Duration of reporter gene expression from naked pDNA in the mouse lung following direct electroporation and development of wire electrodes for sheep lung electroporation studies

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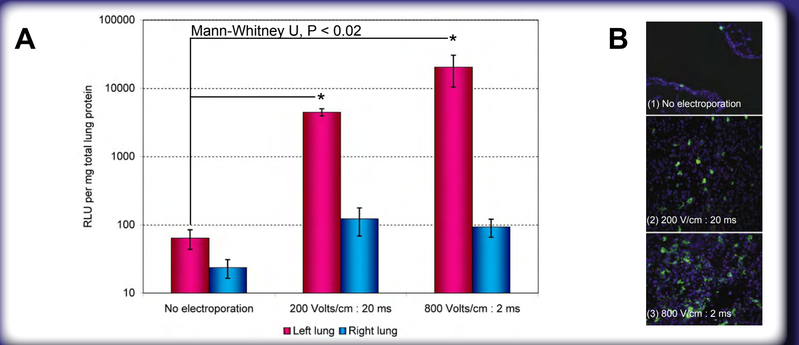
Overview of project.

- Existing non-viral gene therapy for CF lung disease is very ineficient.
- Electroporation is an efficient method for increasing non-viral delivery.
- Lung access makes electroporation technically challenging.
- Electroporation enhances reporter gene expression in the mouse lung (Figure 1).
- We studied the effect of electroporation on the duration of expression (Figure 2).
- Wire electrodes were developed for electroporation in the sheep lung (Figure 3).
- Electroporation increased Lux activity in the sheep lung (Figure 4 & 5).

Figure 1. Electroporation enhances reporter gene expression in the mouse lung.



Mouse lung electroporation model.

(A) Female BALB/c mice were anaesthetised with isoflurane and dosed intranasally with 100 µg pCIKLux (luciferase reporter gene expressed from the CMVIE promoter) in 150 µl water for injection. The mice were ventilated and the lungs surgically exposed. Needle electrodes (0.5 cm apart) (BTX, Holliston, MA, USA) were placed on the surface of the left lung and electric field pulses were applied as indicated using a BTX ECM830 Electroporator. 24 hours post dosing the levels of Lux activity in the right and left lungs was determined using the Promega Luciferase Assay Reagent System (Promega, Southampton, UK). Error = 1 SEM, n = 6-8. (B) Mice were dosed intranasally with pEGFP-N1 (Clontech, Oxford, UK) and electroporated at the conditions described. At 24 hours post-dosing the left lungs were subjected to cryosectioning and cells positive for Green Fluorescent Protein were visualised using standard FITC filters.

Results.

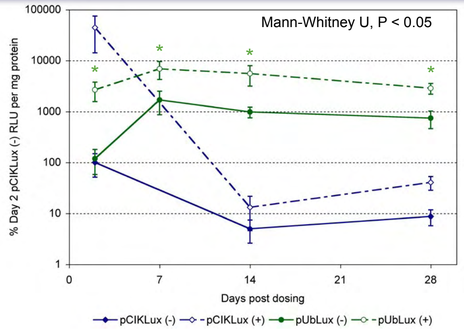
(A) With electroporation at 200 V/cm for 20 ms, the mean Lux activity in the left lung was around 100 fold higher than the no electroporation controls. With electroporation at 800 V/cm for 2 ms, the mean Lux activity in the left lung was around 500 fold higher than the no electroporation controls. No increase was observed in reporter gene expression in the right lungs at either of the electroporation conditions. This suggests that the increase in reporter gene expression was confined to the area contacted directly by the needle electrodes. (B) Naked pDNA delivery in the absence of electroporation was shown to be very ineficient, with very few cells being detected in these sections (1). At 200 V/cm : 20 ms, there was a increase in cells transfected (2) and even more cells could be detected at 800 V/cm : 2 ms (3).

References.

Emerson, M. *et al.*, (2003). Transfection efficiency and toxicity following delivery of naked plasmid DNA and cationic lipid-DNA complexes to ovine lung segments. *Molecular Therapy* 8, 646-653.

Gill, D. R. *et al.*, (2001). Increased persistence of lung gene expression using plasmids containing the ubiquitin C or elongation factor 1 alpha promoter. *Gene Therapy* 8, 1539-1546.

Figure 2. Duration of reporter gene expression is not affected by electroporation.



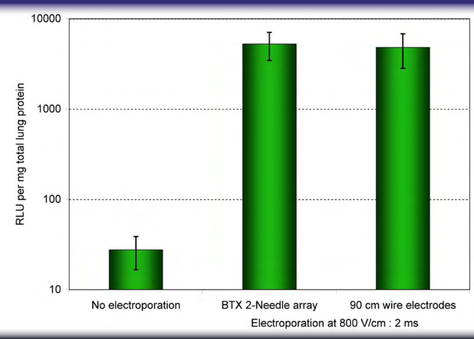
Duration of Luciferase expression in the mouse lung following electroporation.

Mice were dosed with pCIKLux and pUbLux (luciferase reporter gene expressed from the human polyubiqutin C promoter) (Gill *et al.*, 2001). The left lungs (+) were electroporated at 800 V/cm : 2 ms and the right lungs (-) served as a no electropration control. Mice were harvested at 2, 7, 14 & 28 days post-dosing and the levels of Lux activity in the right and left lungs was determined. Error = 1 SEM, n = 6-12.

