Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplemental Information:
Flow through Human Intestinal Organoids with the Gut Organoid Flow Chip (GOFlowChip)

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1. GOFlowChip Assembly and Loading Protocol

A. Sterilization:

- Individual device pieces including acrylic layers, silicone seals, ferrules, glass capillaries, syringes, microfluidic connectors, and microfluidic tubing are immersed in pure ethanol for at least five minutes.
- After sterilization in ethanol, the pieces are dried in a laminar flow biohazard hood. The following assembly procedure is conducted in the hood to maintain sterility.

B. Assembly:

- Two micromanipulator and four screws are attached loosely to platform base to allow for further adjustments (see SI Fig. 1 below).
- The middle acrylic layer, flow-through gaskets, and gasket cap are assembled. The small diameter gasket must be attached to middle layer followed by the bigger diameter gasket and cap (SI Fig. 2A-C).
- Bottom layer consisting 8 screws is placed on top of the platform with 4 screws (SI Fig. 3A).
- O-ring is inserted in place on the bottom acrylic layer of the device followed by the middle layer with side port assemblies, and the over-flow channel gasket (SI Fig. 3B-E).
- Needle holder(s) is attached to micromanipulator leaving not more than a 1 mm gap between the side port entrance and needle tip (SI Fig. 4).

C. Filling with Liquid:

- The luminal inlet flow syringe (glass, 500 μL, Hamilton) fitted with a Luer lock connection is attached to a three-way connector (Kimble, nylon 3-way stopcock), filled with the appropriate liquid (e.g. media or PBS), then attached to the microfluidic tubing (Scientific Commodities Inc. PE/9, 1.40mm I.D. 1.91mm O.D.), before attaching to the pulled glass inlet capillary (c1). Care is taken throughout the process to remove air bubbles.
- The same process above is repeated for the luminal outlet flow syringe and the extraluminal inlet flow syringe.
- The over-flow outlet can remain partially filled with liquid as this channel will be used to remove air bubbles from the device after porting and before pressurization.

D. HIO Loading:

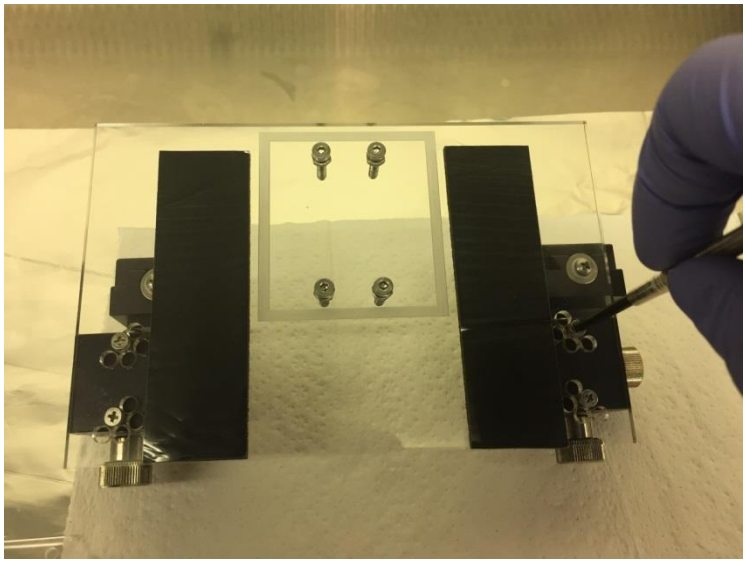
- To coat the bottom of the HIO well, approximately 10-20 μL of pre-chilled liquid Matrigel previously held in an ice bath is added to the center well and allowed to solidify for several minutes at room temperature. The total volume of the well is 60-100 μL, so the gel should only form a thin layer on the bottom of the well.
- The device, including the central well and side ports, is filled with media to prevent air interfaces or bubbles from forming and remaining in the device after sealing.
- Glass needles mounted on micromanipulators are guided through the side ports but are not forced all the way into the well.
- After removing any bubbles, the HIO is loaded into the center well using a P1000 pipette with a truncated tip.
- Once the HIO is placed in the central well, the top layer is placed on top of the middle layer with a silicone gasket between the two. As the top layer is laid onto the device, the end nearest the overflow channel inlet should be brought into contact with the middle layer first. As the opposite end of the upper layer is brought into contact with the middle layer, air will be forced out through the outlet of the overflow channel.
- Nuts should be rotated onto the screws but care should be taken to avoid over compressing the device before the porting procedure.

