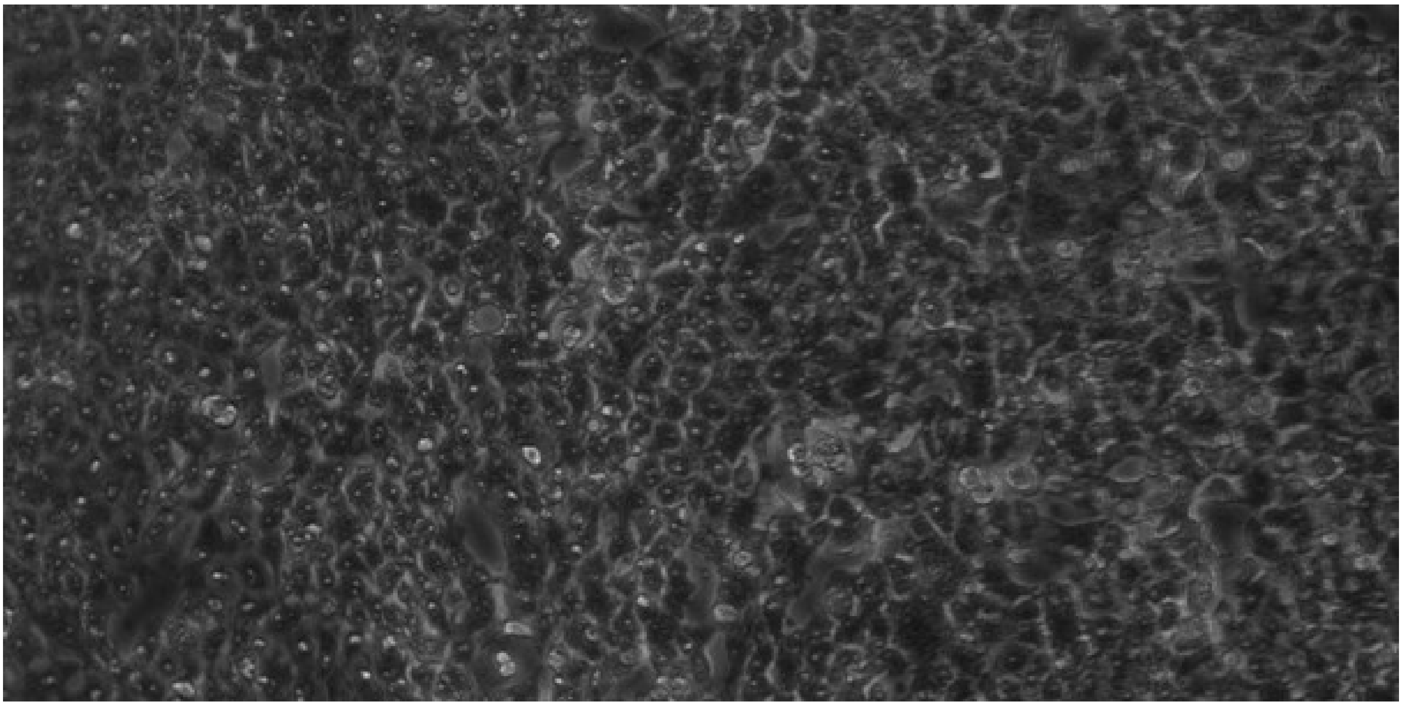
OMI, AUTOMATED ORGAN-ON-CHIP PLATFORM, TO CREATE A GUT-ON-A-CHIP MODEL

Microfluidics for gut-on-chip model (particularly using the Caco-2 cell line) enable precise control over the microenvironment, mimicking the complex physiological conditions of the human gut. Combining the BE-FLOW microfluidic chip with Fluigent's Omi platform creates a system for long-term, automated gut-on-a-chip setups, ensuring precise flow control and continuous nutrient supply for cell differentiation.



1. INTRODUCTION TO GUT-ON-A-CHIP MODELING

Organ-on-chip technology artificially reproduces the physiological complexity of organs using microfluidic and cell culture devices. Gut-on-a-chip models are a powerful tool for investigating the physiology and the pathology of intestines. One major cell type used in this approach is the Caco-2 cell line, which is an immortalized human colorectal adenocarcinoma cell line.

