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623.1.HTC_Precision_Cut_Lung_Slices V.1

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We use this protocol and it's working

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Abstract

Purpose and Scope of the Procedure or Laboratory Assay

1. Provide high quality, viable, 500 µm sections of human lung for culture and other in vitro assays
2. Current preparation has limited ability to produce quality sections with larger cartilaginous airways or sections containing certain pathologic features (ex. metaplastic bone).

Diseases of the respiratory system are diverse in their onset, progression, and have an effect on all levels of complexity of an animal, from a subcellular level to the organism as a whole. Therefore, one could argue that a similarly diverse range of models should be available to researchers looking to study particular questions. One tool which has shown utility is Precision Cut Lung Slices (PCLS). PCLS have been used to investigate a variety of research questions in both human and animal studies. This includes mechanisms regulating airway contractility (1), and induction of pulmonary fibrosis (2), in addition to testing the toxicity of exposure to various chemical compounds (3).

At the University of Rochester Medical Center, we are in a unique position to prepare PCLS from donors in various states of disease progression and across a spectrum of ages and demographics. By providing sections which can be reliably thawed and placed into culture, researchers can begin to ask questions about how the tissue responds to exposure at different life stages or between groups with differing risk factors. In addition, PCLS have shown to be compatible with various -omic techniques making these a valuable tool for gathering a wide range of information about a particular sample.

Guidelines

Record details of procedure in Worksheet or Directly in BRINDL. The contents of the sample specific Worksheet is transferred to the BRINDL Database within the week. If documenting directly in BRINDL Database, please mark and file a worksheet with the Sample ID and statement of direct entry.

Materials

Personal Protective Equipment (PPE):
1. Grossing Station / Fume Hood
2. N95 Mask
3. Lab Coat
4. Nitrile Gloves
5. Safety Googles
6. Biohazard Waste Bags
7. Sharps Container
8. OxyVir TB
9. Disinfecting Bleach

Other Equipment/Materials:
1. Tissue-Tek Replaceable Blade Scissors – Sakura, Catalog # 4794
2. Replaceable blades (Sharp/Sharp) – Sakura, Catalog # 4795
3. 50mL conical tubes
4. Leica VT1000S Vibratome
5. Costar 24-well culture plates – Corning, Catalog # 3526
6. Dialysis tubing clamps (150mm) – Repligen, Catalog # 142253
7. Sterile cotton tipped applicators – Medline, Catalog # MDS202000
8. Sterile syringe (NO NEEDLE, at least 15 mL) –
9. Suture / small zip ties
10. Cyanoacrylate glue (super glue)
11. Gauze – Fisherbrand, Catalog # 22-246-069
12. Kimwipes – Kimberly-Clark, Catalog # 34155
13. Cryovials – Thermo, Catalog # 5000-1020
14. Stainless Steel PTFE-coated Vibratome Blades – Ted Pella, Catalog # 121-4
15. Stericup Vacuum Filter (150 mL) – Millipore, Catalog # SCGPU01RE
16. SureOne Filtered 1000µL Pipette Tips – Fisherbrand, Catalog # 02-707-404

Chemicals:
1. Hanks’ balanced salt solution (HBSS) – Corning; Catalog # 21-022-CV
2. Low-gelling point agarose – Sigma-Aldrich, Catalog # A9045
3. Dulbecco’s Modified Eagle’s Medium (DMEM), phenol-free – Gibco, Catalog # 21063-029
4. Antibiotic/Antimycotic – (Penicillin, Streptomycin, Actinomycin D) Corning, Catalog # 30004136
5. Fetal Bovine Serum (FBS, low endotoxin, preferably certified)
6. Dimethyl Sulfoxide – Sigma-Aldrich, Catalog # D8418-500ML

Safety warnings

The tissue that is being used for preparation of PCLS is unfixed and therefore potentially infectious. Personal protective equipment (PPE) should be utilized to reduce the likelihood of exposure to any known or unknown pathogens within the sample. When cutting the PCLS, the vibratome blade is extremely sharp and should be handled with caution. Remove the blade holder when changing samples or replacing blades to reduce the risk of injury. DMSO, used in the freezing media, has the potential to increase the absorption of other chemicals into the body. Therefore, proper PPE should be used, particularly gloves and a lab coat. Change gloves immediately if contaminated. All institutional biosafety measures are followed in any manipulation of these human tissues.

Reagent Preparation

1 **Preparation of 2% (w/v) Agarose**
The amount of agarose prepared will depend on the size of the lung being inflated. Typical volumes are between 200 mL – 500 mL.
As an example, for a lung requiring 200 mL of 2% agarose, 4g of low gelling-point agarose is added to a clean glass beaker and then enough DMEM is added to bring the volume up to 200 mL. This solution can be heated in a microwave in pulses. Being careful not to boil out of the beaker, until the agarose has melted into the DMEM. Place the beaker in a clean heated (37°C) water bath to keep the agarose **ABOVE 37°C** and **BELOW 40°C** to prevent, respectively, premature gelling of the agarose and damage to the cells when instilled into the lung.