E. Preparation for Porting:

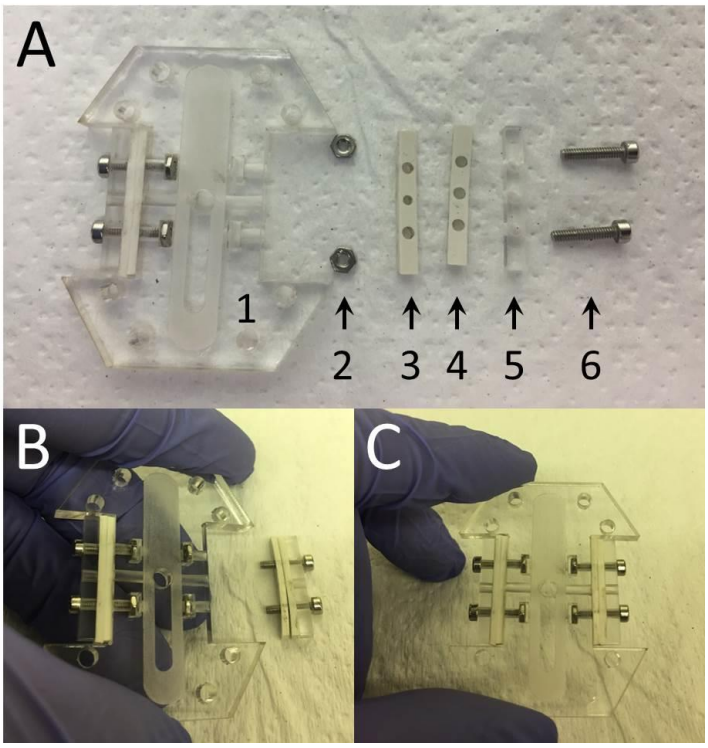
- The device is moved to a microscope with an environmental chamber.
- Syringes are loaded into syringe pumps and syringe pumps are pre-programmed.
- Liquid is flushed through overflow channel to ensure the remove of trapped air bubbles. During this step, a small amount of liquid may leak from the side ports as the device is not yet completely sealed.
- A glass capillary (50 μm tip diameter) is attached to the end of the overflow channel outlet tubing to slightly pressurize the device when flow is initiated.

