Precision Air Curtain Technology for a Dual Purpose Cell Culture Incubator-Biosafety Cabinet Enclosure

Department of Biomedical Engineering

A Major Qualifying Project to be submitted to the faculty of Worcester Polytechnic Institute in partial fulfillment of the requirements for the Degree of Bachelor of Science

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Abstract

There is a need for an environmental chamber in the cellular and tissue engineering fields that combines the characteristics of a cell culture incubator and a biosafety cabinet for long-term maintenance of viable cell populations for complex cellular printing applications and live cell imaging under sterile culture conditions. In order to meet this need, we have developed novel air curtain technology and tested its effectiveness at preserving the conditions within a standard cell culture incubator. The air curtain design was selected based mainly on its low cost. Its ability to maintain environmental conditions (temperature, humidity, and CO_2) and prevent permeability of CO_2 forced through the air curtain from outside was experimentally quantified. Our results indicate that the air curtain was able to maintain CO_2 levels and prevent mixing of extraneous CO_2. The temperature and humidity levels dropped to some degree. We explain the reasons and suggest improvements that can be incorporated, in future studies, to improve the technology.

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Chapter 1: Introduction

The next frontier in tissue engineering is the three dimensional (3D) printing of tissues and organs using cultured cells. Currently, small scale printing is performed inside a biosafety cabinet and the constructs transferred to incubators for long-term culturing. For large scale tissue/organ printing, the process can take several hours to complete. It is therefore imperative to perform printing in sterile enclosures capable of maintaining a controlled environment similar to a cell culture incubator while allowing researchers to access the printing set up as and when necessary (Calvert, 2007). In the current marketplace, there are no enclosures that meet these requirements, thus restricting the use of 3D cell printing.

In order to address this issue, Digilab, Inc. sponsored a Major Qualifying Project (MQP) at Worcester Polytechnic Institute (WPI) where a team of four students designed an enclosure that can house a cell printer, and provide the environmental conditions suitable for prolonged cell viability. Digilab, Inc. is a biotechnology company specializing in manufacturing devices for spectrometry and photonics. One of Digilab's newest products is the CellJet, a first-generation cell dispenser capable of 2D arraying of cells. It can be used for a wide range of applications, including stem cell research, oncology, cell-cell interaction studies, tissue engineering, and regenerative medicine.

The project was aimed at combining the features and convenience of a biosafety cabinet and a cell culture incubator into one enclosure that would pave the way for large scale and long term 3D cell printing without the risk of contamination or cell death. In order to perform a proof of principle experiment, we developed a novel air curtain technology and tested its effectiveness at preserving the environmental conditions inside a conventional cell culture incubator. The specific goals of the air curtain design were (1) to actively prevent contamination of the incubator from outside particulates, and (2) to ensure the preservation of the temperature, humidity, and CO_2 levels within the incubator.

The air curtain was designed to provide a constant stream of moving air across the incubator door. In order to test the effectiveness of the air curtain, the stability of temperature,

humidity and CO_2 levels and CO_2 infiltration from outside were tested. The test results and future improvements are discussed.

1.1 Needs Analysis

Digilab's CellJet printer is marketed as a 2D cell printer that uses Digilab's proprietary liquid handling technology. In order to use the printer to dispense cells, the equipment has to be housed in a laminar flow hood to provide a sterile environment. However, any cells dispensed from the cell printer are viable only for short periods of time because of inadequate control of cell culture conditions in biological safety cabinets, specifically the air flow, lack of humidity, CO_2 concentration, and temperature control. Cells used for printing are dispensed in nanoliter to microliter quantities. Due to the laminar flow of air and lack of environment control, especially humidity control, the media evaporates soon after cell dispensation and therefore the cells die within a short period of time due to dryness and hyperosmolarity. In order to provide an environment sufficient for prolonged cell viability, several environmental conditions need to be controlled, including the temperature, humidity, gas content, while maintaining sterility.

Although this project is focused on designing an enclosure for cell printing, there exist alternative applications that may benefit from this device. Specifically, live cell imaging could theoretically be performed within the enclosure if a small microscope was placed inside. Similar to cell printing, live cell imaging requires a sterile environment with controlled temperature, humidity, and CO_2 to maintain cell vitality (Frigault *et al*, 2009). Utilizing this enclosure could be a cost effective solution for some live cell imaging applications.

To address these needs, the enclosure was designed to combine the aspects of a laminar flow hood, which provided a sterile environment, with the aspects of a CO_2 cell incubator, which provided controlled temperature, humidity, and gas content. By doing this, all of the necessary environmental factors could be controlled, allowing for long-term use of the cell printer, resulting in healthy cells for experiments.

Chapter 2 – Literature Review

2.1 Physiochemical Conditions and their Influence on Cell Culture

Cell printing applications are limited because there is no standardized method for maintaining ideal environmental conditions around the cells being printed. In order to determine which environmental conditions have the greatest influence on cells, a better understanding of how different physiochemical factors affect cells is necessary. The research in this section will help determine where the greatest efforts should be focused in order to build an enclosure that achieves its objectives most efficiently and cost-effectively.

2.1.1 Temperature

Mammalian cell lines are generally cultured at temperatures between 36°C and 37°, as most mammals' body temperatures fall within that range. The exact temperature, however, can differ based on location in the body of the tissue the cells are derived from. For example, skin cells require a slightly lower temperature than muscle cells. The temperature must be precisely controlled in order to maintain optimum protein function within the cells. Temperatures too high or too low will cause proteins to denature and lose functionality. Additionally, cells are more sensitive to overheating than under heating, so measures must be taken to ensure the cells are not overheated. This is typically accounted for by setting incubation temperatures 1°C below the optimal temperature (Zhong and Yoshida, 1993).

2.1.2 Humidity

The CellJet printer deposits cells suspended in as little as 4 µL of fluid onto a substrate - a tiny volume of fluid that evaporates very rapidly. To prevent the fluid from completely drying up, it is vital that the surrounding air is nearly saturated with moisture. This effectively decreases the evaporation rate to an insignificant value, allowing the cells to retain their moisture. It is also important to prevent the surrounding air from becoming over-saturated with moisture, because the resulting condensation may accumulate on the printing deck and wash out freshly printed cells (Calvert, 2007).

2.1.3 CO_2 Concentration

The control of gas content is very important to the wellbeing and growth of cells. Carbon dioxide (CO_2) in particular can have negative effects on cells if there is too much, or

even too little. This is because the carbon dioxide content in the surrounding atmosphere can affect the pH of the cell solution. Our body contains about 5% carbon dioxide, so this is the ideal carbon dioxide content for most cell types during cell culture to maintain a neutral pH; however depending on the cell type or experiment being run, the range can vary from 4-10%. In the cell solution, carbon dioxide exists in the form of bicarbonate ions, which act as a pH buffer that allows for gas and nutrient exchange without causing pH fluctuations. As the cells release carbon dioxide and other ions, the pH of the solution can change. In response to this change, carbon dioxide is taken from, or released into, the atmosphere to maintain the equilibrium between the two. Because of this, it is important to maintain a 5% carbon dioxide atmosphere during culturing so the solution will remain at about 5% as well. While there are other options for controlling the pH of the solution, such as adding a buffering medium like sodium phosphates, these can affect cell growth (Schulz *et al*, 2012).

2.2 Means of Controlling Cell Culture Environment

This section investigates the different means of sensing and manipulating temperature, humidity, and CO_2 concentration within a confined space, as well as different methods to sterilize and subsequently maintain the sterility of the enclosure. Current cell incubator and clean room technologies are of particular interest.

2.2.1 Temperature Control

There are several options for achieving the desired temperature in cell incubators currently on the market. Most commonly, cell incubators are heated by a water jacket, forced air, or direct heat. Descriptions of these heating systems are shown below:

Water Jacket

In a water-jacketed incubator, there are two chambers. The inner chamber is where the samples being incubated are placed while the outer chamber, which surrounds the inner chamber, is filled with water. The water is heated and moves through the jacket via natural convection, providing uniform heat throughout. The heat from this water jacket radiates through to the inner chamber, providing the necessary heat for incubation. The water jacket is advantageous because of the insulating properties of water, as heat can be maintained even without power for several hours. Disadvantages include the lack of mobility associated with its

heavy weight, the amount of time required to heat the water, and difficulties with cleaning and maintenance (Triaud *et al*, 2003).

Forced Air

In a forced air incubator, a heating element is located in the incubation chamber, commonly in the rear. A blower is placed in front of the heating element to move the heated air throughout the chamber, providing uniform heat. While this method of heating is effective, the constant stream of air blowing through the chamber could pose a problem for cell culture, as the medium used could potentially evaporate more rapidly (Okken et al, 1982).

Direct Heat

Many labs are switching from water-jacketed incubators to direct heat incubators, as they are much lighter and easier to maintain. In a direct heat incubator, all six walls of the inner chamber have heating elements behind them, allowing for heat to radiate through the walls to the inner chamber. Direct heat incubators show very uniform heating and are able to heat up quicker than water-jacketed models. The lack of water in the surrounding chamber also eliminates the possibility of condensation causing problems (Triaud *et al*, 2003).

2.2.2 Humidity Control

Humidity can be controlled by several different means, but the principle behind the process includes either increasing the surface area of water in order to facilitate faster evaporation, such as wick, ultrasonic, and impeller humidifiers, heating the water to create steam, or a combination of both (forced air humidifiers). The different types of humidifiers, namely evaporative, steam, ultrasonic, impeller, and forced air humidifiers, are described in the following sections. The Environmental Protection Agency (1991) describes some of the advantages and disadvantages of the aforementioned humidifier types.

Evaporative

An evaporative humidifier has a filter that absorbs water from reservoir and provides a large surface area to evaporate from. The filter is usually made of a fabric or foam material that absorbs water up by capillary action. The advantages of such a filter are that it is self-regulating and requires no energy. Disadvantages include the filter becoming moldy slow humidity regulation, and the fact that it is not controllable. It is prone to contamination.

Steam

In a steam humidifier, water is brought to its boiling point with heating elements to create steam. Simple electric heating coils may be used to generate the required heat. Advantages of this humidifier are that it is clean, produces and extra-fine mist resulting minimal condensation, and requires only a low cost heating element. The main disadvantage is the requirement for high temperatures, which would likely necessitate a means of cooling the vapor so as not to damage cells.

Impeller

An impeller humidifier consists of a rapidly rotating disc that flings water at a diffuser generating a mist by forcing the water into small particles. This also provides more surface area for water to evaporate from. Advantages are that the system is clean, the temperature of mist can be controlled within effective range, and the mechanism is simple. One disadvantage is that condensation of mist on cell deck could be an issue. This may be alleviated by pre-heating the water.

Ultrasonic

An ultra-high frequency piezo-electric transducer is driven by an alternating current to sonicate water at high frequency in order to produce a fine mist (EPA, 1991). Similar to an impeller humidifier, this device creates a high surface area of water to facilitate more rapid evaporation. This type of humidifier has several advantage: (1) it is clean, (2) the temperature can be controlled within an effective range, (3) it is relatively cheap, (4) it is ideal for small enclosure, (5) it requires little energy, and (6) the mechanism is simple. A disadvantage may be the size of water particles generated – condensation of mist on the cell deck can pose a problem.

Forced Air

Forced air humidifiers pump hot air through a waterlogged substrate to generate moist air. This method can be very effective; however it requires moving air which can potentially harm cells. Three commercially available forced air humidifiers are drum, disc-wheel, and bypass flow-through humidifiers. They are described below.

Drum

A foam drum rotates in pan of water as hot air is forced through the drum (similar to wick humidifier), which is closed off at one end, forcing the air to pass through the wet foam drum. Advantages include low cost and inexpensive maintenance. Disadvantages include the requirement for high temperature and lack of output control.

Disc-Wheel

Water from a reservoir is pumped over a grooved plastic disc as hot air passes over it, evaporating the water to generate moist air. Advantages include low maintenance requirements, high output, and consistent efficiency. Disadvantages are that it is relatively expensive and requires high temperature.

Bypass Flow-Through

Water is pumped over a coarse, porous ceramic-coated aluminum "biscuit," through which hot air is then forced. Advantages include low maintenance requirements, adjustability, and energy efficiency. Disadvantages are that it is relatively expensive and requires high temperature.

2.2.3 CO_2 Concentration Control

Currently, there are several options for controlling carbon dioxide levels in enclosed spaces like incubators, greenhouses, and grow houses. This section reviews the most commonly used devices.

Thermal Conductivity Sensor

When the thermal conductivity sensor was introduced, it was the first method that could measure and adjust carbon dioxide concentration based on a set point. It can't, however, measure carbon dioxide directly. The thermal conductivity sensors are made up of two matched thermistors in brass housing, which are hooked up to a small electric board. These thermistors measure the thermal conductivity of the air. One is encased in a sealed chamber in the sensor head, while the other is exposed to the enclosure's environment. The two readings are compared, and the carbon dioxide reading is calculated off of the difference in the thermal conductivity readings. Seeing as this sensor cannot measure carbon dioxide directly when

temperature and humidity are not stable this method wouldn't generate accurate readings. The time required for temperature and humidity to reach the desired levels makes this option unfavorable. Furthermore, in applications in which the door is being opened, it takes time for the environment to readjust, meaning it will take even longer before carbon dioxide can be recovered (Tardy et al, 2004). An example of the thermal conductivity sensor is shown in Figure 1.

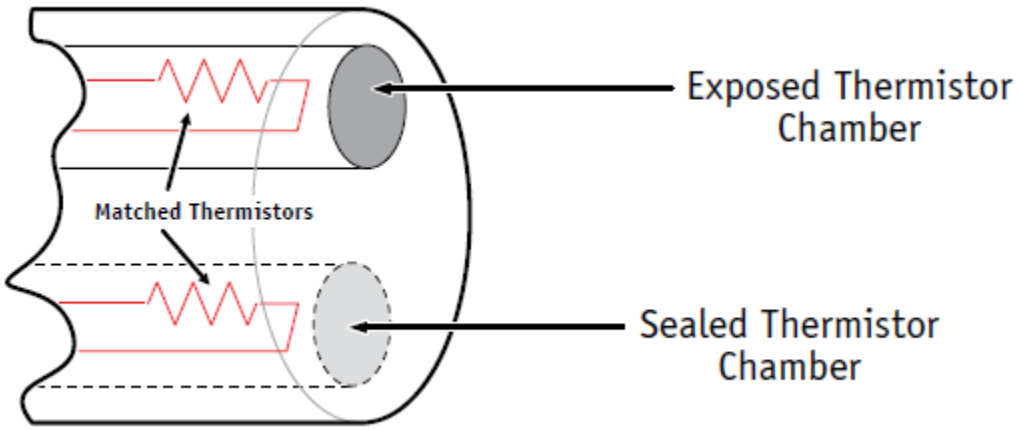

Figure 1: Thermal Conductivity Sensor

Source: www.shellab.com

Infrared Sensor

Infrared sensors are the most sophisticated means of monitoring and controlling carbon dioxide levels, because they directly measure carbon dioxide content in enclosures. The sensor uses a broad spectrum infrared light source in conjunction with a specialized sensor. This sensor reads a specific infrared wavelength that is affected by the presence of carbon dioxide. The air in the enclosure passes through a channel on the sensor located in between the infrared light and the sensor. The amount of light emitted by the light source is known, so the concentration of carbon dioxide is measured as the difference between this known quantity and the amount of light that reaches the sensor. This provides higher accuracy, a quicker recovery of lost carbon dioxide, and means this method is unaffected by changes in temperature or humidity. These properties make it ideal for applications in which doors are frequently opened, or the

environment is undergoing changes. The drawback to this method is that gradually the intensity of the light bulb fades, which leads to a weaker signal being read. This means the sensor is reading that there is an increase in carbon dioxide, when there is not. This can be remedied by either continually calibrating the sensor, or replacing the bulb every few years which can be expensive (Mayrwöger *et al*, 2010). An example of an infrared sensor is shown in Figure 2.

