Microscopic Cell Culture Incubator
Preliminary Report

BME 200/300 Design
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Abstract

The team was tasked with creating and testing a cell culture incubator that will maintain a specific internal environment while being compatible with an inverted microscope. The internal environment must be 37°C, 95%+ humidity, and contain 5% CO_2 in the air. There are current designs on the market that meet this criteria, but the inverted microscope is encapsulated into the incubator making it bulky and inconvenient to disassemble. The team is going to design a cell culture incubator that will be portable and small enough to fit on the inverted microscope stage, allowing the user to view live cells inside of the incubator. The incubator will include a heated water pump and CO_2 pump in order to reach the clients criteria. Transparency, heating, and insulation testing will be conducted on various materials to find the optimal combination for the incubator.

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Body of Report

I. Introduction

A cell culture is a commonly practiced laboratory method for the use of studying cell biology, replicating disease mechanisms, and investigating drug compounds [1]. Due to the use of live cells during this process, incubators are necessary to keep the cells viable for the amount of time being studied. Incubators allow for live cell growth because they maintain a highly regulated internal environment of 37℃, 5% CO_2, and 95% humidity, without compromising the integrity of the microscope. The COVID-19 pandemic has allowed for the CO_2 incubator market to increase 7.69% with an estimated market growth acceleration of 8% over the next decade [2]. Major disadvantages of current commercially available systems are that they tend to be large and bulky enclosing the entirety of the microscope making it difficult to assemble and remove between uses, while also hindering the use of the microscope in general, and they are often expensive; Fisher Scientific's Enviro-Genie cell incubator is priced at $6,510.68 [3]. This project will focus on developing a low cost cell culture incubator that allows for interchangeable culture plates, is compatible with an inverted microscope, allows for easy disinfection, and is capable of live cell imaging via maintenance of the internal environment needed for cell growth.

II. Background

Cell Cultures in Lab

Cell cultures are mainly used in the study of cell biology due to their ability to easily manipulate genes, molecular pathways, and culture systems to remove interfering genetic and environmental variables [4]. Cell cultures follow BioSafety Level 2 [5], which describes the safety procedures for working in a lab that can be associated with human diseases, and any incubators being used in conjunction with cell cultures must follow ISO Class 5 air quality standards [6]. Cell cultures have the ability to work with three different cell types: primary, transformed, and self-renewing cells. Primary cells are directly isolated from human tissue. Transformed cells are those that can be generated naturally with changes to the genetic code, or genetically manipulated. Self-renewing cells are cells that carry the ability to differentiate into a variety of other cell types with long-term maintenance in vitro. An example of self-renewing cells are embryonic stem cells [1].

Incubators used in cell cultures have to maintain a very stable microenvironment and can achieve this via regulated temperature, CO_2, O_2, and pH levels. Controlling these factors is critical for the viability and growth of the cultured cells, as the incubator is aiming to replicate the cells' natural conditions (37℃ with a pH of 7.2-7.4) [7]. CO_2 is needed as a buffer to help with the pH along with a culture medium. The medium most commonly used is a Basal medium, with occasional serums added (such as fetal bovine serum), which controls the physicochemical properties of the cell cultures pH and cellular osmotic pressure [1].

Incubator Types

There are two types of commonly used methods to maintain temperature in industry incubators. Many employ the electric coils method which tends to give off heat through metal coils that surround the body of the incubator, programmed to the desired temperature. The other method is the water-jacketed incubators which use a controlled circulating water bath cabinet around the body of the incubator for even heating throughout the entirety of the chamber.

Clinical Significance

There is a significant need for live cells to be cultured via the assistance of an incubator. Pharmaceutical companies often use these methods for drug development and testing as live cell imaging can be used to screen chemicals, cosmetics, and other drug components for their efficacy [8]. Pharmaceutical companies can also access the drug cytotoxicity in different cell types. Virology and vaccine products benefit from live cell cultures as it can be used to study viruses in order to make new vaccines, such as in the product of the SARS-COVID19 vaccine [1]. Embryonic stem cells are widely studied for their regeneration properties due to cell cultures and genetic engineering/gene therapy using cultures to study the expression of specific genes and the impact they have on cells in the body.

Client

The client for the Microscopic Cell Culture Incubator is Dr. John Puccinelli, an undergraduate advisor and professor in the Department of Biomedical Engineering at the University of Wisconsin-Madison. The client will be using this product for educational purposes in tissue labs for up to a week at a time.

