Isolation and culturing of primary human colonocytes

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Summary. In our laboratory, human cells and tissues are used as experimental models to understand the pathogenesis of human diseases. A reproducible method for the isolation and culturing of colonocytes from human surgical specimens has been developed. This method involves the dissection of the colon specimen to expose the mucosa, cleaning of the mucosa surface, and digestion of the mucosa with a mixture of pronase and collagenase. The dissociated colonocytes are then quantified and cultured on collagen-coated plastic. Our procedure yields approx. 30–40 million viable colonocytes per gram of tissue, with a viability of approx. 90%. We believe that the primary colonocyte cultures can be used to study important human diseases such as Crohn's disease and ulcerative colitis.

Key words: Colonocyte culture, Crohn's disease, Human colonocytes, Ulcerative colitis

1. Introduction

Tissue culture of the bowel mucosa has been attempted for over 20 years [1]. Originally researchers studied organ culture or explant culture models [2, 3]. Numerous techniques have been attempted to isolate and culture colonic mucosal cells, but usually with only variable success [4, 5]. Colonocytes have been isolated by both mechanical [6–9] and enzymatic [10, 11] methods. However, more viable cells have been isolated from smaller surgical specimens using various enzymatic protocols [12]. Chelating agents have been used in some studies but decrease cell viability [12]. This is felt to be secondary to a loss of plasma membrane integrity caused by the chelating agent and decreased calcium [13].

In our Surgical Research Institute, a concerted effort has been made to establish a routine source of human tissues for research. We believe that the data obtained with human cells and tissues are valuable for mechanistic understanding of xenobiotic metabolism, toxicology, and the pathogenesis of diseases in human, and to aid the extrapolation of data obtained from nonhuman laboratory animals to human. We have recently established a procedure for the isolation and culturing of human hepatocytes [14], and human gall bladder epithelial cells [11]. We report here a reproducible procedure for the isolation and culturing of human colonocytes. Our procedures are modified from a combination of procedures published by others [6–12], involving both surgical removal of the mucosa from the colon and enzymatic digestion of the isolated mucosa. Our primary research objective with the primary human colonocytes is to understand the cell and molecular biology of the colonic epithelium's role in inflammatory processes, especially in relation to the pathogenesis of Clostridium difficile colitis and the inflammatory bowel diseases [15].

2. Materials

A. Equipment
1. Waterbath, Dubnoff metabolic shaker incubator, No. 66722.1
2. Bright line hemocytometer.2
3. Inverted phase contrast microscope, Nikon Diaphot.3
4. Bright field Microscope, Nikon Labophot-2.3
5. Laminar flow hood, model NU 425-400.4
6. Incubator, model NU-1600.4
7. Refrigerated centrifuge, model PR-7000.5
8. Pipet-Aid, No. 13-681-19.2
9. pH meter, No. 240.6
10. Weighing balance, Mettler AM100.7

B. Plastic, Glassware, and other miscellaneous materials
1. Beakers, 150 ml, Pyrex, No. 14005.2
2. Erlenmeyer flask, 500 ml, Pyrex, No. 26500.2
3. Media bottles, 500 ml, Pyrex, No. 06-414-1C.2
4. Petri dishes, 100 mm x 15 mm sterile polystyrene, No. 8-757-12.2
5. Centrifuge tubes
   a) 15 ml, polypropylene, No. 05-539-5.2
   b) 50 ml, polypropylene, No. 05-539-6.2
6. Pipettes
   a) 1 ml, polystyrene, No. 4012
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b) 5 ml, polystyrene, No. 4051
c) 10 ml, polystyrene, No. 4101
d) 25 ml, polystyrene, No. 4251

7. Culture vessels
   a) 24-well sterile polystyrene, No. 3524.8
   b) 12-well sterile polystyrene, No. 3043.9
   c) 25 ml, polystyrene, No. 4251

