LONG-TERM RECIRCULATION FOR ORGAN-ON-A-CHIP APPLICATIONS

INTRODUCTION

Organ-on-a-chip technology requires a well-controlled and stable fluid perfusion in a microfluidic system to best mimic a physiological microenvironment leading to gaining advanced insight into cellular responses *in-vitro* resembling those *in-vivo* [1].

Going a step further in the complexity of the system would require the integration of a continuous flow through the chip, allowing the recirculation of the medium.

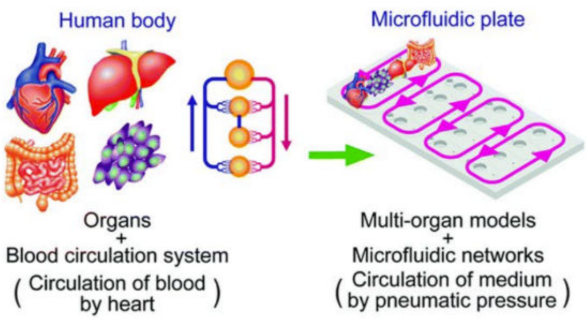

Figure 1: From living organs to organs on chip [1].

THE ADVANTAGES OF RECIRCULATION OF MEDIUM

This technique has several advantages and impacts our understanding of the underlying physiological processes:

- Cells in the living body are always under some kind of shear stress (gastric fluid, urine, blood, etc.)(Figure 2), so this configuration of dynamic cell culture creates more relevant biological explanations.

- On top of providing continuous nutrients and oxygen for the cells, the culture media will be enriched in cell secretion factors over time, allowing simulation of cell communication *in-vivo.*

- The setup of recirculation of media in the chip allows long-term experiments (a few hours/few days/few weeks).

