Caco-2 Transport Assay Using New HTS Transwell®-96 Permeable Supports

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Overview:

Purpose: To demonstrate the performance of Corning HTS Transwell-96 (96 wells) permeable supports in Caco-2 drug transport assay, and compare the results with those from HTS Transwell-24 (24 wells) permeable supports.

Methods: Grow Caco-2 cultures under standard conditions for 21 day after seeding Transwell supports with 35K cells/cm^2. Cultures were fed with fresh medium every other day. Caco-2 drug transport assay was performed on day 21 using 7 compounds with known permeability.

Results: Good Caco-2 monolayer integrity was obtained for both PET and PC 96-well HTS Transwell permeable supports as indicated by TEER values and Lucifer Yellow transport rates; Ratio of Vinblastin transport rates in the presence and absence of Verapamil was ~ 1.5 for both PET and PC 96-well Transwell supports, indicating acceptable level of differentiation of the monolayers; Ranking of transport rates for reference drugs using these new 96-well Transwell permeable supports is comparable to that reported for HTS Transwell-24 permeable supports in the literature.

Introduction

Background:

Caco-2 cell monolayers grown on permeable Transwell supports are frequently used as an in vitro model for evaluating absorption properties, permeability and efflux transport properties of drug candidates in the drug discovery process. Such evaluations, as part of the ADME-TOX screening, are usually performed in the 24-well format. Recent advances in combinatorial chemistry and genomics have generated an unprecedented number of compounds needed for such testing, and have led to an increasing need for higher assay throughput. In this poster, we will describe the use of new HTS Transwell permeable supports in 96-well format using the Caco-2 drug transport assay

New HTS Transwell-96 Systems:

These new HTS Transwell-96 systems come with either PET or PC membranes.

Advantages:

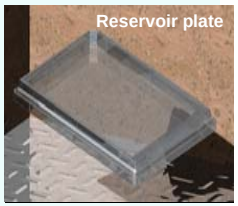
- Greater membrane surface and growth areas (25% more than the largest currently available)
- Decreased basolateral volume – small volume ratio of BL:AP chambers
- Large access ports by each well
- 8 side access ports allow easy access to reservoirs
- Low evaporation lid
- Meet SBS standards ⇒ Automation compatible

Critical Attributes:

Pore size (μm): 1.0 for PET
0.4 for PC

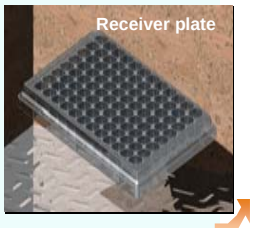
Pore density (cm^2): 1.6 x 10^6 for PET
1.0 x 10^8 for PC

Growth area (cm^2): 0.143



Recommended Working Volumes:

Apical (μL)	75
Basolateral (μL)	235
Reservoir (mL)	25



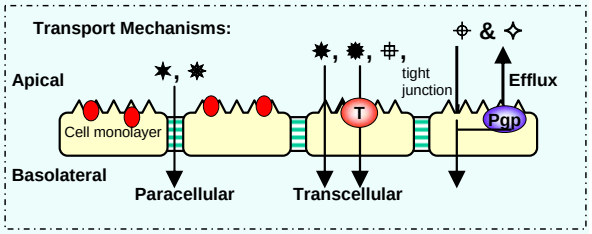
Maximum Working Volumes:

Apical (μL)	100
Basolateral (μL)	275
Reservoir (mL)	30

Materials & Methods

Materials

- Polyester (PET) and polycarbonate (PC) HTS Transwell-96 supports
- Seeding/Growth medium: 20-10% FBS in IMDM + 1% antibiotics
- HBSS without Ca^{++} & Mg^{++}
- TEER Voltmeter for 96-well Transwell supports
- Testing compounds: ✶ Lucifer Yellow, ✸ Warfarin, ✹ Propranolol, ❋ Zantac, ⌗ Digoxin, ⌖ Verapamil, and ✧ Vinblastin

