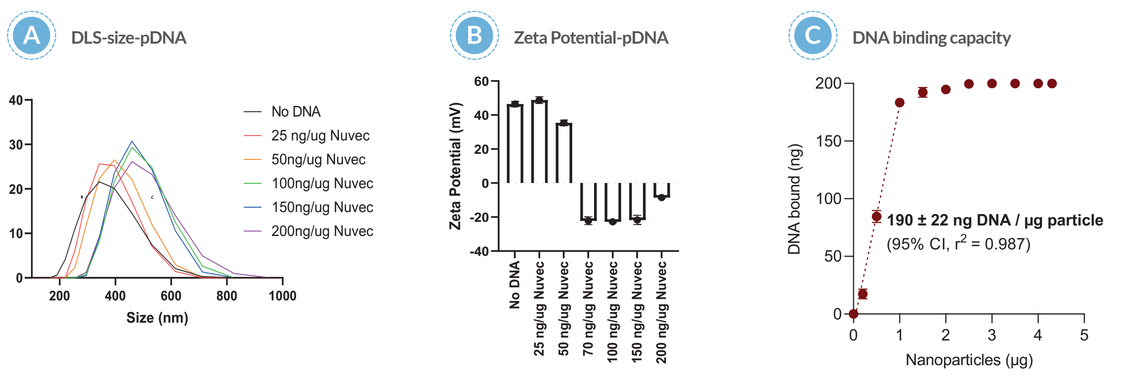


Figure 1. Plasmid DNA (pmaxGFP) loading on Nuvec particles at various mass ratios (ng of plasmid per ug of Nuvec). A: Size distribution analysis of pmaxGFP:Nuvec particles by DLS. B: Assessment of zeta potential of particles by zeta nanosizer. C: Mass spectrometry analysis of binding of plasmid DNA to Nuvec particles.

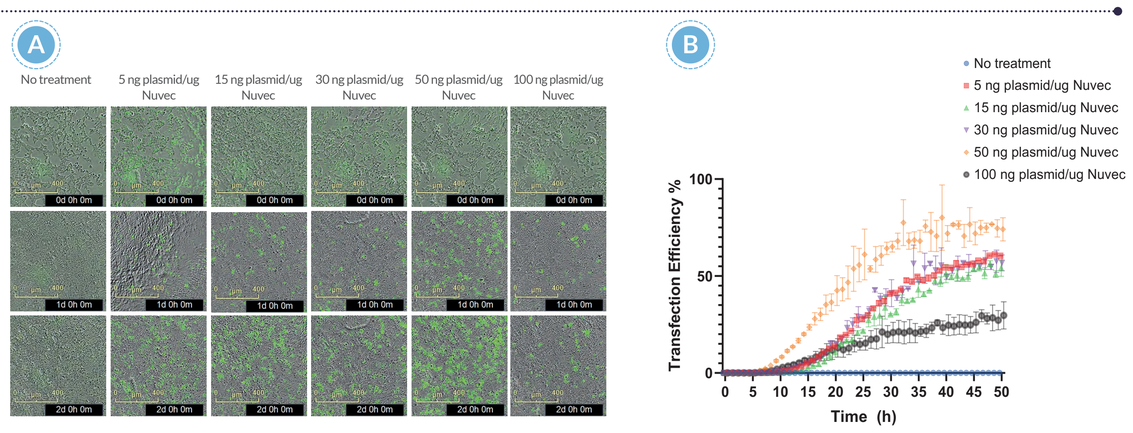


Figure 2. Representative microscopy images (A) & graph showing the transfection efficiency (B) of HEK293T cells transfected with plasmid:Nuvec at different mass ratios over a period of 48 h.