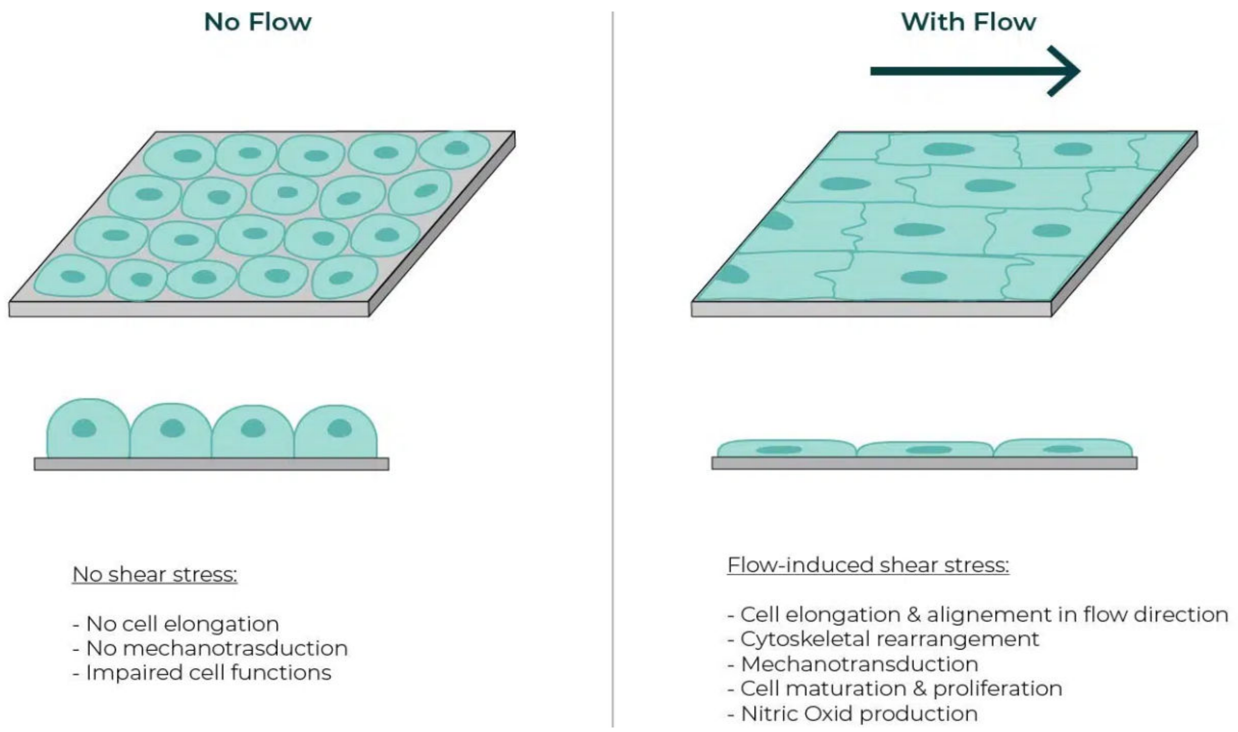

Figure 2: Dynamic cell culture to better mimic cells in-vivo.

TECHNOLOGIES FOR RECIRCULATION OF MEDIUM

Recirculation of the medium may be achieved by using different technologies [2] depending on the application.

- With gravity-driven flow, for example, the use of a tilting rocker platform could be simple and effective in the case of refreshing the culture medium. However, the flow rate is uncontrolled and physiological relevance is limited.

- Syringe pumps are easily used in some cases of unidirectional flow, but the main limitation in OoC applications is the limited volume of the syringe hindering long-term experiments.

- Peristaltic pumps enable recirculation from and toward the same reservoir. However, the induced flow is pulse-like with oscillations of more than 40% of the set flow rate value (Figure 3). This may have increased risks on cell membrane integrity and physiological functions, and on adherent cell detachment.

- Pressure-based pumps provide high accuracy and stability of the flow profile and an improved response time compared to other technologies.

With Fluigent's new Omi, dedicated to OoC applications, medium recirculation is easily performed for long-term cell culture under flow to generate controlled ideal shear stress conditions.

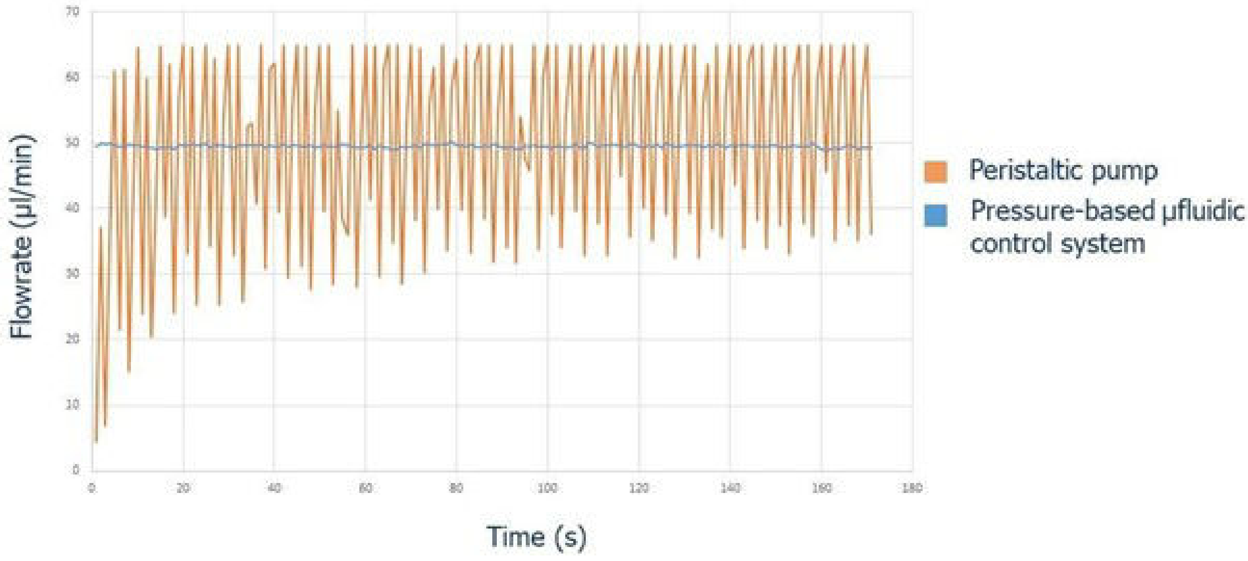

Figure 3: Flow rate stability using a peristaltic pump and a pressure controller.