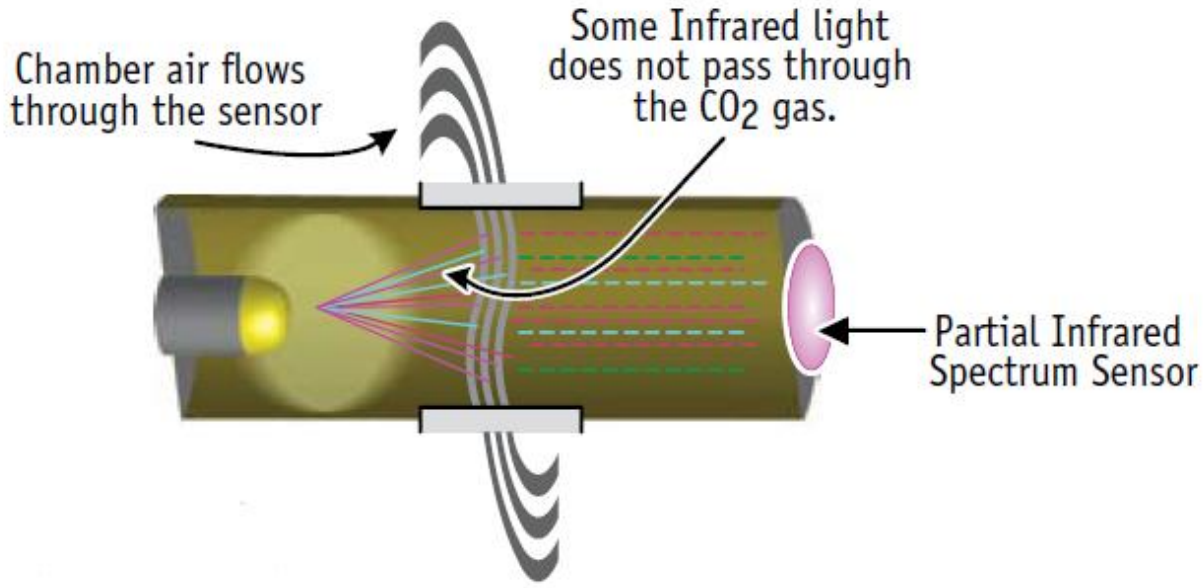

Figure 2: Infrared Sensor

Source: www.shellab.com

Figure 3: Example of Infrared Sensor

Source: www.process-worldwide.com

Dual Infrared Sensors

Dual infrared sensors are a costly option for maintaining accurate CO_2 data acquisition. This system has a second sensor that only reads a wavelength not affected by carbon dioxide. This second sensor is used to compare the spectrum reading to the intensity of the bulb, so as the bulb fades this sensor measures the reduction in total light emitted and compares it to the infrared light detected by the other sensor. This allows for the reduction in light to be compensated for in the calculation of carbon dioxide concentration. Although this eliminates the need for any maintenance on the bulb, the second sensor is costly; the bulb eventually will need to be replaced regardless (Mayrwöger *et al*, 2010). An example of a dual infrared sensor is shown in Figure 4.

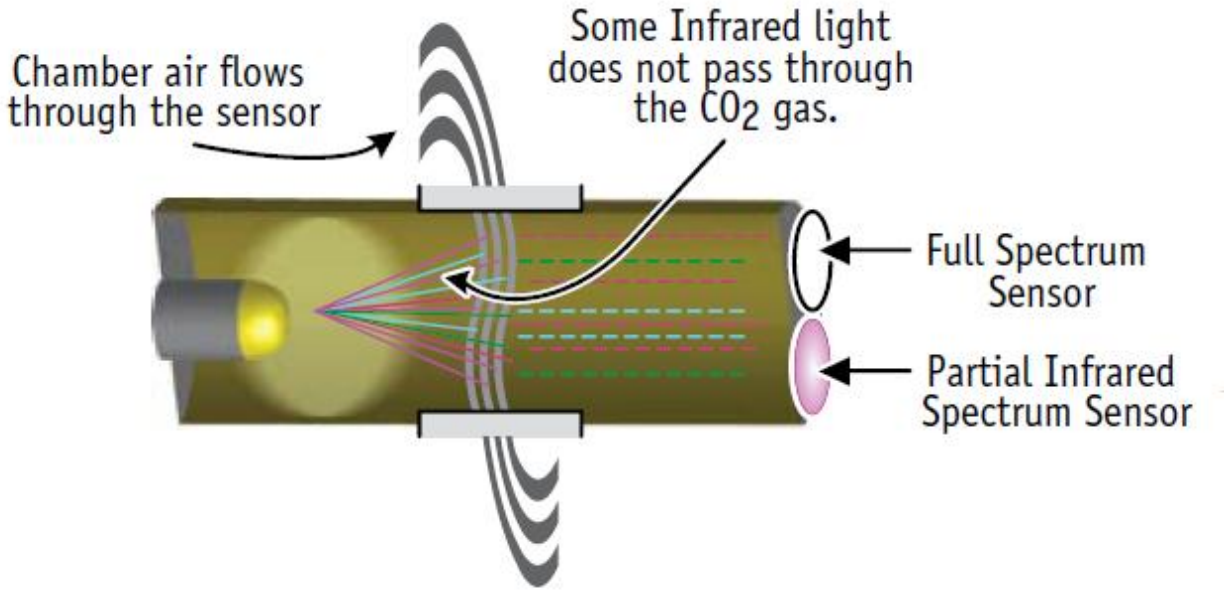

Figure 4: Dual Infrared Sensor

Source: www.shellab.com

2.2.4 Contamination Prevention

Sterility is a term used to describe the state of being free of living microorganisms. This is a very important factor for research involving cell culture because of the inherent threat of biological contamination. This occurs when unwanted biological factors invade a cell culture. Bacteria and fungi spores are the most common contaminants and they usually travel by air and are found on unsterilized surfaces. Bacterial and fungal contamination is easily detectable by visual inspection, and it generally succeeds in fouling the cell culture, thereby making it useless. In most cases, contaminated cell cultures are disinfected and then disposed (Lincoln and Gabridge, 1998). The severity of cell culture contamination ranges from minor annoyances like an occasional contaminated flask which causes no serious hindrance to work, to major catastrophes which involve contamination that casts doubt on the accuracy of current or past work. Although cell culture contamination is usually caused by improper aseptic techniques and human error, it also can occur rather spontaneously. In the laboratory setting, it is impractical to eliminate all contaminants, so even with an impeccable aseptic technique microorganisms can occasionally infect cell cultures. Disadvantages associated with cell culture contamination are inaccurate experimental results, loss of time and materials, and, in some cases, a damaged

reputation (Fogh *et al*, 1971). In order to reduce contamination, the design team produced an enclosure that functions to actively prevent the entrance of contaminants.

Aseptic Technique

Currently, there are well-defined guidelines for contamination prevention in cell culture. This is accomplished by wearing personal protective equipment (PPE), disinfecting work surfaces and materials, using sterile disposable pipette tips, working slowly and methodically, and being aware of contaminated surfaces. Common PPE include latex gloves, protective glasses, and lab coats. These items function to prevent the introduction of contaminants from the skin of the researcher. Disinfection of work surfaces and materials is generally performed using ethanol wipes to remove microorganisms (Lincoln and Gabridge, 1998). Additionally, to promote an environment free of living microorganisms, autoclaving, UV radiation, and gaseous chemicals can be used. Autoclaving is a procedure that takes place in a vacuum sealed chamber and uses high pressure steam to sterilize the sample. UV radiation can be emitted by a lamp and destroys biological contaminants by attacking their DNA. Gaseous chemicals sterilization generally involves the use of ethylene oxide, a highly toxic gas (Vinay *et al*, 2010). By practicing aseptic technique the likelihood of cell culture contamination can be greatly reduced, however, in most cases, the addition of a working environment that can actively maintain its sterility is required. The design team generated an enclosure that satisfies this requirement when used with aseptic technique.

Laminar Flow Hoods

Currently, there exist many laboratory devices designed to help to provide a sterile environment for cell work. Some of the most commonly used devices for basic cell culture applications are laminar flow hoods. Laminar flow hoods are classified as either horizontal or vertical, depending on their air flow pattern. This section of the review is

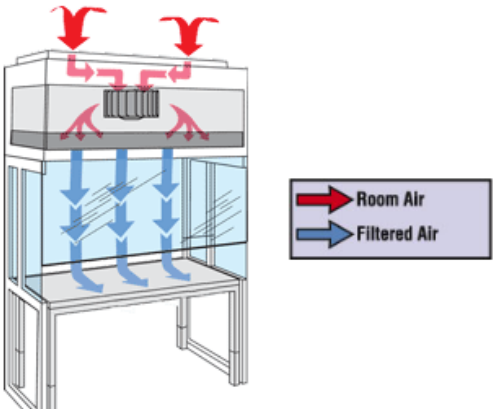

Figure 5: Laminar Flow Hood
(www.terrauniversal.com)

focused on vertical laminar flow hoods. Vertical laminar flow hoods are enclosures that come in a variety of different sizes and they can be either positioned on a bench top or on caster wheels. These devices provide a sterile working environment by utilizing a UV lamp, and a fan/filter unit. UV radiation from the lamp is able to kill microorganisms residing within the chamber by damaging their DNA (Jacobs , 1985). The fan/filter unit is located on the roof of the enclosure and is able to blanket the working surface with a steady laminar flow of filtered air. See Figure 5 for a corresponding air flow pattern. This generates a positive pressure within the enclosure with reduced turbulence, forcing laminar air flow out through the window, thereby inhibiting airborne contamination. Most vertical laminar flow hood utilize either a High-Efficiency Particulate Air (HEPA) filter, which is 99.97% efficient with particles >0.3um, or a Ultra-Low Penetration Air (ULPA) filter, which is 99.99% efficient with particles >0.12um (Sanda *et al*, 1992; Kimman *et al*, 2008). These filters consist of a micro porous polymer membrane that serves to remove particles from flowing air. However, due to the design of these filters they are not able to filter infectious diseases or gaseous chemicals. Although, these flow hoods provide a sterile working environment within the enclosure, it is important to understand their limitations. Specifically, these hoods are not safe for applications involving diseased cell lines or volatile chemicals.

Isolation Chambers

Isolation chambers, as seen in Figure 6, are devices that are generally used for applications requiring controllable pressure or Class 1 sterility, or involving highly dangerous chemical or biological materials. These devices consist of two chambers: the main chamber and the transfer chamber. The main chamber is a flat workspace designed to provide a sterile inert atmosphere for cell work. Additionally, this chamber is airtight and can only be accessed by rubber glove located on the front wall, thereby maintaining sterility. Some isolation chambers include a pressure gauge with allows for pressure control within the main chamber. The transfer

Figure 6: Isolation Chamber
(www.laboratory-supply.net)

chamber allows for materials to be transported in and out of the enclosure without jeopardizing the conditions within the main chamber (Aranki and Freter *I*, 1972).

Air Curtains

Design

Current designs of air curtains contain two elements, a fan or blower and a nozzle. This system allows for a sheet of air to be directed across the face of a door or enclosure opening to minimize any movement of heat, moisture, or particles through the opening. Current air curtains draw air in and use their fan/blower to accelerate it through the nozzle. Because the air curtain is moving at a higher velocity than the ambient atmosphere, there is an increased resistance to any air or particles attempting to pass though the opening.

Advantages

There are many advantages that air curtains provide. They eliminate the need for a physical barrier, increasing visibility and physical movement through the opening. Air curtains also minimize the natural convection flow of the air, which increases the resistance to any air or particle penetration, even with the freedom of movement it provides. They also provide more flexibility than a standard door, as it is possible to adjust the angle and speed of the air curtain, and also heat the air if necessary. Commercially, air curtains also reduce the costs incurred from mechanical door maintenance.

Current Applications

There are several applications that air curtains are being used in. The first is a thermal barrier, shown in Figure 7, which is the air curtain being used to separate spaces with temperature differences. A difference in temperature between two spaces also creates a difference in air densities and pressures. This imbalance causes colder, denser air to move through the bottom part of the opening, while the warmer, lighter air moves through the top of the opening. The air curtain creates moving wall of air that prevents flow across it, and also works to suck in and recycle the air from each side back into their respective systems.

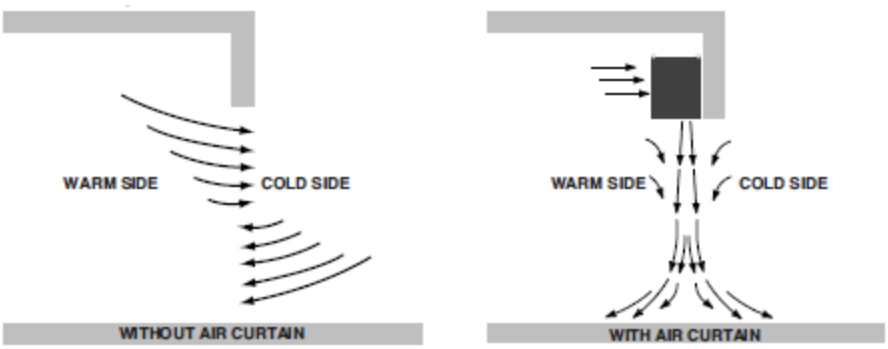

Figure 7: Air Curtain 1

Source: www.marleymep.com

Air curtains are also used for wind resistance into an environment, as demonstrated in Figure 8. Wind passing into an environment can disrupt the inside environment as well as bring in outside particles and contaminants. The air curtain blocks and deflects the wind, directing the wind back away from the air curtain. By adjusting the angle and velocity of the air, this can be made effective for different speeds of wind.

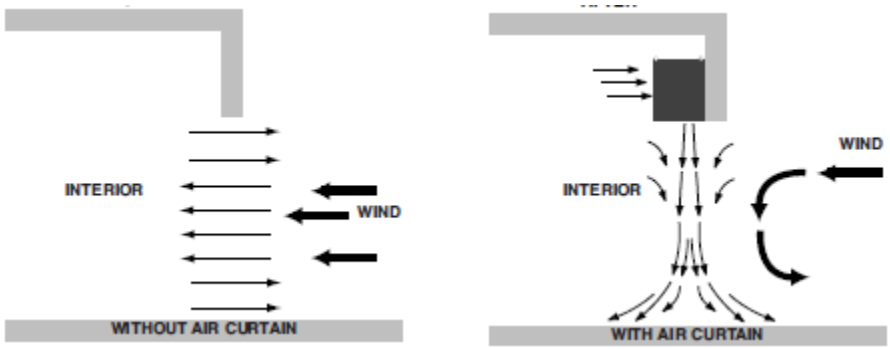

Figure 8: Air Curtain 2

Source: www.marleymep.com

Finally, air curtains are used for interior separation from unwanted fumes or dust, as demonstrated in Figure 9. In many manufacturing settings it is necessary to keep a room or environment "clean" from any manufacturing byproducts. The air curtain repels these particles while still allowing for movement between areas (Anonymous, 2000).

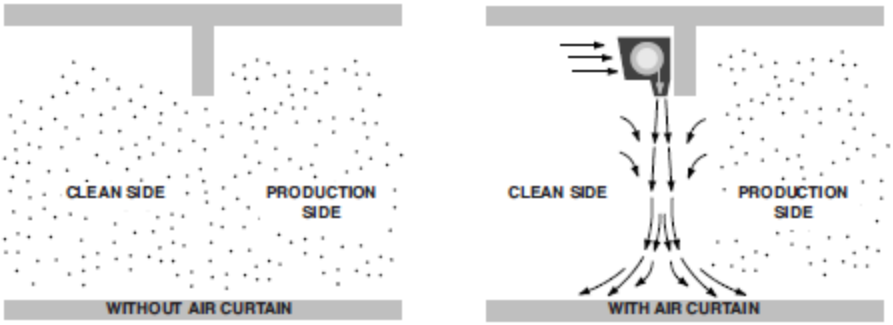

Figure 9: Air Curtain 3

Source: www.marleymep.com

The advantages provided by air curtains, as well as their current applications show that air curtains are a viable option for maintaining a sterile, physiological environment in a biosafety cabinet while still allowing the transfer of media.

Standards and Regulation

Currently in the United States, the federal government (FED-STD) and the International Organization for Standardization (ISO) have established standards for airborne particulate cleanliness in clean zones. These standards group vertical laminar flow hoods and isolation chambers into classes based upon the concentration of airborne particles present inside the enclosure. Figure 10 displays Bionics® Laminar Air Flow System. This device utilizes a HEMA fan/filter unit; therefore, it is labeled a Class 100 hood by FED-STD and a Class 5 hood by ISO. Essentially, these two classes indicate the same degree of airborne cleanliness. Specifically, these class labels mean that 100 is the maximum number of particles with a diameter of 0.5um or greater allowed, per cubic foot of air inside the enclosure. Vertical laminar flow hoods that use HEPA filters are Class 100 (5 ISO), and those

Figure 10: Vertical Flow Hood
(http://www.bionicsscientific.com/)

that use ULPA filters are Class 10 (4 ISO). Isolation chambers use an inert atmosphere, therefore they are capable of achieving the highest level of sterility, Class 1 (3 ISO) (International Standards Organization, 2001).

