An *in vitro* intestinal platform with a self-sustaining oxygen gradient to study the human gut/microbiome interface

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Supplementary information

Supplementary Movie 1.
Simulated oxygen profile over time in the device. The diffusion coefficient of oxygen through the media was set to 3×10^{-9} m^2/s. The diffusion coefficient of oxygen through collagen and the oxygen consumption rate (OCR) of the colonic cells were set to the values calculated below (in the Supplementary methods). The simulation was performed using COMSOL Multiphysics.

Supplementary Movie 2.
Simulated total magnitude of oxygen flux over time in the device using COMSOL Multiphysics. The simulation employed the same conditions as that of Supplementary Movie 1.

Supplementary figures

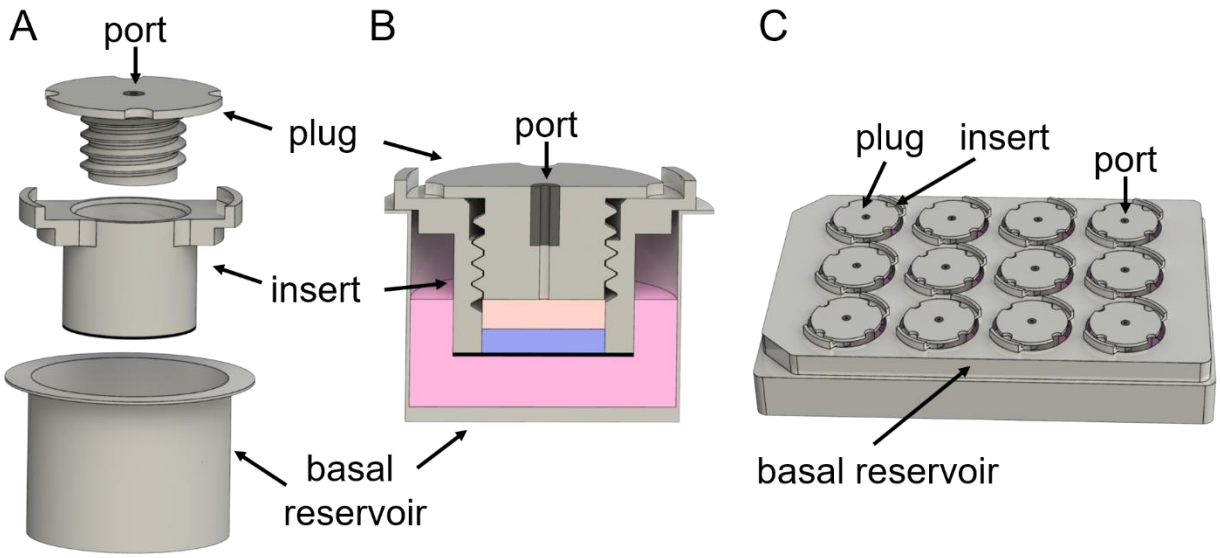

Figure S1. Assembly of the device for generating the oxygen gradient. A) The device is comprised of three parts including the plug (top), the cell culture insert (middle), and the basal reservoir (bottom). The bottom can be a single milled component or one well within a 12-well plate. B). The device assembled for the cell culture. C) Shown is a12-well plate with each well possessing a plug and cell-culture insert. The lid that covers the entire plate is omitted for clarity.

