IMPROVING THE TRANSDUCTION EFFICIENCY OF AN AEROSOL-DELIVERED LENTIVIRAL VECTOR FOR CYSTIC FIBROSIS LUNG GENE THERAPY

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BACKGROUND:

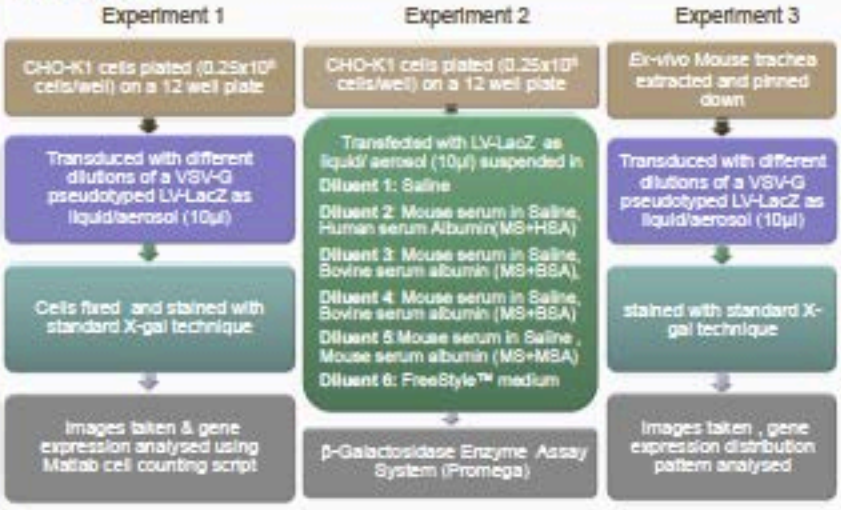
Gene therapy is a potential treatment for cystic fibrosis (CF) lung disease, whereby the therapeutic gene is delivered to the lung to produce functional correction. Aerosol delivery of a gene vector to the lung is an ideal treatment approach because it is non-invasive, easy to administer and less cumbersome compared to liquid delivery.

It is thought that the virus particles are subjected to destructive surface tension and shear stress effects during aerosolization [1]. We have utilised a vibrating mesh nebuliser (Aeroneb®Pro) (Figure 1) for *in-vitro* studies as it it thought to produce minimal shear stress on our lentiviral (LV) gene transfer vector carrying the reporter gene LacZ (LV-LacZ).

The aim of this study was:
1)To test a range of protective agents in which the LV-LacZ is suspended to improve the viability of the LV and in turn gene transduction.
2)To study the distribution pattern of gene expression using liquid delivery vs aerosol delivery of LV-LacZ in *in-vitro* and *ex-vivo* experiments.

Figure 1: Aeroneb®Pro used to aerosolize LV-LacZ

METHODS:



RESULTS:

- The transduction obtained via aerosol was 33% to 51% of the number of cells compared to liquid control, for 1:250 to 1:1000 dilutions of the LV-LacZ (Figure 2).
- Virus suspended in FreeStyle™ medium showed significantly higher levels of transduction (58%) when compared to virus suspended in other diluents (Figure 3).
- Delivery of LV-LacZ aerosol of different VMD (3.01 , 3.61 and 13.61 µm) showed no statistical difference in levels of gene expression produced (not shown)

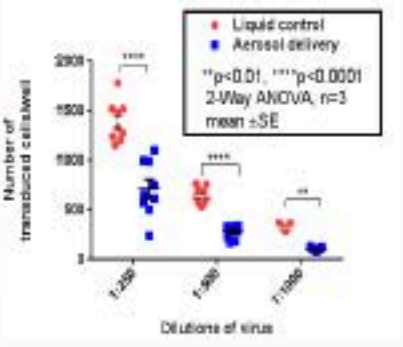


Figure 2: 33% - 51% of CHO-K1 cells were transduced by LV-LacZ aerosol compared to liquid delivery

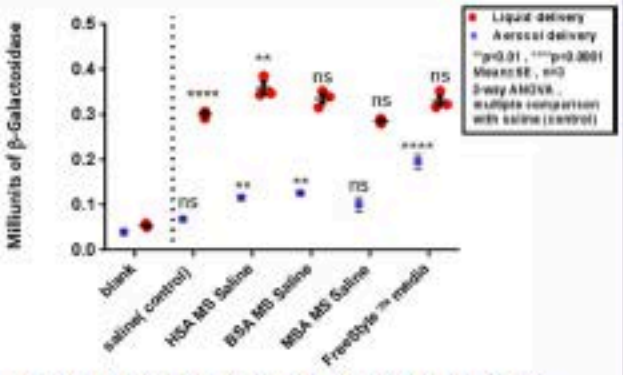


Figure 3: Aerosols of virus suspended in FreeStyle™ medium showed highest level of gene transduction (58%) compared to liquid delivery

- *In-vitro* tests showed that a homogenous distribution of the gene expression was produced by lentiviral vector aerosol (Figure 4a) compared to the more discreet clusters (arrows, Figure 4b) observed after liquid bolus vector delivery.
- *Ex-vivo mouse* trachea was transduced by LV-LacZ aerosols, with a more uniform distribution of gene expression along the trachea observed (arrows on Figure 4c) compared to the patchy distribution normally observed in-vivo (not shown).
- In liquid bolus delivery more gene transduction was observed along the intra-cartilage region of the trachea (arrows on Figure 4d).

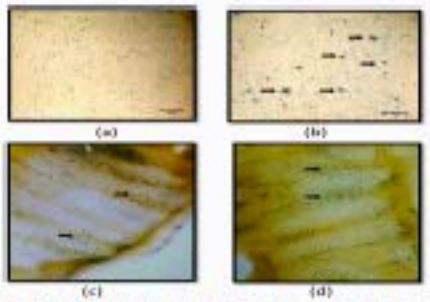


Figure 4: First data (n=1) of planned LV-LacZ transduction studies in CHO-K1 cells by (a) aerosol (b) liquid bolus delivery LV-LacZ , transduction of ex-vivo mouse trachea by (c) aerosol (d) liquid bolus delivery

CONCLUSION:

- *In-vitro* experiments showed that LV-LacZ aerosol was able to transduce about 33% to 51% of CHO-K1 cells
- We speculate that the presence of FreeStyle™ media aids in protecting the LV from shear stress compared to other diluents.
- *Ex-vivo* transduction of mouse trachea via LV-aerosol showed a well distributed ~~uniform~~ distribution of gene expression along the length of the trachea.
- To improve the levels of gene transduction we plan to test different nebulization platforms.
- These findings assist in our understanding of LV aerosolization characteristics and provide practical information for future testing into the lungs of animal models and ultimately for CF airway disease.

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1. Yang W, Marr LC. Mechanisms by Which Ambient Humidity May Affect Viruses in Aerosols. Appl Environ Microb. 2012;78(19):6781-8.