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PCLS: Effective Ex Vivo Platform For Lung Physiology

PRECISION CUT LUNG SLICES: AN EFFECTIVE EX VIVO PLATFORM FOR STUDYING LUNG PHYSIOLOGY

This document highlights experimental considerations and reviews case studies of translational pulmonary research based on Precision-Cut Lung Slices (PCLS). PCLS are thin organ slices prepared from fully developed animal or human lung that closely resembles the morphology and functionality of the respiratory system.

The information contained herein was primarily obtained from a recent seminar held at the 2023 American Thoracic Society meeting with renowned panelists Dr. Jane Bourke from Monash University, Dr. Yan Bai from Harvard University and Liah Fereydoonzad from SCIREQ.

- During her presentation, Dr. Yan Bai provided a historical perspective of the PCLS technique and reviewed recent advances in PCLS preparation, culture, tissue cryopreservation and the simultaneous study of airways and pulmonary vasculature.
- Dr. Jane Bourke provided an overview of the wide range of research applications offered by PCLS studies, including LPS models of lung injury, idiopathic pulmonary fibrosis, and the evaluation of novel bronchodilators and pulmonary vasodilators.
- Liah Fereydoonzad discussed how each step of the PCLS experimental process must be carefully executed to optimize outcomes and minimize experimental variability. She showed how refining processes surrounding lung preparation and filling, slicing, slice selection and lumen area measurements significantly reduced the coefficient of variation.

Finally, this document further discusses the automation of PCLS data collection and analysis using a novel research platform; the physioLens.

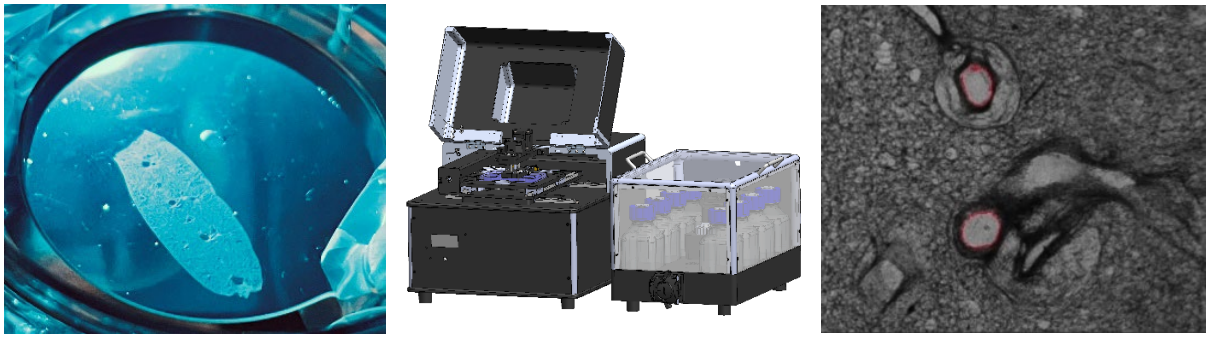

Figure 1 - PCLS Slice, physioLens and physioWare lumen detection

Learn more about the physioLens here!

1. UTILIZING PRECISION-CUT LUNG SLICES TO STUDY THE INTRAPULMONARY AIRWAY AND VASCULAR BIOLOGY IN HEALTH AND DISEASE

PCLS BRIDGES A GAP IN THE STUDY OF LUNG BIOLOGY

Dr. Yan Bai explained how the study of pulmonary vascular biology has been limited by poor access to intrapulmonary vessels, especially for small pulmonary arteries. Current methods involve indirect measurements or isolating extrapulmonary arteries for *ex vivo* testing. However, these approaches do not target diseased vessels or provide cellular-level functional evaluation. Isolated cells, such as primary cells, have their limitations, lacking the lung's natural microenvironment. PCLS bridge the gap between *in vivo* and *in vitro* approaches by preserving the *in vivo* lung microenvironment and enabling functional and molecular mechanistic studies on a variety of cell types, thereby providing valuable insights into pulmonary vascular pathogenesis.