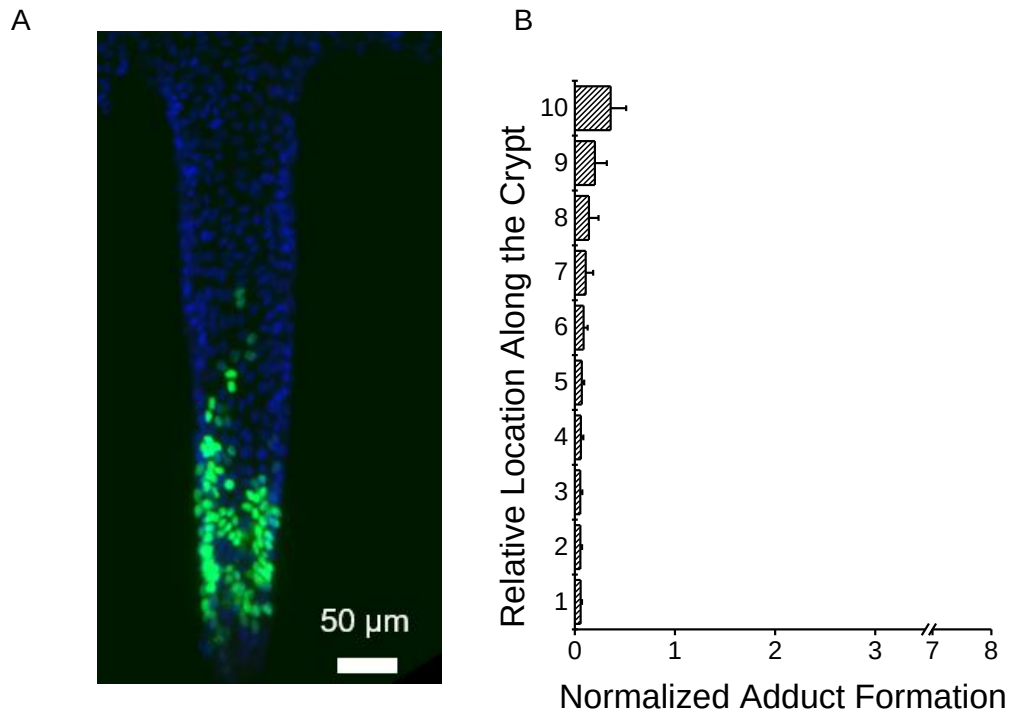

Figure S2. Measurement of nonspecific staining of the fluorescent antibody used to label pimonidazole adducts. Crypts were formed using a chemical gradient of growth factors and then placed under an oxygen gradient. A) A representative fluorescence image of an *in vitro* crypt stained with EdU (green), Hoechst 33342 (blue), and the antibody against pimonidazole adducts (red). No pimonidazole was added to the tissue. No red fluorescence is present indicating the antibody against pimonidazole adducts did not have significant nonspecific binding to the tissue. B) The normalized red fluorescence (from the antibody against pimonidazole adducts) is plotted on the x axis with the distance along the crypt along the y axis. zero is the basal crypt end and 10 is the luminal end of the crypt. The scale of the X-axis matches that of the data of Fig. 5C for facile comparison.

Table S1. Composition of the media used in this study.

	Expansion Medium (EM)	Stem Medium (SM)	Differentiation Medium (DM)
Component (Source)	Concentration	Concentration	Concentration
Advanced DMEM/F12 (Thermo Fisher)	50% (v/v)	50% (v/v)	50% (v/v)
L-WRN conditioned medium[1]*	50% (v/v)	50% (v/v)	-
Fetal bovine serum (FBS) (Atlanta Biologicals)	-	-	10% (v/v)
GlutaMAX (Thermo Fisher)	1×	1×	1×
HEPES (Thermo Fisher)	10 mM	10 mM	10 mM
Murine epidermal growth factor (EGF) (Peprotech)	50 ng/mL	50 ng/mL	50 ng/mL
B27 (Thermo Fisher)	1×	1×	-
N-Acetyl cysteine (MP Bio)	1.25 mM	1 mM	1.25 mM
Gastrin (Anaspec)	10 nM	10 nM	-
Y-27632 (ApexBio)	10 µM	10 µM	-
A83-01 (Sigma Aldrich)	500 nM	-	500 nM
Prostaglandin E2 (PGE2) (Cayman chemicals)	10 nM	-	-
Nicotinamide (Sigma)	10 mM	-	-
SB202190 (Selleckchem)	3 µM	-	-
Primocin (InvivoGen) Not used for antibiotic free formulation	50 µg/mL	50 µg/mL	50 µg/mL

*The L-WRN medium was prepared following the previous publication.[1] All the media above were prepared as previously described[2] with slight modification.

Supplementary Methods

Measuring the diffusion coefficient of O_2 in the collagen gel.
The diffusion coefficient of O_2 through water was taken to be 3×10^{-9} m^2/s at 37˚C as measured by others.[3] The diffusion coefficient of O_2 through cross-linked collagen was determined experimentally by measuring the oxygen concentration with a needle-type fiber optic oxygen probe within the setup shown in Fig. S1.

