Microscope Cell Culture Incubator

Biomedical Engineering 200/300 - Preliminary Report, October 11th, 2022

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Abstract

Cell cultures are a common laboratory practice. To maintain them, cell cultures must be cultivated at specific temperatures, humidity, and carbon dioxide levels. To aid in this, labs will typically use an incubator to maintain a specific climate. Current incubators that are on the market are incredibly expensive. Furthermore, the incubators are either too large to use with an inverted microscope, or the incubators specifically designed for inverted microscopes do little to combat condensation buildup. The team plans to improve upon the previous year's incubator by implementing specific anti-condensation features. The team will use an ITO heating film to maintain constant temperature of the top viewing glass, and the team will use a thin layer of water on the bottom of the incubator to create a uniform medium for the light to travel through, effectively removing the condensation problem from previous designs. Experimental success for the design will be determined by how successful the incubator is at maintaining cell cultures and by the optical clarity of the incubator. The team hopes that by developing an effective low-cost incubator that the economic burden of research in laboratories is lessened.

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Introduction

Motivation & Global Impact

As biomedical technology progresses, the need for innovative solutions in research becomes more pronounced. Creating an affordable microscope cell culture incubator is a compelling endeavor, given its potential to democratize access to crucial research tools.

In a landscape where cutting-edge equipment often comes with a hefty price tag, the development of an inexpensive microscope cell culture incubator is a game-changer. Such a device has the potential to empower a broader range of researchers, including those in resource-constrained environments, by providing them with access to essential incubation capabilities.

Cell incubation research is also essential for many different processes, many of which are used in life-saving therapies against bloodborne illnesses and tissue engineering. Creating a more affordable alternative will give access to this research in those resource-constrained environments

Existing Devices & Current Methods

While there are cell incubators on the market, they are often too large and unportable for use with an inverted microscope. The *Heracell™ Vios 160i CR CO2 Incubator, 165 L* [1], for example, controls for temperature, CO_2, oxygen, and humidity but its dimensions are 637 x 901 x 881 mm and weighs 95kg. This is too large for any microscope even if the vessel was see-through. Designs that aim to specifically work with microscopes, like the patented *Controlled environment incubator for light microscopy* [2] , struggle to solve the issue of condensation build up on the inside of the viewing chamber. Many other solutions are only able to last for short periods of use before needing to be cleaned, like the design shown in the patent *Transparent thermostatic culture vessel for microscope observation* [3].

Problem Statement

This project team serves to develop a low cost cell culture incubation chamber that is compatible with an inverted microscope and capable of live cell imaging. This incubation chamber must be able to maintain an internal environment of 37 C, 5% CO2, and 95-100% humidity over a long duration of time, without compromising the integrity of the microscope's optics or functionality. Special consideration should be taken to maintain even heating and humidity across the chamber as gradients can result in evaporation from low volume cultures such as microfluidic devices. Current commercially available systems are prone to these issues and are extremely expensive. Commercial systems also tend to be large and enclose the entire

microscope making it difficult to assemble and remove and between uses. Because of their size, they also hinder use of the microscope in general.

Background

Anatomy & Physiology

Cell culture is one of the major tools used in cellular and molecular biology. Removing cells from a plant or animal and placing them in a favorable artificial environment causes subsequent growth. Cell culture provides excellent model systems for studying the normal physiology of cells and the effects of drugs and toxic compounds on the cells [3]. Cell cultures fall under Biosafety Level 2 guidelines [4] due to the safety procedures associated with working in a Lab with human diseases. The major types of cells used in culture media are primary cells, transformed cells, and self-renewing cells [5].

Incubators have to maintain very stable environments with regulated temperatures, humidity, light, pressure, CO_2, O_2, and pH levels, and other conditions [5]. Incubator's are designed to maintain this environment in order to replicate the cell's natural conditions in the body. These factors are critical for the growth of the cultured cells. CO2 is primarily used as a buffer in incubators, assisting in maintaining the pH of the medium. The medium that the cell culture resides in can be made of either natural or synthetic media. Natural being medium such as plasma, serum, and embryo extract [6]. Synthetic being made of basal medium and supplements, such as serum, growth factors, and hormones [6].

Client Information

Our client is Dr. John Puccinelli, Associate Chair of the Undergraduate Program, BME Design Curriculum Coordinator, and our professor for BME 200/300. Dr. Puccinelli teaches a tissue engineering course in ECB 2001 and plans to use this device for up to two weeks each year for education purposes.

Product Design Specifications

There are 4 major criteria that must be followed during the design process of the product. The first criteria is cost effectiveness, ideally less than $100. The second criteria is accuracy and reliability. This means that the incubator must be able to maintain internal conditions of 37°C ± 0.5°C, humidity > 95%, and CO2 levels must be 5% ± 0.1% for up to two weeks at a time. The third of the criteria is durability, which consists of the incubator ideally not leaking and easily sterilized by ethanol. The last criterion is size, the final incubator must fit in the maximum constraints of 310x300x45mm in order to fit on the stage of the client's microscope. More detailed information can be found in APPENDIX I.

