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Representing Intestinal Drug Transport In Silico: An Agent-Oriented Approach

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Abstract—**A prototype Epithelio-Mimetic Device (EMD) was developed and tested. EMD components are designed to map logically to biological components at multiple levels of resolution. Those components are engineered to represent actual components within an *in vitro* cellular system used to study intestinal drug transport. Our goal is that the behaviors of the EMD closely match observed behaviors of the *in vitro* systems for a wide variety of drugs. Early stage system verification is achieved. The general patterns of experimental results from the EMD for a set of hypothetical drugs having a variety of physicochemical properties reasonably match observed patterns for a wide range of experimental conditions.**

Keywords—**Agents, computational biology, drug transport, modeling, simulation**

I. Introduction

Emerging, advanced computational methods [1] are expected to provide drug development and basic biomedical researchers with improved abilities to make useful predictions and to better understand how the small intestine, and other barriers to drug absorption, function in the presence and absence of various stresses, including disease, at multiple levels of organization. The envisioned methods will improve R&D efficiency. Here we present such a method. It is based on a completely new technology that is comprised in part of new tools for simulating complex biological processes, and a completely new class of biological analogue models that we refer to as biomimetic in silico devices (BISDs). The in silico components of these devices are designed specifically to be assembled into BIDS that represent behaviors of *in vitro* cellular systems or the intact intestine *in vivo*. Our approach consists of methods for developing, testing, and refining BISDs, as well as means for early stage system verification and evaluation. Early stage system verification is intended to verify that the BISD and its components perform as anticipated, to characterize their available modes of operation and their performances, and to become suitable objects for scientific experimentation, analogous to *in vitro* systems that they mimic. The devices are designed to generate behaviors and are constructed from software components that are specifically designed to map logically to biological components at multiple levels of resolution. The objective of this study has been to systematically collect evidence to support the feasibility of a prototype device designed to provide in silico experimental results for known and new compounds that, in time, will be experimentally indistinguishable from results from the current high through-put *in vitro* cellular systems, such as the industry standard, the Caco-2 cell line in use for studying intestinal drug absorption and metabolism.

II. Methodology

A. The In Vitro Membrane System

The class of BISDs described here is intended to mimic Caco-2 *in vitro* cell culture system that, in turn, is a biological model taken to represent the mature epithelia lining the villi of the small intestine (Fig. 1). Caco-2 cells are typically grown to confluence (e.g., 21 days) on the microporous membrane insert of the Transwell diffusion cell system, and are then used in drug transport studies (Fig. 2a). The Transwell insert is taken to represent the apical lumen compartment of the small intestine, and the cell culture well (into which the insert fits) represents the basolateral side of the intestine. The structure of our prototype epithelio-mimetic device (EMD), diagrammed in Fig. 2b and discussed more below, mimics the essential features of the Transwell system.

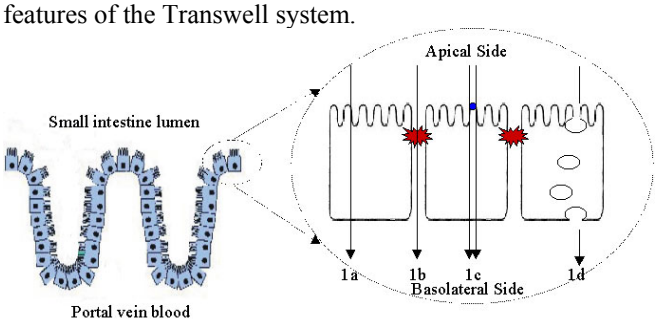

Fig. 1. Small intestine villi structure. The mature epithelia lining the villi are the active absorptive area. The enlargement shows the four processes involved in drug intestinal absorption: 1a: Passive transcellular diffusion; 1b: Passive paracellular diffusion; 1c: Transporter mediated active transcellular transport; and 1d: Transcytosis.

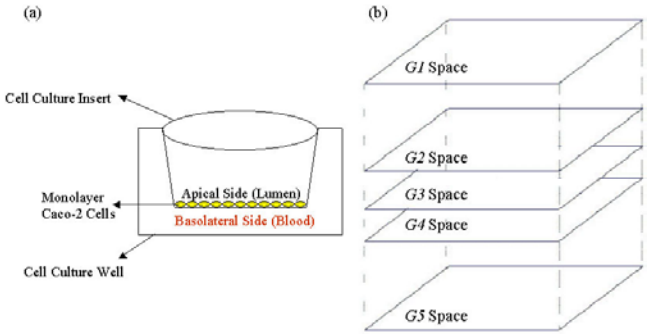

Fig 2. *In vitro* and in silico experimental devices for the study of intestinal drug transport. (a). Caco-2 in vitro Transwell system. (b). Our prototype epithelio-mimetic device (EMD).

