International Journal of Scientific Engineering and Applied Science (IJSEAS) – Volume-6, Issue-11, November 2020
ISSN: 2395-3470
www.ijseas.com

Automated, Controlled and Portable Mini Incubator for Passive Microfluidic Platforms in Biological Applications

Pushparani Micheal Raj[1], Valpapuram Immanuel[1]

[1]Department of Experimental and Clinical Medicine, University Of Catanzaro, Italy

Abstract

The present work summarizes development of mini incubator for biological applications in combination with passive microfluidic devices. There is a pivotal need to automate and control the microenvironment inside the incubator due to the biological samples in use. With PID feedback algorithm in place, the incubator has highly controlled environment. Paramount importance is given to the portability of the equipment to make it a microscope stage top incubator and portable to different locations to carry out research at specific conditions without the need to carry the samples, instead. In addition, imaging is crucial in any cell-based applications, hence the incubator is adapted to minimize auto-luminescence effects of PMMA. The automated, controlled and portable incubator is used with tumor cells for cell culture and chemotaxis studies which proved to be effective and efficient in its goal.

Keywords: Incubator, Passive Microfluidics, Biological Applications, Imaging, Automation

1 Introduction

Incubator is an important laboratory equipment to nurture and preserve microbiological cultures. The incubator sustains optimal temperature, humidity and other conditions such as carbon dioxide (CO_2) and oxygen (O_2) content of the ambience inside. Incubators are crucial for a lot of cell experimental work, microbiology and molecular biology and are used to culture bacterial as well as eukaryotic cells [1-4]. An incubator imitates a three-dimensional (3D) microenvironment that suits almost all cellular activities including cell culture, chemotaxis, drug screening, ageing, cancer research, production of vaccine for lethal diseases like polio, rabies, hepatitis B and C, toxicity testing, production of genetically engineered protein like insulin, tissue replacement and re-engineering, genetic counselling like karyotyping, gene therapy and more [5-7]. Cell culture is a basic routine in many laboratories for its ability to study toxicity, tissue engineering, gene therapy and more. The cell cultures not only rely on media but also the environmental factors like temperature, CO_2, pH, osmolality and relative humidity. Therefore, incubators were expected to deliver an optimal atmosphere for cell studies [8]. Conventionally, cell culture was a manual procedure that demanded high skill and repetitive protocols to perform meticulous and laborious manipulations every day. Hence, the conditions posed a high risk of contamination which is replaced with the proper and efficient usage of incubators, and automated [9, 10]. An incubator reproduces ideal conditions required for cell proliferation and growth, and conducive for high-throughput screening. Thereby, the invention of modern-age automated incubators has driven the cell culture towards robotic and man-free handling of cells. A passive device is self-assembled where fluid is handled internally with reservoirs; pressure difference generates a flow in the microchannels to perform desired cellular activities. By and large, the environment remains contamination-free and simpler in terms of setup. Thereby, a device can be compact enough to place a Petri dish without any external connections to avoid any potential impurities. Anyway, the incubator to contain a passive device is expected to behave more precise and accurate with its ambience to provide best conditions for cell growth and maintenance, including pH, relative humidity, CO_2, oxygen and constant temperatures.

Another crucial factor in designing an incubator for cellular studies is effect of microenvironment ambience. Incubators ideally maintain a constant temperature, however additional features are built in. Humidity, CO_2, O_2 and pH are also monitored and controlled in latest systems. There is a range of incubators, of which, most interestingly, shaking incubators offer a stage movement for mixing of

cultures, gas incubators regulate gas composition and are sourced with power banks not to be disrupted with frequent power outages [11]. Incubators come in a variety of sizes, from table-top to warm rooms, based on the requirements like BioCompare, ThermoFisher and Thomas Scientific.

1.1 Mini Incubator – Integration – Automation Capabilities

Automated incubators are preliminary requisites for fully automated cell culture systems and other cellular studies which are able to monitor cell activities without human interaction. In the last decade, "Lab-on-a-Chip" (LOC) is the most sought-after technique in the field of micro- and nanofluidic as it shrinks a biochemical assay onto a miniaturized chip, mostly comparable to a coin [12-15]. Though the commercial availability of such LOC appliances is far from reality, the expectation on this technology still remains high. Automation and integration are of fundamental importance in LOC. Automation ensures data accuracy and precision, irrespective of loaded data cycles. Unlike manual data collection, automated systems eliminate any parallax errors introduced by humans. The advantages of automation are manifold, among these the possibility of replacing the machine-to-man in monotonous, repetitive and hazardous activities, or in assignments that challenges with human precision, in terms of size, weight and speed. Moreover, this could be translated into economic and resources conservation [10, 16, 17]. Nevertheless, most commercial incubators are designed with a specific software which does not allow any customizations. As in any technique, automation also renders a few limitations like requirement of skilled expertise to design the system, high-definition of sensors, durability of data acquisition systems and bulky data storage facility for long-term processes [11]. Integration of a LOC platform varies widely from integrating sensors of different types to complex systems like micro-arrays of incubators [18, 19] and optical waveguides integrating femtosecond laser [20-23]. A platform with multiple sensing elements ensures high reliability on the data acquired and thereby gives precise conditions of the microenvironment under test.

