

## White Paper

# VitroFlow.Bio - An Integrated and Standardized Platform for Organ-on-a-Chip Applications

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### Abstract

The utilisation of animal models, such as mice, remains an established experimental standard in biomedical research, and their continued use is necessary in specific contexts. Nevertheless, their translational predictive value is limited in several applications, and their use entails substantial time, cost, and ethical considerations. Non-animal methods (NAMs), including Organ-on-a-Chip (OoC) technologies, represent a promising technological advancement by enabling controlled, human-relevant in vitro models under dynamic conditions. Despite significant technological progress, the OoC market remains highly fragmented. Many available systems are either restricted to proprietary chip formats or consist of individually assembled setups that lack standardisation and reproducibility. At the same time, the field is evolving towards increasingly complex and disease-specific models, which require flexible integration of diverse chip architectures, controlled microenvironments, and compatible analytical readouts. However, a universally applicable platform capable of reliably operating this diversity of models within a standardised experimental framework is currently lacking. VitrofluidiX addresses this gap with VitroFlow.Bio, a hardware platform designed to operate multiple chip architectures within a unified and standardised control environment. The system integrates incubation control, temperature regulation, and programmable perfusion into a consolidated setup, enabling reproducible microenvironmental conditions across different experimental configurations. By combining architectural flexibility with standardised operational control, VitroFlow.Bio supports the implementation of diverse OoC and organoid-on-chip applications within routine laboratory workflows while reducing system complexity. It is important to note that this document has not undergone peer review and therefore does not provide external validation of the VitroFlow.Bio v2.3 hardware subsystems. Results from dedicated biological validation studies will be presented separately.

**Keywords:** Microfluidics, Organ-on-a-Chip, 3D cell culture, Organoids-on-Chips, Lab-on-a-Chip

### 1. Introduction

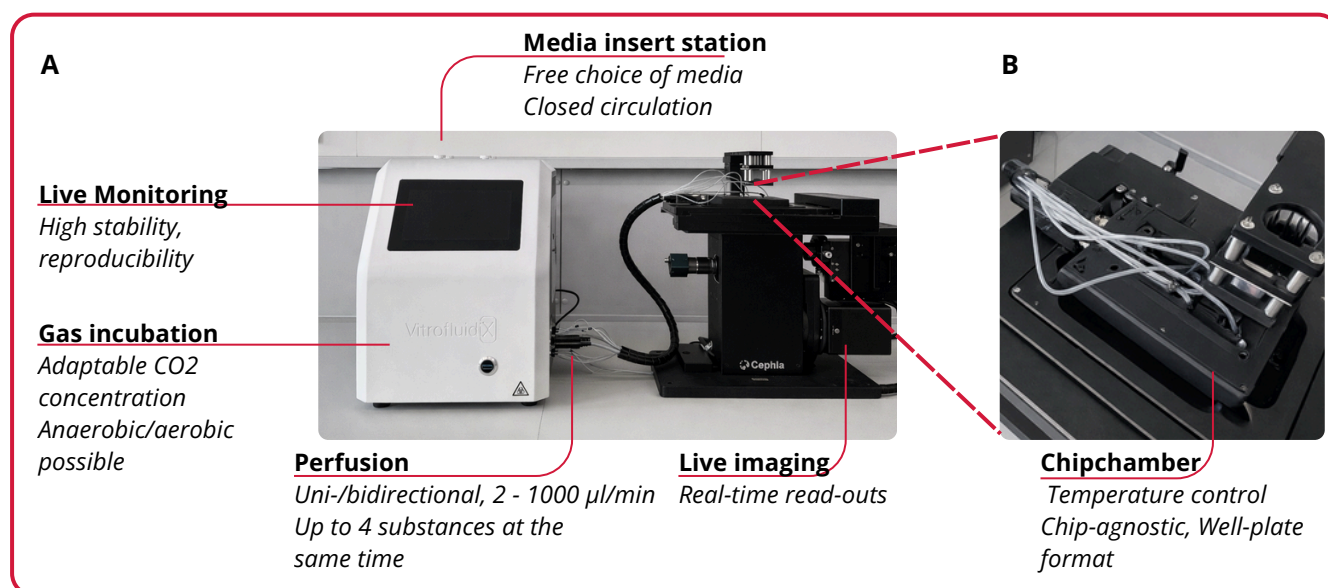
Organ-on-a-Chip (OoC) technologies are increasingly used to generate dynamic, human-relevant in vitro microenvironments [1]. By combining microfluidic perfusion with defined spatial organisation and controlled environmental parameters, these systems enable more physiologically relevant cell behaviour compared to static culture conditions [2]. The field is evolving rapidly towards increasingly complex and disease-specific chip architectures [3]. This diversification of models is a desired development, reflecting scientific

progress and the need for application-specific solutions [4]. However, while chip designs continue to advance, the peripheral control infrastructure required to operate them often remains fragmented and insufficiently standardised.

