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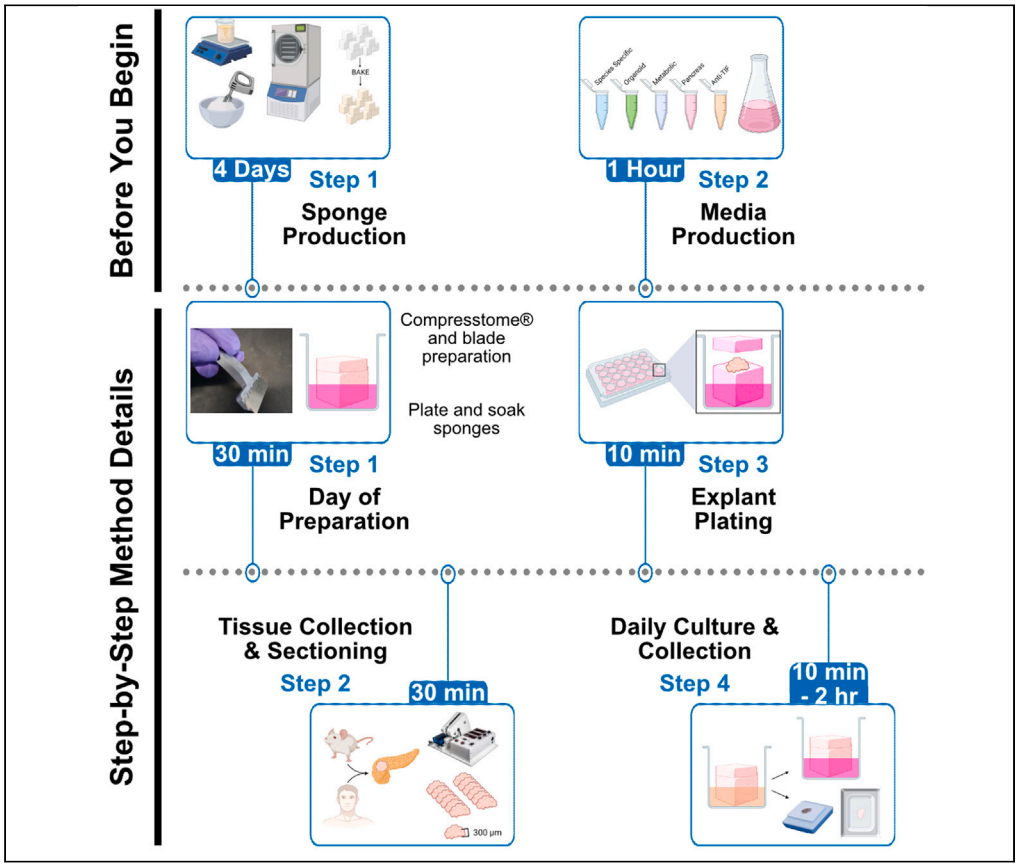
Generation and *ex vivo* culture of murine and human pancreatic ductal adenocarcinoma tissue slice explants



Traditional 2D/3D co-culture models typically do not reflect the cellular heterogeneity of pancreatic ductal adenocarcinoma (PDAC) tumors, while *in vivo* models can be slow and ill-suited to mechanistic investigations. Here, we present a protocol for culturing murine PDAC explants and a corresponding human PDAC model using tissue slice explants. We describe steps for sponge production, preparation of media and materials, tissue collection, and sectioning. We then detail procedures for explant plating, daily culture, and collection of samples.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Protocol for *ex vivo* explant generation and culture

Multi-day culture of human or murine-derived pancreatic tumor

A single tumor sample can provide, on average, 20 separate explants

Explants allow for study of complex phenomena, such as signaling cascades

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Generation and *ex vivo* culture of murine and human pancreatic ductal adenocarcinoma tissue slice explants

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SUMMARY

Traditional 2D/3D co-culture models typically do not reflect the cellular heterogeneity of pancreatic ductal adenocarcinoma (PDAC) tumors, while *in vivo* models can be slow and ill-suited to mechanistic investigations. Here, we present a protocol for culturing murine PDAC explants and a corresponding human PDAC model using tissue slice explants. We describe steps for sponge production, preparation of media and materials, tissue collection, and sectioning. We then detail procedures for explant plating, daily culture, and collection of samples.

BEFORE YOU BEGIN

Work using animal tissues or human patient samples will require appropriate regulatory approval. All animal research experiments were approved by the Columbia University Irving Medical Center (CUIMC) Institutional Animal Care and Use Committee. KPC (LSL-KrasG12D/+;LSL-Trp53R172H/+;Pdx-1-Cre) mouse colonies were bred and maintained with standard mouse chow and water, *ad libitum*, under a standard 12 h light/12 h dark cycle. All work using patient samples were performed with approval from the CUIMC Institutional Review Board (IRB).

