ADME Research Services Brief

Cell Permeability

Predict intestinal absorption, blood-brain barrier penetration, and tissue distribution of drug candidates

Cell permeability of a drug candidate can affect its absorption and distribution in the body and can pose a major challenge for drug development especially for orally administered drugs. Cell permeability assays measure the extent to which drug candidates diffuse across cell membranes and primarily evaluate intestinal absorption.

The assay designs allow the measurement of unidirectional or bidirectional permeability and inhibitors can be included to evaluate the effect of transporters on the permeability of a drug candidate.

BioIVT offers the following test systems to assess cell permeability:

- **Caco-2 cells:** This human colon carcinoma cell line is used to evaluate cell permeability and is a model for intestinal absorption and assessing whether a drug candidate is suitable for oral administration.
- **MDCKII-MDR1 cells:** This canine cell line expresses human P-gp (MDR1) and is often used to evaluate the potential of a drug candidate for blood-brain barrier penetration.

Study Design

Element	Feature		Standard
Design	Incubations performed in Transwell plates		✔
	Preliminary assessment	Stability	optional
		Cytotoxicity	
		Non-specific binding	
	Permeability assessment options*	Bidirectional A → B and B → A	✔
		± Inhibitor	optional
	Drug candidate concentrations		2 or 4
	Incubation time points		1 or 3
	Cultured Caco-2 cells or MDCKII-MDR1		✔
	Replicates		3
	Positive controls for cell permeability and transporter substrate		✔
Deliverables	Preliminary assessment (if included), permeability (P_{app}), % recovery		✔
	Standardized report with summary, materials/methods, results/conclusions, data tables/figures, supporting information		✔

*Rather than using a bidirectional assay the study can be designed to investigate unidirectional permeability (A → B or B → A)

Methodological Considerations and Test Systems

Caco-2 and MDCKII-MDR1 cell permeability assays are conducted in Transwell plates, which can be thought of as a cup within a cup as shown in Fig. 1 on page 2. A polarized monolayer of Caco-2 cells is grown on a permeable membrane that separates the two compartments. This creates a basal and an apical section on either side of the monolayer of cells. The drug candidate is then added to either the apical or the basal side to measure permeability in the apical to basal (A-B) or basal to apical (B-A) direction, respectively. To assess the bidirectional permeability of a drug candidate, both directions, i.e., A-B and B-A, are measured allowing calculation of P_{app} and the efflux ratio. Treatment with a known P-gp-specific inhibitor is included to separate active transport from true membrane permeability of the drug candidate.

Caffeine and mannitol are included in the assay as high and low permeability controls, respectively, and digoxin serves as the transporter substrate positive control as shown in Table 1.

Table 1. Assay Selection

Cell line	Positive control / probe substrate	Positive control inhibitor	Study type
Caco-2	Digoxin	Valspodar	definitive
MDCKII-MDR1	Digoxin	Valspodar/Verapamil	definitive

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Study Deliverables

BioIVT's cell permeability assay reports include the data for the preliminary assessment (stability, cytotoxicity, non-specific binding) and the results for permeability (P_{app}) and % recovery in graphic and tabular form as illustrated in Fig. 2 and Table 2.

Fig. 1. Illustration of a polarized monolayer of Caco-2 cells cultured in a Transwell plate to assess the cell permeability of a drug candidate (represented as yellow circles).

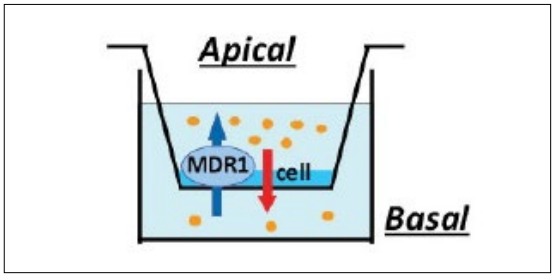

Fig. 2. Cell permeability of a drug candidate in a monolayer Caco-2 culture evaluated at three different concentrations.

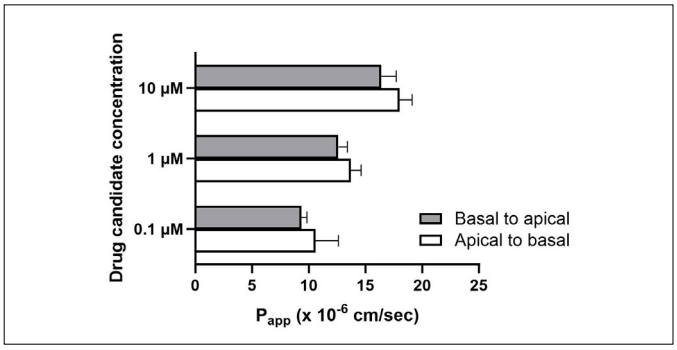

Table 2. Cell permeability of a drug candidate in a monolayer Caco-2 culture evaluated at three different concentrations.

Substrate (drug candidate)	Time (h)	Drug candidate transported (pmol) (mean ± SD)		P_{app} ($x10^{-6}$ cm/sec) (mean ± SD)		Efflux ratio
		Apical to basal	Basal to apical			
0.1 µM	0.25	0.565 ± 0.25	0.165 ± 0.030	10.6 ± 1.0	9.34 ± 0.49	0.883
	0.5	0.481 ± 0.204	0.469 ± 0.013			
	2	2.23 ± 0.19	2.39 ± 0.27			
1 µM	0.25	0.869 (n = 2)	1.97 ± 0.49	13.7 ± 0.9	12.6 ± 0.8	0.921
	0.5	5.61 ± 1.38	5.94 ± 1.08			
	2	26.2 ± 2.2	33.3 ± 3.4			
10 µM	0.25	18.7 ± 10.5	17.1 ± 2.1	18.0 ± 1.1	16.4 ± 1.3	0.910
	0.5	66.8 ± 18.7	65.3 ± 9.1			
	2	342 ± 36	401 ± 57			

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