Optimisation of Bronchoscopic Gene Vector Delivery for Direct Lobe Targeting in Rat Lungs

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Introduction

- Current gene therapy delivery methods produce variable distribution within the lungs.
- Covering the entire lung has proven inefficient, requiring large quantities of gene vector to uniformly dose all desired regions.
- Our new bronchoscopic method targets specific rat lung lobes, improving gene vector transduction efficiency in isolated regions.
- The mini-bronchoscope (see Figure 1) has a camera and light source allowing it to be inserted to at least the 3rd generation of the rat airway tree, and deliver gene vector via a side channel (0.45 mm diameter).
- Shear stresses imposed on the gene vector during bronchoscopic delivery likely reduce gene vector delivery efficiency.
- The aim of this study was to determine the effects of a range of factors on transduction efficiency, when delivered using a bronchoscope. These factors included the draw up method, gene vector protectant, and the fluid delivery speed.

Methods

- NIH-3T3 cells were transduced with a gene vector carrying a green fluorescent protein (GFP) reporter gene re-suspended in our standard diluent (0.9% saline/0.1% rat serum). Several experiments were used to determine which delivery method resulted in the smallest reductions in gene vector gene vector efficiency (Figure 2):
  - Gene vector was directly pipetted onto cells as a bolus delivery (control).
  - Gene vector was drawn up into a 28G metal needle (1.84 mm diameter) and delivered without the bronchoscope, to determine the effect of the long metal delivery channel on gene vector function.
  - **Method 1 and 2:** Gene vector was drawn up into a standard 1 mL syringe or a zero-dead-volume 1 mL syringe using a 28G needle, respectively. The needle was then removed and the fluid was delivered through the bronchoscope side channel.
  - **Method 3:** Gene vector drawn up directly into the bronchoscope side channel from the tip using a 1 mL zero-dead-volume syringe.
  - **Method 4:** Gene vector was pipetted directly into a 1 mL zero-dead-volume syringe barrel and then delivered via bronchoscope side channel.
- The most effective delivery method from above was used for all subsequent experiments:
  - The effect of serum concentration in gene vector diluent was tested (Figure 3).
  - Gene vector delivery speed was then assessed using a Micro4 MicroSyringe Pump Controller (World Precision Instruments) to accurately alter the speed of gene vector delivery (Figure 4).
- After each experiment, NIH-3T3 cells were collected and fixed in 4% paraformaldehyde and GFP positive cells analysed using flow cytometry (FACSDIVA 8.0 and FlowJo_V10 software).