Sponge production

Timing: 4 days

In this protocol, explants are cultured on gelatin sponges to facilitate diffusion gradients of nutrients and waste products. Best practice is to prepare large batches of sponges well in advance of any anticipated experiments. Sponges will keep for at least a month when stored in airtight containers at room temperature (20°C–22°C).

1. Combine 30.0 *g* of porcine gelatin and 500 mL deionized water (diH_2O) (6% w/v) in a 1 L glass beaker. Cover and place on a hot stir plate.
2. Heat at 60°C and mix with a sterile stir bar until gelatin is fully dissolved (approximately 45–60 min).

Note: The solution should become clear with a slight yellow hue.

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Table 1. Freeze dryer program for gelatin sponge preparation

Step	Temperature (°C)	Pressure (mbar)	Time (h)
1	4	Ambient	2
2	−20	Ambient	8
3	−15	0.14	24
4	25	0.02	24

The temperature, pressure, and incubation settings for lyophilizing gelatin sponges in a commercial freeze dryer, as previously reported.

3. Transfer approximately half of the solution into a clean, 500 mL plastic beaker. Keep the remaining solution covered and mixing on the hot plate.
4. Mix using a hand mixer with one wire-whisk at room temperature (22°C) as demonstrated in Method video S1.

Note: For an initial volume of 250 mL, this process should take approximately 20 minutes. The mixture will solidify at different rates depending on ambient temperature and humidity and should be watched carefully.

5. The correct consistency is liquid enough to smoothly mix but runny enough to drip off of the whisk or spatula as demonstrated in Method video S1.
6. Layer silicone mats on a clean freeze dryer tray. Pour the mixed gelatin onto one half of the mat.

Note: The whipped gelatin will form a broad mound that will spread only minimally across the tray and will "jiggle" rather than spread when manipulated with a spatula.

7. Press an inverted 1 × 1 × 1 cm silicone mold into the whipped gelatin firmly until the mold is flush with the silicone mat as demonstrated in Methods video S1.

⚠ CRITICAL: To ensure that the wells are completely filled, cut a small hole in the base of each well and press the mold until gelatin emerges from the holes.

8. Repeat steps 4–5 for the remaining gelatin solution.
9. Transfer tray to freeze-dryer and run Freeze Dryer Program as previously reported[1] described in Table 1.
10. Remove tray from freeze-dryer and transfer silicone mat and mold directly to convection oven tray. Bake batches of sponges in a convection oven to cross-link polymers.
    a. Baking Settings – 3 h at 300°F (~150°C), bottom burner only, set to lightest "toast" setting possible).

⚠ CRITICAL: This step is crucial. Without cross-linking, sponges will melt when soaked in liquid at 37°C.

11. Once baked, carefully remove the sponges from mold and silicone mat. If still connected, use sterile forceps and scalpel to trim into 1 cm cubes.
12. Transfer cut and baked sponges back into glass jars with a desiccant packet and seal tightly. Store at room temperature (22°C) until use.

Media preparation

Timing: 1 h

While human PDAC explant cultures have been previously described,[2–4] here we present murine-derived PDAC explants and a corresponding protocol for human PDAC explants. The final media

composition takes into account pancreas specific,[3] select organoid,[5,6] cell culture metabolites, and additives related to tumor interstitial fluid components.[7]

Note: Media preparation should be done in a sterile environment, either in a tissue culture hood or on benchtop with a Bunsen burner flame. Media can be made fresh for each experiment but can also be made in larger batches in advance. Prepared media can be kept for up to 1 month at 4°C.

Note: All stocks should be prepared, aliquoted, and stored according to manufacturer's instructions. For best results, aliquot reagents into smaller volumes based on anticipated usage.

Note: RSPO-1 conditioned media should be prepared ahead of time from a validated cell line and according to the manufacturer's protocol (Trevigen, Cultrex Rspo1 cells[5]).