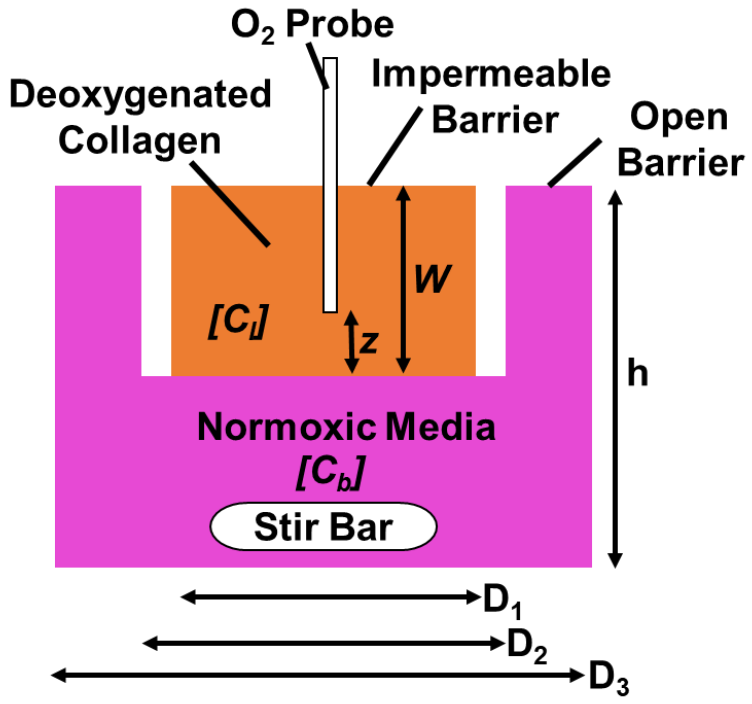

Figure S3. Initial setup for determining the diffusion coefficient of oxygen through cross-linked collagen.

Table S2. Dimensions of device used to determine diffusion coefficient through cross-linked collagen.

h	9 mm
D_1	10.2 mm
D_2	15 mm
D_3	22.1
W	4.5 mm
z	2.5 mm

The luminal reservoir was completely filled with cross-linked collagen (368 µL) and deoxygenated by nitrogen purging. A needle-type oxygen probe was inserted into the deoxygenated collagen, and the top of the luminal reservoir was sealed with an impermeable oxygen barrier. The basal reservoir was filled with normoxic media and a stir bar was placed in the bottom of the basal reservoir. The basal reservoir remained in contact with atmospheric oxygen which, along with rotation of the stir bar, enabled the basal reservoir to be modelled as an infinite oxygen source. Oxygen measurements were taken within the cross-linked collagen every 5 s for a 1 h period. The resultant measurements were fit to the following equation derived from Fick's second law of diffusion:[4]

$$f(t) = (C_b - C_l)\left(\text{erfc}\left(\frac{z}{2\sqrt{D \cdot t}}\right) + \text{erfc}\left(\frac{-z + 2W}{2\sqrt{D \cdot t}}\right)\right) + C_l$$

where C_b and C_l are the starting O_2 concentration within the basal media and luminal collagen plug, respectively. z is the location at which the O_2 measurements were taken or the height of the probe above the luminal/basal reservoir interface. W is the height of the collagen plug. W and z were set to 4.5 mm and 2.5 mm, respectively. t is the time at which the oxygen measurement was taken, and D is the diffusion coefficient of oxygen in m^2/s. The above equation assumes that the diffusion through cross-linked collagen is isotropic and that the basal reservoir is acting as an infinite oxygen source. A representative fit is shown in Fig.S4. The diffusion coefficient of oxygen through cross-linked collagen was $1.2 \pm 0.1 \times 10^{-9}$ m^2/s at 37˚C (obtained from 3 independent experiments).