Product Design Specifications

The client has asked the team to create an incubation chamber that must be able to maintain an internal environment of 37℃ ± 0.5°C, 5% ± 0.1% CO_2, and 95-100% humidity with even heating and humidity across the chamber. The incubator must also fit on an inverted microscope stand (roughly 310x300mm) without interfering with the microscope's optics and functionality. The aim for this project is to be able to make a device that is easily assembled/disassembled, disinfected, and can be moved between uses. The market for this product is teaching labs, but if more successful, could be marketed towards other laboratories and pharmaceutical companies. For more information, see the Full PDS in Appendix A.

III. **Preliminary Designs**

Design #1 Past Project Refurbished

This semester is the sixth semester a group has worked on this project for the client, however Figure 1 displays a past project that may work with more alterations and/or improvements [9]. No group has been successful at fabricating a fully functional microscope cell culture incubator. For this reason, continuing to work on this design to further test the product, improve materials, and fix coding errors regarding the sensors was a realistic option. Every previous design involved a rectangular box for the incubation chamber. The design also included a glass top that minimized optical impairment and allowed the incubator to go through sterilizations while extruding less heat loss. The bottom part of the chamber had a transparent heating element. The CO_2 input tube was linked on both sides of the chamber [10]. Lastly, sensors that controlled CO_2, temperature, and humidity were connected to an Arduino microcontroller. The disadvantages of this design were finding quality materials that could keep CO_2 levels and temperatures constant while being within a low-cost budget.

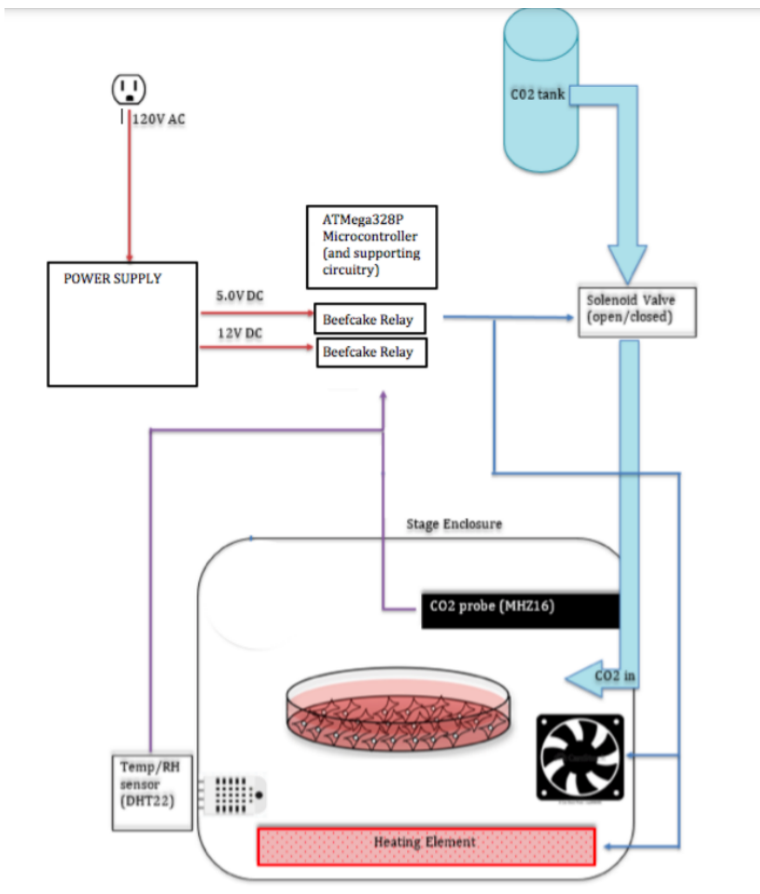


***Figure 1**: Past BME Design project schematic for incubator design*

Design #2 Heated Water Pump Incubator

The heated water pump incubator (Figure 2) will consist of an outer and inner box. The inner box will be where the cell plate is placed and stabilized. There will be transparent glass on the top and bottom of the cell culture incubator to incorporate the inverted microscope. Design #2 received its name based on the heating mechanism used in this incubator. A conducting metal tube will be wrapped around the inside of the incubator and connected to a heated water pump that will be set to 37°C. The inside of the incubator will be filled with water, submerging the metal tube, allowing the internal environment to be heated by conduction as well as increase the humidity to 95%+. The incubator box will also include a tube connector to allow CO_2 gas to be pumped in. Lastly, a separate box will be placed inside the incubator to allow for wiring and sensors to be inside the internal environment. The sensors will be connected to an Arduino microcontroller where temperature, humidity, and CO_2 levels will be collected and analyzed.