HISTORY OF PCLS

Thin organ slices were first used by Dr. Otto Warburg in 1923. In 1987, Drs. Placke & Fischer reported manually slicing agarose filled lung sections. In 1988, with the arrival of automated microtomes, Dr. Stefaniak published the first PCLS as we currently know and use them. Since then, many researchers, including the late Dr. Mike Sanderson, have further refined and democratized the PCLS technique.

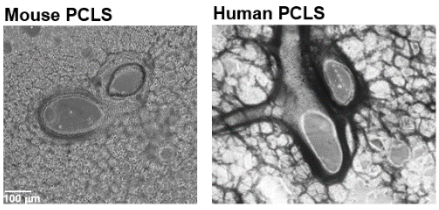

Figure 2 - Mouse vs Human PCLS

PREPARATION OF MOUSE AND HUMAN PCLS

The procedures for preparing mouse PCLS (mPCLS) and human PCLS (hPCLS) are relatively similar. Delivering agarose to mouse lungs can be achieved with ease and consistency by via the trachea. Human lung resections must often be filled in a multi-step process, by cannulating small airways and overcoming agarose leaks. Other key differences between mPCLS and hPCLS studies include tissue size, tissue processing, slice thickness, slice viability and other experimental considerations as outlined in Table 1. During her presentation, Dr. Bai highlighted a critical element for pulmonary vasculature studies using lung slices: prior to slicing, the blood vessels must be filled with gelatin to stabilize and protect them. The gelatin will eventually be dissolved during subsequent steps.

Table 1 - Comparison Between Mouse and Human PCLS Preparation

	Human	Mouse
Concentration of LMP agarose	0.8%-1.5%	1.5%-2%
Volume of agarose	2L for one side	~ 1.2 ml (adult)
Thickness of PCLSs	250-500 µm	100-250 µm
Viability of PCLSs (DMEM/F12)	Weeks, airway contractility 4 weeks	Days, airway contractility 5-7 days
Pulmonary vasculature preservation	No well-formed protocol	Gelatin ± vasodilators

OPTIMIZATION OF CULTURE CONDITIONS

It was noted that by optimizing the culture conditions of the PCLS, for example by adding a low dose of insulin, extends the viability of the slices and their ability to contract to 15 days. It is essential to optimize the culture conditions for the outcomes of interest and the study at hand.

CRYOPRESERVATION OF PCLS

The cryopreservation process for precision-cut lung slices (PCLS) involves freezing and thawing. For freezing, a mixture of DMEM/F12 and 10% DMSO is used, and the PCLS are placed in cryovials within a container filled with isopropyl alcohol. PCLS are then stored at -80°C overnight and can be transferred to liquid nitrogen for long-term storage. Thawing is performed rapidly at 37°C, and the PCLS are subsequently incubated overnight. This cryopreservation method allows for the preservation and storage of PCLS for future experiments.

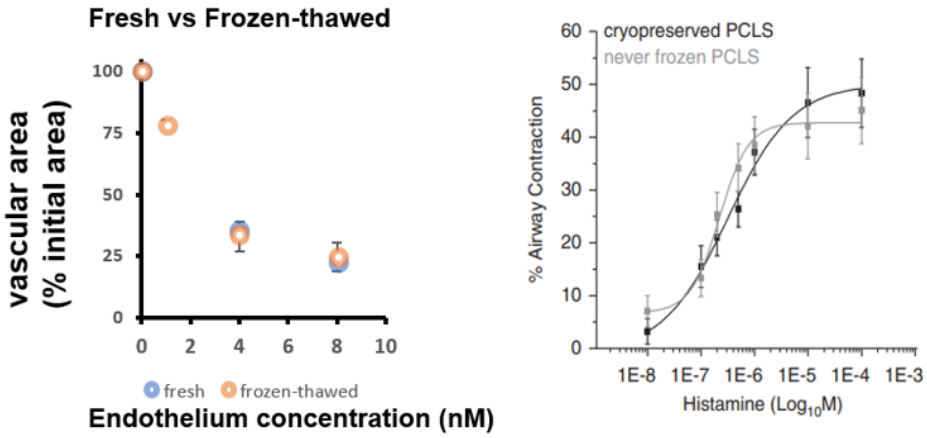

Figure 3 - AMJRCMB, Vol 54(3) - May 2016

SOME LIMITATIONS OF PCLS

While precision-cut lung slices (PCLS) offer valuable advantages for studying pulmonary vascular biology, there are several limitations to consider:

