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PCLS from human lung tissue

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Alsafadi HN, Staab-Weijnitz CA, Lehmann M, Lindner M, Peschel B, Königshoff M, Wagner DE. An ex vivo model to induce early fibrosis-like changes in human precision-cut lung slices. Am J Physiol Lung Cell Mol Physiol. 2017 Jun 1;312(6):L896-L902. doi: 10.1152/ajplung.00084.2017. Epub 2017 Mar 17. Erratum in: Am J Physiol Lung Cell Mol Physiol. 2020 Apr 1;318(4):L844. doi: 10.1152/ajplung.zh5-7811-corr.2020. PMID: 28314802.

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We use this protocol and it's working

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Abstract

This protocol outlines a standardized workflow for generating and cultivating precision-cut lung slices (PCLS) as an ex vivo model that preserves the native structure and cellular complexity of lung tissue. The method describes how to prepare intact lung tissue for sectioning, obtain uniform slices, and maintain them in culture for downstream analyses. PCLS allow functional, mechanistic, and drug discovery studies in the field of respiratory research.

Guidelines

All steps involving human tissue must be conducted under BSL2 safety guidelines.

Materials

- Vibratome (e.g Ci 7000smz2 Campden Instruments, Hyrax V50)
- Knifes (7550/1/SS stainless steel blades Campden Instruments)
- cyanoacrylate glue (e.g. Loctite 406, Henkel, Germany)
- syringes 25 ml, sterile
- 15cm petri dishes, sterile
- 96 well-plates
- low gelling point agarose (sigma A9414-250G)
- Cultivation medium (DMEM/F12, 11330057)
-FBS Good (PAN Biotech P40-37500)
-Pen-Strep (Life technologies 15140122)
-Amphotericin B (Sigma A2942-100ML)
- Scissors + forceps
- Scalpel
- Disposable Biopsy Punch 4mm (pfmmedical 48401)
- 20g cannula/plastic tubing (VasoVet 4269115)
- Surgical drape (Foliodrape 277 500)

Safety warnings

Do not over-inflate since this may cause irreversible damage to the cells or alveoli due to non-physiologic stretch of the matrix.

Ethics statement

Tissue collection for this protocol requires prior approval by the users' Institutional Ethics Board or equivalent ethics committee.

Before start

Before beginning the procedure, ensure that all necessary preparations and equipment are in place. Start by preparing the cultivation medium and autoclaving all surgical tools to maintain sterile conditions. Set up the vibratome by equipping it with the appropriate knife and filling the tissue bath with medium, allowing the system to equilibrate before introducing the lung tissue.

Inflating lung

1h 30m

1 Dissolve low gelling point agarose in Cultivation medium to prepare a 3% (w/v) solution by cooking (e.g. microwave, medium 30sec. repeat till dissolved) and keep at 49°C in a water bath.

1.1 Place a surgical drape inside the hood and place all the needed tools inside the hood (sterile).

1.2 Transfer tissue into a 15 cm petri dish.

1.3 Carefully insert a cannula into the bronchi/ bronchiole while lifting the tissue up gently with a forceps.

1.4 Once the cannula is in place, fill a 25ml syringe with the agarose solution and connect it to the cannula.

1.5 Carefully inject the agarose into the lung until it is inflated. Do not over-inflate since this may cause irreversible damage to the cells or alveoli due to non-physiologic stretch of the matrix.

1.6 Transfer the lung tissue to the cold transport medium and keep there for a minute, until the agarose starts solidifying.

1.7 Repeat the procedure until the whole tissue is filled.

1.8 Transfer the filled tissue back to the transport container.

1.9 Set the agarose filled lung segment at 4°C for at least 30 min (ideally overnight).

30m

Slicing tissue into precision-cut lung slices (PCLS)

1.10 Prepare vibratome: Place the metallic bath in the white plastic ice bath. Then, carefully as much ice as possible and fill the metallic bath with culturing medium.

1.11 Carefully place the knife onto the vibratome head and fix it properly with the screws as instructed by the manufacturer.

1.12 Transfer the filled lung tissue to a fresh 15cm petri dish and with the scalpel remove as much as possible of the pleura and any non-filled tissue (usually very soft) that is left.

1.13 Cut 1.0-1.5 cm^2 blocks with the scalpel (if possible, keep pleura on one of the cube sides).

1.14 Add 100-200ul of cyanoacrylate glue on the holder for the vibratome.

1.15 Mount one block on the holder by carefully placing on top of the glue (pleura facing the glue).

1.16 Place the magnetic metallic holder into the metallic bath and position the tissue in the desired position.

1.17 Start the vibratome with the button on the back left.

1.18 Use program settings for human tissue: thickness: 500 µm, frequency: 100 Hz, amplitude of the knife: 1.2 mm.

1.19 Set up “Slice Window”

1.20 Slice the lung segment with the vibratome by repeating the “slice window”

1.21 Carefully transfer the slice by lifting with forceps from the vibratome tray into an 10 cm petri dish with cultivation medium.

1.22 Use a biopsy puncher to prepare 4mm punches.

1.23 Transfer one 4mm punch per well into a 96 well plate with 100ul of cultivation medium per plate.

Important considerations

1.24 Slices can be produced until the knife gets close to the holder (i.e. 2-3 mm of tissue should be left unsliced since the cyanoacrylate glue is toxic to the cells and may influence experimental outcome)

1.25 If more slices/punches are needed repeat it with new pieces of inflated lung

1.26 If the knifes/Puncher are used too much, please replace them for a new/sharper one.

Cultivation

1w

1.27 For treatment, day 0 is the next day of slicing.

1.28 Change medium every 2-3 days until final time point.