LAMINAR FLOW HOOD SYSTEM DESIGN

BY

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ABSTRACT

In 2011 health care costs for transplants were over 12 billion dollars in the United States for evaluation, procurement, facilities use, physicians, and post-transplant check-ups. In 2012 burns hospitalized 40,000 people and over 17,000 people received organ transplants. Sadly, the number of organ donors greatly lags the number of people on the transplant waiting list and the gap has widened over the decades. While preventative health care is extremely important, researching tissue engineering (TE) to treat patients in fatal condition provides alternatives for replacing or repairing a variety of damaged tissue. These alternative treatments can considerably reduce the supply-demand gap for transplant organs and hopefully reduce the cost of such procedures in the future. Breakthroughs in TE such as tracheal transplants, porcine heart valves, dermal tissue, and others show substantial potential in the medical field. However, technology for this relatively new field of research limits its progress. For example, growing tissues require a specific environment for stability and optimum growth. In this project a laminar flow hood is built which sustains an ideal tissue environment and can assist TE research and development. This paper outlines the control system for a laminar flow hood, also referred to as a tissue engineering hood, in which scientists could engineer and investigate tissues. The laminar flow hood keeps tissues alive by regulating CO_2 concentration, temperature, airflow, and humidity to provide the optimum environment for tissues to thrive.

I. Introduction

Due to the problem of a short supply of human transplant tissue, the popularity of tissue engineering has grown in the medical field. In 2009 over 100,000 people needed organs with only 28,000 available for transplant. Tissue engineering can help alleviate this gap thanks to major prospects for future development of artificial organs for transplant. However, because tissue engineering is an emerging field of research, a lack of supporting technology hinders its progress. Cell organization and tissue incubation affect the viability of the artificial tissues. Without techniques to accomplish these requirements, test engineering becomes much more difficult.

The advent of 3D printers has significantly helped the structural progress of TE. This, in combination with a specially designed laminar flow hood, makes engineering tissues possible. Tissues and organs have distinct shapes and cell organizations, making 3D printing the perfect structural tool due to its high degree of precision. 3D printers can be controlled to accurately produce the multidimensional scaffold required by tissues for growth. Placing a 3D printer inside a laminar flow hood solves the challenge of sustaining an environment conducive to tissue growth. The laminar flow hood controls several factors that affect cell viability such as carbon dioxide concentration, temperature, and humidity. The flow hood seeks to mimic the levels of these three factors in a human, as well as sterility from the outside environment.

Optimum cell viability requires a temperature of 37°C, a humidity of 95%, and a CO_2 level around 5%. The laminar flow hood control system measures these three parameters using calibrated sensors, determining whether they are within acceptable ranges of their ideal level. Parameters falling outside of their ranges trigger heaters, a humidifier, or a CO_2 solenoid, which opens or closes to control the flow of gas, to maintain the balance of the hood environment. Sensor values exceeding their desired value disable the relative system while values under the targeted value enable the system. When the tissues are not being printed or incubated, a UV light-based sterilization can be run in the hood.

II. System Requirements

The laminar flow hood maintains user-selected temperature, humidity, CO2 concentration, and water temperature to sustain the ambient environment ideal for tissue growth. These four functions of the laminar flow hood can be completely enabled or disabled by toggle switches. When a function is enabled, a status LED should light up on the control panel. The airflow, however, is always controlled by the position of the vertically sliding door on the front of the hood. An open door activates high airflow and closed door activates low airflow. UV sterilization is manually controlled by the user.

The first four functions mentioned are all automated to achieve target settings selected by a sliding potentiometer which displays values to an LCD via analog-to-digital conversion and ATMEGA processing. The ATMEGA then actuates the corresponding output, such as a heater, humidifier, CO2 solenoid, or water pump, to achieve the selected setting by comparing the set value to values acquired from several sensors throughout the hood. At least six temperature sensors are averaged to find the ambient hood temperature. The average of two humidity sensors determines the hood humidity and the average of two CO_2 sensors determine the CO_2 concentration. All of the sensors are placed at various locations around the inside hood for the best results.

The laminar flow hood has two main operating modes. The first is stand-by mode where only the blower motor in its high setting and UV light are available for use. This mode is available as soon as the hood is plugged in. To enable the second mode, the incubation mode, an AC-DC power converter is powered on via a toggle switch to support all of the supporting systems, humidity, temperature, etc., for tissue incubation. The AC-DC converter also has a status LED on the control panel that lights up when it is active.

Table 1 below summarizes the system specifications for the range, accuracy, and setting increments of the four incubation functions.

Table 1: System Specifications: Range, Setting Increment, and Accuracy of Each Laminar Flow Hood System

Function	Range	Increment	Accuracy
Ambient Temperature	20°C - 50°C	1°C	±3°C
Humidity	20% - 100%	1%	±2%
CO2 Concentration	0.2% - 10%	0.1%	±0.1%
Water Temperature	4°C - 50°C	1°C	±3°C

III. Design

Depending on sensor readings, ATMEGA 2560 controls turn devices on, off, high or low via MOSFETs and relays to maintain appropriate living tissue environments which are set using potentiometers and internal ATMEGA analog to digital converters. The block diagram in Figure 1 shows a block diagram of the system.

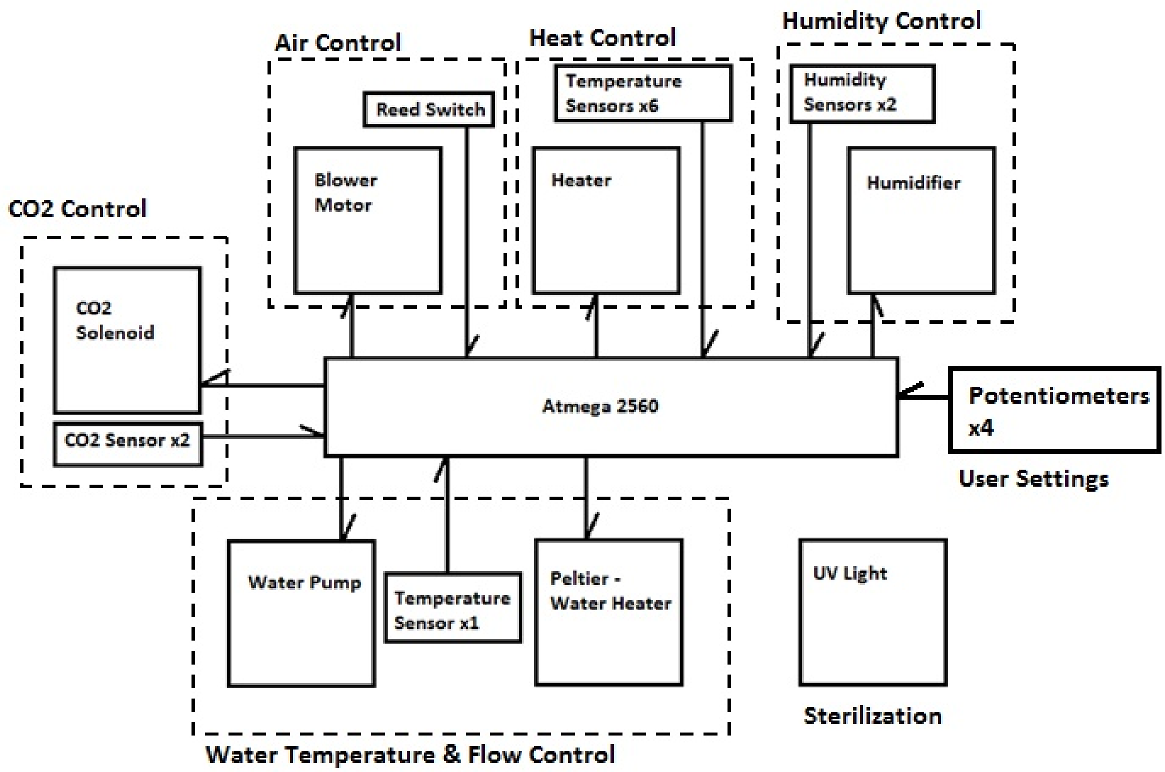

Figure 1: Tissue Engineering Hood Block Diagram

In addition to reading sensors and controlling the different functions of the hood control system, override toggle switches enable or disable certain functions of the laminar flow hood to increase the customizability of the system. An LED indicates whether a function of the hood is active or not. More in-depth diagrams for each system can be found in the subsequent chapter. The bill of materials to implement all electrical systems can be found in Appendix E.