13. Combine all media components as described in Table 2.
14. Filter media into 50 mL aliquots with Steriflip Vacuum-driven Filtration System (0.22 μm).
15. Store at 4°C for up to 1 month.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Patient tumor samples. Pancreatic cancer: adults (>18) from both sexes	NewYork-Presbyterian/Columbia University Irving Medical Center	N/A
Chemicals, peptides, and recombinant proteins		
4% Paraformaldehyde	Santa Cruz Biotechnology	sc-281692
2-Oxoglutarate (α-ketoglutarate) sodium salt	Sigma-Aldrich	K1875
3-Hydroxyisobutyric acid	Sigma-Aldrich	164976
3-Methyl 2-oxopentanoic acid sodium salt	Sigma-Aldrich	198978
70% Ethanol	Fisher Scientific	BP82031
Advanced DMEM/F12 with glutamine	Thermo Fisher Scientific	12634010
Allantoin	Sigma-Aldrich	#05670
Cis-aconitate	Sigma-Aldrich	A3412
Cyanoacrylate (super glue)	Bazic Products	2007
D-citrulline	Sigma-Aldrich	27505
Dexamethasone	Selleck Chemicals	S1322
DL-indole-3-lactic acid	Sigma-Aldrich	I5508
DMEM	Life Technologies	12430062
D-tryptophan	Sigma-Aldrich	T9753
Fetal bovine serum, heat inactivated	Thermo Fisher Scientific	10-438-026
Gastrin I (human)	Sigma-Aldrich	G9020
Gastrin I (mouse)	Phoenix Pharmaceuticals	#027-05
D-(+)-glucose	Sigma-Aldrich	G6152
Hank's balanced salt solution (HBSS)	Thermo Fisher Scientific	14-025-092
HEPES	Thermo Fisher Scientific	15-630-080
Hydrocortisone	Sigma-Aldrich	H0888
Insulin from bovine pancreas	Sigma-Aldrich	I0516
Isoflurane	Henry Schein	29405
L-arginine	Sigma-Aldrich	A5006
L-cystine	Caisson Labs	C018
L-glutamine	Thermo Fisher Scientific	25030164
L-homocitrulline	Sigma-Aldrich	SML2645

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Low-melting a	Sigma-Aldrich	A9414
MEM non-essential amino acids solution	Fisher Scientific	11-140-050
N-acetyl-L-cysteine	Sigma-Aldrich	A9165
N-acetylglycine	Sigma-Aldrich	A16300
Nicotinamide	Sigma-Aldrich	N3376
Orotic acid, anhydrous	Sigma-Aldrich	O2750
Penicillin-Streptomycin	Caisson Labs	PSL01
Porcine gelatin	Sigma-Aldrich	G2500
Recombinant human EGF	PeproTech	AF-100-15
Recombinant human FGF-10	PeproTech	100-26
Recombinant mouse EGF	Thermo Fisher Scientific	PMG8041
Recombinant murine FGF-10	PeproTech	450-61
RPMI 1640	Gibco Life Technologies	21870-076
Sarcosine	Sigma-Aldrich	131776
Sodium bicarbonate	Thermo Fisher Scientific	BP328
Sodium pyruvate	Thermo Fisher Scientific	11360070
Soybean trypsin inhibitor	Thermo Fisher Scientific	17075029
Tissue-Tek OCT compound	Sakura	4583
γ-L-glutamyl-L-alanine	Sigma-Aldrich	483834
Zeocin	Thermo Fisher Scientific	R25001
Experimental models: Organisms/strains		
Mouse: KPC (LSL-KrasG12D/+;LSL-Trp53R172H/+;Pdx-1-Cre), male and female mice, 8–10 months	Bred in-house	N/A
Other		
20 mm specimen tubes	Precisionary Instruments, LLC	VF-SPS-VM-20-BOS
2 mL Eppendorf tubes	Fisher Scientific	14-666-313
50 mL Steriflip-GP filters, 0.22 µm	EMD Millipore	SCGP00525
60 mm plates	Thermo Fisher Scientific	FB0875713A
Chilling block	Precisionary Instruments, LLC	VF-VM-CB-20-BOS
Compresstome vibrating microtome	Precisionary Instruments, LLC	VF-310-0Z
Cultrex R-Spondin1 (Rspo1) cells	Trevigen	3710-001
Disposable scalpel	Exel International	29550
Dissection scissors	Roboz Surgical Instruments	RS-5882
Double-edge prep blades	AccuTec Blades, Inc.	74-002
Falcon 24-well tissue plates	Corning	353047
Forceps	Roboz Surgical Instruments	RS-8254
Freeze dryer bulk tray	Labconco	7756200
FreeZone 2.5 L benchtop freeze dryer	Labconco	700201000
FreeZone tray dryer	Labconco	780701000
Hand mixer and whisk	Hamilton Beach	62636
Isotemp ceramic combination stirring hotplate	Fisher Scientific	11-100-100SH
Magnetic stir bar	Fisher Scientific	16-800-512
Plastic beaker	Corning	1003P-500
Pyrex glass beaker	Corning	1000-1L
Removable blade holder	Precisionary Instruments, LLC	VF-BH-VM-310-0Z-BOS
Rotary vane vacuum pump	Labconco	1472100
Silica gel desiccant packet	Dry & Dry	X001C8I6DD
Silicone baking mold	Art & Beauty (Amazon)	B07PWPCD34
Silicone baking quarter sheet mat	Last Confection	KITCH-MAT-LC21_02
Spatula	Fisher Scientific	21-401-5
Toaster oven	Cuisinart	TOB-40N