This system is primarily used as a **model of the intestinal epithelial barrier.** In culture, TC7- Caco-2 cells spontaneously differentiate into a heterogeneous mixture of intestinal epithelial cells under the correct flow culture conditions. The cells can create locally 3D structures specific to gut "villi" [2]. The local forces exerted onto the intestinal epithelium range from 0.02-0.08 dyne/cm^2.

Devices that generate fluid flow and shear stress have been reported to promote accelerated differentiation of intestinal epithelial cells, formation of three-dimensional villus-like structures, and enhanced intestinal barrier function, mimicking the complex functions of the normal human intestine [3].

Having flow control for long periods of time is key to enable cellular differentiation. By combining a BE-FLOW microfluidic chip with Fluigent's Omi platform for long-time recirculation experiment, one has a complete system approach to create a gut-on-a-chip model.

Omi can tackle recirculation mediums following an automated protocol set by the user, such as monitored shear stress applied on the cells and continuous nutrient supply to the cells for several days and up to three weeks.

2. MATERIALS AND METHODS: CREATE A GUT-ON-A-CHIP MODEL

Microfluidic Chip

The design of the BE-FLOW consists of 2 independent channels with threaded inlets and outlets facilitating connections to fluidic controllers. It is possible to apply an independent flow rate to each channel. To avoid culture media evaporation during the onset of the experiment (before perfusion has started), water-filled reservoirs are positioned next to the medium reservoir (cf scheme).

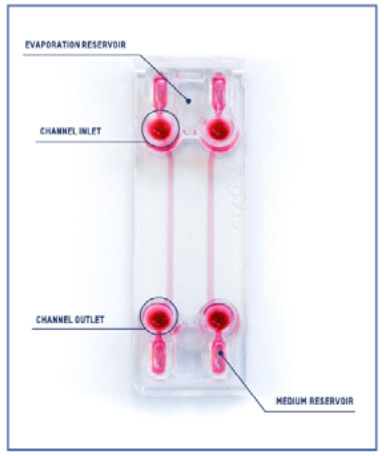


Figure 1: BeOnChip microfluidic chip.

	Height	Width	Lenght	Total volume
Each channel	375 µm	1,5 mm	43 mm	28,5 µL
Inlet/Outlet	7 mm	UNF 1/4''-28		130 µL
Medium Reservoir	5 mm	3,6 mm	8,8 mm	185 µL

* The volumes presented on the table are theorical values calculated for the standard products. Changes in the device features of custom chips may modify the exact channel volume.

Cell Culture

Step 1: Fibronectin coating of the chip

- **Pipette 100 µL** of working solution of fibronectin at 50 µg/mL **and inject it directly** into the channel's inlet. By capillarity the flow is enough to flush air, otherwise tilt the chip a little.
- **Incubate** at room temperature for **1 hour and 30 minutes** under a hood.
- **Remove** the solution from the channel + inlet/outlet using a pipet.
- **Wash** the channel with DPBS two times by tilting the chip to help the flow.
- **Wash** the channel using a small amount of **culture medium.**
- **Aspirate** the medium before seeding.

Step 2: Cell seeding

- Briefly **rinse** the cell layer with **DPBS.**
- Add **Trypsin-EDTA solution to a T25 culture** and incubate at 37°C until the cell layer is dispersed.
- Inhibit the trypsin with **of complete growth medium (DMEM + 10% FBS + P/S).**
- **Centrifuge** the cells to have a pellet at the bottom of the tube, **discard the supernatant.**
- Resuspend the cells to a **medium volume** to reach **a 3M cells/mL** concentration**.**
- **Pipet/introduce** cells at the inlet on the chip and let flow them by **capillarity** through the channel.
- Check that the cells are in the same amount in the inlet and outlet (not in the reservoir).
- **Pipet the excess** of cells in the **inlet** and **outlet (at screw thread level).**
- **Gently add fresh medium, to fill up both sides quickly** to avoid movement of fluid, and the reservoirs.
- Check the channels under the microscope to ensure cell adhesion and morphology.
- Close the petri and incubate the cells **at 37°C over night.**

For more information, see: Be-on-chip website and protocols.

