Supplementary information

1) Kirchhoff matrix approach

The equivalent circuit model, as depicted in figure 3 of the paper can be solved using two approaches: the Kirchhoff matrix approach and the analytical model approach. Each of these approaches has its own benefits and drawbacks.

To calculate the equivalent resistance of the membrane area as depicted in fig. 3 (not including the inlet and outlet channel resistance) of the chip, depicted by l_{mem}, a Kirchhoff matrix M of 2n nodes can be made. For the easiest case where n=2, this matrix looks like:

$$M = \begin{bmatrix} (G_{T1}+G_{M1}) & -G_{T1} & -G_{M1} & 0 \\ -G_{T1} & (G_{T1}+G_{M2}) & 0 & -G_{M2} \\ -G_{M1} & 0 & (G_{M1}+G_{B1}) & -G_{B1} \\ 0 & -G_{M2} & -G_{B1} & (G_{M2}+G_{B1}) \end{bmatrix}$$

with G the conductance (reciprocal) of the corresponding resistance. The nodes are numbered left to right, top to bottom. The circuit looks as follows for n=2, the numbers 1 to 4 (in red) indicate the nodes:

Scheme S1 Example of the circuit shown in Fig. 3, for the easiest case where n=2 for explanation purposes.

From the Kirchhoff matrix M, the conductance matrix C can be derived, assuming node 4 (connected to outlet channel B) is connected to ground.

$$C = \begin{bmatrix} (G_{T1}+G_{M1}) & -G_{T1} & -G_{M1} \\ -G_{T1} & (G_{T1}+G_{M2}) & 0 \\ -G_{M1} & 0 & (G_{M1}+G_{B1}) \end{bmatrix}$$

Next, a boundary conditions matrix Q is defined, which is in this case the applied current i to port A (node 1).

$$Q = \begin{bmatrix} i \\ 0 \\ 0 \end{bmatrix}$$

Finally, the potential (V) at each node can be calculated using Ohms law.

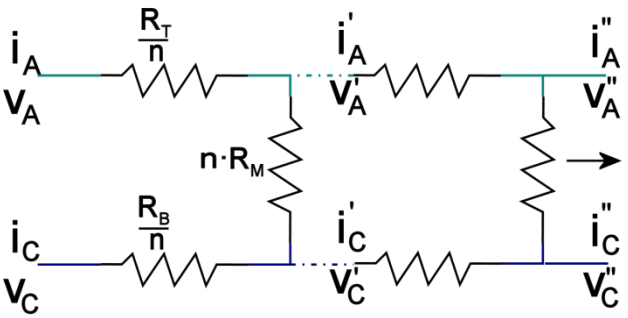
$$V = Q \cdot C^{-1}$$

Finally, the equivalent resistance between node 1 and 4 (port A to B) is the voltage drop between nodes 1 and 4, divided by current i.

For each geometry, the amount of TEER resistors n is varied until n is sufficiently large that the results converge to a stable value. Typically that occurs at n=1000 for the chip geometries discussed in this paper. Also, the Kirchhoff matrix approach is verified using the analytical model discussed below.

2) Analytical model approach

Instead of approximating the network by n resistors, it is also possible to solve this model by approaching n to infinity. Assume the following network:



Scheme S2 Equivalent circuit model used in the analytical model.

For simplification purposes, we introduce the following resistances:

$$R_m = R_M \cdot n$$

$$R_t = R_T \cdot n^{-1}$$

$$R_b = R_B \cdot n^{-1}$$

The circuit shown is basically a ladder network and for which the following is true for the currents and voltages on the first step of the ladder, as denoted by the single accent (′):

$$V'_A = V_A - R_t \cdot i_A$$

$$V'_C = V_C - R_b \cdot i_C$$

$$i'_A = i_A - \frac{V'_A - V'_C}{R_m}$$

$$= i_A - \frac{V_A - V_C - R_t \cdot i_A + R_b \cdot i_C}{R_m}$$

$$i'_C = i_C + \frac{V'_A - V'_C}{R_m}$$

$$= i_C + \frac{V_A - V_C - R_t \cdot i_A + R_b \cdot i_C}{R_m}$$

These equations can be combined into a matrix:

