

Aerosol delivery of concentrated PEI formulations to the sheep lung

Lee Davies

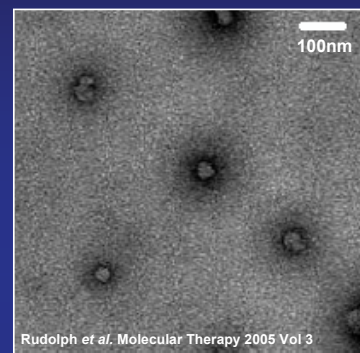
Gene Medicine Research Group

Oxford University

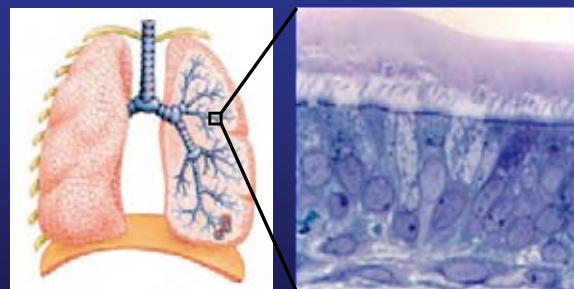
& United Kingdom Cystic Fibrosis Gene Therapy Consortium

Polyethylenimine

- Highly cationic polymer
- Readily complexes with pDNA
- Potent GTA *in vitro* and *in vivo*
- Range of PEI polymers
- 25kDa branched PEI

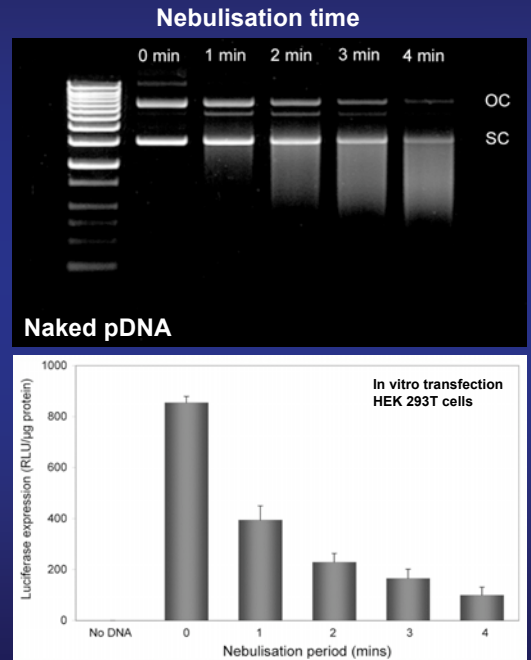


- Introduce functional CFTR gene
- Ciliated epithelial cells
- Topical aerosol delivery of GTAs



Aerosol delivery of non-viral GTAs

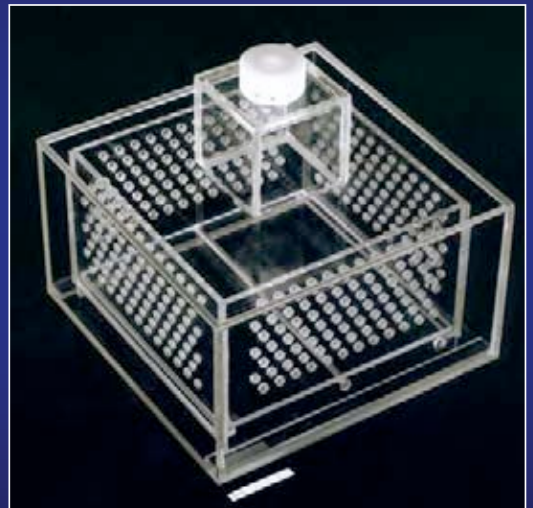
- Generate GTA aerosols
- Clinical nebulisers
- Shear degradation of pDNA
- Some GTAs can protect pDNA
- 25kDa branched PEI
- *In vivo* models



Davies et al Pharm Res 2005 Vol 22

Aerosol delivery to the mouse lung

- Mouse lung aerosol model
- Rapidly assess formulations
- Whole body exposure
- Aqueous DNA/PEI formulations
- N:P ratio of 10:1
- Clinical jet nebuliser
- Luciferase reporter