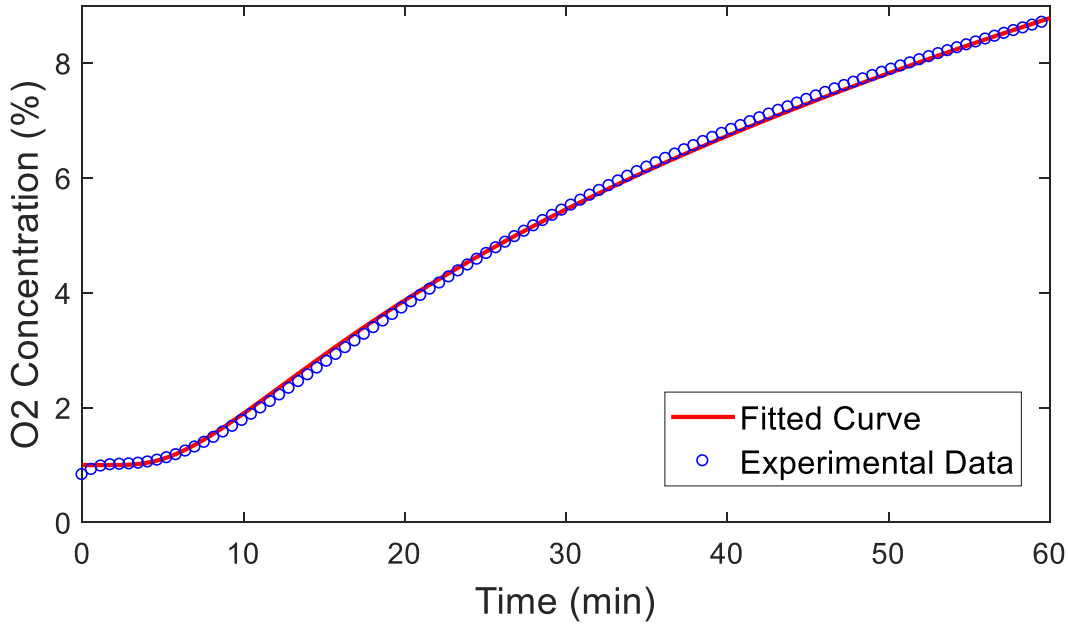

Figure S4. Fit of the experimental data to Fick's Law of Diffusion. The resultant fit possessed an R^2 of 0.999 and gave a diffusion coefficient (D) of 1.4×10^{-9} m^2/s.

Measuring cellular oxygen consumption rate in the media

A simple device was designed to measure the oxygen consumption rate (OCR) of primary human colonic epithelial cells (Figure S5). Neutralized collagen gel (250 µL) was prepared as described in the Methods (1 h, 37°C), crosslinked with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) in 0.1 M of 2-(N-morpholino)ethanesulfonic acid (MES) at final concentrations of 6 µM and 1.5 µM, respectively, (500 µL total volume) for 1 h at 25°C. The crosslinked collagen was washed with PBS at least 4 times. Then the human colonic epithelial cells were cultured on the crosslinked collagen gel in a standard tissue culture incubator (5% CO_2, 69.3% N_2, 18.6%O_2) in expansion media (EM) for 6 days. The cells were then cultured in differentiation media (DM) for a further 4 days under the same environmental gases. The device was then rotated 90 degrees and a stir bar was placed in the chamber. A fiber optic oxygen sensor was inserted into the device and sealed. The device was then placed on a stir plate to uniformly mix the media within the chamber. The oxygen concentration was measured for 24 hours within the chamber.

Previous studies have shown that oxygen consumption rate in cells can be modeled using Michaelis-Menten kinetics.[3, 5, 6] An integral of the Michaelis-Menten kinetics equation was used to fit the measured data:[7 8]

$$C_{O_2} = K_m \cdot W\left(\frac{C_0}{K_m} \cdot exp\left(\frac{C_0 + OCR_{max} \cdot t}{K_m}\right)\right) + C_{min}$$

