

Isolation, Culture and Differentiation of Primary Acinar Epithelial Explants from Adult Murine Pancreas

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[Abstract] The adult pancreas possesses an intrinsic developmental plasticity whereby acinar cells can convert into ductal structures under some pathological conditions. Acinar tissue can be isolated from murine pancreas and kept in three-dimensional collagen culture. Acinar to ductal metaplasia can be induced in primary acinar epithelial explants by treatment with growth factors. This method can be utilized in *ex vivo* studies involving pancreatic epithelial differentiation.

Materials and Reagents

1. Mice (4-7 weeks old). As a positive control you can always use wild type mice and induce differentiation of acinar explants by treatment with EGF as described. Depending on your application you may use transgenic mice.
2. McCoy's 5A medium (Sigma-Aldrich, catalog number: M8403)
3. Waymouth medium MB 752/1 medium (Genaxxon, catalog number: C4119)
4. BSA (Sigma-Aldrich, catalog number: A4503)
5. Soybean trypsin inhibitor (SBTI) (Sigma-Aldrich, catalog number: T6522)
6. Collagenase Type VIII (Sigma-Aldrich, catalog number: C2139)
7. 80% EtOH
8. Penicillin-streptomycin (P/S) (Life Technologies, Gibco®, catalog number: 5140-122)
9. Amphotericin B (not critical, for example from Biochrom, catalog number: A2610)
10. FCS (Life Technologies, Gibco®, catalog number: 10270)
11. Bovine pituitary extract (BPE) (Life Technologies, Gibco®, catalog number: 13028-014)
12. Insulin, Transferrin, Selenium, Ethanolamine solution (ITS-X) (Life Technologies, Gibco®, catalog number: 51500)
13. Rat Tail Collagen Type I (BD Biosciences, catalog number: 354236)
14. Sterile 10x PBS (BIOCHROM, catalog number: 182-50)
15. Sterile dH₂O
16. Sterile 1 N NaOH
17. rhTGF α (R&D systems, catalog number: 239-A)
18. mEGF (BD Biosciences, catalog number: 354010)

19. SBTI stock solution (see Recipes)
20. Collagenase VIII stock solution (see Recipes)
21. Wash solution (see Recipes)
22. 0.1% Culture medium (Waymouth's medium) (Genaxxon, catalog number: C4119) (see Recipes)
23. 30% Culture medium (Waymouth's medium) (Genaxxon, catalog number: C4119) (see Recipes)

Equipment

1. Stericups filter units (EMD Millipore)
2. 50 ml Falcon tubes
3. Tissue culture dishes (10 mm)
4. 6-well plates
5. 24- or 48-well plates
6. Dissecting forceps and scissors
7. Disposable scalpels
8. 100 μ M cell strainer (BD Biosciences, Falcon®, catalog number: 352360)
9. Syringe plungers

Procedure

A. On the bench:

Mice should be between 4 and 7 weeks of age. Isolation of acinar tissue does not work with older mice. Because time is a critical step for the viability of acinar cells, we usually do not process more than 2 mice at the same time.

From one mouse it is possible to obtain about 1,000 acinar-explants. Acinar tissue can be seeded for example in 15 wells of a 48-well plate, thus the optimal density for differentiation of acinar tissue is obtained when you see about 30 acinar explants in a well through a 10x objective. If density is too high differentiation will take longer, if too low acinar cells will die.

1. Put forceps and scissors into 80% EtOH.
2. Anesthetize the mouse with isoflurane, sacrifice the mouse by cervical dislocation and resect the pancreas as fast and possible (*time is a critical step*).
3. Place the resected pancreas into a tissue culture dish containing ice cold sterile PBS.

B. Up to now all protocol steps should be performed under sterile conditions.

Centrifugation steps at 720 x g (18 °C) are a critical step!

