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In Silico Representation of the Liver -- Connecting Function to Anatomy, Physiology and Heterogeneous Microenvironments

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Abstract—**We have built a collection of flexible, hepato-mimetic, in silico components. Some are agent-based. We assemble them into devices that mimic aspects of anatomic structures and the behaviors of hepatic lobules (the primary functional unit of the liver) along with aspects of liver function. We validate against outflow profiles for sucrose administered as a bolus to isolated, perfused rat livers (IPRLs). Acceptable in silico profiles are experimentally indistinguishable from those of the *in situ* referent based on *Similarity Measure* values. The behavior of these devices is expected to cover expanding portions of the behavior space of real livers and their components. These in silico livers will provide powerful tools for understanding *how* the liver functions in normal and diseased states, at multiple levels of organization.**

Keywords—**Drug metabolism, in silico, agent-based, modeling and simulation, computational biology, hepatic.**

I. Introduction

The liver is primarily responsible for the conversion of xenobiotics and endogenous compounds into more water-soluble, excretable forms. Liver disease alters the hepatic microcirculation and can impair the disposition kinetics of many drugs. Liver disease also complicates pharmacological treatments of other diseases, as well as itself, and can influence pharmacotherapeutic decisions. Modeling and simulation helps scientists and medical decision makers predict the likely consequences of various options, and thereby make better decisions. Multiple, different traditional models have been developed and validated ([1] and references therein). They include well-stirred compartments, the single tube, convection-dispersion, and interconnected tube model. Those models work well in accounting for the specific data and system conditions to which they are applied. However, they also have limitations, and among them are the following. Because the models are data-centric, considerable knowledge about hepatic structure and function are mostly ignored. Model components do not map well to specific biological components. In addition, their boundary conditions and parameterizations are restricted to one level of organization. Therefore, it is hard to reuse a model developed for one set of experimental conditions (or for one drug) under new circumstances (or for a different drug). Also, related models on the same system are hard to plug together.

When we discover something important about the transport and metabolism of a new set of drugs and wish to make intelligent decisions based on the likely clinical implications, we will need an already existing, validated model into which we can seamlessly import the new data in order to address the what-if questions of interest. No such models exist. Clearly, new ideas and new approaches are needed. From what direction should new modeling and simulations methods approach the biology: from the bottom-up or the top-down? Noble makes the case for a "middle-out" approach that focuses on the "functional level between genes and higher level function" [2]. We used such a middle-out approach in developing the silico liver described here. We use data drawn from multiple sources, including time series data from perfused liver studies. We focus more on aspects of the structures and behaviors that give rise to the data. We test and affirmed the hypothesis that perfused liver outflow data, in conjunction with other data, can be used to specify and parameterize a novel, physiologically recognizable hepato-mimetic device. Furthermore, that device can generate outflow profiles that are experimentally indistinguishable from the original *in situ* data.

II. Model Design

A. Histological and Physiological Considerations

The in situ liver perfusion protocol is detailed in [3]. A compound of interest is injected into the entering perfusate of the isolated liver. For each drug outflow profile there is a corresponding sucrose profile and it is the latter that we focus on first [1, 4]. Such profile pairing allows us to use a two-step feature extraction procedure for building hepato-mimetic devices: first, from the sucrose data extract information to represent the extracellular environment. That process leads to unique parameterizations for 1) vascular and sinusoidal graphs, 2) the arrangement and attributes of the sinusoids, and 3) the extracellular topography within sinusoids. We then expand that representation with essential intracellular details to account for the outflow data of different drugs.

The lobule is the primary structural and functional unit of the rat liver. Hepatic intralobular heterogeneity is well documented [5]. No two lobules are identical. Sinusoids separate meshworks of one cell-thick plates made up of hepatocytes that perform the major metabolic functions of the liver. Hepatocytes exhibit location-specific properties within lobules, including location-dependent expression of drug metabolizing enzymes [6]. Sinusoids experience

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different flows and have different surface to volume ratios within different zones. In addition to hepatocytes, lobules contain several specialized cell types. Endothelial cells line sinusoids that contain Kuppfer, stellate, and other cell types.

