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Human-Derived Precision-Cut Lung Slices (hPCLS): Agarose Filling, Coring and Slicing Protocol

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TriState SenNet

Cellular Senescence Net...

1 more workspace



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We use this protocol and it's working

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Abstract

Human Precision-Cut Lung Slices (hPCLS) are uniform tissue slices generated from human lungs. These slices contain all resident lung cells and their interactions, including alveolar macrophages and dendritic cells, present in the tissue at the time of slicing, representing the natural complexity of the lung's 3-dimensional architecture and organ function physiology.

hPCLS has become one of the most relevant *ex vivo* organotypic models for studying the pathophysiology of human lungs. Thanks to specialized tissue slicers, a single lung can provide hundreds of slices for both control and experimental conditions. Additionally, hPCLS can be kept in culture for up to 4 weeks, making possible to mimic the onset and progression of lung diseases.

hPCLS has proven valuable in multiple applications such as RNA and protein analysis, biochemistry, metabolism, toxicology, and chronic and acute lung diseases. They have also been used for highly specialized downstream applications such as transcriptomics, proteomics and high-resolution live microscopy.

The following protocol is a guide on how to generate PCLS from human lungs.

Image Attribution

CLAR (Center for Lung Aging and Regeneration) Logo designed by Nayra Cardenes, PhD.

Guidelines

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

Materials

 PR1MA™ Agarose, Low Melt Temperature **MIDSCI Catalog #KCA20070**

Amphotericin B Solution. 250 ug/ml, filtered. Millipore-Sigma, catalog #A2942

Penicillin-Streptomycin 100X solution. Millipore-Sigma, catalog #P00781-100ml

500 ml Pyrex round media storage bottles. Millipore-Sigma Cat# CLS1397500

Sterile physiological solution

Cyanoacrylate gel glue

Dow Corning high vacuum grease

Double Edge, breakable style, stainless steel, razor blades/ Ted Bella, Cat# 121-6

Equipment

Compressstome® VF 310-0Z Vibrating Microtome^{NAME}

NBT BRAND

HVD-751104 SKU

<https://nbtltd.com/> LINK



Protocol materials

 PR1MA™ Agarose, Low Melt Temperature **MIDSCI Catalog #KCA20070** Materials, Step 3

 DMEM/F-12, powder, HEPES **Thermo Fisher Scientific Catalog #12400024** Step 1

 Amphotericin B solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A2942** In 2 steps

 Penicillin-Streptomycin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P0781** In 2 steps

Safety warnings

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Before start

Set a water or bead bath at 42°C

DMEM/F12 Preparation for Agarose or Complete media

- 1 Due to the large amounts of media required, we recommend the use of reconstituted powder DMEM/F12.

 DMEM/F-12, powder, HEPES Thermo Fisher Scientific Catalog #12400024

- 1.1 Dissolve the content of one DMEM/F12 pouch in 700 ml of dd-water (the solution will be yellow).
- 1.2 Add 1.2 g of sodium bicarbonate. Stir and bubble with carbogen (a gas mixture of 30% CO₂ and 70% oxygen) until pH is about 6.8-7.0 (The solution will turn red at this point). Then, stop bubbling.
- 1.3 Adjust the pH with NaOH 1N to 7.2.

- 2 If inflating lungs continue to steps on preparing Agarose (LMPA). If treating or storing the PCLS, continue to next steps for complete medium (DMEM/F12).

Note

Do NOT add antibiotics, antimycotics or serum if preparing LMPA for lung inflation.

- 3 Weigh 15g of LMPA for a final concentration of 2.5% LMPA in 600 ml of DMEM/F12.

 PR1MA™ Agarose, Low Melt Temperature MIDSCI Catalog #KCA20070

STEP CASE

Low Melting Point Agarose (LMPA) 45 steps

Do NOT add antibiotics, antimycotics or serum if preparing LMPA for lung inflation.

- 4 Add the LMPA to 400 ml of DMEM/F12 media in a clean, sterile, wide-mouth bottle (we recommend the 500 ml Pyrex round media storage bottles), and mix. Let the agarose hydrate for about 5 minutes. A magnetic bar can be included in the bottle.