OMI TECHNOLOGY DESCRIPTION

Omi is an innovative platform giving access to multiple possibilities for OoC applications.

It's a pressure-based flow controller that allows highly responsive and stable pulseless flow.

It's a compact device integrating all the crucial components for a microfluidic recirculation setup: pressure source, pressure-based flow controller, flow sensor (for flow rate regulation), reservoirs, as well as all the tubing and electrical connections (Figure 4).

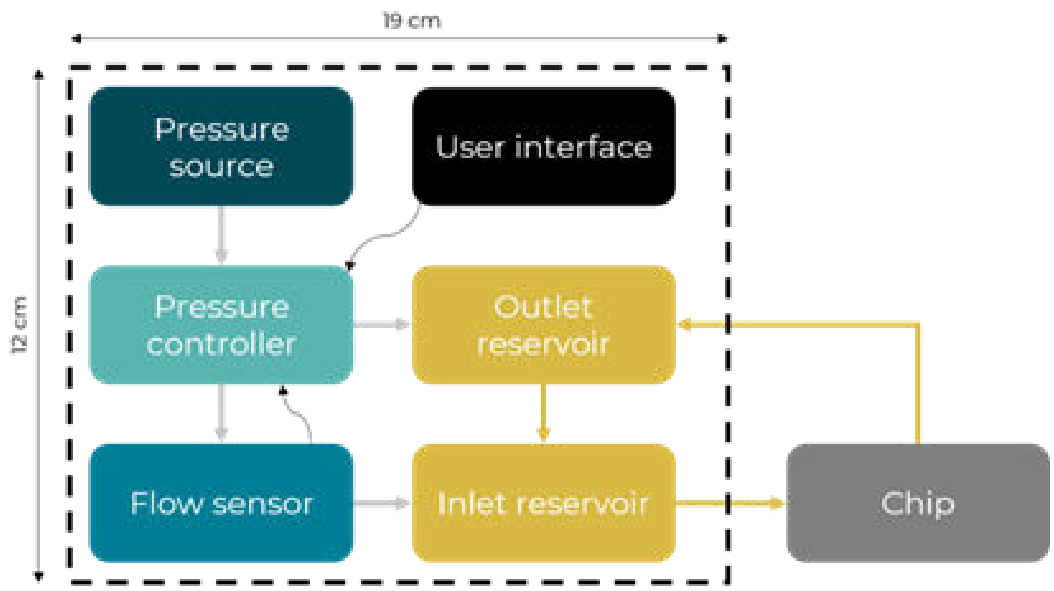

Figure 4: Omi's all-integrated recirculation system.

In Omi's recirculation system, there are two fluidic paths: the first one goes from the inlet reservoir through the microchip to the outlet reservoir, and the second links the two reservoirs directly (Figure 5).

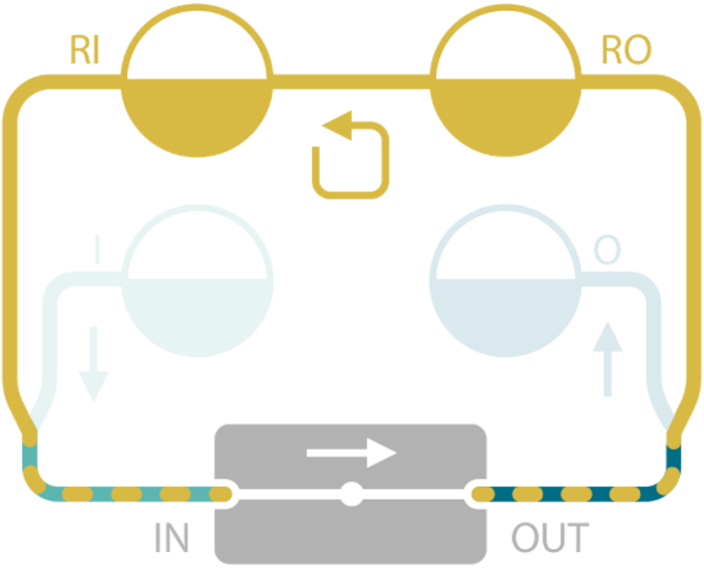

Figure 5: Recirculation system and flow paths in Omi.