1. In the hood, transfer the pancreas into a culture dish containing 10 ml of PBS.
2. Immediately put the pancreas in culture dish with 5 ml collagenase VIII solution (see Recipe 4) and cut the organ in very small pieces (less than 1 mm of diameter) with a scalpel within 2 min. Pipette the tissue up and down.
3. Incubate the dish at 37 °C for 10 min. Shake from time to time.
4. Transfer the sample into a 50 ml falcon. Pipette the solution 3x up and down.
5. Wash the dish with 10 ml wash solution and transfer to the 50 ml falcon.
6. Centrifuge, 720 x g, 5 min, 18 °C.
7. Carefully remove the supernatant.
8. Resuspend the pellet in 5 ml collagenase VIII solution. Put in a culture dish. Pipette the tissue up and down.
9. Incubate at 37 °C for 10 min. Shake from time to time.
10. Aspirate the sample and filter through a 100 µm nylon cell strainer positioned on a 50 ml falcon.
11. Macerate the tissue pieces through the cell strainer with a syringe plunger.
Note: This step is difficult to describe, try to press the tissue through the strainer with the syringe plunger without applying a shear stress. It is possible you have to optimize this step in your hands.
12. Wash the mesh with 10 ml wash solution to carefully collect any remaining cell.
13. Centrifuge, 720 x g, 5 min, 18 °C.
14. Carefully remove the supernatant.
15. Aspirate the pellet in 20 ml wash solution, and transfer the solution in a fresh tube. Do not aspirate up and down!
16. Centrifuge, 720 x g, 5 min, 18 °C.
17. Resuspend the pellet in 2 ml culture medium 30% and transfer it on a well of a 6-well culture dish.
18. Check the quality of isolated acinar tissue under the microscope. Isolation is good if you see clusters of acinar cells (epithelial explants, the morphology is the same as in the Ctr. of the figure below) and only rare isolated acinar cells swimming around. Sometimes damaged acinar cells agglomerate together, remove such sticky agglomerates with a forceps.
19. Let the isolated acini recover for 60 min in the incubator (37 °C with 5% CO₂).
20. Meanwhile precoat a cell culture plate of the desired size with collagen (see below). Calculate the required volume of collagen - medium mixture required for step 25. The volume depends from your application. Calculated 100 µl volume/well for a 48-well plate or 80 µl volume/well for an 8 well chamber slide. Typically you will seed the acinar explants obtained from one wild type mice in 15 wells of a 48 well plate. In this case you

- need $100\ \mu\text{l} \times 15/2 = 750\ \mu\text{l}$ collagen and $100\ \mu\text{l} \times 15/2 = 750\ \mu\text{l}$ medium 0.1%.
21. Collect the acinar suspension from the plate and transfer it to a fresh 15 ml tube.
 22. Centrifuge acinar suspension, $720 \times g$, 5 min, $18\ ^\circ\text{C}$.
 23. Carefully aspirate the supernatant.
 24. Resuspend the pellet in a mixture of collagen and culture medium 0.1% (1: 1 vol. /vol.)
 25. Pipet the acini/collagen-medium suspension mixture into each coated well in the plate (at least in triplicate). For the typical 48-well example you pipet $100\ \mu\text{l}$ of the mixture in 15 wells.
 26. Wait until solidification of the collagen (about 30 min)
 27. Add some more collagen ($100\ \mu\text{l}$ /well for the 48 well plate). You may skip this step when you will perform immunofluorescence as a downstream application. Wait until solidification (about 30 min).
 28. Add culture medium 0.1% with/without the desired supplements to each well. Typically $400\ \mu\text{l}$ /well for a 48 well plate.
 29. To induce transdifferentiation of acinar explants, add TGF α (10 ng/ml) or EGF (25 ng/ml) into the culture medium 0.1% at day 1-3-5 where day 1 is the day of isolation.

