Enhancing adenovirus gene transfer using novel nanoparticles complexes

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1. Background

Adenoviruses (Av) are effective gene delivery vehicles for vaccination and have been recently successful in providing immunity against COVID-19. However, a strong antibody mediated immune response is associated with these vectors especially in their 1st generation form. Av rely on infection of host cells using CAR, a 46-kDa protein that also mediates infection by group B coxsackieviruses (Arnberg, 2009). Low concentration of CAR on target cells results in poor vector attachment for internalisation, which translates into a higher vector titre being required for effective gene delivery. To overcome this limitation, nanoparticles have been used that complex both the vector and host receptors. Nanoparticles work by reducing the overall negative charge between the vector and cell surface, hence increasing vector attachment and internalisation (Sapet et al., 2011). Hydrophobic viral vectors can also be encapsulated in the pores of hydrophilic nanoparticles for improved transportation to the cell providing faster kinetic absorption on target cells, lowering the risk of their degradation, reducing their toxicity and, therefore, enhancing vector transduction efficiency (Wu et al., 2014, Swaid and Jawaid, 2019).

In this study, we investigated the effect of using novel silica-based Nuvec® silica nanoparticles (SiNPs) developed by N4Pharma Ltd to improve Av gene transfer. To identify the optimal concentration of SiNPs to aid Av infection, we compared Nuvec® SiNPs/Av complexes to Av only and Nuvec®/Av complexed with low concentration of PEI polycation. We show that Nuvec® complexed Av can significantly improve Av transduction enabling a reduced vector dose required for effective gene transfer with low cytotoxicity on a range of target cells. We propose that Nuvec® enhancement of Av gene transfer should reduce the burden of Av production required for safe vaccination.

2. Aims and Objectives

1. To visualise Nuvec® SiNPs particle size using transmission electron microscopy (TEM).
2. Test the toxicity of Nuvec® SiNPs on different cell lines; hepatocellular carcinoma (HepG2), primary endometrial (ULTR) cells and breast cancer cells (MCF7).
3. Explore the potential of Av enhancement after complexation with Nuvec®.
4. Compare PEI free Nuvec® and PEI/Nuvec® SiNPs to determine differences in transduction efficiency when complexed with Av.
5. Determine the effects of Nuvec® on the stability of Av when complexed with PEI/Nuvec® and PEI free SiNPs compared to Av only at different laboratory temperatures.

3. Workflow

We used AdRSVβGal (termed Av for this study) with a RSV promoter to drive β-galactosidase gene expression as a reporter gene to indicate successful transduction. To analyse the potential of Nuvec SiNPs to enhance Av efficiency, we used MOIs to achieve approximately 20% infectivity for each cell line. Nuvec® SiNPs developed by N4Pharma Ltd were reconstituted and complexed with Av at the chosen MOI. Different concentration of Nuvec®/Ad complexes were used to infect liver, breast cancer cells and primary muscle cells. Nuvec® SiNPs/Av were compared with Nuvec® free Av and Nuvec®/Av complexed with low concentration of PEI polycation to measure gene transfer efficiency and cell toxicity. A trypan blue exclusion assay was used to determine cells viability over 72 hours. Transduction efficiency was quantified by staining cells with a β-galactosidase expression staining solution shielded from light for 24 hours. As the β-galactosidase in Av catalysed the hydrolysis of X-gal present in the staining solution, cells expressing the transfected gene appeared blue enabling quantification. Ad stability complexed with Nuvec® SiNPs was assayed for infectivity after storage at room temperature and 4° over 14 days.

4. Results

Visualisation of Nuvec® SiNPs

Nuvec® SiNPs were visualised by Transmission Electron Microscopy (TEM) (fig.1). Differences in particle morphology were found when PEI was loaded onto SiNPs compared to PEI free Nuvec® SiNPs. PEI free Nuvec® SiNPs were identified to be ~100nm, whereas PEI coated Nuvec® SiNPs are ~300nm in size (n=30).

