Development of an oxygen sensor integrated microfluidics and its online monitoring system

Capstone report 1

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Abstract:

This paper designs and analyzes a microfluidic oxygen sensor toward the development of organ-on-chip (OoC) technology. It was designed to establish a system that allows real-time observation of oxygen uptake rate by mammalian cells, highly important during the investigation of changes in behavior and viability of cells under different conditions. This project developed and fabricated a microfluidic chip with cell precision monitoring using frontier microfluidic technology and fiber optic sensing.
A 3D CAD software-designed chip, integrated with a PICO-O2 oxygen sensor developed by PyroScience GmbH, and an experimental setup have been calibrated and tested with respect to controlled experimental conditions carried out. Calibration and testing of the developed experimental setup were carried out for controlled experimental conditions, and therefore it is very important for the reliability and accuracy of data. Data was collected at a continuous level, measuring the O2 level and cellular responses using PyroScience Workbench for comprehensive visualized plotting.
The results from the experiments indicated that the chip could support a viable cell culture; in addition, the cell culture measured oxygen uptake accurately.
This really does illustrate the potential to provide tremendous insight into cellular dynamics, which are essential for the success of most biomedical research and therapeutic development, from such integrated systems. They further illustrate that, to help translate this out of the laboratory and into real applications, really high-precision engineering and calibration were going to be needed. This advance does not set any limit for organ-on-chip technologies but rather allows opening new venues in cellular biology research, where in due course, importance has to be given to real-time data acquisition. Further works can be done to integrate more numbers of sensing elements and scale the system to make it work for broader applications.

Contents

Preface

The development of microfluidic chip and the integration of fiber optic sensor have emerged as pivotal areas of exploration. This project represents a significant endeavor into the realm of advanced biomedical instrumentation.

Moreover, this project embodies my commitment to pushing the boundaries of scientific innovation and addressing critical challenges in the field of cell biology and medical research. Through meticulous design, experimentation, and interdisciplinary collaboration, I would like to create an online platform that bridges the gap between traditional cell culture methods and more physiologically relevant in vitro models.

I extend my heartfelt gratitude to professor Gulsim Kulsharova and research assistant Sanzhar Shakarim for their invaluable guidance and unwavering support throughout my capstone project. Their expertise, mentorship, and dedication have been instrumental in shaping the success of this endeavor. I am profoundly appreciative of their insights, encouragement, and commitment to fostering my growth as a researcher. Their mentorship has been transformative, and I am immensely thankful for the opportunity to learn under their guidance. This project would not have been possible without their mentorship, and for that, I am truly grateful.

Nazarbayev University, April 26, 2024

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Chapter 1

Introduction

Microfluidic technologies have emerged as enabling and transforming forces in a gamut of scientific disciplines, most notably biological and medical sciences. Emerging in the early 1980s, microfluidic technologies have revolutionized the ability for precise manipulation and analysis of minute volumes of fluids compared to how researchers have traditionally handled and processed their biological samples [10].

Microfluidics is the technology that deals with miniaturization and automation of biochemical analysis processing, with significant impact touching on genomics, proteomics, and drug discovery fields[1]. It led to the development of the first microfluidic devices, which can control a tiny volume of fluids and therefore can primarily find applications in analytical applications. Integration of microfluidic devices with electronic and optical components further broadened its application to the realization of multi-functional laboratories on a single chip. This innovation not only reduces the volume of reagents and samples but also quickens the process of analysis, making it indispensable in applications of high-throughput screening [2].

A more recent advance in microfluidic technology has further integrated such a system with digital and computational tools, hence allowing much greater opportunity for more intricate and functional devices. Modern innovations in the field include digital microfluidics, where droplets on a surface are manipulated using electrical signals, and paper-based microfluidics, in which capillary forces draw fluids into an absorbent paper. This has given room for new developments to come in that seek to improve diagnostic platforms and environmental monitoring.

More importantly, microfluidics have enabled an immense breakthrough in synthetic biology, where it allows researchers to design and construct new parts, design and construct a new system, or redesign natural-existing ones for useful purposes. Thus, the microfluidic systems humanize the setting, whereby one can deal with the environmental conditions under a controlled and repeatable way. This has vast importance in the field of drug testing, particularly in microfluidic devices that can mimic human tissues and organs' environments in a 3D setting [3].

The use of microfluidic devices is enormous and covers mostly the part of the medical field for diagnostic methods and personalized medicine. The ability to work with really small volumes of biological fluids opens a way for the point of care even for the most complicated kinds of biochemical analyses within the subject. This, therefore, becomes very crucial in resource-limited settings where traditional laboratory infrastructure is unavailable [4].

In addition, microfluidics places a new trend to the field of personalized medicine, where treatments are designed for an individual patient. Integration of microfluidic systems with cell culture technologies is going to help in developing a patient's physiological model of a particular disease. This will permit in vivo predictions by an individual of responses to

various therapeutic agents and treatment regimens.

"Organ-on-a-chip" (OoC) technology represents one of the most promising applications of microfluidics. They are considered devices that mimic whole organs, composed of cells and tissues cultured in controlled microenvironments and able to recapitulate a wide range of characteristics and functions of a physiological context. With technology, it gives key paths towards better drug testing with improved precision, a better disease model with less reliance on animal testing, and predictability for human responses against new drugs.

Looking at microfluidics, the industry seems set to keep growing, with innovations that will further revolutionize this area of medical diagnostics, environmental monitoring, and industrial processes. A further coupling of microfluidics with artificial intelligence and machine learning could humanize these adaptive systems to make them smarter and carry out complex decision-making in the course of processing real-time data.

The development of microfluidic technologies has advanced from its birth repeatedly and to a level that is very advanced; it finds applications across numerous fields. Continual developments in these technologies are destined to increase precision, efficiency, and applicability, thereby ensuring that microfluidics always remains at the leading edge of scientific advancement and technological innovation.