Table 2. Explant media components

Reagent	Final concentration	Amount	Murine media	Human media
Species specific (murine,[4] human[2])				
DMEM (base media)	N/A	100 mL	✓	
Fetal Bovine Serum	1% v/v	1.1 mL	✓	
Dexamethasone	1 μg/mL	22 μL	✓	
Glucose	11 mM	484 μL	✓	
Soybean Trypsin Inhibitor	0.1 mg/mL	1.1 mL	✓	
RPMI 1640 (base media)	N/A	100 mL		✓
Fetal Bovine Serum	10% v/v	11 mL		✓
Glutamine	1% v/v	1.1 mL		✓
HEPES Buffer	14.5 mM	1.76 mL		✓
L-Cystine	20 mg/L	30 μL		✓
Sodium Bicarbonate	1.134 g/L	3.267 mL		✓
Penicillin-Streptomycin	1% v/v	1.1 mL	✓	✓
Select organoid[6]				
Murine Epidermal Growth Factor (EGF)	50 ng/mL	55 μL	✓	
Murine Fibroblast Growth Factor (FGF)	100 ng/mL	110 μL	✓	
Murine Gastrin	10 nM	110 μL	✓	
Human Epidermal Growth Factor (EGF)	50 ng/mL	55 μL		✓
Human Fibroblast Growth Factor (FGF)	100 ng/mL	110 μL		✓
Human Gastrin	10 nM	110 μL		✓
Nicotinamide	10 mM	1.1 mL	✓	✓
RSPO-1 Conditioned Media	10% v/v	10 mL	✓	✓
Metabolic supplements				
N-acetyl L-cysteine	1 mM	275 μL	✓	✓
Non-Essential Amino Acids	1% v/v	1.1 mL	✓	✓
Sodium Pyruvate	1 mM	1.1 mL	✓	✓
Pancreas supplements[3]				
Hydrocortisone	10 μg/mL	55 μL	✓	✓
Bovine Insulin	10 μg/mL	110 μL	✓	✓
Anti-TIF supplements[7]				
2-oxoglutarate (α-keto-glutarate)	15 μM	110 μL	✓	✓
3-methyl 2-oxopentanoic acid	15.5 μM	110 μL	✓	✓
3-hydroxyisobutyric-acid	30 μM	110 μL	✓	✓
Acetylglycine	2.5 μM	110 μL	✓	✓
Allantoin	253 μM	845 μL	✓	✓
Arginine	97 μM	110 μL	✓	✓
Cis-aconitate	6.5 μM	110 μL	✓	✓
Citrulline	103 μM	110 μL	✓	✓
Cystine	111 μM	110 μL	✓	✓
γ-glutamyl-alanine	5 μM	110 μL	✓	✓
Homocitrulline	2.2 μM	110 μL	✓	✓
Indole-lactic acid	1.5 μM	110 μL	✓	✓
Orotic Acid	1.5 μM	110 μL	✓	✓
Sarcosine	15 μM	110 μL	✓	✓
Tryptophan	60 μM	110 μL	✓	✓
Total Volume	N/A	**Murine** – 120 mL **Human** – 135 mL	N/A	N/A

A comprehensive list of media additives to create both murine and human PDAC explant media as described.

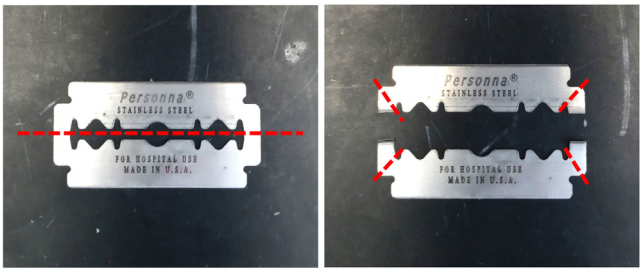

Figure 1. Sectioning blade preparation – Visual representation of step 3

MATERIALS AND EQUIPMENT

Compresstome setup

Refer the Compresstome user manual[8] for more detailed setup and operating instructions. For PDAC, section tissue using the following Compresstome dial settings: Continuous, speed – 8.4, oscillation – 4.6, slice thickness – 300 μm.

STEP-BY-STEP METHOD DETAILS

Compresstome preparation

Timing: 30 min

To ensure consistency, explant slices are cut using a Precisionary Compresstome, which facilitates even, smooth sectioning of the tissue. The steps described below prepare the instrument for use.

Note: Leave the sharp blade edge wrapped in wax-paper wrapper and use forceps while preparing the sectioning plate to prevent injury.

Note: To maintain sterility throughout the protocol, all tools should be sterilized via autoclaving and 70% ethanol spray and users should wear all relevant sterile PPE (sterile gown or lab coat, mask, and surgical gloves).