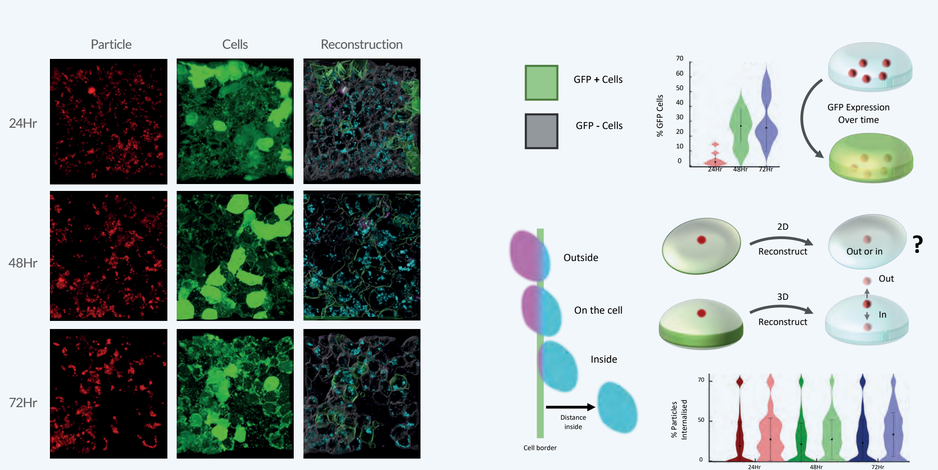


Figure 3. High resolution microscopy images of HEK293T cells treated with Alexa-568 labelled Nuvec particles. 3D reconstruction of images were carried out and percentage of cells expressing GFP and number of Nuvec particles internalised were assessed

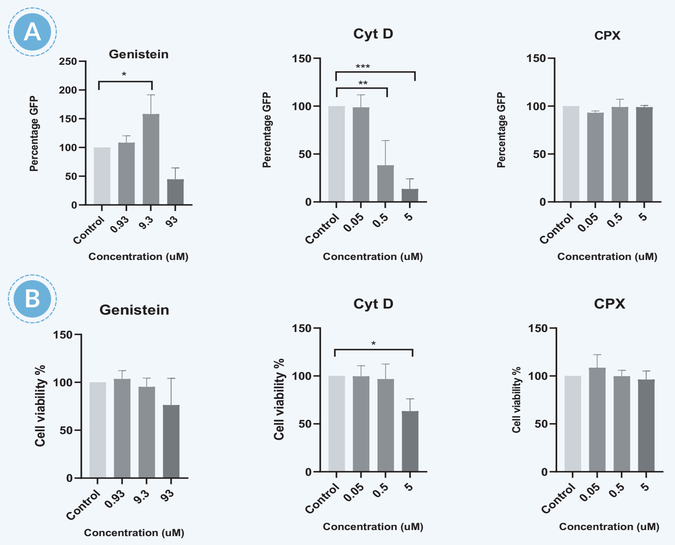


Figure 4. Effect of endocytic inhibitors on the transfection efficiency of plasmid DNA loaded Nuvec (A) & viability of cells (B). Control are the HEK293T cells with no endocytic inhibitor.

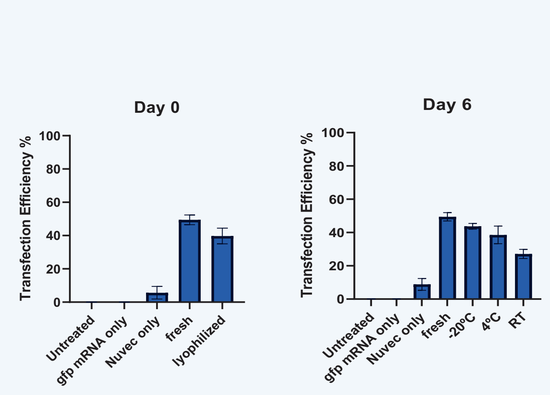


Figure 5. Transfection efficiency of freshly lyophilized GFP mRNA:Nuvec at day 0 and that stored at different conditions for 6 days after lyophilization

Conclusions

- Nuvec particle efficiently binds to nucleic acids – plasmid DNA & mRNA, and mediates their intracellular delivery mainly through macropinocytic pathway
- The cells transfected with GFP plasmid loaded Nuvec particles exhibit up to 70% transfection efficiency
- Our data for transfection efficiency of lyophilized GFP mRNA loaded Nuvec stored at -20°C indicates the ability of Nuvec particles to protect the nucleic acids from degradation. The freeze-dried formulations of Nuvec-based nucleic acid therapeutics would be convenient and cost-effective

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