Chapter 2

Background

The present invention of microfluidic technology has witnessed significant developments since its birth in the 1980s, mainly motivated by progress in MEMS (MicroElectroMechanical Systems) and microfabrication techniques. They had expertise in manufacturing methods and integration from the semiconductor industry to the biological sciences, making it possible to precisely manipulate fluids at the microscale [5]. This control is significant in biochemical experiments where the exact dosage, mixing proportions, and timing of reactants are required to simulate many complex biochemical processes.

The past decade has been when microfluidic device production was increasingly sophisticated and integrated. New materials emerged on the scene, such as PDMS (Polydimethylsiloxane), and novel fabrication approaches included soft lithography, 3D printing, and nanofabrication. These devices carry out, on a highly compact chip, many functions that occur in the laboratory—culture of cells, separation, detection, and analysis [1]. These include miniaturization and integration that allow for reducing sample volumes, reagent costs, and analysis times—allowing, in effect, high-throughput experiments, all of which are essential in fields like drug development and molecular diagnostics.

Flexibility is proven as the microfluidic device has been taken up by many domains in biomedical research. Microfluidics allows the development of portable devices to carry out complex analyses in point-of-care diagnostics with quick turnaround times, such as blood glucose monitoring or infectious disease testing [4]. This is very important in resource-limited settings without conventional infrastructure support for laboratories [10].

The microfluidic systems have changed the field of drug discovery and development. High-throughput screening of drug candidates and biomarkers is carried out with microfluidic devices in many conditions on a single chip. This high-throughput capability increases the discovery process for drugs relatively and reduces the costs related to the development of the medicine[11]. Furthermore, within synthetic biology, microfluidics has become essential for the construction and analysis of synthetic biological circuits. Scientists will one day be able to create, build, and test synthetic biological pathways with a new level of precision and efficiency through controlled cellular environments and regulation in the delivery of genetic and chemical stimuli [12].

The organ-on-a-chip is a significant invention and innovation in tissue engineering and regenerative medicine. It represents an exact mimicry of all structural and functional properties of human organs, say, lung, liver, or heart, on a chip. One such device is the lung-on-a-chip, which can replicate mechanical breathing motions and the biochemical functions of a human lung, thus enabling the study of any respiratory disease or testing of new drugs within an organ-specific context [13].

Not only are these massively valuable for drug testing and disease modeling, but they

also enable an understanding of human physiology and pathology without dealing with many ethical issues and practical complications related to in vivo studies. They represent the opportunity to convert basic biomedical research into clinical therapies at an accelerated rate, allowing enhanced effectiveness and safety treatment.

Integrating sensors into microfluidic devices is not without challenges. Besides, these had to be looked into for the measurement to be correct: stability, biological medium interference, and calibration of sensors. And all these have been sorted recently with the new advancement in sensor technology.

For instance, with the development of chip-integrated wireless sensor networks, human physiological parameters can be monitored without interfacing physically with the system, thus reducing the risk of contamination and interference [14]. The worthiness of microfluidic platforms integrated with oxygen sensors has been hugely highlighted by colossal studies and their implication in cellular and physiological processes related to life.

For instance, previous studies using these platforms have generated new knowledge about the metabolic response of cells under a hypoxic condition and increased our understanding of diseases such as cancer and metabolic disorders [15]. Microfluidics has fundamentally changed the approach to cellular biology by enabling control and manipulation of the cellular environment at the microscale.

The ability of the researchers to isolate, manipulate, and study individual cells within micro-environments that are controlled by using microfluidic droplet-based, single-cell analysis technologies offers great potential for understanding behaviors in cellular disease mechanisms and therapeutic responses [16]. Interfusing front-line technologies with the future of microfluidic research, such as Artificial Intelligence (AI) and Machine Learning, can process the intricate datasets microfluidic devices produce, serving as tools. The offering will be deeper insight and prediction capabilities across realms as varied and vital as personalized medicine, disease models, and drug discovery [17].

Chapter 3

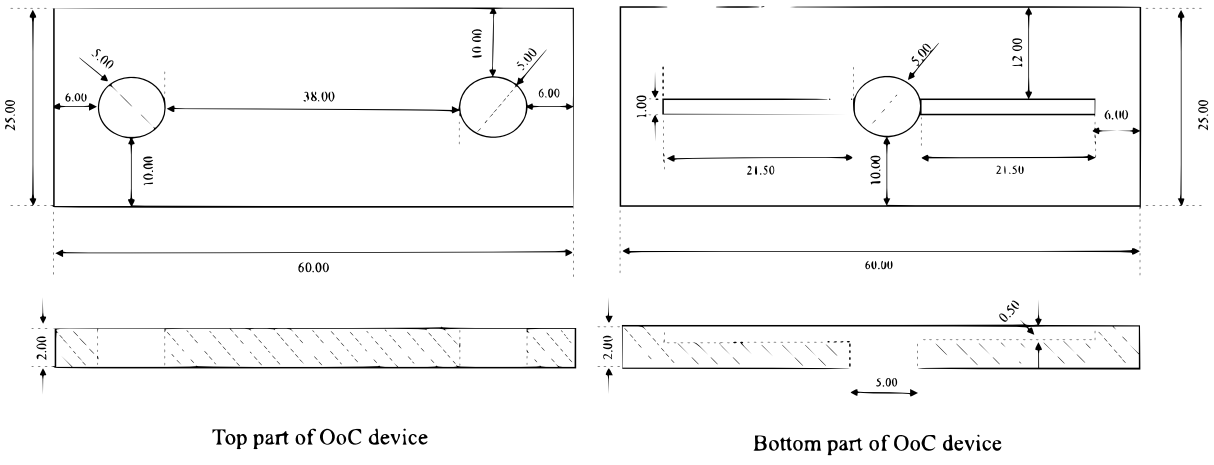
Methodology

3.1 3D Model Design

The design of organ-on-chip (OoC) was drafted in 3D CAD software, SOLIDWORKS, complete with detailed dimensions, and subsequently printed.The microfluidic device, measuring 55mm by 25mm, is composed of two distinct parts crafted from cyclic olefin copolymer (Chip-Shop, Germany). The lower section of the chip includes a microchannel that is 48 mm long, 1 mm wide, and 1 mm deep. The upper part features a smooth surface with holes designed for the inlet and outlet, complementing the bottom part.