1. At least 30 min prior to tissue collection, chill a bottle of Hank's Balanced Salt Solution and the Compresstome chilling block in a −20°C freezer.
2. Weigh out 0.25 *g* low-melting agarose into a 50 mL flask.
3. Prepare the sectioning blade as following:
   a. If necessary, remove any residual glue residue from the Compresstome blade holder using acetone.
   b. Cut a standard razor blade along the dotted lines marked in Figure 1, first horizontally to create a single-sided blade, then remove the top corners.
   c. Adhere the prepared blade to the flat side of the Compresstome blade holder, flush against the lip of the blade holder, using a small amount of super glue.
   d. Allow at least 20 min to dry and set.

Sponge preparation

Timing: 30 min

The culture platform for this protocol is stored long-term as dried, cross-linked sponges. Prior to sectioning and plating the explants, these sponges should be soaked in the species specific media to soften and take up the media components.

Note: This step should be done in a sterile environment, either in a tissue culture hood or on benchtop with a Bunsen burner flame. Likewise, all instruments (forceps and scalpels) should be sterile, either autoclaved or washed with 70% Ethanol and sterilized with flame.

4. Using forceps and a scalpel, trim sponges to cubes approximately 1 cm thick (base sponge). Cut thin sponges 2–3 mm thick (top sponge).
5. Transfer base sponges into 24 well plate(s) and group top sponges by treatment into 60 mm plates.
6. Soak each base sponge with 750 μL of the species specific explant media per well and soak each set of top sponges in approximately 2 mL of the species specific explant media.

Note: Any drugs or relevant treatment additives should be added to the species specific explant media prior to addition to the sponges.

⚠ CRITICAL: Pipet the species specific explant media on top of sponge, rather than directly into well to facilitate sponge soaking from both the top and bottom.

7. Place plates in 37°C incubator to soak until explant are ready to plate.

Note: Sponges should be fully soaked within 30 minutes. If sponges are cut larger, more time and/or media might be necessary.

Tissue collection and preparation

Timing: 10 min

This protocol accommodates culturing of both human and murine derived tissue. Steps 8–12 are specific for collecting tissue samples from mice. If working with human patient samples, please proceed directly to Step 13. If possible, resected patient tissue should be in the shape of a cylinder or wedge, with 2–3 mm width and a longer axial dimension (> 1 cm if possible) in order to facilitate stable embedding into the sample holder and sufficient cross-sectional area for analysis.

⚠ CRITICAL: Speed is of the essence to maximize tissue viability. The entire process from collection to plating should take less than an hour.

Note: Although it may not be possible to collect the tumor tissue under completely sterile conditions, all instruments (forceps and scalpels) should be sterile, either autoclaved or washed with 70% Ethanol and sterilized with flame.

Note: Just prior to tissue collection, fill wells of a 6-well plate halfway with ice cold Hank's Balanced Salt Solution. Keep plate on ice.

8. Humanely euthanize the animal as per your approved IACUC protocol.
9. Place the mouse on a dissection stage on its back and spray the torso with 70% ethanol to sterilize the skin and fur.
10. Cut through the skin and expose the abdominal wall (peritoneum) through blunt dissection with scissors, then cut the peritoneum to open the abdominal cavity and locate the tumor.
11. Use sterile dissection scissors and/or scalpel to remove the tumor from the surrounding tissue. Place in a sterile petri dish.
12. If necessary, remove any healthy pancreas tissue from the tumor, then transfer to the 6-well plate with ice-cold Hank's Balanced Salt Solution and store on ice.
13. Embed tissue in specimen tube (Figure 2).

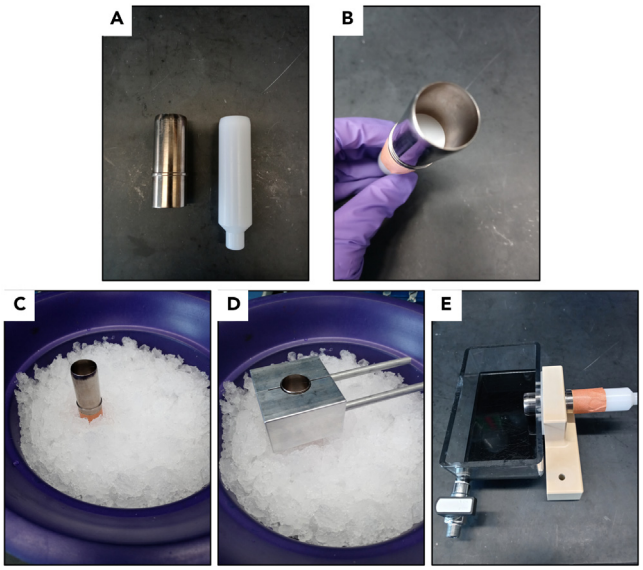

Figure 2. Specimen tube preparation and use – Visual representation of steps 13-14

(A) The two components of the specimen tube, including the plunger (white) and the casing (grey metal).