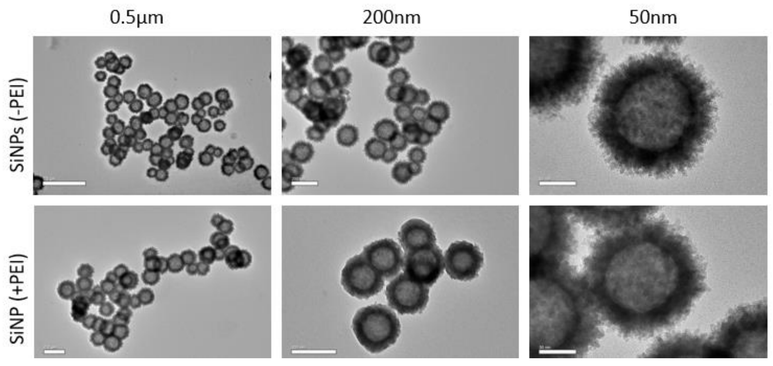
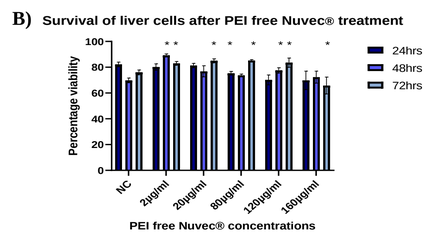
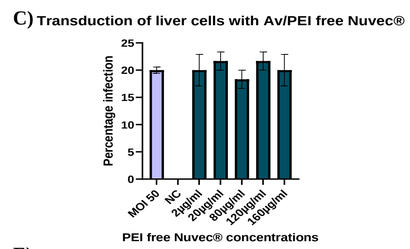


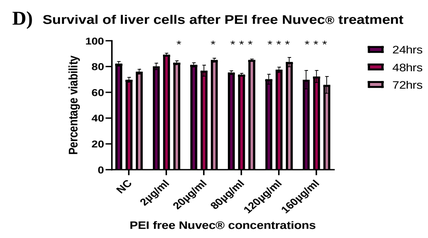
***Fig. 1 Visualisation of SiNPs by Transmission Electron Microscopy**. PEI free and PEI/Nuvec® SiNPs were visualised to compare the differences in structure due to the presence of PEI on the surface of SiNPs. Pictures were captured at scale of 0.5µm, 200nm and 50nm.*

Cytotoxicity and transduction efficiency of Nuvec SiNPs in Liver cells

HepG2 liver cells express high levels of CAR receptors and are therefore, highly permissive to Av infection. To determine whether complexation of Nuvec® can further enhance transduction efficiency, liver cells were treated with Nuvec® SiNPs over 72 hours. Cell viability and recovery was measured to be high post treatment with PEI free Nuvec® SiNPs, and reduced only at the high SiNPs concentration of 160µg/ml (fig.2B). In contrast, PEI/Nuvec® treated cells did not recover as well over this time course, with inconsistent viability detected over the different concentrations of Nuvec ® used (fig.2D). Infection of HepG2 cells by Av or Av complexed with Nuvec® at an MOI of 50 was not changed unless virus particles were complexed with PEI where a 3-fold increase infection was observed when 20µg/ml of this SiNP was used (fig.2E). No difference in infectivity was detected between PEI free Nuvec® and Av only on these cells (fig.2C).







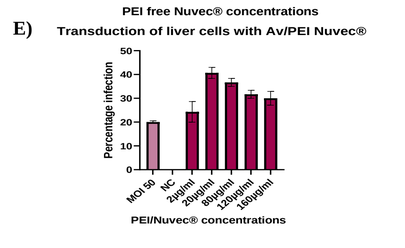
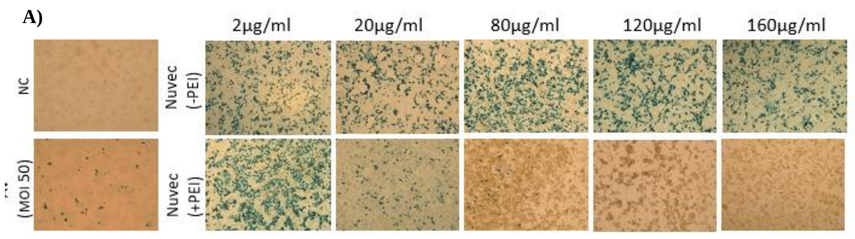
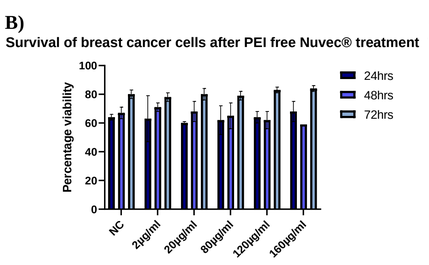