B. A Constructive Approach to Modeling

Our approach to modeling includes some new and novel features. We use a constructive approach that focuses more on the aspects of biological system structure and behavior that give rise to data. We conceptually deconstruct the system into biologically recognizable components and processes that can be represented as software objects, agents, messages, and events. Next, we reconstruct using these objects within a software medium that handles probabilistic events and message passing, and can represent dynamic spatial heterogeneity. The process makes in silico *devices* capable of biomimetic behaviors. We use *device* (rather than model) to stress their modular, constructive nature, to emphasize the essential and desired properties described next, and to distinguish them from traditional equation-based (TEB) models. BISDs are not intended as substitutes or replacements for TEB models. They do not do the same things. We expect that they will dramatically expand the repertoire of modeling and simulation options available to researchers.

C. Essential and Desired Properties

Reaching our goals requires stipulating in advance several essential and desired properties that we believe EMDs need to have. Key among them is the following: EMDs and their components must be reusable, revisable, and easily updateable without having to re-engineer the whole device. Drug interactions with tissue and cell components are characterized by probabilistic events. So, our EMDs are exclusively event driven and most events can be probabilistic. They need to be flexible and adaptable to be useful in a variety of contexts for addressing a broad range of research questions and capable of exhibiting a broad range of behaviors. They should function at multiple levels of resolution, from subcellular to organ. EMD components must logically map to their biological counterparts. A goal is for EMDs to have more in common with *in vitro* and some ex vivo laboratory models, such as tissue cultures and perfused tissues, than they do with TEB models. So, evolvability is also essential. Spatial heterogeneity is a quintessential characteristic of the intestine at each organizational level. So, it is essential that device components be capable of representing *dynamic spatial heterogeneity* at different levels of resolution as required by the problem.

D. Epithelio-Mimetic Devices: Design and Function

Our methods depend upon Object-Oriented (software) Design. Objects are instances of classes with both state and behavior. Some objects can be agents. Agents are objects that have the ability to add, remove, and modify events. Philosophically, they are objects that have their own motivation and agenda; they can *initiate* causal chains, as opposed to just participating in a sequence of events something else initiated. An EMD is an agent whose purpose is to mimic aspects of the behavior *in vitro* Transwell cell culture devices. Agents can contain spaces, objects and other agents. When representing a biological system one might use agents to represent a tissue, cells within that tissue, and components within cells, such as macromolecules or networks of molecules.

An EMD has a minimum of five parallel spaces, *G1* – *G5*, as illustrated in Fig. 2b. Components are not introduced into a device unless it is required to solve the current problem or account for current or past data. Each space is currently represented as a $N \times N$ 2D grid. *G1* represents the apical lumen compartment and the fluid in the Transwell cell culture insert. *G2* represents the apical cell membrane and junctions between cells (viewed from the apical side). *G4* represents basolateral membrane and junctions between cells (viewed from the basolateral side). *G3* represents everything that is intracellular. *G5* represents the basolateral compartment and the fluid in the cell culture well. Solutes and drug molecules are represented by mobile objects that can move within and between spaces. Each mobile object is identified by type and has its own identity. In this study each object type represents a specific drug. Each MOLECULE[1] type has its own list of properties (applied to each object of that type). In this study the properties list includes three physicochemical properties: MW, pK_a, and P (partition coefficient). The list can be reduced or extended to include any number of properties.

The movement of an object is subject to the set of rules[2] and those rules can be adjusted based on properties of objects and space they are in. Within each space movement is governed by a simulated diffusion algorithm. Between different spaces, the movement is governed by probabilistic transition rules.

E. Early Stage EMD Verification

Our goal is that when a drug object representing a specific drug such as Alfentanil is placed in *G1* its in silico permeability in the EMD will be experimentally indistinguishable from its permeability measured *in vitro* in a particular experimental system. To accomplish this the grid point properties and the within and between space transition probabilities and rules need to be specified and calibrated so that the behavior of the EMD reasonably matches the accumulated literature evidence. That process requires an iterative sequence of adjustments, experiments, and comparisons. Early stage system verification is achieved when the general patterns of experimental results from the newly constructed EMD for numerous in silico drug objects reasonably match observed patterns for a wide range of actual drugs that cover a wide range of the physicochemical properties of interest.

F. Representing Permeation

When DRUG i is placed in *G1* an apparent permeability coefficient can be calculated from the appearance of DRUG in *G5* according to $P_{EMD,i} = (dQ_i/ds)/a \bullet c_0$, where $P_{EMD,i}$ is the

[1] The same word may be used to refer to a molecule or event in, or component of, the referent system and the corresponding in silico object or event. In the latter case the word is written using small caps.