I followed a step by step approach to collect data for sensor technology project focusing on studying the effects on the human respiratory system.

The schematic illustration of the whole design of the microfluidic chip, in both the upper and the lower parts, is so highly detailed that Figure 1 has been made using Microsoft PowerPoint. This is an essential referential figure providing a very detailed description of the dimensions outlining the spatial architecture of the chip. Every measurement presented was well annotated in millimeter, producing a clear, unambiguous picture of the scale and geometrics of the chip. This will provide a better understanding of the construction and operation of the chip towards replication and analysis.



DIMENSIONS ARE IN MILLIMETERS

Figure 3.1: Schematic design of OoC device

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Such detail ensures the functional integrity of the chip and contributes much to the reproducibility of the chip. Reproducibility of the chip will be able to be quickly done by the use of CAD software, such as SolidWorks. The construction of such a detailed component in SolidWorks will be multistep and will be as follows:

1. Sketch the Base Rectangle

Step 1.1: Open the Sketch module and select the Rectangle tool on the toolbar. Click on the point of origin and drag over it to sketch the rectangle. Release the mouse at a point visually approximating the size you desire.

Step 1.2: Having sketched the rectangle, now apply the 'Smart Dimension' to assign the exact dimensions of the chip base set at 60mm by 25mm.

2. Define the Central Channel

Step 2.1: While still in the sketch of the base rectangle, select the 'Line' tool from the toolbar and draw the path of the central channel directly on the sketch of the base rectangle.

Step 2.2: Go to the "Features" tab and choose the "Extruded Cut" tool. Select the sketch lines indicating the path of the channels.

Step 2.3: Set the direction and depth of the cut. If the option 'Through All' is selected, it will make a limitation for the channel not to span through the whole thickness of the base rectangle, hence yielding an open channel.

3. Creation of Circular Spots

Step 3.1: On the same sketch, locate the circles you draw as ports for fluid injection and extraction at some places along the channel.

Step 3.2: Use the Smart Dimension tool to space the circles from the rectangle edges and each other at the exact required distances so that there may be symmetry for functional use.

Step 3.3: Exit this sketch, open the same sketch, and using the 'Extruded Cut' tool with the selection of circles, set the extrusion type to 'Through All' for the ports to cut through the whole base depth. Through this, functional inlets and outlets of the ports are created.

This structured approach in SolidWorks facilitates precision manufacturing of the microfluidic chip and contributes significantly to the documentation process that can be utilized for replication or modification in future endeavors. Both of these aspects maintain the integrity of the design and ensure successful fabrication.

