In vitro methods could be described as systems where chemicals require use of multiple tissue types connected with a common perfusate. This arrangement allows for integration of absorption, metabolism, and toxicity data over extended times in vitro and provides a novel, animal-free tool for chemical, cosmetic, and pharmaceutical testing. In order to test this, a study on the uptake and distribution of acetaminophen (APAP) in a human dynamic multi-organ plate (HuDMOP™) with three tissue-surgeries arranged in series: first absorption across a human 3D intestine (Epithelial, MatTek Corp.) then to a liver surrogate with human primary hepatocytes in sandwich culture and then to a kidney preparation (human renal proximal tubule cells) was developed. A common perfusate with human albumin connected the three compartments. APAP was placed on the apical side of the intestinal surrogate at 0 and 24 hr. Samples were collected from all three compartments over time and analyzed for APAP by LC/MS/MS and cytoxicity by LDH leakage. The APAP in the apical reservoir peaked to 60 µM at around 4 hours with a total uptake of 72% of the applied dose entering the first reservoir. A simple PK model was developed to describe the three cellular platforms and their physiological arrangement. Mass balance equations were fit to experimental data to estimate uptake and transport characteristics. The inter-chamber flow rates and fitted experimental absorption rate constant. 0.79 hr⁻¹, were consistent with a Cₚ₀ of 42.0 µM and time of maximum concentration between 3 and 4 hr in the intestine compartment. With the current platform flow rates, much lower concentrations were present in the subsequent two compartments (liver and kidney) with maximum observed concentrations of 4.5 and 2.5 versus 3.1 and 0.9 µM predicted. The interplay between platform modeling and model-directed technical improvements will make the HuDMOP™ results more directly applicable to in vivo behavior of various chemicals.

Preparation of Plates: HuDMOP™ custom designed plates (Figure 1) were used and equipped with a simulated blood system. The simulated vascular system consisted of tubing connected to a semipermeable membrane. The section of semipermeable membrane was 3 cm in length. The tubing was custom fit into the plate, such that only the semipermeable membrane was in contact with each organ compartment. A perfusion rate of 5 µl/min was used in each experiment.

Cell Culture

Intestinal Compartment: The Epithelial™ 3D human tissue from MatTek Corp. was used for the intestinal chamber. Tissues were cultured under standard conditions on transwell inserts. Tight junctions were assessed by transepithelial electrical resistance (TEER). The Epithelial™ tissues were placed into the HuDMOP™ plates (Figure 1) and connected to the liver compartment via simulated blood system (Figure 4).

Liver Compartment: The liver compartment was cultured with Transporter Certified™ human primary hepatocytes from BDIVT in sandwich culture. The cells were added to the HuDMOP™ cup in culture media at a density of 300,000 cells/well and incubated at 37°C, 5% CO₂ for 48 hr prior to beginning the experiments.

Kidney Compartment: To simulate a kidney human renal proximal tubule cells from Lonza were used. The cells were added to the HuDMOP™ cup in culture media at a density of 1.1 x 10⁶ cells/well and incubated at 37°C, 5% CO₂ for 5 days prior to beginning the experiments.

Dosing Regimen: After equilibration, the test material was added to the apical side of the intestinal chamber to simulate an oral exposure at time 0 and 24 hr. For acetaminophen (APAP) the dose was from a 2500 µM stock, while for cycloheximide (CyHex) the dose applied was 100 µM from a 100 µM stock.

Analytical Procedures: APAP and CyHex were measured by LC-MS/MS. Standard curves and QC samples were prepared in PBS and compared to standard curves and QC samples in media with and without serum.

In order to better understand the in vitro system a pharmacometric model was developed. Absorption across the HIE into the intestine compartment was simulated as a first-order absorption process. Intestine, kidney, and liver compartments were described by a volume, flow rate, and clearance rate. The final collection compartment was simulated as a sink accumulating any compound not retained or removed by the previous compartments. Compartment volumes were from 2.5 to 3 mL, and the flow through the system was 5 μl/min. The compartments are assumed to be well-mixed and in equilibrium with the semipermeable tubing perfusing the system. APAP and cycloheximide experiments were used to fit the absorption and clearance rates:

1. Concentration in compartment 1 (Intestine) → First-order absorption rate constant (ka)
2. Concentration in compartment 2 (Liver) → Michaelis-Menten mechanism (Vmax and Km). Partition between media and tissue (Pm)
3. Concentration in compartment 3 (Kidney) → First order elimination constant in the kidney (ku)

The model outputs for all compartments and the model output in the collection were exported and plotted using Microsoft Excel.

Figure 2: Schematic of the Model with Equation

Table 1: Chemical parameters fitted or used in the model.

![Table Image](image)

RESULTS

![Figure 4: Amount collected the collection compartment for APAP](image)

![Figure 5: Amount collected the collection compartment for cycloheximide](image)

CONCLUSIONS

In vitro methods capable of describing systemic effects of chemicals require use of multiple tissue types connected with a common perfusate. This arrangement allows for integration of absorption, metabolism and toxicity data over extended times in vitro and provides a novel, animal-free tool for chemical, cosmetic, and pharmaceutical testing. Integration with computational modeling is key to transitioning these unique data to in vivo application, and the interplay between platform modeling and model-directed technical improvements will make the HuDMOP™ results more directly applicable to expected in vivo behavior of various chemicals. The current data provide a basis for in silico modeling of the in vitro system. The computational model predictions represent the data well, though there appears to be more abrupt appearance in the final perfusate collection for both chemicals, and the case is under investigation. Current thoughts for computational probing include the possibility for non-specific binding to plastic IMS system, and rate-limiting uptake into and out of the semipermeable membrane perfusing the compartments.

The computational modeling approach is increasingly used as a way of mathematically representing, interpreting, and extrapolating experimental data from in vitro (and in vivo) systems. The development and model-based interrogation of such novel in vitro systems to better inform chemical kinetics and toxicity in future testing of chemicals holds significant promise for reducing animal use, time, and money, with the ultimate goal of predicting human kinetics and toxicity without animal testing.

FUTURE DIRECTIONS

The ultimate goal of this partnership of a novel in vitro system and computational modeling is to predict the human health effects of chemical exposure. Through iterative computational and laboratory innovation, we can achieve this goal.