Figure 2 depicts the planned flow diagram of the microcontroller. A one-time operation initializes any communications systems required for any hardware, for example, the LCD display. The ATMEGA then reads and stores the values of all the sensors of the system to determine its current environmental conditions. These values are displayed on the LCD display for the user to read. The ATMEGA analog-to-digital ports then read and store all of the control panel potentiometers which correspond to either the CO_2, humidity, temperature, or water temperature settings. If these settings change, they are displayed to the LCD so the user can accurately define their desired hood environment. The microcontroller then checks the control panel toggles to see which functions of the tissue engineering hood should be activated and lights a corresponding LED to show the user that function is active. Finally, the active function actuators, the humidifier, CO_2 solenoid, heater, or water heater, activate until their function is turned off or their target setting is achieved.

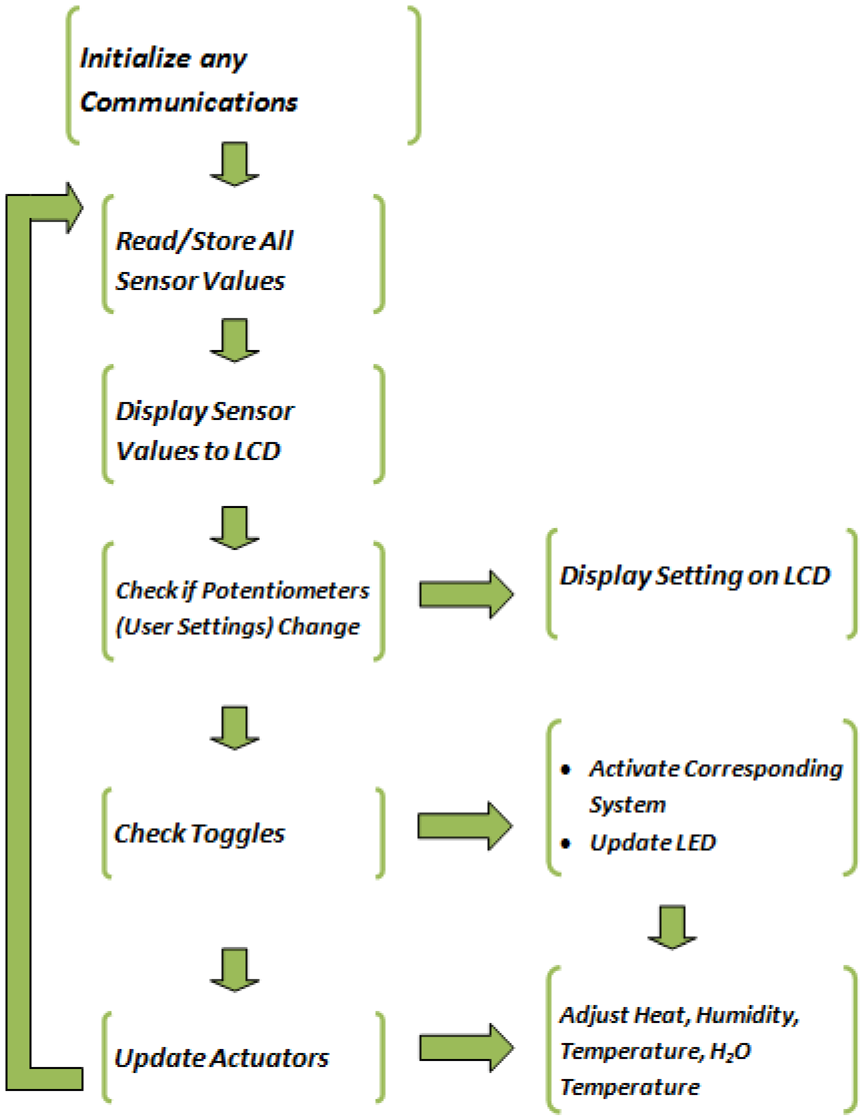

Figure 2: Initial Flow Diagram

IV. Implementation

1. *Blower Motor Control*

The blower motor operates on either a high setting or a low setting, affecting the amount of airflow within the hood. When the vertically sliding door of the hood closes, airflow is low. If the door slides open, the airflow is high. Figure 3 describes the blower motor schematic.

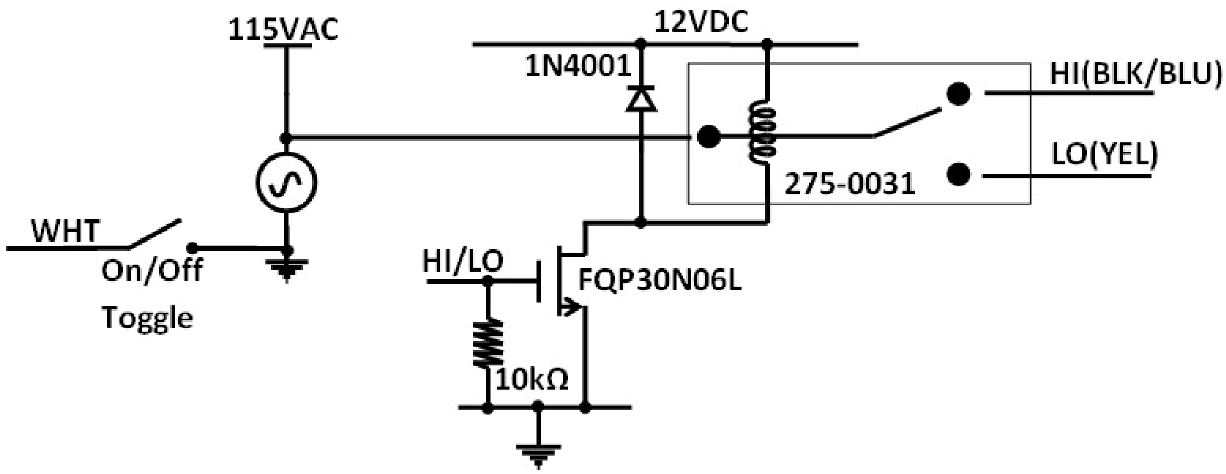

Figure 3: Blower Motor Schematic

In Figure 3 a HI/LO control signal from the ATMEGA 2560 switches power between high and low blower motor control lines. A high signal turns the MOSFET on allowing current to run through a relay coil which switches the 115VAC source voltage from the HI line of the blower motor to the LO line, thereby decreasing the laminar flow hood airflow. A flyback diode protects the MOSFET from reverse voltage spikes. An additional on/off toggle switch disconnects the neutral line of the blower motor, completely powering off the blower motor.

Figure 4 describes a schematic for detecting when the laminar flow hood door opens using a Reed switch.

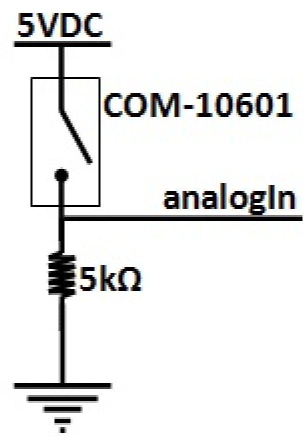

Figure 4: Blower Motor Control Arduino Input Schematic

When the flow hood door is open, the Reed switch opens and the microcontroller reads a low voltage and pulls the blower motor control MOSFET gate low resulting in high air flow. Otherwise, a magnet built into the flow hood door closes the Reed switch when the door is in the closed position and the microcontroller pulls the MOSFET gate high, switching the blower motor power to low.

Otherwise, when the switch between the 115V source and converter opens (see Figure 13) power to the relay cuts off and the blower motor relay defaults to its normally open position of high.

2. *WATER PUMP AND CO_2 SOLENOID CONTROL*

The water pump system is used as extra temperature control in the hood. The temperature of the water within the pump system is read by a temperature sensor and then adjusted between 4°C and 50°C by a Peltier heating and cooling unit controlled by the ATMEGA. The setting for the water temperature can be adjusted in 1°C increments and should have a temperature accuracy of ±3°C. Once the water temperature has reached the target setting the water pump is activated. The CO_2 solenoid controls the carbon dioxide concentration within the hood by first calculating the average of two CO_2 sensor outputs and then opening or closing a solenoid attached to a CO_2 tank. The carbon dioxide concentration can be adjusted between 0.2% and 10% in 0.1% increments ideally with ±0.1% accuracy. Both the water pump and the CO_2 solenoid can be controlled the same way. Figure 5 shows a schematic of the water pump and solenoid controls.