Chapter 3 – Project Strategy

3.1 Initial Client Statement

Design and build an enclosure for Digilab's CellJet that can provide and maintain the environmental conditions needed for cell culture and cell viability, namely temperature, humidity, CO_2 concentration, and sterility.

3.2 Objectives and Constraints

The design team came up with several objectives for the project by meeting amongst us as well as meeting with our client. The major objectives and sub-objectives for this project can be seen in the objectives tree in Figure 11. The design team decided on three major objectives, environmental control, marketability, and a bench-top design. The environmental control aspect deals with obtaining the conditions needed for cell culture and cell viability. This includes temperature control, humidity control, and gas control. Controlling these environmental conditions will improve cell viability after culture, and provide a broader range of applications for the cell printer. These factors are crucial for the success of the design. The marketability aspect of our objectives can be split into two sub-objectives, reproducibility, which includes a cost-effective design as well as a design comprised of commercially available components, and user-friendliness, which includes a simple user-interface as well as easy maintenance. For our client, lab space is a premium, and therefore we decided that a bench-top design would be ideal to suit this need

The design team decided that both the temperature control and humidity control were the most important objectives. If our enclosure did not meet these objectives, the design would have been considered a failure. Gas control was ranked the next most important objective. Controlling the level of CO_2 in our enclosure does make for a better environment for cell culture, but cells wouldn't be in immediate danger if this control were not present. Reproducibility was ranked next; we felt as though it was not as important as the environmental controls, but more important than being user-friendly and a bench-top design. The bench-top design was ranked least important, as the size of the enclosure would not affect the success of the design. A bench-top design would be convenient, but it was not essential.

3.3 Constraints

There were several design constraints the project team established to ensure our device would be successful. This is an important part of the design process, as it allows for accurate and effective design development.

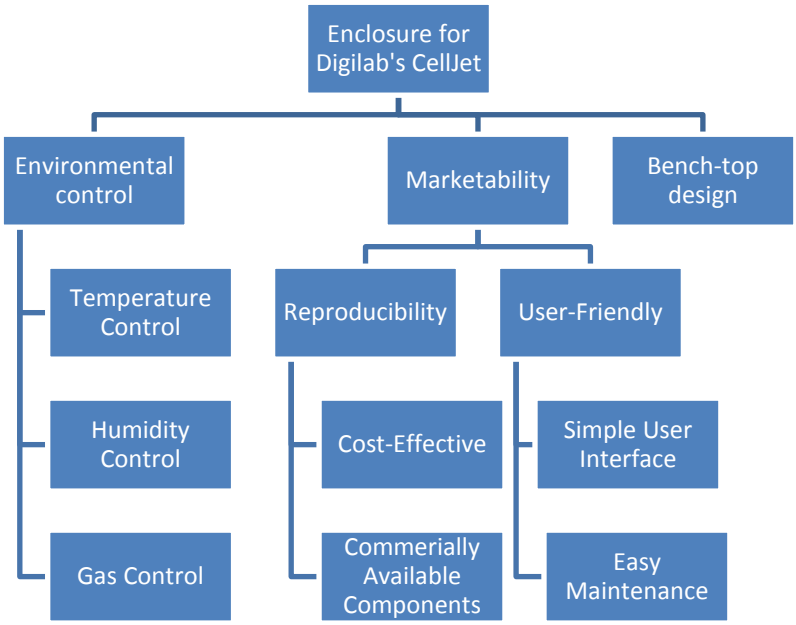

Figure 11: Objective Tree

The first constraint was time. The deadline for our project was project presentation day, which fell on April 18, 2013. Therefore, the design team had a timetable of roughly seven months to complete the project.

The second of these constraints was accessibility of the cell printer. One of the key reasons the cell printer was housed in a laminar flow hood was the fact that it had a large glass front panel that allowed for easy access to the printer and cells during experiments. The client felt this was extremely important, which is why this was viewed as a constraint rather than an objective.

Thirdly, our design had to provide a sterile enclosure for the cell printer. In any biomedical experiment, a sterile environment is important not only to prevent contamination, but also to ensure reproducibility. If the environment is not sterile, then the experiments and data are invalid, making this possibly the most important constraint.

The fourth constraint concerned the safety of cells. While our goal was to create a physiological environment ideal for cell survival, we had to ensure that the methods we used to control the conditions did not have any adverse effects on the cells.

Finally, our design could not impede the performance of the cell printer. The cell printer has a wide range of movement, so our device needed to accommodate this.

3.4 Revised Client Statement

The revised client statement was generated by reevaluating the initial client statement with input from the client and the design team. The revised client statement is as follows:

Design a sterile, bench-top enclosure for housing Digilab's cell printer that provides the internal conditions necessary for cell culture. This enclosure must be sterilizable and able to inhibit the contamination of the printing deck from outside factors. Its dimensions must be at least 4.5ftx2ftx3.5ft, and it must be bench top compatible. Its internal temperature, humidity, and CO_2 concentration must be controllable within the following ranges: 0-40 °C, ambient->80%, and ambient-15%, respectively. Additionally, the enclosure must provide visibility and easy access to the cell printer.

3.5 Project Approach

This section outlines the design team's strategy for meeting the client's expectations on time and within our budget. The main steps in our project plan included the following:

- conducting background research
- revising the initial client statement
- generating several plausible design alternatives
- refining and finalizing our selected design with help from the client
- building and validating a prototype

Once the client's expectations were broadly defined (in the form of objectives and constraints), we proceeded to conduct background research in order to narrow down possible functions, means, and specifications. Next we identified all plausible combinations of means in order to generate several alternative designs. These designs were then presented to our client and subsequently tailored according to their feedback. This process included determining exact device specifications. Once the preferred design had been decided upon (after several iterations of presentations and alterations), a prototype was built and validated. Any necessary adjustments were made throughout the validation process prior to creating a final, manufacturable design.

Chapter 4 – Preliminary Design Process

4.1 Functions and Specifications

In order to be considered a success, the enclosure must maintain and adjust (as needed) the temperature, humidity, and CO_2 concentration without contaminating or otherwise adversely affecting the cells being printed. The functions of sterility, humidity control, temperature control, and CO_2 control are described in more detail in sections 4.1.1, 4.1.2, 4.1.3, and 4.1.4.

4.1.1 Sterility

In this context, sterility is a term used to describe the state of being free of living pathogenic microorganisms. This is a very important factor when dealing with tissue or cellular engineering experiments. With most of these experiments contamination will result in failure. The most common forms of *in vitro* contamination are bacteria and fungi. In these cases bacteria or fungi spores will inhabit a cell or tissue culture. These contaminants can be found on any unclean surface and they usually travel by air (Mycoplasma Contamination). In a laboratory setting, sterile operating procedures are commonly followed in order to reduce the likelihood of contamination. Common aseptic practices include wearing rubber gloves, general cleanliness, autoclaving tools, and utilizing ethanol wipes and ultraviolet lamps. Additionally, devices called laminar flow hoods or biological safety cabinets are often used to provide sterile environment to work with cells. These devices are essentially metal bench tops with a fan/filter

hood. These hoods actively maintain sterility of the bench top by filtering incoming air and using controlled airflow patterns. For most of these devices air is pushed through a HEPA (High Energy Particle Air) filter at a velocity of 90 ft/min which removes all particles that are >0.3micrometers from the air. There are many different airflow patterns that can be used to maintain sterility. The type of airflow pattern is determined by the experiments conducted within the hood. If volatile substances are being used, then the hood is generally designed so that all air entering and exiting the hood is filtered. If contamination is the primary concern then positive pressure generated by a blower is sufficient. It is crucial that the interior of the enclosure described in this paper is capable of being sterilized and maintaining sterility.

4.1.2 Humidity Control

Providing a humid environment within the enclosure is essential for cell vitality. The ideal humidity for cell survival *in vitro* is 99% or just beneath saturated. This degree of humidity is preferred for cell culture because it helps prevent evaporation of cell media and the subsequent concentration of salts, therefore, keeping cells healthy.

4.1.3 Temperature Control

Temperature control is very important for cell vitality. The most common temperature for mammalian cell culture is body temperature (37 °C). At this temperature cell growth is optimized. Depending on the experiment being conducted and the cell type being cultured, the desired temperature during incubation may vary. For instance, temperature can be used as a factor for triggering specific differentiation of specific cell types (Buzin, 1978). Therefore, temperature within the enclosure must be controllable in order to provide the best condition for cells.

4.1.4 Carbon Dioxide Control

CO_2 concentration is a very important factor to consider when dealing with cell culture because it directly influences the pH of a solution. As the CO_2 concentration of surrounding air increases, the pH of a solution decreases (becomes more acidic). The standard pH range for most mammalian cell culture is 7.4-7.7. In order to achieve a media with a pH level within this range a CO_2 concentration between 4-10% is used. This range is so broad because it considers the differences of CO_2 diffusivity into different medium with different concentrations of sodium

bicarbonate. Like temperature, a media's pH plays an important role in cell differentiation (Schulz, 2012). Therefore, CO_2 concentration within the enclosure must be controllable.

4.2 Design Alternatives

The enclosure design can be broken up into two components: the enclosure and the climate control system. The different possible enclosures and climate control systems are first discussed separately in sections 4.2.1 and 4.2.2, and then as complete design assemblies in sections 4.2.3 and 4.2.4.

4.2.1 Enclosure

The purpose of the enclosure itself is to prevent contamination of the cells being printed without adversely affecting them i.e. exposing them to harmful wavelengths of light, excessive airflow, etc. Four different types of enclosures were considered as feasible options for meeting this objective – a vertical laminar flow hood with filtered exhaust, a vertical positive pressure hood, a sealed glove-box, and a custom air curtain incubator. Each of these options met the objectives of contamination prevention and accessibility to different extents. The following three sections investigate each enclosure in more detail.

Vertical Laminar Flow Hood with Filtered Exhaust

A vertical laminar flow hood provides superior accessibility for the user when compared to the other two aforementioned enclosure options. The fact that air is partially recycled through the hood and filtered before it is exhausted potentially allows for an enclosure design that can maintain the desired environmental conditions within while the sash is open, allowing the user to manipulate the contents of the

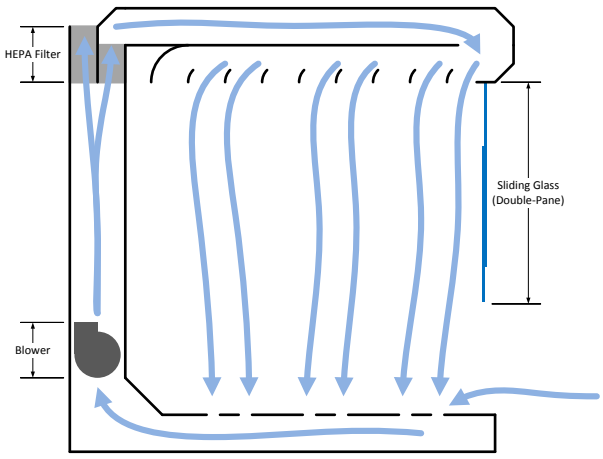

Figure 12: Vertical laminar flow hood with

enclosure without severely altering the internal conditions. The mechanism by which conditions may be maintained while the sash is open is illustrated in Figure 12. The heated and humidified air within the enclosure is circulated through the system rather than being expelled immediately as it would be in a simple positive pressure hood, which means the heat and moisture in the circulated air can be recycled by means of a heat pump and condenser respectively.

Although this enclosure option may potentially provide a high level of accessibility for the user, it relies on an extremely ineffective means of heating and humidifying the incoming air and then cooling and dehumidifying the outgoing air. This would make precise environmental control inefficient and difficult to achieve. In addition, the cells would be exposed to moving air and therefore a higher evaporation rate whenever the sash is open, potentially causing them to dry up. The ducts in the enclosure would also be difficult to sterilize effectively.

Vertical Positive Pressure Hood

A positive pressure flow hood is a simple and inexpensive yet effective enclosure option. The principle behind this type of enclosure is that a continuous stream of filtered air is blown through the enclosure and out the sash, preventing any external unfiltered air from entering, as illustrated in Figure 13. A positive pressure flow hood does not have any ducts and requires only one HEPA filter, keeping the overall design simple and inexpensive in terms of material, operating, and maintenance costs. The drawback with this enclosure option is that whenever the sash is opened, the air inside is evacuated into the atmosphere and air of ambient temperature and humidity is blown in. This makes it impossible to maintain cell-culture appropriate conditions within the enclosure while

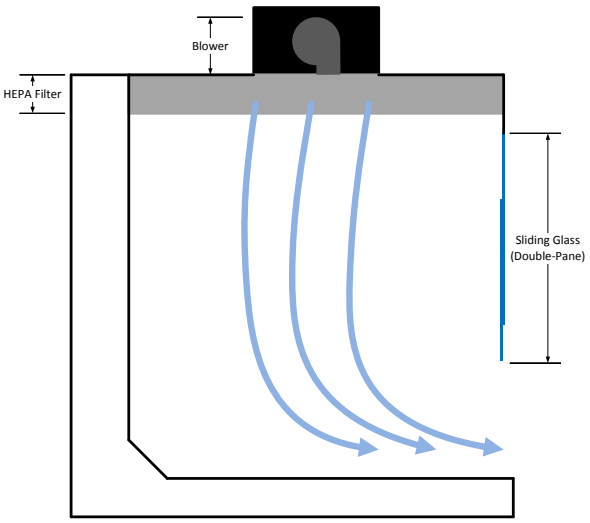

Figure 13: Vertical positive pressure hood

the sash is up, and necessitates an effective method of readjusting the temperature, humidity, and gas concentration within after the sash is closed.

Sealed Glove-Box

The glove box enclosure, illustrated in Figure 14, consists of two parts: the main chamber and the transfer chamber. The main chamber has an airtight seal and is completely closed off from the outside. Gloves located on the front enable the user to work within the enclosure without jeopardizing the interior environmental conditions or sterility. The transfer chamber functions to allow for materials to be brought in and out of the main chamber without contaminating the cells in the main chamber. The transfer chamber has two doors: one that leads to the outside, and one that leads into the main chamber. Airborne contaminants are prevented from crossing the threshold into the main chamber by maintaining a negative pressure within the transfer chamber. The negative pressure is created by the exhaust blower located on the ceiling. All airborne contaminants entering though the outside door are exhausted back outside by the blower, thereby maintaining sterility in the main chamber. The user utilizes this chamber by placing work materials inside the transfer chamber and closing the outside door. Next the user uses the gloves to open the inside door and transport the materials into the main chamber.

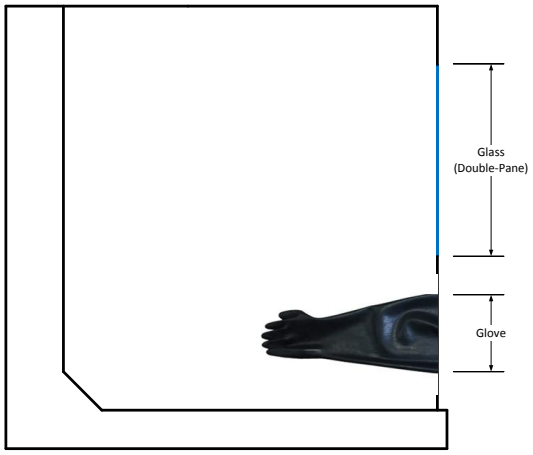

Figure 14: Sealed glove box

Advantages of the glove box design include the sterile and inert atmosphere that is established inside the main chamber. This eliminates the need for a HEPA filter and a complicated airflow pattern within the enclosure. A disadvantage is the limited accessibility to the contents of the enclosure and impeded dexterity and range of motion due to the gloves. Also, the user is restricted to bringing only materials that can fit within the transfer chamber to the main chamber.

Air Curtain Incubator

The air curtain incubator enclosure is essentially the combination of a cell incubator and laminar flow air curtain. The enclosure consists of an incubation chamber with an attached air curtain, as illustrated in Figure 15 – this air curtain functions to serve as a barrier between the internal and external environments. Once ready, samples may then be transferred by the user through the air curtain to the incubation chamber, without risk of contamination.