Equipment

Pyrex® round media storage bottles, wide-mouth

NAME

Millipore Sigma

BRAND

CLS1397500

SKU

[https://www.sigmaaldrich.com/US/en/search/cls1397500?
focus=products&page=1&perpage=30&sort=relevance&term=CLS1397500&type=product](https://www.sigmaaldrich.com/US/en/search/cls1397500?focus=products&page=1&perpage=30&sort=relevance&term=CLS1397500&type=product)

LINK

500 ml capacity

SPECIFICATIONS



- 5 Microwave the media/LMPA mix on high for about 30 seconds and then mix. Repeat the heating and mixing occasionally until the agarose starts to dissolve (this can be done with a magnetic stirrer). DO NOT BOIL. When the agarose is completely dissolved and the media is transparent, let cool to approximately 50-55°C.



Note

Do NOT add antibiotics, antimycotics or serum to the DMEM/F12 hot media until the agarose is completely dissolved and it has cooled down to close to 40-45°C.

- 6 Add 6.0 ml of FBS (final concentration 1%), 12 ml of Pen/Strep 10x (final concentration 2X), and 9 ml of Amphotericin B stock solution (final concentration 1.5X, optional). Mix well.

Amphotericin B solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A2942**

Penicillin-Streptomycin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P0781**

- 7 Complete the volume to 600 ml with DMEM/F12 media at room temperature. Keep stirring. Warm it up a little if necessary to keep the agarose from gelling.



- 8 Keep this solution at 42°C in a water or bead bath until use.

Lung Filling and Coring

9



Safety information

ALL STEPS MUST BE PERFORMED UNDER BSL2 (Biosafety Level 2) CONDITIONS.

It is recommended to take pictures of the lung tissue in different planes before agarose inflation for future records. Include a ruler for size reference.

- 10 If the lung has not been flushed or has too much blood or mucus in the airways, it will be necessary to flush it with saline and antibiotics before agarose inflation.
Fill a 60 ml syringe with sterile saline containing 2x Pen/Strep and 1.5x Amphotericin B (optional but recommended). Gently push the saline through airways and blood vessels to force blood and mucus out. Gently squish the saline out of the lung by compressing the tissue. Do not apply excessive force. Repeat the process until most of the blood and mucus have been cleared out. For the mid and lower left lobes, about 1 L of saline should suffice.
- 11 After flushing, rinse the tissue in a clean bucket twice with sterile saline and antibiotics.
- 12 Find the main bronchus and examine the opening to determine how many airways branch from it. This will guide the positioning of the cannula through the different airways to fill the lung.
- 13 Use hemostats to clamp shut all arteries, exposed blood vessels, and any areas where the pleura has been cut or compromised. Fill a 60ml syringe with warm agarose and attach it to the end of a cannula or flexible tubing, insert it into the main airway following its natural track being careful not to use excessive force or forcing the cannula in.
Using constant pressure and speed, inject the agarose solution into the lung.



Note

IMPORTANT: the media/LMPA mix should be kept at 42°C at all times to prevent agarose solidification, which could affect tissue inflation.

- 14 While injecting the agarose solution, check for leaks at the pleura or through blood vessels/airways. Use hemostats to clamp shut any leaks. If the agarose solution starts leaking out through the same airway it was injected, clamp it closed with a hemostat keeping the injecting cannula tightly fixed in place inside the airway.



- 15 Once one region of the lobe is full, relocate the inflating cannula to a different airway and continue filling with warm agarose until full inflation. Do not overfill.
Complete agarose filling will ensure structure preservation and will facilitate coring and slicing.
- 16 After filling the lung lobe, clamp shut all airways and blood vessels that may leak. Place the tissue into a clean, new zip-lock plastic bag. Close it. Transfer the bag to an ice bucket and ensure complete coverage with ice for 45-60 minutes for the agarose to be completely solidified.
Be careful not to allow ice or ice water to leak into the plastic bag.
- 17 After 45-60 minutes transfer tissue to a cutting board and remove all clamps.
- 18 Orient the tissue according to your experimental plan and slice the lobe into thick slices of about 2-2.5cm thick with a long trimming blade.
- 19 Take pictures of the lung slices for future records.
- 20 With a tissue coring tool, punch out cylindrical cores from the areas of interest.
- 21 Collect the cores in a container with DMEM/F12 supplemented with 1% Pen/Strep, 1X of Amphotericin B (optional) and 1% FBS. Keep at 4°C until slicing. (Check Step-case 2 for Complete medium recipe).

Note

It is important to note that cores must be processed within the next 72 hours.

