Miniature Devices for Controlled Airway Surface Perturbation in Rats: Which Device Produces the Best Lentiviral Vector Gene Transfer?

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Introduction

Airway gene-addition therapy offers a potential treatment for a range of inherited and acquired respiratory diseases.

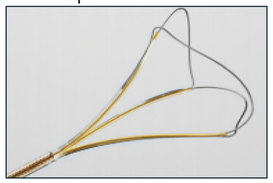
Natural airway defences are known to impede the efficacy of viral vector-mediated gene transfer.

Airway surface preparation or "conditioning" methods performed prior to vector delivery can disrupt these barriers, improving viral vector access to target receptors and airway stem cells.

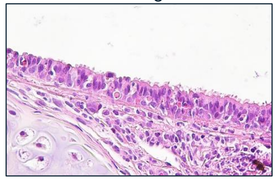
The aim of this study was to assess and quantify the histological and gene transfer effects of novel physical perturbation devices in rat airways to identify the most effective approach.

Experimental design

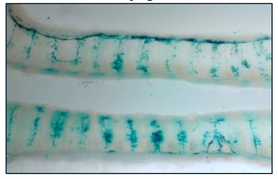
Select perturbation devices

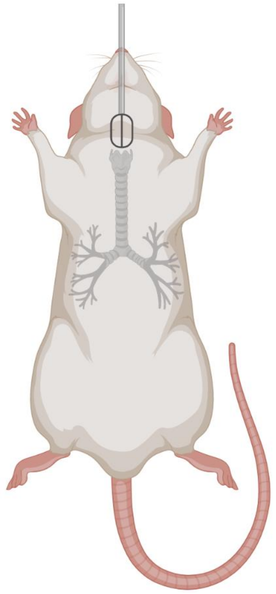


Examine histological effects



Assess airway gene transfer





Methods

Stage 1: Examine airway histological effects produced by devices

A Brush, biopsy forceps, balloon catheter and a variety of flexible wire baskets with varying configurations including the NCircle®, NCompass®, NGage® (Cook Medical) and 11582M Stone Extractor (Storz) were evaluated in n=3 rats/device.

Rats were anaesthetised, intubated, and controlled physical perturbation performed in the trachea via the endotracheal tube. Ten minutes later, tracheas were excised, fixed, embedded and sectioned to produce hematoxylin and eosin (H&E) stained slides. Tracheal regions flattened to one cell layer thick, or completely devoid of cells on the basement membrane were measured.

Stage 2: Assess device airway gene transfer enhancement

A subset of devices were tested to assess whether they could enhance gene transfer (n=3-6 rats/group). Anaesthetised rats underwent airway perturbation followed ten minutes later by 50 µL of lentiviral vector carrying the *LacZ* reporter gene (5 x 10^8 TU/mL). After 7 days, excised tracheas were X-gal processed to quantify the area of LacZ staining. Immunohistochemistry for cytokeratin 5 identified basal cells, a known airway stem cell type.

Statistics:

Data was analysed using one-way ANOVA with Dunnett's post-hoc or Kruskal-Wallis test with Dunn's post-hoc.

Results

The devices produced observable histological effects on the airway epithelium **(Fig 1)**. Significant tracheal epithelium removal was produced by the Brush, and the NCircle®, NCompass® and NGage® wire baskets ($p<0.05$) **(Fig 2)**. Based on the epithelium removal data and histological observations the NCircle®, NCompass®, NGage®, Brush, and 11582M Stone Extractor were tested for their ability to enhance LV vector-mediated gene transfer. The forceps and balloon catheter were not pursued because they produced inconsistent histological effects and were difficult for the operator to employ.

Quantification of LacZ staining found that only the NGage® wire basket significantly increased gene expression ($p=0.00025$), showing an 8-fold increase in the proportion of LacZ-stained area when compared to rats that did not receive perturbation **(Fig 3)**. All devices produced LacZ positive basal cells **(Fig 4)**.