$$\begin{bmatrix} i'_A \\ i'_C \\ V'_A \\ V'_C \end{bmatrix} = \begin{bmatrix} 1+\frac{R_t}{R_m} & -\frac{R_b}{R_m} & -\frac{1}{R_m} & \frac{1}{R_m} \\ -\frac{R_t}{R_m} & 1+\frac{R_b}{R_m} & \frac{1}{R_m} & -\frac{1}{R_m} \\ -R_t & 0 & 1 & 0 \\ 0 & -R_b & 0 & 1 \end{bmatrix} \cdot \begin{bmatrix} i_A \\ i_C \\ V_A \\ V_C \end{bmatrix}$$

For infinite amount of steps n in the ladder, the result is:

$$\begin{bmatrix} i_A^\infty \\ i_C^\infty \\ V_A^\infty \\ V_C^\infty \end{bmatrix} = \lim_{n\to\infty} \left(I + \begin{bmatrix} \frac{R_t}{R_m} & -\frac{R_b}{R_m} & -\frac{1}{R_m} & \frac{1}{R_m} \\ -\frac{R_t}{R_m} & \frac{R_b}{R_m} & \frac{1}{R_m} & -\frac{1}{R_m} \\ -R_t & 0 & 0 & 0 \\ 0 & -R_b & 0 & 0 \end{bmatrix} \right)^n \cdot \begin{bmatrix} i_A \\ i_C \\ V_A \\ V_C \end{bmatrix}$$

Using the mathematical identity

$$e^A = \lim_{n\to\infty} \left(I + \frac{1}{n} \cdot A \right)^n$$

and the notion that $i_A^\infty = i_D$, and $i_C^\infty = i_B$, and $V_C^\infty = V_B$, and $V_A^\infty = V_D$, the result can be simplified to:

$$\begin{bmatrix} i_D \\ i_B \\ V_D \\ V_B \end{bmatrix} = \exp \begin{bmatrix} 0 & 0 & -\frac{1}{R_M} & \frac{1}{R_M} \\ 0 & 0 & \frac{1}{R_M} & -\frac{1}{R_M} \\ -R_T & 0 & 0 & 0 \\ 0 & -R_B & 0 & 0 \end{bmatrix} \cdot \begin{bmatrix} i_A \\ i_C \\ V_A \\ V_C \end{bmatrix}$$

Assuming a two-port measurement, a current i can be applied between ports A and C, resulting in a voltage drop between A and C, equal to the voltage over the entire membrane area. The resulting membrane area resistance can be calculated using Ohm's law. This membrane area resistance is the equivalent resistance of the entire network shown in scheme S2. The 'measured' TEER value can be calculated by multiplying this membrane area resistance by the width and length of the channel.

The following graph (Fig. S3) shows the result of the analytical model for varying TEER values. The geometry of the Gut-on-a-Chip (table 1) is used for these calculations.

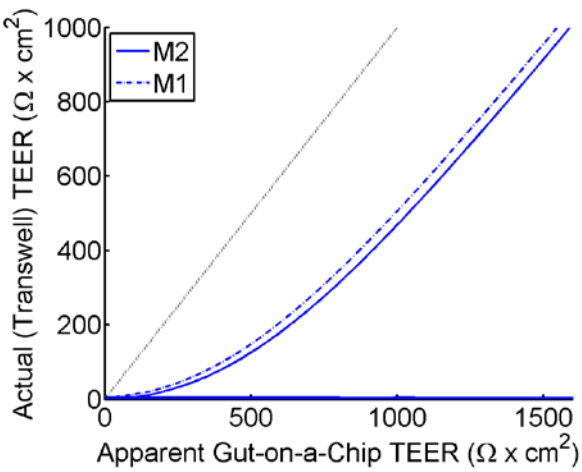


Fig. S3 Measured TEER as a function of actual TEER solved using the analytical model (M2, solid lines) compared to the Kirchhoff matrix model (M1, dashed lines).

As can be seen from the graph, the result is similar to the Kirchhoff matrix result shown in the paper. Note that the major drawback of the analytical model is the difficulty in solving the equation for small TEER values, as the results at TEER close to zero are inaccurate. Note that the horizontal line at the x-axis is resulting from errors from the analytical model.