Preliminary Designs

Design 1: Layer of Water Design

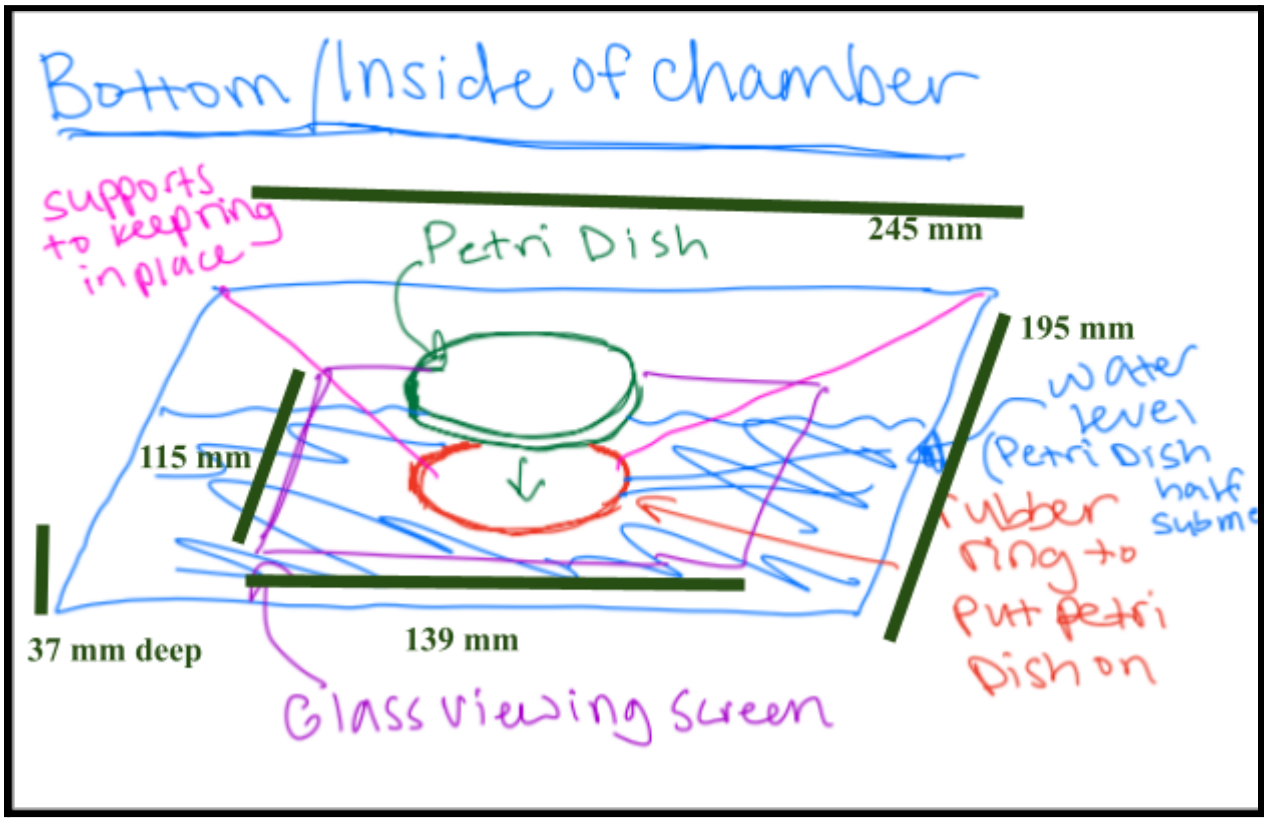

Figure 1. Layer of Water Design

Design one, depicted in Figure 1 consists of a 245x195x37mm black acrylic chassis manufactured by the team from previous semesters. It has two viewing screens made of glass. There will be a thin layer of water at the bottom of the chamber. This layer of water will prevent condensation on the bottom viewing screen because there is a uniform medium. A rubber ring will be secured at the bottom of the chamber and the petri dish will be placed on a rubber ring to prevent movement and prevent water from affecting the cell culture media. Set up for this design is relatively simple; the user just has to add a layer of distilled water at the bottom of the well, then place the cell culture in, and then start the code for the incubator to run.

This method greatly depends on the ability of the rubber ring to immobilize the petri dish containing the cell culture and media. An issue that may occur due to human error may come from unintentional moving the incubator with too much force or jostling it, causing movement of the layer of water and possibly causing the water to flow into the petri dish, potentially causing issues with cell proliferation or reliable data collection.