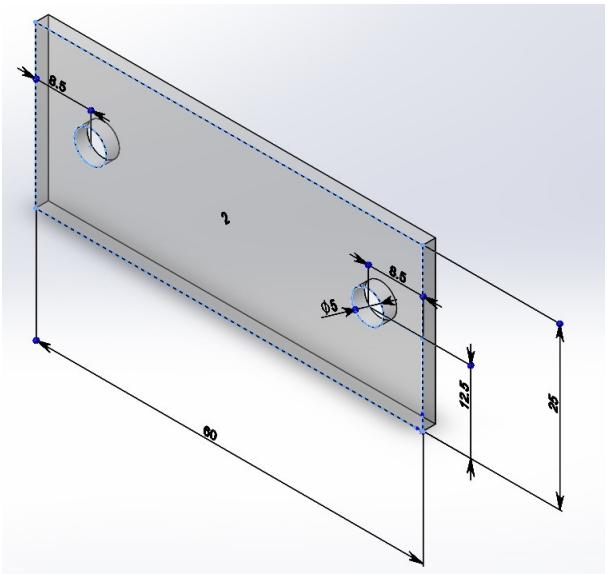

Figure 3.2: The side view of OoC - Top Part

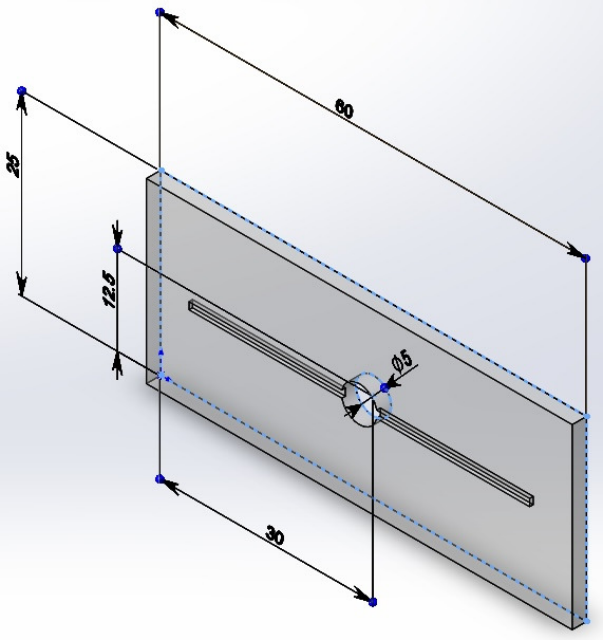

Figure 3.3: The side view of OoC - Bottom Part

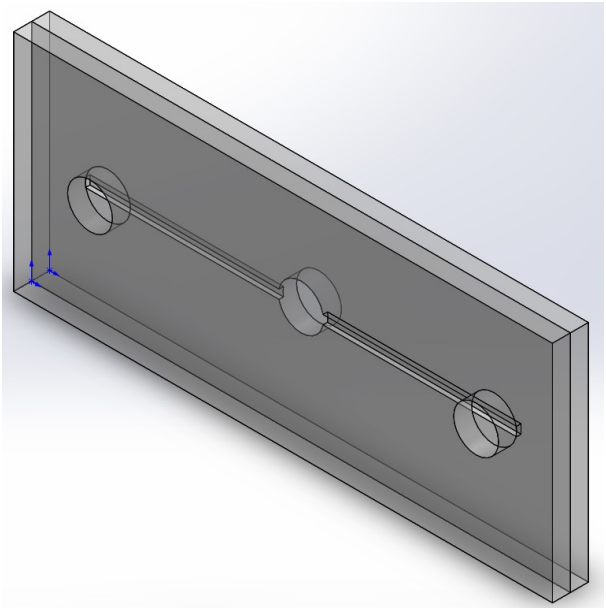

Figure 3.4: The side view of OoC

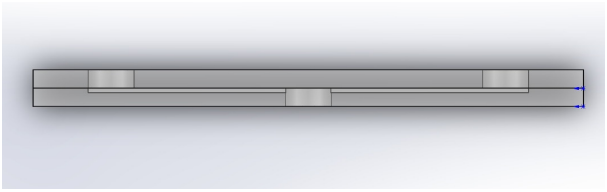

Figure 3.5: The side view of OoC

3.2 Fabrication

Fabrication of Polydimethylsiloxane (PDMS) Chips involves precise steps to ensure the functionality and accuracy of the final product. Here's the detailed process:

Preparation of PDMS Mixture: PDMS chips are fabricated using SYLGARD 184 Silicone Elastomer Base and SYLGARD 184 Silicone Curing Agent, known for their biocompatibility and mechanical properties, suitable for microfluidic devices.

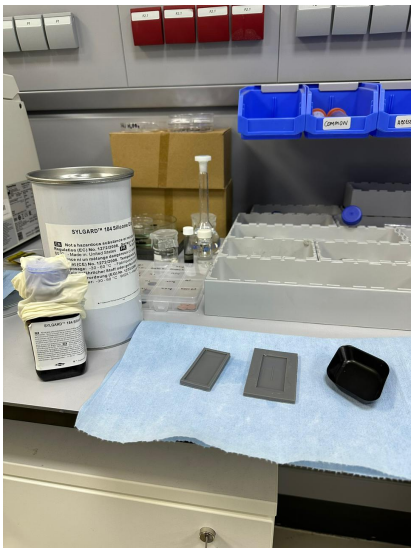

Figure 3.6: The setup to make polydimethylsulfoxide chips.

Mixing Method: Measure SYLGARD 184 Silicone Elastomer Base and Silicone Curing Agent in a 10:1 ratio by mass. Accurate measurement is crucial to ensure the chemical properties of PDMS remain consistent.

Thoroughly mix the components by hand to achieve a homogeneous mixture, which is vital to avoid inconsistencies in the polymer's structure after curing.
Mold Preparation and Filling: Use a UV lamp placed 10-15 cm above the molds to remove any residual materials or contaminants from previous experiments, ensuring the purity of the PDMS structures.
Carefully pour the mixed PDMS into the molds up to the edges, avoiding air bubbles that can affect the structural integrity. Use a spatula or a similar tool to level the surface and remove excess material.

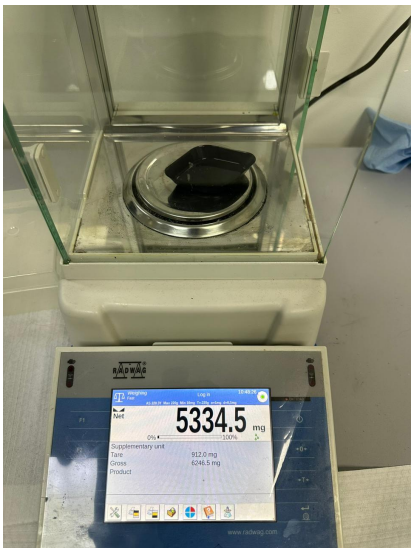

Figure 3.7: Selection of the desired mass.

Curing Process: Cure the PDMS in an oven preheated to 100 degrees Celsius for 1.5 to 2 hours to achieve optimal cross-linking of the polymer chains. Monitor the curing process closely to avoid overheating, which can cause deformation or defects in the chips.

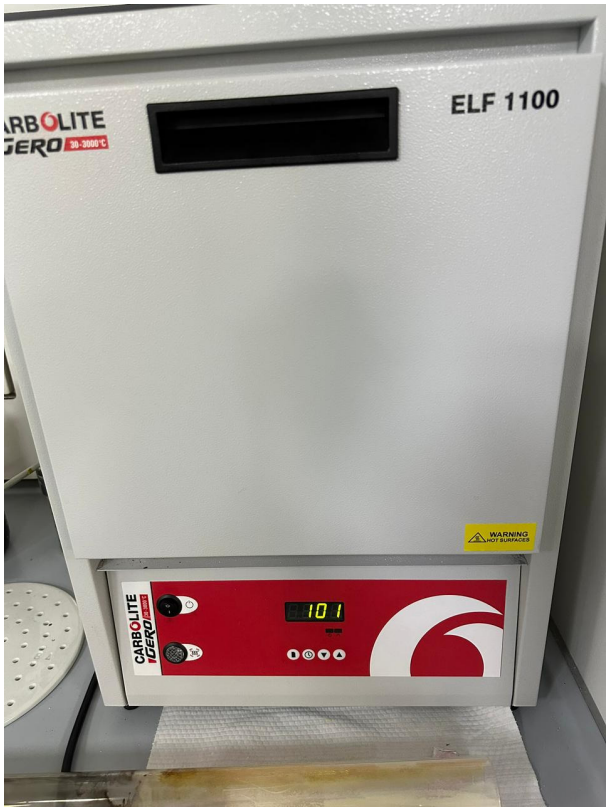

Figure 3.8: Put the molds to the oven

Figure 3.9: Completed PDMS

This method outlines the controlled and repeatable steps necessary for fabricating PDMS chips, ensuring reliable and uniform microfluidic devices.