Mouse aerosol exposure chamber

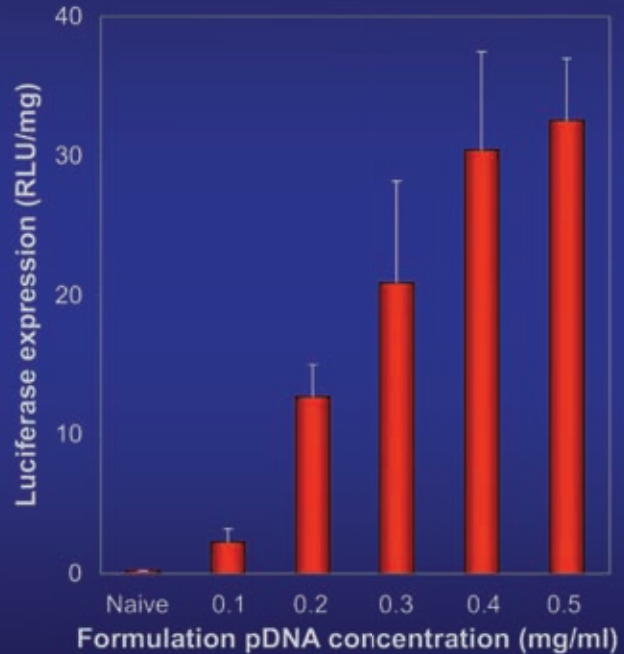
Aerosol delivery of PEI complexes

- Dose dependent lung expression
- Maximum concentration 0.5mg/ml
- Compares poorly to other GTAs
- Clinical viability
- Estimate require >50mg per patient

Delivery time

Delivery volume

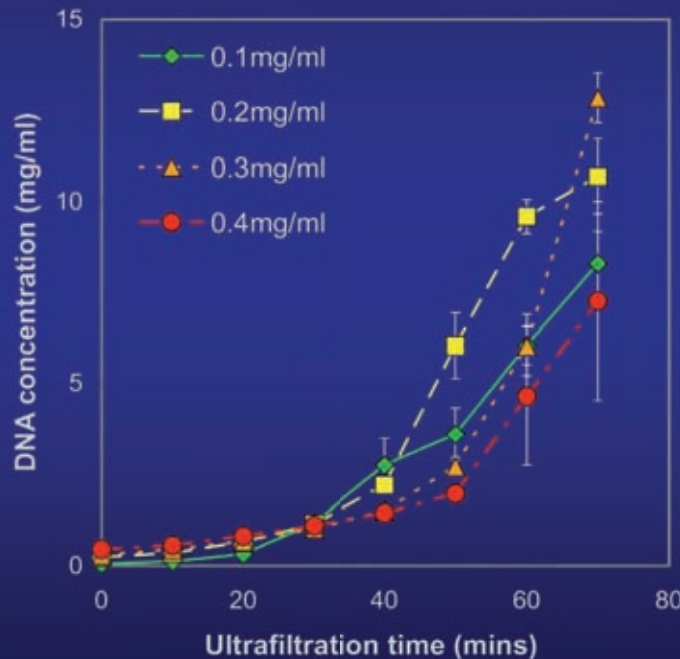
- Higher concentrations required
- Ultrafiltration



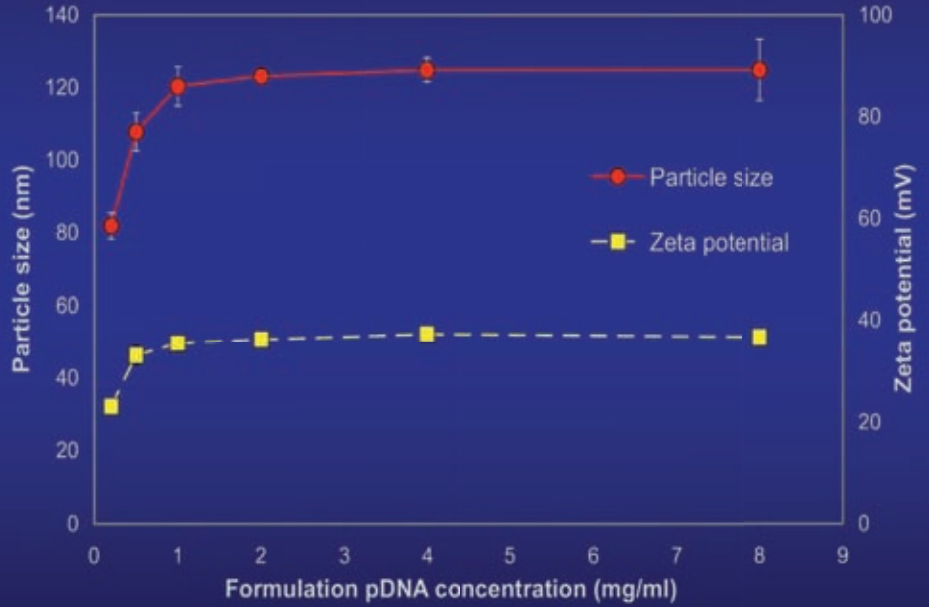
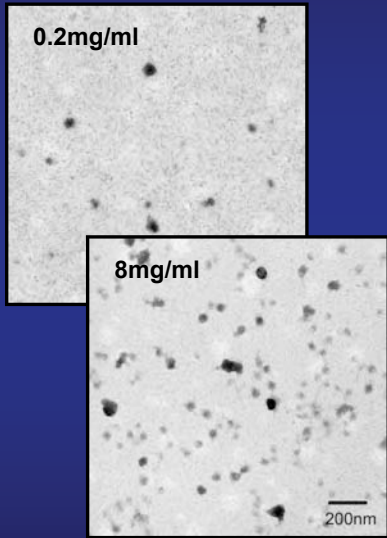
BALB/c mice (n=6)
10ml aerosol