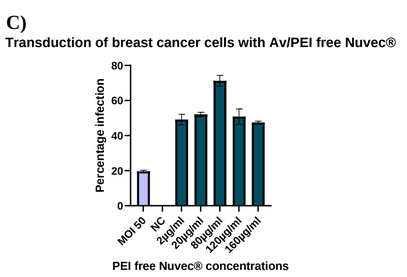
***Fig.2 Time course cell recovery and transduction efficiency after Nuvec® SiNPs complexation in HepG2 liver cells**. A range of PEI free and PEI/SiNPs concentrations were analysed over 72hrs to assess cytotoxicity on HepG2 cells (n=3). The percentage of infection of HepG2 after treatment with Av complexed with Nuvec® was determined by quantification of β-galactosidase expression and compared to HepG2 transduced with Av alone. Mann-Whitney U Test was performed to analyse the statistical difference. If significant difference was identified between the control and treated samples * is stated (p<0.05). A) Bright field images (x5) of HepG2 exposed to Av (MOI 50) with a range of concentrations of PEI free Nuvec® and PEI/Nuvec® SiNPs. B) Viability of cells treated with PEI free SiNPs. C) HepG2 were treated with Av complexed with PEI free Nuvec® and infectivity was compared between Av alone and the different concentration of Nuvec®. D) Viability of PEI/Nuvec® treated cells. E) Percentage infectivity of HepG2 after treatment with Av complexed with PEI/Nuvec® and Ad on its own.*

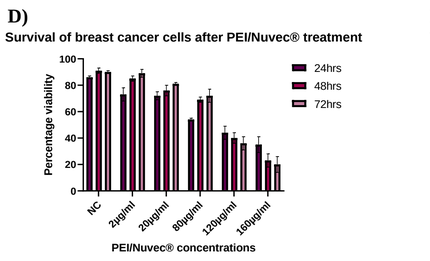
Cytotoxicity and transduction efficiency of Nuvec® SiNPs in breast cancer cells

Av is also used in cancer therapy due to its efficiency in transduction of several cancer cells types. We, therefore tested the cytotoxicity of Nuvec® on breast cancer cells by exposing MCF7 cells to SiNPs with and without PEI for 72 hours. PEI free SiNPs, showed cell recovery to be high at all concentrations over time (fig.3A/B). However, PEI/SiNPs treated cell viability did decrease at high SiNP concentration (120-160µg/ml) most likely due to PEI associated toxicity (fig.3d). At all concentrations of PEI free Nuvec tested, percentage infectivity of complexed Av was 2-fold greater than Av only (MOI 50) (fig.3C). In contrast, cells exposed to Av complexed with PEI/Nuvec only resulted in a modest increase in infectivity except for at 2µg/ml SiNPs, that resulted in a 3-fold increase in infectivity above Av alone (fig.3E).