F. HIO Porting:

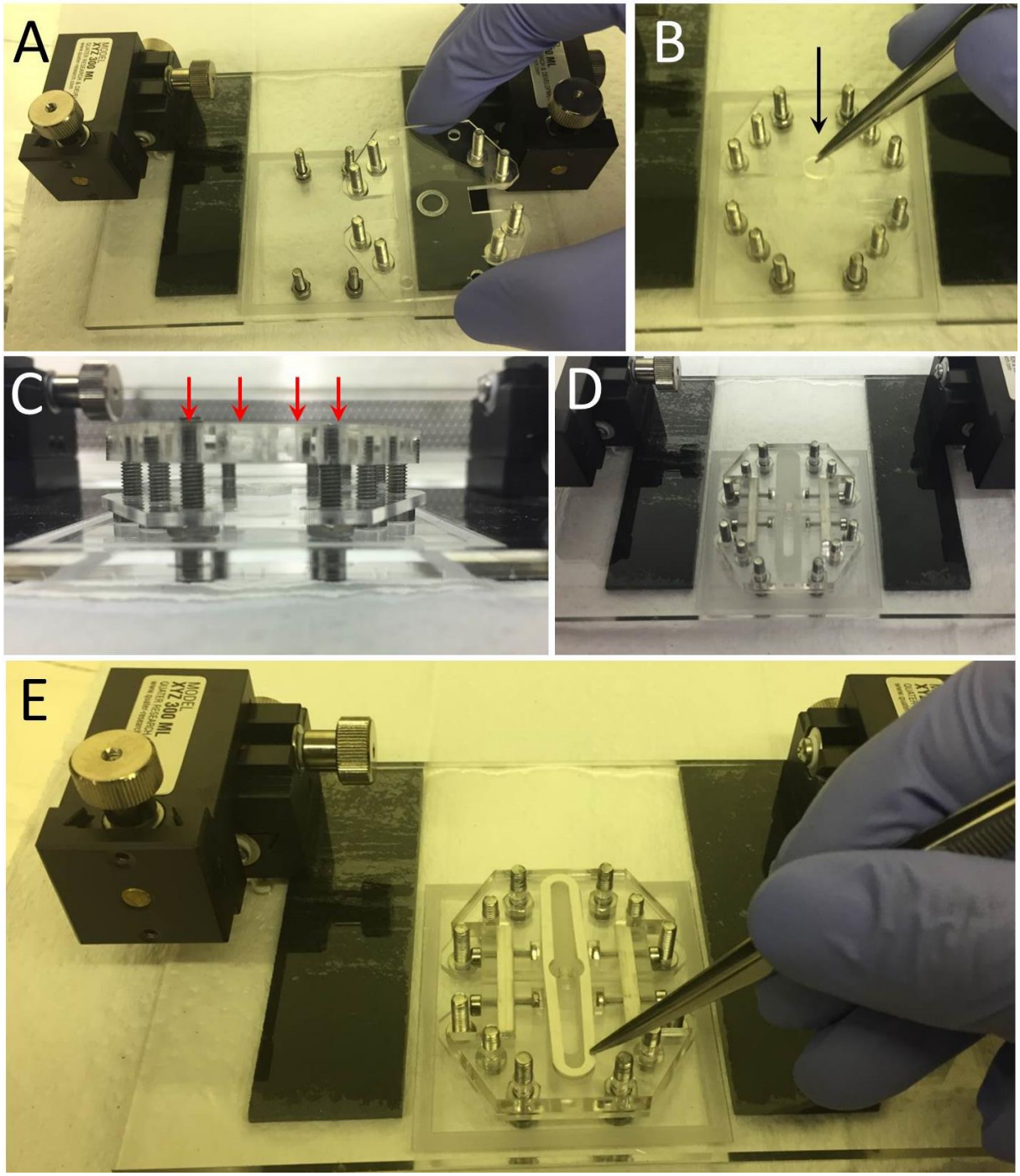
- The glass inlet capillary (c1), mounted on a micromanipulator, is translated in through the side port until the HIO shell is punctured. Successful puncturing of the shell is readily observable as the shell pops back from being deformed by the capillary.
- The glass outlet capillary (c2) is inserted similarly to c1. c2 is larger in diameter than c1, so greater pressure is required to puncture the HIO shell; however the Matrigel and c1 provide enough support for the larger diameter needle to puncture the organoid without allowing it to be pushed to the wall of the organoid chamber.
- At this point, the nuts are tightened to compress the silicone and seal the device.
- Over flow is initiated by starting the syringe pump.
- The syringe pump driving flow through c1 should be initiated with 1 μL pulsating injections. Swelling of the HIO should be observed to confirm successful porting of the HIO.
- Similarly, the syringe pump withdrawing flow through c2 should be initiated with 1 μL pulsating withdrawals to confirm successful porting of the HIO.
- If porting is confirmed in c1 and c2, luminal flow through is initiated by setting $q1 = q2$.



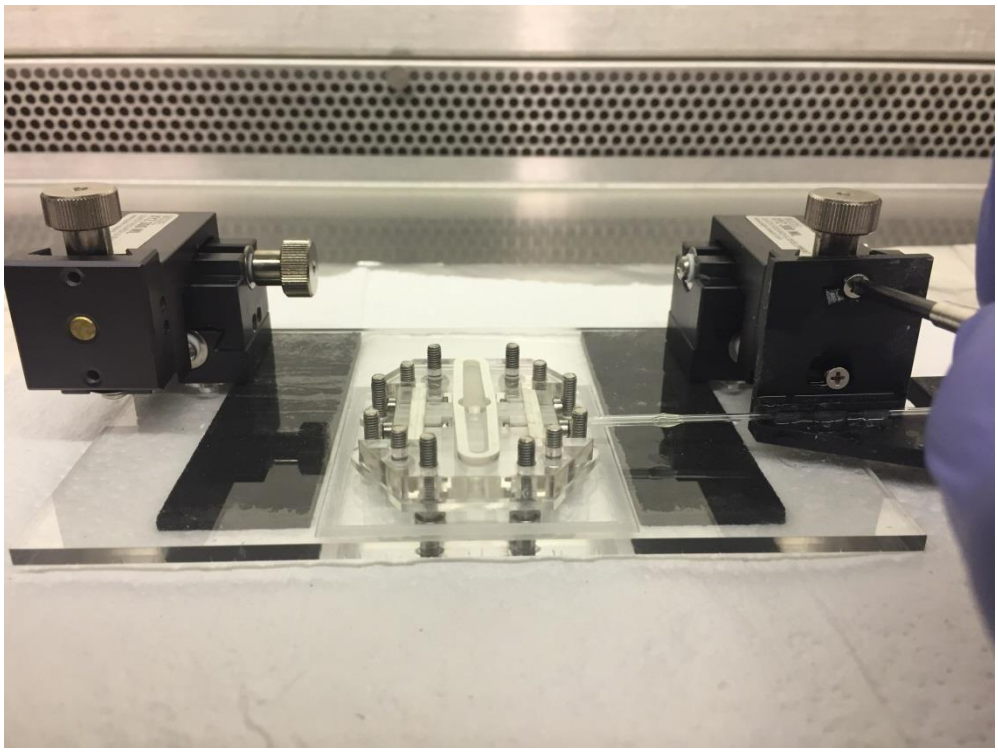
SI Fig. 1: Image of platform base (upside down) with four screws inserted.