Item NO.	Item Description	Dimensions (mm)	QTY.
1	Top glass plate	250 x 200 x 5	1
2	Sealed glass plate holder	260 x 210 x 6	1
3	Metal tube for water	d = 7.16	1
4	Outer box of incubator	250 x 200 x 28	1
5	Inner box of incubator to hold cell plate	140 x 96 x 18	1
6	Lower glass plate	250 x 200 x 5	1

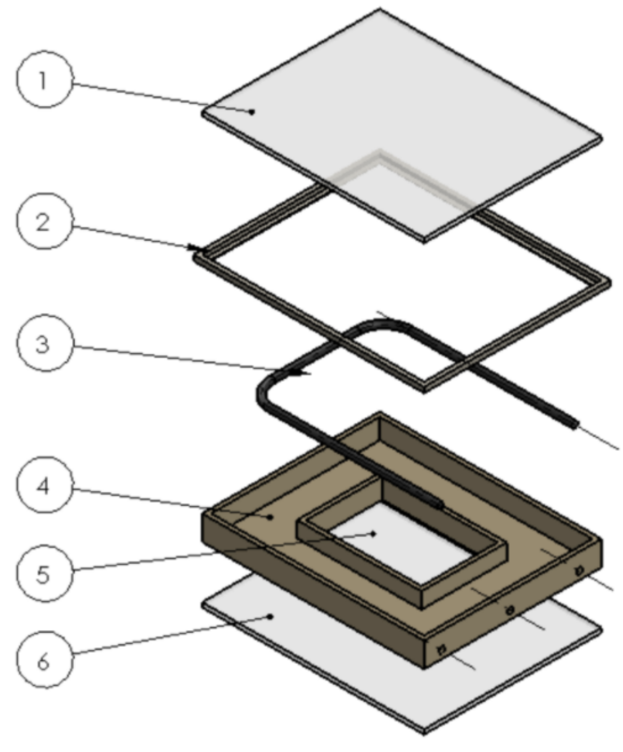


***Figure 2**: Exploded view of SOLIDWORKS drawing of heated water pump with item descriptions*[1]

[1] See Appendix B for SOLIDWORKS Drawing

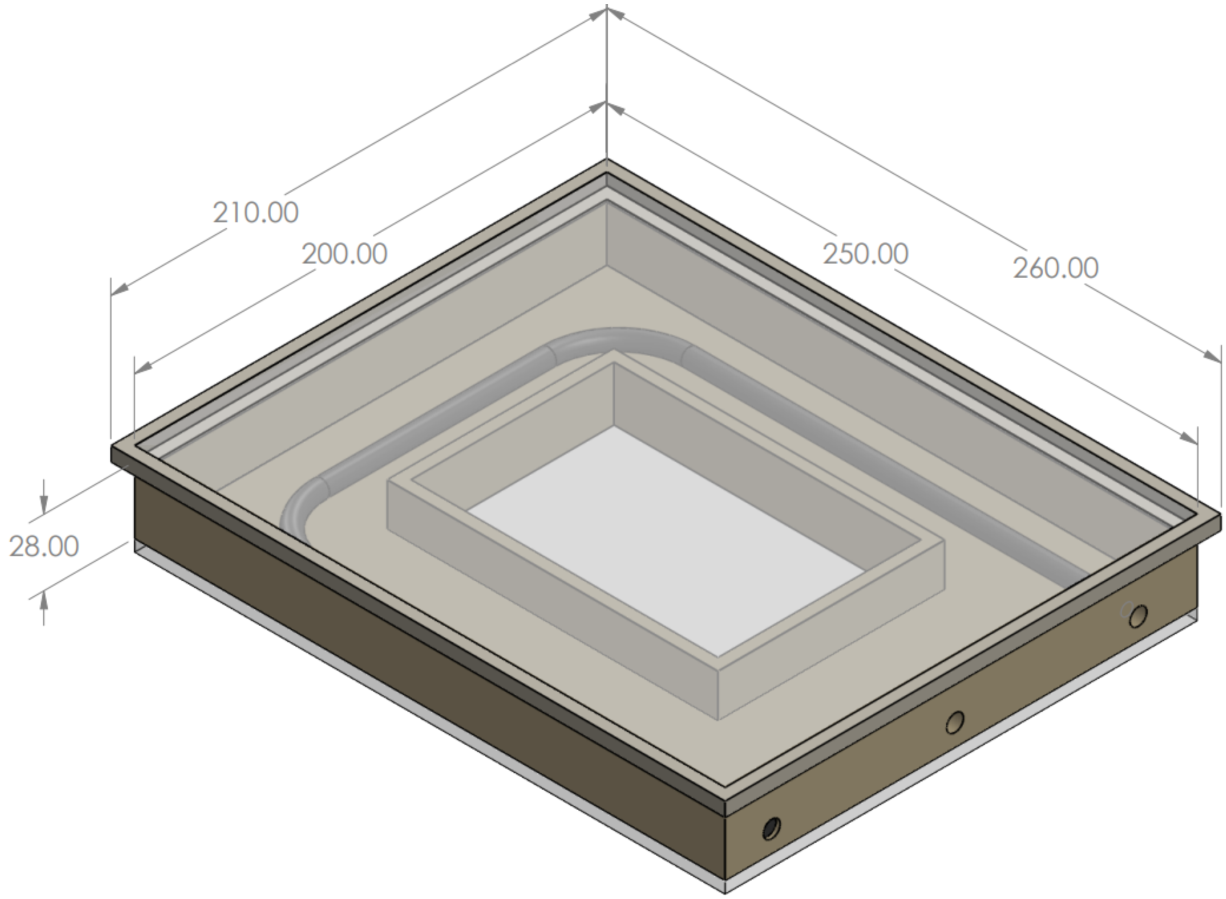


***Figure 3**: Collapsed view of Heated water pump incubator design with dimensions in mm.*

Design #3 Shelving Incubator

The shelving incubator design (Figure 4) utilized a multi shelf system that could hold multiple different well plates within it. A door panel would most likely be placed to contain the well plates (an opaque or glass material) that would serve as a simple observation or containment