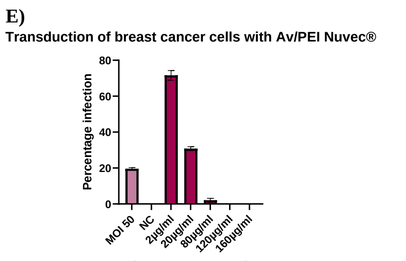
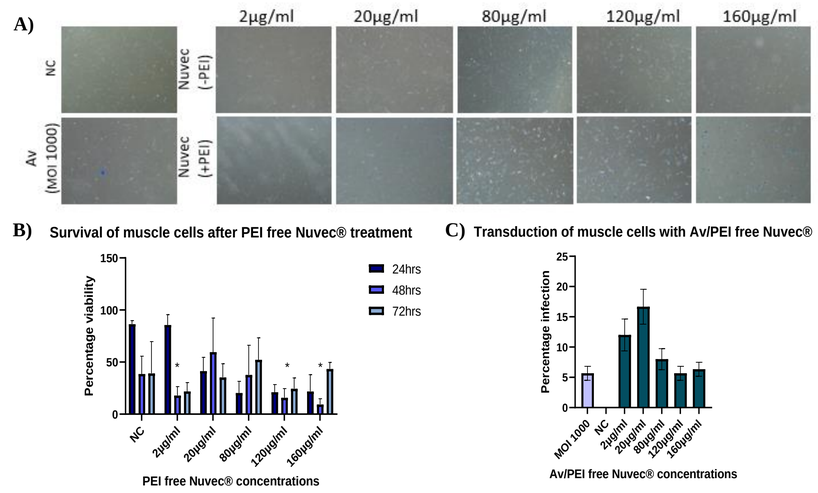


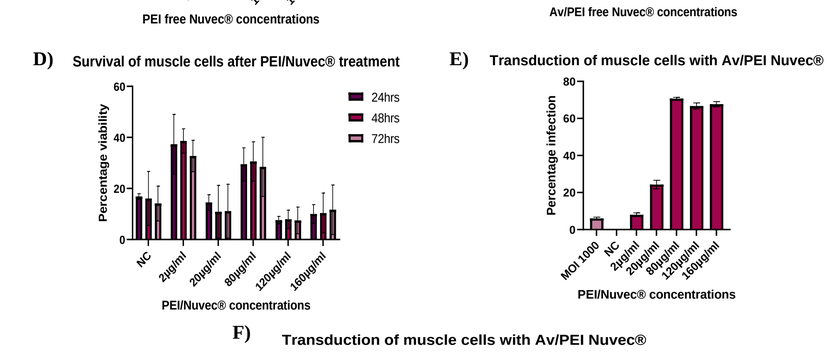
Fig.3 Time course cell recovery and transduction efficiency after Nuvec SiNPs complexation in MCF7 breast cancer cells. A range of PEI free and PEI/SiNPs concentrations were analysed over 72 hrs to assess cytotoxicity on MCF7 cells (n=3). The percentage of infection of MCF7 after treatment with Av complexed with Nuvec® was determined by quantification of β-galactosidase expression and compared to MCf7 transduced with Av alone. Mann-Whitney U Test was performed to analyse the statistical difference. If significant difference was identified between the control and treated samples * is stated (p<0.05). **A)** Bright field images (x5) of MCF7 exposed to Av (MOI 50) with a range of concentrations of PEI free Nuvec® and PEI/Nuvec® SiNPs. **B)** Viability of cells treated with PEI free SiNPs. **C)** MCF7 were treated with Av complexed with PEI free Nuvec® and infectivity was compared between Av alone and the different concentration of Nuvec®. **D)** Viability of PEI/Nuvec® treated cells. **E)** Percentage infectivity of MCF7 after treatment with Av complexed with PEI/Nuvec® and Av alone.

Cytotoxicity and transduction efficiency of Nuvec SiNPs in Muscle cells

We next used a primary endometrial smooth muscle cell line (ULTR) that resembles primary human myometrial cells as surrogate muscle cells to determine the efficiency of Nuvec® SiNPs mimicking Av vaccine delivery to muscle. Recovery of ULTR primary cells was reduced compared to HepG2 and MCF7 cells and these cells also appeared poorly transduced when using Av alone even at a high MOI (MOI 1000). However, we observed that in presence of Nuvec®, the infectivity of Av was greatly enhanced. Using 20ug/ml of PEI free SiNPs infectivity by Av was 3-fold greater than Av alone (MOI 1000) (fig.4C), while at the same concentration of PEI/Nuvec® the percentage of Av infectivity resulted to be 4-fold greater compared to Av alone (MOI 1000) (fig. 4E). Moreover, at 80-160ug/ml concentrations of PEI/Nuvec®, infectivity of Av resulted in a 10-fold increase in infectivity compared to Av alone (MOI 1000) (fig. 4E).