Many OoC experiments rely either on proprietary chip-specific devices or on individually assembled peripheral setups composed of external pumps, incubators, tubing systems, and imaging solutions [5].

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**Figure 1:** **A** Overview of the VitroFlow.Bio (v2.3) with indicators for the accessible external parts of the device seen in **A**, **B** Image of chip chamber connected to the VitroFlow.Bio (A) as it is placed under an inverted microscope.

Variations in perfusion control, gas conditioning, tubing configuration, internal volumes, and temperature regulation can significantly influence shear stress, residence time, and overall microenvironmental stability. As a consequence, experimental reproducibility and cross-laboratory comparability may be compromised. From an engineering perspective, increasing system integration reduces potential sources of variability. Consolidating perfusion control, environmental conditioning, and thermal regulation within a unified architecture enables more stable operating conditions and simplifies experimental workflows. Standardised control of critical parameters becomes particularly important as chip architectures grow more sophisticated.

VitrofluidiX GmbH has developed the VitroFlow.Bio platform to address these technical challenges (see Fig. 1). The system is designed to support multiple chip architectures within a standardised hardware environment. It integrates controlled perfusion, gas conditioning, and temperature regulation into a unified setup, while remaining adaptable to diverse chip geometries. The platform further enables direct implementation of live imaging modalities without reliance on specialised incubator-compatible microscopy systems. Its open architecture is intended to facilitate future sensor integration and methodological

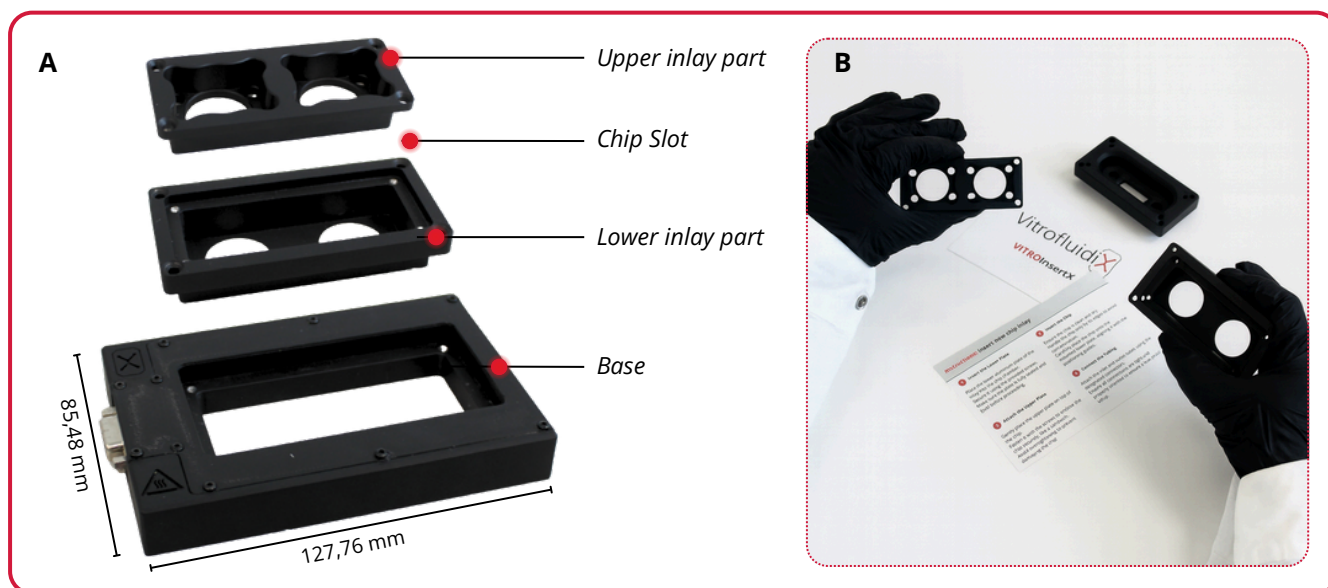
expansion while maintaining automated and reproducible operation.

This white paper focuses exclusively on the technical and functional validation of the VitroFlow.Bio v2.3 hardware subsystems. The data presented herein evaluate temperature stability, gas conditioning precision, and perfusion control performance. Dedicated biological validation studies and application-specific data will be presented separately.