(B) The plunger is inserted partially into the casing, leaving a 2–3 cm well. Plunger and casing are then taped together (here with orange tape) to secure.

(C) The completed specimen tube should be inserted vertically into ice for tumor embedding into agarose.

(D) The agarose and tumor tissue are rapidly cooled by placing the cooling block over the specimen holder.

(E) Once the agarose is fully solidified, the specimen holder is inserted into the collection tray, then the tape can be removed (not shown).

a. Create a 2.5% w/v solution of sterile distilled water and low-melting agarose (10 mL H_2O and 0.25 *g* agarose) and microwave until the agarose is fully dissolved (approximately 30 s).

⚠ CRITICAL: Let cool approximately 1 minute until the flask is comfortable to touch (around 50°C/120°F).

b. Insert Compresstome white plastic plunger into metal casing, leaving an approximately 2–3 cm chamber.
c. Tape casing to plunger with lab tape and place the completed construct vertically in ice, as illustrated in Figures 2A and 2B.
d. Use a transfer pipet to fill the specimen tube with agarose solution.
e. Using forceps, fully submerge the tumor tissue into the liquid agarose solution.
f. Insert the cold chilling block over the specimen tube and allow to incubate on ice for several minutes until the agarose solidifies.

Note: The agarose will solidify and turn slightly opaque. Remove the sample from the cooling block before the agarose freezes, as indicated by crystals forming on the edges of the agarose.

Tissue sectioning

Timing: 20 min

Bulk tissue is first embedded in an agarose matrix, then sectioned using the Compresstome into explant slices.

14. Remove the specimen tube from chilling block and ice, then insert tube into Compresstome tray through the hole in the side wall until flush with raised rim on tube. Then remove tape to release the plunger.
15. Connect the Compresstome tray to the base with the provided screw.
16. Add ice cold Hank's balanced salt solution (HBSS) to tray to fully submerge the specimen tube.
17. Attach the blade holder to the vibration head with blade-side facing the control box and secure using the provided Allen wrench.
18. Press "Start" to begin sectioning.

Note: Speed and oscillation settings will depend on the characteristics of the bulk tissue. The settings listed above are appropriate for autochthonous pancreatic ductal adenocarcinoma (PDAC) but may need to be adjusted slightly for other tissue types, such as orthotopic or subcutaneous.

19. Collect slices into cold HBSS in a 6-well plate on ice.
20. Once the necessary number of explant slices have been obtained for the experiment, remove the remaining bulk tissue from the specimen holder with forceps and preserve as desired as the "day 0" sample.
    a. **Formalin Fixed** – transfer into a 2 mL microcentrifuge tube with 1 mL 4% paraformaldehyde (PFA) and incubate at 4°C for 2 h, then transfer to 70% ethanol at room temperature (20°C–22°C) until paraffin embedding.
    b. **Frozen** – fill sample mold halfway with Optimal Cutting Temperature Compound (OCT), transfer explant tissue and flatten as much as possible, fully cover with additional OCT, store at −80°C.

Tissue transfer to sponge plate

Timing: 10 min/plate

The following steps describe an efficient method of transferring thin, malleable tissue slices from buffer to the culture sponge platform.

Note: This step should be done in a sterile environment, either in a tissue culture hood or on benchtop with a Bunsen burner flame. Likewise, all instruments (forceps and spatulas) should be sterile, either autoclaved or washed with 70% ethanol and sterilized with flame.

21. Remove sponge plate from incubator to a sterile environment.
22. Use forceps to slide explant flat onto a spatula.
23. Transfer the explant slice to the sponge by touching the edge of the spatula to the sponge and using the forceps to slide the tissue onto the sponge, as demonstrated in Methods video S2.

Note: If necessary, use the forceps to flatten the tissue onto the sponge top surface as much as possible.

24. Use forceps to place the thin top sponge over the tissue.
25. Place plate into 37°C, 5% CO_2 incubator.

Daily culture and final samples collection

Timing: 10 min/plate for daily media changes, or 2 h to formalin fix samples

Explant media should be replaced daily, until the last time point and final collection.

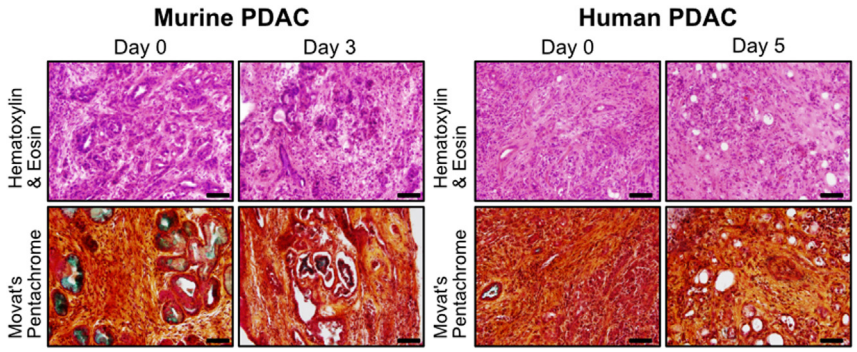

Figure 3. Histological staining of cultured explants

Representative images of hematoxylin & eosin and Movat's pentachrome staining demonstrate maintenance of key histological structures of PDAC, including ductal structures and stromal components.
Scale bar 50 μm.