Given the remarkable enhancement in infectivity of Ad in muscle cells with PEI/Nuvec®, cells were transduced with a lower Av (MOI of 100) complexed with the same range of SiNPs concentrations. Results showed that the percentage infectivity with Av (MOI 100) complexed with PEI/Nuvec® was up to 9-fold greater than Ad alone, demonstrating that PEI/Nuvec improved Ad efficiency with a lower MOI in ULTRs





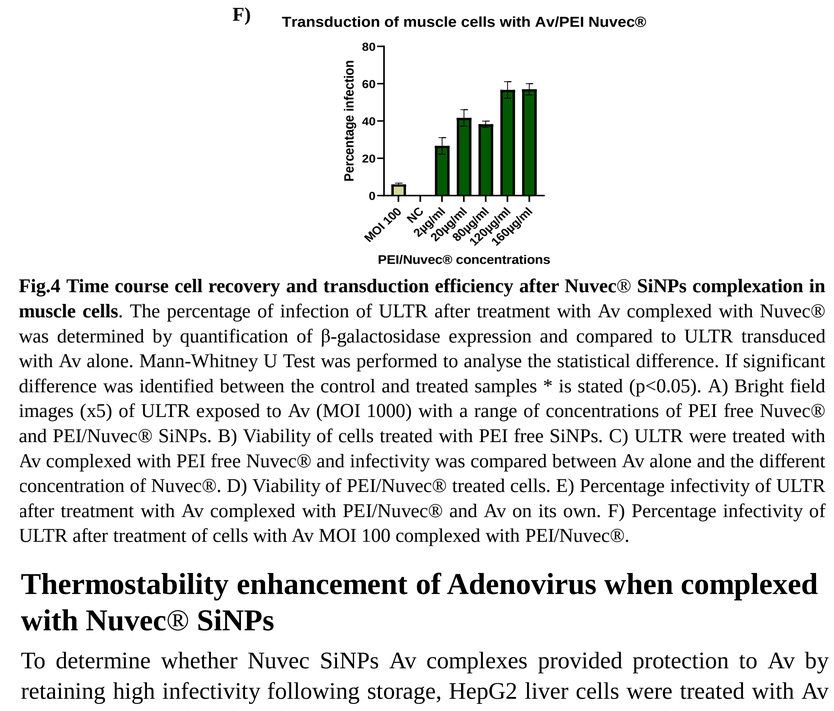


Fig.4 Time course cell recovery and transduction efficiency after Nuvec® SiNPs complexation in muscle cells. The percentage of infection of ULTR after treatment with Av complexed with Nuvec® was determined by quantification of β-galactosidase expression and compared to ULTR transduced with Av alone. Mann-Whitney U Test was performed to analyse the statistical difference. If significant difference was identified between the control and treated samples * is stated (p<0.05). A) Bright field images (x5) of ULTR exposed to Av (MOI 1000) with a range of concentrations of PEI free Nuvec® and PEI/Nuvec® SiNPs. B) Viability of cells treated with PEI free SiNPs. C) ULTR were treated with Av complexed with PEI free Nuvec® and infectivity was compared between Av alone and the different concentration of Nuvec®. D) Viability of PEI/Nuvec® treated cells. E) Percentage infectivity of ULTR after treatment with Av complexed with PEI/Nuvec® and Av on its own. F) Percentage infectivity of ULTR after treatment of cells with Av MOI 100 complexed with PEI/Nuvec®.

Thermostability enhancement of Adenovirus when complexed with Nuvec® SiNPs

To determine whether Nuvec SiNPs Av complexes provided protection to Av by retaining high infectivity following storage, HepG2 liver cells were treated with Av (MOI 50) complexed with 20µg/ml Nuvec® SiNPs and stored at 4° or room temperature (RT) for 24 hours, 7 days and 14 days (fig.5A). After 14 days, both RT and 4° storage showed successful Av infectivity could be achieved when Av was complexed with PEI/Nuvec®, at 80% and 40%, respectively. Compared to infectivity of Av alone a decrease of 70% was observed following incubation at 4° and complete loss of infectivity after incubation at RT for over this period of time (fig.5B).