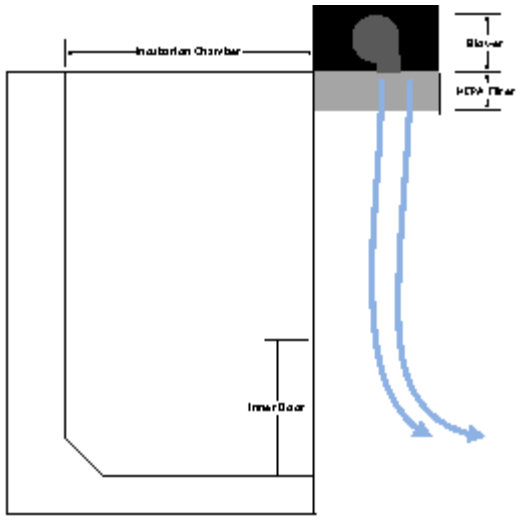

Figure 15: Dual-chamber flow hood

The advantages of this enclosure option are that the incubation chamber need only be opened for extremely short periods of time, allowing the conditions within the incubation chamber to adjust completely before the sample is placed inside. Also, because the incubation chamber is only opened for a quick transfer of the sample, the conditions within the chamber will not be compromised. Additionally, the air curtain design functions to support the preservation of the environmental conditions within the incubator while the chamber is exposed to the external atmosphere. The main disadvantage is that the air curtain flow may enter the interior environment and adversely affect the conditions present.

4.2.2 Climate Control System

Climate control in the context of this project refers to adjusting and maintaining the physiochemical conditions within the enclosure, namely temperature, humidity, CO_2 concentration, and O_2 concentration. Temperature and humidity can be adjusted by two distinct means – conduction and convection – whereas gas concentration adjustments are carried out by injecting more of a desired gas (in the case of carbon dioxide, where the desired concentration is above the ambient concentration) or by diluting the desired gas by injecting a neutral gas, such as nitrogen (in the case of oxygen, where the desired concentration is below

the ambient concentration). The conductive and convective means of controlling temperature and humidity are explained in the following two sections.

Conductive Temperature and Humidity Control

Conductive heating and humidification was an attractive option because of its simplicity and predictability. Direct heat, for example, relies on a relatively simple heating element built into the walls of the enclosure that dissipates heat over time. As for humidification, a water pan is an extremely simple yet effective means of maintaining high humidity. In addition to being simple, a water pan is self-regulating and unlikely to over-saturate the air with moisture. A conductive control system was preferable to a convective system because it is far simpler, does not require contamination-prone ducting, does not rely on potentially harmful air flow, and creates a more uniform heat and moisture distribution. The main disadvantage, however, is adjustment time – conduction is slow compared to convection.

Convective Temperature and Humidity Control

Convective climate control is a plausible alternative to a conductive system. A convective climate control system utilizes delocalized forced air heating coupled with forced air humidification. Forced air heating and humidification is rapid and efficient, although this method would require additional ducting, increasing the potential for contamination, would expose the cells to air flow, and would not disperse heat and moisture uniformly throughout the enclosure. This option would thus necessitate accommodations to address the issues associated with it, which translates into higher material as well as manufacturing costs.

4.2.3 Design Assemblies

Numerous alternative design assemblies were generated by combining compatible components described in sections 4.2.1 and 4.2.2. The plausible combinations are shown in Table 1. Note that all designs utilize the same gas control mechanism – infrared CO_2 sensor with CO_2 tank. The designs were then graded relative to one another based on the following metrics: temperature control, temperature uniformity, humidity control, humidity uniformity, contamination prevention, maintenance of cell viability, gas concentration control, accessibility, and cost. The metrics were assigned weights representative of their importance. The grades are calculated in Table 2.

Table 1: Summary of possible design assemblies

	Conductive Climate Control	Convective Climate Control
Vertical Laminar Flow Hood with Filtered Exhaust	X	Design 1
Vertical Positive Pressure Hood	Design 2	Design 3
Sealed Glove-Box	Design 4	Design 5
Dual-Chamber Flow Hood Incubator	Design 6	Design 7
Precision Air Curtain on Incubator	Design 8	X

Table 2: Design evaluation matrix

	Weights	**Design 1**	**Design 2**	**Design 3**	**Design 4**	**Design 5**	**Design 6**	**Design 7**	**Design8**
Contamination Prevention	*x8.5*	5	6	6	7	7	7	7	8
Maintenance of Cell Viability	*x8.5*	4	6	5	7	6	7	6	8
Temperature Control	*x5.5*	5	6	7	7	8	7	8	4
Temperature Uniformity	*x5.5*	4	6	5	7	6	7	6	8
Humidity Control	*x5.5*	5	6	7	7	8	7	8	7
Humidity Uniformity	*x5.5*	4	6	5	7	6	7	6	8
Gas Concentration Control	*x3*	7	7	7	8	8	8	8	8
Accessibility	*x2*	7	6	6	4	4	5	5	8
Cost	*x1*	4	6	5	7	6	7	6	8
TOTAL SCORE		**200.5**	**259**	**249.5**	**298**	**288.5**	**300**	**290.5**	**332.5**

As shown in Table 2, designs 4, 6, and 8 received the highest scores. The advantages and disadvantages of these designs are summarized in the following four sections.

Sealed Glove Box with Conductive Climate Control

This design utilizes a sealed glove box enclosure with a conductive climate control (i.e. direct heat and water pan) as means of meeting the client's objectives. The design is illustrated in Figure 16. The advantages of this design assembly are uniform temperature and humidity distribution, no loss of internal conditions during active use, and a high degree of sterility. The disadvantages are mainly the limited accessibility and range of motion provided to the user. The gloves may severely impair the user's ability to work with fine instruments within the enclosure.

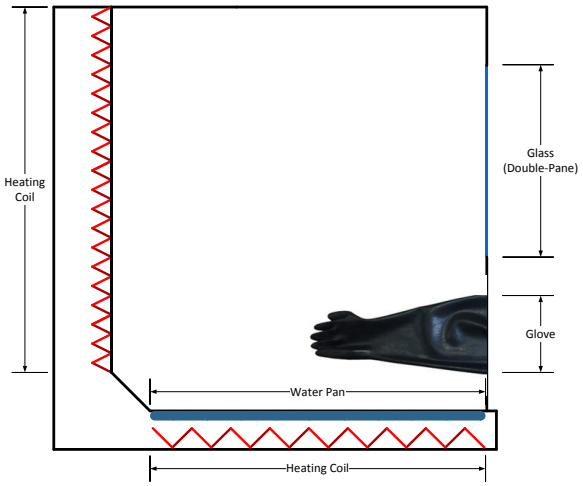

Figure 16: Sealed glove box with conductive climate control

Sealed Glove Box with Convective Climate Control

This design is similar to the previous in that it utilizes the sealed glove box enclosure, although it incorporates a convective climate control system (i.e. forced air heating and humidification), as seen in Figure 17. The convective climate control system provides a rapid method for adjusting internal temperature and humidity at the expense of uniformity. Otherwise, this design is still capable of

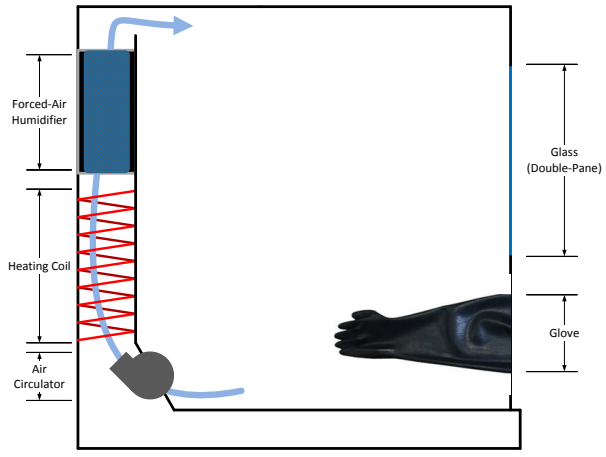

Figure 17: Sealed glove box with convective climate control

maintaining sterility and internal environmental conditions during use. Limited user accessibility and range of motion are still problematic in this design. The duct work necessitated by this system also poses the threat of contamination, as the duct system would be difficult to sterilize. Additionally, the convective climate control system requires the movement of air within the chamber, which can be potentially harmful to the cells being printed.

Dual-Chamber Flow Hood Incubator with Conductive Climate Control

The dual-chamber flow hood incubator design provides the advantages of the sealed glove box design, but improves the user's accessibility and range of motion by incorporating a laminar flow chamber rather than crude gloves, as seen in Figure 18. The incubation chamber (which houses the cell printer) remains closed during use, so the internal environmental conditions can adjust completely before a sample is placed inside. Additionally, because the incubation chamber is opened only for the purpose of inserting a sample, there is a minimal loss in temperature and humidity that can easily be corrected with the conductive climate control system in a short amount of time. The conductive climate control system also helps preserve a static atmosphere inside the incubation chamber, as there is no need for air movement.

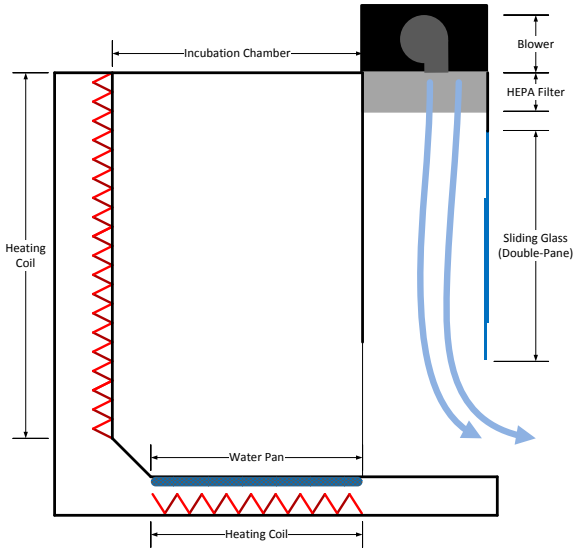

Figure 18: Dual-chamber flow hood incubator with conductive climate control

Dual-Chamber Flow Hood Incubator with Convective Climate Control

This design utilizes the same enclosure as the previous design with the addition of a convective climate control system, as seen in Figure 19. The climate control system used in this design allows for more rapid adjustments of environmental conditions within the incubation chamber, but also exposes the cells in the chamber to moving air. The duct work necessitated

by this system also poses the threat of contamination, as the duct system would be difficult to sterilize.

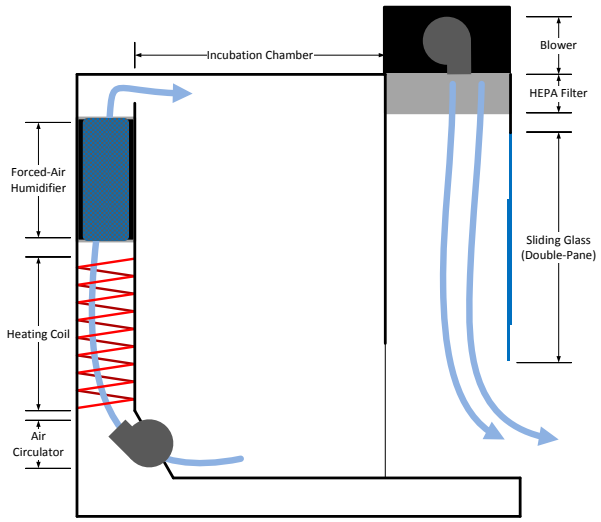

Figure 19: Dual-chamber flow hood incubator with convective climate control

Air Curtain Incubator with Conductive Climate Control

The air curtain incubator design, shown in Figure 20, is a slight modification of the dual chamber design. This design provides almost all of the advantages found in the dual chamber design, but also decreases the bench top footprint. This curtain of air, when positioned over the threshold of a cell incubator, helps maintain internal conditions (temperature, humidity, and CO_2) while the door is open. This would allow the user to access the inside of the incubator/cabinet with minimal effects on the conditions and without allowing the entrance of contaminants. This device would make it possible to convert large cell incubators into models appropriate for the CellJet, allowing the user access to the device when it is inside. The disadvantage with this design includes the lack of a sterile work bench space. Users have to utilize a biological safety cabinet to prepare their samples prior to placing them on the deck of the cell printer.

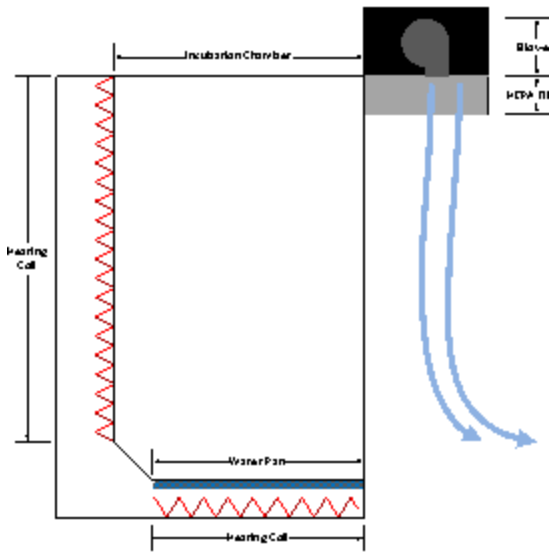

Figure 20: Air curtain incubator with convective climate control

4.2.4 Tentative Final Design

Based on the evaluation matrix in Table 2, the air curtain incubator with conductive climate control was the most promising design (Design 8). The design consists of an enclosure with a pressurized air curtain positioned above the threshold of door of the incubation chamber. An ultraviolet lamp inside of the chamber and a standard HEPA filter blower unit are used to maintain sterility within the enclosure. The UV lamp kills microbial contaminants within the chamber and the HEPA filter cleans the ambient used in the air curtain to maintain the sterility. The HEPA fan/filter unit is the current gold standard in laminar flow hoods, and the air curtain generate a barrier that actively prevents contamination. Direct heating coils, a water pan, and an infrared carbon dioxide sensor with carbon dioxide supply tank are used to control temperature, humidity, and carbon dioxide concentration, respectively. The direct heating coils are currently used in many commercial incubators, and consist of coils in the walls of the enclosure behind a conductive metal. As the coils heat up, the heat is emitted into the enclosure. Heat distribution is uniform because the heat originates from many sources, not just one central source. The heating coils are located in every wall of the incubation chamber. A water pan humidifier is a simple and convenient way to produce humid air. Water evaporates from the pan by means of conductive mass transfer. While this method wouldn't allow the

humidity level to be controlled, it should still be able to raise the humidity to above the required 80%. Infrared carbon dioxide sensors are the current gold standard in low carbon dioxide incubators, and even in other applications such as greenhouses. This sensor monitors a specific infrared wavelength that is affected by the presence of carbon dioxide, and dispenses carbon dioxide from a supply tank to adjust it to the desired level. This device was ideal for this particular design because changes in temperature or humidity have little effect on it readings.

These components were incorporated into the final design, shown in Figure 21, thereby generating an enclosure that fulfills the client's needs and objectives. The combination of the ultraviolet lamp and HEPA fan/filter unit allow for the enclosure to be initially sterilized, and then remain sterile through the entire experimentation procedure. The direct heating coils produce an even heat distribution, and can be controlled to maintain a specific temperature. The heat emitted also provides an ideal environment for the water pan humidifier to produce warm, humid air consistently. The IR carbon dioxide sensor allows for accurate CO_2 reading and enables controlled carbon dioxide levels even during rapid changes in the internal environment. The feasibility experiments were discussed in section 5.1.

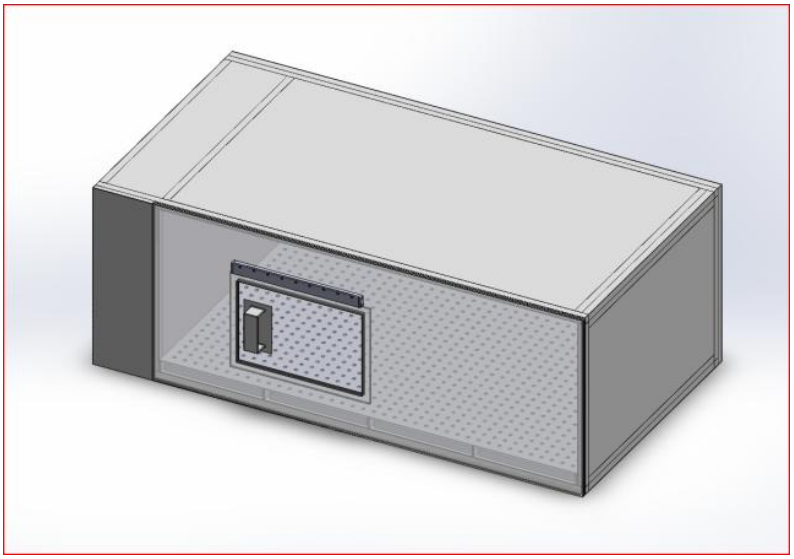

Figure 21: Final Design: Air curtain incubator with convective climate control

4.2.5 Experimental Design

After discussing the options for developing our tentative final design with Digilab, Inc. and the project advisor, the decision was made to borrow a water-jacketed cell culture incubator (Forma Scientific, model # 3110) from WPI to serve as the incubation enclosure for experimentation. This was decided because temperature, humidity, and CO_2 concentration control is achieved ubiquitously in the biotech field through the means of readily available cell incubators. The incubator that the team was loaned was able to actively sense and control temperature and carbon dioxide concentration. A water pan and hygrostat were incorporated into the incubator to generate and sense humidity.