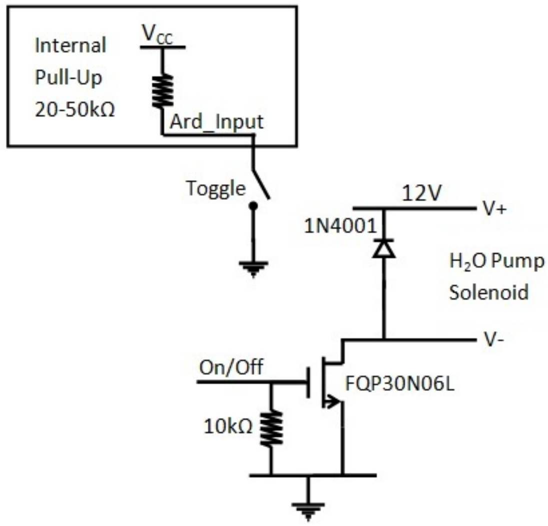

Figure 5: Water Pump and Solenoid Controls

Depending on user-input, water temperature, or CO_2 concentration, the ATMEGA sends high or low On/Off signals to a MOSFET, allowing current to power a water pump or CO_2 solenoid from a 12V source. Toggle switches, as shown in the upper left of Figure 5, enable or disable the use of either of these two features at any time. Closing a toggle switch corresponding to the solenoid or water pump, or switching it "on", grounds a digital ATMEGA input and signals an ATMEGA output to automate control of its respective device. Opening the switch, or turning it "off", sets a corresponding digital ATMEGA input to "high" via an internal pull-up resistor which the ATMEGA interprets to pull the gate of the desired MOSFET low, cutting off power and disabling automated control via software.

The ATMEGA controls the CO_2 solenoid based on the average of two COZIR CO_2 sensor readings. These communicate using universal asynchronous receiver transmitter communication, also called UART. Both CO_2 sensors have a Tx and Rx line through which bytes of data control or read the COZIR sensors. If the average reading is less than the desired level the CO_2 solenoid opens as the ATMEGA pulls the controlling MOSFET gate high. When CO_2 levels are equal to or greater than the desired level the ATMEGA pulls the gate low, closing the solenoid and cutting off the CO_2 supply.

A temperature sensor controls when the water pump turns on and off in addition to a Peltier heating and cooling unit. Once the Peltier has achieved the desired water temperature of the water pump, the ATMEGA activates the water pump via its controlling MOSFET. Figure 6 below shows a schematic of the Peltier control circuit.

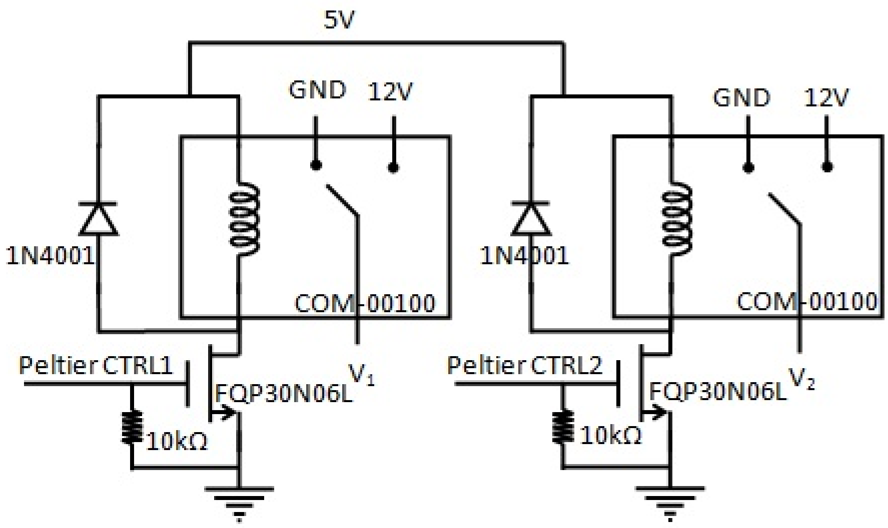

Figure 6: Schematic of Peltier Controls

Both Peltier control relays default to ground, disabling the Peltier. Depending on the temperature sensor readings, positive 12V across Peltier V_1 and V_2 lines enables the heating function to increase the water temperature to the desired setting. Negative 12V across V_1 and V_2 enable the cooling feature of the Peltier. Whenever the Peltier is heating or cooling, the ATMEGA activates an on-board fan via a MOSFET to disperse excess heat. Figure 7 shows a schematic for the Peltier fan.

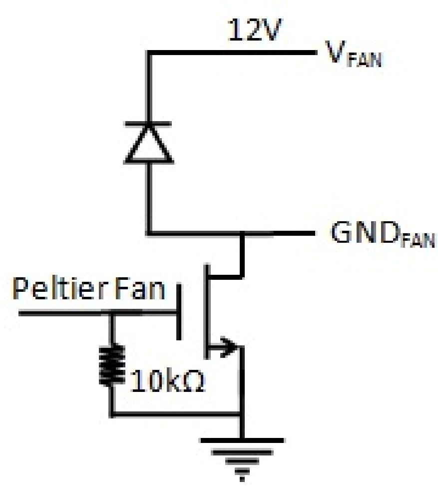

Figure 7: Peltier Fan Control

3. *HEATER CONTROL*

Using a the average of at least 6 temperature sensors scattered throughout the hood, the ATMEGA controls a heating unit consisting of a large heat sink with four heating pads adhered to its surface to increase the temperature of the hood between 20°C and 50°C. The set temperature can be adjusted in 1°C increments and should have an accuracy of ±3°C. Figure 8 shows a heater control schematic.

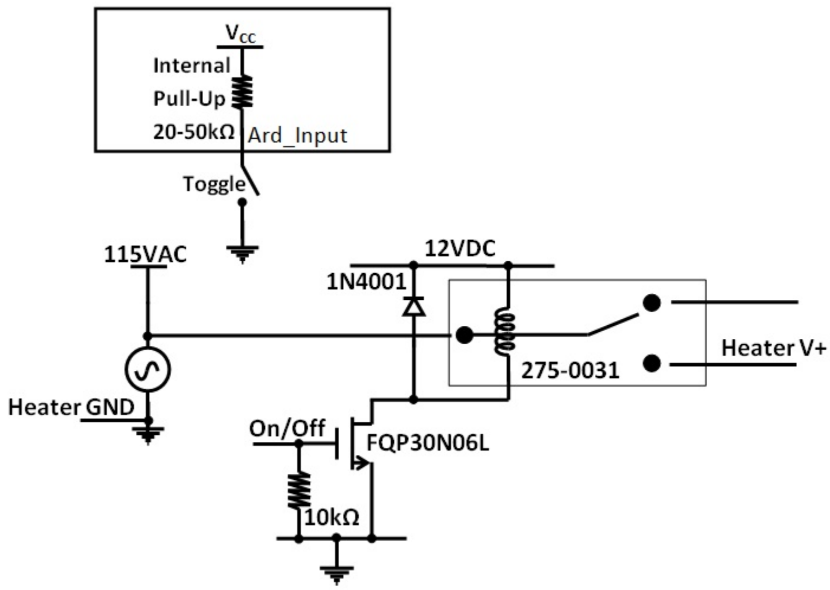

Figure 8: Heater Control Schematic

A MOSFET controlled by a digital ATMEGA output is pulled high when the hood temperature, determined by eight temperature sensors, drops below a certain range. This allows current to flow through the relay coil which switches to allow the 115V source to connect to the source terminal of the heater. When the digital output is pulled low, the relay switches to an unconnected terminal which disengages the heaters from their source. An ATMEGA input pin with an internal pullup resistor controls the override feature of the heaters. When the toggle switch is open, or "off", the digital input pin pulls high and the ATMEGA interprets it to deactivate automated heating. Otherwise when the toggle switch closes in the "on" position, the digital input pin pulls low which the ATMEGA interprets to activate automated heating. Figure 9 below shows the temperature sensor pinout.

