Cell culture-based models for intestinal permeability: a critique

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The model systems that are currently used to determine the intestinal permeability characteristics of discovery compounds often represent a combination of high-throughput, but less predictive, *in silico* and *in vitro* models and low-throughput, but more predictive, *in vivo* models. Cell-based permeability models have been integrated into the discovery paradigm for some time and represent the 'method of choice' across the industry. Here, in addition to an objective analysis of the utility of cell culture models for permeability screening, anticipated future trends in the field of cell culture models are discussed.

Recent reports have put the final price of bringing a drug to the market at approximately US$1 billion dollars, with an estimated research time running into multiple years [1]. Considering the tremendous amount of time, effort and money that goes into discovering and developing medicines, it is imperative that the pharmaceutical industry constantly reinvents itself to stay afloat and grow in the competitive marketplace. Combinatorial and *in silico* chemistry, proteomics, genomics, robotics and miniaturization have all been steps in the right direction to reducing costs and expediting the drug discovery cycle. In parallel with these technological advances, a pragmatic conceptual awakening is also helping the industry to perform drug discovery with better economic sense. Compared with the old paradigm of drug discovery that was linearly oriented, the smarter drug discovery practiced today is matrixed and parallel in design. In the earlier linear design, new chemical entities were initially selected on the basis of their pharmacological activity, followed by sequential profiling to assess their ADMET characteristics. Such a strategy left only a small margin for error, and was generally more rigid, as well as more time- and resource-intensive. Newer drug design efforts incorporate a parallel matrixed approach to drug discovery, where the pharmacological efficacy is screened parallel to the initial ADMET profiling of compounds, providing more information for selecting superior quality drugs for further development. However, one of the cornerstones of such an approach is the availability of highly accurate, low-cost and high-throughput techniques that can provide fast and reliable read-outs on the developability characteristics of discovery compounds. Such screening techniques facilitate the selection of compounds with a greater probability of succeeding in the clinic, and also provide guidance to chemists on the design of better compounds. Thus, the task of screening discovery compounds for biopharmaceutical properties (e.g. solubility, intestinal permeability and metabolic stability) is now a major challenge facing the industry. Assessing permeability properties is a crucial step in determining the fate of an administered drug. This has provided a great impetus within the pharmaceutical industry to implement appropriate screening models that are high-capacity, cost-effective and highly predictive of *in vivo* permeability and absorption.

For a compound to be a successful medicine, it should have pharmacological activity coupled with adequate structural properties that enable it to reach

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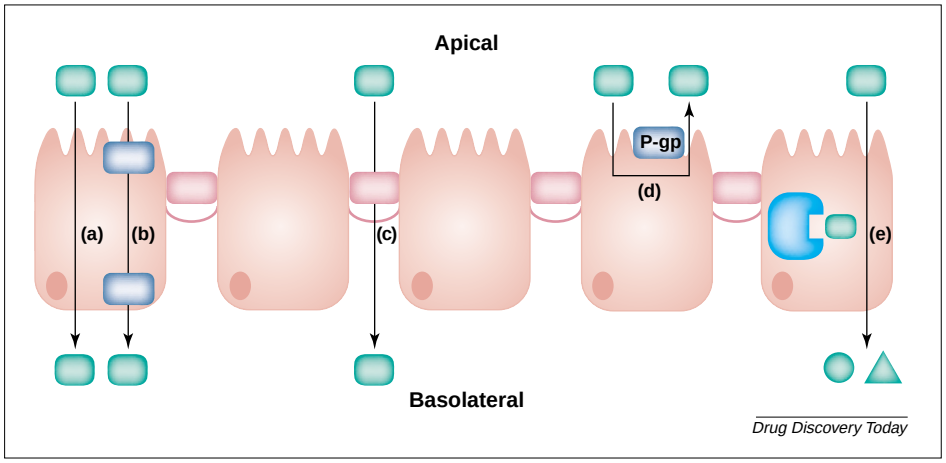