Note: This step should be done in a sterile environment, either in a tissue culture hood or on benchtop with a Bunsen burner flame. Likewise, all instruments (forceps) should be sterile, either autoclaved or washed with 70% Ethanol and sterilized with flame.

26. Change media daily by aspirating spent media and replacing with 500 μL of fresh media directly into the well.
27. As with the initial media prep, any drugs or treatment additives should be mixed in with the media prior to adding to the well.
28. Samples can be collected at the end of the experiment and processed as normal tissue.
    a. **Formalin Fixed** – transfer explant into 2 mL microcentrifuge tube with 500 μL 4% paraformaldehyde (PFA) and incubate at 4°C for 2 h, then transfer to 70% ethanol at room temperature (20°C–22°C) until paraffin embedding.
    b. **Frozen** – fill sample mold halfway with Optimal Cutting Temperature Compound (OCT), transfer explant tissue and flatten as much as possible, fully cover with additional OCT, store at −80°C.

EXPECTED OUTCOMES

In adapting human-derived PDAC explant protocols for murine tissue, we found that KPC-derived explants required additional media supplements to improve viability, including compounds associated with normal functions of PDAC, such as insulin,[3] and select supplements known to support PDAC organoid growth and development.[6] Most strikingly, we saw the largest improvement in explant viability with the addition of what we've named 'anti-TIF' supplements. Analysis of Tumor Interstitial Fluid (TIF) from KPC PDAC tumors[7] revealed a distinct signature of both metabolites with both higher and lower concentrations in KPC tumors compared to plasma, thus providing a comprehensive recipe for recapitulating the metabolic environment present in murine PDAC. Rather than supplementing the explant media with the metabolites enriched in KPC TIF (TIF), we posited that the depleted metabolites (anti-TIF) are likely highly utilized compounds, and thus would support explant survival. Not only did the addition of anti-TIF supplements greatly improve murine PDAC explant survival, it also supported our position that these PDAC explants were responding to the extracellular environment similarly to *in vivo* tissue.

As recently characterized,[9] explants produced using this protocol maintain stable tissue architecture, confirmed by histological staining demonstrating maintenance of ductal, stromal, and ECM structures over time. Explants cultured using this protocol have also been shown to respond comparably to *in vivo* drug treated tissue, both in terms of cellular viability, cellular populations, and behavior[9] (Figure 3). Therefore, explants cultured using this protocol are expected to be a reasonable complement to *in vivo* mechanistic studies.

One major advantage to utilizing murine explants is the ability to correlate *in vivo* and *ex vivo* experimental results, an opportunity not readily possible with human samples. In this way, *ex vivo* murine explants can provide the opportunity for both pre-clinical therapeutic screening prior to moving into *in vivo* studies and deeper interrogation of *in vivo* findings. Additionally, this "medium-throughput" method rapidly decreases the amount of animals and time necessary to generate an experimental cohort. A single KPC tumor can yield upwards of 20 explants and allow for testing of many variables (drugs, concentrations, time points, etc.) simultaneously.

Explants are an advantageous model system for at least two types of experiments. First, as upwards of twenty explants can be obtained from a single parental tumor, this system is well-suited for screening multiple drugs, concentrations, and time points simultaneously, while minimizing the use of animal models. Second, as explants maintain the complex tissue architecture and cellular connections of the parental tumor, this system is useful for interrogating paracrine signaling pathways across multiple cell types, something not achievable with monolayer or many co-culture platforms.

LIMITATIONS

The most significant limitation of this protocol is stromal drop-out over time, particularly for murine explants. Based on our analysis of cellular changes over time,[9] murine PDAC explants can best be utilized up to five days and human explants up to seven. Therefore, this model is recommended for use for short-term cellular response treatments, rather than bulk tissue response (i.e., protein expression rather than development of resistance mechanisms).

Similarly, this model, is not capable of supporting and replenishing peripheral cell types, such as immune cells, long term (>3 days). Preliminary studies not described here, have suggested that immune cells isolated from matched peripheral tissues such as the spleen can be co-cultured with activating supplements such as PMA/ionomycin, whereupon these immune cells infiltrate into the explant tissue.