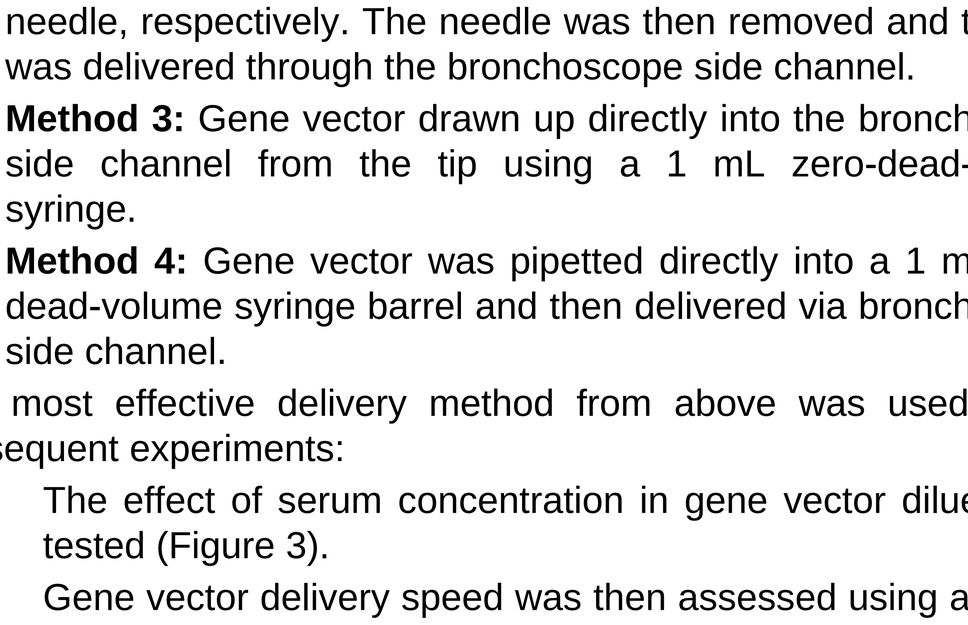

Figure 1: Bronchoscopic lentiviral delivery system used to determine effects of delivery speed on functional gene vector including A) pump to control delivery speed, B) syringe connecting the pump and bronchoscope, C) bronchoscope, D) tube for gene vector collection and E) NIH-3T3 cells for viral transduction of collected gene vector.

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Results

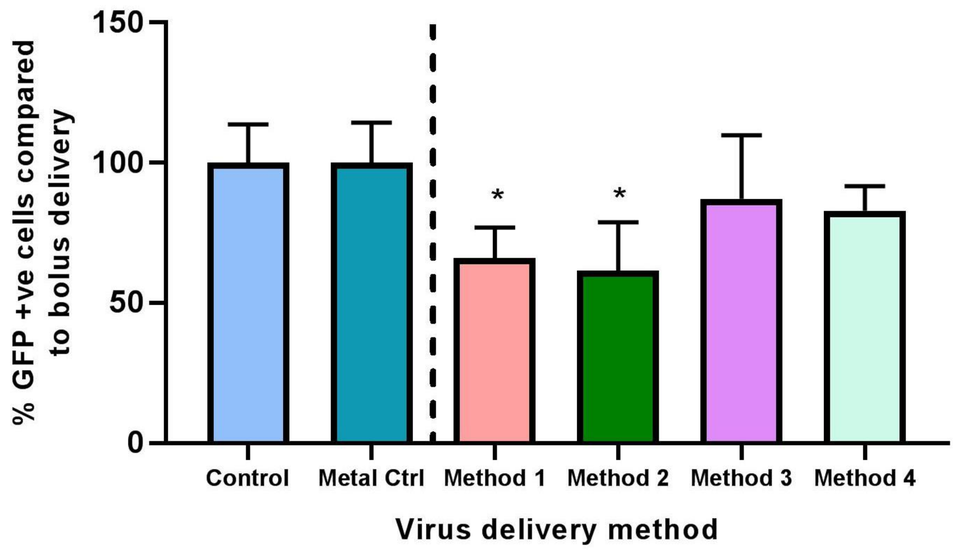

Figure 2: Effect of bronchoscope-based gene vector delivery methods on functional gene vector when delivered via the bronchoscope. The bolus delivery method was used for comparison (control). The effects of the long metal delivery channel on functional gene vector (Metal Ctrl) were determined. Gene vector was then expelled through the bronchoscope followed by a 200 µL volume of air to ensure full extrusion of the dose. Results are represented as the mean + SD percentage of GFP-positive cells compared to standard bolus delivery (n=3). *p<0.05 based on a one-way ANOVA with post hoc Tukey test.