Design 2: ITO Film Design

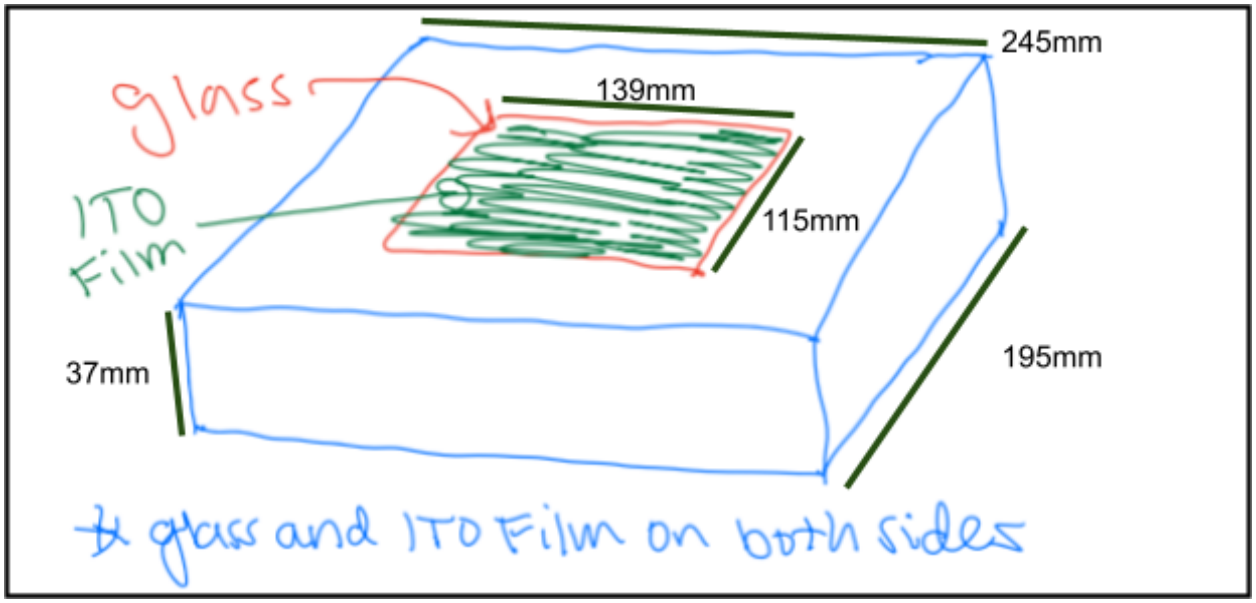

Figure 2. ITO Film Design

Design two consists of the same base chassis used in Design 1, as shown in Figure 2 . Both viewing screens will be made out of glass but with an added layer of indium-tin-oxide (ITO). ITO is a transparent and conductive material. Both layers will be connected to an arduino that will run charge through the material whenever it is activated. This will heat the ITO and as a result heat the glass underneath it. The heated glass will then no longer be able to form condensation due to uniformed heating. However, heating the glass too quickly may also lead to shattering. This problem will be remedied by running a duty cycle that slowly ramps up to constant charge running through the ITO.

This method suffers from being the most expensive solution. Although, its connection to the arduino and evenly distributed heating make it hypothetically the most reliable. The cost of this solution might make other additions to the design much more difficult to fit into the team budget.

