ELECTRONIC SUPPLEMENTARY INFORMATION

Human Gut-on-a-Chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow

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SUPPLEMENTARY EXPERIMENAL

Calculation of shear stress

Shear stress (τ, dyne/cm^2) was calculated based on the following equation,[1] $\tau = 6\mu Q/h^2 w$, where μ is viscosity of the culture medium (g/cm·s), Q is volumetric flow rate (cm^3/s), and w (cm) and h (cm) are width and height of the microchannel, respectively.

Measurement of β-galactosidase activity

For measuring the catalytic activity of β-galactosidases, *O*-nitrophenyl-β-D-galactopyranoside (ONPG; Sigma, St. Louis, MO) was used as described in Experimental in the main text. LGG cell culture was carried out in MRS medium, then LGG cells were harvested at exponential phase. After LGG cells were washed twice in antibiotic-free cell culture medium, cell density was adjusted to ~1.0×10^8 CFU/mL in ONPG-containing antibiotic-free cell culture medium (30 μg/mL, final ONPG concentration), then samples were immediately incubated at 37°C under 5% CO_2 in a humidified atmosphere. Samples intermittently taken for 12 hours were centrifuged,

and optical density of supernatant was measured at 420 nm (SpectraMax M5, Molecular Devices); fresh culture medium was used as a reference. Caco-2 cells were cultured in an ECM-coated 24-well plate (Falcon, BD) for 2 weeks, and culture medium was then switched to antibiotic-free medium for 12 hours prior to performing ONPG assay. After ONPG solution (30 μg/mL) was applied to the Caco-2 culture plate, sample aliquots were intermittently taken from the culture medium, and optical density was measured at 420 nm.

Table S1. Design Parameters for the Gut-on-a-Chip

DESCRIPTOR	VALUE
Cell microchannel	
width × length × height	1,000 × 10,000 × 150 μm
volume of the top microchannel	~1.2 μL
growth surface area	0.11 cm^2
effective pore area	0.021 cm^2
porosity of a PDMS membrane	19.5%
residence time of fluid (at 30 μL/h)	~4.51 min
Vacuum Chamber Dimensions	
width × length × height[a]	1,684 × 9,089 × 330 μm
Physiological Parameters	
range of volumetric flow rate	10-100 μL/hr
range of shear stress[b,c]	0.006-0.06 dyne/cm^2
range of cyclic mechanical strain	0-30% (in cell strain)
frequency of cyclic mechanical strain	0.15 Hz

[a]The height of the vacuum chamber was estimated by considering the total height in the hollow area, in which the height of an upper and lower layer (150 μm) and the thickness of a porous membrane (30 μm) were all taken into account.
[b]The range of shear stress corresponds to the range of fluid flow rate designated in this table.
[c]Shear stress was calculated by the equation in ESI Experimental.

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Fluid flow is a critical factor for the control of cell shape and polarity in Caco-2 cells. Confocal fluorescence views of a vertical cross section through a Caco-2 monolayer cultured in the Gut-on-a-Chip using flow rates of 10 μL/hr (**a**) or 100 μL/hr (**b**) for 20 hours in the absence of cyclic strain, confirming that higher flow rates (30-100 μL/hr) specifically induce polarization and formation of a columnar epithelium. (**c**) Quantification of the mean heights of Caco-2 cells cultured at either 10 μL/hr or 100 μL/hr without mechanical strain (*, $p<0.0001$; bar, 20 μm).

Fig. S2. Assessment of β-galactosidase activity in live LGG cells and Caco-2 cells cultured independently. Live LGG cells actively cleaved the β-galactosidase substrate, ONPG, and produced a progressive increase of optical density of the *O*-nitrophenol product (closed circles), whereas human Caco-2 epithelial cells did not exhibit any specific β-galactosidase (closed squares). Differences in activity expressed by LGG versus Caco-2 cells were statistically significant ($p < 0.001$) at all time points.

ESI REFERENCES

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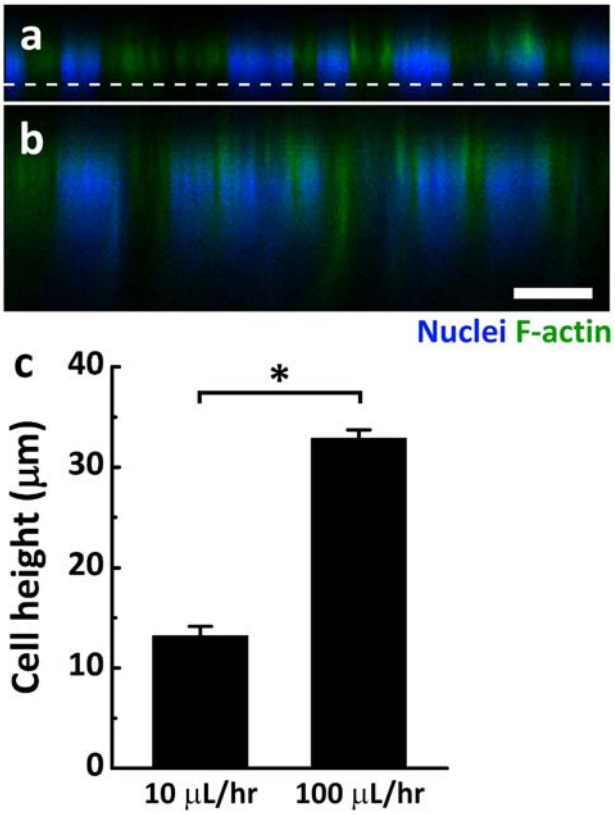

Fig. S1

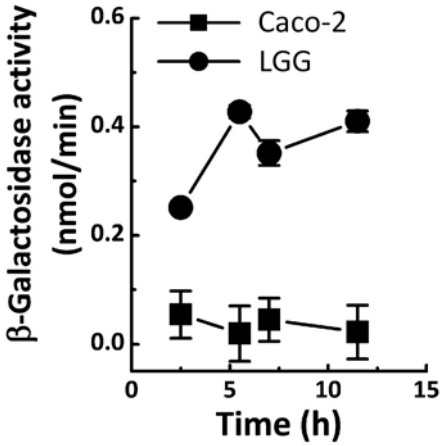

Fig. S2