FIGURE 1

Different pathways for intestinal absorption of a compound. The intestinal absorption of a compound can occur via several pathways: **(a)** transcellular passive permeability; **(b)** carrier-mediated transport; and **(c)** paracellular passive permeability. However, there are also mechanisms that can prevent absorption: **(d)** intestinal absorption can be limited by P-gp, which is an ATP-dependent efflux transporter; and **(e)** metabolic enzymes in the cells might metabolize the compound.

the site of action intact. Consequently, it is also required to have reasonable permeability characteristics (i.e. it can freely travel through the multiple lipid bilayers in the system). Transport of drug substances across the intestinal membrane is a complex and dynamic process that includes the passage of compounds across several functional pathways in parallel. Passive transport occurs through the cell membrane of enterocytes (transcellular) or via the tight junctions between the enterocytes (paracellular). There are various functional influx and efflux mechanisms (via carriers and transporters) that dictate the permeability of compounds. Moreover, several different pathways are available via which molecules can travel from the lumen in to the systemic circulation (Figure 1).

Drug discovery scientists use many techniques when evaluating the intestinal permeability of drug candidates during the drug selection process [2–9]. The most pervasive preclinical methodologies currently used throughout the industry are: *in vitro* methods, for example, animal tissue-based Ussing chamber or membrane vesicles; cell-based assay systems such as Caco-2 cells and Mardin-Darby canine kidney (MDCK); artificial lipid-based systems such as parallel artificial membrane permeability assay (PAMPA) or immobilized artificial membranes (IAM); *in vivo* methods (whole animal pharmacokinetic studies); *in situ* methods (single-pass perfusion); and *in silico* (computer-aided drug design) methods. One, or a combination of these models, is routinely used in permeability assessment in drug discovery. A tiered approach is frequently used, which involves high-throughput (but less predictive) models for primary screening followed by low-throughput (but more predictive) models for secondary screening and mechanistic studies. Cell culture models strike the right balance between predictability and throughput and thus are the method of choice for permeability assessment across the pharmaceutical industry.

TABLE 1

Biological and physical characteristics of the human intestinal tract

Gastrointestinal segment	Surface area (m^2)	Segment length (cm)	pH of segment
Stomach	3.5	0.25	1.0–2.0
Duodenum	1.9	~35	4.0–5.5
Jejunum	184.0	~280	5.5–7.0
Ileum	276.0	~420	7.0–7.5
Colon and rectum	1.3	~150	7.0–7.5

Anatomy and physiology of the small intestine

The human small intestine is ~2–6 m in length and is loosely divided into three sections – duodenum, jejunum and ileum, which comprise 5%, 50% and 45% of the length, respectively: the biological and physical parameters of human intestinal tract are listed in Table 1 [10–12]. Approximately 90% of all absorption in the gastrointestinal tract occurs in the small intestinal region, the surface of which has various unique projections that significantly increase the potential surface area available for digestion and absorption. Macroscopic valve-like folds, called circular folds, that encircle the inside of the intestinal lumen are estimated to increase the surface area of the small intestine threefold. In addition, the presence of villi and microvilli increase the surface area by 30-fold and 600-fold, respectively.

The key function of the small intestine is the selective absorption of major nutrients. In addition, it serves as a barrier to digestive enzymes and ingested foreign substances. The epithelial cells in the intestinal region are a heterogeneous population of cells, which include enterocytes or absorptive cells, goblet cells (secrete mucin), endocrine cells, paneth cells, M cells and tuft and cup cells. Enterocytes are the most common epithelial cells and are thus responsible for the majority of the absorption of nutrients and drugs in the small intestine. Because enterocytes are polarized, having distinct apical and basolateral membranes that are separated by tight junctions, molecules are predominantly absorbed via mechanisms such as passive diffusion (paracellular and transcellular) and carrier-mediated processes (facilitated and active).

Cell culture-based permeability-screening models

Varieties of cell monolayer models that mimic *in vivo* intestinal epithelium in humans have been developed and currently enjoy widespread popularity. Unlike enterocytes, human immortalized (tumor) cells grow rapidly into confluent monolayers that exhibit several characteristics