2 **Culture Media**
Prepare culture media by briefly thawing a 50 mL aliquot of FBS at 37°C. Filter this aliquot through a 0.22 µm vacuum filter into a new 500 mL bottle of DMEM. Finally, add 5 mL of the antibiotic/antimycotic reagent and mix the prepared media by closing the DMEM bottle and shaking. Store the culture media at 4°C for **no more than 1 month** from the date of preparation.

3 **Freezing Media**
Freezing media is made up of 10% (v/v) of FBS and DMSO. The FBS should be filtered **BEFORE** adding DMSO, as the DMSO can cause problems with filtering.

Inflation of the Lung

4 Dissect the lobe of interest (typically one of the upper lobes) away from the en bloc lung as needed and cannulate its main bronchus with a large endotracheal tube, or similar catheter, to fit the size of the main bronchus. Secure the tube in place with suture or zip-tie to prevent dislodgement and leakage. The tube should be inserted as deeply as possible, but do not bypass the first airway branchpoint to ensure uniform inflation of the lobe. For larger adult lobes, a dialysis clamp may be used to reduce the region of the lobe which will be agarose inflated.

5 Place the cannulated lobe into the warmed HBSS for 5 minutes to bring the lobe up to temperature prior to inflation to avoid the agarose gelling upon entry to the lung.

6 When 5 minutes have elapsed, keeping the lobe in the warm HBSS, fill your syringe with the prepared 2% agarose and connect this to the end of the endotracheal tube. Slowly apply pressure to begin inflation, refilling the syringe with warm agarose as needed. Being sure to keep 2% agarose solution at 37°C, while trying to move quickly and gently.

7 When the lobe (or lobe part) has become moderately firm by inflation, the cannulating tube is removed as the suture / zip-tie is tightened to secure the airway and prevent

leakage and deflation. If a clamp was used, leave this in place.

8 Place the lobe into ice-cold HBSS and keep any floating portions of the lobe moist with gauze for at least 40 minutes, until the lobe solidifies.

Blocking of the Lung

9 Remove the lobe from the ice-cold HBSS and slice the lobe according to already established protocol.

10 When selecting blocks for culture, make sure that the blocks are well inflated. Having minimal cartilaginous airways (unless requested). For simplified vibratome sectioning, select blocks containing one side of pleura.

11 Place the blocks into 50 mL conical tubes containing at least 10 mL of culture media.

12 Store overnight at 4°C until sectioning. This step has been shown to improve subsequent viability.

Sectioning of the Lung

13 Cool HBSS to 4°C by placing it in the refrigerator the night before or in the freezer for about 15 minutes while setting up the vibratome (avoid freezing the HBSS).

14 Set up the vibratome in a room with minimal interruptions. For safety it is suggested that anyone entering the room wears at minimum a lab coat, gloves, safety goggles and an N-95 mask due to the risk of aerosol generation from the sectioning.

15 Place ice in the lower chamber of the vibratome followed by the specimen well. Fill specimen well with HBSS.

16 Remove a block from their respective 50 mL conical tube and blot dry the pleura with a kimwipe (make sure the remainder of the block does not dry).

17 Add a drop of cyanoacrylate glue to the specimen holder and gently spread it around to cover at least the size of the block.

18 Place the block onto the specimen holder, pleura side down on the adhesive making sure it is firmly attached, and cover with a single piece of gauze for a few minutes until the glue has dried enough.

19 While you wait, place a blade into the blade holder (DO NOT PUT ON VIBRATOME YET).

20 Place the sample onto the vibratome such that the shorter edge is parallel with the direction of the blade.

21 Place the blade holder (with blade) onto the vibratome. IMPORTANT: Remove the blade mechanism before changing samples to reduce the possibility of injury.

22 With the blade above the sample, set your sectioning window. This is the region that the vibratome will use for sectioning and should consist of your entire block from end to end.

23 **Use the following when sectioning:**
i. Frequency - 100 Hz
ii. Amplitude - 1.2 mm
iii. Forward Speed - 0.3-0.12 mm/s
iv. Thickness - 500 micrometers

24 The first section will be a bit thicker than normal so your subsequent sections are even on both the top and bottom. **Discarded the first section.**

25 Subsequent sections, can be cut in half using replaceable blade scissors, if surface area is more than needed.

26 Use a new sterile cotton tipped applicator to transfer sections from specimen well into a 24 well plate filled with HBSS that is on ice.

Culturing PCLS

1d

27 After a plate is full, the sections will be placed into a fresh, prewarmed in a 37°C incubator, plate with 1 mL of culture media in each well. Incubate overnight. 1d

28 The next day, these sections can be frozen down by placing them in labeled cryovials with 1 mL freezing media per section. These vials will go into a -80°C freezer using a Mr. Frosty to control the rate of freezing.

29 After 24 hours in the -80°C freezer, these sections will be transferred to a liquid nitrogen freezer.

Assessment of Viability

30 The viability of these sections has been analyzed using a variety of techniques. For example, a WST-1 assay or Live/Dead fluorescence imaging.

Protocol references

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