- PCLS cannot maintain slices *in vitro* for chronic disease processes, as they are a static system lacking cyclic breathing motion and blood circulation present *in vivo*.
- PCLS is an isolated system and may not be suitable for studying adaptive immune responses that impact pulmonary structural cells.
- The lack of an intact neural network in PCLS.
- The presence of agarose gel in the alveolar space can potentially affect the experimental conditions. Lastly, ASMCs (airway smooth muscle cells) in PCLS are resistant to gene manipulation, posing challenges for genetic studies.

2. PRECISION CUT LUNG SLICES AS AN INNOVATIVE PLATFORM FOR STUDYING MECHANISMS AND THERAPEUTIC APPROACHES FOR LUNG DISEASES

DR. JANE BOURKE

Dr. Bourke leads the Respiratory Pharmacology group in this division where her research concentrates on therapies for Chronic Lung Diseases such as Asthma, COPD, and Infectious Diseases. She has unique expertise with PCLS Techniques to visualize intrapulmonary airway and artery lumen area.

PCLS serves as a valuable platform for investigating various aspects of lung diseases. By utilizing PCLS from disease models and human lung tissues, researchers can conduct *ex vivo* assessments of airway and artery reactivity, enabling an integrated evaluation of underlying mechanisms and potential therapeutic interventions.

ANALYSIS OF AIRWAY CONSTRICTION

In mouse PCLS, drug perfusion studies capture images at regular intervals, usually every 2 seconds, allowing for a frame-by-frame analysis of time-course responses. Lumen area calculations, performed by summing pixels, provide valuable information about the maximum stable responses observed.

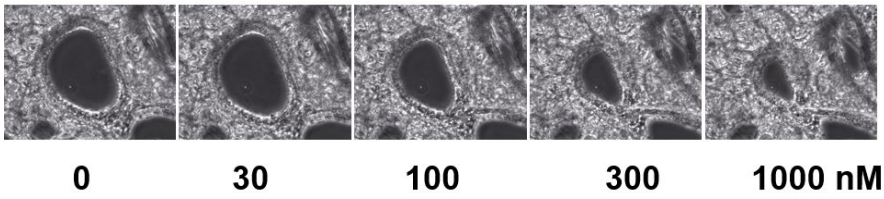

Figure 4 - Analysis of Airway Contraction

LPS EFFECTS IN VIVO AND IN VITRO

Based on a previous study from Donovan et al (2015) demonstrated that lipopolysaccharide (LPS) treatment increased TNFα release 6-fold, but did not alter small airway reactivity as expecting in mouse lung slices. To build upon investigating this further, Dr. Bourke's laboratory retested LPS administration to mice in vivo and in vitro, now looking into both airway and arterial contraction.

These recent studies by Lamanna, et al (2023; *in preparation*) demonstrated that LPS treatment *in vivo* and *in vitro* increased artery, but not airway, contraction.