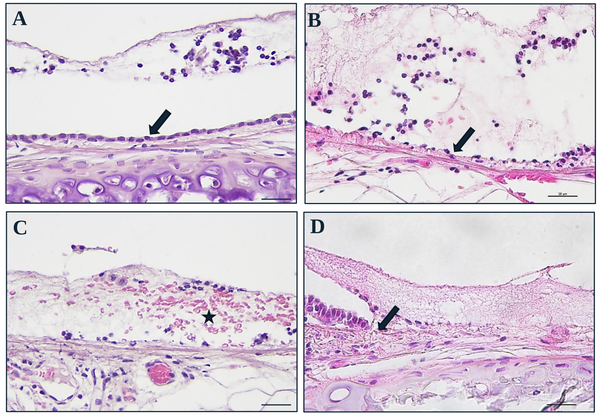


Figure 1: Examples of airway histological effects produced by physical perturbation devices. H&E stained sections demonstrate: (A) Flattened epithelial cells, (B) desquamated cells in the lumen with intermittent basal cells remaining on the basement membrane, (C) extravasated erythrocytes in the airway lumen, (D) complete denudation of the epithelium.

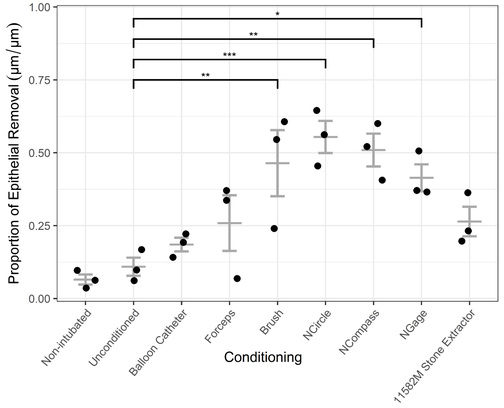
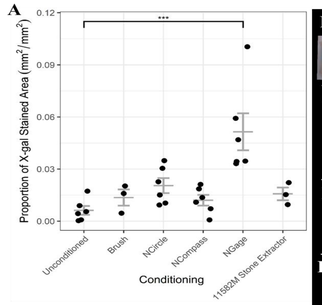


Figure 2: Proportion of removed epithelium across the length of the trachea after conditioning with physical perturbation devices. Mean ± SEM presented, n = 3 rats per group. ANOVA and Dunnett's test vs. unconditioned group (*p<0.05, **p<0.01, ***p<0.001).



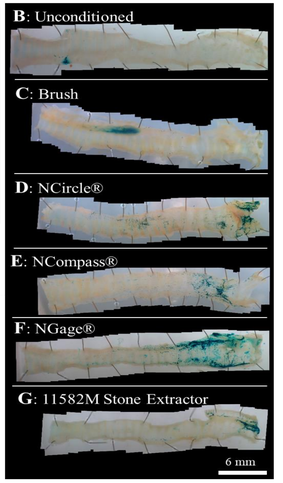


Figure 3: LacZ staining assessments. (A) Quantification of the proportion of *en face* LacZ-stained area across the trachea and bronchi for each device assessed 7 days post-procedure. Mean ± SEM presented, n = 3-6 rats per group. Kruskal-Wallis and Dunn's test vs. unconditioned group (***p<0.001). (B-G) Composite images of *en face* LacZ-stained rat tracheas.

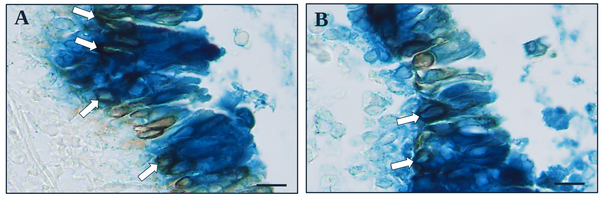


Figure 4: LacZ positive basal cells. Basal cells (coloured brown) were identified by immunohistochemical staining for cytokeratin 5. White arrows: LacZ-positive basal cells.

Conclusions

The NGage® wire basket was the only device to significantly increase LacZ staining, even though other devices also produced significant epithelial cell removal. These findings suggest that factors other than cell removal contribute to perturbation-enhanced gene transfer, such as the location and pattern of disturbance, or type and viability of cells remaining. All devices produced LacZ-positive basal cells indicating the potential for long-term gene expression with this approach. Evidence of airway gene transfer enhancement shown here supports further development of physical perturbation.

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