Omi

Omi Technology Description

The Omi is a **pressure-based flow controller** that creates responsive and stable pulseless flow. It's a compact device integrating all the crucial components of a **microfluidic recirculation setup including** a pressure source, pressure-based flow controller, flow sensor (for flow rate regulation), reservoirs, tubing, and electrical connections.

In Omi's recirculation system, there are **two fluidic paths**: one goes from the inlet reservoir through the microchip to the outlet reservoir, and the other links the two reservoirs directly (Fig. 2).

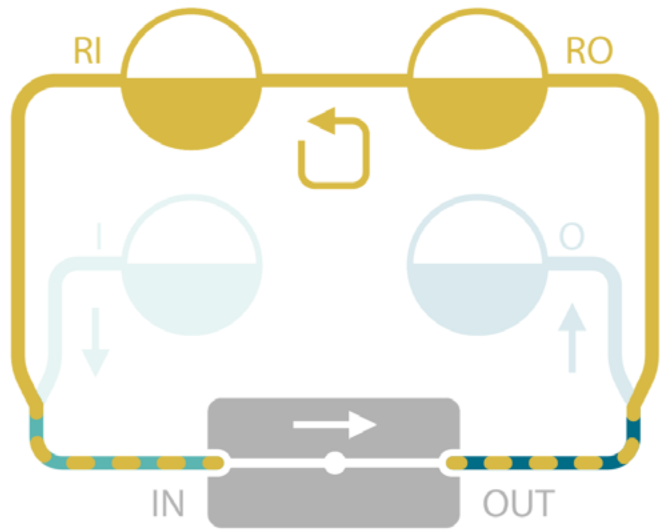


Figure 2: Recirculation system and flow paths in Omi.

Biological and OOC applications require a reproducible, sterile, and contamination-free environment. Consumable, disposable cartridges and chip adaptors have been developed for Omi and are compatible with any type of microfluidic chips (Fig. 3).

Figure 3: Disposable cartridge and chip adaptor.

Protocol

To work with cells, assembling the circuit must be performed under sterile conditions under a biosafety cabinet. Before use, autoclave pneumatic tubes, threaded connections, and ferrules to be used.

Before setting the flow up:

- Sterilize and pre-warm the tubing and the fluidic elements overnight at 37°C.
- Put the system under a laminar flow cabinet.
- Note that the channels and inlets/outlets should never be depleted of culture medium.
- Both inlets and outlets use connectors (1/4' - 28).

Step 1: Preparing Omi.

1. Clean Omi outside and inside with EtOH 70%.
2. **Under sterile conditions,open a new reservoir** and **low resistance adaptor,** and insert them into the Omi bunder the hood.
3. Make the fluidic circuit and launch a **reservoir sensor "calibration" with sterile water.**
4. Run the protocol "**Sterilization**" to ensure your Omi is sterile and ready to use.
5. Replace the low resistance adaptor with a **high resistance adaptor**, as the flow rate used for this application is very low.
6. Run the protocol "Loading" to ensure the whole system is primed with the corresponding culture medium for your gut-on-a-chip model.

Step 2: Connect the chip

1. Check if the Caco-2 cells are ready: **adherent** and **numerous**. Select the channel to perfuse.
2. **Pipette the medium** at the inlet and outlet and in the reservoirs.
3. Add (not directly in the channel) 100 µL of fresh medium to the inlet. Tilt the chip to create a **slow flow** from the inlet to the outlet.

Step 3: Connection and recirculation start

1. Gently insert the connector into the inlet. Screw the PEEK connector snugly. Connect the outlet tubing as previously shown.
2. Fill the reservoir of the second non perfused channel. This channel is useful as a control without flow.
3. Go to the tablet application and load the Caco-2 "recirculation" protocol. It allows users to have a constant low rate of 2µL/min for three days, and then increases to 3 µL/min.
4. Examine the channel under a microscope and turn on recirculation (starting at 2µL/min).
5. Once the flow is stable, place the Omi in an incubator at 37°C for the experimental time.
6. After 4-5 hours of perfusion, pause the protocol and change the medium. This step is to discard the non-adherent cells to the RO medium.