8. Filter system units
   a) 250 ml, No. 8360.8
   b) 500 ml, No. 8340.8
   c) 1000 ml, No. 8350.8

9. Disposable sterile bottles
   a) 250 ml, No. 8390.8
   b) 500 ml, No. 8393.8

10. Pasteur pipettes, No. 13-678-7C.2

11. Sterile Pipetman tips
   a) Redi-tips, 200 µl size, No. 21-197-8H.2
   b) Redi-tips, 100 µl size, No. 21-197-8A.2

12. Scissors, No. 08-940.2

13. Forceps, No. 10-300.2

14. Scalpels, No. 08-927-5D.2

15. Sterile latex surgical gloves, No. 11-394-2C.2

16. Sterile cotton gauze, No. 7624.m

17. Autoclaving bag for biohazardous materials, No. 01-815C.2

C. Chemicals

1. Lactated Ringer’s solution.11
4. Penicillin, No. P-3414.12
5. Streptomycin, No. S-9137.12
7. Dithiothreitol, D-0632J 2
8. Collagenase Type IV, C-5138.12
9. Pronase, P-8811.12
10. Ham’s F-12 medium, N-4888.12
11. Fetal Bovine serum, No. F-2442.12
15. Selenium, No. 40201.13
17. Rat Tail Collagen, No. 40236.13

D. Preparation of collagen coating

1. Prepare plates one to three days before an expected specimen.
2. Dilute stock bottle of rat tail collagen to 0.5 mg/ml with sterile deionized ultra filtered water.
3. Employing sterile technique, pipet appropriate amount of collagen solution into each well of plate (1 ml/well for 12-well plate).
4. Allow solution to evaporate under laminar flow hood, leaving an even and complete collagen coating of the well.

E. Preparation of solutions

1. Lactated Ringer’s (LR) solution. Empty a one liter bag of LR into a one liter graduated cylinder on a mixing plate. To this 125 mg of penicillin, 270 mg of streptomycin, 1.25 mg of Amphotericin B, and 771 mg of dithiothreitol. Then sterilize the mixture by filtration. Store the final mixture at 2-4 °C.

2. Enzyme solution. There should be roughly 20 ml of solution per gram of mucosa and this should be prepared fresh as needed for cell isolation. Our protocol typically yields 4-5 g of mucosa. Therefore, to 83.3 ml of LR solution from above, add 25 mg of pronase (0.03%) and 83.3 mg of collagenase type IV (0.1%).

F. Preparation of medium

To a 500 ml bottle of Ham’s F-12 medium add 250 units insulin, 290 mg L-glutamine, 2 mg hydrocortisone, 8 µg selenium, 12.5 mg ampicillin, 62.5 mg penicillin, 135 mg streptomycin, and 0.625 mg amphotericin B. Sterilize by filtration and add 100 ml fetal bovine serum. Store at 2-4 °C.

3. Procedures

A. Preparation of mucosa

1. A 2-5 cm segment of colon removed during scheduled colectomies is transported to the lab on ice.
2. First, open the specimen along the mesenteric border with scissors, and rinse it thoroughly with ice-cold LR solution.
3. Blot the specimen with sterile gauze to remove residue and mucous. Then place it mucosal surface down in a dish filled with ice-cold LR solution.
4. After two separate soaks over about 10-15 minutes, place the specimen mucosal surface up on a surgical towel soaked with ice cold LR solution. Hold the specimen to the towel with Alice clamps and bluntly dissect the mucosa from the specimen using a hemostat.
5. Then soak this mucosal strip in ice-cold LR solution in a 250 ml beaker on ice. Periodically stir the solution, and after 5-10 minutes transfer the mucosa to a fresh beaker of LR solution on ice.

B. Isolation of colonocytes

1. Prepare the enzyme solution and mucosal strip in a 500 ml erlenmeyer flask. Cover the flask and place it in a 37 °C water bath shaking at 150 cycles/sec.
2. After 90 minutes filter the resulting suspension through two layers of sterile cotton gauze into two 50 ml sterile centrifuge tubes.
3. Centrifuge the suspension at 4000 x g for