### 1.1. VitroFlow.Bio

The VitroFlow system was developed to address current technical limitations in OoC experimentation by providing a controlled, modular, and reproducible microphysiological environment. A major redesign of the core components of conventional OoC setups was undertaken to create an integrated microfluidic platform. This combines circulation, incubation, environmental control, monitoring, and chip imaging within a single system.

The VitroFlow system is characterised by a closed-loop microfluidic circulation architecture. The commencement of each circuit is initiated at the medium insert, where a 6 mL vial containing cell culture medium is positioned within a mechanical elevator mechanism. The cannulas pierce the elastomeric seal of the vial, thereby.



**Figure 2:** **A** detailed overview on the chip chamber. Seen on the left side are the three parts of the chip chamber: The well-plate formatted base of the Chip as well as the upper and lower inlay parts with the slot for the microfluidic chip in between. Both upper and lower part form the Insert product line of VitrofluidiX. **B** Image of an exemplary InsertX with packaging for the Fluidic480 of MicrofluidicChipShop.

establishing a sterile and secure connection to the system. The vial serves as a reservoir from which a peristaltic perfusion pump continuously drives medium through the circuit. The medium is then introduced into the integrated CO<sub>2</sub> incubation module within the primary device, where gas exchange and temperature are meticulously regulated. Subsequent to the process of incubation, the medium is subjected to an inline bubble trap and subsequently exits the primary apparatus via a tubing interface that connects to the external chip chamber.

The chip chamber comprises a well-plate-formatted heated aluminium platform, which has been designed to accommodate microfluidic chips while maintaining stable thermal conditions during perfusion (see Fig. 2). Subsequent to passing through the microfluidic chip that contains the OoC, the medium is returned to the primary device and recirculated into the reservoir vial, thereby establishing a continuous closed-loop system. Integrated sensors are utilised to monitor key actuators, including pumps, heating elements and CO<sub>2</sub> incubation. This enables feedback-controlled operation and ensures stable long-term performance. VitroFlow.Bio integrates four independent circulation loops within a single device, thereby enabling parallel operation of up to four mono-layer chips or, by way of illustration, two double-layer chips.

Each circuit can be subjected to individual control and analysis. The system's modular architecture facilitates scalability through circuit replication, as well as the integration of additional sensors or functionalities in subsequent iterations. This multi-circuit closed-loop configuration provides the foundation for reproducibility, sterility, and long-term stability in advanced OoC applications.

### 1.2 Perfusion System

The VitrofluidiX perfusion system is composed of three primary components that facilitate controlled, bubble-free flow rates ranging from 2  $\mu\text{L}/\text{min}$  to 1000  $\mu\text{L}/\text{min}$  (see Technical Datasheet – VitroFlow.Bio v2.3). The system incorporates a peristaltic pump developed by VitrofluidiX, an SLF3S-0600F flow sensor (Sensirion), and an inline bubble trap (PeciGenome).

The peristaltic pump is subject to continuous monitoring by the flow sensor, thereby providing real-time flow data and enabling precise regulation. The removal of entrapped gas bubbles is facilitated by employed in the peristaltic pump and the flexible outer tubing towards the chip chamber. Stainless steel tubing with an inner diameter of 0.75 mm is utilised in the remainder of the system.

Parameter	Sensor	Method
Flow	EK-SLF3S-0600F	Thermal displacement
Oxygen	OXROB10-HS	Optical Ru(bpy)
	Gravity analog dissolved O <sub>2</sub>	Clark electrode
Temperature	Bosch UniversalTemp	IR emission

**Table 1:** The Table shows the sensory devices used for validating flow rates, oxygen concentrations and the chip chamber temperature as well as the sensing method used by each of the sensory devices.

This dimension represents a compromise between minimising internal volume and maintaining sufficient lumen diameter to limit excessive shear stress, particularly in applications involving circulating immune cells. The selected SLF3S-0600F flow sensor operates on the principle of thermal displacement rather than differential pressure measurement, thereby enabling accurate low-flow detection under recirculating conditions. The present document is an evaluation of the technical capabilities and limitations of the perfusion system.

### 1.3 Incubation Module

Gas conditioning in microfluidic systems is commonly achieved by placing the entire setup within a conventional CO<sub>2</sub> incubator. While this approach has been demonstrated to be effective in the context of environmental control, it does have the disadvantage of occupying laboratory infrastructure and exposing electronic and mechanical components to elevated levels of humidity and temperature, which can increase the risk of failure. Moreover, external incubation frequently necessitates the utilisation of specialised microscopy solutions, thereby restricting imaging flexibility. VitrofluidiX addresses these limitations through an integrated microfluidic gas incubation module. The system employs a gas-permeable medium channel situated within

a regulated gas chamber, enabling precise modulation of CO<sub>2</sub> concentrations ranging from 0 to 100% (v/v) (refer to the Technical Datasheet – VitroFlow.Bio v2.3 for further details). Gas diffuses into the perfused medium without enabling evaporation, which is critical in low-volume microfluidic applications.