Notes:

- a. *We usually quantify transdifferentiation of wild type acinar explants after EGF treatment at day 5 (Figure 1).*
- b. *Culture can be kept until day 8. For longer periods of culture you have to reseed the acinar explants into fresh collagen because cultures become acidic and collagen breaks down.*

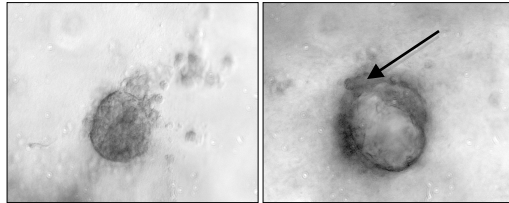
C. Gelation Procedure for Rat Tail Collagen I, 2.5 mg/ml.

1. Place on ice: Collagen, sterile 10x PBS, sterile water, sterile 1 N NaOH, falcon tubes. Keep all reagents on ice.
2. Calculate the total amount of collagen (final concentration of 2.5 mg/ml) needed with the formula: $(\text{final Vol.} \times 2.5) / (\text{collagen concentration in the bottle})$. You need a $100/120\ \mu\text{l}$ collagen layer/well for a 48-well plate or $80\ \mu\text{l}$ collagen layer/well for an 8 well chamber slide.
3. Prepare a tube on ice with the following volume of 10x PBS: $(\text{final Vol.})/10$.
4. Add the following volume of 1 N NaOH to the tube containing 10x PBS: $(\text{Vol. collagen to be added}) \times 0.023\ \text{ml}$.
5. Add to the 10x PBS/1 N NaOH the following volume of sterile ice-cold water: $(\text{final Vol.}) - (\text{Vol. collagen}) - (\text{Vol. 10x PBS}) - (\text{Vol. 1 N NaOH})$.
6. Mix the contents of tube and hold in ice.
7. Add the calculated volume of collagen and mix. Leave on ice (stable for 2-3 h) until ready

for use.

8. Coat tissue culture dishes with collagen (use 24/48 wells for quantification of transdifferentiation, protein or RNA extraction, or use 8 well chamber slides for later staining applications).
9. Allow collagen to solidify at 37 °C (about 30 min).

Note: Downstream applications include immunofluorescence, protein extraction, RNA extraction and cytotoxicity assay.



Ctr

+EGF

Figure 1. Example of acinar explants at day 5. Acinar explants from a wild type mouse were isolated and cultivated for 5 days in collagen as described. Ctr (medium only): a typical acinar explant is composed by a sphere of several acinar cells. EGF: in presence of 25 ng/ml of EGF cell clusters get a more flattened morphology and differentiate to a duct-like structure characterized by a lumen (arrow) lined by several cells.

Recipes

1. SBTI stock solution
10 mg/ml in McCoy medium, store at -20 °C.
2. (optional) Collagenase VIII stock solution
9.6 m/ml in McCoy medium (not fully dissolved), store at -20 °C.
3. Wash solution (40 ml/mouse)
McCoy's medium
0.1% BSA (sterile filtered)
0.2 mg/ml SBTI (stock 1:50)
4. Collagenase VIII solution (10 ml/mouse)
McCoy's medium
0.1% BSA (sterile filtered)
0.2 mg/ml SBTI (Stock 1:50)
1.2 mg/ml Collagenase VIII (critical step, add collagenase just before use, dilute the collagenase VIII stock solution 1:8)

5. 0.1% culture medium
Waymouth's medium
0.1% BSA (sterile filtered)
0.2 mg/ml SBTI (Stock 1:50)
P/S 1:200 (*optional, not necessary for short term culture*)
0.25 µg/ml Amphotericin B (*antifungal, optional*)
ITS-X 1:100
BPE: 50 µg/ml (dilute according the batch concentration) (Aliquots, -20 °C)
0.1% FCS
6. 30% culture medium
0.1% culture medium
30% FCS

Acknowledgments

This protocol for acinar epithelial explants from adult murine pancreas was established by modification previously published protocols (Lisle and Logsdon, 1990; Githens *et al.*, 1994; Means *et al.*, 2005). The work was supported by grants from the Wilhelm Sander Stiftung (2010.021.1).

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