Omi's recirculation device is equipped with liquid level sensors (patented) that detect when the device needs to be refilled, allowing automation of the whole recirculation process.

These optical sensors are a combination of an infrared LED and an infrared photoreceptor integrated into a single component.

Biological and Ooc applications require a reproducible, sterile, and contamination-free environment, therefore, consumable disposable cartridges and chip adaptor have been developed for Omi recirculation system with any type microfluidic chips.

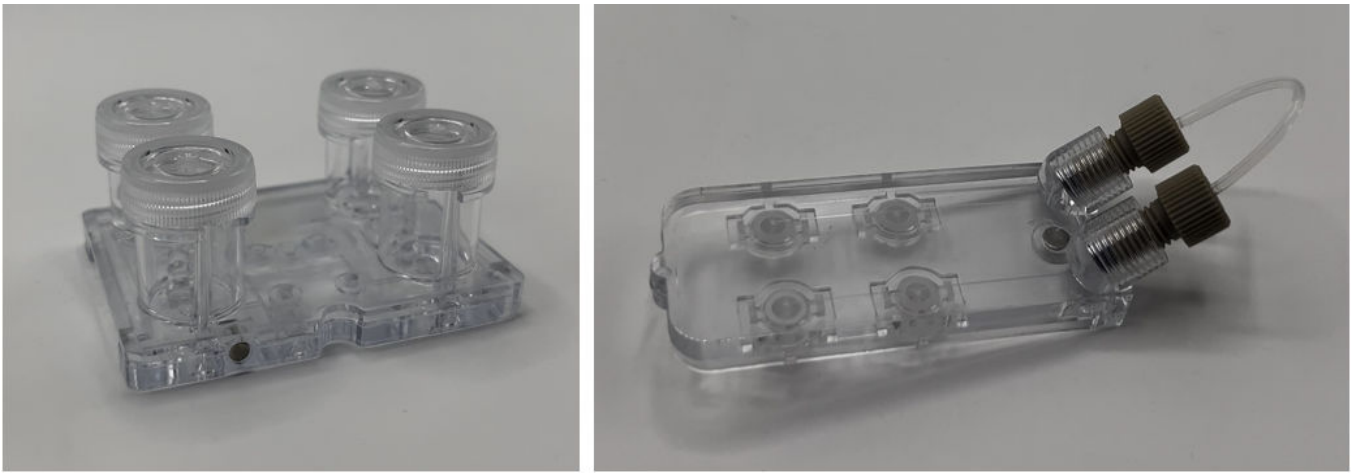

Figure 6: Disposable cartridge and chip adaptor.

RECIRCULATION FOR OOC APPLICATIONS

Omi's microfluidic system can be controlled both manually using an embedded touchscreen or remotely using a Wi-Fi connection. Two options are available for the remote control: either via an application on a tablet or via a web interface from any device supporting a browser.

The associated software (Figure 7) allows for the creation of protocols with predefined steps such as injection, perfusion, sampling, and recirculation, also defining parameters such as volumes, flow rates, durations, or flow patterns. The software allows pairing and registering devices, monitoring experiments, and visualizing data.

The connection to a cloud allows users to collect and store data that can be retrieved when needed.