Given the addition of the cell incubator, the focus of the project shifted from developing an entire enclosure to developing novel air curtain technology to achieve two specific goals: (1) actively prevent contamination of the incubator from outside particulates, and (2) ensure the preservation of the temperature, humidity, and CO_2 levels within the incubator.

Chapter 5: Design Verification

5.1 Feasibility Study

The tentative final design was selected based on the assumption that a conductive climate control system would be able to meet the required specifications as defined by the client. In order to test whether this assumption is true, a feasibility study must be carried out. Two methods have been deemed appropriate for testing the climate control system – computer modeling and scaled experiments.

5.1.1 Scaled Experiments

The scaled experiments are intended to provide a better understanding of the heat and moisture distribution during heating and cooling. A good understanding of these factors was necessary to generate an effective control interface with adequate responsiveness. The ideal way to map heat and moisture distribution is to utilize an array of thermocouples and hygrostats and record the temperature and humidity over a period of time. The experiment requires that a prototype of the climate control system be built. Once the prototype and data-collection array is built, various power outputs can be measured and the associated heat and moisture distribution maps generated.

5.1.2 Preliminary Data

The experimental data we needed to collect was the temperature and humidity distribution rate and uniformity. Because the ultraviolet lamp and HEPA fan/filter unit and infrared carbon dioxide sensor are current gold standards used in laminar flow hoods and incubators, respectively, we believed there was no need to collect experimental data for those components. The temperature distribution rate and uniformity data would allow us to calculate how hot and how long to run the heating coils to achieve our desired temperature, evenly distributed throughout the enclosure. The humidity distribution rate and uniformity data would allow to show whether or not the water pan is effective enough to produce above 80% humidity, evenly distributed throughout the enclosure. The final aspect of this data we needed was the time it took to reach these ideal levels, as our clients provided us with a time limit of 30 minutes to achieve our ideal environment. We expected that these elements will provide the climate we needed within the 30 minute limit, although we were prepared to test more

advanced humidification techniques if the water pan could not raise the humidity fast enough. Preliminary tests were done to prove that the air curtain and rig set up would be an effective way to test. The humidity and temperature data shown in Figures 22 and 23 suggested that not only was our set up effective, but that the air curtain itself would be effective.

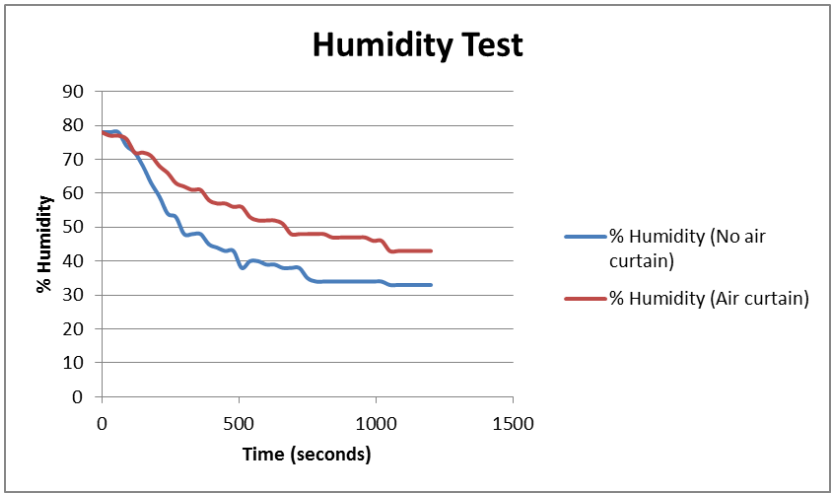

Figure 22: Preliminary Humidity Results

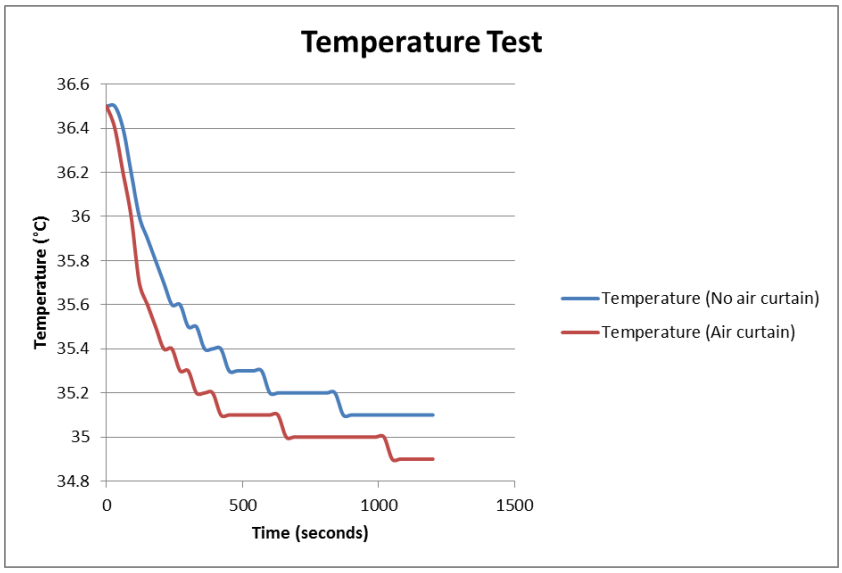

Figure 23: Preliminary Temperature Results

5.2 Temperature

The results of the temperature testing are shown in Tables 3-7 and Figures 24-28. Tests were performed three times for each of the four categories: the control (no air curtain), and the air curtain running at 20 psi, 25 psi, and 30 psi. The average ambient air temperature and humidity in the laboratory where the testing was conducted was 24 degrees Celsius, and 27%.

5.2.1 Control

Table 3: Temperature Data (Control)

Trial 1		Trial 2		Trial 3		Average	
Time (s)	Temperature (°C)	Time (s)	Temperature (°C)	Time (s)	Temperature (°C)	Time (s)	Temperature (°C)
0	36.6	0	36.4	0	36.5	0	36.5
30	36.2	30	36.1	30	36.1	30	36.13333333
60	35.8	60	35.6	60	35.7	60	35.7
90	35.6	90	35.2	90	35.4	90	35.4
120	35.4	120	34.9	120	35.2	120	35.16666667
150	35.2	150	34.7	150	35	150	34.96666667
180	34.9	180	34.5	180	34.9	180	34.76666667
210	34.8	210	34.3	210	34.8	210	34.63333333
240	34.8	240	34.2	240	34.7	240	34.56666667
270	34.7	270	34.1	270	34.6	270	34.46666667
300	34.5	300	34	300	34.5	300	34.33333333
330	34.5	330	34	330	34.4	330	34.3
360	34.6	360	33.9	360	34.3	360	34.26666667
390	34.6	390	33.8	390	34.2	390	34.2
420	34.5	420	33.8	420	34.2	420	34.16666667
450	34.5	450	33.8	450	34.2	450	34.16666667
480	34.4	480	33.7	480	34.1	480	34.06666667
510	34.5	510	33.7	510	34	510	34.06666667
540	34.5	540	33.7	540	34	540	34.06666667
570	34.5	570	33.7	570	34	570	34.06666667
600	34.5	600	33.7	600	34	600	34.06666667

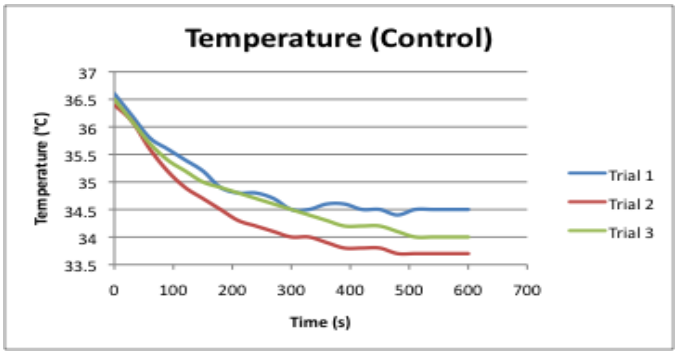

Figure 24: Temperature Data (Control)

5.2.2 20 psi

Table 4: Temperature Data (20 psi)

Trial 1			Trial 2			Trial 3			Average	
Time (s)	Temperature (°C)		Time (s)	Temperature (°C)		Time (s)	Temperature (°C)		Time (s)	Temperature (°C)
0	36.6		0	36.6		0	36.6		0	36.6
30	36.3		30	36.4		30	36.4		30	36.36666667
60	35.9		60	35.8		60	35.9		60	35.86666667
90	35.5		90	35.4		90	35.3		90	35.4
120	35.2		120	35.2		120	35		120	35.13333333
150	34.9		150	34.9		150	34.7		150	34.83333333
180	34.7		180	34.8		180	34.5		180	34.66666667
210	34.6		210	34.6		210	34.4		210	34.53333333
240	34.4		240	34.5		240	34.3		240	34.4
270	34.3		270	34.3		270	34.1		270	34.23333333
300	34.2		300	34.2		300	34		300	34.13333333
330	34.1		330	34		330	33.9		330	34
360	33.9		360	33.9		360	33.8		360	33.86666667
390	33.8		390	33.8		390	33.7		390	33.76666667
420	33.7		420	33.7		420	33.6		420	33.66666667
450	33.6		450	33.7		450	33.5		450	33.6
480	33.6		480	33.6		480	33.4		480	33.53333333
510	33.5		510	33.6		510	33.4		510	33.5
540	33.5		540	33.6		540	33.4		540	33.5
570	33.4		570	33.5		570	33.3		570	33.4
600	33.4		600	33.5		600	33.3		600	33.4

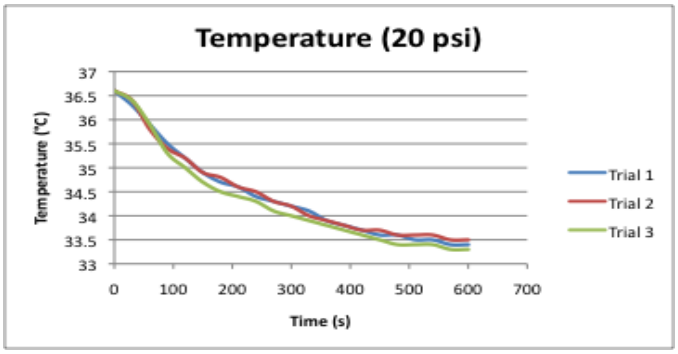

Figure 25: Temperature Data (20 psi)

5.2.3 25 psi

Table 5: Temperature Data (25 psi)

Trial 1			Trial 2			Trial 3			Average	
Time (s)	Temperature (°C)		Time (s)	Temperature (°C)		Time (s)	Temperature (°C)		Time (s)	Temperature (°C)
0	36.5		0	36.6		0	36.9		0	36.66666667
30	36.4		30	36.4		30	36.6		30	36.46666667
60	35.7		60	35.9		60	36.1		60	35.9
90	35.2		90	35.4		90	35.7		90	35.43333333
120	34.9		120	34.9		120	35.4		120	35.06666667
150	34.7		150	34.6		150	35.2		150	34.83333333
180	34.4		180	34.3		180	35		180	34.56666667
210	34.1		210	34.2		210	35		210	34.43333333
240	33.8		240	33.9		240	34.8		240	34.16666667
270	33.6		270	33.8		270	34.5		270	33.96666667
300	33.5		300	33.7		300	34.3		300	33.83333333
330	33.3		330	33.6		330	34.2		330	33.7
360	33.2		360	33.5		360	34		360	33.56666667
390	33.1		390	33.4		390	33.9		390	33.46666667
420	33		420	33.3		420	33.8		420	33.36666667
450	33		450	33.3		450	33.7		450	33.33333333
480	32.9		480	33.2		480	33.7		480	33.26666667
510	32.9		510	33.1		510	33.6		510	33.2
540	32.9		540	33.1		540	33.6		540	33.2
570	32.8		570	33		570	33.5		570	33.1
600	32.8		600	33		600	33.5		600	33.1

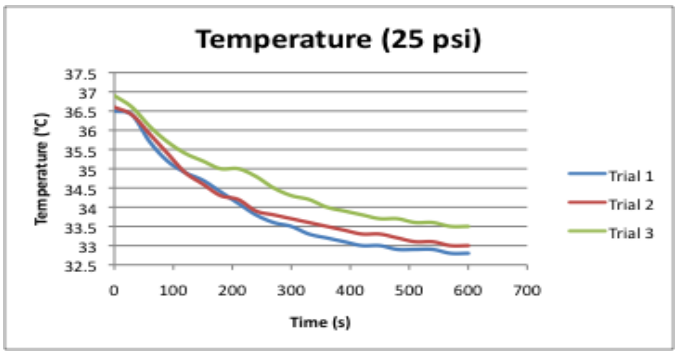

Figure 26: Temperature Data (25 psi)

5.2.4 30 psi

Table 6: Temperature Data (30 psi)

Trial 1		Trial 2		Trial 3		Average	
Time (s)	Temperature (°C)	Time (s)	Temperature (°C)	Time (s)	Temperature (°C)	Time (s)	Temperature (°C)
0	36.8	0	37.1	0	37.1	0	37
30	36.4	30	36.9	30	36.7	30	36.66666667
60	35.8	60	36.4	60	36.1	60	36.1
90	35.3	90	36.1	90	35.9	90	35.76666667
120	35	120	35.7	120	35.6	120	35.43333333
150	34.7	150	35.3	150	35.3	150	35.1
180	34.4	180	35.1	180	35.2	180	34.9
210	34.1	210	35	210	35.1	210	34.73333333
240	33.9	240	34.8	240	34.9	240	34.53333333
270	33.8	270	34.8	270	34.7	270	34.43333333
300	33.6	300	34.7	300	34.6	300	34.3
330	33.5	330	34.6	330	34.6	330	34.23333333
360	33.4	360	34.5	360	34.5	360	34.13333333
390	33.4	390	34.5	390	34.4	390	34.1
420	33.3	420	34.4	420	34.4	420	34.03333333
450	33.3	450	34.4	450	34.3	450	34
480	33.2	480	34.3	480	34.4	480	33.96666667
510	33.2	510	34.3	510	34.3	510	33.93333333
540	33.3	540	34.3	540	34.3	540	33.96666667
570	33.2	570	34.2	570	34.3	570	33.9
600	33.2	600	34.2	600	34.3	600	33.9

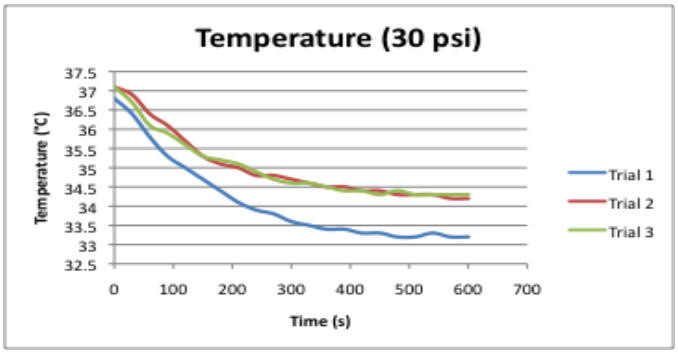

Figure 27: Temperature Data (30 psi)

5.2.5 Average

In control measurements, the temperature dropped by approximately 2.5°C (6.67%) in ten minutes, compared to a drop of 3.2°C (8.74%), 3.5°C (9.73%) and 3.1°C (8.38%) with air curtain set to 20, 25 and 30 psi, respectively (Table 7, Figure 28).