Ultrafiltration of DNA/PEI formulations

- Concentrate standard formulations
- Stirred ultrafiltration cell
- 100kDa nitrocellulose membrane
- Concentrations up to 10mg/ml
- No precipitation
- No DNA in filtrate
- “Free” PEI removed during filtration
- **Concentration effects**

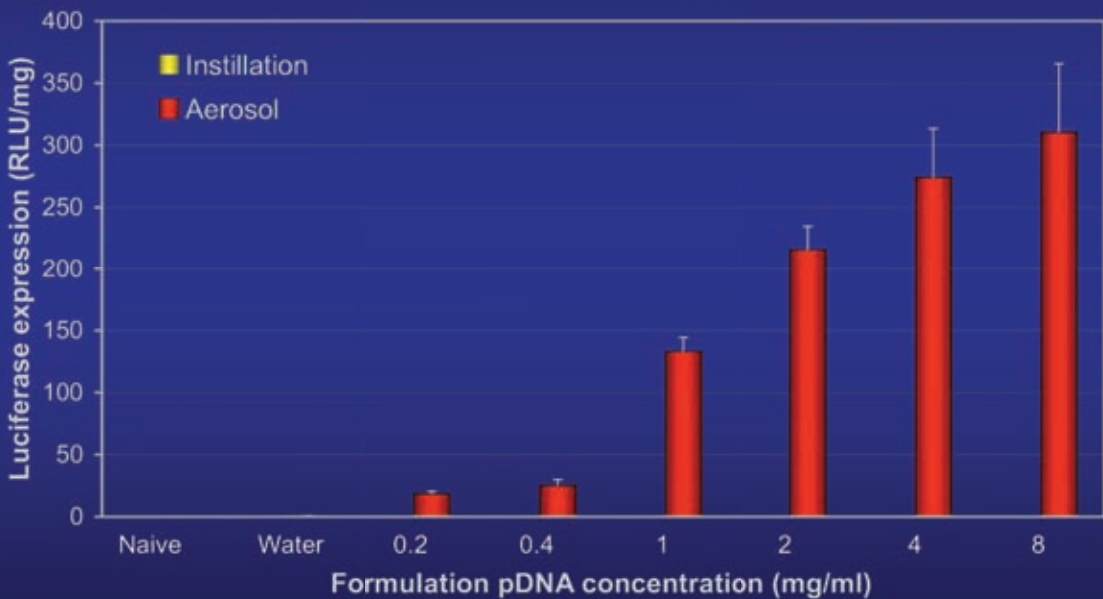


Physical characteristics of cPEI formulations



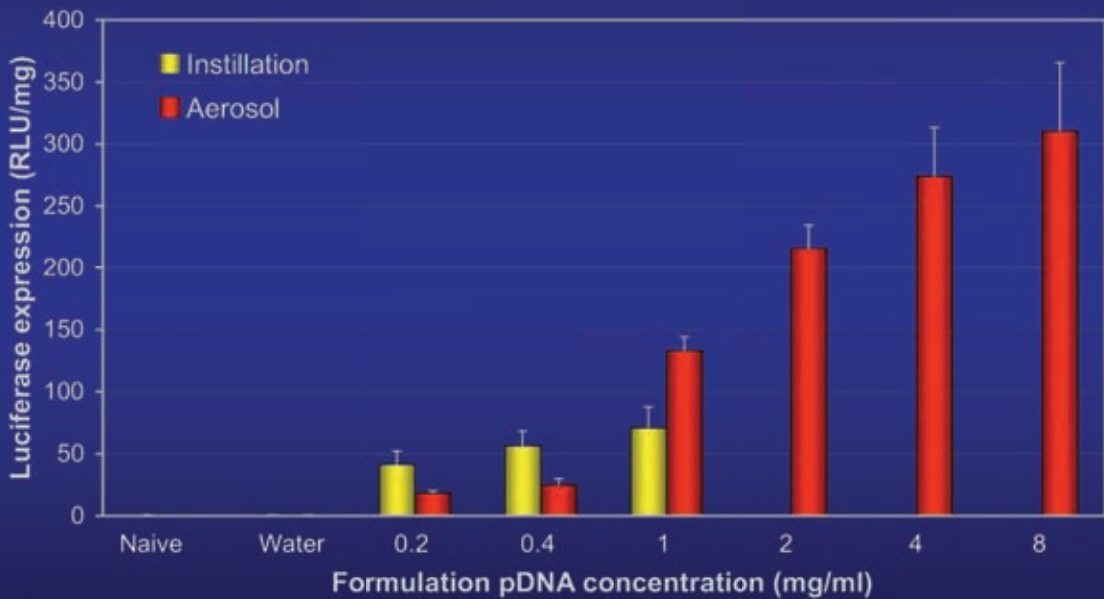