Design 3: Heated Wire Design

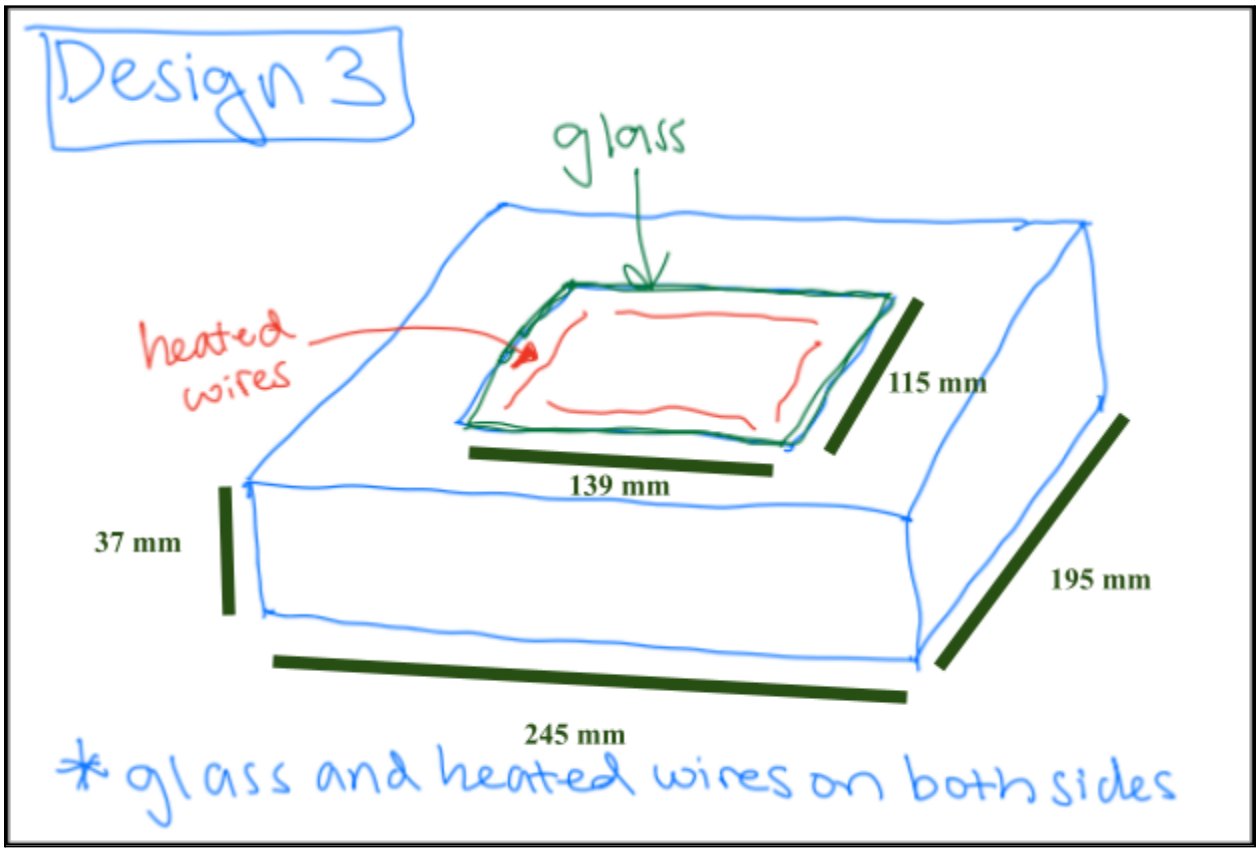

Figure 3. Heated Wire Design

Design three, shown in figure 3, will have the same chassis and glass viewing screens as design one and two. On the viewing screens, there will be copper wires around the edges. These wires will be connected to an Arduino that will produce run current through them and monitor the temperature inside the chamber. This design's benefits come primarily in cost, as it only changes the windows to glass and adds copper wire to the outside rim of the glass. This upgrade to the existing design will be very inexpensive and should fix the condensation problem.

This design, although inexpensive and theoretically effective, suffers from some glaring issues. Some issues of this design are the exposed wires which may lead to accidental burns, the danger of heating the glass too quickly,shattering it, and most importantly, the lack of temperature uniformity due to heating only the outside.

Preliminary Design Evaluation

Criteria	Weight	Design 1 Layer of water + glass on bottom, heating element on top		Design 2 ITO Film + Glass (maintain constant temp) link		Design 3 Heated Wire Design + glass on both sides	
Reliability	40	4/5	32	5/5	40	2/5	16
Cost	25	4/5	20	2/5	10	5/5	25
Durability	10	3/5	6	3/5	6	2/5	4
Ease Of Fabrication	10	3/5	6	3/5	6	5/5	10
Ease of Use	10	3/5	6	4/5	8	5/5	10
Safety	5	5/5	5	4/5	4	3/5	3
Total	100	75		74		68	

Table 1: Preliminary Design Matrix

Summary of the Design Matrix

In order to effectively evaluate each of the preliminary designs, a comprehensive design matrix was created. This matrix analyzed several factors related to the application of designs through the use of each criteria. Each design would be scored on a scale from 1 to 5 with each criteria having different weightedness; A score of 1 being unsatisfactory and 5 being very satisfactory. Six different criteria were defined as the following:

- *Durability*: Considers how long the parts of the design could be expected to last with consistent use. A higher score represents a design that would not be expected to break even with long-term use. A low score means that the design would likely be prone to breaking down.
- *Reliability*: Considers how consistently and to what standard the design would fulfill its intended purpose of defogging the glass, A higher score means that the design is expected to consistently and totally solve the fogging issue. A low score means that the design is expected to infrequently or poorly solve the fogging issue.

- *Ease of Fabrication*: Considers which designs would require the least amount of effort and strenuousness to fabricate correctly. A higher score indicates a design that can be fabricated with less effort while lower scores represent higher effort.
- *Cost*: Considers the amount of money needed to fabricate and maintain each design. Low scores indicate a higher cost and higher scores indicate a lower cost.
- *Safety:* Consider how safe each design is to use. Low scores indicate a less safe design and higher scores indicate a safer design.
- *Ease of Use*: Considers how easily the client will be able to use each design. Low scores indicate a design that will be harder to use (involve more moving parts) and higher scores indicate a design that will be easier to use.

Proposed Final Design

Our proposed final design is a combination of Design 1 and Design 2, using Design 1's layer of water solution as shown in Figure 1 for the bottom viewing port and Design 2's ITO heating element for the top light window, shown in Figure 2. We will use glass for our transparent surfaces and keep all other designs and materials from the predecessor's designs, using copper tubing with a heated water pump to heat the petri dish, an NDIR sensor to detect CO2 levels, a thermistor to detect temperature, and a CO2 pump: exhibited in Figures 4 & 5.