Where W is the Lambert W function, K_m [mol·cell^{-1}] is the Michaelis constant, OCR_{max} [mol·cell^{-1}·s^{-1}] is the maximum rate achieved, C_0 [mol·cell^{-1}] is the initial oxygen concentration, C_{min} [mol·cell^{-1}] is the minimum oxygen concentration that the cells were consuming oxygen, and t is time [s]. C_{O2} [mol·cell^{-1}] is the oxygen concentration and can be related to the measured oxygen concentration by the following equation:

$$C_{O_2} = \frac{[O_2] \cdot V_{Ch}}{n}$$

Where $[O_2]$ is the concentration of oxygen in the chamber in mol·m^{-3}, V_{Ch} is the volume of the chamber, and n is the number of cells. To determine the number of cells within the chamber, the cell density was measured at 10 different locations (640×640 μm each). The density was then multiplied by the total surface area to calculate the number of total cells.

The fit was used to calculate the OCR_{max} (-1.4±0.2×10^{-17} mol·cell^{-1}·s^{-1}) and K_m (1.0±0.4×10^{-13} mol·cell^{-1}) for the colon cells (Fig.S4), which in turn was used to calculate the OCR [mol·cell^{-1}·s^{-1}] as a function of oxygen concentration for the oxygen gradient device:

$$OCR = \frac{OCR_{max} \cdot ([O_2] - [O_2]_{min})}{[O_2] - [O_2]_{min} + K_m} \cdot \delta([O_2] > [O_2]_{min})$$

Where $[O_2]$ is the oxygen concentration within the gradient device, $[O_2]_{min}$ is the minimum oxygen concentration measured in the device over a 24 h period, and δ is a step function.

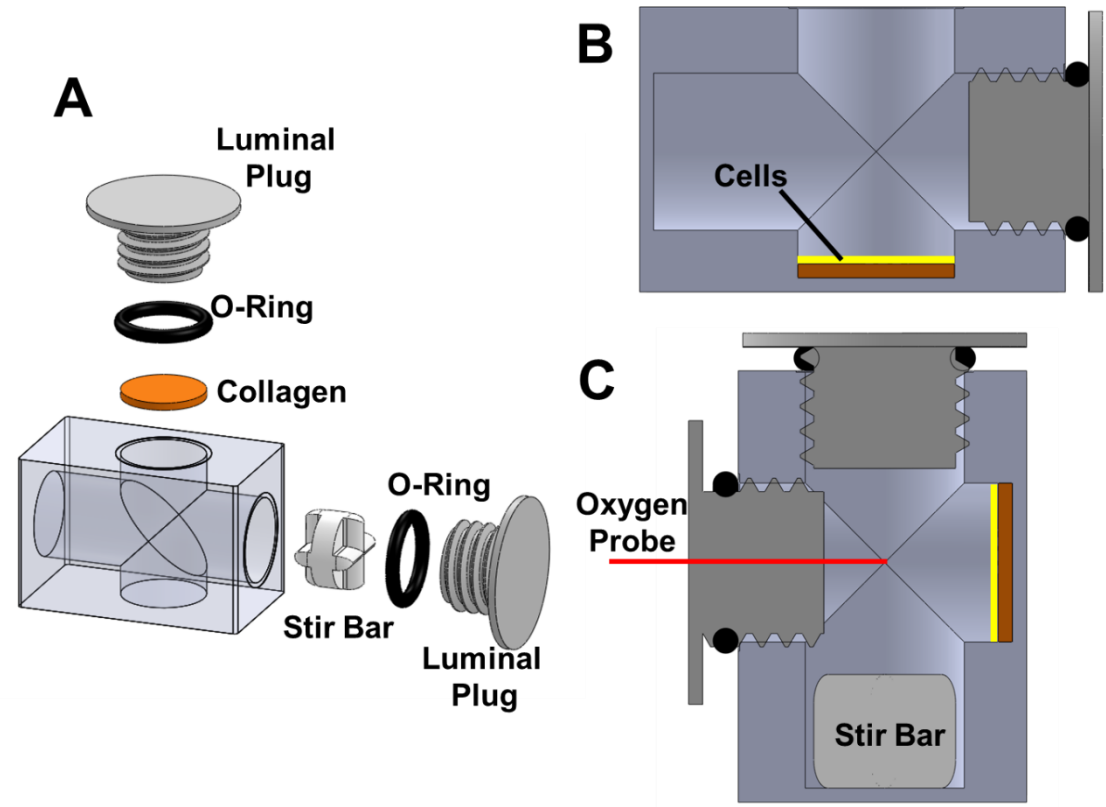

Figure S5. Device for measuring OCR in colon cells. A) Component parts. B) The device configured to culture colon cells. C) The device configured to measure the OCR of colon cells.

