Efficient Intracellular Delivery of Nucleic Acids by NUVEC® Silica Nanoparticles

Aadarash Zia,[1] Nisha Ponnappan,[1] Duygu Yilmaz,[1] Rebecca,[1] Dominic Simpson,[1] Isabel Peset Martin,[1] Emily Offer,[1] David Templeton,[2] Robert Harris,[2] Melody Janssen,[2] Nigel Theobald,[2] Lorna FitzPatrick,[1]

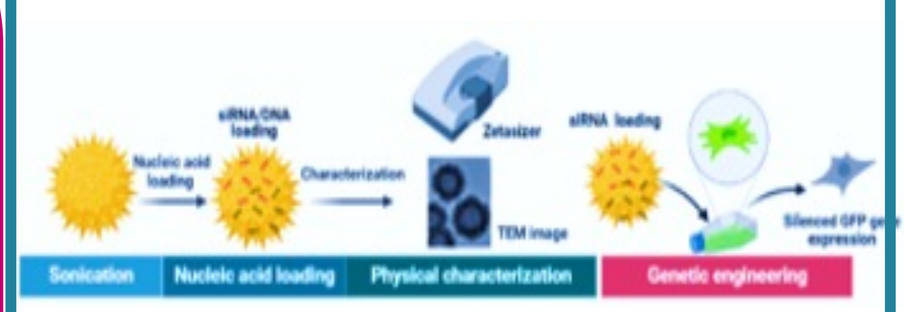
[1] Medicines Discovery Catapult, Block 35, Alderley Park, Cheshire, SK10 4ZF

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

md.catapult.org.uk

In recent years, there has been a surge in the development of a more diverse range of therapeutic modalities to treat disease, initiating a shift from a drug discovery pipeline of largely small and large molecule medicines. This includes but is not exclusive to nucleic acid therapeutics. Although efficacious and target specific when inside the cell, limitations of delivery are restricting translation of therapeutic mRNA, siRNA and DNA to the clinic. It is critical to ensure adequate exposure and penetration in the target tissues and cells. In a quest to tackle this issue, N4 Pharma has developed NUVEC®, a next-generation, complex silica nanoparticle with the capacity to deliver nucleic acids efficiently into a broad spectrum of cell types. These particles have an intricate structure with a unique spiky surface coupled with positively charged polyethyleneimine (PEI) that effectively entrap, deliver, and protect the cargo from nuclease-mediated degradation. At MDC, we have developed a pipeline of advanced techniques and assays that we have applied to characterise NUVEC® bound with either plasmid DNA or siRNA cargo. We assess physical properties via Zetasizer and demonstrate effective loading of cargo on the surface of NUVEC® particles via mass spectrometry. High-resolution microscopy and genetic engineering techniques have been employed to better understand NUVEC® and its mechanisms of intracellular trafficking. Our data demonstrates cellular internalization, endosomal escape followed by release of functional cargo into the cytoplasm. Subsequently, we observe target gene silencing comparable to lipid transfection with a leading commercial reagent; ~80 % knockdown of both endogenous (EHMT2) and overexpressed (GFP) genes. Furthermore, NUVEC® particles have the capacity to simultaneously bind and deliver two different siRNA at an efficacious concentration. Our data demonstrates the therapeutic potential for NUVEC in oncology and other disease areas where dual inhibition of undruggable targets is required to improve clinical outcomes. Furthermore, the techniques and assays used to characterise NUVEC® particles can be applied to the characterisation of other complex medicines.

Methods



- ❑ Loading of different amounts of siRNA/DNA onto NUVEC®
- ❑ Incubation for 20 minutes at room temperature
- ❑ Physical characterisation I.e., size and zeta potential using Zetasizer
- ❑ Transfection of HEK-293T cells with NUVEC®-Nucleic acid complex
- ❑ Flow Cytometry and advanced microscopy characterisation

