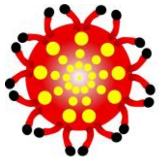
Culturing of polarized Caco-2 BBe cells



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Background

Caco-2 is human colon cancer epithelial cell line. **Caco-2 BBe (brush border-expressing)** is a derivate cell line that is expressing brush-border proteins. Caco-2 cells grow as small colonies, with the edge of each colony expanding by both cell migration and cell division. These two processes are identical to those in epithelial wound healing, a process recapitulated by cell migration and growth to seal the 'wound' of the exposed plastic. Thus, the colonies will ultimately merge to cover the entire flask. When this occurs, Caco-2 cells shift gene expression programs from a "wound healing" mode to a "differentiated function" mode. This is apparent morphologically as well. When cells are in the wound healing mode, they divide rapidly and have a flat "fried-egg" or "pancake-like" shape. The spread to cover as much surface area as possible. When the wound is healed and the cells move towards assuming differentiated functions, they take up less surface area per cell and for tight colony mosaics of tall columnar-shaped cells. The morphology also varies between Caco-2 cell lines.

Morphology is an important judge of cell health and growth and should also be used to determine when to passage cultures.

General tissue culture rules

For good maintenance of tissue culture:

1. Do not let the culture exceed the maximal optimal cell confluence that is appropriate to the cell line. *

2. Do not split the cell culture before it reaches at least 60% confluence.

3. Feed culture with fresh medium regularly according to the protocol, usually it is 2-3 times a week, depending on the cell density. If the media becomes yellow-orange (acidic) it should be replaced sooner.

4. Warm in advance the reagents before use, but do not keep them too long in the 37°C water bath. Thaw Trypsin-EDTA to room temperature (since at 37°C the trypsin will auto-digest rapidly).

*** Caco-2 BBe culture should not grow beyond 80% confluence.**

Caco-2 BBe feeding, plating and culturing

Culturing Reagents

Growth Medium formulation:

High Glucose DMEM (BI 01-055-1A) supplemented with 10% FBS (BI 04-007-1A), 1% L-Gln (Stable Dipeptide, BI 03-022-1B) and 1% PSA antibiotics (BI 03-033-1B).

Splitting Reagents

Trypsin-EDTA Solution A (BI 03-050-1A)

DPBS (BI 02-023-1A)

0.5M EDTA pH=8.0. Sterile filtered.

Splitting and plating for maintenance

1. Aspirate the culture medium and wash the cells once with 5-10 ml DPBS supplemented with 1mM EDTA (10-20 µl of 0.5M EDTA, respectively).
2. Add 1.5-2.0 ml Trypsin-EDTA Solution A and spread it all over the plate.
3. Incubate the cell plate in 37°C incubator for 15-20 min.
4. Ensure that the cells are detached, if needed look under light microscope.
5. Add to the detached cells 8.0-8.5 ml of Growth Medium and collect all the cells in the plate and by pipetting up and down few times disperse the clumps into single cells.
6. Transfer the cells to a sterile conical tube and centrifuge 2-3 min at 1500-2000 rpm.
7. Aspirate the medium and leave the pellet with minimal volume, suspend the cell pellet with fingertip hitting on the cone of the tube.
8. Add 5-10 ml of Growth Medium to the cells and suspend them well and transfer 1/10 volume to a new 10 cm plate with the right volume of Growth Medium (10-12ml).

9. Disperse the cells homogenously over the plate and look under light microscope if the cells look mostly in the form of single cell, if the density of the cells are fine
10. Place the plate in CO_2-incubator at 37°C and replace the medium 3 times a week until culture reach ~80% confluence.

Plating and maintenance of polarized Caco-2 monolayers cultured on Transwell inserts

Background

For optimal plating of inserts, cells should be in the growth (wound-healing) phase. For experiments in which complete differentiation into absorptive cells is desired, the cells should be cultured for at least 18-21 days post-confluence. Ideally, this should be on a semi-permeable support (e.g., Transwell). The monolayers need to be fed three times each week.

Insert preparation and cell plating

Materials

Transwells, coated with collagen at least 24 hours in advance

Freshly-trypsinized cell suspension, as below.

For Transwell collagen coating – See the Protocol**: Collagen Coating of coverslips, or Transwells, or plastic plates.**

The collagen coated inserts should be wash twice with Growth Medium before cell plating.

Splitting for plating on Transwell inserts

1. Take a ~80% confluent Caco-2 plate.
2. Aspirate the culture medium and wash the cells once with 5-10 ml DPBS supplemented with 1mM EDTA (10-20 µl of 0.5M EDTA, respectively).
3. Add 2.0 ml Trypsin-EDTA Solution A and spread it all over the plate.
4. Incubate the cell plate in 37°C incubator for 20 min.
5. Ensure that the cells are detached, if needed look under light microscope.

6. Add to the detached cells 8.0-8.5 ml of Growth Medium and collect all the cells in the plate and by pipetting up and down vigorously against the plate bottom at least 5 times for dispersing the clumps into single cells.
7. Transfer the cells to a sterile conical tube and centrifuge 2-3 min at 1500-2000 rpm.
8. Aspirate the medium and leave the pellet with minimal volume, suspend the cell pellet with fingertip hitting on the cone of the tube.
9. Disperse the cells homogenously in 10 ml Growth Medium and count the cells by Hemocytometer.
10. Prepare a cell suspension of 250,000 cells/ml in a tube.
11. Prepare the inserts with the medium of the basal chamber only, and then add above the insert to the apical chamber the right volume of the cell suspension (250,000 cells/ml) according to the table below.
12. 6 hrs. - 24 hrs. after cell plating replace the Growth Medium for the removal of the floating cells. Keep feeding 3 times a week for 18-21 days.
13.

Table: Transwell Working Details

Transwell Diameter	Surface Area	Basal volume	Cell Volume (250,000 cells/ml)	Total Cell Amount	Costar Cat#
6.5 mm	0.33 cm^2	1.0 ml	0.166 ml		3413
12 mm	1.1 cm^2	1.5 ml	0.5 ml	125,000	3401
24 mm	4.5 cm^2	3.0 ml	2.0 ml	500,000	3412
75 mm	44.2 cm^2	10.0 ml	10.0 ml		3419

Additional information on the Caco2-BBe cell lines can be found in the following references:

1. Peterson MD, Bement WM, & Mooseker MS (1993) An in vitro model for the analysis of intestinal brush border assembly. II. Changes in expression and localization of brush border proteins during cell contact-induced brush border assembly in Caco-2BBe cells. *J Cell Sci* 105 (Pt 2):461-472.
2. Peterson MD & Mooseker MS (1993) An in vitro model for the analysis of intestinal brush border assembly. I. Ultrastructural analysis of cell contact-induced brush border assembly in Caco-2BBe cells. *J Cell Sci* 105 (Pt 2):445-460.
3. Peterson MD & Mooseker MS (1992) Characterization of the enterocyte-like brush border cytoskeleton of the C2BBe clones of the human intestinal cell line, Caco-2. *J Cell Sci* 102 (Pt 3):581-600.