# FOR INTESTINAL PERMEABILITY

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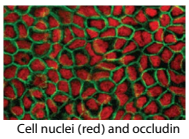
## Role of intestinal absorption assays in drug development.

Powerful methods have recently been developed for the combinatorial synthesis of organic compounds as have methods for high-throughput screening of pharmacological activity. As a result, large number of potential drug candidates are being obtained. This has increased the demand of screening methods for oral drug absorption during preclinical trials, suggesting an interest in **cell culture models** for experimental prediction of intestinal permeability.

### Caco-2 cells.

Standard model for assesing the intestinal permeability and the most extensively characterized. However, its limitations must not be overlooked:

- Lack of correlation for active transported compounds due to underexpression of carrier proteins.
- Lack of correlation for paracellular absorbed compounds.
- Lack of drug metabolic enzimes such as isozyme (CYP)3A4.
- Cacophilicity: non-specific drug binding to Caco-2 cells, causing underestimation of permeability values.



Cell nuclei (red) and occludin fluorescent staining (green) revealing tight junctions.

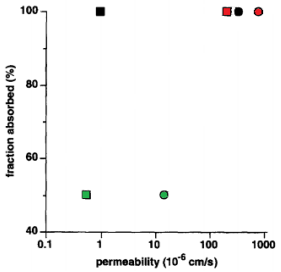
**Comparison of Caco-2 model and *in vivo* situation of typical drugs absorbed through different pathways:**

Squares → Caco-2 model
Circles → Human jejunum

Naproxen → Transcellular route: 2-to 4-fold slower rate

Atenolol → Paracellular route: 30- to 80-fold slower rate

L-dopa → Carrier-mediated: >100-fold slower rate




Data and figure obtained and modified from [1].

### Cell lines used for high-throughput screening.

Ideal cell-based model :

1. High capacity
2. Cost-effective
3. Predictive
4. Simultaneous study of drug transport and metabolism

| Cell line | Origin | Characteristics |
|---|---|---|
| Caco-2 | Human colorectal adenocarcinoma | ▪ Spontaneous enterocytic differentiation after 21 days.<br>▪ Polar cell morphology with an apical brush border and tight junctions between adjacent cells.<br>▪ Elevated trans epithelial resistance (TEER) involves smaller permeability values compared to human intestine. |
| MDCK<br>LLC-PK1 | Canine kidney cells<br>Pig kidney cells | ▪ Require only 6 days of culture to become differentiated.<br>▪ Ideal for transfections (used to study the role of efflux and influx transporters). |
| IEC-18 | Rat small intestine cells | ▪ No carrier-mediated transport of drugs can be determined.<br>▪ Size-selective barrier for paracellularly transported compounds. |
| 2/4/A1 | Rat fetal intestinal epithelial cells | ▪ Requires only 5 days of culture to become differentiated.<br>▪ Ideal for paracellularly absorbed compounds due to have leakier pores: pore radius is 9.0 0.2 Å (similar to human small intestine), comparing to 3.6 0.1 Å pore radius of Caco-2 cell line. |

## Caco-2 modifications.

Some efforts have been developed to overcome Caco-2 model weaknesses and make a further refinement.

### TC-7 Cells

Obtained from cloning passage nº81 of Caco-2 parental cell line. Expression of many metabolic enzymes that are absent in Caco-2.
Non-suitable model to predict absorption of paracellular and carrier-mediated compounds.

Does not represent a significant advantage respect Caco-2 cell line.

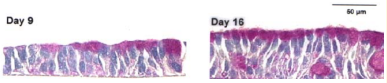
### Transfected Cells

DNA transfection with genes from efflux transporters (MDR1 and MDR2 from Multidrug Resistance Protein family) increasing the predictability of the model.

Has not been implemented to high-throughput screening because it is not enough simple and reproducible as Caco-2 model.

### Caco-2/HT29-MTX

Co-culture with HT29-MTX cell line, which is characterised to be mucin-producer model. Mimics better the *in vivo* situation than monocultures.
However, the role of mucus does not represent an important contribution to determine the permea-bility.

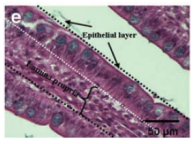



Mucus secretion in HT29/MTX cells as a function of culture period.