[2] The rules can be simple or complicate; they may include an equation or depend on the outcome of running a separate model.

apparent permeability of DRUG *i* measured in silico, dQ_i/ds is the incremental change in the number of drug objects in *G5* following simulation (time) step *ds*, *a* is the area of *G2/G4*, and c_0 is the initial "concentration" of DRUG *i* in *G1* at time step 0. The various parameters and rules of the model, including the transition probabilities, are adjusted over a range of experimental conditions so that for several different DRUGS $P_{EMD} \sim$ {the apparent permeability coefficients measured *in vitro*} [2]. The in silico studies documented the influence of three interrelated physicochemical properties (lipophilicity, ionization, and molecular size [3]) on DRUG permeability, as well as concentration gradient on the permeation rate. We assume that small (≤ 200 Dalton) water-soluble DRUGS having *P* (partition coefficient) values less than 2 [4] pass through cell monolayers only by passive paracellular diffusion through aqueous pores. We also assume that transmembrane movement of the unionized fraction (calculated by environmental pH and the DRUG'S pK_a) of drug increases with increasing log*P* (increasing lipophilicity) values up to 2.5~3.5 and declines thereafter [5, 6]. MW is used as the simplified molecular size descriptor and is related to the in silico DIFFUSION COEFFICIENT ($D_{EMD,i}$): $D_{EMD,i} \propto 1/(MW_i)^n$). In these studies we assume $n = 1$ in lipoid membrane and 0.6 in aqueous fluid.

III. RESULTS

The following experiments and their results show the general relative patterns for results of experiments conducted using the prototype EMD and numerous DRUGS over the full range of EMD operation and over the full range of the three physicochemical properties of interest. The passive paracellular and transcellular transport of the prototype EMD were evaluated over a wide initial concentration range for two hypothetical drugs, both weak bases with $pK_a = 6.5$. One (DRUG I) is hydrophilic (log*P* = 0.2) with MW = 150. The other (DRUG II) is a hydrophobic drug (log*P* = 2) with MW = 500. The in silico pH of *G1* and *G3* was fixed at 7.4. EMD concentration-time profiles were recorded and the initial rate of DRUG permeation was calculated under sink condition (≤ 10% initial DRUG in *G1* is transported into *G5*). Results are presented in Fig. 3. Higher initial concentrations resulted in larger rates of permeation for both hydrophilic and hydrophobic DRUGS, as expected. Transcellular transport had a higher permeation rate than paracellular transport due to the smaller surface area in paracellular pathway (< 1000 fold) [7].

The rate of intestinal absorption is known to be positively correlated with partition coefficient [8]. Membrane permeability as a function of lipophilicity can exhibit different patterns: linear, hyperbolic, sigmoid and bilinear [3]. A set of sigmoidal relationships result from the additional influence of MW [3, 5]. The EMD was initialized to generate experimental results that reflect those

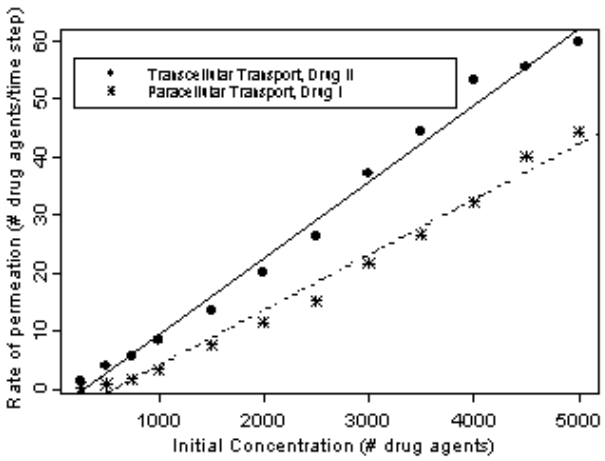

Fig. 3. Rate of permeation across an in silico EPITHELIA from experiments using the prototype EMD at 12 different initial numbers of DRUGS (representing initial concentration).

characteristics. To examine the consequences, the passive permeability of five sets of DRUGS (MW values of 100, 150, 300, 500, and 700) was calculated from EMD experimental results with log*P* values ranging from very hydrophilic (log*P* = – 2) to very hydrophobic (log*P* = 4). For DRUGS with log*P* < 3.5, a set of sigmoidal curves is observed (Fig. 4). For larger DRUGS, the curves are shifted to lower permeabilities. Four patterns are observed, consistent with the cited literature. DRUGS with small log*P* values are too hydrophilic to significantly cross cell membranes, so the passive paracellular pathway dominates for the two sets with MW ≤ 150. For more hydrophobic DRUGS paracellular transport is negligible and transcellular permeability is strongly dependent on lipophilicity. For those drugs with 2.5 ≤ log*P* ≤ 3.5, the expected permeability plateaus are reached. For more hydrophobic DRUGS, permeability decreases with increasing lipophilicity.

