ISOLATION OF RAT PROSTATE CELL CULTURES BY PHYSICAL DISSOCIATION

Submitted by

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I. INTRODUCTION

Using a modification of a previously reported method (1) we have isolated rat prostate cell lines by the outgrowth of cells from explants. This procedure allows the investigator to obtain cultures composed primarily of epithelial cells within 10 to 21 days. Epithelial cells have been identified by their morphology under the phase contrast microscope. We have been able to maintain successfully several lines through 10 passages. The prostate cells are presently being examined biochemically in conjunction with in vivo rat experiments.

II. MATERIALS

Growth medium: Ham F-10 supplemented with 20% fetal bovine serum, 2X L-glutamine, 1X nonessential amino acids (Eagle) and 50 μg/ml gentamicin, Micro[1] (this proved to be the best medium for growing primary and secondary cultures of rat prostate cells)

Dulbecco's Phosphate Buffered Saline (DPBS)[1]

Amphotericin B methylester (AME).[2] Dissolve the AME in dimethylsulfoxide (DMSO) (MCB)[3] at a final concentration of 10 μg of AME per 10 μl DMSO. Add 10 μl of the antibiotic solution per ml of tissue culture medium. The final DMSO concentration is 1.0% and is not harmful to the cells.

Source of tissue: Male Wistar rats about 10 to 12 weeks of age, Carworth.[4] Anesthesize the animals with an injection (0.5 ml), i.p., of nembutal sodium, (sodium pentobarbital injection), Abbott.[5]

ATV: Use ATV (trypsin-versene) to passage the primary cultures (2). Consists of:

	grams/liter
NaCl	0.8g
KCl	0.4g
dextrose	1.0g
$NaHCO_3$	0.58g
trypsin (Difco 1:250)[6]	0.5g
versene	0.2g

to which 1000 ml of distilled water is added. Filter the solution through a 0.22 nm Swinnex filter (Millipore)[7] and store at −20°C until used. Once thawed, it should not be refrozen. (All chemicals are of reagent grade, Fisher.)[8]

Tissue culture flasks: 25 cm^2 (30 cc), No. 3012 Falcon[9]; and 75 cm^2 (250 cc), No. 220-45[8]

Sterile microtiter plates, Flow[10]

Fungizone®, Squibb[11]

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1 Microbiological Associates, Bethesda, MD.
2 Supplied by Dr. W. Mechlinski and Dr. C.P. Schaffner of Waksman Institute of Biology.
3 Matheson, Coleman and Bell, East Rutherford, NJ.
4 Carworth Farms, Wilmington, MA.
5 Abbott Laboratories, North Chicago, IL.
6 Difco, Detroit, MI.
7 Millipore Corp, Bedford, MA.
8 Fisher Scientific, Springfield, NJ.
9 Falcon Plastics, Oxnard, CA.
10 Flow Laboratories, Rockville, MD.
11 Squibb and Sons, Princeton, NJ.

III. PROCEDURE

A. Collection of tissue for planting

1. Inject the rat intraperitonally with sodium nembutal. Sterilize the animal with 70% ethanol at the site of the incision; sacrifice by exanguination and dissect.

2. Clean the ventral rat prostrate lobes of extraneous fatty material, lift the lobes away from the bladder with sterile forceps, and cut the lobes at the base of the bladder. Make sure not to pick up any surrounding tissue. The rat prostate gland is composed of discrete paired ventral lobes at the neck of the bladder and a dorsolateral group of prostatic acini and their ducts. The acini surround dorsolaterally the urethra, base of the bladder, seminal vesicles, and coagulating glands (3).

3. Place the lobes in sterile vials on ice for no longer than 2 hr.

4. Wash the lobes twice in 70% ethanol (5-sec rinses) and twice in DPBS (5-sec rinses).

5. Cut the tissue into 1 mm^2 segments with a sterile scalpel and resuspend in a small amount (3 to 5 ml) of growth medium. The growth medium can contain either AME (10 to 25 μg/ml) or Fungizone ® (1 to 2 μg/per ml) to control fungal or mycoplasmal contamination (4, 5).

B. Planting of tissue

1. Pipette 20 to 50 tissue segments into 75 cm^2 T-flasks and add enough growth medium to cover the bottom (about 4 ml).The greater the number of segments added per flask, the shorter the incubation time necessary to obtain a confluent monolayer.

2. Leave the screw tops of the flasks loose.

3. Incubate the flasks at 37°C in a CO_2 incubator (95% air: 5% CO_2).

C. Fluid renewal and passaging

1. Check the flasks every day until an outgrowth of cells is observed (usually 24 to 72 hr).

2. Remove the medium and add 12 ml of fresh growth medium.

3. Change the medium every 3 to 4 days until a monolayer is formed (about 10 to 21 days). The outgrowing cells are mostly epithelial as seen under the phase contrast microscope. If the explants are allowed to grow longer than 3 to 4 weeks, the cultures become overgrown with fibroblastic cell types.

4. Either use the cultures at once or passage with ATV at a split ratio of 1:2.

5. Wash the cells twice using 5 to 10 ml of freshly thawed ATV, leaving a small residual volume (about 1 to 2 ml) in contact with the cells.

6. Incubate 10 to 15 min at 37°C.

7. Disperse the cells with rapid pipetting in 5 ml of growth medium and dispense into one new plus the original flask.

D. Cloning of cells

1. If the cultures are found to contain many cells of fibroblastic origin, it is necessary to clone the epithelial cells.

2. Disperse the cells with ATV and collect by centrifugation at 600 x *g* at 4° C for 15 min.

3. Determine viable cell number using the trypan blue exclusion technique (6).

4. Dilute the cell suspension with growth medium to about 10 cell per ml.

5. Add 0.1 ml to individual wells in a sterile microtiter plate.

6. Incubate at 37°C in a CO_2 incubator (95% air: 5% CO_2).

7. After 1 week check daily for the appearance of colonies and epithelial

cells (about 50 to 100 cells per colony) in each well.

8. To isolate the cells, add 0.1 ml ATV per well and incubate the plates at 37°C until the cells slough off.

9. Remove the dissociated cells with a sterile pasteur pipette and place in a 25 cm^2 T-flask containing 5 ml of growth medium.

10. Incubate until the cells are confluent, and passage.

IV. DISCUSSION

Using the explant method, we are able to isolate monolayers of epithelial cells within 10 to 21 days. These cultures are 75% to 100% confluent depending on the number of segments initially added to the tissue culture flask. Since "harsh" enzymatic conditions are avoided during the isolation procedure, we consider that these cultures more closely resemble the in vivo state. Cultures left for longer lengths of time without passaging (more than 3 weeks) tend to become overgrown with fibroblastic cells. The cultures are therefore used at once or split with ATV and eventually cloned. AME is used to control mycoplasmal contamination at 10 to 25 μg per ml; Fungizone® may be employed at lower concentrations (1 to 2 μg per ml) to control fungal contamination.

V. REFERENCES

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