3.3 Oxygen Sensor Integration

The oxygen sensor, PICO-O2 developed by PyroScience GmbH, was calibrated within an incubator environment and regulated to human body temperature (37°C).
In this experiment, the chips were functionalized with the addition of sensors that had been specially designed to measure the rate of oxygen consumption. Calibration of sensors was done before any measurement acquisition, following the need to provide information on the required accuracy and precision in every measurement activity. The calibration is done in a straight line with the instructions from the manufacturer. It will involve putting a chip in an incubator and then attaching it to a sensor. The temperature was set at 37 degrees Celsius. This is to mimic the human body and, in so doing, it gives conditions conducive to the environment for the cells under study. The sensors were carefully calibrated with the PyroWorkbench v1.08 software developed specifically for the PICO-O2 (OEM Fiber Optic Oxygen Meter). In this light, the calibration step had to be undertaken to confirm the readings of the sensors before the experiment began.

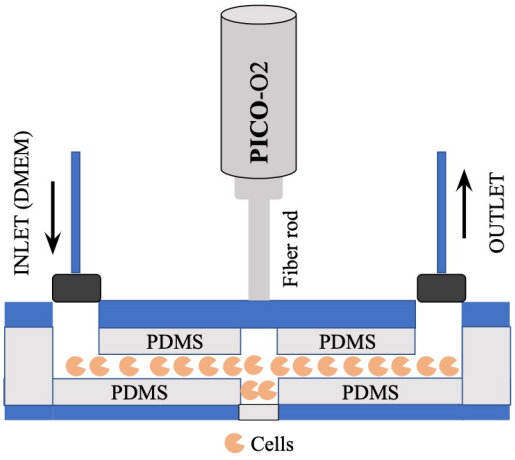

Figure 3.10: The side view of OoC with integrated oxygen sensor PICO-O2

3.4 Oxygen Measurements

Before oxygen consumption measurement, A549 alveolar basal epithelial cells were introduced into the microchannel of the organ-on-a-chip (OOC) system. The prepared device assumes great importance since it will be used to replicate the cellular environment and cellular function present within the respiratory tissues. The channels were then allowed to settle cells to adhere to and functionalize essential surfaces for the accuracy of the subsequent measurements.

Experimentation was carried out under continuous flow conditions, respecting the configuration of the specific experimental set-up detailed in Figure 3.11 of the reference[18]. The most cautious design has been done so that it precisely comes under the control of the experimental conditions and happens in the circumstances justifying the replicability of the results. Key components in this system were an Aladdin Single-Syringe pump, the PICO-O2 oxygen meter, and a device containing an organ-on-a-chip integrated with a bonded polydimethylsiloxane (PDMS) chip.

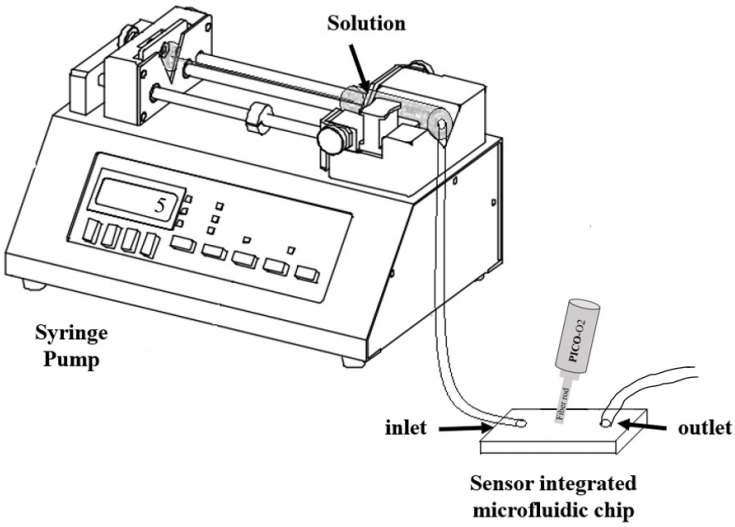

Figure 3.11: The setup [18]

The flow rate at which the medium flowed through the system was controlled using a

single Aladdin syringe pump. It contained a syringe filled with the mixture of Dulbecco's Modified Eagle Medium (DMEM, Sigma, USA) and distilled water provided all the nutrition to cells along with the osmotic balance required for living and functioning.

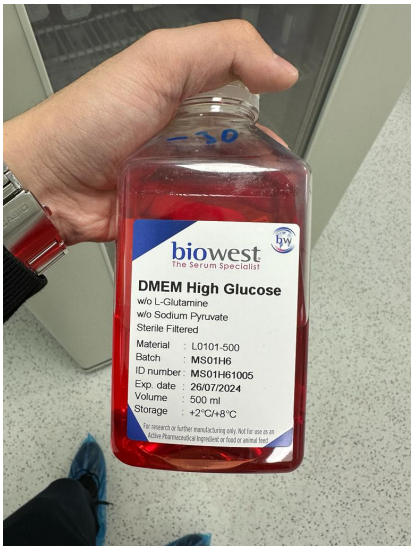

Figure 3.12: The concentration of DMEM

A better relevant experimental model is DMEM, that human biological processes support the cultured cell growth and broadly used medium of the cell; it approximates the physiological medium. The flow rate was set precisely at 10 µL/min, balancing the nutrients and the waste products without overstressing the generated shear force on cells.