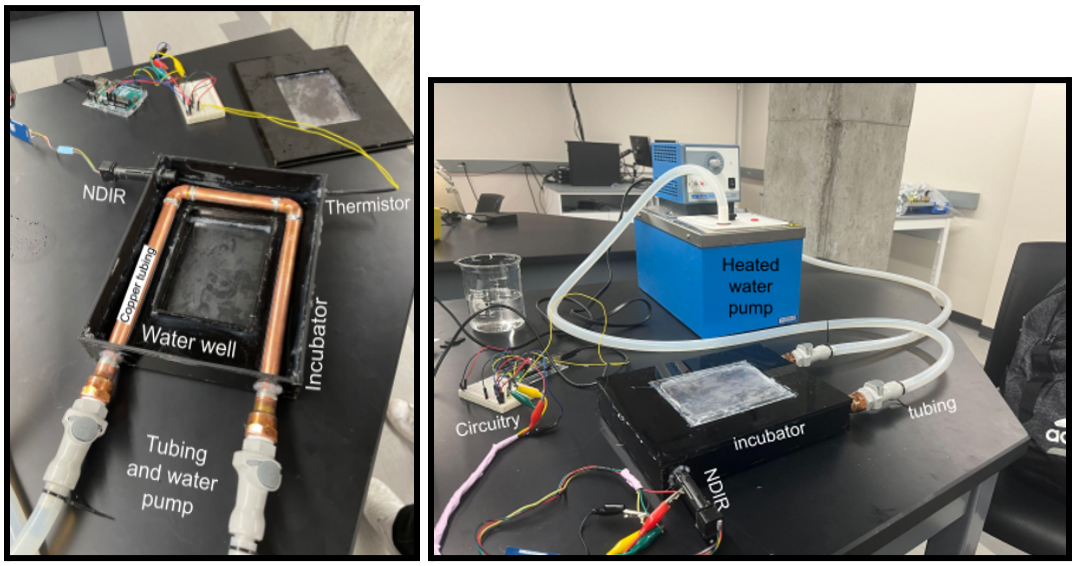

Figure 4 & 5. Previous Team's Final Design

Fabrication and Development Process

Materials

For the viewport, we will be using glass, distilled water, and a rubber ring to hold the petri dish in place above the distilled water, and polycarbonate to hold the water in a basin below

the dish. We will use a layer of Indium Tin Oxide on the glass and use an Arduino to run a variable current through the layer and warm the glass depending on the internal temperature of the incubator.. We will be using a solenoid valve and a NDIR CO2 sensor to regulate the CO2 in the environment. Heat control in the incubator, we will use a copper pipe that is connected to a heated water pump. Lastly, the frame of the device will be acrylic and all parts that connect from outside of the device will be sealed using silicone.

Methods

CO_2 Control

Previous work has determined that the MH-Z16 NDIR sensor part number SEN-000030 from Sandbox Electronics accurately measures CO_2 levels [7], and that a 100% CO_2 tank along with a relay circuit system with a solenoid valve connected to the incubator can be used to regulate carbon dioxide levels [7]. A gas permeable RKI Sensor Cover, part number 33-0172RK from RKI will cover the sensor to make it water-proof in the high-humidity environment of the incubator. Both the sensor and the solenoid valve will be attached to an arduino, which will only permit the solenoid valve to release CO_2 when the sensor detects CO_2 levels within the incubator are below 5%. Tests by the previous team have shown this to be effective over their testing period of 9 hours [7].

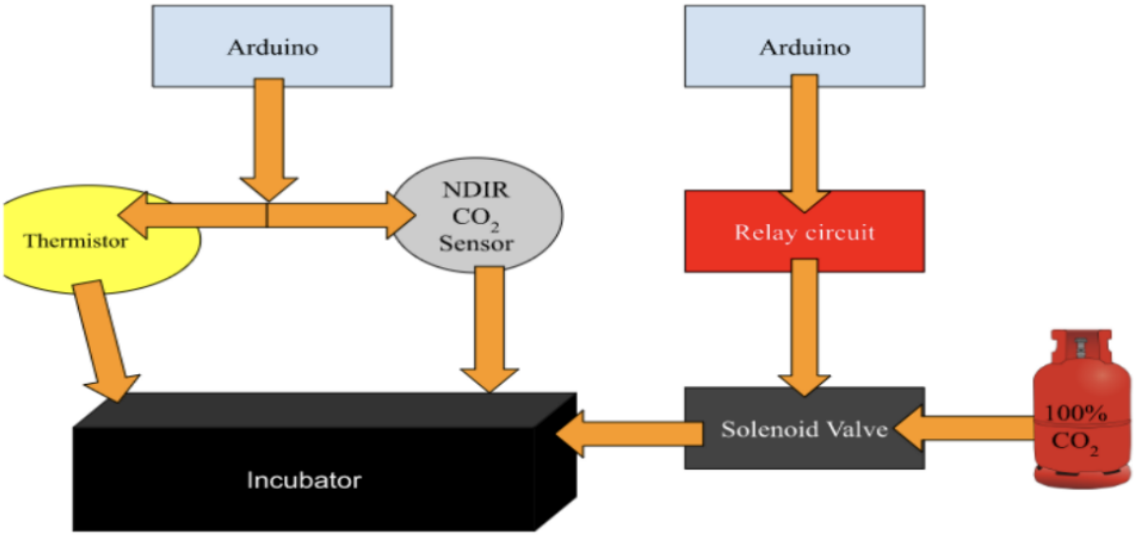

Figure 6. Diagram of CO_2 Maintenance Method by Previous Team

Temperature and Humidity Control

A thermistor will continue to be used to measure temperature and humidity levels as previous work found through a two-sample t-Test assuming equal variances that its accuracy is

comparable to that of the commercially available DHT22 humidity and temperature sensor [7]. Humidity will be calculated with the equation indicated in Figure 7. used by the previous team, which was determined to be accurate through the aforementioned t-test. High humidity was visually evident by previous teams during their tests. The method of placing a water tray at the bottom of the incubator was proven successful by previous teams, and will thus continue to be employed.