Imaging is most important in any cell-based studies, and unfortunately many incubators support little or no real-time imaging. Live cell imaging is widely used in various bioscience experiments for improved understanding and collect more information on dynamic cellular activities such as migration, division, differentiation and organelle-level procedures [24, 25]. It is indeed difficult to integrate a microscope to an incubator unless customized [26]. Perhaps, in utmost studies, the images are fetched before and after incubation whereas the real-time monitoring is considered nearly incredible with conventional large incubators. However, commercial incubators are available with enormous space for live-cell imaging, though it comes along with a high price tag and less customization options like Olympus VivaView™ and Nikon BioStation™ platforms. If, imaging is coupled to affordable portable and transparent incubator, this would beacon a lot of surprising scientific findings during the course of incubation.

In this study, poly-methylmethacrylate (PMMA) is employed in the development of incubator for cell studies for multitude of reasons, of which, optical transparency, thermal properties like glass transition temperature (Tg = 110°C) and biocompatibility benefitted. Although the material sounds good with its list of attributes, prime challenge is insulation, even with an increased thickness of 6 mm was relatively challenging but achievable. The development of mini incubator replaced the work stage of an inverted microscope and can house a passive microfluidic device (PMFD) of specific dimensions. The ambience in the incubator ensures healthy cell life with corresponding values of temperature, humidity and CO_2 that is appropriate for bio samples [27, 28]. Many considerations are made during design phase of the incubator as the cells are quite delicate with increased temperatures or decreased humidity, hypoxia conditions. This incubator will host a closely mimicked 3D microenvironment that is suitable for variety of cellular studies and investigations that include but not limited to cell culture, chemotaxis, drug screening, ageing, cancer research – to study the mechanism and means for conversion of normal cells to cancer cells, thereby develop drugs to selectively target cancer cells [5-7]. Being so persuasive with its applications, the mini

incubator is carefully designed for the cellular microenvironment that influences cell growth and development in a more comparable condition of *in-vivo*.

A microfluidic platform that makes use of pure passive hydrodynamics to run on-chip cell cultures and chemotaxis studies under 3D microenvironment conditions is realized with the development of an automated, controlled and portable mini incubator combined with temperature and humidity control system that significantly reduces cell contamination is successfully published in this work. An integration of incubator with passive microfluidic platforms emerged to be efficient in cell culture and chemotactic behavior of cells, mounted on an inverted microscope. This hugely indicate the quality of microenvironment produced by the mini incubator. This development, by and large, can be used for culture studies, drug discovery, drug screening and other biochemical analytics.

A temperature/humidity sensor is utilized for data collection in both characterization and actual experiments. An open-source 8-bit microcontroller board (ARDUINO UNO) is used to digitize the voltage signals from sensors. The measurement data is communicated to the computer (pc) via Universal Serial Bus (USB) and, the pc stored the received data in NI LabVIEW file format. Data logging, sensor control (via ARDUINO UNO), data processing and pH computation are carried out with NI LabVIEW 2014. Once the signals initiated, no recalibration or maintenance is required, and measurements can be carried out without any special manual attention till the end of the analysis.

2 Design and Fabrication

The system is designed in a software environment using SolidWorks®, which is subjected to theoretical simulations in COMSOL® and then fabricated with six pieces of PMMA using micro mechanical machining and 3D printer with epoxy resin. Once machined, individual faces of the system were assembled together with PMMA screws and silicone to secure tightly. A confirmation of theoretical simulations was performed over experimental tests to verify temperature and humidity conditions inside the incubator.

2.1 From Calculation to Design

The incubator is designed to replace the microscope stage, to avoid any misalignment, and thus resulting in perfect fixation of the incubator above the illumination source. All parts of incubator are fabricated with 6 mm thick PMMA to ensure better isolation from external ambience and improved insulation capabilities, and the humidifier tanks are 3D printed with epoxy resin. The incubator dimensions (210.4 x 102 x 59 mm) as in Figure 1, were evaluated based on the space available on the microscope with particular attention to the lower layer which has a specific pattern so that it can fit on the stage.