Little effect of concentration upon physical characteristics

Concentrated PEI in the mouse lung



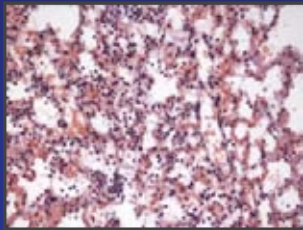
BALB/c mice
n = 8
10ml aerosol
100µl instilled

Concentrated PEI in the mouse lung

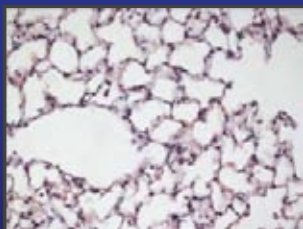


BALB/c mice
n = 8
10ml aerosol
100µl instilled

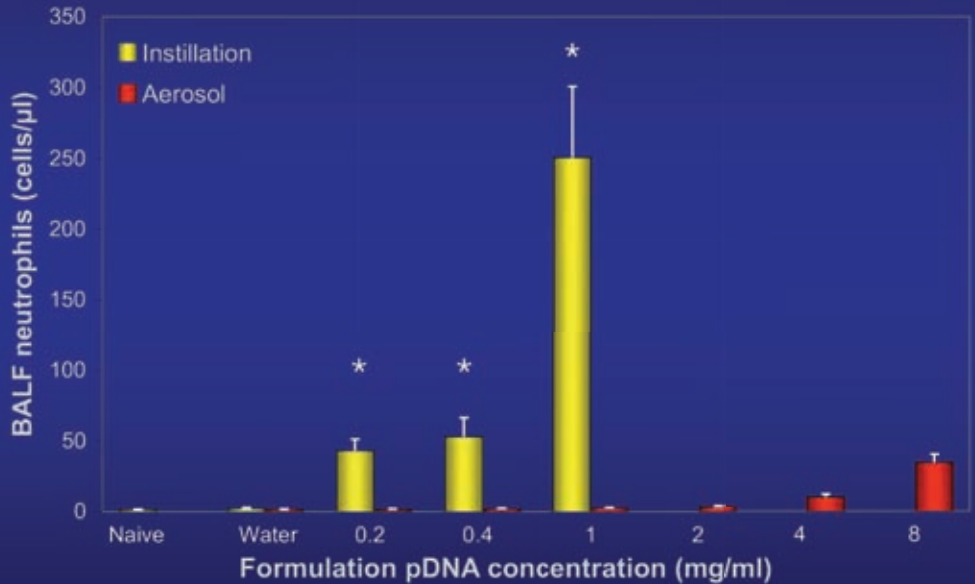
PEI toxicity in the mouse lung



1mg/ml Instillation



8mg/ml Aerosol



Mouse lung sections
H&E (x40)