SI Fig. 2: (**A**) Image of parts needed to put together the side port assembly: (1) middle acrylic layer, (2) nuts, (3) inner, small-diameter gasket, (4) outer, larger-diameter gasket, (5) acrylic gasket cap, (6) bolts. The side port assembly on the left has already been assembled. (**B**, **C**) After assembling parts (3-6), the nuts are placed into recessed holds and the assembly is screwed into place.



SI Fig. 3: (**A**) Bottom acrylic layer consisting 8 screws is socketed on top of the platform that has 4 screws. (**B**) O-ring (arrow) is inserted into recessed holder on top of bottom acrylic piece. (**C**) Middle acrylic layer with side port assemblies attached is placed on top of the bottom acrylic piece, with the o-ring sandwiched between. Red arrows indicate middle piece is moving down. Device assembly before (**D**) and after (**E**) placement of the over flow gasket into the recessed portion of the layer.



SI Fig. 4: Black 3D-printed needle holder(s) with attached capillary is attached to micromanipulator. The tip of each capillary needle should be approximately 1 mm from the side port entrance. The same process is repeated for the left-hand side.

2. Additional Practical Considerations for Porting and Establishing Luminal Flow

Through extensive optimization and device design iteration, and while attempting to port approximately 50-70 HIOs, we identified the two most common problems associated with porting and flow to be (1) issues encountered during HIO puncture and (2) clogging of the exit capillary during long-term flow. These two problems occur in roughly the same frequency.

Puncturing problems occur for two main reasons. First, poor alignment of the capillary needle with the side port and with the HIO can lead to breaking of the capillary or missing the HIO entirely during attempted puncture. Once an HIO has been placed in the device well and adhered to the Matrigel in the bottom of the well, it is difficult to relocate without damaging the HIO or rupturing the epithelial shell. Second, difficulty in puncturing an HIO can arise due to heterogeneities in the mesenchymal layer on the outside of the HIO; regions with a thicker mesenchymal layer provide greater resistance to puncture. Here, pressure applied to puncture the HIO with the capillary may dislodge the HIO, driving it to the opposite side of the well, and often leading to HIO rupture. These problems could be mitigated by the use of optics with improved perception in the z-axis.

As briefly mentioned in the bulk manuscript, clogging problems occur when luminal waste blocks the lumen of the exit capillary. While we have been able to minimize this effect by using a capillary tip diameter of $d \approx 80$ μm and a flow rate $q_{out} \leq 5$ μL/h, clogging still occurs in approximately 50% of ported HIOs over $t = 65$ h, which means that ported HIOs should be monitored and flow rates adjusted if clogging occurs. In the future, clogging could be minimized in a number of ways: (1) by porting HIOs before substantial amounts of luminal waste accumulate, (2) by introducing enzymes or chemicals to degrade the luminal waste, (3) by replacing syringe pumps with pressure-driven flow regulators that can sense and adjust to pressure buildup.

3. SI Video Descriptions

SI Video 1: Time-lapse microscopy video of an HIO embedded in Matrigel and imaged for 25 hours. Cellular debris accumulates over time and settles to the inner, bottom surface of the HIO due to gravity. Video corresponds to the images series in Figure 1a.

SI Video 2: Time-lapse microscopy video of an HIO swelling due to a net influx of liquid (q_{in} = 5 μL/h; q_{out} = 0 μL/h). No leaking from the puncture site is observed. Video corresponds to the images series in Figure 4a, and the length of the video spans 120 minutes.

SI Video 3: Time-lapse microscopy video of an HIO undergoing striking volume changes in response to oscillations in the net influx of liquid. Video corresponds to the images series in Figure 4b, c and the flow conditions plotted in Figure 4d.