Individual designs of each panel and details are illustrated in Figure 2. The bottom panel has a circular window of diameter 35 mm and 38 mm, internal and external respectively, designed to allow high definition and optimal cellular screening in relation to an optical window created on the microfluidic device. On the ridge of this window is placed a ring made of PMMA of 31, 37 and 3 mm, internal diameter, external diameter and height respectively. Above the ring is placed a glass slide of thickness 100 µm and 32 mm Ø, to enrich the visualization effects and to trap the ambience of incubator environment [29]. On the frontal panel of incubator, a window of 120 x 20 mm is designed to allow the placement of PMFD exactly on top of a dent of dimensions 85 x 75 mm to tightly secure the device throughout the experiments.

The mini incubator set up ensures temperature and humidity control suing the temperature sensor DHT22 and k-type cold junction thermocouple. Video Graphics Array (VGA) connector pin placed in the rear panel, connects the internal circuitry to external power source and controller, thereby isolating the

incubator off any wires making it a portable device. The rear panel has an additional slot inside which the micro air pump is placed, housed external to the incubator by a PMMA case designed and built using micro-milling. The panels of entire structure are fastened appropriately and assembled.

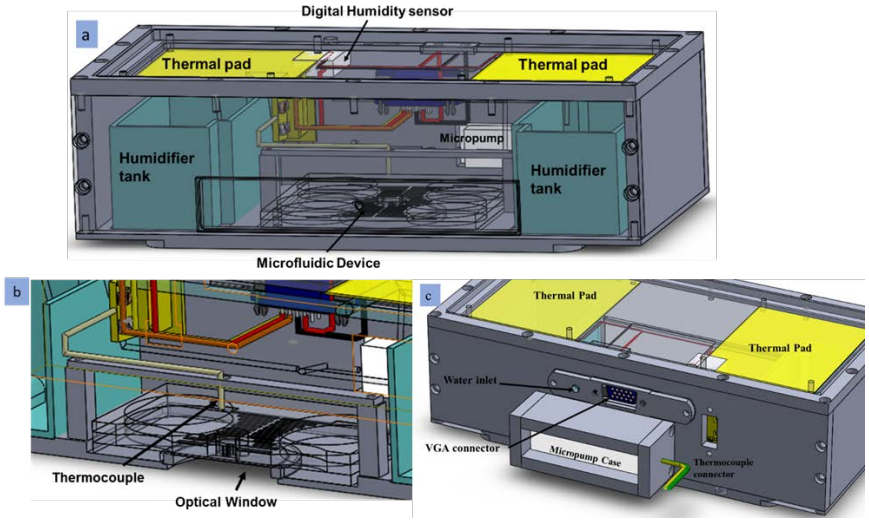

Figure 1. Modular incubator CAD design a-c. a) Full view of the incubator displaying humidifier tanks, thermal pads, micro air pump and thermocouple with the placement of passive microfluidic device; b) Section view of the incubator that clearly displays the optical window at the bottom of the incubator; c) Rear view of the incubator with slots for micro air pump, VGA connector and thermocouple connector

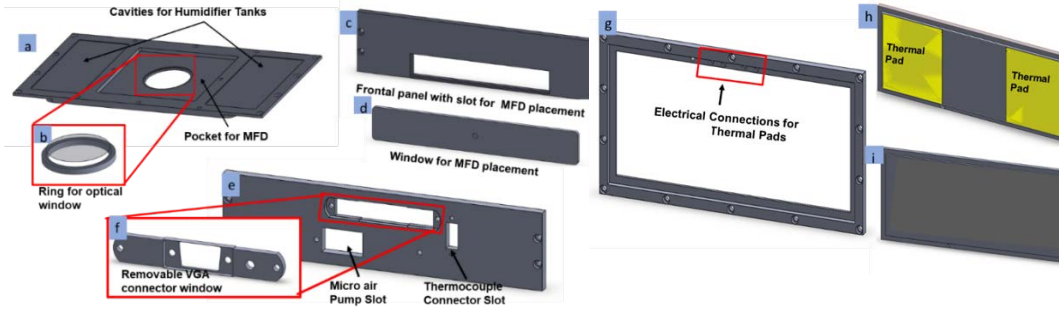

Figure 2. CAD design of panels a - i. a) Bottom layer with cavities for PMFD and humidifier tanks to ensure no displacement during experiments; b) A ring-like structure made of PMMA for placement of PMFD on the OW; c) Frontal panel with slot for entry and exit of the device; d) Window for the frontal slot; e) Rear panel with slots for positioning the micro-pump and VGA window; f) Removable window containing the VGA connector; g) Top panel with space for removable window; h) Removable top window with thermal pads; i) Removable top window

2.2 Humidification System

For best cell culture and other biological studies, proper temperature, gas tension (CO2 / O2 / N2) and humidity should perform collectively, to provide optimum growth. As right temperature boosts cell proliferation rates, high relative humidity (RH) as much as 95% - 99% prevents evaporation of growth media, which is essential in both active and passive systems. All these environmental factors are employed for healthy cells which express precise protein profiles [28, 30, 31]. When RH is the amount of water vapor present in a gas at any temperature, the absolute humidity measures the amount of water vapor present in dry gas at a standard temperature and pressure. Hence, based on the requisite of cell culture conditions, the perfect attention is upon relative humidity [31].