Step 4: Maintenance of the culture

1. Medium should be changed every 1-2 days.
2. Regularly check the cell monolayer.
3. At each medium change, the flow must be paused.

3. RESULTS

Over six days of perfusion, the flow was stopped three times for medium maintenance, and the system is placed under an inverted microscope to monitor cell morphology/behavior/differentiation daily.

As observed in the following figures, cell density has increased after being cultured under flow conditions. Caco-2 started to form 3D structures-a signature of a gut-on-a-chip model- and to colonize the top of the chip after three days, features not seen under static conditions.

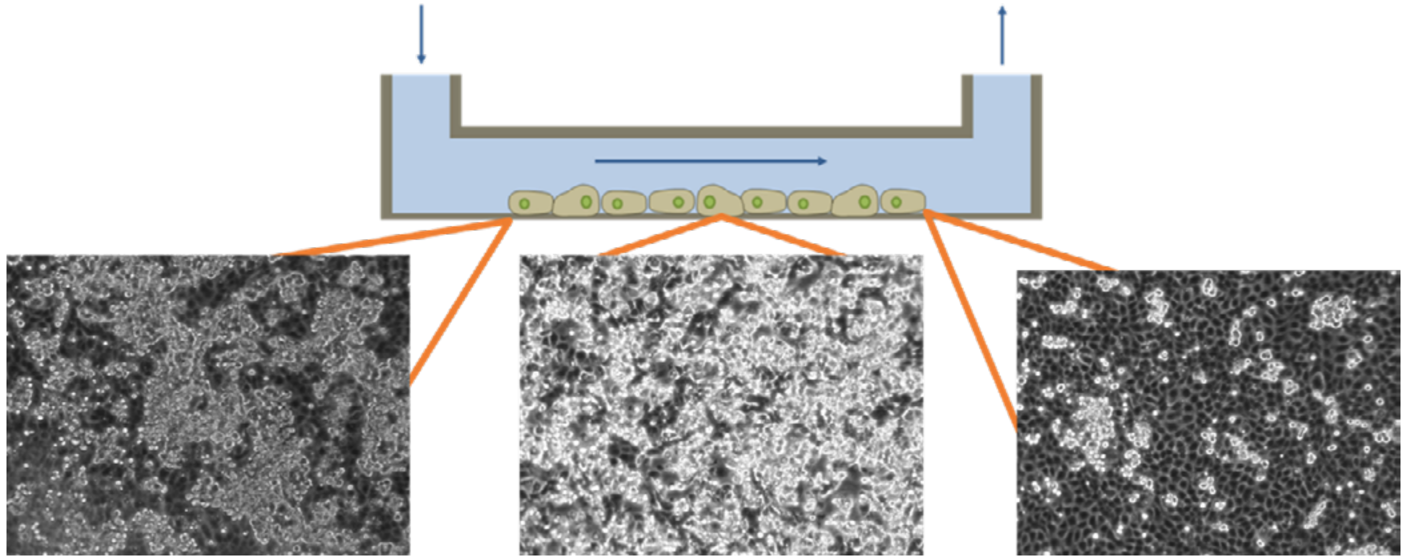


Figure 4. Microscopy photographs (10X) of the perfused channel after initiation of recirculation.