This slow rate keeps the stability of the microenvironment and makes it possible to look in detail at the cellular activity in response to numerous stimuli. In this experiment, the configuration was of great importance—particularly the integration of the PICO-O2 oxygen meter—to monitor in real-time and measure accurately, at steady intervals, the oxygen consumed by the A549 cells. For sure, the interfaced oxygen meter with the OoC device served its prime purpose of detecting and quantifying the levels of oxygen in a microenvironment; hence, it was an essential source of data concerning cellular respiration and metabolic activities.

3.5 Analytics

The experimental procedures use the Pyro Workbench software that interfaces well with the PICO-O2 oxygen sensor. A significant part played by the very advanced software in data acquisition is that it allows one to come up with elaborate graphical presentations for oxygen consumption data. The purpose of these graphs is to have an initial idea of the graphical trends of the data, the general data behavior, and the outcomes from the experiment.

Nevertheless, due to the limited functionalities of the Pyro Workbench software, we usually transfer raw experimental data into Microsoft Excel for further and better-precise analysis. Excel is an advanced method of data processing that can be used to build a detailed scatter plot. This allows further research into the data points concerning establishing underlying patterns or anomalies that might not be visible from the graphical outputs of Pyro Workbench. That step is quite crucial because it yields a detailed view of the experimental results; hence, it increases the reliability and depth of our analysis.

Furthermore, for a detailed data analysis, various microscopic techniques are used to view the cellular activity at a microscale level.

This method will give priceless qualitative insights into the cellular dynamics occurring post-experiment. Qualitative imaging will document the alteration of cell morphologies, interactions, and behaviors under the given experimental conditions. It is from such microscopic examinations that the entire cellular response in the microenvironment of the organ-on-a-chip system can be realized, hence providing a complete understanding of cellular mechanisms and responses. With the integration of both qualitative assessments made through microscopy and quantitative data analysis done via Excel, the experiments not only quantitative oxygen consumption but also precise this complex biological response of the cells under study. This two-pronged approach holds scientific validity and guarantees a holistic view of the cellular processes, enriching our understanding of them.

Chapter 4

Results

4.1 Data Overview

Careful data acquisition has been done using the PyroScience Workbench, interfaced with a calibrated oxygen sensor, to ensure high fidelity in monitoring and recording. The oxygen sensor was calibrated under very controlled conditions where temperatures of 37°C were kept throughout to mimic physiological conditions analogous to the human body. Furthermore, the atmospheric pressure remained constant at 1013 mbar, which equals standard atmospheric pressure at sea level, hence no fluctuation from one data to another due to pressure fluctuation.

Throughout the entire period of the experiment, the measurement data were collected and continued to realize complete records of oxygen levels at all times that the experiment entailed. The measurements were in hectopascals (hPa). This unit of pressure is susceptible and good at giving precise measurements of gases. All measurements in this study were taken at five-minute intervals, approximately, affording a fine-grained temporal resolution that is necessary to precisely capture the dynamics of oxygen consumption by the cellular constructs.

This high spatial and temporal resolution dataset carries a great deal of importance, as it is to be used not only for detecting small changes in oxygen that may point toward cellular response or metabolic activity but also for deriving information on the spatiotemporal dynamics of oxygen at microscale levels. Essentially, this is a compromise between the choice of recording intervals and preciseness in data capturing methodology; both have to provide an adequate amount of data for a detailed analysis but at the same time not flood data processing stages so that they become unworkable. The approach followed by continuous recording not only increases the reliability of data but also helps frame a very strong framework for assessing the efficacy of the experimental setup and the health of cellular systems under study.