To ensure appropriate RH in the incubator presented in this work, a set of experiments were conducted and validated for accurate standards with respect to the system built. Anyway, being a mini incubator, different methods to humidify are possible: bubbling, headspace saturation and permeation. Though difficult, bubbling system is chosen for its feasibility with the incubator design. Subsequent to scrutiny on stability and integration to this system, a humidifier tank of 38 mm height is manufactured with Objet 3D, an additive manufacturing technique. The structure can fill a volume of 100 ml each and thus two tanks of volume 200 ml in total is placed inside the incubator. It is calculated, from previous experiments, a loss of 1 ml volume per hour, hence to withstand a week of incubation. When a stream of gas is bubbled through water, it is easier to achieve 100% relative humidity [31, 32].

3 Simulations

The simulations for temperature and humidity of ideal conditions are designed using COMSOL Multiphysics software. The evaluation was idealized by combining materials, physics and type of study and simulations obtained are comparable to experiments conducted.

3.1 Temperature and Humidity

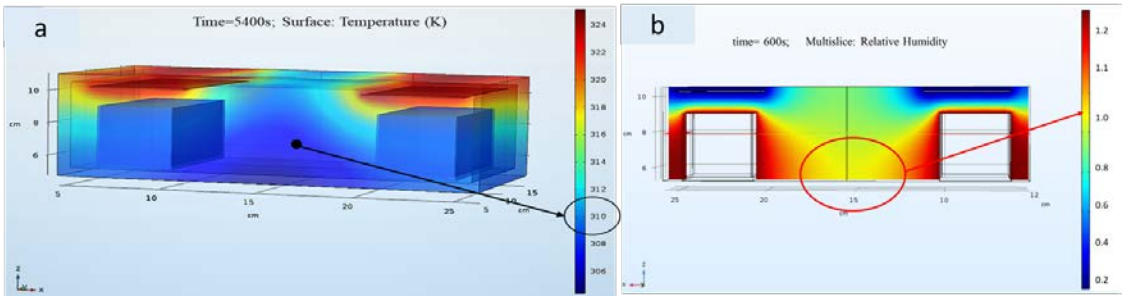

Figure 3. Temperature and Humidity Simulations a-b. Representation of the temperature trend at different time points, a) At 5400 s a temperature of 310 K in the central area of the mini-incubator; b) The system attains the required 100% RH after 600 s

For most biological cells, the ideal temperature for cell growth and proliferation is determined as 37 °C [33-36]. Hence, this study focused on simulating a temporal trend of temperature inside the designed structure with an ultimate aim to evaluate the time required to insulate the structure at 37 °C closer to the PMFD within which the cells act [37]. The water filled in humidifier tanks, thermal pads and external laboratory conditions are at 35, 50 and 23 °C respectively. A transient study was conducted to determine the effect of water temperature, . The temperature near the cells in PMFD reaches 37 °C after 90 minutes and thereafter, remaining stable for the entire duration of experiment. Similarly, transport of humid air is set with the initial value of RH ≈ 0.2 and top faces of humidifier tanks are considered as wet surfaces due to the presence of warm water with an evaporation rate ≈ 100 m/s. With the set values, transient study generated a custom mesh and generated a simulation (Figure 3).

4 Control and automation Unit

Automation and integration play key role in this study as the system had great scope with a variety of components involved, as described in the schematic Figure 4.

4.1 Temperature and Humidity Control

The microfluidic platform demands high precision in temperature, humidity and CO2. Hence the built mini incubator should match and highly tune the microenvironment inside the chamber. The temperature is to be set at a desired point and it needs to be controlled. The entire platform is controlled and processed by Arduino – a microcontroller which offers a prodigious opportunity for customization. Arduino

Interface program, written in C language, integrated the microcontroller and components inside the incubator.