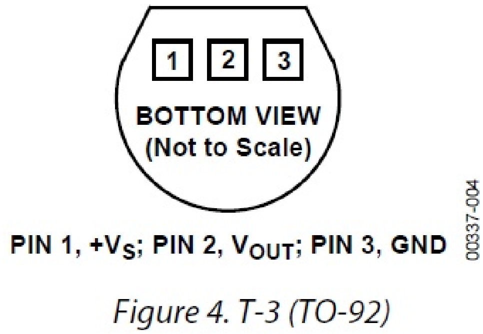

Figure 9: Temperature Sensor Pinout

The V_{OUT} pin of the temperature sensor changes between 0V and 5V which, after analog-to-digital conversion via an analog ATMEGA pin, converts to a temperature using an equation. Using this reading the ATMEGA activates or deactivates the heater via the MOSFET in Figure 8 to acquire the desired air temperature of the laminar flow hood. When the ATMEGA calculates a temperature lower than the desired temperature, it pulls the MOSFET gate high, activating the heater, otherwise the ATMEGA pulls the gate low, deactivating the heater.

4. Humidifier Control

The ATMEGA calculates the average of two humidity sensors and controls a humidifier to adjust the humidity within the hood. The humidity of the hood can be set between 20% to 100% in 1% increments and should have an accuracy of ±2%. Figure 10 shows how the ATMEGA controls the humidifier.

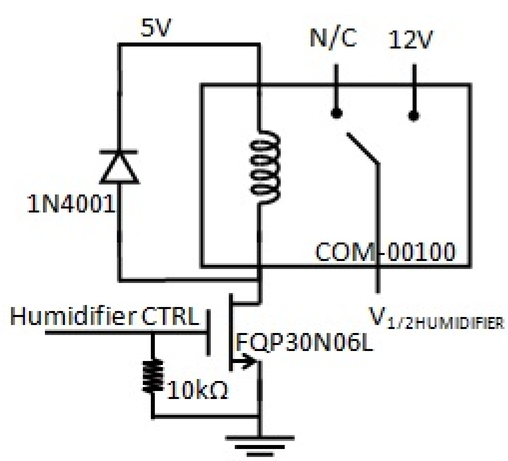

Figure 10: Humidifier Control Schematic

Two supply voltage lines from the humidifier need to connect or disconnect from a 12V source in order to activate a base fan and a dispersion fan. The two fans do not need to function independently and are both tied to the pin of the relay not connected by default. In this way, by default the relay connection is normally open. Otherwise, depending on the current humidity the ATMEGA controls when the humidifier activates by pulling its controlling MOSFET gate high and when the humidifier deactivates by pulling the gate low. The control circuit, however, can be reduced to a MOSFET without a relay.

ATMEGA control of the humidifier depends on the average of two HH10D humidity sensor readings. Figure 11 below shows the sensor pinout.

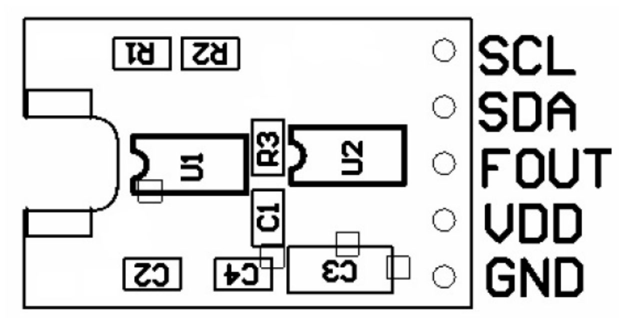

Figure 11: Humidity Sensor Pinout

The humidity sensors communicate with the controller using inter-integrated communications, or I2C. I2C uses a shared data line and clock line, SDA and SCL, to communicate with different devices by transmitting an address before sending data. The HH10Ds both have the same address so they share a data and clock line. Figure 12 shows how ATMEGA controls a MOSFET to switch a relay to read the frequency of one sensor and then the next using a frequency counter library. These frequencies are then used to calculate the humidity read by each sensor. After storing both humidity values the ATMEGA averages them and turns the humidifier on or off to adjust the humidity of the hood as explained earlier.

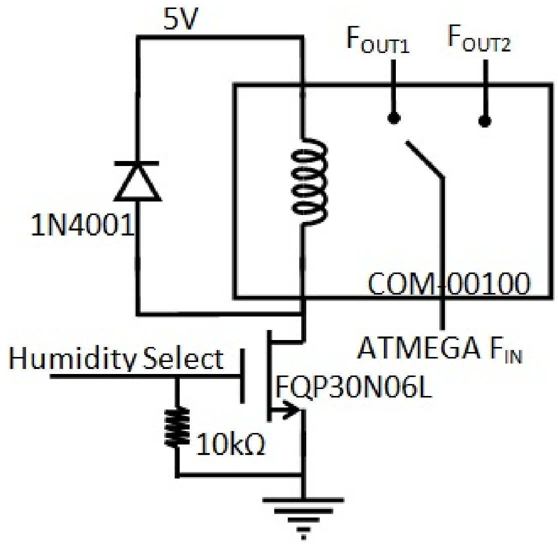

Figure 12: Humidity Sensor Select Schematic

5. *Power Analysis*

Table 2 compiles the power requirements needed to design the system.

Table 2: Power Requirements

AC or DC	Input/Output	Qty (#)	Vin (V)	Iin (A)	Power (W)	Itot (A)	Ptot (A)
DC	Humidity Sensor	2	3.3	0.000018	0.0000594	0.000036	0.000119
DC	CO_2 Sensor	2	3.3	0.033	0.0035	0.066	0.007
DC	Temp Sensor	10	5	0.00005	0.00025	0.0005	0.0025
DC	Water Pump	1	12	1.46	18	1.46	18
DC	Humidifier	1	12	0.6	7.2	0.6	7.2
DC	CO_2 Solenoid	1	12	0.883333	10.6	0.883333	10.6
DC	ATMEGA 2560	1	12	0.002	0.024	0.002	0.024
DC	Peltier	1	12	2.8	33.6	2.8	33.6
AC	Heater	3	115	0.173913	20	0.521739	60
AC	Blower Motor	1	115	4	460	4	460
AC	UV Ballast	1	120	0.45	38	0.45	38
Summary:							
Power Source (V)		**Total Current (A)**			**Total Power (W)**		
DC	3.3	0.066036			0.0071188		
DC	5	0.0005			0.0025		
DC	12	5.745333333			69.424		
AC	115/120	4.97173913			558		

A wall outlet powers the heater, blower motor, and UV light. An AC-DC converter rated for 6 amps provides 12V sources for the Peltier heater, solenoid, humidifier, water pump, and microcontroller, as well as DC-DC converters for the 5V and 3.3V sources. The ATMEGA 2560 has sufficient sources for low power devices such as system status LEDS and humidity sensors which require less than 40mA of current. Figure 13 maps out power distribution for the system.