$$RH = \frac{e}{es}(100) = 100\left(\frac{e^{0.37}}{e^{((17.0625*Tc)/(243.04+Tc))}}\right)$$

RH = relative humidity e = actual vapor pressure e_s = standard vapor pressure Tc = Temperature (℃)

Figure 7. Humidity Calculation Equation

Condensation Control

The methods of the previous team's to combat condensation were ineffective as microscope visibility was still significantly impaired. Previously used methods include anti-fogging spray, wipers, and mini-fans, which were either ineffective or caused issues such as requiring manual intervention. The team will utilize two different approaches to mitigate the effects of condensation, a thin layer of water on the bottom viewing glass, and a heated ITO film attached to the glass on the top viewing surface.

Due to the laws of refraction ($n_1\sin\theta_1 = n_2\sin\theta_2$), light from our eyes that enter droplets refract off the spherical in a multitude of directions. This causes vision through the droplets to be unclear. However, a uniform layer of water would cause all light to refract in the same direction, causing vision to be clear past it. A thin layer of DI water on the bottom viewing glass where condensation forms will create a uniform, transparent layer for users to view the petri dish through. The layer of water will be deep enough for the petri-dish to be half-submerged. A rubber ring will be fixed to the bottom of the incubator with adhesive to keep the petri dish in place.

A heated ITO film will be attached to the top viewing glass with an adhesive and powered by an arduino. The formula for dew point for relative humidity levels above 50% is Td = T - ((100 - RH)/5) where Td = dew point temperature ℃, T = observed temperature ℃, RH = relative humidity (in percent) [8]. Based on the levels of temperature and humidity of the incubator, the arduino will set the temperature of the ITO film to be higher than that of the dew point temperature, preventing condensation from forming.

The plastic viewing ports of the previous design will be removed and replaced with glass, which will be fixed in place by silicone. A study found that dry glass transmitted light better than dry polyethylene, and glass with condensation transmitted light better than polyethylene with condensation [9]. Additionally, glass has better thermal conductivity and may help with temperature uniformity.

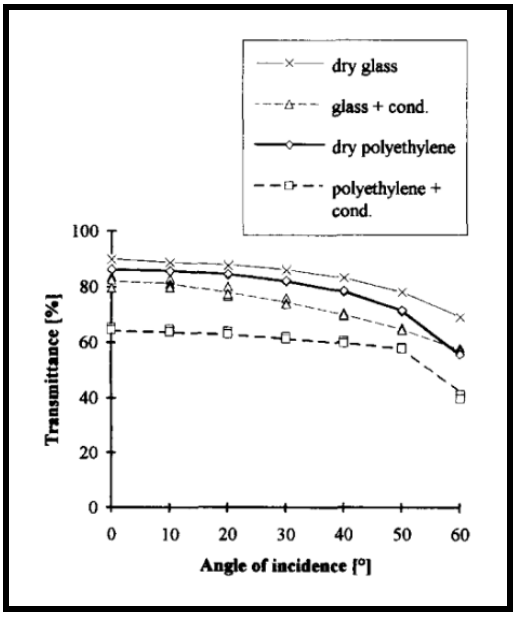

Figure 8. Light Transmission Scatter Plot of Glass and Polyethylene

Final Prototype

MH-Z16 NDIR CO_2 sensor and 100% CO_2 tank with a relay circuit system with a solenoid valve connected to the incubator to maintain necessary CO_2 conditions. There will be a water tray placed at the bottom of the incubator to maintain high humidity. A water heater along with water pipes will be used to heat the incubator, and a thermistor will be used to measure temperature and humidity. An ITO film will be placed on top of the glass of the top viewing port to reduce condensation, and a thin water film will be placed below the petri dish for the same purpose. A rubber ring will be secured to the bottom of the incubator to keep the petri dish in place. An arduino will be connected to each of the sensors along with the CO_2 valve and ITO film to maintain required conditions.

Testing

The team will test our final product's viability in our client's tissue engineering lab and primarily test the incubators ability to maintain the required internal conditions as well as the ability of the cells to proliferate within that environment.