It is imperative to note that all other tubing within the system is gas-impermeable, thereby ensuring that the conditioned medium maintains its defined gas concentration until reaching the chip. The technical performance of gas conditioning across varying CO<sub>2</sub> concentrations is evaluated herein.

### 1.4. Chip chamber

Precise temperature control is imperative for maintaining stable cell culture conditions. The VitrofluidiX chip chamber is equipped with an external thermal regulation system for mounted chips, with a range of 25–92°C (see Technical Datasheet – VitroFlow.Bio v2.3).

The baseplate has been formatted according to standard well-plate dimensions to ensure compatibility with the inline bubble trap and prevent disruption to flow. The tubing, which has an inner diameter of 1.0 mm and is made of thermoplastic polyurethane (TPU), is compatible with conventional microscopy platforms. Incorporating an integrated heating unit ensures uniform temperature distribution.

The chip chamber incorporates the VitroInsertX adapter system, which allows for the secure mounting of chip-specific geometries between the upper and lower adapter elements. This design ensures mechanical stability and adaptability to various chip architectures. Integrating the chip chamber and VitroInsertX with the VitroFlow system enables the continuous perfusion of preconditioned medium into a temperature-controlled chip while concurrently facilitating real-time imaging. Subsequent sections present the functional validation and precision testing of temperature stability and integration performance.

## 2. Materials & Methods

To validate the complex VitroFlow.Bio system, several methods were used, including flow, gas, and temperature measurements, as well as system stability and sterility tests. The devices and sensors required for these tests were calibrated according to the manufacturer's documentation. Meanwhile, raw sensor data obtained using the sensor manufacturers' software was analyzed with R Studio (version 4.0.1) and Excel.

### 2.1. Flow Measurement using Thermal displacement sensor

The SLF3S-0600F Evaluation Kit [6] by Sensirion was used to measure the flow rate created by the VitroFlow perfusion system. Although it uses the same sensor as the VitroFlow.Bio, using the EK-SLF3S-0600F in combination with Sensirion software can minimize possible bias. The sensor operates using a small heater to create a cloud of warm medium. When flow is applied, the cloud moves over an array of thermometers.

The perfusion system was tested at physiologically relevant flow rates ranging from 1 to 50  $\mu\text{L}/\text{min}$ , as well as at the maximum operating speed. The sensor was placed in the position where the microfluidic chip would attach to the VitroFlow.Bio.

### 2.2. Incubator Validation Using Optical and Electrical O<sub>2</sub> Measurement

Since dissolved CO<sub>2</sub> sensors are not regularly available, the measurement of O<sub>2</sub> was chosen as a feasible substitute indicator for dissolved CO<sub>2</sub> concentrations. According to Henry's Law, the concentration of a dissolved gas ( $c_i$ ) is proportional to its partial pressure ( $p_i$ ) in a gas mixture, and the Henry constant ( $k_h$ ) remains the same.

$$c_i = k_H \times p_i$$

Thus, incubating a CO<sub>2</sub>-air mixture instead of air reduces the partial pressure of O<sub>2</sub>, leading to a decreased concentration of O<sub>2</sub> due to the VitroFlow-Incubator's volumetric operation.

$$x_{CO_2} = \frac{\dot{V}_{CO_2}}{\dot{V}_{CO_2} + \dot{V}_{Air}}$$

$$x_{CO_2} = \frac{CO_2 \text{ concentration} \times \dot{V}_{CO_2}}{\dot{V}_{CO_2} + \dot{V}_{Air}} = \frac{\text{Volume flow of } CO_2}{\text{Volume flow of air}}$$

Therefore, the oxygen concentration in a CO<sub>2</sub>-air mixture is defined as the percentage of the mixture's volume that is oxygen, expressed as a fraction of the total volume of the mixture ( $V_{Air} + V_{CO_2}$ ). To measure O<sub>2</sub> concentrations, a Clark electrode by DFRobot was used for high-flow-rate measurements ( $\geq 100 \mu\text{L}/\text{min}$ ) as a proof of concept. An optical sensor by PyroScience was used for measurements at physiologically low flow rates ( $\leq 100 \mu\text{L}/\text{min}$ ).