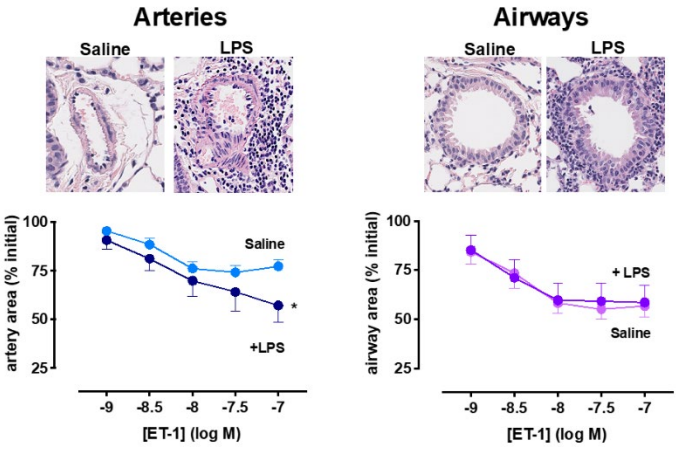

Figure 5 - In Vivo LPS Increased Artery Not Airway Contraction

MODELING ALTERED REACTIVITY USING PCLS

Table 2 - Modeling Altered Reactivity Using PCLS

Model	Disease	Outcomes
Allergen challenge *in vivo*	asthma	Donovan, *Plos One*, 2013; *AJP Lung*, 2015; Liu, *J Path*, 2017
Cigarette smoke *in vivo*	COPD	Donovan, *AJRCMB*, 2015
Poly I:C or virus *in vitro* virus *in vivo*	viral infection	Chitty, Thomas *in progress* FitzPatrick, *Sci Rep*, 2016
Smoke and virus *in vivo*	COPD exacerbation	Donovan, *Clin Sci*, 2016
LPS *in vitro* LPS *in vivo*	bacterial infection	Donovan, *Plos One*, 2015 (airways) Lamanna, *in prep* (airways, arteries)
LPS *in utero* / hyperoxia	bronchopulmonary dysplasia	Royce, *AJRCMB*, 2016 (airways) Bui, *Front Immunol*, 2019 (arteries)
Bleomycin *in vivo*	IPF/PH	Lam, *in progress*
TGFβ overexpression	fibrosis	Chitty, Thomas *in preparation*

RELAXIN

These PCLS enable *ex vivo* assessment of airway and artery reactivity, providing valuable insights into the underlying mechanisms and potential therapeutic interventions. Integrated evaluations of mechanisms and therapies can be performed, focusing on the effects of novel dilators. Among these dilators, Relaxin exhibits bronchodilator and anti-fibrotic properties, making it an excellent tool to study contractility (Lam et al, 2016).

In rat PCLS, relaxin has been identified as a novel bronchodilator that exhibits epithelial-dependent effects and shows greater potency than β2-agonists. These findings suggest that relaxin could serve as an alternative or adjunct therapy for severe asthma. Conversely, in human PCLS, relaxin is also a novel bronchodilator, but its effects are epithelial-independent, and it potentiates the action of β2-agonists. Notably, relaxation is observed under both perfused and static conditions, indicating the potential clinical utility of relaxin. Moreover, relaxin increases the potency of salbutamol, a commonly used β2-agonist, in human PCLS. These findings highlight the therapeutic potential of relaxin in the management of asthma and suggest its ability to enhance the effectiveness of existing bronchodilator treatments.

PCLS TO STUDY FIBROSIS

PCLS facilitates the investigation of fibrosis-related aspects. Human PCLS derived from non-idiopathic pulmonary fibrosis (IPF) lung resections maintain viability over extended periods, enabling exploration of gene expression, protein levels, and localization using techniques such as Western blot and immunofluorescence. Studies conducted on human PCLS treated with a "fibrotic cocktail" (FC) reveal increased expression of fibrotic genes, including FN1, COL1A1, SERPINE1, CTGF, MMP7, and ACTA2, within

24 hours. Notably, relaxin and pirfenidone exhibit the ability to inhibit collagen deposition in FC-treated human PCLS (Alsafadi et al, 2017).

SUMMARY

PCLS represents an innovative platform for studying lung diseases, as it allows researchers to visualize *ex vivo* airway and artery reactivity, quantitate fibrosis and inflammation, assess responses to infections, and evaluate the efficacy of therapeutic interventions. By integrating the assessment of bronchodilation, vasodilation, fibrosis, and inflammation, PCLS offers a bridge to clinical translation, providing valuable insights for advancing our understanding and treatment of lung diseases.

3. SLICING THROUGH THE OBSTACLES: ENHANCING PRECISION IN PRECISION-CUT LUNG SLICES

Liah Fereydoonzad from SCIREQ, presented a comprehensive review of challenges faced in precision-cut lung slices (PCLS) physioLens experiments and outlined the steps taken to optimize protocols and improve precision.

LITERATURE REVIEW AND PROTOCOL OPTIMIZATION:

The initial phase involved conducting a literature review of PCLS dose-response publications, protocols, and results. The target coefficient of variation (COV) for bronchoconstriction studies was established at 40% or better. The review revealed significant variability in bronchoconstriction data obtained using common methodologies, highlighting the need for protocol improvements.