layer. Next, each shelf would be covered by sealed observation tops that would allow the user to observe well plates without breaking their internal atmosphere. Each shelf would be capable of sliding out of the incubator for further inspection. There would also be a track along the sides of the incubator that would allow for movement around a microscope. As a whole, this device would succeed in both observation, growth, and protection of multiple well plates for complex research purposes.

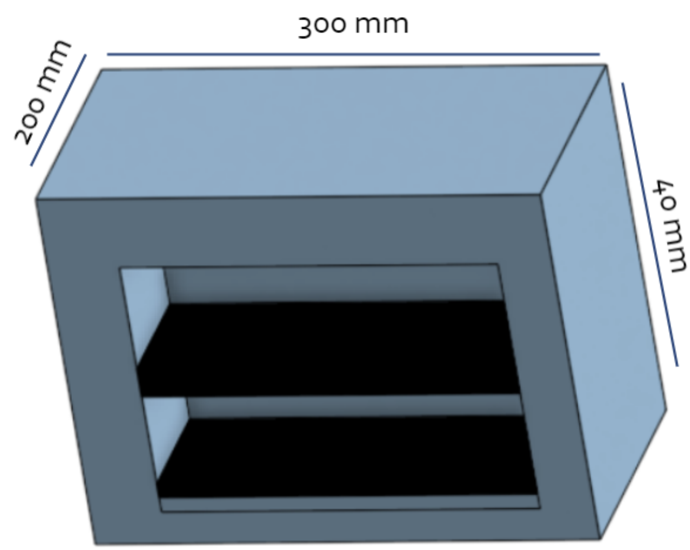
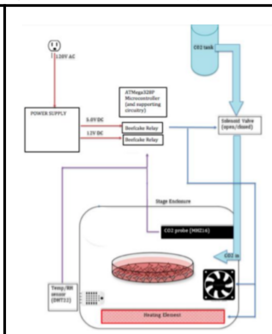
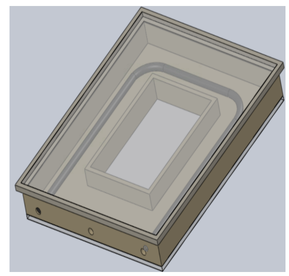
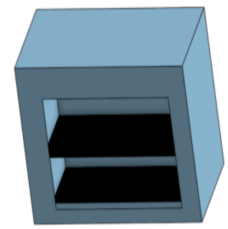


***Figure 4**: Shelving Incubator Design #3*

IV. Preliminary Design Evaluation

Design Matrix

***Table 1**: Design Matrix with all methods scored on internal environment maintenance, microscope compatibility, accuracy and reliability, ergonomics, cost, life in service, and safety.*