This choice was made since Clark electrodes are reliable, but use up  $O_2$  due to their electrochemical reaction [7]:

**Cathode reactions:**

1.  $2H_2O + O_2 + 2e^- \rightleftharpoons H_2O_2 + 2OH^-$
2.  $H_2O_2 + 2e^- \rightleftharpoons 2OH^-$

**Anode reactions:**

3.  $4Ag \rightleftharpoons 4Ag^+ + 4e^-$
4.  $4Ag^+ + 4Cl^- \rightleftharpoons 4AgCl$

At low flow rates, the oxygen consumption rate of the electrode could exceed the medium exchange rate in the measurement element, resulting in imprecise results. For lower flow rates, the optical sensor was used because it does not consume oxygen.

### 2.3. Temperature Measurements Using Infrared Emission

In addition to the internal DS18B20 sensor integrated into the VitroFlows chip chamber, which is employed for temperature regulation, an infrared thermometer manufactured by Bosch (UniversalTemp) was utilized to verify the set temperature within a range of 30-43°C. This range is considered to be within the physiologically relevant parameters for cell culture experiments. IR-based measurements were obtained by placing a Fluidic480 Chip by Microfluidic Chip Shop into the corresponding VitroInsertX for the chip chamber by VitrofluidiX. The measurements were taken at the cell culture area of the chip while water was perfused.

### 2.4. Sterility Test in Non-Sterile Environment

The long-term sterility of the VitroFlow system was assessed by examining the fact that biological metabolism is net acidic, leading to extracellular acidification. This process involves the incorporation of phenol red, a biocompatible pH indicator that is frequently included in cell culture media. Therefore, a contamination, defined as biological metabolism, will acidify the medium, resulting in a color shift from red to yellow.

The VitroFlow System was subsequently filled with high glucose DMEM medium, as established by Roth, and operated at a rate of 100  $\mu$ l/min for a period of seven days. In order to increase the probability of contamination during this timeframe, no antibiotics were added to the medium, and the system was placed in an e. coli laboratory. Following a period of seven days, the medium underwent photometric analysis utilizing a V-730 photometer manufactured by Jasco. The analysis focused on the 560-nanometer wavelength, as this represents the maximum absorption peak of phenol red in its red state. The results demonstrated a significant decline in absorption intensity upon the transition to yellow. A sample of DMEM was aliquoted on day 7 of the experiment to serve as the negative control, ensuring that the sample remained uncontaminated during this period.

### 2.5. Stability Test and Electromagnetic Interference (EMI)

Given the diminished reliability of laboratory equipment, a series of long-term experiments were conducted to assess the reliability of VitroFlows. In this experiment, the system was filled with water and operated at various parameters for a period of 2-4 weeks. The temporal framework was selected through the simulation of the experimental parameters and duration. Subsequently, the system was employed. On weekdays, each subsystem of the VitroFlow was examined on a daily basis. Depending on the specific laboratory environment, particularly in facilities where potentially EM-emitting or EM-sensitive prototypes and equipment are in operation, ENISO61010 conformity can be of paramount importance. Therefore, an Electromagnetic Interference (EMI) test was conducted at Würth Elektronik eiSos to ascertain the company's compliance with EMI standards.

### 3. Results

#### 3.1. Pumping System

As is typical of a peristaltic, the mean flow rate is subject to variations due to the mechanical design. Consequently, a maximum flow rate is attained at an angle of 60° for each of the six ball bearings of the pump, at which point the ball bearings cease to be in contact with the tube. As demonstrated in Figure 3A, the average flow rate (40 minutes at 10 Hz,  $n = 24,000$ ) exhibits a strong linear relationship ( $R^2 = 0.9654$ ) with increasing rotor speed. This finding indicates that the VitroFlow pump is suitable for generating flow rates relevant for organ-on-a-chip applications. Given the variability of chip geometry, which is contingent upon the application and manufacturer of the microfluidic chips, it is impractical to extrapolate a universal flow dynamic and shear-stress profile produced by the VitroFlow perfusion. Therefore, VitrofluidiX has optimized the flow rates to a broad range, aligning with the recommendations of prominent chip manufacturers such as Ibidi or Microfluidic Chip Shop. This allows for the accommodation of diverse shear stress conditions.

#### 3.2. Incubator

The incubator was operated at a high flow rate of 1,000  $\mu\text{L}/\text{min}$  with iteratively increasing  $\text{CO}_2$  concentrations in the VitroFlow incubator to demonstrate its efficacy in incubating various  $\text{CO}_2$  concentrations into a watery medium. As illustrated in Figure 3C, the utilization of the VitroFlow incubation system leads to a consistent decline in the dissolved  $\text{O}_2$  concentration as the volumetric  $\text{CO}_2$  concentration injected into the incubator is augmented. This observation indicates that  $\text{O}_2$  is being substituted by  $\text{CO}_2$  during the course of this experiment. In order to validate that the remaining tubing utilized in the VitroFlow is indeed non-permeable to gases and that no connectors could serve as a source of loss in gas concentration, a measurement was conducted at 100%  $\text{CO}_2$  and 5  $\mu\text{L}/\text{min}$  to create a high diffusion potential at a low enough flow rate to simulate lower limits of OoC applications.