B. In Silico Components: Directed Graphs

A trace of flow within one lobule sketches a network that can be represented by an interconnected, directed graph. Such graphs are the core architectural feature of our lobule-mimetic devices (hereafter, LOBULE[1]). Teutsch et al. [7] subdivide the lobule interior into concentric zones. For now, we impose a three-zone structure on the graph (I, II and III in Fig. 1) and require that each zone contain at least one node and that a shortest path from portal vein tract (PV) to central vein (CV) will pass through at least one node and no more than one node per zone. The insert in Fig. 1 illustrates a portion of a graph network that connects in silico PV outlets through nodes in Zones I and II and then through one Zone III node to the CV. Graph structure is specified by the number of nodes in each Zone and the number of edges connecting those nodes. Edges are further identified as forming either inter-zone or intra-zone connections. Edges specify "flow paths" that have zero length and contain no objects. A solute object exiting a parent node is randomly assigned to one of the available outgoing graph edges and appears immediately as input for the SS located downstream at the child node. Randomly assigned intra-zone connections are allowed but are confined to Zones I and II. Nodes are randomly assigned to each zone so that the number of nodes in each zone is approximately proportional to the fraction of the total lobule volume found in that zone.

C. In Silico Components: Sinusoidal Segments

Agents called sinusoidal segments (SSs) (Fig. 2) are placed at each graph node. There is one PV entrance (effectively covering the exterior of the LOBULE) and one

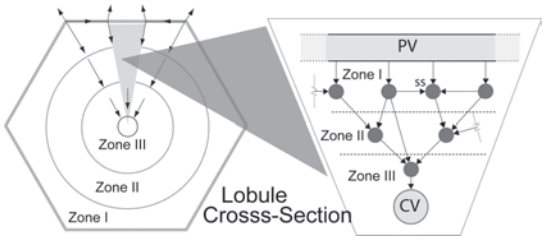

Fig. 1. A schematic of an idealized cross-section of a hepatic lobule showing half an acinus and the direction of flow between the terminal portal vein tract (PV), and the central hepatic vein (CV). SS: Sinusoidal Segment. The insert is an illustration of a LOBULE and corresponds to a portion of an acinus and a small fraction of a lobule.

[1] The same word may be used to refer to a biological molecule, component, or event and the in silico object or event that represents them. In the latter case the word is written using small caps.

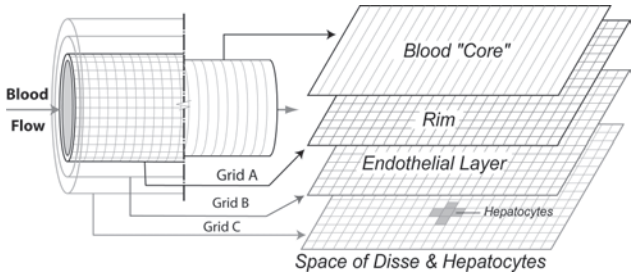

Fig. 2. Schematic of a sinusoidal segment (SS). Two types of SS are discussed in the text. One SS is placed at each node of the directed graph.

CV exit for each ISL. A solute object is a passive representation of a chemical as it moves through the in silico environment. The PV creates solute objects, as dictated by the experimental dosage function, and distributes them to the SSs in Zone I.

Movement of solutes from place to place within a lobule can be modeled as message passing. A solute object moving through the LOBULE represents molecules moving through the sinusoids of the lobule, and their behavior is dictated by rules specifying the relationships between SOLUTE location, proximity to other objects and agents, and the solute's physicochemical properties. Each SOLUTE has dose parameters (mass, constituents, timing, and catheter effects [1]) and a scale parameter (molecules per solute object). The relative tendency of a SOLUTE to move forward within a SS determines the effective flow pressure and this is governed by a parameter called *Turbo*. If there is no flow pressure (*Turbo* = 0), then solute movement is specified by a simple random walk. Increasing *Turbo* biases the random walk in the direction of the CV.

Viewed from the center of perfusate flow out in Fig. 2, a SS is modeled as a tube with a rim surrounded by other layers. The tube and rim are the sinusoidal space and its immediate borders. The tube contains a fine-grained abstract Core space that represents blood flow. Grid A is the Rim. Grid B is wrapped around Grid A and represents the endothelial layer. Another fine-grained space (Grid C) is wrapped around Grid B to collectively represent the Space of Disse, hepatocytes, and bile canaliculi. If needed, hepatocytes and connected features such as bile canaliculi can be moved to a fourth grid wrapped around Grid C. The properties of locations within each grid can be homogeneous or heterogeneous depending on the specific requirements and the experimental data being considered.

