In Vivo Lentiviral Gene Transfer to Airway Surfaces for Cystic Fibrosis is Improved by Magnetic Guidance of Particles

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Introduction

Gene vectors designed to treat cystic fibrosis lung disease should be targeted to the conducting airways for therapeutic benefit.

Transduction efficiency is directly related to vector residence time, but gene vectors rapidly spread to the alveoli during inspiration. Extending residence time in the conducting airways is desirable.

Gene vector conjugated magnetic particles (MP) that can be guided to the conducting airway surfaces could improve targeting. However, the behaviour of small MP on the airway surface in the presence of an applied magnetic field are poorly understood, as is the efficacy of MP-guided vector delivery on conducting airway gene transfer.

The aim was to use synchrotron phase contrast X-ray imaging (PCXI) to visualise the motion of a range of MP *in vitro,* and *in vivo* in the trachea of live rats. In a second series of studies we sought to use that optimal configuration to demonstrate the transduction patterns that result from lentiviral LV-MP delivery into rat airways *in vivo*.

Methods

Stage 1: Phase Contrast X-ray Imaging studies

PCXI was performed at the BL20XU beamline at the SPring-8 Synchrotron in Japan. The motion of a range of MP was visualised *in vitro* (Fig. 1) and in live rat trachea (Fig. 2), to examine the dynamics of individual and bulk MP behaviour. A range of polystyrene and iron oxide containing particles of differing sizes and concentrations (Table 1) were combined with different magnet orientations (Fig. 3).

Stage 2: Lentiviral vector MP studies

In subsequent rat studies (Fig. 4) informed by the results from the PCXI visualisation experiments we then delivered a LV-LacZ reporter gene vector conjugated to MP both with and without an external magnetic field, to assess whether this approach increased transduction efficiency in the rat trachea. Physical perturbation of the trachea (see Poster #606) was performed prior to LV-LacZ delivery.

In these studies we held the magnet in one location to determine whether LV transduction a) could be improved compared to vector delivery without a magnetic field present, and b) if airway cell transduction could be focussed to that magnetically targeted region in the upper airway.

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Experimental setups

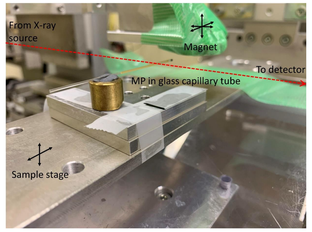

Figure 1: *In vitro* imaging setup with a MP sample in a glass capillary tube on the sample x-y translation stage. The path of the x-ray beam is marked with a red dashed line.

Figure 2: (a) *In vivo* imaging setup in the SPring-8 imaging hutch, with the path of the x-ray beam marked with a red dashed line. (b-c) The magnet positioning over the trachea was performed remotely using two orthogonally mounted IP cameras.

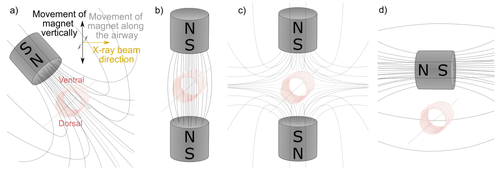

Figure 3: Single and double magnet configurations (attract/repel) for *in vivo* imaging.

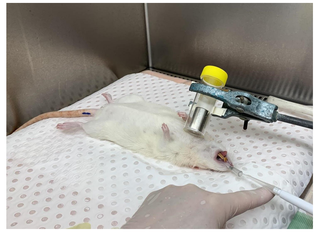

Figure 4: LV-MP delivery setup in a biosafety cabinet.

Phase Contrast X-ray Imaging Results

Table 1: Visibility of the tested magnetic particles using PCXI at SPring-8 BL20XU.

Sample ID	Brief description	Visible *in vitro*	Visible *in vivo*	*In vivo* magnet configurations tested
MP1	18 µm Polystyrene	No	Not tested	-
MP2	0.25 µm Polystyrene	No	Not tested	-
MP3	0.25 µm 10-15% Fe3O4	Weakly	No	-
MP4	0.9 µm 10-15% Fe3O4	Weakly	No	-
MP5	0.25 µm 98% Fe3O4	Strongly	Weakly	a, b, c, d
MP6	CombiMag	Weakly	No	-

PCXI revealed the behaviour of magnetic particles in stationary and moving magnetic fields, both *in vitro* (Fig. 5) and *in vivo* (Fig. 6).