3) Microscope images of cell culture in Gut-on-a-Chip

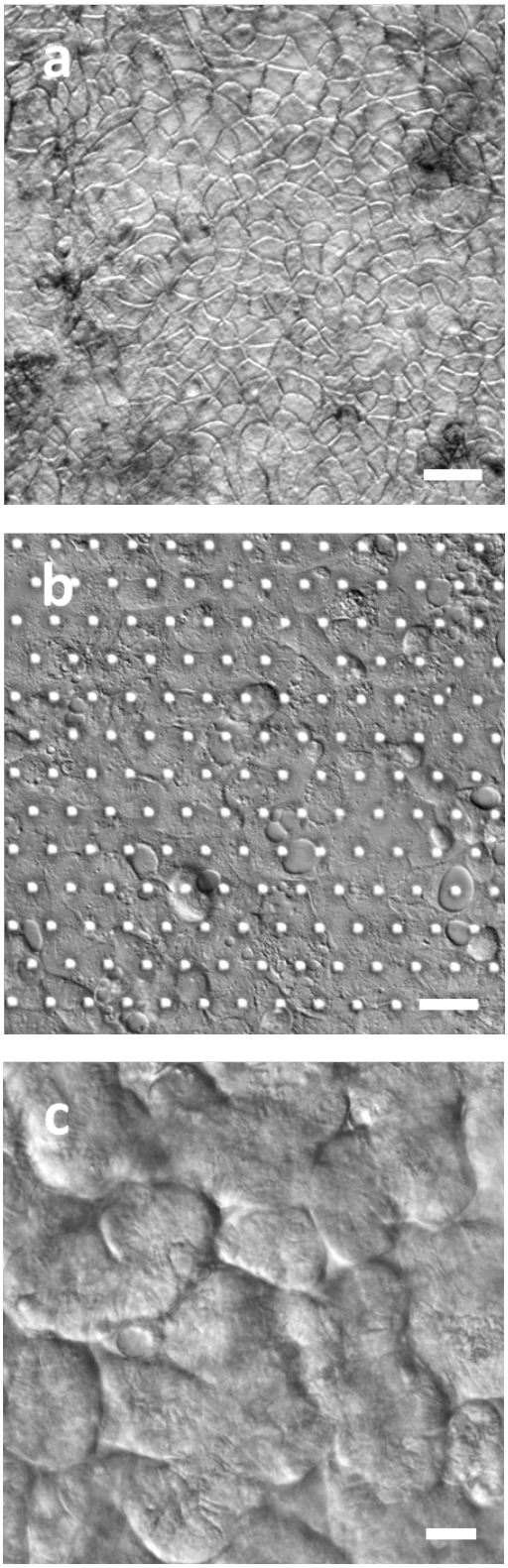


Fig. S4 DIC images of Caco-2 cell morphology. (a) A monolayer of Caco-2 cells grown in a static Transwell for 3 weeks. (b) A monolayer of Caco-2 cells cultured in a Gut-on-a-Chip microdevice for 24 h. (c) Villi formed in a Gut-on-a-Chip device after ~100 h. An array of small white circles in (b) shows the pores visible beneath the Caco-2 cell monolayer. Bar, 50 µm.

Overview of TEER measurements in literature

Table TS1 Overview of literature reporting TEER measurements. See list of references in the main paper for full bibliographic details.