Objects can be assigned to one or more grid points. For example, a subset of Grid B points can represent one or more Kupffer cells. Another subset can map to intracellular gaps and fenestrae for particulate sieving into the Space of Disse. KUPFFER CELL activity can be added to the list of Grid B objects, as they are needed. Objects that move from a location on a particular grid location are subject to one or

more lists of rules that are called into play at the next step.

Within Grid B a parameter controls the size and prevalence of FENESTRATIONS and currently 10% of Grid B in each SS is randomly assigned to FENESTRAE; the remaining 90% represents cells. Similarly, within the grid where some locations map to hepatocytes, there is a parameter that controls their relative density.

D. Classes of Sinusoidal Segments (SS)

To further enable accounting for sinusoidal heterogeneity, including differences in transit time and flow [8], topographic arrangement [9], and the different surface to volume ratios within three zones [5], we defined different classes of SINUSOID, direct SINUSOID (*DirSin*) and tortuous SINUSOID (*TortSin*). Additional SS classes can be specified and used when needed. Relative to *TortSins,* the *DirSins* have a shorter path length and a smaller surface-to-volume ratio, whereas the *TortSins* have a longer path length and a larger surface-to-volume ratio. The circumference of each SS is specified by a random draw from a bounded uniform distribution. To reflect the observed relative range of real sinusoid path lengths, SS length is given by a random draw from a gamma distribution having a mean and variance specified by the three gamma function parameters, α, β, and γ.

E. Dynamics of Solutes within Sinusoidal Segments

Solute objects can enter a SS at either the Core or the Rim. At each step thereafter until it is metabolized or collected it has several options, most are stochastic. In the Rim or Core it can move within that space, jump from one space to the other, or exit the SS. From a Rim location it can also jump to Grid B or back to the Core. Within Grid B it can move within the space, jump back to Grid A or to Grid C. When it encounters an ENDOTHELIAL CELL within Grid B it may (depending on its properties) partition into it. Once inside, it can move about, exit, bind or not. Within Grid C it can move within the space or jump back to Grid B. When a HEPATOCYTE is encountered the solute can (depending on its properties) partition into it or move on. Once inside a HEPATOCYTE it can move about, exit, bind (and possibly get METABOLIZED) or not. Currently all objects within a HEPATOCYTE that bind can also METABOLIZE. The probability of a solute object being METABOLIZED depends on the object's properties. Once METABOLIZED the object is destroyed. The only other way to exit a SS is from the Core, Rim or into BILE (not implemented here). When the object exits a SS and enters the CV, its arrival is recorded (corresponding to being collected), and it is destroyed.

III. SIMILARITY MEASURE

Replicate *in situ* experiments conducted on the same liver provide similar but not identical solute outflow profiles. The same is true for experiments on LOBULES. There are two main contributors to intraindividual variability: methodological and biological. For replicate experiments in the same liver the coefficient of variation for fractional solute outflow within specific collection intervals typically ranges between 10 and 40%. A coefficient of variation can define a continuous interval bracketing the experimental data. A new set of results that falls within that bracketed range and has essentially the same shape is defined as being experimentally indistinguishable. The same should hold even if the data comes from an in silico experiment. There would be no way to determine whether a data set came from an *in situ* or an in silico experiment. This last observation provides the basis for designing and evaluating a Similarity Measure (SM).

The objective of the SM is to help select among various models of the liver, not simply to assume a model and select among variations on that model. Hence, the successful SM targets the various features of the outflow profile that correlate with the generative structures and building blocks inside the model. Neither an instantaneous, per observation, comparison, nor a whole-curve comparison is ideal because the different regions of repeated outflow profiles clearly show different variances. For these reasons, a multiple observation SM seems most warranted. However, for simplicity in these early stage studies we have assumed that the coefficients of variation of repeat observations within different regions of the curve are the same. In that way we can use a simple interval SM. A set of *in situ* outflow profiles, which are generated from different subjects following the same protocol, is used as training data. Calculate the mean of each time point and use this set of means as reference profile (P^r). For each observation in P^r, create a lower, P^l, and an upper, P^u, bound by multiplying that observation by $(1 - D)$ and $(1 + D)$, respectively. The two curves P^l and P^u are the lower and upper bounds of a band around P^r. The two outflow profiles are deemed similar if the second profile, P, stays within the band. The distance D used for sucrose is one standard deviation of the array of relative differences between each repeat observation and the mean observations at that time.