Results

Physical Characterisation

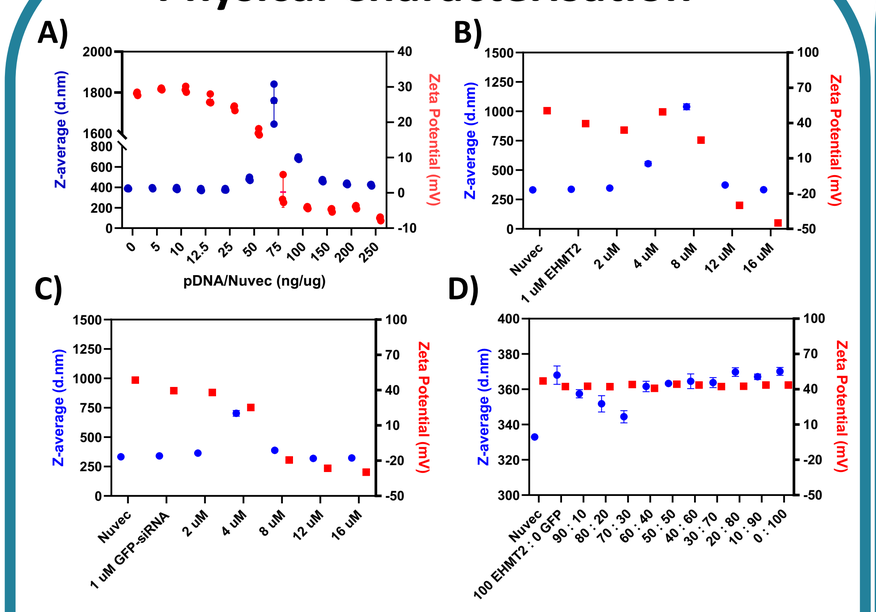


Figure 1. Plasmid DNA, EHMT2 and GFP siRNA loading on NUVEC® particles at various concentrations. **A)** Size distribution analysis and zeta potential assessment of plasmid DNA **B)** EHMT2 siRNA-NUVEC® complex size and zeta potential analysis by DLS. **C)** GFP-siRNA-NUVEC® complex size and potential analysis by DLS **D)** Dual siRNA (EHMT2&GFP) loading onto NUVEC®, size and potential analysis.