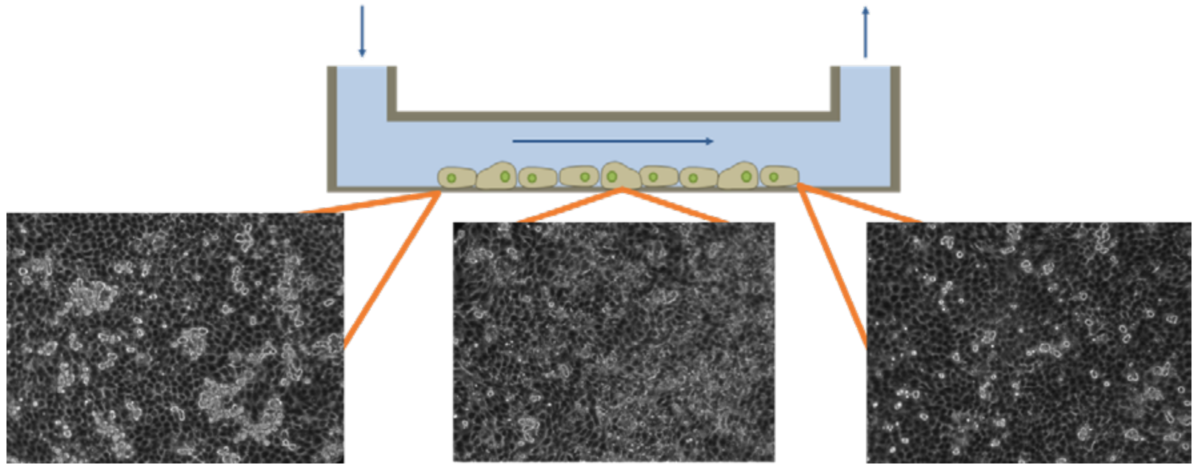


Figure 6. Microscopy photographs (10X) of the perfused channel after 2 days of recirculation.

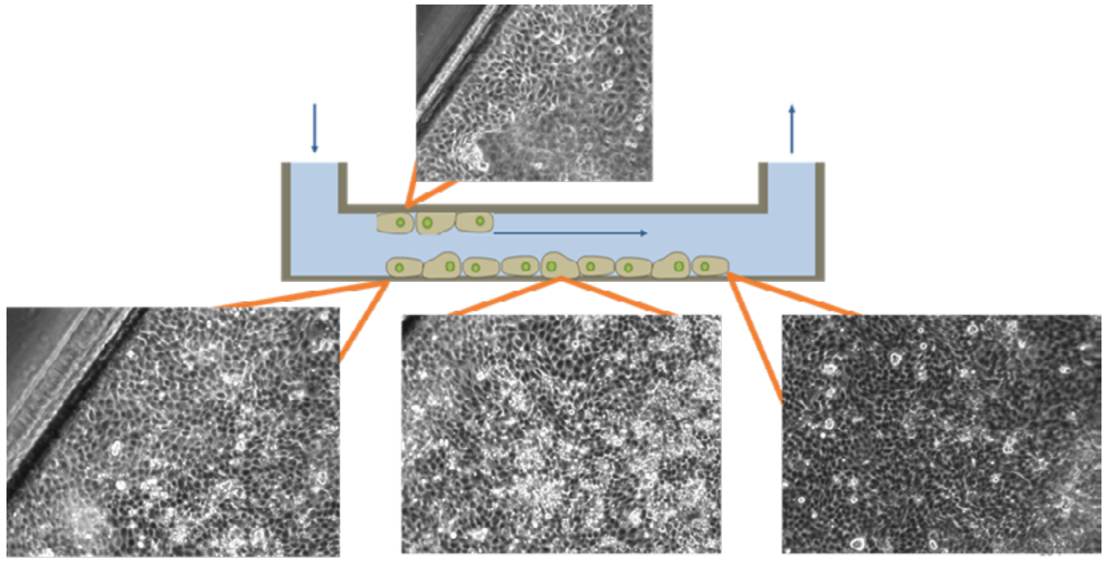


Figure 7. Microscopy photographs (10X) of the perfused channel after 3 days of recirculation.