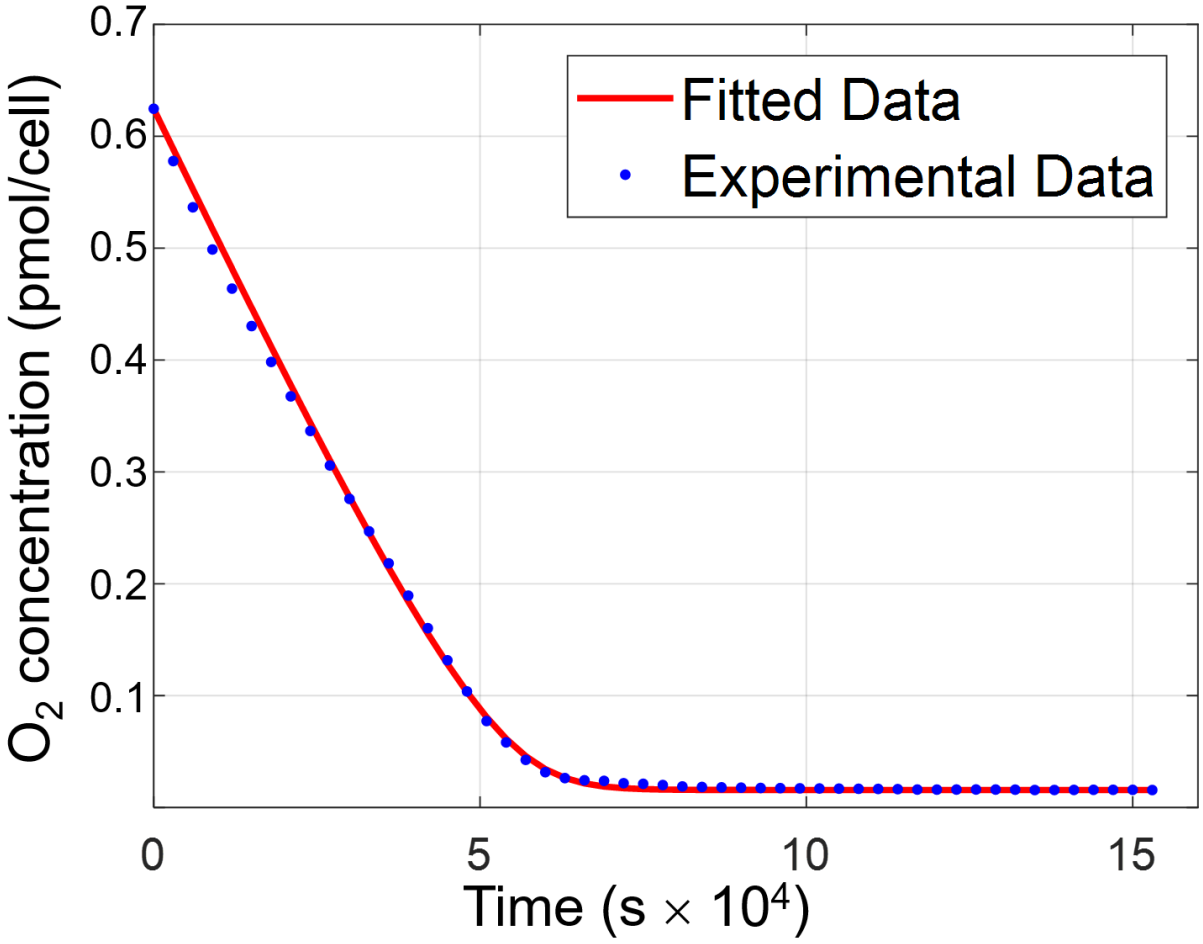

Figure S6. An example of experimental data with the Michaelis-Menten fit. $R^2 = 0.99$

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