Human	BMEC	monoculture	Man et al	2008	Clin and developmental immunology	100	not mentioned	
			Wong et al	2004	Experimental Neurology	200	memb. of purified collagen (Cellagen discs)	
			Lin et al	2013	Brain Research	225	0.4 µm and 8µm	
			Shimizu et al	2012	Neurochem Res	18	0.4 µm	
			Thomsen et al	2013	ACS Chemical Neuroscience	43	Transwell, but size not mentioned	
			Mishiro et al	2012	Neuroscience	140	0.4 µm	
			Wassmer et al	2006	Infection and Immunity	128	3 µm	
			Lippman et al	2012	Nature Biotechnology	200	0.4 µm	
			Chaitanya et al	2011	J neuroinflammation	245	8 µm PETP Transwell inserts (Falcon)	
			Kuo et al	2011	Colloids and Surfaces B: Biointerfaces	175	1 µm	
		cocult.	Lippman et al	2012	Nature Biotechnology	1450	0.4 µm	astrocytes
			Chaitanya et al	2011	J neuroinflammation	232	8 µm PETP Transwell inserts (Falcon)	HFA
			Kuo et al	2011	Colloids and Surfaces B: Biointerfaces	230	1 µm	
	HCMEC	mono	Vu et al	2009	Eukaryote cell	60	8 µm	
			Cucullo et al	2008	J Cerebral Blood flow and metabolism	65	0.4 µm	
			Chaitanya et al	2011	J neuroinflammation	297	8 µm PETP Transwell inserts (Falcon)	
		co	Cucullo et al	2008	J Cerebral Blood flow and metabolism	69	0.4 µm	HA
			Chaitanya et al	2011	J neuroinflammation	176	8 µm PETP Transwell inserts (Falcon)	HFA
	HUVEC	monocult.	Yamada et al	2014	Microvascular research	12	0.4 µm	
			Xie et al	2005	J of controlled release	25	8 µm	
			Man et al	2008	Clin and developmental immunology	80	not mentioned	
			Seok et al	2013	Arch Pharm Res	87	Transwell, but size not mentioned	
		co	Seok et al	2013	Arch Pharm Res	207	Transwell, but size not mentioned	C6
	CaCo-2	monocult.	Kotze et al	1998	J of pharmaceutical sciences	250	Transwell, but size not mentioned	
			Tsuzuki	2007	Lipids	330	0.4 µm	
			Gutsi et al	2014	Physiological reports	400	3 µm	
			Rodriguez-Gaztelumendi	2011	Toxicology letters	700	0.4 µm	
			Smith et al	2004	Pharmaceutical Research	800	0.4 µm	
			Kotze et al	1998	J of controlled release	900	Transwell, but size not mentioned	
			Jevprasesphant et al	2003	Pharmaceutical Research	900	Transwell, but size not mentioned	
			El-Sayed et al.	2002	J of controlled release	1000	3 µm	
			Borchard et al.	1996	J of controlled release	1300	Transwell, but size not mentioned	
Murine	bEnd3	monoculture	Simon et al	2010	Annals of biomedical engineering	20	0.4 µm	
			Booth et al	2012	Lab on a chip	25	polycarbonate, pore size not mentioned	
			Wuest et al	2013	J Neurosci Met	30	0.4 (value based on), 1, 3 and 8 µm	
			Hue et al	2013	J of Neurotrauma	30	0.4 µm	
			Chaitanya et al	2011	J neuroinflammation	276	8 µm PETP Transwell inserts (Falcon)	
			Inamura et al	2013	Neurochem Res	22	0.4 µm	
			Seok et al	2013	Arch Pharm Res	45	Transwell, but size not mentioned	
		co	Simon et al	2010	Annals of biomedical engineering	30	0.4 µm	astrocytes
			Booth et al	2012	Lab on a chip	250	polycarbonate, pore size not mentioned	C8D1A (mouse)
			Chaitanya et al	2011	J neuroinflammation	208	8 µm PETP Transwell inserts (Falcon)	HFA
			Seok et al	2013	Arch Pharm Res	84	Transwell, but size not mentioned	C6
	BMEC	monoculture	Fleegal-DeMotta et al	2009	J Cerebral Blood flow and metabolism	1	0.45 µm	
			Calabria et al	2006	J Neurochemistry	40	0.4 µm Transwell-Clear filter	
			Demeuse et al	2002	J neurociene methods	50	1 µm	
			Dohgu et al	2011	J of neuroinflammation	65	0.4 µm	
			Weidenfeller et al	2007	J Neurochemistry	75	0.4 µm	
			Shayan et al	2011	Eur J Pharm Sci	130	0.4 µm	
			Wuest et al	2013	J Neurosci Met	150	0.4 µm	
			Honda et al	2006	cellualr and molecular neurobiology	158	0.45 µm	
			Xie et al	2005	J of controlled release	200	8 µm	
			Jiang et al	2012	Int J Alzheimer disease	300	0.4 µm	
			Zhou et al	2013	Biomed Envir Sci	125	1 µm	
			Zhu et al	2012	J Ethnopharmacology	40	8 µm	
		cocult.	Calabria et al	2006	J Neurochemistry	200	0.4 µm Transwell-Clear filter	*doping Puromycin+HC
			Demeuse et al	2002	J neurociene methods	425	1 µm	Astrocytes
			Weidenfeller et al	2007	J Neurochemistry	110	0.4 µm	NPC
			Shayan et al	2011	Eur J Pharm Sci	190	0.4 µm	astrocytes
			Wuest et al	2013	J Neurosci Met	200	0.4 µm	astrocytes
			Zhou et al	2013	Biomed Envir Sci	350	1 µm	AC