Table 7: Average Temperature Data

Average	Control		Average	20 psi		Average	25 psi		Average	30 psi
Time (s)	Temperature (°C)		Time (s)	Temperature (°C)		Time (s)	Temperature (°C)		Time (s)	Temperature (°C)
0	36.5		0	36.6		0	36.66666667		0	37
30	36.13333333		30	36.36666667		30	36.46666667		30	36.66666667
60	35.7		60	35.86666667		60	35.9		60	36.1
90	35.4		90	35.4		90	35.43333333		90	35.76666667
120	35.16666667		120	35.13333333		120	35.06666667		120	35.43333333
150	34.96666667		150	34.83333333		150	34.83333333		150	35.1
180	34.76666667		180	34.66666667		180	34.56666667		180	34.9
210	34.63333333		210	34.53333333		210	34.43333333		210	34.73333333
240	34.56666667		240	34.4		240	34.16666667		240	34.53333333
270	34.46666667		270	34.23333333		270	33.96666667		270	34.43333333
300	34.33333333		300	34.13333333		300	33.83333333		300	34.3
330	34.3		330	34		330	33.7		330	34.23333333
360	34.26666667		360	33.86666667		360	33.56666667		360	34.13333333
390	34.2		390	33.76666667		390	33.46666667		390	34.1
420	34.16666667		420	33.66666667		420	33.36666667		420	34.03333333
450	34.16666667		450	33.6		450	33.33333333		450	34
480	34.06666667		480	33.53333333		480	33.26666667		480	33.96666667
510	34.06666667		510	33.5		510	33.2		510	33.93333333
540	34.06666667		540	33.5		540	33.2		540	33.96666667
570	34.06666667		570	33.4		570	33.1		570	33.9
600	34.06666667		600	33.4		600	33.1		600	33.9

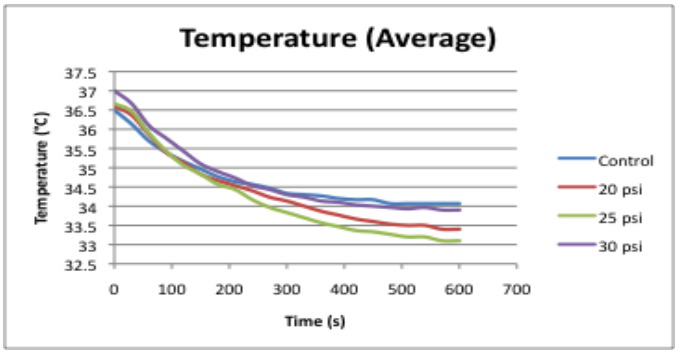

Figure 28: Average Temperature Data

5.3 Humidity

The results of the humidity testing are shown in Tables 8-12 and Figures 29-33. Tests were performed three times for each of the four categories: the control (no air curtain), and the air curtain running at 20 psi, 25 psi, and 30 psi. The average ambient air temperature and humidity in the laboratory where the testing was conducted was 24 degrees Celsius, and 27%.

5.3.1 Control

Table 8: Humidity Data (Control)

Trial 1		Trial 2		Trial 3		Average	
Time (s)	% Humidity	Time (s)	% Humidity	Time (s)	% Humidity	Time (s)	% Humidity
0	80	0	80	0	80	0	80
30	80	30	80	30	78	30	79.33333333
60	66	60	77	60	71	60	71.33333333
90	56	90	76	90	64	90	65.33333333
120	47	120	71	120	61	120	59.66666667
150	41	150	67	150	58	150	55.33333333
180	37	180	62	180	57	180	52
210	37	210	61	210	57	210	51.66666667
240	37	240	56	240	55	240	49.33333333
270	36	270	55	270	54	270	48.33333333
300	31	300	51	300	50	300	44
330	33	330	50	330	48	330	43.66666667
360	33	360	45	360	44	360	40.66666667
390	32	390	46	390	42	390	40
420	32	420	45	420	42	420	39.66666667
450	32	450	42	450	42	450	38.66666667
480	32	480	41	480	41	480	38
510	32	510	41	510	41	510	38
540	32	540	40	540	40	540	37.33333333
570	32	570	35	570	38	570	35
600	32	600	37	600	38	600	35.66666667

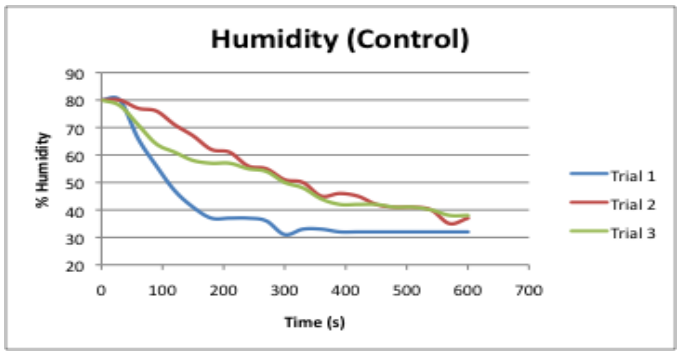

Figure 29: Humidity Data (Control)

5.3.2 20 psi

Table 9: Humidity Data (20 psi)

Trial 1		Trial 2		Trial 3		Average	
Time (s)	% Humidity	Time (s)	% Humidity	Time (s)	% Humidity	Time (s)	% Humidity
0	80	0	80	0	80	0	80
30	80	30	80	30	80	30	80
60	80	60	79	60	80	60	79.66666667
90	80	90	76	90	78	90	78
120	75	120	74	120	75	120	74.66666667
150	71	150	69	150	70	150	70
180	67	180	65	180	68	180	66.66666667
210	65	210	61	210	64	210	63.33333333
240	65	240	60	240	60	240	61.66666667
270	62	270	59	270	60	270	60.33333333
300	57	300	56	300	58	300	57
330	52	330	56	330	57	330	55
360	52	360	52	360	56	360	53.33333333
390	51	390	52	390	54	390	52.33333333
420	46	420	51	420	51	420	49.33333333
450	47	450	51	450	50	450	49.33333333
480	47	480	49	480	50	480	48.66666667
510	46	510	51	510	50	510	49
540	46	540	48	540	49	540	47.66666667
570	41	570	48	570	49	570	46
600	43	600	47	600	49	600	46.33333333

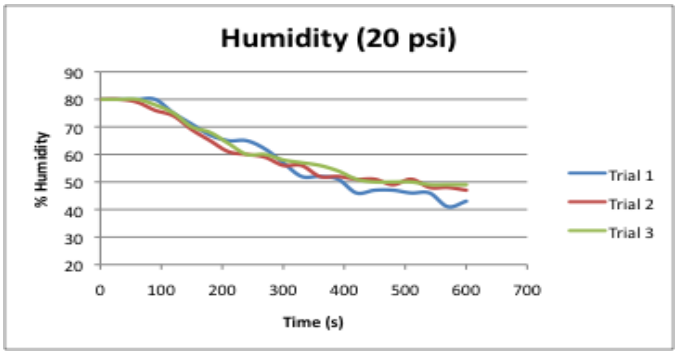

Figure 30: Humidity Data (20 psi)

5.3.3 25 psi

Table 10: Humidity Data (25 psi)

Trial 1			Trial 2			Trial 3				
Time (s)	% Humidity		Time (s)	% Humidity		Time (s)	% Humidity		Time (s)	% Humidity
0	82		0	80		0	80		0	80.66666667
30	78		30	79		30	77		30	78
60	76		60	75		60	76		60	75.66666667
90	66		90	69		90	70		90	68.33333333
120	62		120	64		120	66		120	64
150	57		150	60		150	62		150	59.66666667
180	53		180	58		180	59		180	56.66666667
210	51		210	53		210	54		210	52.66666667
240	47		240	49		240	49		240	48.33333333
270	46		270	46		270	45		270	45.66666667
300	43		300	43		300	42		300	42.66666667
330	42		330	43		330	42		330	42.33333333
360	42		360	43		360	41		360	42
390	42		390	41		390	41		390	41.33333333
420	41		420	40		420	40		420	40.33333333
450	41		450	40		450	40		450	40.33333333
480	38		480	39		480	40		480	39
510	38		510	37		510	39		510	38
540	37		540	37		540	38		540	37.33333333
570	37		570	37		570	38		570	37.33333333
600	37		600	37		600	37		600	37

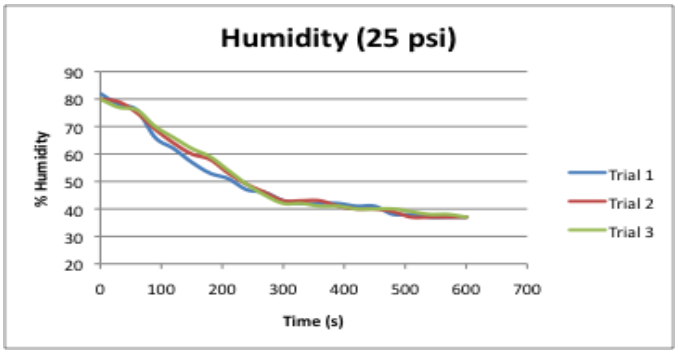

Figure 31: Humidity Data (25 psi)

5.3.4 30 psi

Table 11: Humidity Data (30 psi)

Trial 1		Trial 2		Trial 3			
Time (s)	% Humidity	Time (s)	% Humidity	Time (s)	% Humidity	Time (s)	% Humidity
0	81	0	80	0	80	0	80.33333333
30	78	30	77	30	77	30	77.33333333
60	72	60	74	60	72	60	72.66666667
90	66	90	69	90	65	90	66.66666667
120	61	120	63	120	60	120	61.33333333
150	57	150	58	150	56	150	57
180	52	180	51	180	53	180	52
210	51	210	49	210	51	210	50.33333333
240	47	240	49	240	50	240	48.66666667
270	49	270	47	270	49	270	48.33333333
300	46	300	46	300	47	300	46.33333333
330	43	330	44	330	45	330	44
360	43	360	44	360	43	360	43.33333333
390	43	390	42	390	42	390	42.33333333
420	42	420	42	420	41	420	41.66666667
450	42	450	40	450	41	450	41
480	41	480	40	480	40	480	40.33333333
510	41	510	40	510	39	510	40
540	41	540	38	540	39	540	39.33333333
570	38	570	37	570	38	570	37.66666667
600	36	600	37	600	38	600	37

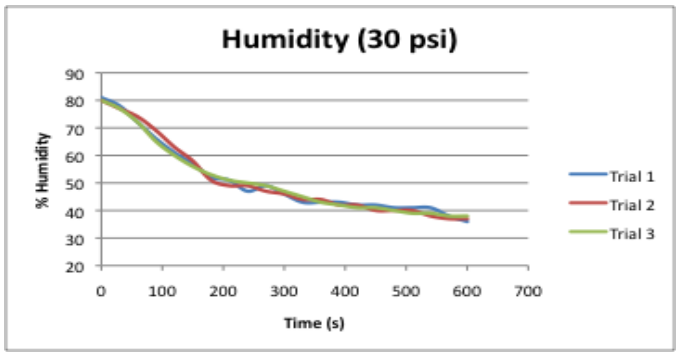

Figure 32: Humidity Data (30 psi)

5.3.5 Average

In control measurements, the relative humidity dropped from 80% (saturation) to 44.4%, compared to a drop to 33.7% 43.6% and 43.3% humidity with air curtain set to 20, 25 and 30 psi, respectively (Table 12, Figure 33).

Table 12: Average Humidity Data

Average Time (s)	Control % Humidity	Average Time (s)	20 psi % Humidity	Average Time (s)	25 psi % Humidity	Average Time (s)	30 psi % Humidity
0	80	0	80	0	80.66666667	0	80.33333333
30	79.33333333	30	80	30	78	30	77.33333333
60	71.33333333	60	79.66666667	60	75.66666667	60	72.66666667
90	65.33333333	90	78	90	68.33333333	90	66.66666667
120	59.66666667	120	74.66666667	120	64	120	61.33333333
150	55.33333333	150	70	150	59.66666667	150	57
180	52	180	66.66666667	180	56.66666667	180	52
210	51.66666667	210	63.33333333	210	52.66666667	210	50.33333333
240	49.33333333	240	61.66666667	240	48.33333333	240	48.66666667
270	48.33333333	270	60.33333333	270	45.66666667	270	48.33333333
300	44	300	57	300	42.66666667	300	46.33333333
330	43.66666667	330	55	330	42.33333333	330	44
360	40.66666667	360	53.33333333	360	42	360	43.33333333
390	40	390	52.33333333	390	41.33333333	390	42.33333333
420	39.66666667	420	49.33333333	420	40.33333333	420	41.66666667
450	38.66666667	450	49.33333333	450	40.33333333	450	41
480	38	480	48.66666667	480	39	480	40.33333333
510	38	510	49	510	38	510	40
540	37.33333333	540	47.66666667	540	37.33333333	540	39.33333333
570	35	570	46	570	37.33333333	570	37.66666667
600	35.66666667	600	46.33333333	600	37	600	37

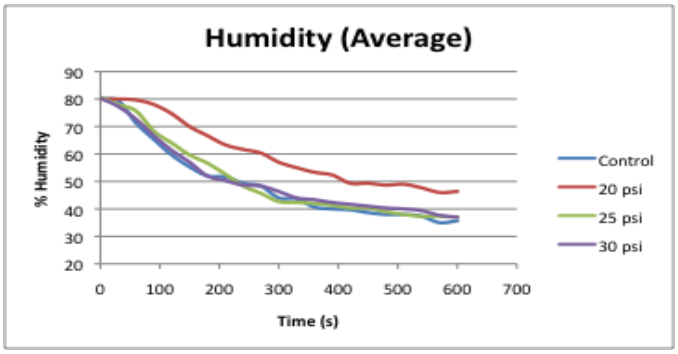

Figure 33: Average Humidity Data

5.4 CO_2 Concentration

The results of the CO_2 testing are shown in Tables 13-17 and Figures 34-38. Tests were performed three times for each of the four categories: the control (no air curtain), and the air curtain running at 20 psi, 25 psi, and 30 psi. The average ambient air temperature and humidity in the laboratory where the testing was conducted was 24 degrees Celsius, and 27%.

5.4.1 Control

Table 13: CO_2 Concentration (Control)

Trial 1			Trial 2			Trial 3	
Time (s)	% CO2		Time (s)	% CO2		Time (s)	% CO2
0	5.8		0	5.9		0	5.9
30	5.8		30	5.6		30	5.8
60	5.4		60	5.3		60	5.4
90	5.3		90	5.1		90	5.2
120	5.4		120	5.1		120	5.2
150	5.6		150	5.3		150	5.3
180	5.5		180	5.4		180	5.4
210	5.4		210	5.6		210	5.6
240	5.3		240	5.6		240	5.6
270	5.3		270	5.5		270	5.6
300	5.3		300	5.5		300	5.5

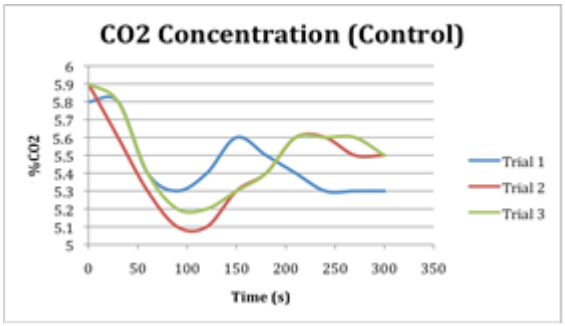

Figure 34: CO_2 Concentration (Control)

5.4.2 20 psi

Table 14: CO_2 Concentration (20 psi)

Trial 1			Trial 2			Trial 3	
Time (s)	% CO2		Time (s)	% CO2		Time (s)	% CO2
0	5.9		0	5.9		0	5.8
30	5.7		30	5.8		30	5.8
60	5.7		60	5.8		60	5.8
90	5.7		90	5.7		90	5.7
120	5.6		120	5.7		120	5.7
150	5.6		150	5.7		150	5.7
180	5.6		180	5.6		180	5.7
210	5.6		210	5.6		210	5.6
240	5.5		240	5.6		240	5.6
270	5.5		270	5.6		270	5.6
300	5.5		300	5.5		300	5.6

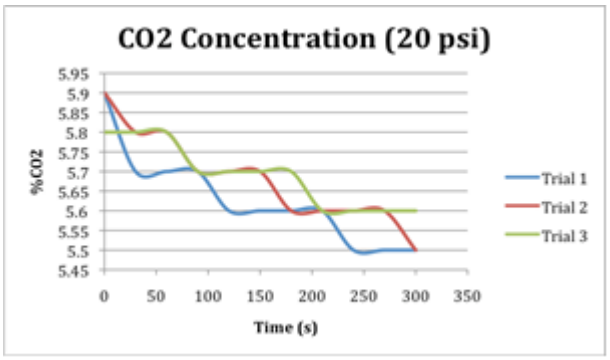

Figure 35: CO_2 Concentration (20 psi)

5.4.3 25 psi

Table 15: CO_2 Concentration (25 psi)

Trial 1			Trial 2			Trial 3	
Time (s)	% CO2		Time (s)	% CO2		Time (s)	% CO2
0	5.9		0	5.8		0	5.9
30	5.8		30	5.8		30	5.9
60	5.8		60	5.8		60	5.9
90	5.8		90	5.8		90	5.8
120	5.7		120	5.7		120	5.8
150	5.7		150	5.7		150	5.8
180	5.7		180	5.6		180	5.8
210	5.6		210	5.6		210	5.7
240	5.6		240	5.5		240	5.7
270	5.6		270	5.5		270	5.6
300	5.6		300	5.5		300	5.6

