A Novel Platform to Study Airway Constriction and Cilia Beating in Mouse Precision-Cut Lung Slices

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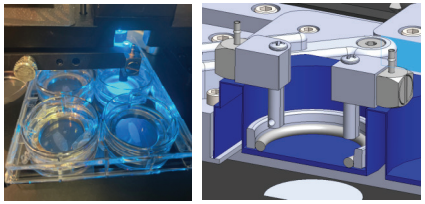
BACKGROUND

- Precision-cut lung slices (PCLS) are an important *ex vivo* research model. However, standardized protocols and commercial systems able to automate difficult and **labor-intensive** tasks in a reproducible manner are lacking.
- Bronchoconstriction experiments require **detecting the beating of micron scale cilia**, **discerning the contour of the lumen of an airway**, and **reliably adding a dose** without perturbing the lung slice.

The physioLens and the dosing module

OBJECTIVES

- Validate the physioLens, an automated microscopy platform for:
  - i. Airway lumen area measurement
  - ii. Cilia beating observation and measurement
  - iii. Automated fluid handling
- Provide guidance on lung dissection and slice selection.

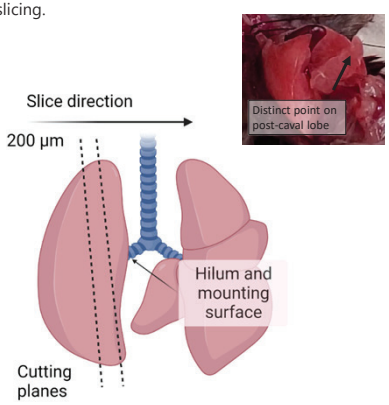


A nozzle changes the media in the well plate, dosing the PCLS

METHODS

Mouse lung dissection and slicing

- Three 22 g female mice (Hb9-mitoEGFP) of more than 12 weeks of age were euthanized by an overdose of Pentobarbital.
- After dissection and tracheal cannulation, 2 % agarose from a 44 °C water bath was used to fill the lungs.
- Lungs were inflated until a distinct point was formed on the post-caval lobe, requiring approximately 1.5mL of agarose.
- ~ 0.5 mL of air was quickly added after agarose inflation was completed.
- It was important to clear tissue around the post-caval lobe, in order to be able to see the formation of a distinct point.
- Compresstome VF-310-0Z (Precisionary) used for slicing.



Guidelines on filling and slicing

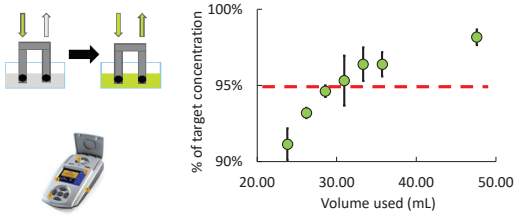
Dose Response

- physioLens with automated fluid handling system.
- Slices were dosed with HBSS with no agonist, then doses of 5 µM and 125 µM of methacholine, for 8 minutes, to ensure max contraction was reached.
- Airways ranging from 100 µm to 500 µm in diameter with intact cilia beating were selected.

RESULTS

Dosing verification

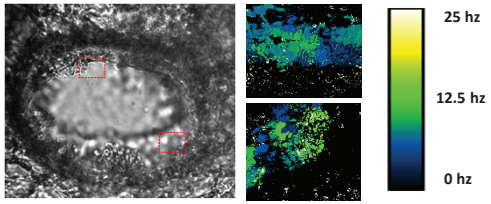
- The data below is for a 2.4 mL/s flow rate in a well of a six well plate with an initial volume of 6.5 mL.



Dosing measurement concept and performance

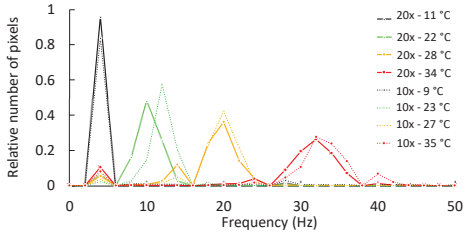
Cilia beating validation

- Cilia beating can be observed as brightness fluctuations which can be analyzed to extract a dominant frequency.
- Maps of beat frequency can be then obtained by analyzing temporal changes in brightness associated with each pixel.



A sample airway with cilia beating frequency maps for regions of interest denoted by square boxes

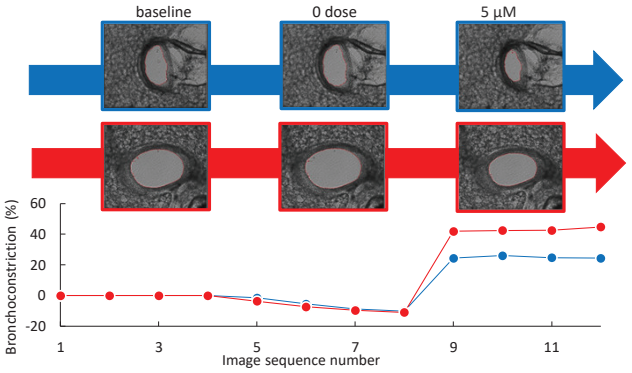
- Cilia beat frequency is known to increase with temperature.
- The correlation between beating frequency and temperature confirms the performance of the hardware and analyzers.



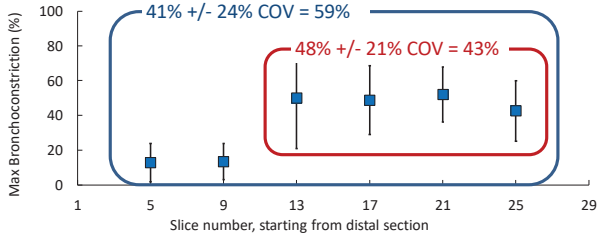
RESULTS

Lumen area measurements

- The slice is not displaced during dosing, as seen by the repeated images of the same airway.
- Some dilation was observed when administering media with no agonist



Bronchoconstriction as a function of lung slice position



Max contraction at 125 µM MCH. Mean +/- stdev for 5-10 airways for 3 mice

SUMMARY

- Automated microscopy and fluid handling can accomplish airway hyperresponsiveness studies in mouse PCLS.
- Proper filling was ensured by tracking the post-caval lobe.
- Cilia beating can be tracked with a 10x or 20x objective.
- The final drug concentration can reach 95% of the target concentration with less than 35mL of solution.
- Selecting slices from generations 4-6 (slices 13-25) reduces variability.

DISCLOSURE

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