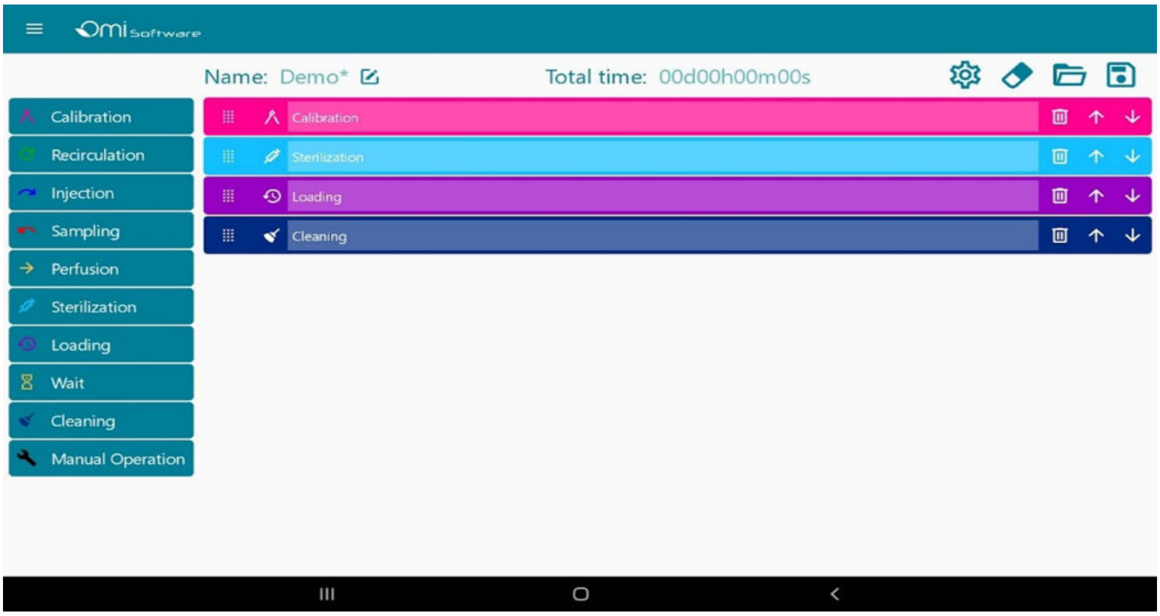

Figure 7: Omi software interface.

MATERIALS & METHODS

Omi (Figure 8) is an automated pressure-based flow controller capable of performing various perfusion protocols: simple perfusion, uni-directional recirculation, sampling, and injection.

Any type of microchip can be used with Omi thanks to the chip adapter, offering possibilities for different cell culture types or organ-on-a-chip models.

It can be placed directly in the incubator for long-term experiments, as it can be easily transported and positioned under the microscope to have a real-time observation of cell behavior.

One Omi is used in the system for one microfluidic chip.

For co-culture types of studies using two microfluidic chips, interconnecting two Omi is possible.

Figure 8: Omi standalone.

Table 1: List of materials included in Omi's uni-directional recirculation package.

OMI standalone	Automated flow controller
OMI tablet	Samsung Galaxy Tab S6 Lite 10.4" - 64 Go Ref SM-P610
Cartridge	3 Disposable sterile cartridges
Low-resistance chip adaptor	3 reusable adaptors
High-resistance chip adaptor	3 reusable adaptors
Connectors and tubing kit, Pneumatic kit & power supply kit	