Temperature Testing

The thermistor's ability to accurately measure temperature inside the incubator will be

tested. The goal being to maintain an internal temperature of 37° C ± 0.5°C. The thermistor will be calibrated with the resistance values provided on the Arduino website. Once the thermistor was calibrated, the accuracy of the thermistor will be tested. The incubator would be run in normal conditions with the thermistor recording temperature via the Arduino IDE and a secondary digital thermometer to compare against. The thermistor will be set up to record temperature every 5 seconds for 10 minutes and it must record temperatures within 1°C in order to pass.

CO2 Testing

The NDIR CO2 sensor's ability to record if the incubator maintains the specified internal environment of 5% CO2 ± 0.5%. The CO2 sensor testing protocol will be the same as the previous groups from Spring 2022 [10]. Since the code will now be changed from hard coding the solenoid valve to open for a specific amount of time, to the valve being fluidly opened and closed based on the internal reading of the NDIR CO2 sensor.

Optical Testing

Optical testing will consist of a protocol that will quantitatively and qualitatively measure the optical clarity of the glass under regular use. For the quantitative test, the incubator will be run normally with a cell culture present and first the incubator will run for 5 minutes to allow any condensation to build up and then a image will be taken through the glass of the cell culture, following that, a image will be taken without the top cover on in order to establish a control. These images then will be imported into imageJ and the cells will be counted in both, ideally there should be the same number of cells in both. Qualitative testing will be done on the same images taken and each group member will rank the clarity of the glass on a scale of 1-10, the control being a 10. With this protocol, the clarity of the glass can effectively be tested. Successful values will be ±5% on the cell count and any score above 7.5 for the qualitative testing. If this criteria is met, the glass is fit for use in the incubator.

Incubator Temperature Uniformity Testing

Temperature uniformity testing will begin by designing a new lid which has added holes in order to put a digital temperature probe through. The incubator will be run in normal conditions, but now the probe will be placed through each hole and record the temperature at different places inside the incubator. With this data, a heat map can be created and will convey the uniformity of temperature inside the incubator. Ideally the heat map should read 37°C in each spot.

Cell Proliferation Testing

Cell proliferation testing will be conducted by comparing the designed incubator to a traditional one. Cell cultures of the same type will be placed in the team's incubator and in the control traditional incubator. The cells will be allowed to proliferate for 5 days and inspected every 24 hours with an inverting microscope in the tissue engineering lab. Images will be captured of each culture at each time point and will be imported to ImageJ to quantify the proliferation. A successful test would be equal proliferation in both cell cultures.

Results

The team has not begun testing yet. When the final design is fabricated, testing will be done. The team anticipates performing a number of tests to determine the reliability and accuracy of the design and how well it is able to maintain the specified conditions.

Discussion

Based on the design matrix, it is shown that the layer of water design is the best design based on the criteria, with the ITO film design following very closely. Due to the similarity of the score, the team will focus on fabricating a design with glass windows, layer of water + rubber ring on the bottom window and ITO film on the top window. With the selected design, the team has the opportunity to pivot to design 2 in Table 1 in case the layer of water design becomes too difficult to fabricate. Upon completion of the prototype, the team will begin testing in order to ensure they meet the client's requirements. If the device meets the requirements for the temperature testing, CO2 testing, optical testing, and temperature uniformity testing, then it will be allowed to be tested with live cell cultures. If the design is proven successful, it will ultimately empower a broader range of researchers, allowing them access to essential incubation technology to power their research and help develop our understanding of cellular biology, pathology, and treatment development. However, ethical concerns also center on making sure that these widely available incubation systems are used ethically and in a safe and high-quality manner. To avoid any compromises in research quality, researchers and developers must uphold strict criteria. There is a moral need to present accurate, trustworthy findings, particularly when those findings have the potential to have an impact on industries like health, biology, and drug development.

Conclusions

Conclusion

Incubators that are currently on the market are either expensive, too large, or don't possess an efficient way to combat condensation on the viewing surfaces. The team will focus on improving the previous year's design by implementing various anti-condensation features. The team's final design will accomplish this by using ITO to heat the top viewing surface and having a thin film of water covering the bottom surface. By doing this, the team hopes to both maximize visibility while also maintaining a stable environment for the culture proliferation.

Future Work

Future work for the team will consist largely of applying solutions to issues of condensations and proceeding with optical testing. Given successful results, the team will move on to temperature, CO_2, incubator temperature uniformity, and cell proliferation testing to confirm the viability of the already existing mechanisms. We also intend on eventually switching the heating mechanism out for a non-water based heating method and potentially creating a box to store wires in to reduce clutter.

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Appendix

Appendix I - Project PDS

Product Design Specifications: Microscope cell culture incubator

Client:	Dr. John Puccinelli
Advisor:	Dr. Joshua Brockman
Team:	Rishi Mereddy *(Leader)*
	Cherry Qui *(Communicator)*
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Date:	September 22nd, 2023

Problem Statement

This project team serves to develop a low cost cell culture incubation chamber that is compatible with an inverted microscope and capable of live cell imaging. This incubation chamber must be able to maintain an internal environment of 37 C, 5% CO2, and 95-100% humidity over a long duration of time, without compromising the integrity of the microscope's optics or functionality. Special consideration should be taken to maintain even heating and humidity across the chamber as gradients can result in evaporation from low volume cultures such as microfluidic devices. Current commercially available systems are prone to these issues and are extremely expensive. Commercial systems also tend to be large and enclose the entire microscope making it difficult to assemble and remove and between uses. Because of their size, they also hinder use of the microscope in general.