AGAROSE TEMPERATURE:

Maintaining an optimal agarose temperature is crucial to prevent tissue damage or incomplete lung filling. The presented protocol involved melting 2% agarose and cooling it in a water bath to 40°C for 15 minutes. The ideal temperature range for agarose was determined to be between 35°C and 40°C. Future efforts aimed at introducing a heated automated agarose injector were discussed to streamline the process further.

FILLING VOLUME:

Precise lung filling is vital for obtaining consistent results. Mouse lung volume is approximately 1 mL, however this is not enough to adequately fill the lungs. Visual cues such as the formation of a pointed postcaval lobe and clear, sharp edges of the lobes were used to assess proper lung filling. Additionally, injecting a small bolus of air helped clear agarose from the airways, ensuring optimal filling.

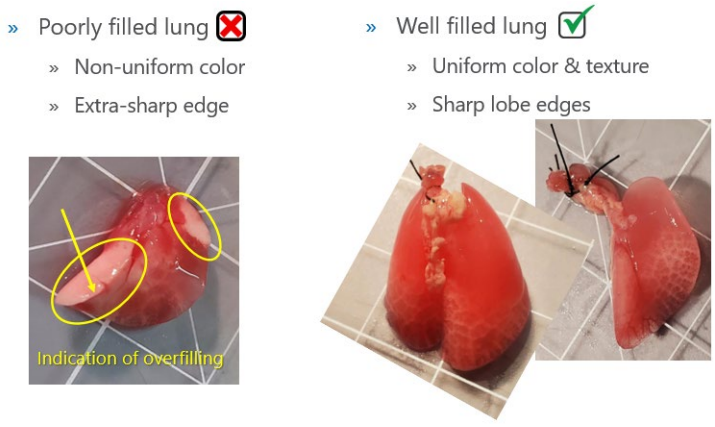

Figure 6 - Lung Filling in PCLS Preparation

SLICE SELECTION - AIRWAY GENERATION:

Studies indicated that airway generations 3, 4, and 5 exhibit the highest levels of bronchoconstriction. To reduce variability, selecting slices within this range (slice 5-15) was found to be effective. By focusing on these specific slices, the variability in bronchoconstriction data was significantly reduced from 57% to 33%. This approach aligns with existing literature and provides a standardized methodology for slice selection.

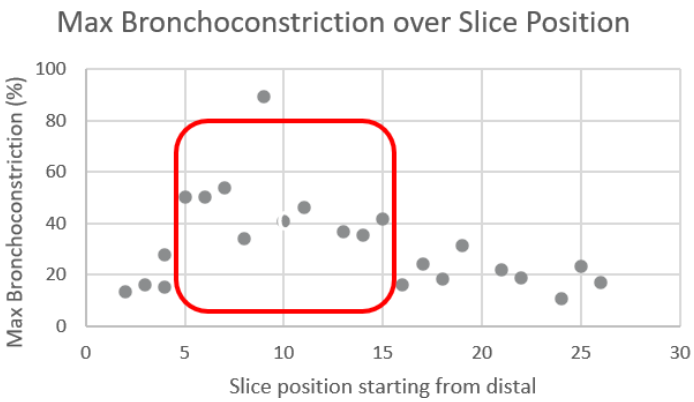

Figure 7 - Maximum Bronchoconstriction Over Slice Position

AIRWAY SELECTION - SCANNING:

A systematic scanning technique was implemented to easily identify airways within the selected slices. By scanning the slice at 4X magnification, a "slice map" was created, allowing researchers to navigate to specific positions for detailed examination. This approach streamlined the airway selection process, enabling researchers to quickly identify the desired airways.