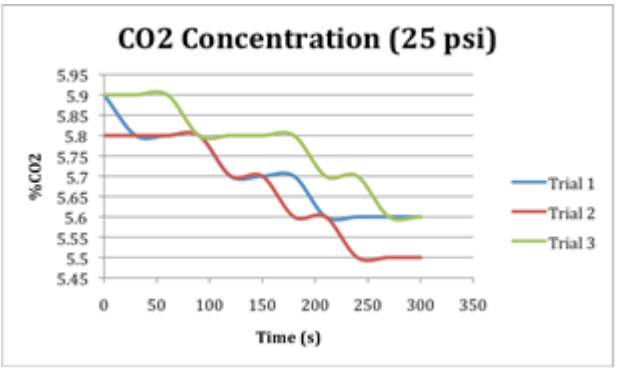

Figure 36: CO_2 Concentration (25 psi)

5.4.4 30 psi

Table 16: CO_2 Concentration (30 psi)

Trial 1			Trial 2			Trial 3	
Time (s)	% CO2		Time (s)	% CO2		Time (s)	% CO2
0	5.8		0	5.9		0	5.9
30	5.8		30	5.8		30	5.9
60	5.8		60	5.8		60	5.7
90	5.7		90	5.7		90	5.7
120	5.7		120	5.7		120	5.6
150	5.6		150	5.6		150	5.6
180	5.6		180	5.6		180	5.6
210	5.6		210	5.6		210	5.6
240	5.6		240	5.6		240	5.6
270	5.5		270	5.6		270	5.6
300	5.5		300	5.6		300	5.6

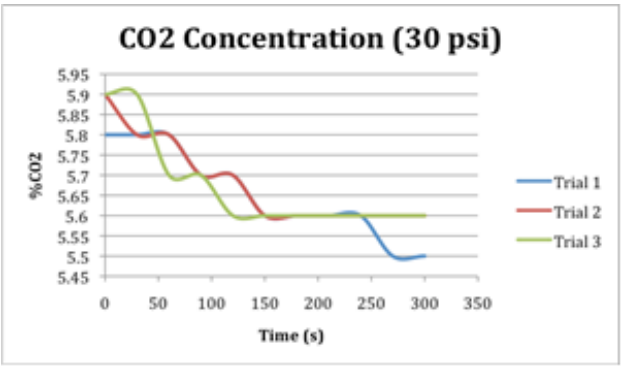

Figure 37: CO_2 Concentration (30 psi)

5.4.5 Average

In control measurements, CO_2 concentration dropped by 0.7% before rising back to a final 5.4%. This compares to drop in CO_2 levels by 0.33%, 0.3% and 0.3% with air curtain set to 20, 25 and 30 psi, respectively (Table 17, Figure 38).

Table 17: Average CO2 Concentration

Average Time (s)	Control % CO2		Average Time (s)	20 psi % CO2		Average Time (s)	25 psi % CO2		Average Time (s)	30 psi % CO2
0	5.866666667		0	5.866667		0	5.866667		0	5.866667
30	5.733333333		30	5.766667		30	5.833333		30	5.833333
60	5.366666667		60	5.766667		60	5.833333		60	5.766667
90	5.2		90	5.7		90	5.8		90	5.7
120	5.233333333		120	5.666667		120	5.733333		120	5.666667
150	5.4		150	5.666667		150	5.733333		150	5.6
180	5.433333333		180	5.633333		180	5.7		180	5.6
210	5.533333333		210	5.6		210	5.633333		210	5.6
240	5.5		240	5.566667		240	5.6		240	5.6
270	5.466666667		270	5.566667		270	5.566667		270	5.566667
300	5.433333333		300	5.533333		300	5.566667		300	5.566667

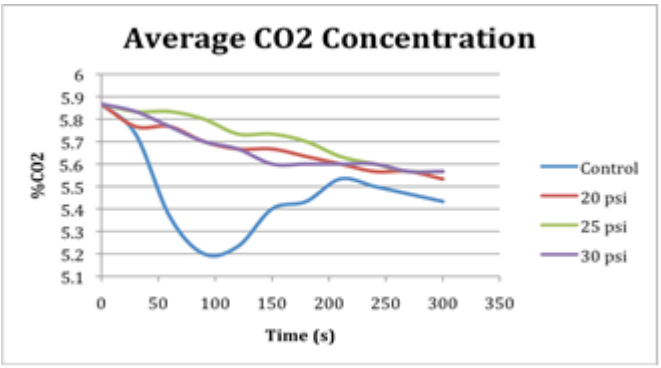

Figure 38: Average CO_2 Concentration

5.5 Permeability

The results of the permeability testing are shown in Tables 18-22 and Figures 39-43. Tests were performed three times for each of the four categories: the control (no air curtain), and the air curtain running at 20 psi, 25 psi, and 30 psi. The average ambient air temperature and humidity in the laboratory where the testing was conducted was 24 degrees Celsius, and 27%.

5.5.1 Control

Table 18: Permeability Data (Control)

Trial 1			Trial 2			Trial 3	
Time (s)	CO_2 (%)		Time (s)	CO_2 (%)		Time (s)	CO_2 (%)
0	4.8		0	5		0	5
30	4.9		30	5.1		30	5.3
60	5.9		60	5.9		60	5.8
90	6.5		90	6.3		90	6
120	6.7		120	6.8		120	6.3
150	6.9		150	6.8		150	6.5
180	7		180	7.1		180	6.9
210	7		210	7.1		210	6.9
240	7		240	7.1		240	6.9
270	7		270	7.3		270	7
300	6.9		300	7.2		300	7

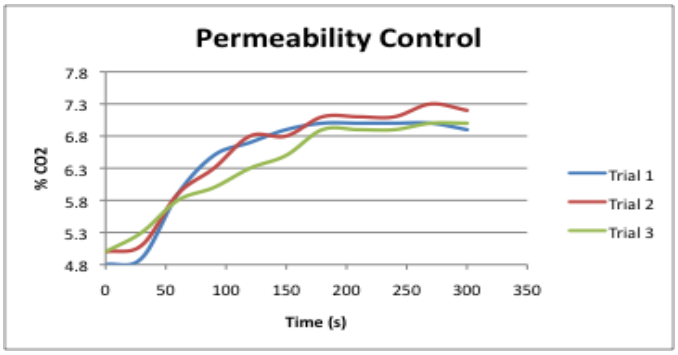

Figure 39: Permeability (Control)

5.5.2 20 psi

Table 19: Permeability Data (20 psi)

Trial 1			Trial 2			Trial 3	
Time (s)	CO_2 (%)		Time (s)	CO_2 (%)		Time (s)	CO_2 (%)
0	5		0	5		0	5
30	4.9		30	5		30	5.2
60	5.2		60	5.2		60	5.4
90	5.4		90	5.3		90	5.4
120	5.5		120	5.4		120	5.5
150	5.5		150	5.4		150	5.5
180	5.5		180	5.5		180	5.6
210	5.4		210	5.6		210	5.7
240	5.7		240	5.6		240	5.7
270	5.7		270	5.6		270	5.8
300	5.7		300	5.7		300	5.8

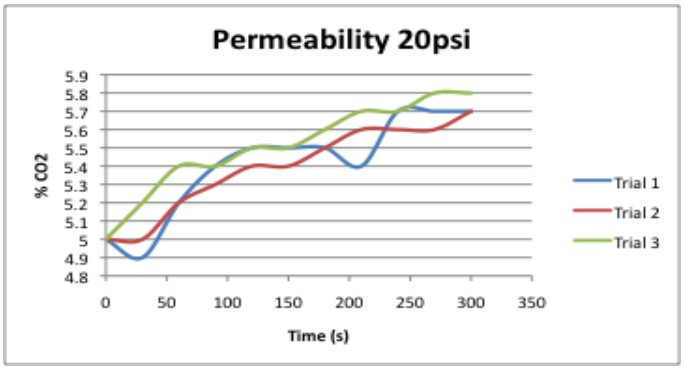

Figure 40: Permeability (20 psi)

5.5.3 25 psi

Table 20: Permeability Data (25 psi)

Trial 1			Trial 2			Trial 3	
Time (s)	CO_2 (%)		Time (s)	CO_2 (%)		Time (s)	CO_2 (%)
0	5		0	5		0	5
30	5.1		30	5.2		30	5
60	5.1		60	5.2		60	5.3
90	5.3		90	5.4		90	5.3
120	5.4		120	5.4		120	5.4
150	5.5		150	5.5		150	5.4
180	5.5		180	5.6		180	5.4
210	5.5		210	5.6		210	5.5
240	5.5		240	5.6		240	5.5
270	5.6		270	5.6		270	5.5
300	5.6		300	5.7		300	5.6

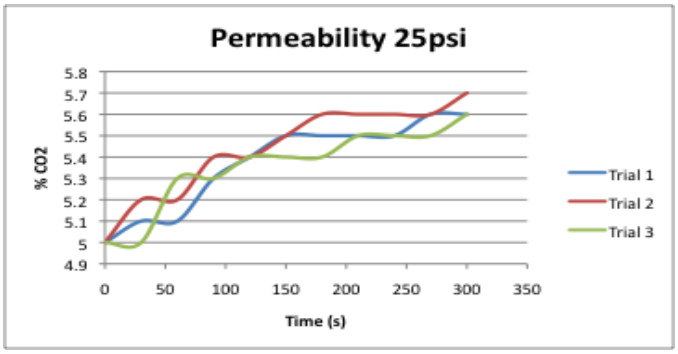

Figure 41: Permeability (25 psi)

5.5.4 30 psi

Table 21: Permeability Data (30 psi)

Trial 1			Trial 2			Trial 3	
Time (s)	CO_2 (%)		Time (s)	CO_2 (%)		Time (s)	CO_2 (%)
0	5		0	5		0	5
30	4.9		30	5		30	5.1
60	5.1		60	5.1		60	5.1
90	5.2		90	5.1		90	5.2
120	5.3		120	5.1		120	5.2
150	5.4		150	5.2		150	5.2
180	5.3		180	5.2		180	5.3
210	5.4		210	5.4		210	5.3
240	5.4		240	5.4		240	5.3
270	5.4		270	5.4		270	5.3
300	5.4		300	5.5		300	5.4

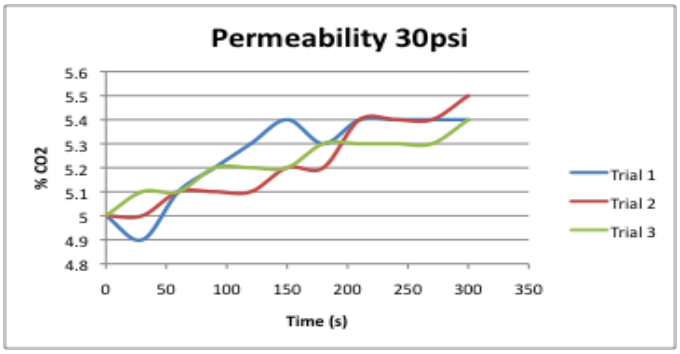

Figure 42: Permeability (30 psi)

5.5.5 Average

In control measurements for CO_2 permeability, CO_2 concentration inside the incubator increased by 2.1%. This compares to an increase in CO_2 levels by 0.73%, 0.63% and 0.43% with air curtain set to 20, 25 and 30 psi, respectively (Table 22, Figure 43).

Table 22: Average Permeability Data

Average Time (s)	Control CO_2 (%)	Average Time (s)	20 psi CO_2 (%)	Average Time (s)	25 psi CO_2 (%)	Average Time (s)	30 psi CO_2 (%)
0	4.933333333	0	5	0	5	0	5
30	5.1	30	5.03333333	30	5.1	30	5
60	5.866666667	60	5.26666667	60	5.2	60	5.1
90	6.266666667	90	5.36666667	90	5.33333333	90	5.16666667
120	6.6	120	5.46666667	120	5.4	120	5.2
150	6.733333333	150	5.46666667	150	5.46666667	150	5.26666667
180	7	180	5.53333333	180	5.5	180	5.26666667
210	7	210	5.56666667	210	5.53333333	210	5.36666667
240	7	240	5.66666667	240	5.53333333	240	5.36666667
270	7.1	270	5.7	270	5.56666667	270	5.36666667
300	7.033333333	300	5.73333333	300	5.63333333	300	5.43333333

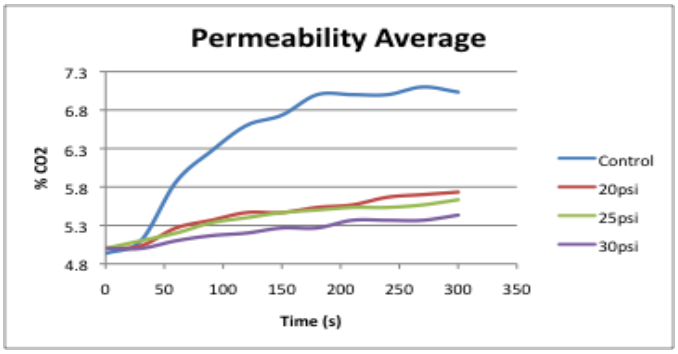

Figure 43: Average Permeability Data

Chapter 6: Discussion

The results of the CO_2 dissipation and impermeability tests demonstrate that the air curtain was effective in reducing CO_2 loss inside the enclosure and creating an impermeable barrier. However, the results of the temperature and humidity dissipation tests demonstrate that temperature and humidity dispersed more rapidly from the incubator with the air curtain functioning than not. This implies that the device formed a barrier acting to inhibit the entrance of particles; however it was unable to effectively preserve all conditions within the incubator.

Overall, our results showed that the air curtain running at 20 psi was the most effective in achieving the goals of the project, followed by 25 psi, and 30 psi respectively. The air curtain running at 20 psi was the most effective in preventing humidity loss by far, was on par in preventing temperature and carbon dioxide loss, and was still effective in preventing air from entering the incubator, as shown by the permeability test. We believe this is due to the slower, more laminar flow that the 20 psi provided, which minimized air flow from the air curtain entering and disrupting the environment inside the incubator. While the 25 and 30 psi air curtain tests were both slightly more effective in the carbon dioxide permeability tests, they were both more ineffective in the humidity, temperature, and carbon dioxide loss tests.

The compressed air used by the air curtain had a minimal impact on preserving the temperature and humidity inside the enclosure, but for most of the data it was observed to have a slight negative effect when compared to the control. Due to the position of the air curtain, compressed air from the device was blown into the interior of the incubator. This compressed air was ambient (~23C) and inherently dry (due to the compression). Therefore when this air was introduced during testing, it reduced the temperature and humidity within the incubator. Additionally, to presence of moving air in the enclosure generated turbulence that contributed to the temp and humidity drop. The results from the CO_2 dissipation test were not negatively impacted by this because temperature and humidity do not significantly affect CO_2 concentration, and the CO_2 present within the incubator was recirculated by the air curtain. When the temperature dropped beneath 37C, the incubator's heaters turned on. This action proved to do little to improve the temperature because the water-jacketed incubator was slow

to adjust for the rapid temperature drop. Despite the water pan and active humidification, the addition of dry moving air caused a substantial humidity drop.

The temperature and humidity drop was also attributed to areas of weak air flow or "dead space" across the incubator opening. "Dead space" was found at the bottom outside edges of the enclosure door. At these locations temperature and humidity from within the incubator is able to escape because a strong laminar flow of air is not generated by the air curtain. The suspected causes of "dead space" include errors in the manufacturing of the air curtain, and the width of the air curtain in relation to the width of the door (in this study, the air curtain extended the enclosure door on each side by only one inch). However, despite the existence of "dead space", it was demonstrated that a stream of CO_2 gas blown perpendicular to the air curtain was incapable of permeating the curtain as evidenced by little or no change in CO_2 levels inside the incubator when the air curtain was on compared to the control experiment (air curtain off). This showcases the air curtain's capabilities at protecting the internal environment from the external environment.

6.1 Economic Impact

The air curtain enclosure that was described in this report would have little impact on the economy if it was introduced into the market. Most of the materials and equipment utilized in our design are common in cell culture incubators and biological safety cabinets, therefore the cost of our proposed design would be similar. Additionally, the starting materials for the air curtain component are commonly available and cost effective.

6.2 Environmental Impact

All materials used in the design are commonly found in similar laboratory equipment. Therefore the environmental impact associated with our design would be equivalent to that of biological safety cabinets and cell culture incubators.

6.3 Societal Influence

The use of our design in biological research could promote the advancement of complex cell printing applications like *in vitro* tissue and organ printing. The progress made in this field could greatly influence the future of healthcare.

6.4 Ethical Concerns

The ethical concerns associated with our design are no different than the ethical concerns associated with the use of biological safety cabinets or cell culture incubators.