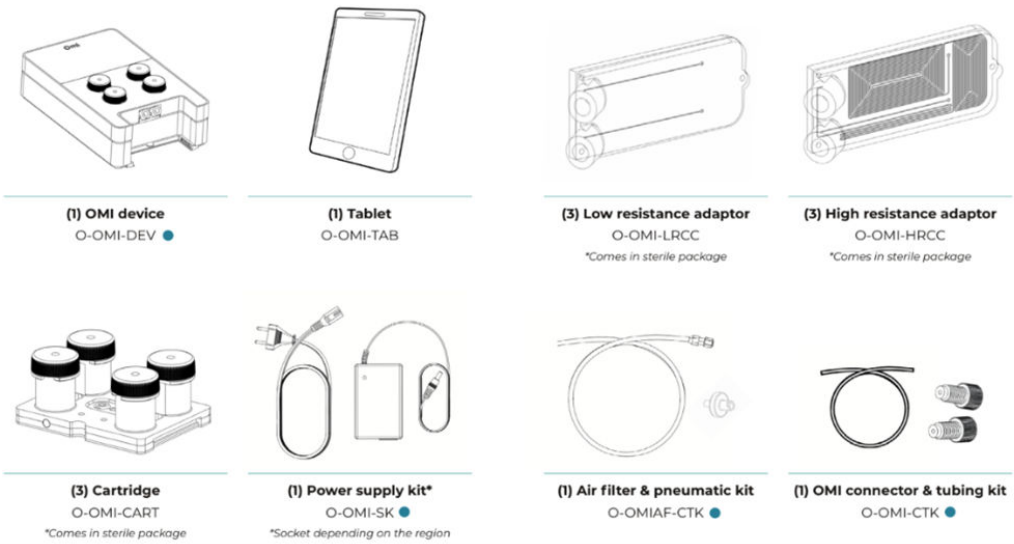

Figure 9: What is included in Omi standalone package.

In order to test the stability of the flow rate in Omi recirculation system, a long-term experiment was conducted using distilled water.

After the calibration, sterilization, and loading of the device with distilled water, a recirculation step of a defined flow rate (5 µL/min, 10 µL/min or 50 µL/min) for 14 days was launched, and the device was placed inside the incubator.

Monitoring the experiment constantly was available via the web interface.

RESULTS

Figure 10 is a close-up representation of the Flow rate in µL/min (orange graph) and the Pressure rate in mbar (purple graph) versus time.

This small section of the experiment shows only a few minutes time-lapse to highlight the recirculation and refilling steps.

During the recirculation cycles, the rates measured by the sensors are very stable.

Once the inlet reservoir is empty, the pressure will drop, the flow will stop and the valves will open, allowing liquid to refill the reservoir, and beginning a new recirculation cycle.