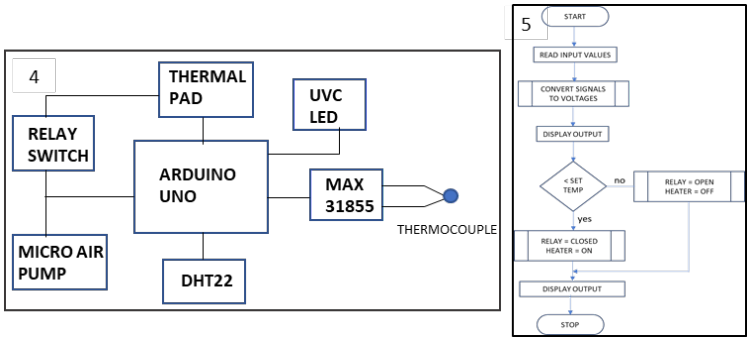

Figure 4: Automation Schematic. The ARDUINO UNO controls the entire system of thermal pads (via relay), temperature and humidity sensor (DHT22) and thermocouple; **Figure 5: Control Flowchart**. The control unit is programmed with microcontroller, which reads signals from sensors and converts to voltages to display temperature and humidity data. Relay works on decisions made with the actual temperature against the set temperature values

A temperature sensor (Digital Humidity and Temperature sensor – DHT22) coupled to a k-type cold junction thermocouple connected to power supply act as control unit. A relay mechanism is in place to turn on/off the thermal pads according to the set temperature values. However, the maximum heating temperature of thermal pads is 50 °C which was sufficient to heat up the entire incubator environment within few minutes. It is also important to note that the heating effect of thermal pads did not cause any damage to the PMMA layers, because the glass transition temperature of PMMA is 110°C [38]. As discussed earlier, RH, in addition to, temperature is controlled with a micro air pump connected to a relay and power supply. The air pump fits tube (TYGON R3607) that is immersed into the humidifier tanks produce bubbles required to humidify the microenvironment, which is precisely monitored by DHT22. A circuit box is designed to contain all the circuitry without any hindrance to the microscope observations. The control unit is designed as a graphical user interface (GUI) using NI LabVIEW, replicating the complex logic of the schematic, where a single user-friendly window to acquire data from DHT22 and thermocouple. A flow chart visually explains the flow of procedures followed in data acquisition, control and display (Figure 5). Moreover, the GUI allows a user to modify the temperature and humidity values according to their specific needs, thus being flexible to be used across cross-platforms.

5 Fabrication and Assembly

The designed mini incubator was manufactured using mechanical micro-machining and 3D printer. The individually developed components were assembled and secured tightly to endure long-term application. The incubator was made of PMMA of 6 and 2 mm thickness for faces and frontal window respectively. OBJET 3D from Stratasys, was deployed to fabricate humidifier tanks as the structure was quite complex to be done with micro-milling. VeroClear (RGD810) – a transparent resin comparable to the properties of PMMA and support material "SUP705" were used for the structure. High-pressure water cutter was used to clear off all the residues from the final clear structure. The components from micromill and 3D printer were hand-finished and cleared off any impurities during the assembly phase (Figure 6).

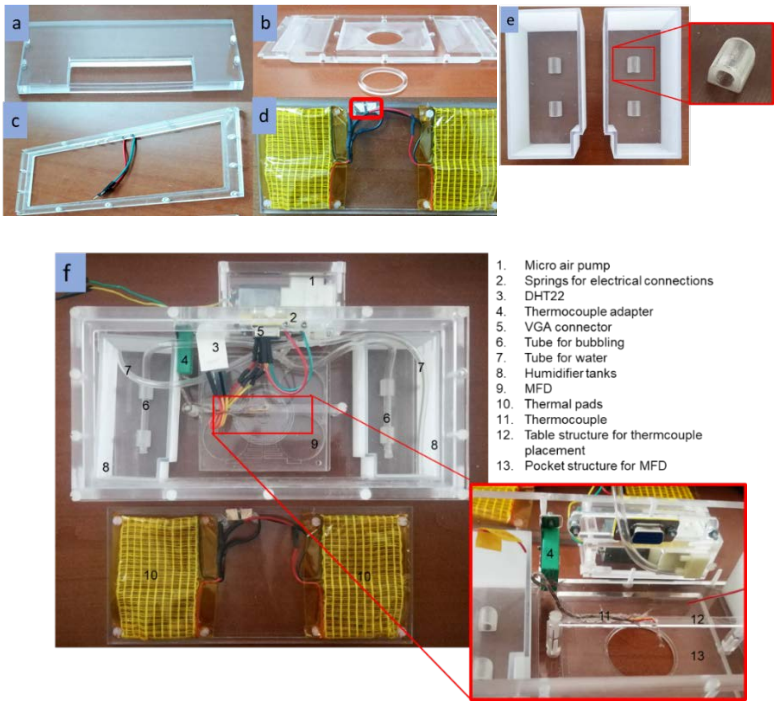

Figure 6. Fabricated Incubator parts a-f. a) Front panel with the slot for window to place PMFD; b) Bottom layer with the window for imaging and ring for placement of PMFD; c) Top window with electrical connections for thermal pads; d) Top removable window with thermal pads and copper connections for electrical conductivity; e) 3D printed humidifier tanks with the structure for fixation of bubbling tubes; f) Final assembled mini incubator

