Intestinal epithelial cells – primary cell culture

1. Flush the contents of the intestine with Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (HBSS) containing 2% glucose, 25 ng of amphotericin B per ml, 100 U of penicillin per ml, and 100 µg of streptomycin per ml.

2. Splice the intestine into small pieces and incubate for 15 min at 22°C on a shaker platform in Ca^{2+}- and Mg^{2+}-free HBSS containing 5mM EDTA, 2% bovine serum albumin, and 0.2 mg of soybean trypsin inhibitor per ml.

3. Take out the supernatant and wash with (DMEM), 100 U of penicillin per ml, 100 µg of streptomycin per ml, and 5% fetal bovine serum (FBS)

4. Cells were cultured in 24-well plates, at a seeding density of approximately 2X 10^6 cells/well

5. One hour before plating cells, culture surfaces were coated with 40 µl of Matrigel (BD Biosciences) per cm^2 diluted 1:2 in phenol-red-free DMEM (Sigma).

6. Epithelial cells were cultured in epithelial cell medium (ECM) containing equal volumes of phenol-red-free DMEM and Ham's F-12 medium (Biowhittaker) with the following additives:

   5 µg of insulin (Sigma) per ml,
   5×10^8 M dexamethasone (Sigma),
   60 nM selenium (Sigma),
   5 µg of transferrin (Sigma) per ml,
   5×10^8 M triiodothyronine (Sigma),
   10 ng of epidermal growth factor (Sigma) per ml,
   20 mM HEPES,
   2 mM glutamine,
   100 U of penicillin per ml,
   100 µg of streptomycin per ml,
   0.2% D-glucose,
   2% FBS.

7. Cells were cultured in 5% CO_2 at 37°C with periodic supplementation of medium to maintain a volume of 2 ml per well.