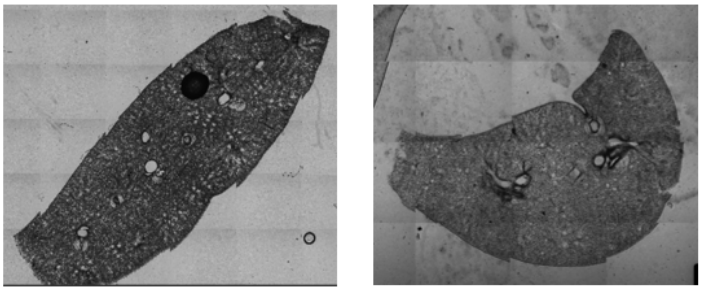

Figure 8 - physioWare Slice Map

CILIA BEATING AND FREQUENCY:

Cilia beating was explored as both a quality control measure and a potential outcome of interest. It was noted that assessing cilia beating frequency can serve as a quality control indicator for airway viability. A cilia beating frequency map was created using both 10x and 20x objectives, capturing approximately 155 frames per second. The observed cilia beat frequencies at various media temperatures aligned with existing literature.

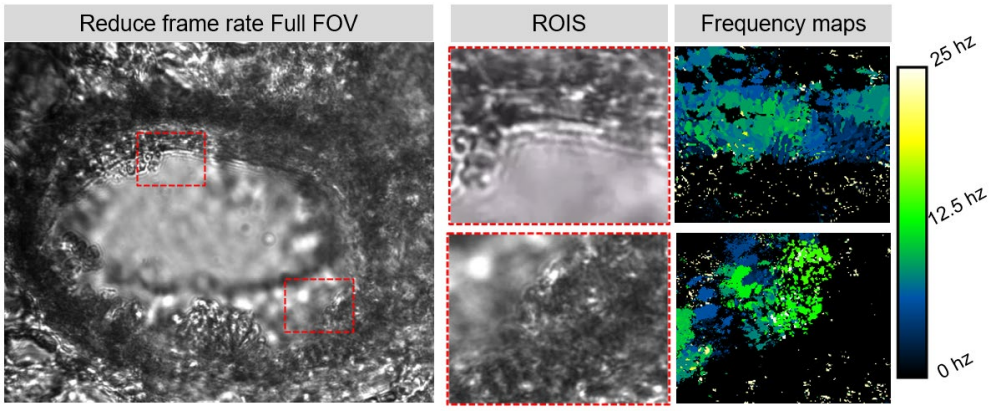

Figure 9 - physioWare Detection of Cilia Beating & Frequency

SLICE IMMOBILIZATION:

Currently, slices are held in place using a harp or by placing a mesh and washer on top of the slice. The development of a transparent mesh slice holder was introduced, which enhances visibility during experimentation.

EXPERIMENT AUTOMATION:

Acknowledging the demand for efficiency, the presentation emphasized the significance of experiment automation. With the aim of studying the maximum number of slices within 48 hours, automation was deemed essential to streamline processes and improve overall productivity. The time needed for a 6 well dose response was reduced from 2h30 manually to 1 hour automated (only requiring 20 minutes of active user intervention).

LUMEN OUTLINE DETECTION:

An automatic software solution for airway lumen detection was proposed. This one-click approach offers rapid and standardized detection, enabling real-time tracking of airways throughout the experiment.

SUMMARY:

Through an extensive literature review and systematic protocol optimization, the SCIREQ team successfully reduced variability in bronchoconstriction studies from 97% to 34% in PCLS experiments. Improvements were made in agarose temperature control, filling volume determination, slice and airway selection, cilia beating frequency assessment, slice immobilization, experiment automation, and lumen outline detection. These enhancements not only addressed the challenges faced but also established standardized methodologies, contributing to the overall precision and reliability of PCLS research.

4. PHYSIOLENS

The physioLens is a Precision-Cut Lung Slice (PCLS) imaging research platform designed, amongst other things, as a turnkey solution for automated airway contractility studies in pulmonary tissues derived from mice, rats, pig or human lungs.

Figure 10 - The physioLens Base Unit & Doser Unit

The physioLens provides:

- A unique ability to combine physiological outcomes and tissue imaging, providing scientist with a wealth of outcomes such as airway contractility, cilia beating, cell viability, inflammation markers.
- A fully integrated PCLS research solution which enables scientists to add translational PCLS outcomes into their studies with ease and confidence.
- Fully automated analysis and dose responses curves obtained from multiple lung slices studied in parallel with minimal user intervention.

Receive a physioLens information package here!