- 22 From different areas of the remaining thick tissue slices, cut a few small pieces of about 2-3 mm³ and distribute them into a 6-well plate. Add fresh DMEM/F12 media with 1% strep, 1x Amphotericin, 1% FBS, and incubate overnight at 37 °C in a quarantine incubator.
- 23 Check for possible contamination the next day.
If contamination is present, discard all cores.

Core Slicing

24



Note

IMPORTANT: Slicing should be done under BSL2 guidelines.

Equipment

Compresstome® VF 310-0Z Vibrating Microtome^{NAME}

NBT BRAND

HVD-751104 SKU

<https://nbtltd.com/> LINK



Materials:

- Agarose 2.0% in DMEM/F12 with antibiotics and fungizone warmed up and kept at 40-42°C
- Double edge razor blades cut in half lengthwise and cleaned with ETOH to remove protective wax and oils.
- Cyanoacrylate Glue (we like Loctite, super glue, gel control).
- Bucket with ice
- DMEM/F12 with 1% penstrep, 2.5 ug/ml amphotericin (optional), and 1.0% FBS
- Fine painting brush (natural fibers, NOT SYNTHETIC)
- Two 100 mm Petri dishes
- Forceps
- Several 3 ml sterile plastic transfer pipettes
- Clean Kimwipes
- Silicon grease

- 25 From the Compresstome, take the white Teflon plunger out of the metal specimen tube and apply a light coat of silicone grease to the first half of it. Reinsert the piston into the metal tube. It should have a tight seal.
- 26 Grab one lung core and remove any liquid excess with a new, clean Kimwipe. Do not overdry the tissue.
- 27 Apply a light coat or a drop of the cyanoacrylate glue gel to the center top of the Teflon plunger keeping the metal specimen tube upright. Gently set the tissue core. Wait a few seconds until the glue is set.
- 28 Pull the piston down into the specimen tube until the whole core is inside it.



Specimen tubes containing lung cores.

- 29 With a plastic transfer pipette fill the specimen tube with enough warm agarose to cover up the tissue.



Specimen tubes containing lung cores and filled with agarose.

- 30 Make a big enough hole in the ice to put the specimen tube with the tissue in it to cool the agarose. Wait for the agarose to solidify.





Note

DO NOT COVER THE SPECIMEN TUBE.

Specimen tubes containing lung cores and agarose, on wet ice.

31



In the meantime, prepare the Compresstome: Put a light coat of silicone grease inside the specimen tube holder of the slicing chamber.

Greased tube specimen tube holder.

32 Attach one-half of the double-sided blade in the blade holder by applying a light coat of cyanoacrylate glue to the holder IF NECESSARY (newer Compresstome models have a magnetic blade holder). Center the blade and let the glue cure.

33

Once the agar in the canister is set and cool, take the specimen tube out of the ice, dry the metal surface, and apply a light coat of silicone grease around the center of the canister.



Specimen tube canister properly greased for posterior slicing.

34 Insert the specimen tube into the holder of the slicing chamber making sure not to push the agarose with the tissue out. It should have a tight, waterproof seal.

- 35 Check that the metal guide from the Compresstome control panel is completely retracted into the control panel, otherwise the sample holder piston won't fit in place.
- 36 Secure the slicing chamber with the safety screw.



Slicing chamber properly secured with the black safety screw at the bottom of the image.

- 37 Fill the chamber with DMEM/F12 up to cover the sample holder.
- 38 Press the “fast forward” button to move the metal guide forward until contacting the base of the Teflon plunger.
- 39 Attach the blade holder to the vibratory arm and secure it firmly with the hex screw.
- 40 Check the parameters for slicing PCLS: Speed 4.0, Oscillation 4.0, and slice thickness at 300 um.
- 41 Press the “fast forward” button again to push the agarose without tissue out of the sample holder. About 1 mm.
- 42 Press the “continuous” key down and press “start”.
- 43 The first slice should contain mostly agarose and if so, a little piece of tissue. This piece can be discarded.
- 44 Slicing should be continuous and shouldn't present any major problems.

Note

PCLS will slide into the buffer tray. They can be fished out with a brush. DO NOT USE FORCEPS.

- 45 Transfer the PCLS into a petri dish kept on ice containing DMEM/F12 with antibiotics and 1.0% FBS.
- 46 If some agarose remains attached to the PCLS, hold the PCLS down by the agarose using a pair of forceps and gently “pull away” the tissue from the agarose matrix with the brush.
- 47 Once slicing is done, transfer the PCLS to a 24-well plate. One PCLS per well, with complete DMEM/F12 (see Step-case 2 for recipe).

Protocol references

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