Additionally, as this protocol relies on the use of live tissue, efforts should be made to minimize the time between collection of the bulk tissue and final plating. Preferably, the entire process (bulk tumor collection to plating) should be completed in less than 1 h.

Finally, while this protocol utilizes the Compresstome instrument, other vibratome models are likely suitable. Similarly, gelatin sponges are commercially available through Patterson Veterinary (Vetspon Absorbable Hemostatic Gelatin Sponge, #07-849-4032) if access to a freeze-drying system is not available.

TROUBLESHOOTING

Problem 1

In-house produced sponge consistency is heterogeneous, related to before you begin steps 1–12.

Potential solution 1

The most common issue with sponge consistency is a base that is very dense with a flaky, insubstantial upper layer. This can be due to insufficient air incorporation during gelatin solution mixing (step 4). This can be improved by mixing the gelatin longer. Ideally, the mixture should not spread or sink when poured onto the freeze-dryer tray.

Problem 2

The bulk tumor tissue dislodges from the agarose and falls out of the specimen holder during sectioning, related to steps 14–20.

Potential solution 2

The tissue can be re-embedded using fresh melted agarose (return to step 8), with careful attention to the orientation of the tissue when embedded, which will help to anchor the tissue. The sample

should be oriented to produce axial sections when sliced (i.e., cutting along the smallest plane). If this is a recurring issue, it may be helpful to increase the percentage of agarose slightly to 3%. It should be noted though, that in order to achieve a consistent and smooth cut, the agarose density should not be too different from the tissue density. Alternatively, damaged razor blades may catch and pull the tissue out, so replacing the blade might also help.

Problem 3

Sponges disintegrate or "melt" during incubation at 37°C, related to steps 26–28.

Potential solution 3

Occasionally, sponges can breakdown during incubation, resulting in partially or fully disintegrated base sponges. Sponge structural stability is largely impacted by the extent of polymer cross-linking. If sponges are regularly melting at 37°C, increase the length of time that the sponges are baked. Do not increase the temperature, as this will only burn the sponges. If you wish to continue the experiment, soak newly baked sponges in fresh media in a new plate and transfer the explants to the new sponges.

Alternatively, bacterial contamination can sometimes lead to digestion of the sponges. If the media in the well is excessively yellow in color and cloudy, bacterial contamination might be the causative factor. Continuation of the experiment with those conditions is not recommended.

Problem 4

Wells appear heterogeneous with respect to the color and volume of media remaining in the well after overnight culture, related to Steps 26–28.

Potential solution 4

Sponges significantly larger than the recommended 1 × 1 × 1 cm are capable of absorbing all of the initial 750 μL overnight, leaving no media remaining in the well. As long as both sponges (base and top) are thoroughly wet, this will not have an impact on the explant viability. After changing the media as described (adding 500 μL fresh media), the issue will be resolved.

Explants of significantly different sizes will exhaust the supplied media at different rates, such that larger explants will result in a more depleted media (yellow) compared to smaller explants (pink). Such differences are expected. To prevent discrepancies, the explant slices can be trimmed to similar sizes prior to plating. Alternatively, if certain samples are depleting media faster than others, the media for those wells could be changed more frequently, if desired.

Additionally, despite a broad spectrum antibiotic in the media, individual wells can become contaminated through non-sterile practices or contamination from the explant tissue itself. Contaminated media usually appears bright yellow and opaque, but we have observed a dramatic color change is not always present with contamination. If a single well is contaminated, the sample should be removed from the experiment and the well bleached to prevent additional contamination in the plate. If the entire plate appears to be contaminated, that is an indication that the media stock is contaminated and a fresh batch should be made.

Problem 5

Explant tissue is of poor quality, resulting in low viability of vehicle tissue even at early time points, related to steps 26–28.

Potential solution 5

The success of explant culturing depends on the viability and health of the initial tissue. Samples with reduced starting viability (for example, human samples exposed to neoadjuvant therapy) will yield non-viable explants. Samples, especially if obtained from patients or animals that have previously undergone *in vivo* treatment, can be screened with a "touch prep" H&E stain, where the sample

tissue is pressed against a standard glass slide to leave a residue, followed by a standard H&E stain.[10] This process should give a reasonable indication as to the initial health of the sample tissue, prior to following through with the full experiment.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the lead contact, Dr. Kenneth P. Olive (kenolive@columbia.edu).

Materials availability

This study did not generate new unique agents.

Data and code availability

This study did not generate any dataset nor analyze code.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2023.102711.

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AUTHOR CONTRIBUTIONS

A.R.D.-F. developed and optimized media composition and the sponge platform, with assistance from A.M. and F.L. The Muir group provided KPC mouse tumor interstitial fluid media[7] for testing during the protocol development process, which influenced the development of the murine media formulation. A.R.D.-F. prepared the manuscript. K.P.O. provided resources and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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