4.2 Results

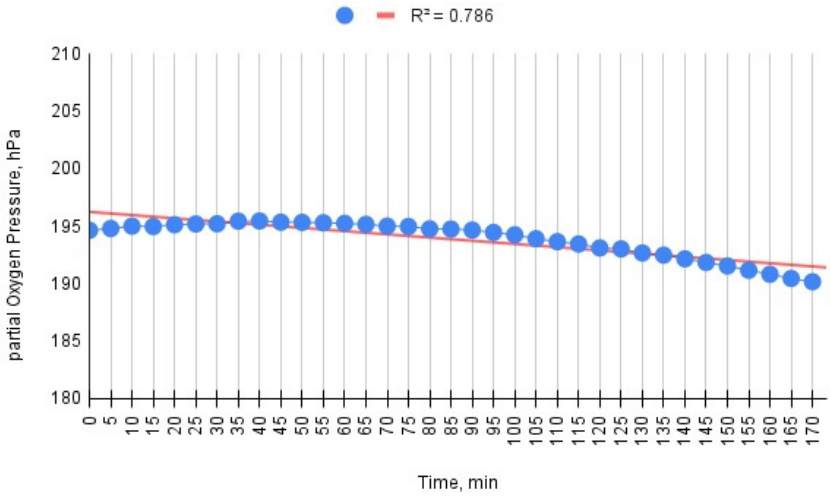

Figure 4.1: Result of the experiment №1

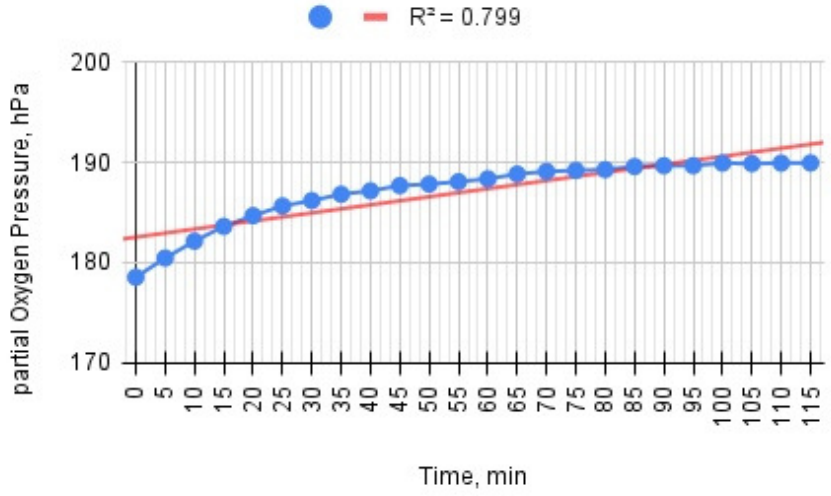

Figure 4.2: Result of the experiment №2

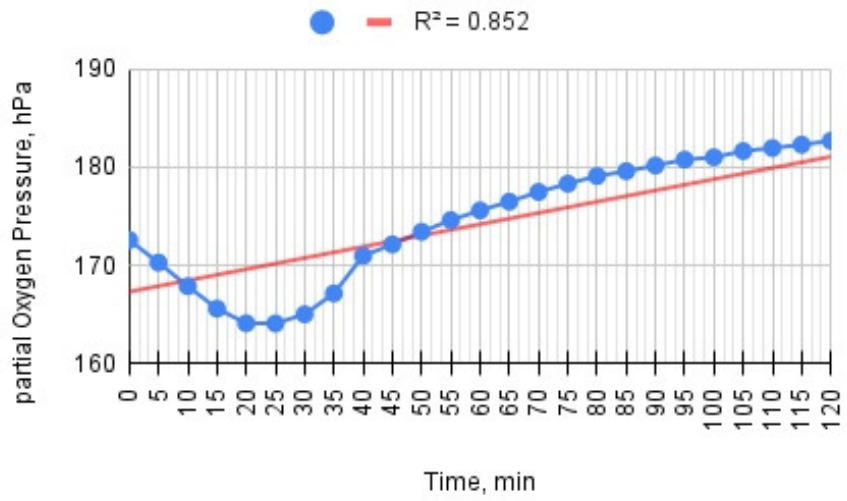

Figure 4.3: Result of the experiment №3

4.3 Interpretation of Results

Only a slight increase in the oxygen level was detected as early as within the microfluidic device. Perhaps this was related to the adjustment of cells to their new environment. Most likely, metabolic adaptation occurs during this time to optimize energy production based on the existing oxygen levels. This temporarily changed rate of oxygen consumption is attributed to mechanisms of cells that help in adjusting to sustain life and function under potential stress.

The adaptation might involve the shift into more anaerobic processes, such as the process of glycolysis, until cells get stable and adjust the oxygen availability.

The net oxygen concentration has generally decreased throughout the whole experiment, an indication that the cells were metabolically active in reducing oxygen for the production of energy.

The active respiration observed is a positive indication of cell health on the microfluidic chip and suggests that cells survive in a functionally active state. They undertake the energy metabolism required to maintain cellular processes and responses.

4.4 Limitation of the Project

Mechanical Setup Errors

Mechanical errors, though it may be factual that they exist in the case of microfluidic devices in the setup, are a great source of negativity against the reliability and repeatability of the experiments. Such errors may come from a variety of sources.

Fabrication errors may cause differences in the dimensions of the microchannel, thus leaving discrepancies within the fluid flow and shear stress; such differences may affect the behavior of cells and, eventually, the result of the experiment. Little changes in channel width or depth would change the flow velocities and shear forces of the cells, which might affect these cells' metabolic activities and possibly physiology in general.

Any slight misalignment, more particularly in the process of integrating the PDMS and COC chips or placement of the oxygen sensor, would lead to leakages or blockages. This misalignment, therefore, may disrupt the intended pathways of the fluidics and, in turn, bring out poor distribution of the waste products, nutrients, oxygen, and withdrawal.

Poor sealing of the device will result in the evaporation of the medium; this will, therefore, increase changes in concentration and osmolarity. This may impact stress on the cells and, in turn, modify the normal physiological responses of the cells, affecting the outcome of the experiment.

Lack of sufficient oxygen

Oxygen supply in microfluidic systems might be problematic when higher-density culturing of cells and tissues with high metabolic activity is required.

Highly metabolic active cells may even use up the available oxygen much faster than it can be restored, hence creating an uninformed, hypoxic environment. This may play a significant role in changing cellular behavior since it results in the turning of cells to anaerobic metabolism, therefore producing less energy and changing growth and function.

The measurement of oxygen is measured accurately, and calibration drift, response time, and sensitivity of the oxygen sensors can also affect the measurement. Any of these factors can thus bring in inaccuracies with the readings of oxygen levels that would thereby even mislead the interpretation of cellular metabolism and health.

Chapter 5

Conclusion

The present project aimed to design, fabricate, and validate a microfluidic device using PDMS and COC materials integrated with a PICO-O2 optical sensor to conduct oxygen uptake studies within cellular environments. The research pinpoints the potential and challenges presented by microfluidic systems with avant-garde technologies and methodologies used in biomedical engineering. The following section sums up the primary outcomes of the project and reflects on the implications these could have on future work within the area.

A 3D CAD model of the microfluidic chip was developed to allow exactness in the production and assembly of all the necessary parts. The design process was adopted with the help of state-of-the-art software tools that would ensure high accuracy and reproducibility to achieve the consistent device production of microfluidics.

Microfluidic chips have succeeded in being fabricated with PDMS and COC by combining these good features from two materials. The PDMS allows flexibility and penetrability of gas to keep the environment inside the cell alive, while COC supplies structural hardness to resist chemical actions that are needed for functionality.

