A CULTURE SYSTEM FOR THE STUDY OF PANCREATIC ORGANOGENESIS

George K. Gittes and Philip E. Galante

Department of Surgery, University of California San Francisco, San Francisco, California 94143

SUMMARY: Methods of microdissection and culture are described for the establishment and short-term maintenance of cultures derived from early embryonic mouse pancreas. Varying culture conditions may be employed to study aspects of epithelio-mesenchymal interactions in the developing pancreas.

Key words: embryonic pancreas; organ culture; epithelium; mesenchyme; organogenesis.

I. INTRODUCTION

Organogenesis consists of two components: morphogenesis, the physical shaping of an organ, and cytodifferentiation, the expression of specialized cellular functions in an organ. The embryonic pancreatic epithelium in the mouse undergoes morphogenesis and cytodifferentiation to form both endocrine and exocrine tissue in vitro (7). These processes are thought to be induced by the overlying pancreatic mesenchyme (2,3,5). Mesenchyme from various other embryonic (but not adult) tissues, however, is thought to be able to induce the same processes (6). It has also been shown that the inductive effect of mesenchyme can be exerted across a membrane filter (4). Thus, interactions between the mesenchyme and the epithelium of the pancreas may be mediated by the release of some "mesenchymal factor(s)" (5,6). The nature of these factors, as well as the relationship of different components of mesenchyme to differentiation of the pancreatic anlage into the various pancreatic cell types (acinar, islet, ductal) is not known.

One obstacle to a successful study of epithelio-mesenchymal interactions in the pancreas has been the inability to completely isolate the epithelium from the mesenchyme. Mesenchymal contamination can occur despite mechanical removal of the mesenchyme and further trypsinization due to pockets of mesenchyme trapped among buds of pancreatic epithelium.

With improved dissecting microscope optics, filter insert systems, and defined media additives, we have been able to improve the embryonic culture systems developed 20 to 30 yrs ago. Our method of microdissection and culture allows for the study of epithelio-mesenchymal interactions using transparent Millipore cell inserts with either mesenchyme-free pancreatic epithelia, whole embryonic pancreas, or even recombinant (mesenchyme and epithelium separated, then recombined) pancreases. The filter inserts allow the tissue to be maintained at the air-media interface, providing optimal access to oxygen from above and medium through the membrane filter below.

Various substrates, growth factors, or gel matrices can be added to the culture system to study the mechanisms involved in pancreatic organogenesis and epithelio-mesenchymal interactions. The tissues can be observed directly, while growing, under phase or dissecting microscopy, and later fixed for histologic and immunohistochemical staining.

II. MATERIALS

A. Equipment
1. Biohazard hood VBM-400, Baker
2. Incubator, CO₂, Napco no. 4300, Baxter
3. Stereo microdissection microscope, no. SV8, Zeiss
4. Phase contrast, inverted microscope, Nikon Diaphot-TMD equipped with 4 X 10 and 10 X 100 objectives and a Nikon AFX-II photographic attachment, Technical Instrument Co.

B. Dissection tools
1. Microdissecting forceps, Dumont no. 4, Roboz
2. Microdissecting forceps, straight no. RS-5280
3. Microdissecting scissors, Vannas 3 in. straight no. RS-5610
4. Microdissecting knife, 4.5 in. spear point no. RS-6210
5. Operating scissors RS-6750

C. Cultureware and incidentals
1. Tissue culture plates, 24-well, Falcon 08-772-1, Fisher
2. Petri dishes, 35 X 10 mm, Falcon 08-757-100A
3. Pipettes, serological
   a. 1-ml, 13-675-3A
   b. 25-ml, 13-676-10K
4. Polaroid B+W film 6676
5. Millipore CM insert, 0.4-μm pore size, 12 mm diameter no. PICM 012 50, Millipore
6. Pipetman
   a. 20-μl, no. P-20, Rainin
   b. 200-μl, no. P-200
   c. 1000-μl, no. P-1000