Intracellular Delivery and Endosomal Escape

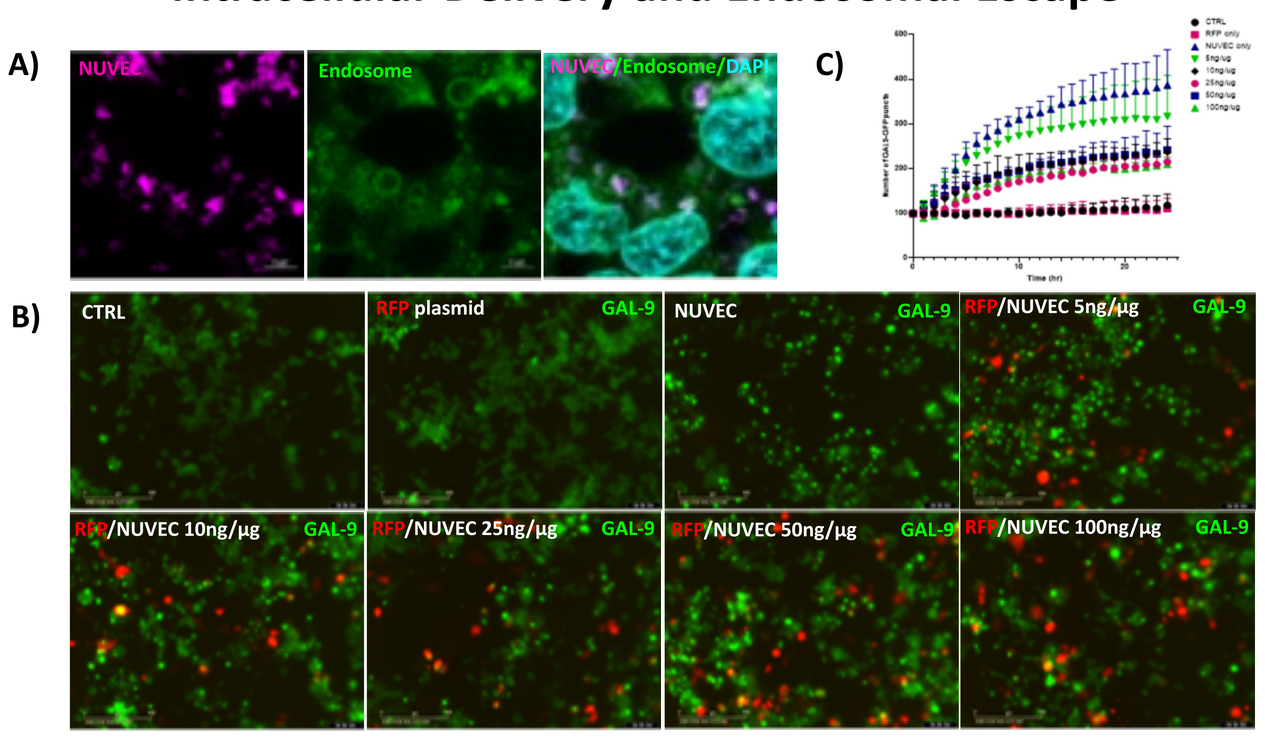


Figure 2. Endosomal uptake and escape of NUVEC particles. **A)** Intracellular delivery of NUVEC® particles (pink) located inside the endosomes (green) after 6hr incubation. **B)** Lentiviral Galectin9-GFP reporter as sensor of endosomal escape. GAL-9 puncta (green) form following endosomal rupture of the NUVEC® alone and RFP/NUVEC® complex at various concentrations. **C)** Quantitative analysis of number of GAL-9 puncta appeared as a result of endosomal escape of RFP/NUVEC® complex overtime.

Transfection Efficiency and Gene Knockdown

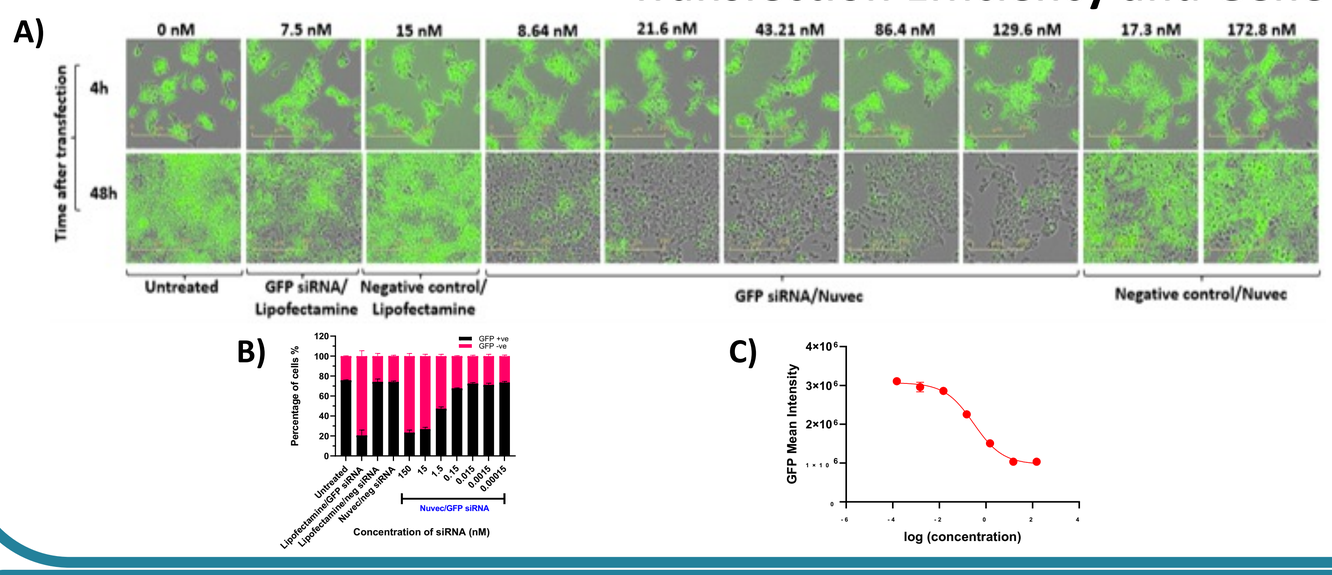


Figure 3. Transfection efficiency and gene knockdown. **A)** siRNA loaded NUVEC® particles showed GFP gene knockdown after 48 h of transfection by incucyte analysis **B)** Flow cytometry data also showed prominent GFP-gene knockdown when treated with GFP-siRNA/NUVEC complex. **C)** IC50: 0.298 nM of siRNA (with 70 ug/ml of NUVEC®) **D)** Western blot analysis of endogenous gene knockdown i.e., EHMT2 using EHMT2 siRNA-NUVEC® complex in HEK293T cells.

Conclusion

- •NUVEC® particles efficiently bind to nucleic acids – plasmid DNA/siRNA and mediates their intracellular delivery and endosomal escape.
- •HEK293T cells exhibit excellent transfection efficiency when transfected with GFP plasmid loaded NUVEC® particles.
- •NUVEC® particles loaded with GFP-siRNA/EHMT2-siRNA causes gene silencing in both endogenous (EHMT2) and overexpressed (GFP) genes in HEK293T cells.
- •NUVEC® particles have the capacity to simultaneously bind and deliver two different siRNA at an efficacious concentration.

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