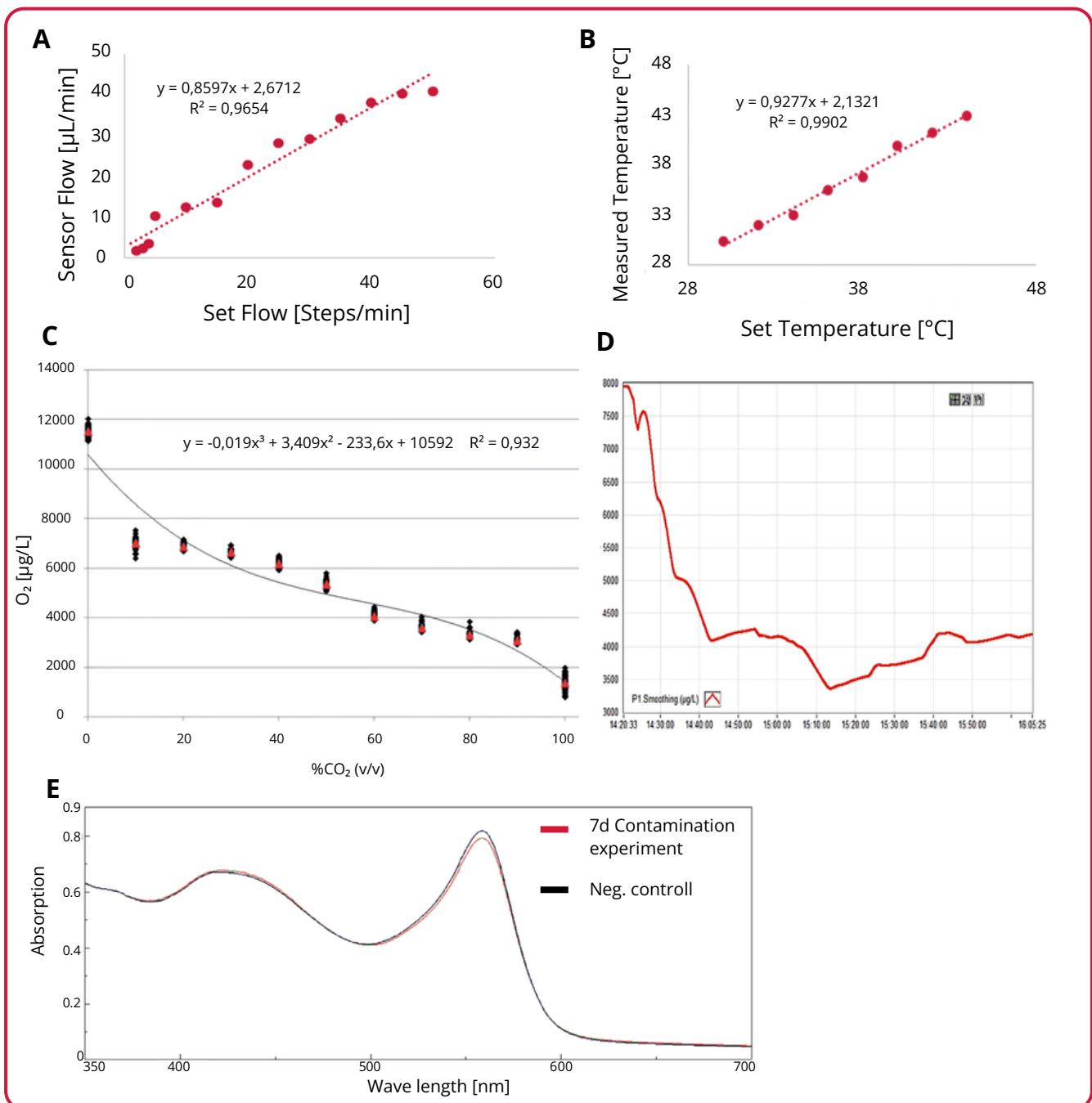
As illustrated in Figure 3C, the VitroFlow incubator demonstrated its capacity to maintain its ability to produce concentrations of approximately 2600  $\mu\text{g}/\text{L}$   $\text{O}_2$ . A marginal rise was observed in the proof of concept, reaching 1800  $\mu\text{g}/\text{L}$  under identical conditions when compared to the initial value. Concurrently, the variance of both measurements at 100%  $\text{CO}_2$  remains constant. Figure 3D illustrates the anticipated logarithmic decline in oxygen levels in water, initiated upon commencing the experiment, as predicted.