Thus, the system could be in a position to quantify the exact oxygen level within the chip by integration with a commercial-available PPCO-O2 optical sensor. This setup was, therefore, paramount with respect to real-time data acquisition, which would illuminate dynamic changes characteristic of oxygen consumption by the cultured cells.

For the instrument tests, one set of cells used is the A549 alveolar basal epithelial cell since they represent an ideal model used in pulmonary studies. A test in this phase, for example, showed that the chip could easily support all sorts of cellular activities and effectively measure metabolic rates, laying out the promise for the device in biomedical applications.

The results of this project reflect the possibility that with modern microfluidic systems, on one side, cellular functions could be supported and controlled. In contrast, on the other, precision in system design and interpretation of the data is tremendously important. Some of the most important considerations for future developments include:

The design and fabrication of microfluidic devices are exact. Possible future advancements in this area would include improved reproducibility and reliability of the experimental outcome through more accurate devices components and further perfecting assembly methods.

The proper utilization of microfluidic technologies demands accurate interpretation of obtained data. Efforts, therefore, should be made to develop more sophisticated analytical tools that will be able to interpret the complex datasets by these systems effectively and accurately.

In the future, the research could be extended to scale this technology in a wide range of other types of cellular studies. The design could be made adaptable in all kinds of cells and

conditions. Hence, there is a need for its adaptation to expand the application scope in much more comprehensive scientific and clinically researched fields.

Finally, this project represents a quite relevant advancement for the use of microfluidic devices in biomedical applications. It means precisely how these technologies could eventually revolutionize the field of cellular biology, as an ever-increasing effort is devoted to improving precision and scalability.

Bibliography

[1] G. M. Whitesides. "The origins and the future of microfluidics". In: *Nature* 442.7101 (2006), pp. 368–373.

[2] E. K. Sackmann, A. L. Fulton, and D. J. Beebe. "The present and future role of microfluidics in biomedical research". In: *Nature* 507 (2014), pp. 181–189.

[3] D. E. Ingber. "Reverse engineering human pathophysiology with Organs-on-Chips". In: *Cell* 164.6 (2010), pp. 1105–1109.

[4] P. Yager et al. "Microfluidic diagnostic technologies for global public health". In: *Nature* 442.7101 (2006), pp. 412–418.

[5] Bernhard Müller et al. "Measurement of respiration and acidification rates of mammalian cells in thermoplastic microfluidic devices". In: *Sensors and Actuators B: Chemical* 334 (2021), p. 129664. DOI: 10.1016/j.snb.2021.129664.

[6] Yong Zhao et al. "Applications of fiber-optic biochemical sensor in microfluidic chips: A review". In: *Biosensors and Bioelectronics* 166 (2020), p. 112447. DOI: 10.1016/j.bios.2020.112447.

[7] Stefanie Fuchs et al. "In-Line Analysis of Organ-on-Chip Systems with Sensors: Integration, Fabrication, Challenges, and Potential". In: *ACS Biomaterials Science & Engineering* 7.7 (2021), pp. 2926–2948. DOI: 10.1021/acsbiomaterials.0c01110.

[8] K. Eyer et al. "Implementing Enzyme-Linked Immunosorbent Assays on a Microfluidic Chip To Quantify Intracellular Molecules in Single Cells". In: *Analytical Chemistry* 85.6 (2013), pp. 3280–3287. DOI: 10.1021/ac303628j.

[9] David Bovard et al. "Organs-on-a-chip: A new paradigm for toxicological assessment and preclinical drug development". In: *Toxicology Research and Application* 1 (2017), p. 2397847317726351. DOI: 10.1177/2397847317726351.

[10] H. A. Stone, A. D. Stroock, and A. Ajdari. "Engineering Flows in Small Devices: Microfluidics Toward a Lab-on-a-Chip". In: *Annual Review of Fluid Mechanics* 36 (2004), pp. 381–411. DOI: https://doi.org/10.1146/annurev.fluid.36.050802.122124.

[11] P. S. Dittrich and A. Manz. "Lab-on-a-chip: microfluidics in drug discovery". In: *Nature Reviews Drug Discovery* 5.3 (2006), pp. 210–218. DOI: https://doi.org/10.1038/nrd1985.

[12] A. S. Khalil and J. J. Collins. "Synthetic biology: applications come of age". In: *Nature Reviews Genetics* 11.5 (2010), pp. 367–379. DOI: https://doi.org/10.1038/nrg2775.

[13] D. Huh et al. "Microengineered physiological biomimicry: organs-on-chips". In: *Lab on a Chip* 11.12 (2011), pp. 2156–2164. DOI: https://doi.org/10.1039/c2lc40089h.

[14] A. Mata, A. J. Fleischman, and S. Roy. "Characterization of polydimethylsiloxane (PDMS) properties for biomedical micro/nanosystems". In: *Biomedical Microdevices* 7.4 (2010), pp. 281–293. DOI: https://doi.org/10.1007/s10544-005-6070-2.

[15] T. H. Park and M. L. Shuler. "Integration of cell culture and microfabrication technology". In: *Biotechnology Progress* 19.2 (2003), pp. 243–253. DOI: http://dx.doi.org/10.1021/bp020143k.

[16] E. Z. Macosko et al. "Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets". In: *Cell* 161.5 (2015), pp. 1202–1214. DOI: https://doi.org/10.1016/j.cell.2015.05.002.

[17] D. E. Ingber. "Is it time for reviewer 3 to request human organ chip experiments instead of animal validation studies?" In: *Advanced Science* 7.22 (2020), p. 2002030. DOI: https://doi.org/10.1002/advs.202002030.

[18] Sanzhar Shakarim, Daniele Tosi, and Gulsim Kulsharova. "Integration of fiber optic sensors in organ-on-a-chip devices towards label-free cell viability assays". In: (2022), pp. 1–6. DOI: 10.1109/SAS54819.2022.9881355.