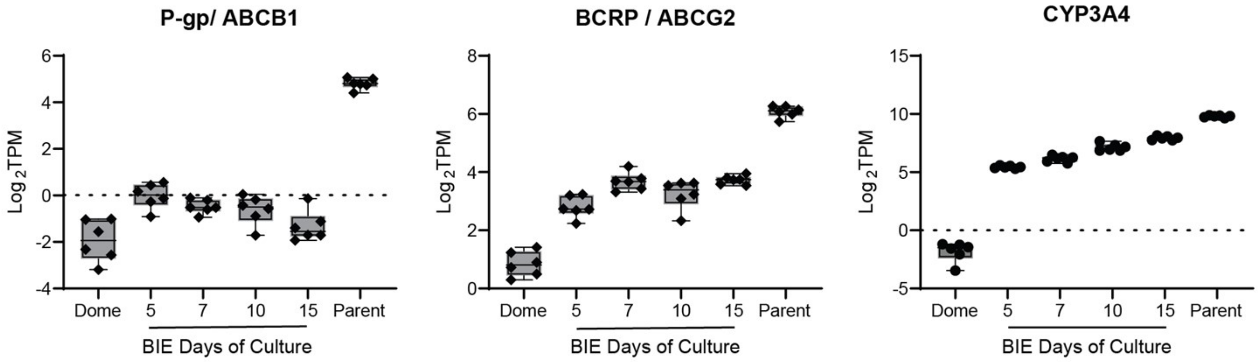
Supplementary information

Initial bulk RNA-Sequencing characterisation of DT/DME in one donor



Supplementary Figure 1: Initial bulk transcriptomic characterization in one organoid donor across a time course was used to refine a culturing window for later functional characterization across multiple organoid donors. Box plots show key DME and DT genes of interest: CYP3A4, P-gp and BCRP.

Bulk RNA-sequencing Heatmap of DME and DT genes of interest

Mean of log2 TPM per Condition and Gene

Gene	Parent Tissue	Caco-2 Day 21	Dome culture D0	TW Day 7	TW Day 10	BIE Day 7	BIE Day 10
ABCB1 (P-gp)	4.30	1.98	1.55	3.33	3.81	2.68	2.78
ABCG2 (BCRP)	4.76	3.55	1.69	4.05	4.40	4.23	4.44
ABCC1 (MRP1)	1.50	3.50	4.58	3.26	3.30	3.77	3.36
ABCC2 (MRP2)	6.10	7.22	2.60	3.81	4.18	4.41	4.59
ABCC3 (MRP3)	6.02	7.57	7.67	7.65	7.32	8.27	8.11
SLC10A2 (ASBT)	2.01	3.35	−6.08	−3.44	−2.90	−4.72	−4.66
SLC15A1 (PEPT1)	8.43	7.11	3.85	7.51	7.72	7.08	7.35
SLC16A1 (MCT1)	2.87	5.50	6.07	5.13	4.97	5.44	5.36
SLC22A1 (OCT1)	−2.00	0.25	−1.66	−0.34	−0.66	−0.31	−0.94
SLC22A3 (OCT3)	−1.40	2.53	2.64	2.39	2.21	2.42	2.14
SLCO4A1 (OATP4A1)	−0.26	5.38	4.57	2.97	3.35	4.27	4.13
SLCO2B1 (OATP2B1)	5.10	8.90	4.59	6.58	6.60	6.53	6.42
CYP3A4	8.98	−0.40	0.03	7.25	8.22	7.35	8.38
CYP3A5	7.99	2.73	6.06	7.81	8.11	8.35	8.07
CYP2C9	7.28	4.49	5.80	7.57	7.32	7.57	7.31
CYP2C19	6.10	5.19	4.93	6.45	6.21	6.74	6.56
CYP1A2	−6.64	−5.28	−5.31	−1.92	−3.07	−3.27	−3.60
CYP2D6	5.72	2.19	1.00	4.78	4.84	4.81	4.38
UGT2B7	4.42	3.93	−0.39	5.25	5.85	4.86	5.66
UGT1A1	3.34	−0.17	4.70	5.24	4.33	5.21	4.99
CES1	−0.75	8.64	−2.06	−0.43	0.21	1.63	3.39
CES2	8.73	6.16	8.12	10.52	10.61	10.47	10.47
ALPI	8.53	5.12	−0.44	6.55	6.65	5.87	6.38
SI	8.77	5.42	4.92	7.77	7.63	8.50	9.00