Each portion of the human gastrointestinal tract typically has a different pH. In the major absorptive part of small intestine duodenum, pH 6.0 - 6.5 favoring absorption of weak base drugs. We obtained results from EMD experiments for one weak base DRUG Alfentanil with properties (Table I) specified. We changed pH in *G1*(corresponding to a large variation in the degree of ionization for Alfentanil) and used Monte Carlo simulation for 8 experiments per pH value. Results are shown in Fig. 5. The rescaled permeability obtained in our EMD is in the range of 81.9 to 101.8×10^{-6} cm/sec when the *G1* properties correspond to pH 8.0 and the patterns observed are consistent with expectations [9].

IV. DISCUSSION

These results verify that for the conditions tested, the prototype EMD exhibits patterns of drug permeability behavior that are similar to those reported for *in vitro*

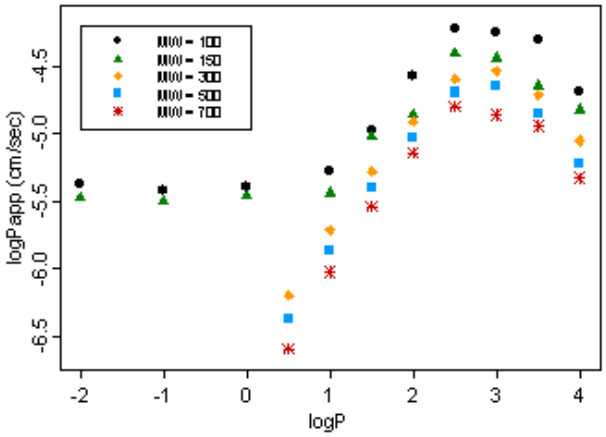

Fig. 4. Influence of log*P* and MW on DRUG permeability (logPapp) from experiments using the prototype EMD.

TABLE I
PHYSICOCHEMICAL PROPORTIES OF DRUG ALFENTANIL

Basic drug	MW	logP [a]	pKa [a]	$P_{app}(10^{-6}cm/sec)$ [b]
Alfentanil	416	2.16	6.5	93.5

[a] Data from [9]. There is slight difference from those obtained in Chemical Abstract Database, where Alfentanil's pKa is 7.59, and logP is 2.033.
[b] In Ref [9], 1.2 mM Alfentanil transported across Caco-2 cell monolayers at pH 8.0 at a low stirring rate of 100 rpm.

experiments. Additional experiments are underway to further verify that the EMD can generate experimental results that are experimentally indistinguishable from *in vitro* results for a variety of compounds. These verification studies have focused on passive transport through paracellular and transcellular pathways, the routes for a majority of drugs. However, in the intestine active transport and biotransformation of many important drugs do occur. These processes are part of the body's robust defense system against potentially harmful xenobiotics. P-glycoprotein, present on apical epithelial membranes, actively transports

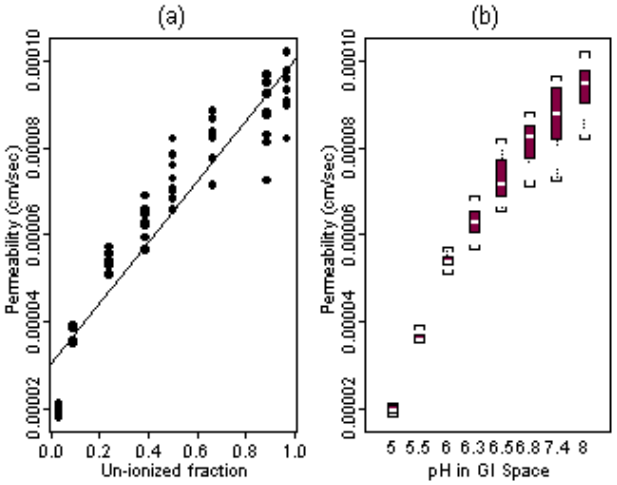

Fig 5. In silico pH-dependent transport of a DRUG Alfentanil within the EMD. (a) Simulated permeability has a linear relationship with fraction of un-ionized DRUG. (b) EMD permeability as a function of *G1* pH.

xenobiotics out of epithelial cells back into the lumen where they can be degraded by bacteria. CYP3A4, the dominant isoform of cytochrome P450 in epithelial cells, plays an important role in the first pass effect for many drugs. Our EMD is designed to easily add (and remove) components (agents, objects and spaces) that can map to transporters and enzymes to enable such an upgraded EMD to account for an increasing fraction of observed drug transport properties. Subsequently, guided by appropriate data, additional components can be designed and added to account for localized heterogeneous system properties including intracellular transport systems and gene up- and down-regulation.

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