			Author	year	Journal	TEER	Support/pore size	Remarks
Murine	ECV304	mono	Easton et al	2002	Brain Research	70	0.4 µm	
			Neuhaus et al	2011	Brain Research	120	1 µm	
			Chaitanya et al	2011	J neuroinflammation	353	8 µm PETP Transwell inserts (Falcon)	
		co	Easton et al	2002	Brain Research	225	0.4 µm	C6
			Chaitanya et al	2011	J neuroinflammation	327	8 µm PETP Transwell inserts (Falcon)	HFA
	RBEC	monoculture	Hayashi et al	2004	Regulatory peptides	8	0.4 µm	
			Nakagawa et al	2009	Neurochem International	100	Corning life science Transwells 12	
			Dohgu et al	2011	Microvascular Research	150	0.4 µm	
			Yamada et al	2014	Microvascular research	175	0.4 µm	
			Toyoda et al	2013	Cell Mol Neurobiol	281	8 µm and some 0.4 µm	
			deVries	1996	J Neuroimmunology	150	0.4 µm	
		cocult.	Hayashi et al	2004	Regulatory peptides	100	0.4 µm	pericytes
			Nakagawa et al	2009	Neurochem International	200	Corning life science Transwells 12	astrocytes
			Dohgu et al	2011	Microvascular Research	280	0.4 µm	pericytes
			Toyoda et al	2013	Cell Mol Neurobiol	329	8 µm and some 0.4 µm	pericytes / astrocytes
Porcine	BMEC	monoculture	Smith et al	2007	J of drug targeting	80	0.4 µm	
			Kuhlmann et al	2009	Neuroscience letters	218	0.4 µm	
			Franke et al	2000	Brain Research Protocols	400	0.4 µm	
			Franke et al	1999	Brain research	500	0.4 µm	
			Zhang et al	2006	Drug Metabolism and disposition	550	0.4 µm	
			Patabendige et al	2013	Brain research	595	0.4 µm pores	
			Neuhaus et al	2008	Drug discovery interface	149	1 µm	
		cocult.	Smith et al	2007	J of drug targeting	834	0.4 µm	C6
			Kuhlmann et al	2009	Neuroscience letters	278	0.4 µm	astrocytes
			Franke et al	1999	Brain research	800	0.4 µm	*serumfree
			Patabendige et al	2013	Brain research	779	0.4 µm pores	astrocytes
Bovine	BMEC	mono	Colgan et al	2008	Brain research	30	0.4 µm	
			Rubin et al	1991	J Cell Biol	61	0.4 µm	
			Haorah et al	2005	Alcoholism: Clinical and experimental	250	0.4 µm	
		co	Colgan et al	2008	Brain research	45	0.4 µm	C6
			Rubin et al	1991	J Cell Biol	115	0.4 µm	astrocytes
	BCEC	mono	Dehouck et al	1990	J Neurochemistry	416	0.4 µm	
			Gaillard et al	2001	Europ J Pharma Sci	131	0.4 µm	
			Salmeri et al	2013	Cellular Microbiology	70	0.4 µm pores also 3µm	
			Boveri et al	2005	Glia	150	0.4 µm	
		co	Dehouck et al	1990	J Neurochemistry	661	0.4 µm	Astrocytes
			Gaillard et al	2001	Europ J Pharma Sci	857	0.4 µm	
			Salmeri et al	2013	Cellular Microbiology	267	0.4 µm pores also 3µm	pericytes