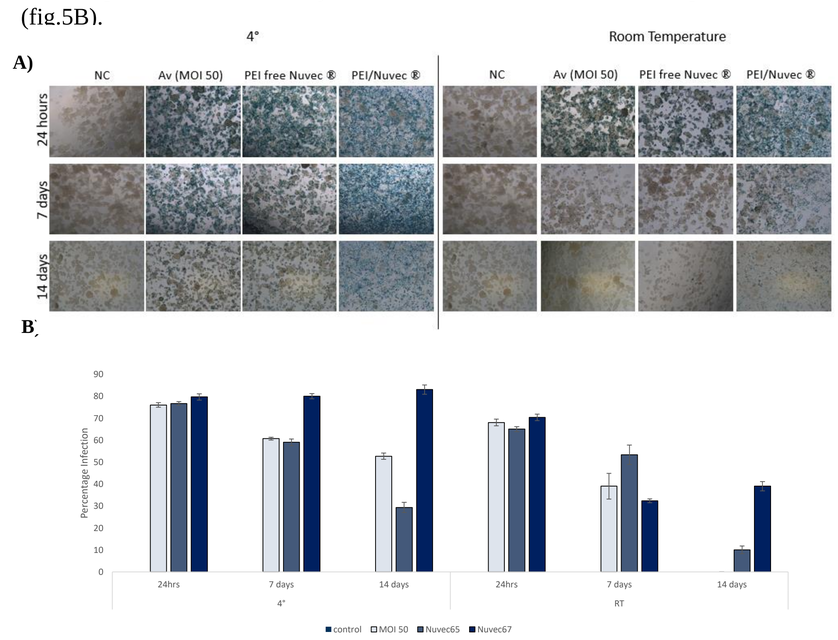


Fig.5) Quantification of Av expression over time at 4° and room temperature (RT) after treatment with Nuvec® nanoparticles. A) HepG2 were transduced with Av (MOI 50) complexed with 20µg/ml of PEI free and PEI/Nuvec® SiNPs, along with an untreated control and one sample transduced with Av alone and kept at 4° and RT for 24hrs, 7 days and 14 days. B) At 24hrs cells transduced with Av complexed with PEI/Nuvec® showed an increase in Av expression compared to Av alone and Av complexed with PEI free Nuvec® both at 4° and RT. At days 7 at 4° there was an increase in Av expression complexed with PEI/Nuvec® compared to Av alone and PEI free Nuvec®. While at the same time point at RT there was in increase in β-galactosidase expression with Av complexed with PEI free Nuvec® compared to PEI/Nuvec® and Av alone. At 14 days at both RT and 4° there was increase in Av expression when Av was complexed with PEI/Nuvec®.

5. Conclusion and future work

Our data shows that nanoparticles enable increased Av delivery of different cell types at low SiNP concentration compared to Av alone with low observable cytotoxicity. Overall, concentrations of Nuvec® SiNPs between 2µg/ml-80µg/ml provided lower cytotoxicity and improved transduction compared to high doses of 120µg/ml-160µg/ml. PEI coated Nuvec® also provided significantly enhanced Av transduction at lower MOI and should enable a reduction in the amount of vector required for therapy. Polycations such as PEI are useful to enhance Av gene transfer most likely because the positive charge on PEI reduces the repulsion between negative charges on the cell surface and the vector. Importantly, we will test our observations in vitro with SiNP bound Av in vivo.

We have also found that Nuvec® SiNPs also improve lentivirus gene transfer (data not shown) and studies are ongoing to determine the potential of Nuvec® when complexed with adeno-associated-virus. Hence, we propose that N4Pharma's silica nanoparticle Nuvec® may be a useful universal agent to improve vector mediated therapeutics with reduced side effects.

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