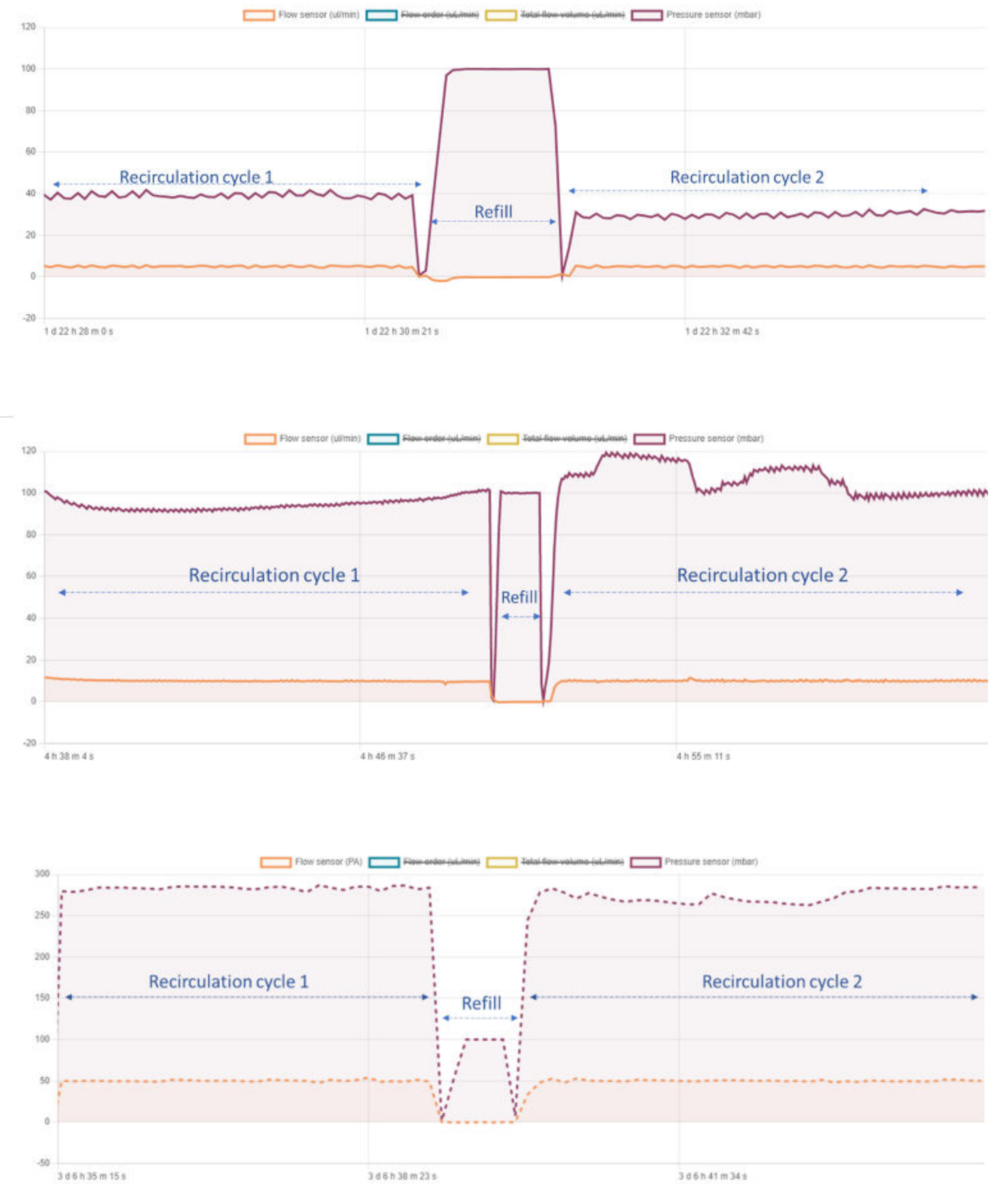

Figure 10: Flow rate and Pressure rate monitoring over time for 5 µL/min, 10 µL/min or 50 µL/min, respectively.

Figure 11 shows the flow rate and the pressure measured by the sensors over an 11-hour portion of the 14 day experiment (Input flow rate 50 µL/min).

As shown, Omi device is able to maintain a stable flow rate (5/10/50 µL/min) with regular refill steps for several days without any issues in the emptying and refilling of the reservoirs.

Omi is a suitable solution for many biological applications, ranging from underflow cell cultures and live cell imaging [3] to studying cell secretome [4] and organ-on-a-chip applications [5].

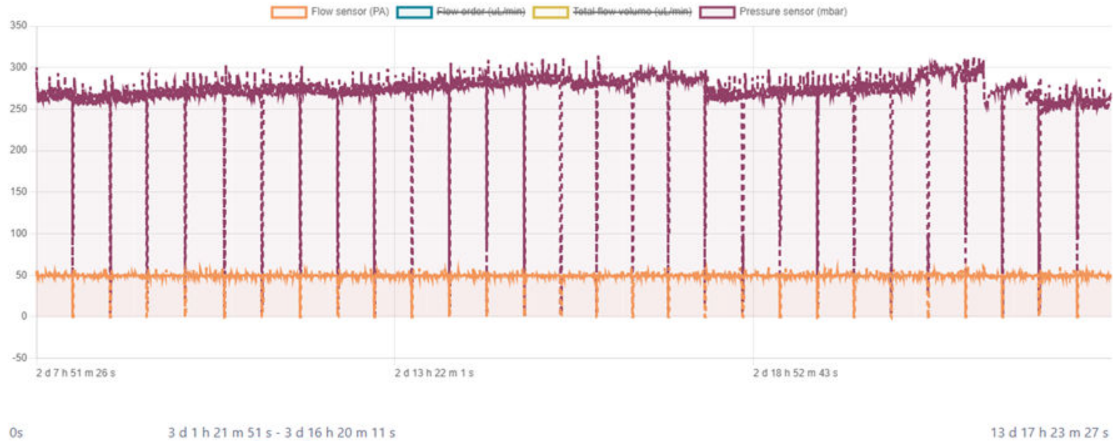

Figure 11: Recirculation performed over several hours with Omi recirculation system.

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