cPEI formulations *in vivo*

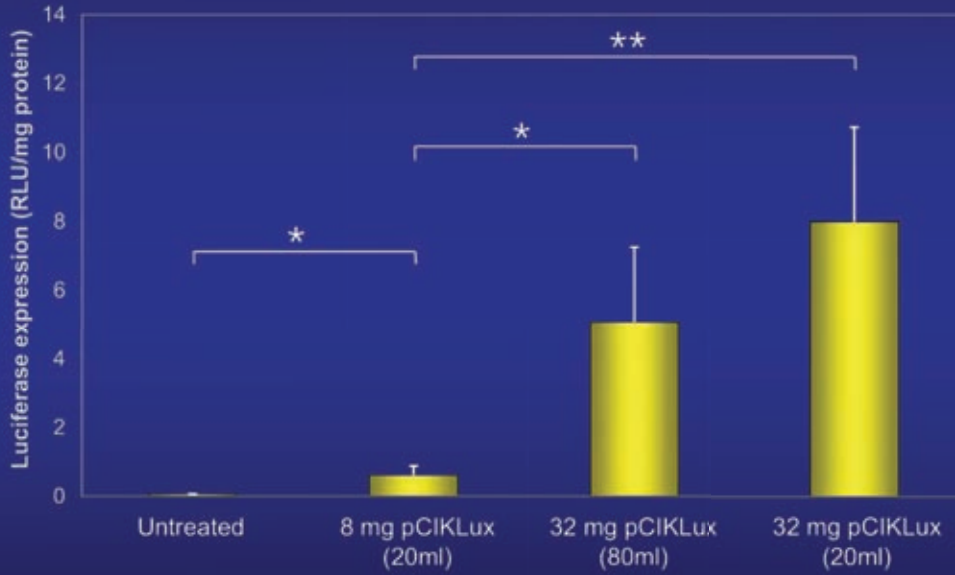
- **Mouse lung model**
- Good gene expression from cPEI aerosols with minimal toxicity
- Contrast to instillation
- Delivery methodology important in expression and toxicity
- **Large animal aerosol delivery model**
- Sheep lung
- More clinically relevant delivery and expression model

Sheep aerosol model

- Suffolk cross ewes
- Anaesthetised
- Intubated
- Negative pressure ventilation
- 3 breath-actuated nebulisers
- 20ml aerosol
- 1 hr delivery time



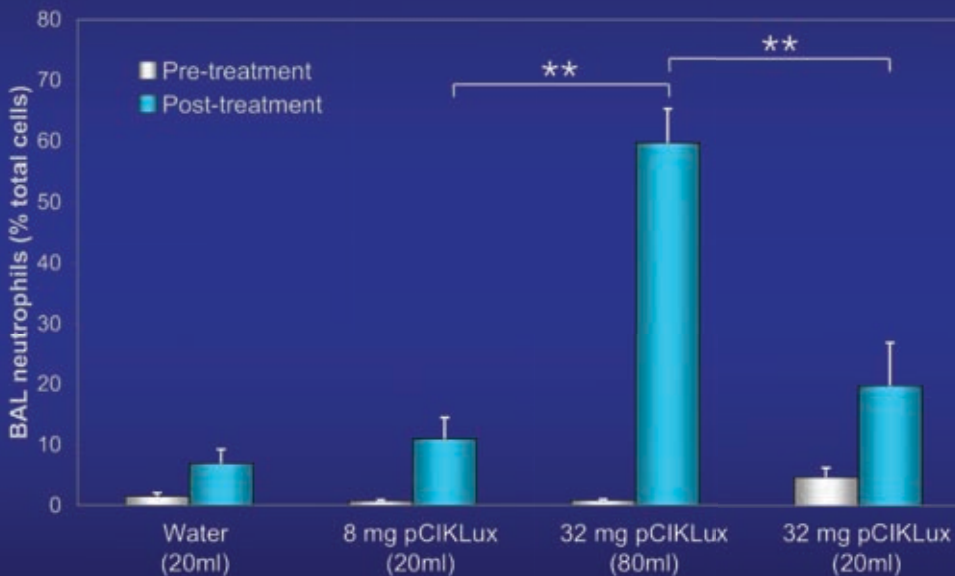
Sheep aerosol delivery - gene expression



Suffolk-cross ewes
35-60kg
n=6 per group

Equivalent expression with reduced volume and delivery time

Sheep aerosol delivery - BAL neutrophils



Suffolk-cross ewes
35-60kg
n=6 per group

cPEI aerosol formulations exhibit lower toxicity

Summary

- Ultrafiltration reliable method to concentrate PEI formulations
- Improved clinical viability
- Relatively high expression in absence of significant toxicity

- 25kDa PEI very basic vector
- Encouraging results
- Novel synthetic polymers
- Suitability for aerosol delivery

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