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D. Animals
1. Mice B6D2F1 4 to 6-wk old, Simonsen
2. Serum-containing media
3. Molding cup trays nos. 17177A and 16643A
4. EBH-2 block holders no. 1589913
5. JB-4 Embedding kit no. 00226, Polysciences
6. Trypsin inhibitor, type I-S no. T-9003
7. Trypsin, type XII-S no. T-2271, Sigma
8. Hanks' balanced salt solution (HBSS) BClO0-N181
9. Presterilized tips
10. Mice B6D2F1 4 to 6-wk old, Simonsen

III. PROCEDURE
A. Tissue procurement
1. Mate female B6D2F1 mice in estrous, as determined by swollen, erythematous external vaginal mucosa, with male B6D2F1 mice, the latter having been singly-housed for at least 2 wk. Check for vaginal plugs the morning after mating and define noon of that day as gestational age 0.5 days.
2. Kill pregnant females at gestational age 11.0 to 11.5 days by cervical dislocation.
   Note: Exsanguinate for 3 to 5 min (Fig. 1 A).
3. Remove the uterus from the mother under sterile conditions and place in a sterile petri dish containing HBSS. Deliver the embryo from the uterus by enlarging the uterine opening. Transfer each embryo to its own dish with HBSS and allow the embryos to exsanguinate for 3 to 5 min (Fig. 1 A).

B. Microdissection
1. Under the Zeiss dissecting microscope, with only light from above and with the embryo resting on its right side, cut the embryo transversely by placing the prongs of the no. 4 forceps on the dorsal and ventral sides of the thorax, just below the forelimb buds, and squeezing gently. Discard the upper half.
   Note: The lobes of the liver and the stomach should be visible as a solid mass in the abdomen. Be sure to make the aforementioned transverse cut so that these structures are in the lower half of the embryo.
2. Using the left hand, pin the lower half of the embryo down by placing the prongs of the forceps through the dorsal curve of the embryo. Make sure that the embryo is still resting on its right side. Using the microdissecting scissors cut the left lateral abdominal wall starting at the superior cut edge and extending inferiorly below the hindlimb (Fig. 1 B).
3. Now, using only transmitted light, pull the skin flap back with the forceps. Identify the liver lobes and the stomach. Using the microdissection scissors or the microdissection knife remove the stomach and intestine.
4. With the stomach resting on its dorsal side the pancreas should be adherent to the posterior and inferior surface of the stomach as a slightly translucent bud surrounded by its more opaque mesenchyme (Fig. 1 C). Pin the stomach down with the forceps and remove the pancreas and its mesenchyme with the microdissection knife (Fig. 1 D).
5. Remove as much of the mesenchyme as possible by placing the microdissection knife between epithelium and mesenchyme and gently pulling away.
   Note: Older epithelia can be identified because of a more lobulate appearance with buds or nodules on the surface. These older epithelia contain pockets of mesenchyme trapped among the epithelial buds. This mesenchyme cannot be removed and therefore these epithelia should not be used for studies of isolated pancreatic epithelium.
6. Place epithelia in 1% trypsin in HBSS for 20 to 30 min at 4 ° C. Neutralize trypsin by adding excess serum-containing medium or, if a serum-free environment is required, by adding trypsin inhibitor in serum-free medium. Strip away remaining mesenchyme gently with the forceps or the microdissection knife. With the Zeiss dissecting microscope used here, even individual clusters of a few cells of mesenchyme are readily visible (Fig. 1 E).
C. Tissue culture
1. Preparation of the plate: In the biohazard hood place Millicell inserts individually in 24-well plates. These inserts become transparent when wetted with medium. Add 300 µl of medium to each well making sure no medium is placed inside the filter insert.
2. Place the isolated epithelia, free of mesenchyme, on the filter (Fig. 2 A). For recombinant controls, place mesenchyme on the filter and position it on either side of the epithelium (Fig. 2 B).
3. Maintain cultures in a humidified incubator at 37 ° C with 5% CO₂ for 10 days. Replace medium every