		 [11]					
		Past Project Refurbished		**Heated Water Pump Incubator**		**Shelving Incubator**	
Criteria	Weight	Score (10 Max)	Weighted Score	Score (10 Max)	Weighted Score	Score (10 Max)	Weighted Score
Internal Environment	25	9	23	7	18	5	13
Microscope Compatibility	20	10	20	10	20	10	20
Accuracy and Reliability	20	7	14	8	16	4	8
Ergonomics	15	5	8	8	12	4	6
Cost	10	2	2	4	4	3	3
Life in Service	5	10	5	10	5	10	5
Safety	5	10	5	10	5	10	5
Sum	**100**	**Sum**	**77**	**Sum**	**80**	**Sum**	**60**

Scoring Criteria

Internal Environment: The internal environment maintenance was weighted the highest due to the client's request that these standards be met as close to industry standards as possible, with some leeway provided the internal environment is viable with live cells. Since live cells are being used in the cell cultures, the incubator must be able to meet 37℃ ± 0.5°C, 5% ± 0.1% CO_2, and 95-100% humidity, in order to survive for the duration of the teaching lab.

Microscope Compatibility: Many currently available incubators are not compatible with inverted microscopes as a result of their size and price. The team needed to design an incubator to fit onto an inverted microscope stand, roughly 310x300mm. The team's current designs are much smaller than current incubators. The final product must not interfere with the microscope's optics, allowing for transparency for top and bottom viewing of the cells, along with a maximum thickness of 32.40mm so that the product does not come in contact with the lens of the scope.

Accuracy and Reliability: Due to the importance of the internal environment for cell growth, the incubator must be able to regulate the conditions within a small margin of error. The accuracy and reliability of the device will be evaluated and monitored using temperature, humidity, and CO_2 sensors connected to the device via an Arduino microcontroller.

Ergonomics: The device must be within a size and weight that the average user can safely handle and move with ease.

Cost: The total cost of the product has a budget of $100, although the client has said that more funds may be provided based on the success of the initial prototype.

Life in Service: The final product will need to be used for one week out of the semester in the client's teaching lab. The shelf life of this product has a minimum of 10 years.

Safety: The product needs to adhere to FDA and OSHA standards and regulations [12][13]. Due to the use of tissue cells, the incubator must abide by Biohazard Safety Level 2 and ISO Class 5 air quality standards [14][15].

Proposed Final Design

The team chose to move forward with the second design, The Heated Water Pump Incubator. This design fits the clients needs the best because it will produce an accurate and reliable internal environment. The use of a heated water pump containing the desired temperature of 37°C throughout a smaller space not only ensures homogeneous temperature, but also helps maintain humidity. The design is also relatively small allowing for easy assembly, ease of use, and can be readily disassembled and interchanged for another type of cell culture. The Heated Water Pump Design was also the lowest in cost, with materials from past semesters being used to ensure that the target budget is not exceeded. Overall, this method won over the other two due to its compactness, accuracy and reliability, and low production cost.

V. **Fabrication/Development Process**

Materials

Arduino Materials

The device will be made with an arduino sensing unit for the purpose of measuring CO_2 levels and temperature during incubator usage. The materials needed for the arduino circuit are an Arduino Uno, and Alphanumeric LCD, Texas Instruments General Quad Op-Amp, a temperature sensor with thermistor VMA-320, MQ-135, SD card logging shield VMA-304, and a pack of resistors, specifically 10k ohm, 1k ohm, and 221 ohm.

Incubator Materials

The incubator part of the cell culture will be made using 3D printing materials, either ABS or Black, at the University of Wisconsin Makerspace. The team will also be using Frosted Polycarbonate roofing sheets and/or High Transparanted PC Lexan Polycarbonate sheets for the top and bottom of the incubator. The polycarbonate glass with the best tested optical properties will be chosen for the final design. Tubing inside of the incubator will be a cheap, conducting metal, compatible with higher temperatures and water. Steel will be most likely used. The team may also use a waterproof silica aerogel mat inside the water box of the incubator for the purpose of insulation.