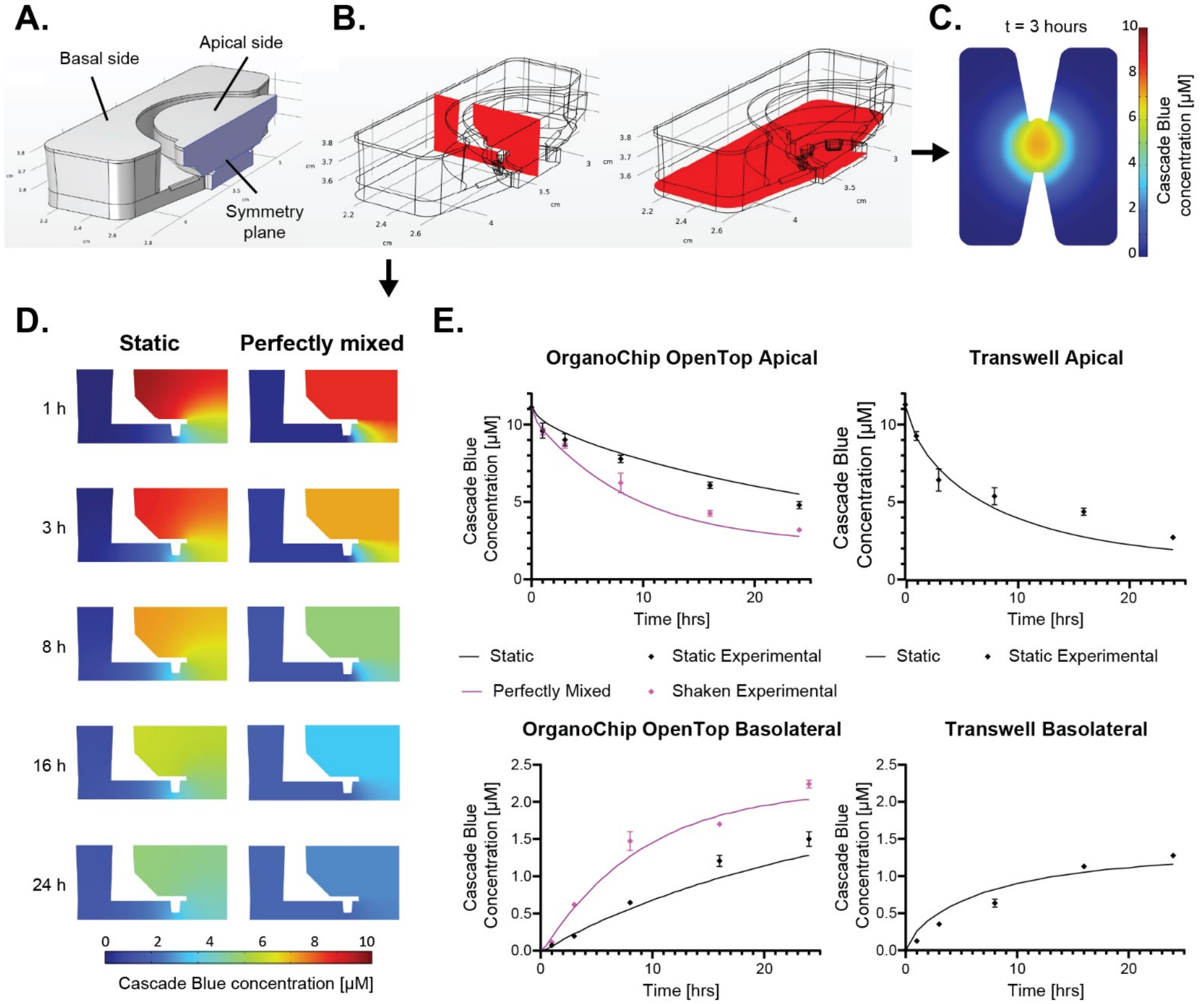
Supplementary Figure 2: Heatmap of genes of interest related to DTs and DMEs. Values displayed are the mean across all technical and biological replicates, with higher log_2TPM (transcript per million) values appearing in red and lower values appearing in blue. Parent values are from a single donor with 6 technical replicates, Caco-2 values are from 2 separate experiments with 6 technical replicates each, dome culture samples are from 3 organoid lines, with 2-6 technical replicates for each donor. All TW and BIE samples are an average of 3 organoid donors, with 6 technical replicates for each.

Modelling Small Molecule Diffusion through the Micropatterned Hydrogel

To design compound permeation experiments with appropriate timing, detection sensitivity, and similar applicability to classical TW barrier models, it is necessary to characterize molecule transport through the OpenTop OrganoChip. First, the volumes at which the apical and basolateral compartment share the same liquid level were determined experimentally via measuring the efflux ratio of cascade blue. This was important to isolate the effect of passive molecule diffusion from gravitationally induced hydrostatic pressure flows and surface tension effects (100 µL apical, 210 µL for each basolateral). These volumes, the exact geometry from the computer aided design files of the OpenTop OrganoChip and the standardized hydrogel volume of 21 µL were used to create a COMSOL model.