6 Experiment and Results

Time [min]	RH [%]	Tmp [°C]	Tc [°C]
0	64	27	28
1	69	27	28
3	93	27	28.75
5	100	28	29
10	100	29	30
30	100	32	32.25
60	100	34	34.50
120	100	35	35.25
180	100	36	36.25
240	100	36	37

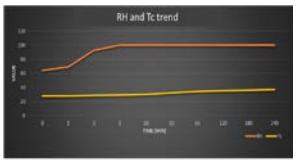

Figure 7. Data sheet of incubator performance and RH and temperature graph. (Left) Values obtained with the initiation of the circuit is listed in the table. It is evident that RH attained 100% in 5 min whereas temperature reached 37°C after 4 hours; (Right) from the experiments, the system had a better initiation and recovery protocol as the circuit was programmed with a feedback mechanism to avoid fluctuations

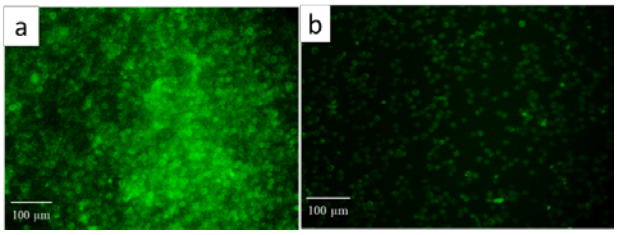

Figure 8. Fluorescence imaging through incubator from an inverted microscope a-b. A passive microfluidic device placed inside the incubator atop the optical window for cellular screening studies; a)

Jurkat cells labeled with FITC, viewed under fluorescence microscope through the optical window of the incubator. A PMFD without optical window exhibits high auto-fluorescence resulting in poor visibility of cells; b) Optical window created on a PMFD, with an optimal fluorescence imaging

The assembled mini incubator was placed on the microscope stage to carry out an experimental validation of the parameters to be checked to mimic the cellular microenvironment. The humidification system was improvised as follows: Holes were punctured with a 0.4 mm Ø needle at 30 mm distance in the tube. The tubes were connected from micro air pump positioned on the rear panel and hence an air flow of 0.7 l/min is generated inside the tanks. In addition, two tubes filled water at 35 °C in the humidifier tanks from rear panel, connected to a syringe. An external cylinder of 5% CO2 is connected to the system, thereby fulfilling the basic conditions of cell environment.

Once the electrical circuits are connected, DHT22 and thermocouple started to acquire initial data and displayed on LCD panel, mounted on the circuit holder box, specially fabricated for this system. When the top removable window holding the thermal pads was positioned atop the upper panel, the circuit started to function according to the set parameters on GUI, and the entire system was isolated and ideal microenvironment developed inside the mini incubator. A graph is generated to represent the initial conditions of the ambience inside the mini incubator illustrated in Figure 7. With the desirable conditions acquired, like temperature of 37 °C and RH of 100%, a set of cell experiments were performed inside the mini incubator. Moreover, the incubator was coupled to an inverted microscope and hence, imaging was a challenge using a 6 mm thick PMMA for incubator, that produces autofluorescence. There was no possibility of compromise on the thickness because insulation of the incubator ambience from the external is of prime concern. This was later overcome with the optical window milled at the rear side of the incubator in relation to a similar window created in the PMFD. The depth of the optical window allowed full imaging resolution as it was determined carefully considering the working distance of the microscope objectives. As an outcome, fluorescence imaging was also possible with high resolution and better details (Figure 8).

7 Conclusions

Incubators predominantly maintain a constant temperature, however additional features are built in. In this study, a mini incubator is designed and developed with automation capabilities that houses passive microfluidic devices for cellular screening and cultures. The most attractive feature comes with the low-cost and yet transparent material of fabrication, PMMA. This makes the device more affordable and expandable. Reproducibility is extraordinary with the incubator, by which, devices of any cellular investigations could be integrated as there is tight control over the environment and flexibility in ambient conditions, provided by automation. Robustness is another feature of this incubator as it resists against perturbations and yet maintains the ambient conditions. This incubator could be easily handled by biologists, with no much hassle in placement of loaded PMFDs, electrical circuitry and GUI.

The experiments carried out on the final product confirm its reliability and efficiency at attaining the temperature at 37 °C after 240 minutes and the relative humidity at 100% after 5 minutes. The incubator is totally portable because all the electrical cables are carried outside only through connectors fixed on the rear panel, and the circuit box is connected via USB port to computer. Therefore, the experimental confirmations have respected the design features set initially, and the results are compliant with computational simulations and experiments. With a microscope stage-top incubator discussed in this work, it is easier to share the microscope for other research activities just by lifting it away whereas a dedicated system has little scope for portability and sharing.

Acknowledgments

The author gratefully acknowledges Italian Ministry of Health for financial support to this project.