Methods

The fabrication of the cell culture incubator will begin with 3D printing the box at the University of Wisconsin Makerspace. Next the transparent glass will be cut to the correct dimensions in order to fit underneath, as well as on top of the 3D printed box. Before the glass is inserted into the crown of the top of the incubator, a thin layer of rubber will line the incubator. This will help prevent leaking through cracks from the internal environment to the external as well as removal of the glass to exchange cell plates. The bottom glass will be glued to the bottom of the incubator box, not allowing any leaks to occur. Next, the metal tube will be bent to 90 degrees twice in order to wrap around the inside of the incubator. The tubing will be inserted through holes in the side of the box in order for the heated water pump to be fastened to the tube and ultimately heat the incubator. Furthermore, the CO_2 gas pump and gauge will be connected and inserted into the incubator via another hole in the side of the box to provide the 5% CO_2 levels. Lastly, one last hole will be drilled into the incubator in order to allow wiring from internal sensors to be connected to an Arduino Microcontroller and computer to produce live data. All of the holes will be surrounded and sealed with glue or insulation to prevent any leakage of the internal environment.

The Arduino sensing unit will be developed using the materials recommended by the Arduino website in order to build a basic circuit that has both temperature and CO_2 testing. The team will use the sample code provided by Arduino with some minor modifications in order to also output the humidity readings.

Final Prototype

Final prototype has not been fabricated yet.

Testing

The team will be testing the accuracy of the proposed design in the client's cell culture lab in order to determine if the internal environment is stable and if the microscope optics are not corrupted.

Internal Environment Testing

The team will be employing sensors inside the incubator in order to measure the internal CO_2, temperature, and humidity. For CO_2, the tank employed in the current lab has a sensor to check the CO_2 levels, but a CO_2 sensor will be placed inside the incubator as well. The measurement of CO_2 recorded by the Arduino sensors should be within 2% of the pressure gage on the CO_2 tank. The measurements of the humidity and temperature will be obtained by an AOSONG DHT22 Arduino compatible sensor. The team will test to make sure that the code and the AOSONG are working correctly by first measuring the temperature and humidity of the working environment to gauge if they are both working as expected. Afterwards the team will measure the temperature inside the incubator with a thermometer and the sensor. The test will be considered successful if the sensor value is within 2℃ of the thermometer temperature.

Optical Testing

The team will test two types of glass, Frosted Polycarbonate Roofing Sheet Transparent Thermal Insulation sheets and High Transparent Lexan Polycarbonate sheets to determine which best matches the optical properties of well plates. Well Plates have a gloss percentage of 75-90, a haze percentage of 11, and a transparency percentage of 85-90 [16]. The team has researched that the transparency percentage of polycarbonate is 88-89 and the haze% is 1 [17]. The team will determine through live cell imaging, either by fluorescent microscopy or bright field microscopy depending on the client's cell cultures, whether 88% transparency is acceptable.

Recovery Testing

The team will test the recovery time of the incubator after it has been opened by timing how long it takes for the incubator to return to performance conditions (37°C, 5% CO_2, and >95% humidity). The maximum recovery time should not exceed five minutes after a 30 second exposure to the external environment.

VI. **Results (Future Work for Now)**

Now that a final design has been proposed, the prototyping and testing stages of the project can begin. The group plans to break into three teams Materials, Arduino Coding, and Incubator Fabrication which will each work independently to streamline the design process. The materials group will determine and purchase necessary materials. The incubator fabrication group will begin prototyping and creating testing protocols. The arduino coding group will begin writing and testing their code for the sensors.

VII. Discussion

Discussion will be written once results have been collected.

VIII. Conclusion

The client is in search of a microscopic cell culture incubator compatible with an inverted microscope that is lightweight, maintains a stable internal environment, and is cost effective for the purpose of using it in a teaching lab during the semester. The team has proposed a design that is lightweight, cost-effective, and able to maintain the desired internal environment. The proposed final design will include a metal tube that is wrapped around the inside of the incubator and connected to a heated water pump that will regulate the internal incubator conditions and keep them at their optimal values. The incubator box will also contain a hole for CO_2 to be pumped in. Additionally, it will include a separate box to house sensors to monitor and modulate temperature, humidity, and CO_2 levels to ensure that the device meets the design requirements. Moving forward, the team will begin the prototyping and purchasing stages of the design process, before moving onto the testing phase.

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X. <u>Appendix</u>

Appendix A: Product Design Specifications (PDS)

Function: Develop a low cost cell culture incubation chamber with interchangeable culture plates that is compatible with an inverted microscope and capable of live cell imaging.