#### 3.3. Chip Chamber

A comparison of the measured temperature at the cell culture area of the Fluidic480 to the set temperature of the VitroFlows chip chamber reveals a strong linear correlation, as depicted in Figure 3B. The correlation coefficient,  $R^2$ , is 0.9902, indicating a high degree of linearity in the data. This outcome demonstrates the VitroFlow's capacity to produce reliable temperature incubation and OoC.

#### 3.4. VitroFlow: Bio stability and sterility

Following a seven-day circulation of the DMEM medium and subsequent photometric comparison with the negative control, the spectral analysis revealed no statistically significant decrease in the medium's absorption at 560 nm (see Fig. 3E). This finding indicates that the VitroFlow System is fully sealed, making it resistant to long-term contamination. With regard to EMI compliance, the results of the tests indicated adherence to the EN ISO 61010 standards. It has been demonstrated that electromagnetic (EM) devices are not affected by the VitroFlow. Furthermore, electromagnetic devices that emit EM radiation do not affect the VitroFlow, provided that they are also compliant. In preparation for biological experiments, each VitroFlow is subjected to a standard testing period of at least two weeks, with the duration extending as long as the application requires. Consequently, a series of VitroFlows were subjected to extended periods of testing, with no technical malfunctions observed at  $n=6$  for a duration of 2-4 weeks.



**Figure 3:** **A** Graph shows the measurement of flow rates created by the VitroFlow.Bio, x-axis = set values, y-axis = resulting flow rates as average over 1ml perfused volume. The line graph shows a linear approximation of the values. **B** Graph shows measurement of temperature of the chip chamber, x-axis = set temperature, y-axis = resulting temperature measured at the microfluidic chip inside the chip chamber as average over 5 measurements. **C** Graph shows the resulting Oxygen concentrations inside the cell culture medium while incubating at different  $\text{CO}_2$  concentrations, x-axis = set  $\text{CO}_2$  concentration, y-axis = resulting  $\text{O}_2$  concentrations. Black dots indicate all measurements taken while red dots indicate the mean value. **D** Graph shows a measurement of  $\text{O}_2$  concentration when using  $\text{O}_2$  saturated water inside the VitroFlow.Bio and incubating at 100%  $\text{CO}_2$  (vol./vol.). The decrease in  $\text{O}_2$  concentration (left y-axis) demonstrates the efficacy of the VitroFlow incubator over time (x-axis) and the measured water temperature during this experiment (right y-axis). **E** Graph shows the measured absorption rate (y-axis) over the measured wavelength (x-axis) of high-glucose DMEM:F12 medium w/o antibiotics. The Black graph shows the negative control of the experiment while the red graph shows the same cell medium but circulated in the VitroFlow.Bio for 7 days.

## 4. Discussion and Outlook

The results demonstrate that all subsystems of the VitroFlow.Bio v2.3 run with VitroFlow.Control v5.5.8 are well suited to obtain and maintain all parameters required for OoC experiments in a physiologically relevant range and in various laboratory environments. Nonetheless, VitrofluidiX identified numerous areas for enhancement that will culminate in the formulation of VitroFlow.Bio v2.4 and VitroFlow.Control v6.0, which will be developed as a result.

### 4.1. Pumping System

The peristaltic pump developed by VitrofluidiX demonstrates the capacity to generate flow rates within the physiologically relevant range of OoC experiments with a high degree of precision. Notwithstanding, the peaks in the flow profile, which are characteristic of peristaltic pumps, have the potential to impede the functionality of certain OoCs that are highly sensitive in nature. Consequently, in VitroFlow.Control 6.0, the integrated flow sensor will be utilized not only for the monitoring of flow rates but also for the adjustment of motor speed according to real-time flow measurements. Consequently, a robust linearization of the flow profile can be accomplished. The findings indicate that the sensor utilized in the VitroFlow system exhibits optimal performance in measuring low flow rates at elevated readout frequencies. This particular type of sensor is especially well-suited for measuring flow in an OoC experiment, as it relies on thermal displacement rather than partial pressure. The utilization of partial pressure sensors necessitates the employment of small junctions, which have the potential to inflict harm upon circulating cells by means of sudden increases in shear stress as the cells traverse the sensor.