References

[1] Y. J. Dong, M. D. Varisanga, N. R. Mtango, M. Aono, T. Otoi, and T. Suzuki, "Improvement of the culture conditions for in vitro production of cattle embryos in a portable CO2 incubator," Reprod Domest Anim, vol. 36, no. 6, pp. 313-8, Dec 2001.
[2] S. Ts, "[Working Conditions in Incubator Stations]," Gig Sanit, vol. 29, pp. 101-2, Apr 1964. Usloviia truda na inkubatornykh stantsiiakh.
[3] J. Dancis and H. M. Cardullo, "Incubator care of the premature infant; discussion of optimal conditions and the current methods for their provision," Pediatrics, vol. 6, no. 3, pp. 432-40, Sep 1950.
[4] M. Bernardi and S. Nasso, "[Influence of surrounding conditions on immature newborns; a type of incubator recently imported into Italy]," Minerva Pediatr, vol. 2, no. 4, pp. 158-62, Apr 1950. Influenza delle condizioni ambientali sul neonato immaturo;
[5] H. Yuan, K. Xing, and H. Y. Hsu, "Trinity of Three-Dimensional (3D) Scaffold, Vibration, and 3D Printing on Cell Culture Application: A Systematic Review and Indicating Future Direction," Bioengineering (Basel), vol. 5, no. 3, Jul 23 2018.
[6] T. Imura, K. Nakagawa, Y. Kawahara, and L. Yuge, "Stem Cell Culture in Microgravity and Its Application in Cell-Based Therapy," Stem Cells Dev, vol. 27, no. 18, pp. 1298-1302, Sep 15 2018.
[7] T. Hori and O. Kurosawa, "A Three-dimensional Cell Culture Method with a Micromesh Sheet and Its Application to Hepatic Cells," Tissue Eng Part C Methods, Nov 9 2018.
[8] A. E. Fadda M., Guidi G., Cesarini M, "A new cell culture incubator and management system," White Paper - Comecer Group.
[9] J. D. Haynes and J. K. Moch, "Automated synchronization of Plasmodium falciparum parasites by culture in a temperature-cycling incubator," Methods Mol Med, vol. 72, pp. 489-97, 2002.
[10] F. Carpignano et al., "A new cell-selective three-dimensional microincubator based on silicon photonic crystals," PLoS One, vol. 7, no. 11, p. e48556, 2012.
[11] D. Brunner, H. Khawaja, M. Moatamedi, and G. Boiger, "CFD modelling of pressure and shear rate in torsionally vibrating structures using ANSYS CFX and COMSOL Multiphysics," (in English), International Journal of Multiphysics, vol. 12, no. 4, pp. 349-358, 2018.
[12] S. Yalcin et al., "In Vivo Comparison of "V-Loc 90 Wound Closure Device" With "Vicryl" and "Monocryl" in Regard to Tissue Reaction in a Rabbit Bladder Model," Urology, vol. 116, pp. 231 e1-231 e5, Jun 2018.
[13] M. Sigl, J. Jung, H. Kolble, and K. Amendt, "Multiple Stent Delivery System "Multi-LOC": Focal self-expanding nitinol stenting of complex femoropopliteal lesions-A monocenter feasibility pilot study," JRSM Cardiovasc Dis, vol. 8, p. 2048004018822618, Jan-Dec 2019.
[14] A. Collignon et al., "(R)-GEMOX chemotherapy for unfit patients with refractory or recurrent primary central nervous system lymphoma: a LOC study," Ann Hematol, vol. 98, no. 4, pp. 915-922, Apr 2019.
[15] T. Kinoshita et al., "Long-term Outcomes of Laparoscopic Versus Open Surgery for Clinical Stage II/III Gastric Cancer: A Multicenter Cohort Study in Japan (LOC-A Study)," Ann Surg, vol. 269, no. 5, pp. 887-894, May 2019.
[16] D. W. Cook, "Automatic incubator for use with modified A-1 test for enumerating fecal coliform bacteria in shellfish growing waters," J Assoc Off Anal Chem, vol. 64, no. 3, pp. 771-3, May 1981.
[17] A. V. Beran, T. Doniguian, W. F. Taylor, and D. R. Sperling, "An automatic incubator oxygen monitor and control system," Med Biol Eng, vol. 10, no. 1, pp. 1-8, Jan 1972.
[18] SciGene, "Hybex®Microarray Incubation System - USER MANUAL " 2012.
[19] M. Cornaglia et al., "An automated microfluidic platform for C. elegans embryo arraying, phenotyping, and long-term live imaging," Sci Rep, vol. 5, p. 10192, May 7 2015.
[20] M. Xu, W. Yang, T. Hong, T. Kang, J. Ji, and K. Wang, "Ultrafast all-optical flip-flop based on passive micro Sagnac waveguide ring with photonic crystal fiber," Appl Opt, vol. 56, no. 16, pp. 4577-4584, Jun 1 2017.
[21] W. Shin and K. Oh, "Novel micro-optical waveguide on micro-actuating platform for reconfigurable wavelength selective optical switch," Opt Express, vol. 12, no. 19, pp. 4378-89, Sep 20 2004.