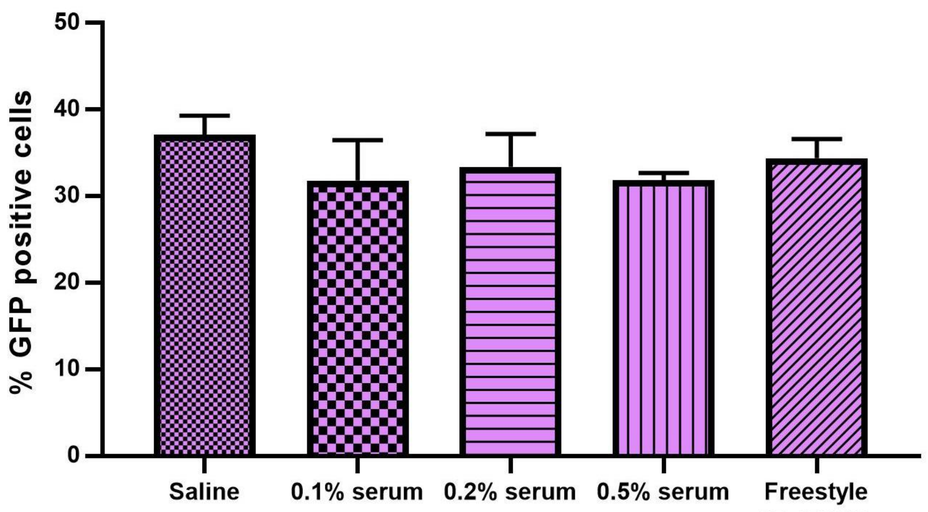

Figure 3: The effect of gene vector diluent on functional gene vector when delivered using method 3 'draw gene vector up directly into the bronchoscope from the tip using a 1 mL zero-dead-volume syringe'. Gene vector was expelled through the bronchoscope followed by a 200 µL volume of air to ensure full extrusion of the dose. Results are represented as the mean + SD percentage of GFP-positive cells (n=3). No statistically significant differences were observed.

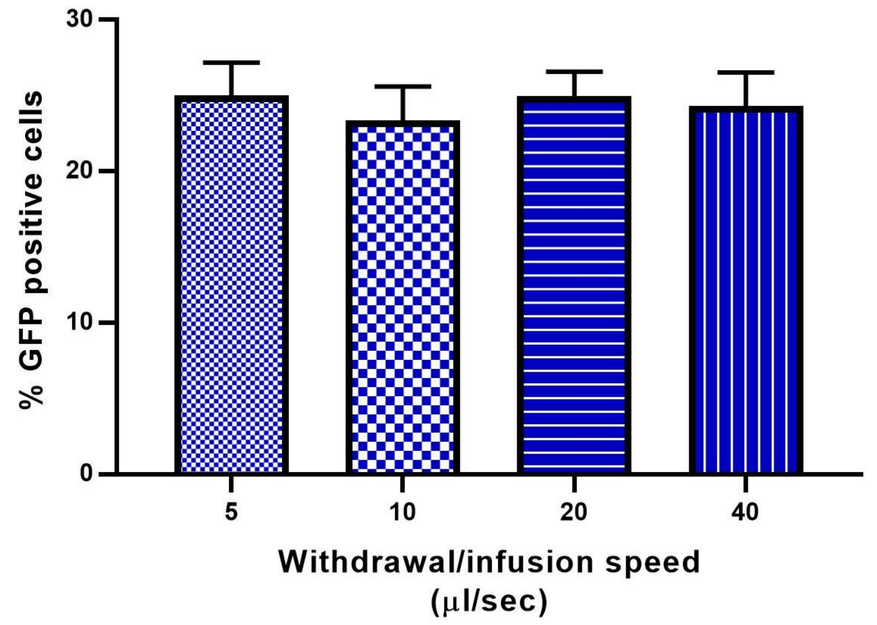

Figure 4: The effect of gene vector withdrawal and delivery speed on functional gene vector using delivery method 3, connected to a pump to control delivery speed (Figure 1). Results are represented as the mean + SD percentage of GFP-positive cells (n=3). No statistically significant differences were observed.

Conclusions

- The most efficient LV delivery method we tested was method 3, directly drawing up the gene vector into the bronchoscope side channel.
- The addition of serum to stabilise the gene vector did not significantly affect the amount of functional gene vector.
- The speed of gene vector withdrawal and delivery did not affect the amount of functional gene vector in the range tested.
- We recommend using delivery method 3 with gene vector resuspended in as little serum as possible, as serum could induce immune responses.

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