### 4.2. Incubator

The results of the oxygen measurement demonstrate that the VitroFlow incubator is capable of diffusing CO<sub>2</sub> into a medium. Furthermore, it is well suited for supplying stable gas concentrations at physiological levels

to a microfluidic device attached to the VitroFlow. The device has an internal volume of 330 µL and a diffusible surface area of 17 cm<sup>2</sup>, which allows for precise control over gas concentrations in the medium perfused. In addition to cell culture, other applications are conceivable, such as the diffusion of other gases into different media. This approach enables the introduction of gases into microfluidic applications without the loss that typically occurs through evaporation, thereby ensuring the integrity and efficacy of the system. The data also reveals that the oxygen concentration at 100% is also apparent.

The concentration of carbon dioxide (CO<sub>2</sub>) was found to be 800 µg/L higher at a flow rate of 5 µL/min than at a flow rate of 1,000 µL/min. The phenomenon under scrutiny can be attributed to two possible rationales. It is hypothesized that the oxygen consumption of the Clark electrode could have utilized the differential of 800 µg/L. Secondly, the tubing and tube connectors located outside the incubator, while exhibiting relatively low permeability, lack sufficient inertness to withstand the substantial pressure differential between the interior and exterior of the circulation system. In order to compensate for the aforementioned issue, improvements were made to the VitroFlow. Specifically, all Luer-based tube connectors, which are susceptible to evaporation, were replaced with UNF-based connectors. Additionally, the tubing within the VitroFlow was transitioned from TPU to stainless steel tubing.

### 4.3. Chip Chamber

It has been demonstrated that the VitroFlow chip chamber possesses the capacity to heat a microfluidic chip with a high degree of precision, exhibiting a high degree of accuracy in maintaining the set temperature. It is evident from Figure 3B that the calibration of the +2.1°C to the linear function demonstrates the potential for effective compensation for the distance between the heating unit of the chip chamber and the microfluidic chip within the chip chamber. In general, the technology created with the chip chamber has demonstrated its capacity to function

as a universal adapter for microfluidic chips in the microscope slide format. This capability enables the connection of any chip in this format. VitrofluidiX has interpreted these results as substantiation of the hypothesis that a well-plate formatted adapter is suitable for use with microfluidic chips. A significant advantage of the microfluidic chip is its ability to be imaged while subject to thermal stimuli, a process that can be accomplished using any microscope suitable for well plates. In subsequent steps, VitrofluidiX intends to expand its product line to include chip chambers capable of accommodating various chip formats, including those in accordance with ISO 22916:2022 and PDMS-based chips. The latter will include a CO<sub>2</sub> incubator in the chip chamber. This will allow for the exploitation of PDMS's property of being gas-permeable.

#### **4.4. VitroFlow.Bio**

The subsystems of the VitroFlow, as well as the device in its entirety, have demonstrated efficacy in regulating the various parameters of microfluidic cell culture over a period of at least four weeks. Evidence of this phenomenon has been observed in low-risk environments, as is typical in most cell culture laboratories. Furthermore, experimental results have shown that this phenomenon is also present in high-risk environments, including areas with a high risk of contamination, such as E. coli laboratories, and EMI critical environments. Despite the fact that the most extensive evaluation was conducted over the course of four weeks, it is imperative to acknowledge that the VitroFlow.Bio is a novel product. The most recent evaluations extend up to the day of publication and beyond. Conversely, conventional OoC experiments generally require 1-2 weeks, with occasional instances extending beyond 4 weeks.

This finding, when considered in conjunction with the absence of any indicators of sudden system failure over the four-week period under observation, suggests that the VitroFlow system is well-suited for the stable and reliable creation of these parameters over extended periods.

Therefore, it can be concluded that the VitroFlow.Bio is a robust instrument for the study of organs-on-chips and other cell culture-based microfluidics, including organoid-on-a-Chip and perfused trans-well experiments.

The initial cell culture experiments, encompassing a HEK293-based Kidney-on-a-Chip and a Colorectal-cancer-on-a-Chip, substantiate these observations (refer to the VitrofluidiX Whitepaper for experimental data and a comprehensive methodology, to be announced). This renders VitroFlow.Bio the inaugural fully integrated, fully adjustable Organ-on-a-Chip platform device, capable of supplying four lines of flow as demonstrated here. Concurrently, the adaptability of the chip chamber within the VitroInsertX product line affords researchers a broad array of microfluidic chips, which is currently unparalleled for fully integrated devices. VitrofluidiX offers researchers a variety of possible adaptations in the biological, optical, and sensory design of OoC experiments, thereby opening this technology to a wide variety of research questions, ranging from off-the-shelf solutions to individual experimental designs. This is achieved through the manifold aids provided by VitrofluidiX. Therefore, an ecosystem for OoC experiments is created, and a standard is established in the perfusion setup, thereby ensuring high standardization at the critical points of OoC experimentation.

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