IV. IN SILICO EXPERIMENTAL RESULTS

In a normal liver each lobule is functionally similar. Consequently, a model representing the liver using one *average* lobule or even a portion thereof may be adequate to account for a specific drug outflow profile. For the first outflow profile we find the simplest LOBULE design, given restrictions: one that is comprised of the minimum components needed to generate an acceptably similar profile. An initial unrefined parameterization is chosen based on available hepatic physical and anatomic information. Components are added according to that initial parameterization. If the behavior of the resulting LOBULE is not satisfactory, any given piece of the LOBULE may be

discarded and replaced, or modified or reparameterized with minimal impact on the other components within the device. This process continues until the LOBULE generates outflow profiles that are experimentally indistinguishable: after averaging several runs, the result falls between P^l and P^u. Once that parameterization is found, the parameter space is searched further [10, 11] for additional, possibly better-performing, solution sets[2].

The typical *in situ* IPRL outflow profile is an account of approximately 10^{15} drug molecules percolating through several thousand lobules. The typical in silico dose for *one* run with one LOBULE is on the order of 5,000 drug objects, where each drug object can represent a number (≥ 1) of drug molecules. Thus, a single outflow profile will be very noisy and will be inadequate to represent the referent *in situ* profile. In the latter part of such an outflow profile it is increasingly possible to encounter a collection interval during which no drug objects are collected. Another independent run using that same LOBULE, parameter settings, and DOSE will produce a similar but uniquely different outflow profile. Changing the random number generator seed alters the specifics for all stochastic parameters (e.g., placement of SSs on the digraph), thus providing a unique, individual version of the LOBULE, analogous to the unique differences between two lobules in the same liver. One in silico *experiment* combines the results from 20 or more independent runs using the same LOBULE thereby producing an outflow profile that is sufficiently smooth to use the SM. It takes one or more *experiments* to represent an outflow profile for an in silico liver. Note that an experimental result comprising 20 runs of the LOBULE is analogous to results one might obtain if one could conduct an *in situ* perfusion experiment on only 4-to-5 liver lobules.

Fig. 3 shows results from one parameterization of a LOBULE against an IPRL sucrose outflow profile. The shaded region is a band enclosing the mean fraction of dose collected for each collection interval. The width of the band is ± 1 std about the mean. The filled circles are results obtained using a LOBULE parameter vector that provides an acceptable solution set according to the SM.

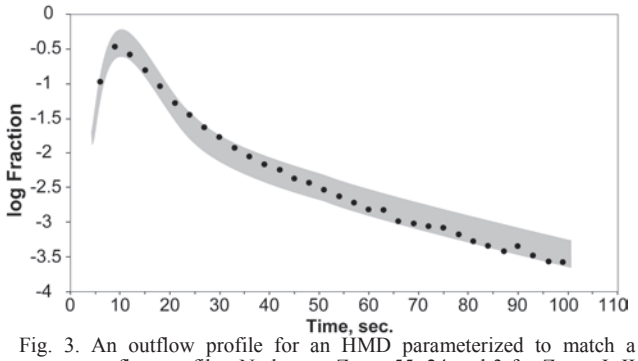

Fig. 3. An outflow profile for an HMD parameterized to match a sucrose outflow profile. Nodes per Zone: 55, 24 and 3 for Zones I, II and III, respectively; total edges: 60; intra-zone connections: Zone I = 10, Zone II = 8, Zone III = 0; inter-zone connections: I→II = 14, I→III = 4, II→III = 14; SSs: 50% S_A and S_B; Number of runs = 100

V. Conclusion and Discussion

We have tested and affirmed the hypothesis that IPRL outflow data obtained following bolus administration of sucrose can, in conjunction with other data, be used to specify and parameterize a physiologically recognizable hepato-mimetic device, an in silico liver. Furthermore, that device can generate outflow profiles that are experimentally indistinguishable from the original *in situ* data. This new technology is intended to provide powerful tools for optimizing the designs of real experiments and for challenging our understanding for *how* mammalian systems, such as the liver, function in normal and diseased states, when stressed, or when confronted with interventions. These biomimetic devices are expected to evolve to become suitable experiment platform to test hypothesis.

[2] *Solution set* is the combination of the inputs and parameters that result in the biomimetic behavior, plus the actual device that is biomimetic.

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