The physics module used was transport of diluted species with a time dependent study. For computational simulation efficiency, a plane of symmetry was used as depicted in suppl. Figure 3A, while no flux conditions were selected for all other wall conditions. The model mesh contained 39793 mesh vertices and 196843 tetrahedra. A diffusivity of 3.81 x 10-10 m^2/s was estimated using the Stokes–Einstein–Sutherland equation and a molecular diameter of 1.083 nm in a DMEM medium of viscosity 0.011 Pa.s at 37°C. To simulate static conditions, this diffusivity was applied to all the geometrical domains, including the hydrogel, as it was postulated that its porosity is high, leading to a negligible effect on small molecule permeation. To simulate perfect transport conditions, this diffusivity was limited to the hydrogel where no mixing or flow is assumed to occur due to shaking due to the very high surface to volume ratios in the gel porosity. In this case a comparatively infinite diffusivity (1 m^2/s) was applied everywhere else. The experimental set-up of a routine Cascade blue barrier permeability was applied to the model, with 10 µM applied apically in base medium (Advanced DMEM, Gibco) with concentration profiles generated in the XY and XZ plane (Suppl. Figure 3B). The model was simulated over 24 hours, covering a relevant time-frame for a range of different drug permeability and metabolism studies. Suppl. Figure 3C shows the simulated XY concentration gradient near the base of the OpenTop OrganoChip at 3 hours, illustrating the radial diffusivity of cascade blue from the hydrogel to the surrounding basolateral volume. Supplementary figure 3D displays the XZ cross-sectional concentration profile at 5 timepoints, under static and perfectly mixed conditions, allowing to visualize the transfer of molecules from the apical to the basolateral volumes. Experimental data was collected to compare to the computational simulation (Suppl. Figure 3E). For the sampling of experimental samples, the entire volume of each apical and basolateral compartment was thoroughly mixed to ensure the sampled concentration was an accurate measure of the average concentration to enable comparison to the COMSOL simulation. The perfectly mixed experimental condition had the OpenTop OrganoChip slides placed in a standard cell culture orbital shaker. To determine a diffusion coefficient for cascade blue in our base media the experiment was completed in parallel with standard 24-well TWs (Greiner). Direct comparison of apical and basolateral concentration over time is not possible between the OpenTop OrganoChip and TWs as the geometry and experimentally determined apical/ basolateral volume ratio where gravitational flow is avoided is significantly different. Applying the same diffusion coefficient that fits the TW experimental data to the COMSOL simulation of the OpenTop OrganoChip, including the hydrogel, also fits the corresponding experimental data reasonably well under both static and mixed conditions.

This would indicate that cascade blue diffuses through the hydrogel component of the OpenTop OrganoChip at a similar passive rate to an equivalent volume of basement media and the hydrogel does not significantly impede its permeation. Although cascade blue is an appropriate molecular size to benchmark small molecule compounds, this simulation does not model physicochemical properties such as non-polar and hydrophobic molecules that might have a propensity to bind to the hydrogel. Further work could investigate a range of different compound properties and large molecules to determine broad applicability of the platform across compound classes.



Supplementary Figure 3: Simulation of Cascade Blue diffusion across the OpenTop OrganoChip. (A) COMSOL model geometry and plane of symmetry. (B) XZ and XY planes to generate profile views. (C) XY concentration profile of Cascade Blue after 3 hours of passive permeation. (D) XZ concentration profiles under static and perfectly mixed simulation conditions at a sample of time-points. (E) Simulations of apical and basolateral concentration-time curves in static or shaken conditions together with experimental data (depicted by data points).

Supplementary Materials & Methods:

Patient-derived intestinal organoid donor information

Supplementary Table 1: Organoid Donor Information

Donor	Age	Gender
1	76	Female
2	83	Female
3	83	Female
4	63	Male
5	71	Male
6	61	Female

Primary Antibodies

Supplementary Table 2: Primary antibodies

Antibody	Dilution	Species	Vendor	Catalog #
MDR1/ABCB1 (E1Y7B) mAb	1:100	Rb	Cell Signalling Technology	13342
CYP3A4 mAb (3H8)	1:200	Ms	Invitrogen	MA5-17064
Anti-BCRP/ABCG2 antibody [EPR20080]	1:200	Rb	Abcam	ab207732
Anti-Villin antibody [SP145]	1:100	Rb	Abcam	ab130751
Anti-SOX9 antibody [EPR14335-78]	1:800	Rb	Abcam	ab185966
MUC2 Monoclonal Antibody (996/1)	1:100	Ms	Invitrogen	MA5-12345
FABP1 Polyclonal Antibody	1:100	Rb	Invitrogen	PA5-28945
Ki-67 Monoclonal Antibody (SolA15), eBioscience™	1:500	Rat	Invitrogen	14-5698-82
E-Cadherin Mouse Monoclonal (36)	prediluted	Ms	Ventana	790-4497
DAPI 4',6-diamidino-2-phenylindol	1:1000		Roche	