Client Requirements

- No condensation can form on the viewing screen of the incubator
- Cannot hinder the optics of the microscope
- Must be able to be cleaned with ethanol
- Make the temperature across the incubator and cell culture as uniform as possible
- Limit the amount of wires if possible

Design requirements

This device description should be followed by list of all relevant constraints, with the following list serving as a guideline. (Note: include only those relevant to your project):

1. Physical and Operational Characteristics

a. *Performance requirements*: The device must fit on the inverting microscope used by the client, have a window that allows the user to see the cell culture as well as if there was no incubating chamber there. The device must maintain an internal environment of 37℃, 5% CO2, and 95-100% humidity. Condensation build up due to humidity must be removed in order to preserve clarity for the user.

b. *Safety*: The device should operate without harming the user in any way. Some issues that we may encounter include the heat of the object surface coming into contact with the user, improper gas connections leaving CO2 gas to fill the room, and breaking of glass due to improper usage or drastic changes in temperature.

c. *Accuracy and Reliability*: The device must be able to maintain a temperature of 37°C ± 0.5°C [1] throughout the entire internal environment. The humidity must be kept above 95% humidity [2]. CO_2 levels must be 5% ± 0.1%. The incubator must be able to maintain these conditions constantly for at least two weeks. The device must also be able to reach these conditions after the incubator has been opened and exposed to the external environment within five minutes of interruption.

d. *Life in Service*: The device should be able to sustain two weeks of active use with a viable cell culture. This includes humidity, CO_2 levels, and temperature that are all within the optimal range. Optimal use will be for one week at a time as part of a tissue engineering lab.

e. *Shelf Life*: The shelf life of this device will be ten years.

f. *Operating Environment*: The device will be used in a clean room and there are no adverse effects caused by this environment. The incubation chamber will contain internal conditions of 37 °C, 5% CO2, and 95-100% humidity over a long duration of time.

g. *Ergonomics*: This device is being reinvented in part due to the lack of ergonomics for devices that are currently on the market. In the other devices, our client described difficulties in setting up the microscope to work with the cell cultures despite being experienced with microscope usage. Therefore, our device must function in such a way that it is easy to take on and off of the microscope while still maintaining its primary function. The device must also

be easy to set up and start the process so that it can be done by a user with minimal experience [3].

h. *Size*: The device must have a maximum size of 310x300x45mm [4]. This size constraint will allow the device to effectively interface with the current set up used by the client. In general, this device needs to fit with all inverting microscopes.

i. *Weight*: The device must have a maximum weight of 30kg [4]. This weight will allow for the device to be put on the microscope without damaging it.

j. *Materials*: The device may use stainless steel as its core and incorporate copper wirings as a variety of sensors. The device may use a water heater as its heating source and may thus also use a water pump.

k. *Aesthetics, Appearance, and Finish*: The device may look rectangular in shape, with a water heater pipe running along its perimeter. The device may be filled with water, with a glass window for the microscope to see through.

2. Production Characteristics

a. *Quantity*: Only one device is necessary. However, two would be preferable for the tissue engineering class it is designed for. The device should ideally be able to be produced at a larger scale if required.

b. *Target Product Cost*: The target cost for the whole project is under $100. The goal is to utilize other models, previous prototypes, and used sensors to minimize cost so that we can effectively accomplish the tasks we set out to accomplish. It is currently estimated by the client that most of the budget for this project will be allocated to sensors and the devices needed to make use of the sensor functions.

3. Miscellaneous

a. *Standards and Specifications*:

i. Product should follow the standards and regulations set by the FDA in CFR Title 21

1. The incubator must have multiple chambers or compartments filled with water in which controlled environmental conditions, particularly temperature, are maintained [5].
2. As a class one medical device, the device is exempt from premarket notification procedures and good manufacturing practice requirements [5].

b. *Customer*: The primary customer is John Puccinelli, professor at UW-Madison using this for the education of future BMEs. It may also be used by other professors and researchers that are looking for a more affordable option for a microscope cell culture incubator.

c. *Patient-related concerns*: It is recommended that the incubator be sterilized with ethanol occasionally, approximately one or two times a month [6]. There is no storage of patient data that must be safeguarded for confidentiality. The main concern of the patient is the accuracy of the temperature, CO_2, and humidity levels.

d. *Competition*:

i. Current inverted microscope incubators and standard incubators are priced from around $500-$40000. These prices are costly in comparison to the client's demands. [4]

ii. Previous UW-Madison BME design team designed an incubator with a copper tube heating element that had water flowing through the copper tube. The design also had a solenoid valve to manage the CO2 levels. One main issue with the design was the humidity impaired visibility of the microscope.

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