6.5 Health and Safety Issues

Our design would not significantly impact laboratory safety. The health issues associated with our design are no different than those associated with the use of biological safety cabinets or cell culture incubators.

6.6 Manufacturability

Both the enclosure and the air curtain designs are highly manufacturable. All materials used and machines utilized are commonly available in the manufacturing industry. Additionally, the tolerances for the design specifications are easily achievable using technology that already exists.

6.7 Sustainability

All materials and equipment used in the design are commonly found in similar laboratory equipment. Therefore the impact on sustainability associated with our design would be equivalent to that of biological safety cabinets and cell culture incubators.

Chapter 7: Final Design and Validation

This chapter describes the materials and methods that were used by the design team in order construct the air curtain prototype and carry out experiments which test the effectiveness of the prototype.

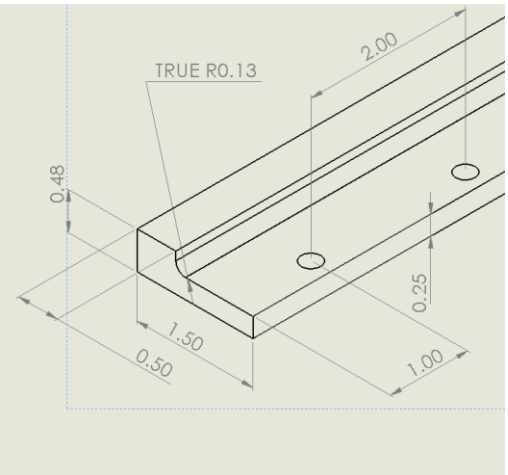

Figure 44: Air curtain bottom plate specifications

7.1 Air Curtain Fabrication

The air curtain design involved a long aluminum blade with inlets at both ends, which attached to laboratory tubes which joined each other via a two way hose splitter. The splitter was attached to a pressure gauge which was directly connected to a source of compressed air.

The blade portion of the air curtain design was comprised of two custom milled aluminum plates fixed together with 1/4in nuts and bolts. A 72 x 1.5 x 0.5 inch rectangular bar of multipurpose aluminum stock was used as raw material. Two 18.25in lengths of aluminum were cut from the 72in stock using a horizontal band saw. These two lengths served as the materials for the top and bottom plates of the air curtain design. Each length of aluminum was then milled using a VM3 milling machine. Figure 44 shows the specifications of the bottom plate. Three different milling operations were used to generate the bottom plate. First, a 1/2in end mill was used to cut the groove in the plate. Second, a 1/8

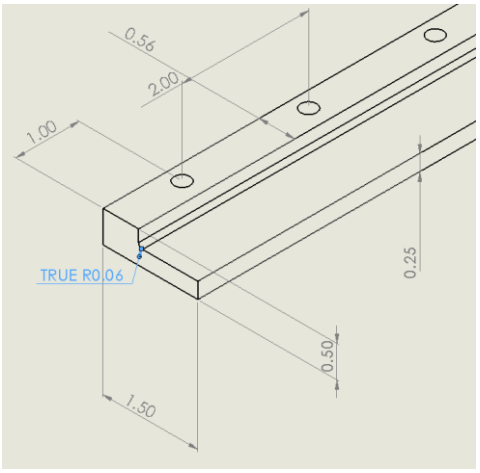

Figure 45: Air Curtain Top Plate Specifications

ball end mill was used to cut the radius at the corner of the groove. Third, a 1/4in drill bit was used to drill the nine 2in spaced holes 1/4in from the edge of the back side of the plate. Figure 45 shows the specifications of the top plate. Like the bottom plate, the same three milling operations were used on the top plate. However, there were three notable differences. First, during the operation using the 1/2in end mill, an additional 5/1000in of aluminum was removed from the surface after the initial 1/4in cut. Second, a 1/4in ball end mill was used to cut the radius at the corner of the groove instead of the 1/8in ball end mill. Third, the nine 1/4in holes were drilled 1/4in from the edge on the thinner side of the plate.

In order to seal the two ends of the air curtain and create a suitable interface for integrating compressed air into the design, end caps were made. The end caps were 0.5 x 1.25 x 0.25 inch rectangular pieces of acrylic with a 1/4in hole cut in the center of the largest face. These end caps were manufactured by using a laser cutter and scrap acrylic. Figure 46 shows the exploded assembly of the two plates including end caps and nuts and bolts. Super glue and electrical tape were used to secure the end caps to the plates.

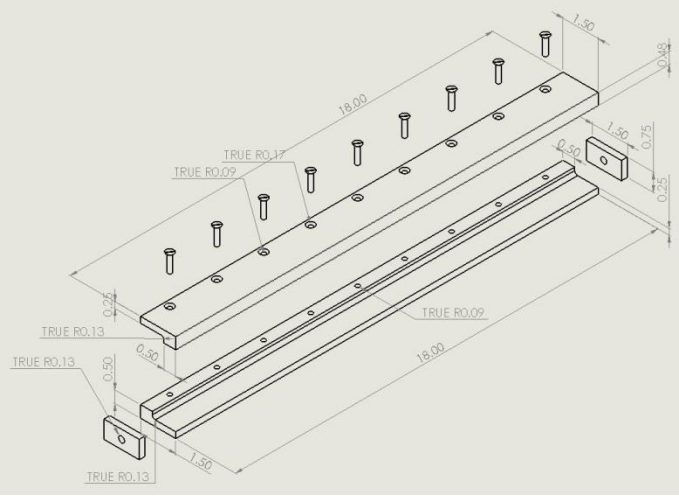

Figure 46: Exploded Air Curtain Assembly with End Caps

In order to create a sealed inlet which successfully interfaces with laboratory tubing two 1in long pieces of 1/4in diameter copper pipe were cut using a handsaw. These pieces of copper pipe were placed in the end caps and secured using Teflon tap and super glue. Approximately half the length of each pipe was left protruding from each of the end caps, thereby forming inlets for compressed air.

The remaining portion of the air curtain design involved configuring a pressure gauge with a two-way hose splitter and two lengths of tubing that interfaced with the inlets of the air curtain. A pressure gauge and two-way hose splitter were purchased and screwed together. Three 1/4in barbed hose fittings were purchased. These fittings were used to interface the ends of the two-way splitter and the pressure gauge with 1/4in laboratory tubing. Two 3ft lengths and one 4in length of laboratory tubing were cut. The three foot lengths connected the outlets of the two-way splitter to the inlets of the air curtain. The 4in length of tubing connected the pressure gauge to the compressed air supply.

7.2 Incubator Door and Air Curtain Mounting Rig

In order to carry out experiments that tested the effectiveness of the air curtain prototype, a CO_2 incubator coupled with a custom acrylic door and air curtain mounting rig was used. The CO_2 incubator belonged to the Biomedical Engineering Department at Worcester Polytechnic Institute. The acrylic door and air curtain mounting rig was custom made by the design team.

A 1/4in thick 20 x 26 inch clear acrylic sheet was used as raw material for the incubator door. Using a vertical band saw, the acrylic sheet was cut in two. The dimensions of the two resulting panels were 20 x 17in and 20 x 9in. Using a laser cutter, a 16 x 10in rectangular hole was cut into the 20 x 17in panel along with an series of thirteen 1/4in holes positioned 1in from the edges of the bottom and the two sides of the panel. Figure 47 shows a drawing of the 20 x 17 inch panel.

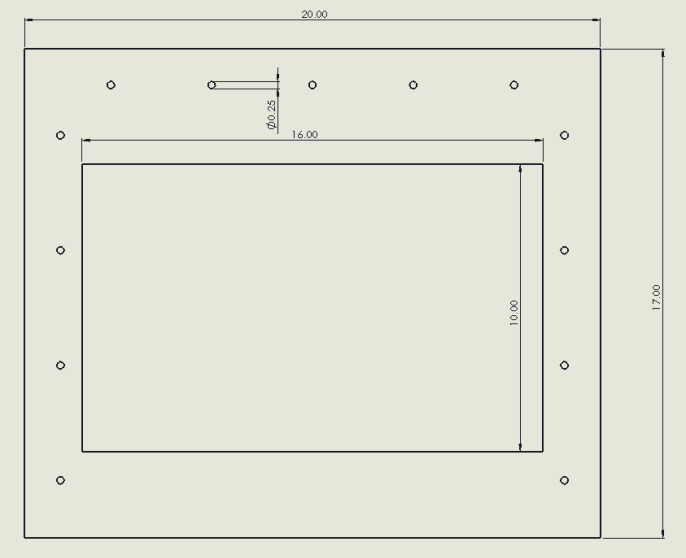

Figure 47: 20 x 17in Incubator Door Panel

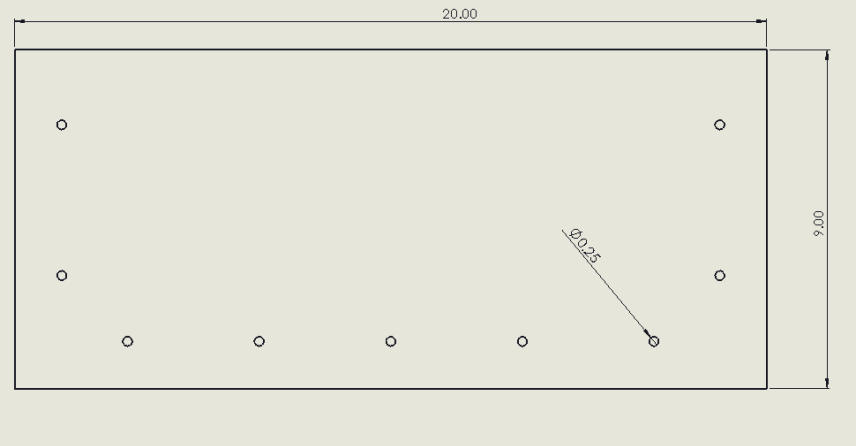

Figure 48: 20 x 9in Incubator Door Panel

For the 20 x 9in panel, a series of nine 1/4in holes was cut 1in from the edges of the top and the sides. Figure 48 shows a drawing of the 20 x 9in panel.

Using super glue and electrical tape, the top of the first panel was joined with bottom of the second, thereby resulting in an acrylic door which extends the length and width of the

incubator's opening. Acrylic tabs were made using a laser cutter in order to be used with 1/4in nuts and bolts to effectively secure the door to the incubator. Figure 49 shows the drawing of the tabs.

In order to secure the air curtain directly above the 16 x 10in opening in the acrylic door and to allow for the angle of the air blade to be easily adjusted, a mounting rig was generated. The rig was comprised of two plates that interfaced with the aluminum blade. The first plate was attached on the inner face of the aluminum blade and had hinges that allowed for angle adjustment. The second plate was attached on the outer face of the blade and functioned to secure the air curtain at whatever angle the user chose. The hinges of the first plate were secured to two rectangular spacers that were firmly fixed to the surface of the acrylic incubator door via superglue and screws. The spacers were positioned so that the plane of the bottom of the air curtain was the same as the top of the opening in the door. With the exception of the metal hinges, screws, nuts and bolts, all pieces of the mounting rig were made from acrylic using a laser cutter. Figure 50 shows the assembled incubator door and mounted air curtain.

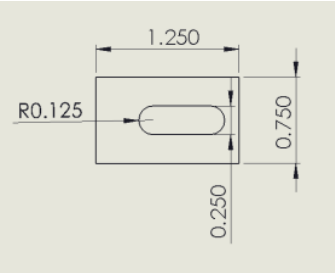

Figure 49: Door Tab

Figure 50: Air Curtain Rig with Attached Air Curtain

7.3 Experimental Procedure

7.3.1 Air Curtain Permeability Test

The permeability of the air curtain prototype to external ambient air must be quantified in order to demonstrate its degree of effectiveness. In order to do this, a steady, gentle stream of carbon dioxide aimed at the center of the experimental rig's door originating from a set distance of 36" in front of the center of the opening was utilized in conjunction with the carbon dioxide sensor inside the incubator.

Materials

- Incubator
- Air curtain prototype and rig
- Compressed CO_2
- Regulator valve
- Needle/ball valve

Protocol

1. The compressed CO_2 was disconnected from the incubator and connected to a regulator valve (if necessary) to control the back pressure in the CO_2 line and subsequently to a needle/ball valve to control CO_2 outflow.

2. The needle/ball valve outlet was positioned at a set distance in front of the incubator rig opening, aimed directly at the door.

3. The ideal starting back pressure for the stream of CO_2 was determined by increasing the pressure until a significant increase in CO_2 concentration inside of the incubator over the duration of approximately 20 minutes is sensed (e.g. ambient to 5-10% CO_2). This was done with the air blade OFF.

4. Once a suitable starting back pressure is determined, the test was conducted with and without the air curtain present for approximately 20 minutes.

5. Several iterations was performed with and without the air curtain present, with pressure being increased by an increment of 10% of the starting pressure with each iteration.

6. The CO_2 sensor reading was recorded every 30 seconds for each test.

7.3.2 Temperature Test

In order to show the effectiveness of our air curtain in maintaining the temperature of the enclosure's environment, the incubator was heated and the door was opened with the air curtain on at various pressures and with it off.

Materials

- Incubator
- Air curtain prototype and rig

Protocol

1. The incubator was set to 37 degrees Celsius.
2. When the incubator reached this temperature, testing began.
3. The door was taken off, and temperature readings were taken every 30 seconds for 10 minutes for each test. This was done with the air curtain off.
4. When the test is over, the door was replaced and the incubator was allowed to return to 37 degrees.
5. This test was repeated two more times, for a total of three tests.

6. This testing procedure was repeated with the air blade running at 20 psi, 25 psi, and 30 psi.

7.3.3 Humidity Test

Maintaining humidity was another important test for validating our air curtain. This was done by placing a water pan in the incubator, and recording the humidity loss with the air curtain on at various pressures and with it off.

Materials

- Incubator
- Air curtain prototype and rig
- Water pan
- Humidity sensor

Protocol

1. The incubator was set to 37 degrees Celsius.
2. The humidity sensor was placed in the incubator.
3. A water pan was placed in the incubator and filled with approximately 500 mL of hot water.
4. When the humidity reaches about 80% testing began.
5. The door was taken off, and humidity readings were taken every 30 seconds for 10 minutes for each test. This was done with the air curtain off.
6. When the test is over, the door was replaced and the humidity was allowed to return to about 80%.
7. This test was repeated two more times, for a total of three tests.
8. This testing procedure was repeated with the air blade running at 20 psi, 25 psi, and 30 psi.

7.3.4 Carbon Dioxide Test

With carbon dioxide concentration being an important factor for cell culturing and viability, our air curtain needed to maintain the concentration within the enclosure. To validate

this, we opened the enclosure and recorded carbon dioxide loss with the air curtain on at various pressures and with it off.

Materials

- Incubator
- Air curtain prototype and rig

Protocol

1. The incubator was set to 5.6% carbon dioxide.
2. When the carbon dioxide reaches 5.6% testing began.
3. The door was taken off, and carbon dioxide concentration readings were taken every 30 seconds for 10 minutes for each test. This was done with the air curtain off.
4. When the test is over, the door was replaced and the carbon dioxide concentration was allowed to return to 5.6%.
5. This test was repeated two more times, for a total of three tests.
6. This testing procedure was repeated with the air blade running at 20 psi, 25 psi, and 30 psi.

Chapter 8: Conclusion and Recommendations

Through our testing we demonstrated that the air curtain we designed was successful in maintaining the CO_2 levels inside a cell culture hood and prevented external conditions from affecting the internal environment, as evidenced by CO_2 permeability data. The air curtain was also effective is preserving the humidity levels within the incubator. However, it was not effective in preventing temperature loss due to the inherent inefficiency of a water jacketed incubator and the limitations presented by our prototype. Overall, this was a successful project that demonstrates the great potential that air curtain technology has biomedical engineering field.

In order to improve the functioning of air curtain in the final design, we propose 5 modifications: (1) Precondition the compressed air used in air curtain to 37^{o}C and >90% humidity. This could be accomplished easiest by developing a system to recycle the incubator's air through an air compressor. (2) Manufacture the air curtain to have an ultra-polished finish on its slit surfaces. This could be achieved by using a metal polishing and buffing machine. (3) Ensure that the air curtain extends the width of the incubator door on each side by at least 3in. This could be achieved either by redesigning the air curtain to be wider, or by reducing the width of the incubator door. (4) Use a direct heat incubator as opposed to water-jacketed incubator to improve the time it takes for the temperature to recover. (5) Use two identical air curtains mounted in reverse orientation above and below the threshold of the incubator door. This should improve the preservation of internal conditions as well as the impermeability of the curtain.

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