Results.

The duration of expression from pCIKLux was not enhanced by electroporation. At day 2, Lux activity from electroporated (+) pCIKLux was 500 fold higher than the no electroporation (-) control, but by day 14 the activity from both conditions had fallen to background levels. Electroporation increased the level of Lux activity from pUbLux at each time-point of the study. By 28 days post dosing, the Lux activity from pUbLux (+) was 30 fold higher than the pCIKLux (-) at 2 days post-dosing. Therefore while electroporation does not improve the limited duration of expression from the CMV promoter, when approrpriate long duration constructs are used, activity may be maintained at elevated levels for at least 1 month.

Figure 3. Teflon insulated wires function as electroporation electrode devices.



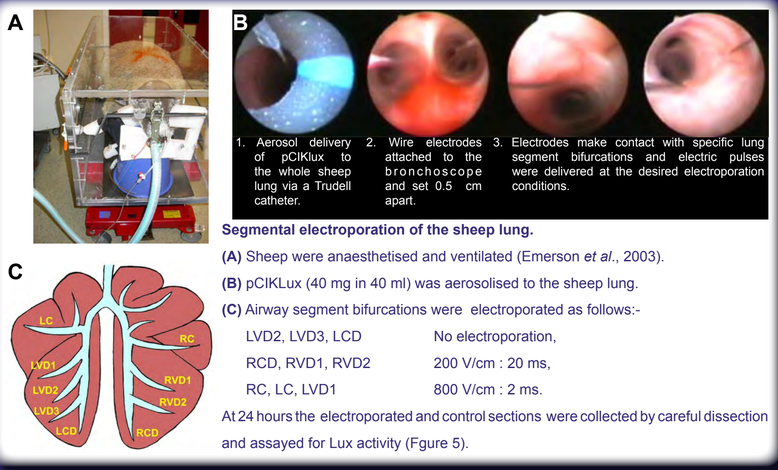
Wire electrode test.

Teflon insulated stainless steel wire (330 µm thick) (A-M Systems, Carslborg, WA, USA) was cut into 90 cm sections and soldered to universal electrical connectors to allow connection to the ECM830 Electroporator. Mice were dosed with pCIKLux and the lungs were immediately removed and electroporated at the condition shown with the Teflon insulated wire electrodes (0.5 cm gap) or with stainless steel needle electrodes (0.5 cm gap) (BTX). Lungs were maintained in cell culture medium overnight (37 °C, 1.5 atm O_2) and Lux activity was determined 24 hours post-dosing. Error = 1 SEM, n = 6 lungs per group.

Results.

Both of the electrode designs resulted in approximately 200 fold higher levels of Lux activity compared to the no electroporation control. The Teflon wires were easy to manouver and safe to use. This demonstrated that the wires could be used in conjunction with a bronchoscope to electroporate targeted areas of the sheep lung.

Figure 4. Electroporation of sheep lung segments with wire electrodes.



Segmental electroporation of the sheep lung.

(A) Sheep were anaesthetised and ventilated (Emerson *et al.*, 2003).

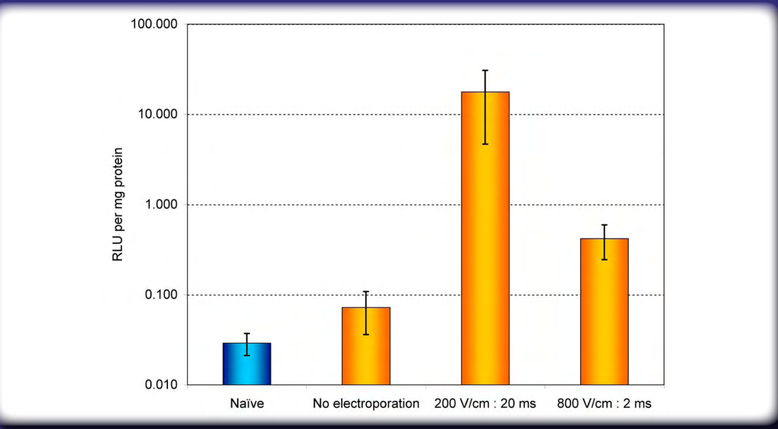
(B) pCIKLux (40 mg in 40 ml) was aerosolised to the sheep lung.

(C) Airway segment bifurcations were electroporated as follows:-

LVD2, LVD3, LCD	No electroporation,
RCD, RVD1, RVD2	200 V/cm : 20 ms,
RC, LC, LVD1	800 V/cm : 2 ms.

At 24 hours the electroporated and control sections were collected by careful dissection and assayed for Lux activity (Fgure 5).

Figure 5. Electroporation enhances reporter gene expression in the sheep lung.



Results.

The procedure was well tolerated by the sheep. Lux activity from naked pCIKLux in the absence of electroporation was around the level of naive samples (taken from an untreated sheep). Electroporation at 200 V/cm : 20 ms resulted in a 250 fold increase in activity relative to no electroporation and electroporation at 800 V/cm : 2 ms resulted in modest 5 fold increase in activity. Error = 1 SEM, n = 3 samples per condition.

Conclusions.

- Electroporation increased Lux activity for 1 month in the mouse lung.
- Wire electrodes are safe and perform as well as needle electrodes.
- Electroporation increased Lux activity by 250 fold in the sheep lung.
- Successful translation of electroporation to a large animal model.

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