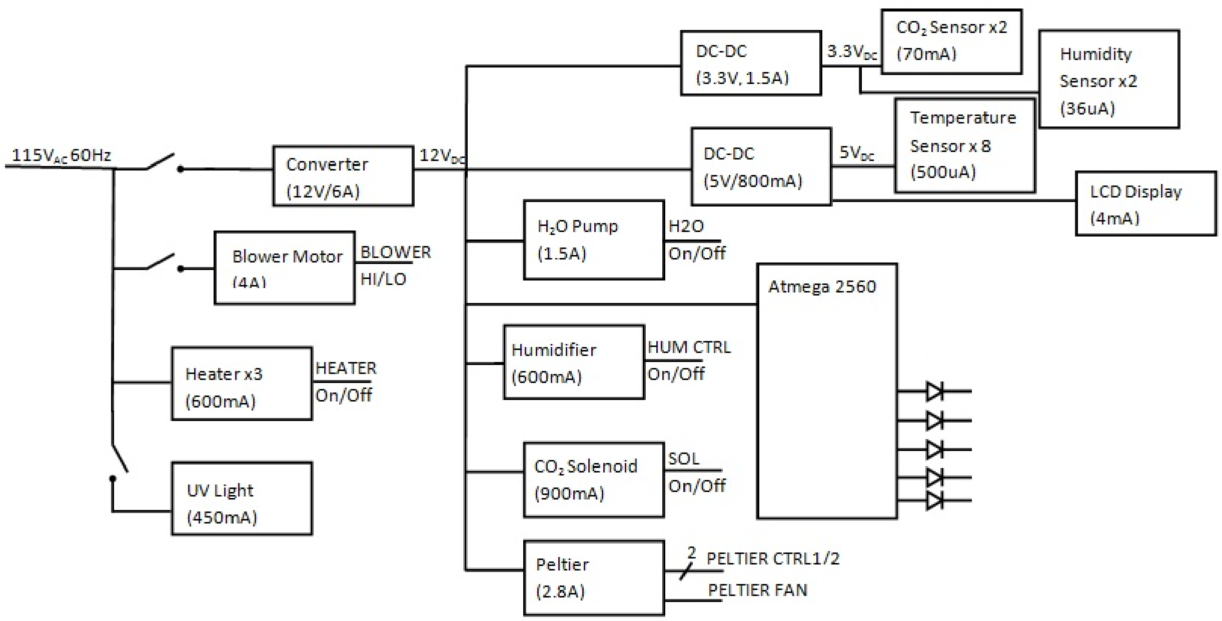

Figure 13: Power Distribution

The switch between the 115V source and converter facilitates constant blower motor airflow during long periods of time in which the hood idles and other hood functions are unnecessary. This means the laminar flow hood turns all power off only when unplugging it from the wall. The UV light operates independently of all other hood functions and requires its own switch. MOSFETs control power to all other devices.

The ATMEGA 2560 has several analog pins, which are preceded by an “A”, digital pins, and communication pins that function specially for I2C or UART. The microcontroller also has a 3.3V low-power voltage sources in addition to a few 5V sources which are useful for buttons and switches. The numerous pins available on the 2560 make it a valuable tool for controlling the many actuators, sensors, LEDs, potentiometers, and more. Table 3 shows the ATMEGA pinout for the system with a legend in the lower left indicating which LED, toggle, or potentiometer number corresponds to what system function.

Table 3: ATMEGA Pinout

ATMEGA Pin	Designation	ATMEGA Pin	Designation	ATMEGA Pin	Designation
IOREF	N/C	AREF	N/C	5V	N/C
RESET	N/C	GND	N/C	5V	N/C
3.3V	N/C	13	N/C	22	SCRN11
5V	5V Pots/Toggles	12	SCRN4	23	SCRN12
GND	Reed Switch Ground	11	SCRN6	24	SCRN13
GND	Arduino Power Ground	10	N/C	25	SCRN14
VIN	12V Arduino Power	9	N/C	26	HUM CTRL
A0	TEMP0	8	N/C	27	N/C
A1	TEMP1	7	N/C	28	HUM SEL
A2	TEMP2	6	N/C	29	PELTIER FAN
A3	TEMP3	5	N/C	30	PELTIER CTRL1
A4	TEMP4	4	N/C	31	PELTIER CTRL2
A5	TEMP5	3	N/C	32	H20 ON/OFF
A6	TEMP6	2	TOG0	33	HEATER ON/OFF
A7	TEMP7	1	TX	34	SOL ON/OFF
A8	TEMP8	0	N/C	35	BLOWER HI/LO
A9	POT0	14	TX2	36	REED
A10	POT1	15	N/C	37	LED4
A11	POT2	16	N/C	38	LED0
A12	POT3	17	N/C	39	LED1
A13	N/C	19	N/C	40	LED2
A14	N/C	19	N/C	41	LED3
A15	N/C	20	SDA	42	N/C
		21	SCL	43	TOG1
				44	TOG2
				45	TOG3
				46	N/C
				47	FOUT
				48	N/C
Toggle/LED/Potentiometer Legend				49	N/C
Number	**Control Panel Function**			50	RX2
0	**Temperature**			51	N/C
1	**Humidity**			52	RX
2	**CO_2 Concentration**			53	N/C
3	**Water Pump**			GND	LED Ground
4	**AC-DC Converter**			GND	Toggles & Potentiometers Ground

6. *FLOW DIAGRAM*

Figure 14 describes the basic flow diagram of the program which controls all of the systems. After initializing the LCD, sensors, and UART and I2C communications, a counter is incremented. This counter is used to control how often the humidity sensor is read partially due to the audible noise of the relay switching, but also to avoid severe delays in the loop of the program. After the counter increments, all of the sensor values are read and averaged. These are the values which are displayed on the LCD so the user can read what the current hood chamber environment is. The potentiometers which are used to set the desired system values are then checked to see if they have been changed. Changing the setting results in an LCD update with the new set value so the user knows exactly what value will be used to implement any system of the hood. After this, the toggles are checked to determine which system (heater, humidifier, CO_2 solenoid, or water pump) to activate. If a system is active, a flag is set so the following function knows which actuators should be activated to begin adjusting their respective output. The counter is then checked to see if it has exceeded 5, and if it does the counter is reset and the humidity sensors read the current humidity. The loop is then repeated from the point the humidity counter is incremented.

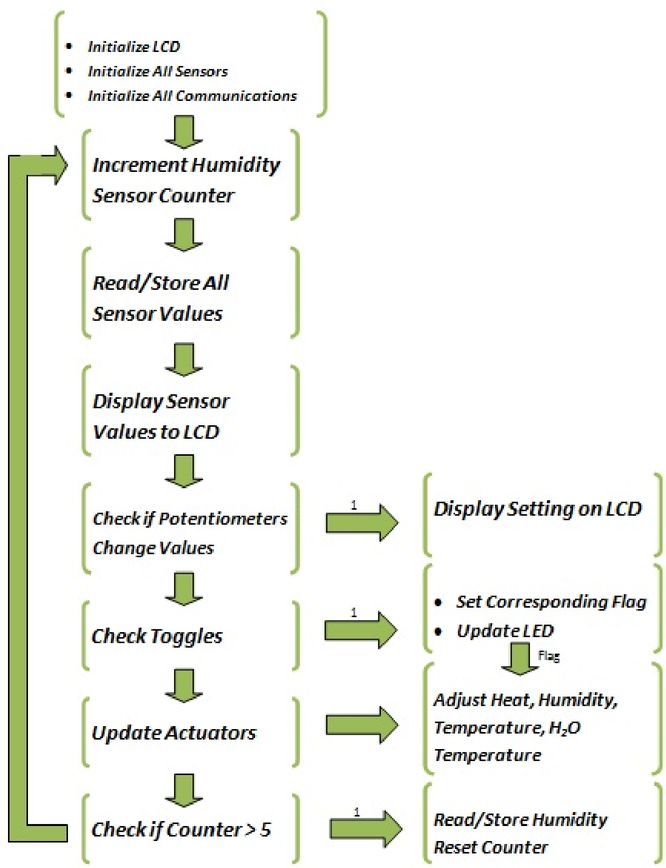

Figure 14: Basic Program Flow Diagram

V. Testing and Results

Before integrating any of the sensors they were bench tested. The temperature sensors showed special sensitivity to the length of wire, several feet, between the ATMEGA's analog-to-digital converter and the sensor. The result was an overall reduction of the detected temperature by a couple of degrees Fahrenheit, shown in Figure 15 below.

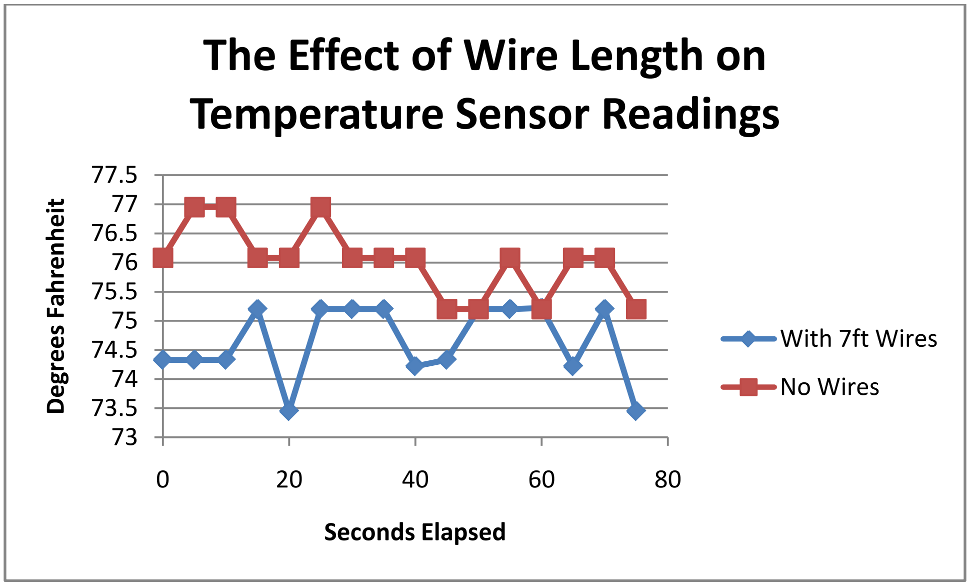

Figure 15: The Effect of Wire Length on Temperature Sensor Readings

Additionally, the humidity sensors were tested to ensure accurate measurement compared to two analog humidity meters. Figure 16 shows the resulting sensor readings when both analog meters read 40%. The results agree with the humidity sensor's data sheet which specifies an accuracy of 3%.