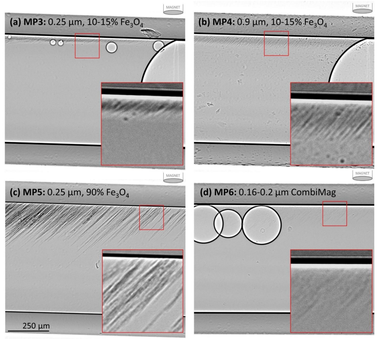

Figure 5: The visibility of the MP under PCXI varied between the samples. As the magnet was translated to the right the angle of the MP strings changed. The distinct large circular shapes are air bubbles trapped in the capillary tube. Red boxes contain a contrast-enhanced enlargement.

During delivery (Fig. 6), MP deposition was focused within the field of view where the magnetic field was the strongest. MP could be concentrated and aligned by magnetic field manipulation, but MP could not be dragged along the live airway surface.

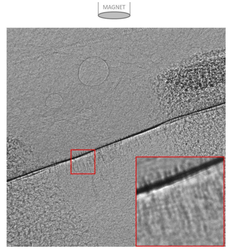

Figure 6: Image immediately after delivery of MP5 into the trachea of a rat, with the magnet located directly above the imaging region.

Example videos

In vivo video of the trachea of a live anaesthetised rat at four locations within the airway. Long strings of MP5 are visible in the centre of the airway and move as the magnet is translated overhead to the left and right of the imaging plane.

MP5 particles are present on the airway surface and the strings moved as the magnet was translated right and left. The magnet was then translated across and above the trachea.

Lentiviral Vector MP Results

The LV-LacZ titre was 1.8 x 10^8 TU/ml, and after mixing 1:1 with CombiMag MP (MP6) animals received a 50 µl tracheal dose of 9 x 10^7 TU/ml LV vector (i.e., 4.5 x10^6 TU/rat). Transduction efficiency in rat trachea was improved six-fold when the LV-MP were delivered in the presence of a magnetic field (Fig. 7).

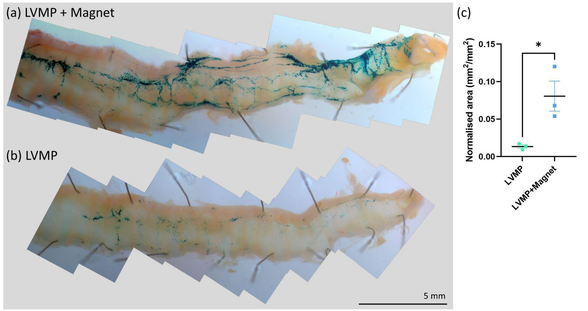

Figure 7: Example composite images showing the tracheal transduction produced by the LV-MP (a) in the presence of the magnetic field, and (b) without the magnet present. (c) There was a statistically significant improvement in the normalised LacZ transduced area within the trachea when using the magnet (*p=0.029, t test, n=3 per group, mean±SEM).

Neutral fast red stained sections demonstrated LacZ stained cells were present in similar patterns and locations to those we have previously reported (Fig. 8).

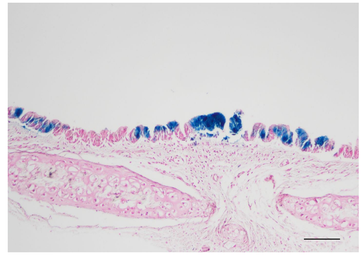

Figure 8: Neutral fast red stained image showing LacZ transduced cells (blue staining) in the trachea of a rat that received LV-MP in the presence of a magnetic field. Scale bar 250 µm.

Conclusions

These results show that conjugating gene vectors to MP may be a valuable approach for improving gene vector targeting to the conducting airways *in vivo*.