[22] X. Lin, A. Hosseini, X. Dou, H. Subbaraman, and R. T. Chen, "Low-cost board-to-board optical interconnects using molded polymer waveguide with 45 degree mirrors and inkjet-printed micro-lenses as proximity vertical coupler," Opt Express, vol. 21, no. 1, pp. 60-9, Jan 14 2013.
[23] I. Valpapuram et al., "Waveguiding and SERS Simplified Raman Spectroscopy on Biological Samples," Biosensors (Basel), vol. 9, no. 1, Mar 3 2019.
[24] T. E. Angelini, E. Hannezo, X. Trepat, M. Marquez, J. J. Fredberg, and D. A. Weitz, "Glass-like dynamics of collective cell migration," Proc Natl Acad Sci U S A, vol. 108, no. 12, pp. 4714-9, Mar 22 2011.
[25] R. Srinivasan et al., "Pharmacological chaperoning of nicotinic acetylcholine receptors reduces the endoplasmic reticulum stress response," Mol Pharmacol, vol. 81, no. 6, pp. 759-69, Jun 2012.
[26] J. P. Roberts, "Live-Cell Imaging: Controlling the Environment," BioCompare, 2016.
[27] M. Takagi and K. Ueda, "Comparison of the optimal culture conditions for cell growth and tissue plasminogen activator production by human embryo lung cells on microcarriers," Appl Microbiol Biotechnol, vol. 41, no. 5, pp. 565-70, Jul 1994.
[28] R. G. Ham and T. T. Puck, "A regulated incubator controlling CO2 concentration, humidity and temperature for use in animal cell culture," Proc Soc Exp Biol Med, vol. 111, pp. 67-71, Oct 1962.
[29] C. Ince, D. L. Ypey, M. M. Diesselhoff-Den Dulk, J. A. Visser, A. De Vos, and R. Van Furth, "Micro-CO2 -incubator for use on a microscope," J Immunol Methods, vol. 60, no. 1-2, pp. 269-75, May 27 1983.
[30] W. Dick, "Aspects of humidification: requirements and techniques," Int Anesthesiol Clin, vol. 12, no. 4, pp. 217-39, Winter 1974.
[31] A. R. Chamney, "Humidification requirements and techniques. Including a review of the performance of equipment in current use," Anaesthesia, vol. 24, no. 4, pp. 602-17, Oct 1969.
[32] C. F. Forney and D. G. Brandl, "Control of Humidity in Small Controlledenvironment Chambers using Glycerol-Water Solutions," HortTechnology, vol. 2(l), 1992.
[33] B. L. Ekerdt et al., "Thermoreversible Hyaluronic Acid-PNIPAAm Hydrogel Systems for 3D Stem Cell Culture," Adv Healthc Mater, vol. 7, no. 12, p. e1800225, Jun 2018.
[34] J. Yang et al., "Cell delivery in regenerative medicine: the cell sheet engineering approach," J Control Release, vol. 116, no. 2, pp. 193-203, Nov 28 2006.
[35] T. Iwata et al., "Cell sheet engineering and its application for periodontal regeneration," J Tissue Eng Regen Med, vol. 9, no. 4, pp. 343-56, Apr 2015.
[36] Y. Haraguchi et al., "Cell sheet technology for cardiac tissue engineering," Methods Mol Biol, vol. 1181, pp. 139-55, 2014.
[37] Z. Zhang, P. Boccazzi, H. G. Choi, G. Perozziello, A. J. Sinskey, and K. F. Jensen, "Microchemostat-microbial continuous culture in a polymer-based, instrumented microbioreactor," Lab Chip, vol. 6, no. 7, pp. 906-13, Jul 2006.
[38] C. B. Roth and J. R. Dutcher, "Glass transition temperature of freely-standing films of atactic poly(methyl methacrylate)," Eur Phys J E Soft Matter, vol. 12 Suppl 1, pp. S103-7, Nov 2003.

Pushparani Micheal Raj is currently a Postdoc researcher at MAX IV Laboratory in Lund University, Sweden. She received her PhD in Molecular Oncology and Translational Medicine at University of Catanzaro, Italy in 2019. Her research interest are in micro/nano-fluidics, LOC, optofluidics, biosensors, micro/nano fabrication, spectroscopy and mechatronics. She has published in several International Journals and presented her work in 7 International Conferences in the form of posters and oral presentations. She is an active resource person in International webinars, sharing her expertise in the field of microfluidics, automation and X-ray spectroscopy.