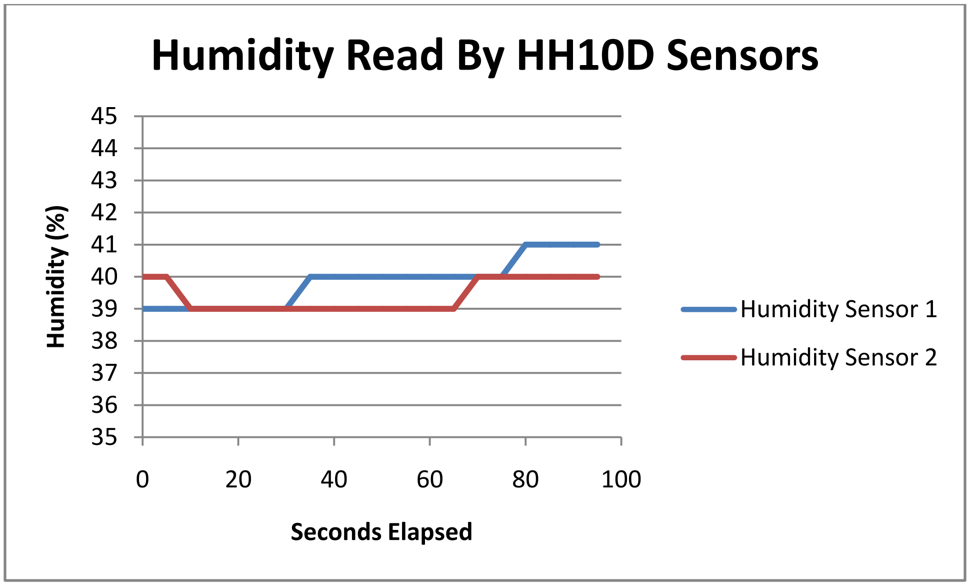

Figure 16: Humidity Sensor Accuracy Test Results

Pictures of the assembled tissue engineering hood can be found in Appendix D. After the hood was assembled, data was taken for the rate at which the temperature increased or decreased, the accuracy of the temperature readings, the accuracy of CO_2 concentration control, the rate at which the water heated or cooled, as well as how well the laminar flow hood was able to incubate living cells.

Figure 17 shows the rate at which the ambient temperature of the hood increased with four heating pads on. The resulting rate was 0.122°C per minute, which is very slow, over a range of 22°C to 29°C.

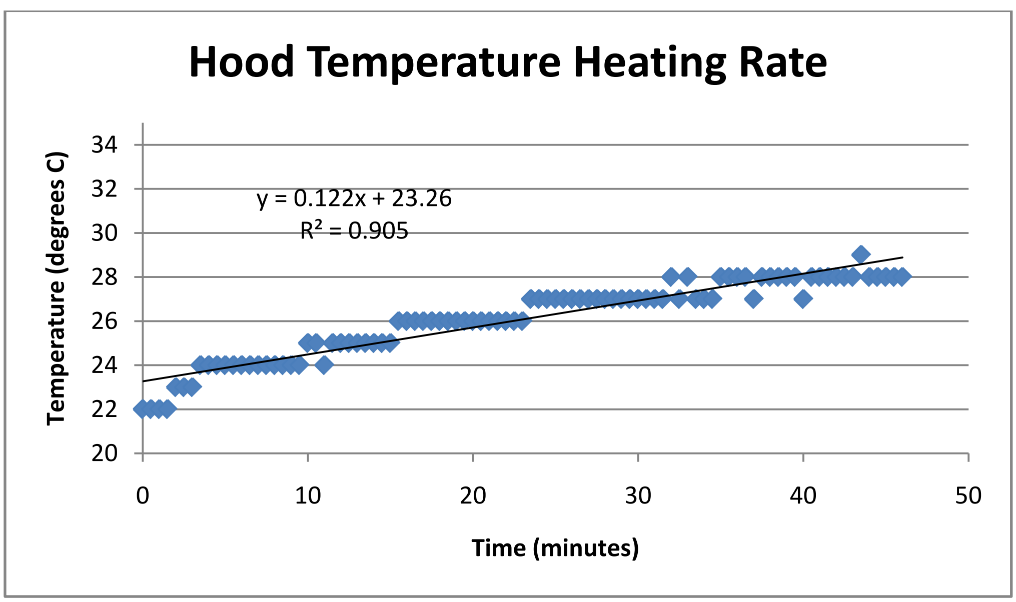

Figure 17: Hood Temperature Heating

The rate that the laminar flow hood ambient temperature decreased was much slower than the heating rate. Figure 18 shows a rate of -0.04°C over a range of 30°C to 26°C.

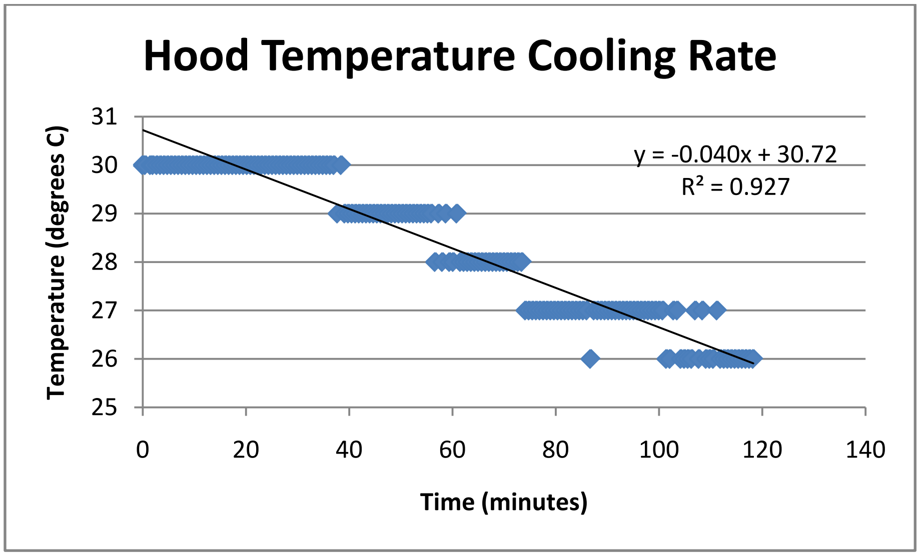

Figure 18: Hood Temperature Cooling Rate

The heating rate of the laminar flow hood was affected by the number of heaters used. Although the total number of heating pads was increased from three to four, it was still not enough to significantly speed up the rate of heating. The rate of cooling was decreased greatly by the ambient temperature of the room as well as excess heat generated by the blower motor and converter. In addition, this excess heat attributed to an increased rate of heating.

The accuracy of the temperature while the hood was set to maintain a temperature of 31°C is shown in Figure 19 below. The temperature oscillated between 31°C and 30°C with an outlier at 29°C.