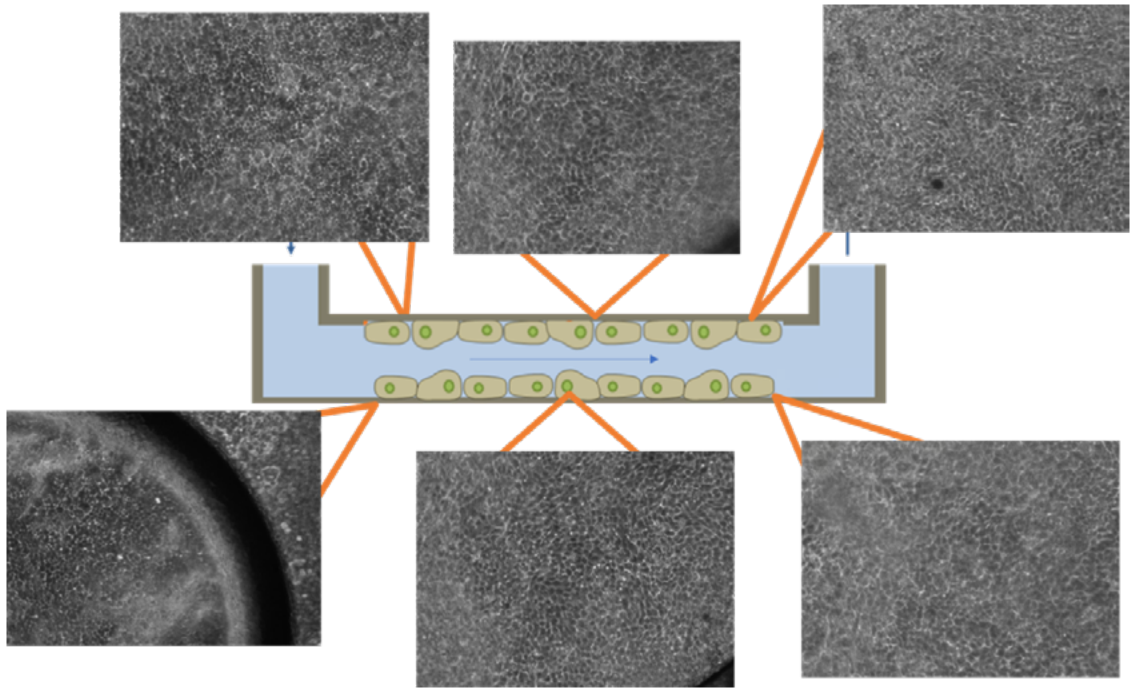
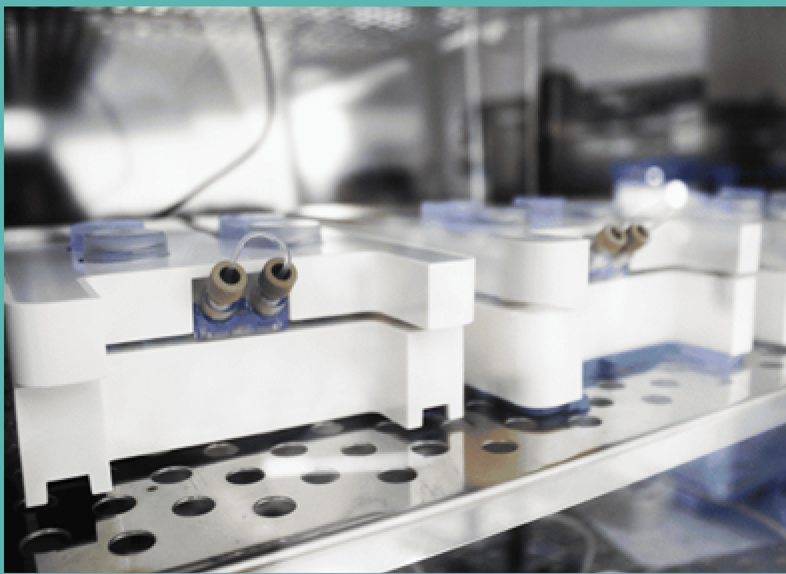


Figure 8. Microscopy photographs (10X) of the perfused channel after 6 days of recirculation.

CONCLUSION

Omi, affiliated with BE-FLOW, is a suitable solution for the long-term recirculation of cell culture media to create cellular gut-on-a-chip model. It provides precise flow delivery, which has allowed users to obtain a monolayer for further experimentation. Omi is easy to use, and can fit under a microscope, making it the perfect companion for on-chip biology experiments.



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FLUIGENT SMART MICROFLUIDICS

BEOnChip Biomimetic Environment On Chip