Client requirements:

- Incubation chamber must be able to maintain an internal environment of 37℃, 5% CO_2, and 95-100% humidity
- Microscope's optics and functionality must not be damaged
- Maintain even heating and humidity across the chamber
- Create device that stays within a budget of $100
- Ensure that the device can be easily assembled and removed between uses

Design requirements:

1. Physical and Operational Characteristics

a. ***Performance requirements:*** The device must be able to sit on a microscope stand, be transparent on the top and bottom to allow for optical visualization with an inverted microscope, and maintain an internal environment of 37℃, 5% CO_2, and 95-100% humidity.

b. ***Safety:*** The incubator and the cell culture environment must be in corporation with BioSafety Level 1 Standards [1]. Any material and electrical or mechanical machinery must be sterilizable and waterproof.

c. ***Accuracy and Reliability:*** The device must be able to maintain a temperature of 37°C ± 0.05°C throughout the entire internal environment. The humidity must be kept above 95% humidity. CO_2 levels must be 5% ± 0.1%. The incubator must be able to maintain these conditions for extended periods of time and be able to reach these conditions after the incubator has been opened and exposed to the external environment in an efficient manner.

d. ***Life in Service:*** The device must be able to be used for two weeks, but optimal usage will occur for one week at a time for teaching purposes in the client's tissue lab.

e. ***Shelf Life:*** The shelf life of this product should be ten years.

f. ***Operating Environment:*** The operating environment is a clean room. The incubation chamber must be able to maintain an internal environment of 37°C, 5% CO_2, and 95-100% humidity over a long duration of time, without compromising the integrity of the microscope's optics or functionality. Even heating and humidity across the chamber must be maintained to ensure that evaporation does not occur.

g. ***Ergonomics:*** The device should be portable in that one should be able to carry and store the device easily. Wires should not be hanging freely out of the device, and it should be easy to pick up and put away when needed.
h. ***Size:*** The size constraints for this device are that it must sit on the microscope stage and hold a well plate that also doesn't interfere with the optics or functionality of the microscope. It would be ideal if all sides are transparent, but it is a requirement that the bottom and top are transparent. Overall, the product must be compatible with an inverted microscope.
i. ***Weight:*** There are no specific weight requirements. However, minimizing weight would be ideal to promote incubator mobility and usability.
j. ***Materials:*** There are no specific materials that are required for development of this device. However, it is important to examine different material properties to determine which materials hold heat effectively and have a transparent appearance.
k. ***Aesthetics, Appearance, and Finish:*** The client does not have a preference in color. Well plates are clear, black (to stop contamination), and white (to increase light). Using materials that would block out external light sources would be ideal, but this is not a requirement for the device. Finish should exclude messy elements, such as long wires, and be transparent on both the top and bottom.

2. Production Characteristics:

a. ***Quantity:*** Only one device is necessary to produce, but ideally, it would have the capacity to be produced on a larger scale to be used repeatedly in the teaching labs.
b. ***Target Product Cost:*** The target product cost for this device is $100. It will be paid for via UW BME Departmental teaching funds.

3. Miscellaneous

a. ***Standards and Specifications:*** The incubator would need to adhere to the ISO 13485 regulation which outlines requirements for regulatory purposes of Medical Devices [2]. The incubator would also need to follow the FDA's Code of Federal Regulations Title 21, Volume 8 where it outlines the requirements for Cell and Tissue Culture products [3].
b. ***Customer:*** The client, Dr. John Puccinelli, is an undergraduate advisor in the Biomedical Engineering Department at the University of Wisconsin - Madison. Dr. Puccinelli is asking for the cell culture incubator in order to amplify the teaching curriculum in his classroom environment. Having an incubator that is easy to disassemble and compatible with an inverted microscope would result in efficient classroom lessons.

c. ***Patient-related concerns:*** The accuracy of the temperature, humidity, and CO_2 concentration is of utmost concern for the client. Humidity must be 95-100%, otherwise cells will begin to dry out. Having a set temperature of 37°C will replicate optimal cellular environments. Lastly, ease of disassembly and disinfecting of the incubator was of concern.
d. ***Competition:*** There are currently multiple inverted microscopes and cell culture incubators on the market ranging from $500-$40,000 [4]. ThermoFisher, NuAire, and New Brunswick all have incubators currently on the market. ThermoFisher and NuAire are more popular as they have both direct heat and water jacketed incubators. The most popular ThermoFisher design is the Heracell VIOS 160i CO2 Incubator with Copper Interior Chambers, which has HEPA filtration for ISO Class 5 air quality and an overnight Steri-Run for total sterilization [5]. Others have also attempted to design low-cost live-cell imaging platforms using 3D printed and off the shelf components. A team of researchers from Australia were able to successfully design a portable low-cost long-term live-cell imaging platform for biomedical research and education for under $1750 [6]. This low-cost incubator also monitored and regulated temperature, CO_2, and humidity as per the parameters for successful mammalian cell culture. Past BME 200/300 design projects have attempted to build incubators for this client, but none have been completely successful.