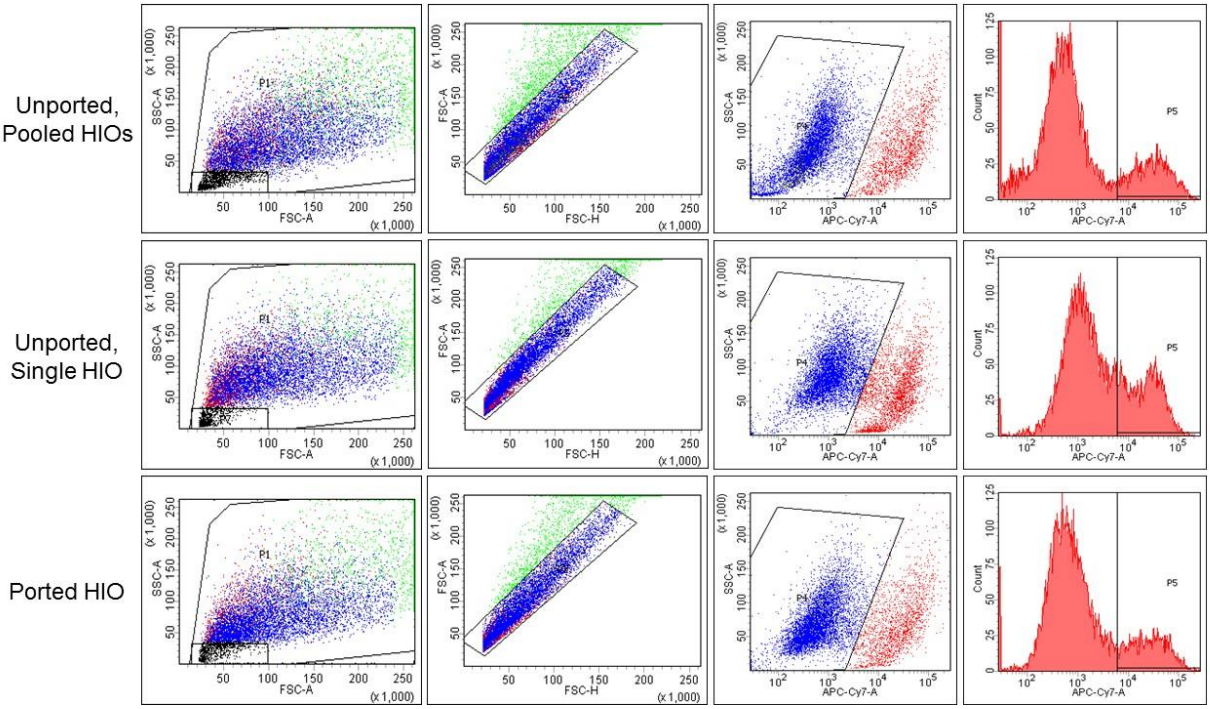
SI Video 4: Time-lapse microscopy video of steady state luminal flow through an HIO for $t \geq 65$ h using both a single, double-lumen capillary device.

SI Video 5: Time-lapse microscopy video of steady state luminal flow through an HIO for $t \geq 65$ h using both a double, single-lumen capillary device.

SI Video 6: Time-lapse microscopy video of waste being swept from the luminal space of an HIO over a time period of approximately 27 hours. At the 20 h mark, the image was refocused revealing the removal of waste from the HIO. Video corresponds to the images series in Figure 5a-d.

SI Video 7: Time-lapse microscopy video of a digitally-zoomed region from Supplementary Video 6 reveals the movement of large objects moving out through the exit capillary as they are carried by liquid flow. Video corresponds the series of microscopy images in Figure 5e, f, and the length of the video spans 152 minutes.

4. SI Flow Cytometry



SI Fig. 5. Additional flow cytometry plots to demonstrate gating decisions and live/dead stain analysis. (**Panel 1, left**) Cells are first analyzed and gated based on forward scatter area (FSC-A) and side scatter area (SSC-A) profiles. Cells that have very small FSC-A and SSC-A profiles are considered mostly cell fragments, cell debris, and dead cells that are captured as a sub-population, shown in black on the bottom left. (**Panel 2, mid-left**) FSC-height (FSC-H) and FSC-A are then used to gate around singlets and also to exclude doublets and clumps of cells. (**Panel 3, mid-right**) The intensity of the emitted infrared staining of single cells after excitation is indicated on the x-axis using the APC-Cy7-A setting and plotted against SSC-A. (**Panel 4, right**) A histogram of the counts of single cells across the spectrum of APC-Cy7-A emitted infrared staining. Dead cells were gated based on the manufacturer's guidelines and an overall assessment of the staining profiles of all data. The exact same gating parameters were applied to all datasets.