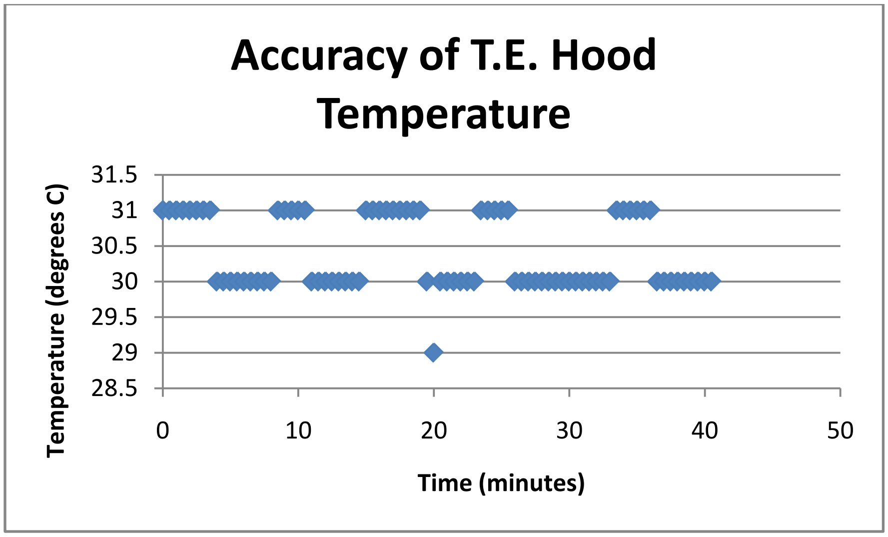

Figure 19: Accuracy of Test Engineering Hood Temperature

The CO_2 percentage readings while the hood was set to maintain a 5% concentration is illustrated in Figure 20 below. The hood was able to maintain an oscillating concentration around 5% with a maximum deviation of ±0.27%. This results from a delay in how long it takes for the CO_2 gas to reach the main hood chamber and therefore the CO_2 sensors as well.

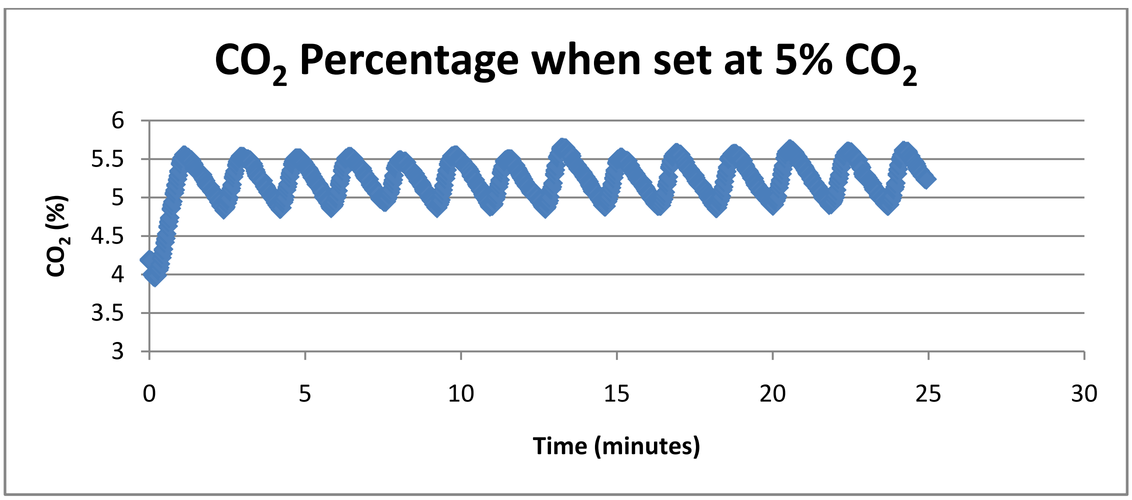

Figure 20: CO_2 Percentage Readings at 5% CO_2

Due to leaks in the ideally air-tight hood, a five pound CO_2 tank maintained a CO_2 concentration of 5% for only two hours before exhausting itself. Overall, however, the CO_2 system was capable of achieving up to 10% CO_2 concentration. The rate of CO_2 increase was found to be 1.16% per minute which could be increased or decreased by adjusting the pressure setting of the gas regulator.

Figure 21 and Figure 22 show graphs of the water in the pump system cooling and heating respectively. The pump system temperature was controlled by a Peltier. The figures show that the Peltier was better at heating water at a rate of 0.497% per minute than cooling water at a rate of -0.331% per minute.

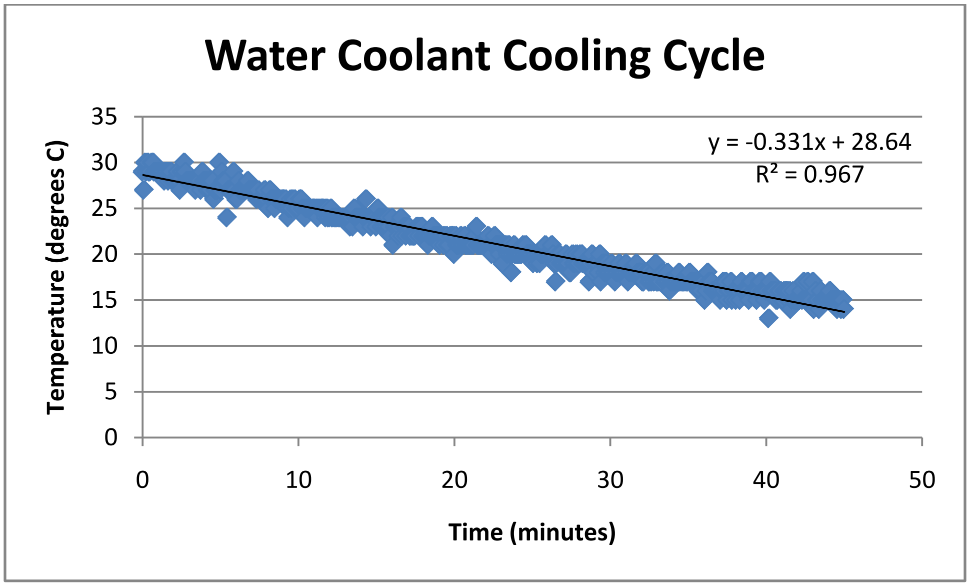

Figure 21: Water Coolant Cooling

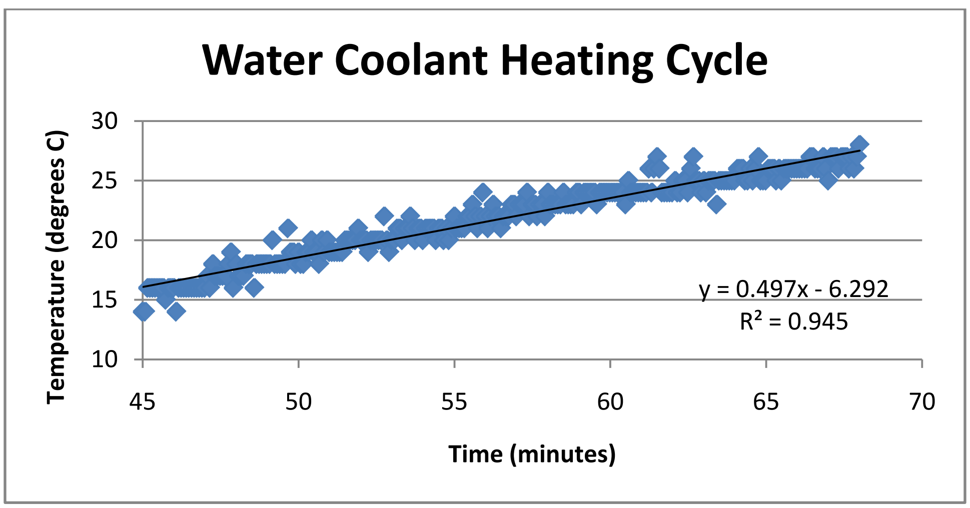

Figure 22: Water Coolant Heating

Once the water reached the desired temperature the water pumping engaged. In order to keep the water pump from turning on and off in case the temperature fluctuated, once the water pump engages the system has to be turned off to deactivate it.

The humidity system had limited success. While the humidifier is rated for 60 cubic feet, it was unable to humidify the 47 cubic feet of the hood with the blower motor on. The system was able to achieve over 70% humidity while the blower motor was off. Once the blower motor was activated the humidity reduced significantly to the initial humidity of the hood at around 40%. Data was also collected on the humidity sensors comparing them to multiple analog humidity gauges inside the hood. The results of the sensors were consistently 4% above from the analog hygrometers over a wide range of humidity, so a 4% offset was subtracted from the logic that calculates the humidity from the sensors.