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Appendix B: SOLIDWORKS CAD Drawing of Cell Culture Incubator

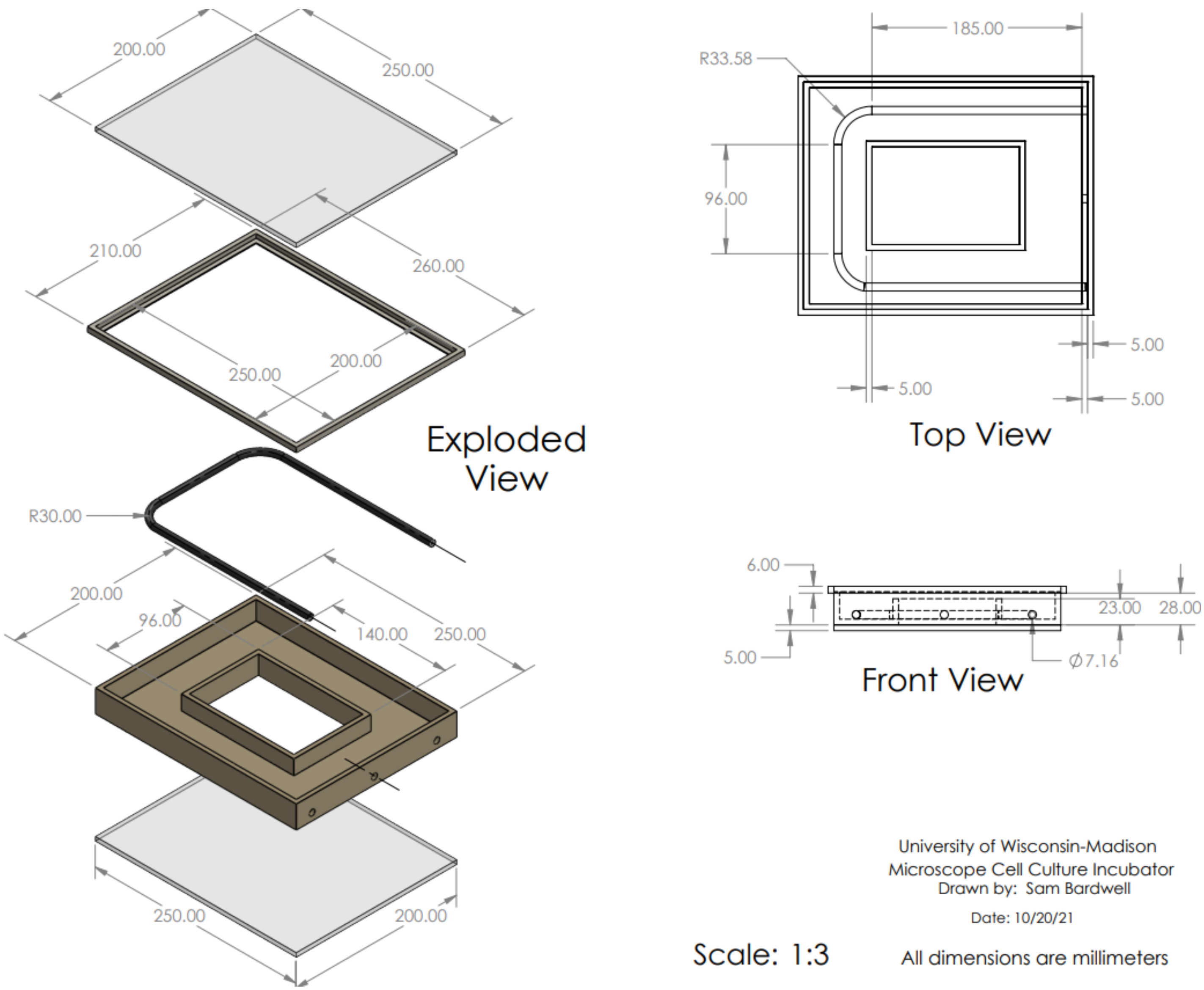

***Figure 1**: SOLIDWORKS Drawing of Design #2*