Chromatography & MS/MS Parameters

Supplementary Table 3: Additional LC-MS/MS info for bidirectional transport studies

Samples from bidirectional transport studies	
Total flow (mL/min)	0.6
Column	Ascentis Express C18 2.7 µm, 2.1 x 20 mm
Column Temp [°C]	60
Injection volume [µL]	2
Solvent A	0.5 % formic acid in 95 % water and 5 % acetonitrile OR 20 mM ammonium bicarbonate in 95 % water and 5 % acetonitrile*
Solvent B	100 % acetonitrile

Time [min]	% Solvent B
0	0
0.26	0
0.90	95
1.23	95
1.33	0
1.40	0

* only atenolol samples were analyzed with solvent A containing ammonium bicarbonate

Supplementary Table 4: MS/MS parameters in MRM acquisition mode and retention time (RT) for bidirectional transport study

Compound	Q1 [Da]	Q3 [Da]	DP [V]	CE [V]	RT [min]
Edoxaban	548.1	366.1	106	27	0.63
PhiP	225.0	210.2	136	41	0.59
Atenolol	267.2	190.2	66	25	0.56
Propranolol	260.1	116.1	61	25	0.54
Internal standard (Oxazepam)	287.1	241.1	51	31	0.71

Supplementary Table 5: Chromatography program for analysis of midazolam and its metabolites

Midazolam & its metabolites	
Total flow [mL/min]	0.7
Column	LC column: Waters Acquity UPLC BEH C18 1.7 µm, 2.1x50 mm
Column Temp [℃]	50
Injection volume	5 µL
Solvent A	0.1% formic acid in water
Solvent B	0.1% formic acid in acetonitrile and 10% Mobile phase A
Time [min]	% Solvent B
0	5
0.2	5
2	50
2.01	95
2.5	95
2.51	5
3	5

Supplementary Table 6: MS/MS parameters in MRM acquisition mode and retention time (RT)

Compound	Q1 [Da]	Q3 [Da]	DP [Volts]	CE [Volts]	RT [min]
Midazolam	326.099	249.161	150	55	2.25
1-OH-Midazolam	342.066	203.115	130	55	2.21
1-OH-Midazolam-Glucuronide	518.161	324.139	140	35	1.99
Internal Standard (Labetalol)	329.2	162.1	76	37	2.01

Comment [|]: Reviewer 1, minor comment 2. Correction of 106 to 10^6

BIE input intestinal DMPK parameters

Supplementary Table 7: BIE and input intestinal DMPK experimental parameters

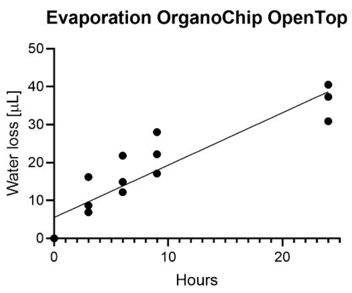
Parameter	Units	Value
BIE surface area	cm2	0.195
BIE cell number	10^6 cells	0.356
OpenTop OrganoChip evaporation constant	µL/min	0.0077
Initial (MDZ) concentration	µM	1
Sampling volume	µL	16
Apical volume	µL	100
Apical sampling times	min	60, 240, 480
Basolateral volume	µL	386
Basolateral sampling times	min	30, 60, 90, 240, 360, 480
Fraction of unbound midazolam in incubation media: $F_{u,inc}$ (MDZ)	-	0.0845
Fraction of unbound 1'-OH- midazolam in incubation media: $F_{u,inc}$ (1'-OH-MDZ)	-	0.0872
Fraction of unbound 1'-OH-midazolam-glucuronide in incubation media: $F_{u,inc}$ (1'-OH-MDZ-Glu)	-	0.613

Evaporation Constant

A constant term for the rate of evaporation (k_{ev}) was determined by measuring milligram changes in weight over time in three OpenTop OrganoChip slides under experimental incubation conditions (Supplementary Figure 4). Weight changes were taken as a proxy measurement for the change in volume over time.

$$k_{ev} = \frac{V_i - V_f}{incubation\ time}$$

where V_i and V_f refer to the initial and final volume.



Cell Number

Cell number in the BIE was quantified via directly peeling the tissue and dissociation in TrypLE express solution (Gibco) containing 250 U/ml DNaseI (Roche) and 10µM Y-27632 (Tocris) for 15-30 min at 37 °C. Once a single cell suspension was obtained, a cell count was estimated using the Countess automated cell counter.