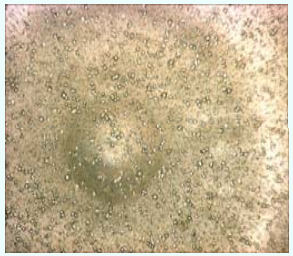


Procedure

- Soak Transwell supports in seeding medium for 1 hr at 37ºC
- Seed Transwell supports with Caco-2 cell suspension (~35K cells/cm^2)
- Grow cultures under 5%CO_2, 37ºC and high humidity
- Change medium every other day: 100μL - Apical); 25mL - Reservoir
- Perform transport assay on Day 21:
  - Wash Transwell insert 2-3 times with HBSS;
  - Add HBSS/compound solutions to basolateral/apical chambers or vice versa using recommended volumes
  - Incubate at 37ºC on shaker (50rpm) for 1 hr
  - Take out Transwell supports and quantify compounds by either fluorescent reader or HPLC
  - Calculate apparent permeability coefficients (Papp)

Results

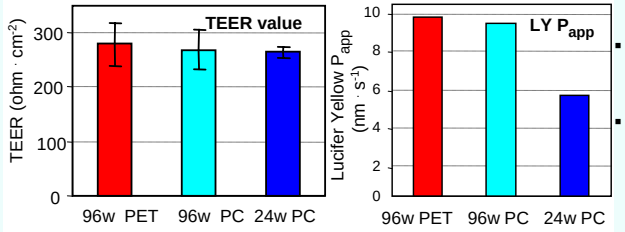
I. Microscopic observation:



Two hours after seeding (4x) Showing the density of cells on the membrane (35K cells/cm^2);

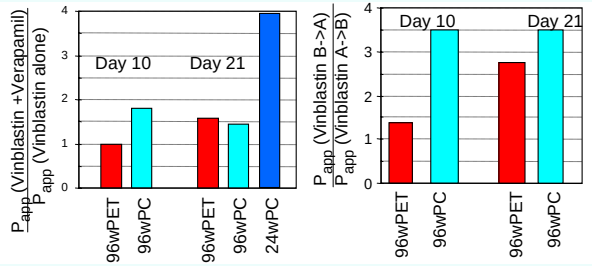
One day after seeding (4x) Showing well attached cells spreading and growing outward;

II. Evaluation of membrane/cell monolayer integrity: -21 day cultures



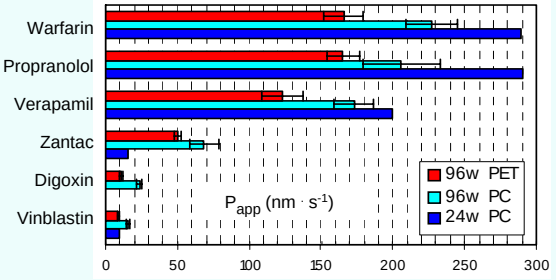
- TEER values -- comparable between 96-well and 24-well Transwell supports
- LY permeability – 2x higher for 96-well supports than for 24-well Transwell supports

III. Evaluation of cell differentiation:



- Similar trends obtained using both ratios/indices
- 96-well PC Transwell insert appears to differentiate faster than 96-well PET Transwell supports
- Higher ratio for 24-well Transwell supports than for 96-well Transwell supports

IV. Drug Transport Properties: - Ranking of drugs with known permeability



- P_{app} values are slightly higher for 96-well PC Transwell supports than for 96-well PET Transwell supports
- Similar ranking obtained for both 96-well Transwell supports
- These rankings were similar to those found in literatures for 24-well Transwell supports

Conclusions

- Acceptable TEER values and LY permeable rates were obtained with 21-day cultures using both 96-well PET and PC Transwell supports, indicating good membrane/cell monolayer integrity
- Acceptable level of cell differentiation were also obtained with the 21-day cultures grown on both PET and PC 96-well Transwell supports.
- Transport rates for reference drugs using these new 96-well Transwell supports ranked similarly to those reported for 24-well Transwell supports in the literature.