### Three-Dimensional model

Co-culture with Caco-2 and HT29/MTX is indirectly seeded with collagen gel and stromal cells (immunocytes and fibroblasts).

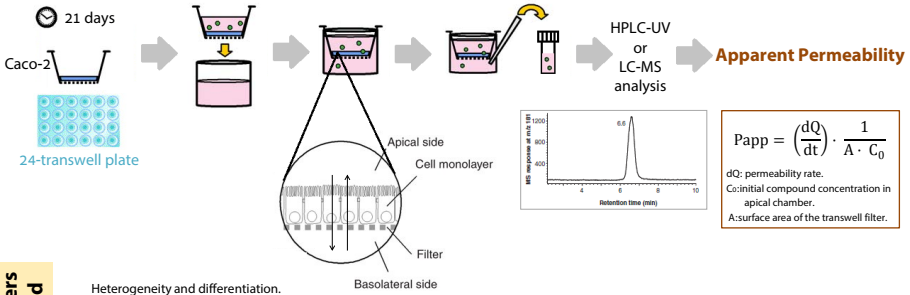
Lower TEER values and increased permeability values provides an improved correlation with *in vivo* situation.




SEM image displayed the uniform surface topology of the epithelial layer in the 3D coculture.

## Utility of cell culture assays to measure drug absorption into the discovery paradigm of new pharmaceuticals.

**1 Cell culture phase** **2 Transport experiment** **3 Data analysis**






$$Papp = \left(\frac{dQ}{dt}\right) \cdot \frac{1}{A \cdot C_0}$$

dQ: permeability rate.
$C_0$:initial compound concentration in apical chamber.
A:surface area of the transwell filter.

**Parameters assessed**

1. Heterogeneity and differentiation.
   Monolayer age and confluency .
   Cell passage number (25-100 passages).
   Cell seeding density (n2,5·10⁵ - 4·10⁵ cells/cm²).
   Filter membrane type, size and coating.
   Culture conditions (95% air, 5% $CO_2$; 37°C; 7,4pH).
2. Drug concentration.
   Agitation.
   Sampling method and schedule.
   Monolayer integrity measuring TEER.
3. Permeability equation.
   Method of drug analysis.
   Calculation from initial slope or entire plot.
   Measure appearance in receiver chamber.
   Measure disappearance from donor chamber.

### Higher throughput: Optimization.

Several approaches have been persued to screen major number of compounds in a short period of time:

- Automation of cell culture phase.
- Miniaturization by using 96-transwell plates.
- N-in-one analysis: 5-10 co-incubated compounds are simultaneously analysed. Potential dug interactions can occur and should be taken into account.

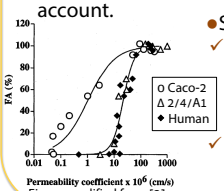



Figure modified from [3].

- Shorter is better:
  - ✓ Use of improved culturing media and modified well plates (BD BioCoat™) → 3 days of Caco-2 culture to reach confluency.
  - ✓ Use of another cell line → 2/4/A1 reduces culture time and posses better correlation than Caco-2 model.

**Conclusion.** Although intestinal aborption is a well known physiological process, a perfect model wich mimics perfectly its properties does not exist. However, Caco-2 model can be used to identify drugs with potential absorption problems, and also to select drugs with optimal passive absorption characteristics from series of pharmacologically active compounds generated. Then, **the principal challenge to reach an improved scenario is to optimize cell-based permeability assays by reducing costs and enable even greatrer applicability to drug discorvery programs.**

**References.**

[1] H. Lennernäs, et al., *"Comparison between active and passive drug transport in human intestinal epithelial (caco-2) cells in vitro and human jejunum in vivo."* International Journal of Pharmaceutics, vol. 127, no. 1, pp. 103–107, Jan. 1996.
[2] P. Artursson, K. Palm, and K. Luthman, *"Caco-2 monolayers in experimental and theoretical predictions of drug transport."* Advanced drug delivery reviews, vol. 46, pp. 27–43, 2001.
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[4] P. Balimane and S. Chong, *"A critique of cell culture models for intestinal permeability"* Drug discovery today, vol. 10, no. 5, pp. 335–343, 2005.
[5] R. van Breemen and Y. Li, *"Caco-2 cell permeability assays to measure drug absorption"* Expert opinion on drug metabolism toxicology, vol. 1, no. 2, pp. 175–185, 2005.

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