Lastly, the laminar flow hood's ability to keep T3T mouse fibroblast cells alive was tested using the procedure outlined in Appendix B. Cells are tested in three environments to compare the viability of each to the other. The first environment is an incubator which is made to keep cell samples alive at 37°C, 95% humidity, and 5% CO_2 concentration. The second environment is a bench test where the ambient room conditions are not conducive to cell viability at 25°C, 40% humidity, and 0.05% CO_2 concentration. The last environment is in the tissue engineering hood which was able to reach 32°C, 30% humidity, and 5% CO_2 for the first two hours of the test and 0.05% for the remainder of the test. The cells were visually checked at the beginning of the test and every two hours after until all of the bench test cells appeared deceased. Once all of the bench test cells appeared dead, a sample dyed cells are put in a hemocytometer which distributes the cells on a two dimensional surface in order to count how many are still alive through a microscope. Table 4 summarizes the results of the cell viability test when the majority of the bench test cells were dead after 10 hours.

Table 4: Cell Viability Results

	# Live	# Dead	# Total	Total # of Cells	% Viability
Incubator	44	21	65	520000	67.69
Bench	8	51	59	472000	13.55932
T.E. Hood	24	36	60	476000	40

Figure 23 shows images of one ninth of the area living cells are counted from to calculate cell viability. The leftmost image is the result of 10 hours of incubation, the center image is from the bench test, and the rightmost image is from the laminar flow hood. The brightly lit circular dots are all considered viable cells. From this ninth of the total area used to calculate cell viability, it is obvious the bench was unable to incubate the cells while the incubator did the best job. The tissue engineering hood fell between the two.

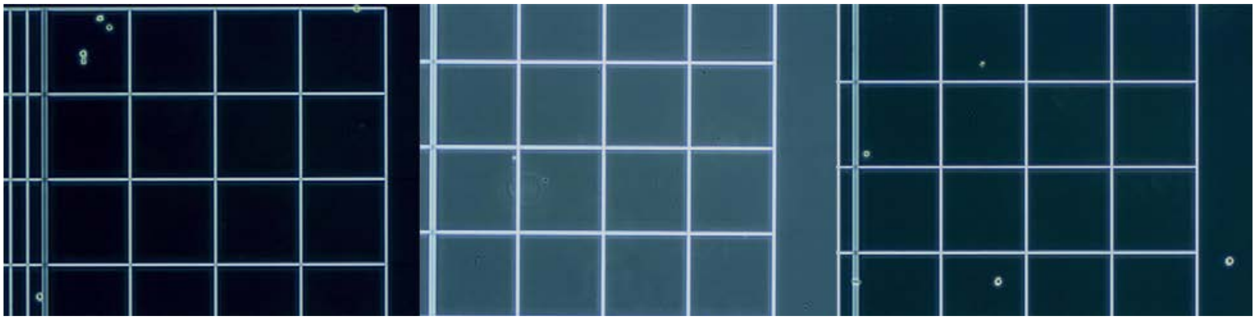

Figure 23: Incubator, Bench, and Tissue Engineering Hood Hemocytometer Images (From Left to Right)

Images taken every hour for the 10 hour duration of the experiment can be found in Appendix C.

VI. IMPROVEMENTS

The flow hood losses waste the energy and resources it takes to maintain the desired environment. One improvement would be to seal the hood more thoroughly to decrease the amount of CO_2 and humid air that leaks. The CO_2 tank would last longer as it would not have to offset the large CO_2 loss. The humidifier should be able to humidify the chamber volume adequately, but the loss of humidity due to the blower motor circulating air might benefit from an additional humidifier. The hood would also benefit from better insulation to achieve higher temperatures than 32°C, since 37°C is the ideal hood temperature. The inability to decrease to a very low temperature is less important since tissues generally need a warmer climate to thrive.

The amount of time it takes for the hood to realize the desired environment is also problematic. Adding more heaters could improve the rate at which the chamber heats and decrease the time it takes to reach the desired hood temperature, which currently takes around an hour. This also applies to the amount of time it takes for the water to heat or cool. Multiple Peltier temperature adjusters could greatly benefit the time it takes to warm or cool the water and engage the water pump. However, the power converter would have to be upgraded to a higher wattage to handle the additional power draw; a single Peltier draws around 33 Watts of power.

The code could also optimize the amount of resources used when using multiple systems. For example, the CO_2 is the most limited resource of the flow hood and should be engaged closer to the time the ambient chamber temperature, water temperature, and humidity reach their desired levels unless those systems are deactivated. If the heating system is active, the humidifier should also activate at higher temperatures to help maintain the humidity of the chamber. More data would greatly improve these results by predicting when the best time to stagger activation is.

Other improvements could be made to the control circuit itself. Solid state relays would greatly reduce the amount of audible noise, especially in regards to switching between the two humidity sensors for measurements which happens every few seconds. The humidifier control can be simplified to a single MOSFET instead of using a relay to switch between no connection and the supply voltage. The wire management would also improve if a printed circuit board was designed to route the various signals. The current control system is spread across three separate prototype boards, and a printed circuit board could potentially reduce them to a single board. The signals could then be carried via ribbon cables to the microcontroller development board. Also, a more efficient way to implement the controls for the system would be designing a custom microcontroller. This would reduce the hardware and software required to control the whole system, in addition to optimizing the amount of time it takes to run the program. Given 10 weeks to complete the project, many of these issues could not be addressed.

Lastly, the user interface could use improvement. Upgrading the display to an LCD with more character cells would improve the spacing of the sensor readings and allow more information to be presented. Also, when changing the settings via the sliding potentiometers there is a lag between updated sensor value settings displaying. This is due to the delays in some functions which slow the continuous loop of the program in which the potentiometers are read. For example, all of the sensor values have to be read and the actuators have to be updated before the program can loop around to display the updated sensor value again.

VII. Conclusions

The laminar flow hood has mediocre results. Current incubators generally result in a cell viability of 70% to 80%. The flow hood cell viability of 40% needs improvement before a 3D printer can be implemented to print tissues. Drawbacks include the time it takes to achieve the desired environment as well as heat, humidity, and CO_2 loss. The control panel could also be more user-friendly.

The laminar flow hood was a successful prototype in that it allowed shortcomings to be found which can be addressed in later editions. The ideal environment for tissue growth has 95% humidity, 5% CO_2 concentration, and is 37°C. This project's tissue engineering hood could maintain 5% CO_2 concentration for a limited amount of time, two hours, could only increase to a maximum of 32°C, and could not maintain its humidity while the blower motor was on. Considering the lower-than-ideal humidity, CO_2 concentration, and temperature, 40% cell viability is still a big improvement over the 13% resulting from the bench-top experiment. Once the ideal tissue environment can be achieved, the laminar flow hood will be ready to support a 3D printer to ensure the tissues printed survive in a sterile environment.

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APPENDICES

APPENDIX A: ABET ANALYSIS

Project:

Student: Sophie Schneider   Signature:

Advisor: Tina Smilkstein   Initials: Date:

Summary of Functional Requirements:

This system controls the environment of a tissue engineering hood to maintain 97% humidity, 5% CO_2 concentration, and 32°C.

Primary Constraints:

The primary constraint on the project was the 10 week timeline. Joining the project late in its development cost time to get up to speed with its requirements. Furthermore, the control system design and implementation had to match the building timeline of the laminar flow hood. The secondary constraint stemmed from the fact that the parts were already purchased for the control system, which limited the design.

Economic:

Project economics necessitated time commitments from various people. Tina Smilkstein advised this project and Malcolm Lapera managed the project and assembled the hood. Malcolm Lapera also provided the project capital, $5,000. The manufacturing capital consisted of machining, soldering, assembling, sealing, coding, and power. Natural resources included water and carbon dioxide to supply the ideal tissue environment. The user must replenish these resources.

Analyzing manufacturing economics, the design and test stages of the tissue engineering hood accrue the most cost. When the hood satisfies conditions for tissue viability, implementing a 3D printer and developing tissues has potential for great profits.

The project budget of $5,000 funded the entire project. The initial estimated total cost of the project was $4,070. The final cost of the project was $4,744. Table 5, the bill of materials, summarizes the final costs of the project. Cal Poly facilities provided the extra equipment required to machine and assemble the various parts.

Table 5: Bill of Materials

Bill of Materials					
Quantitiy	Description	Product #	Supplier	Unit	Subtota

				Price	l
Electronics					
2	Humidity sensor - HH10D	SEN-10239	Sparkfun	$9.95	$19.90
12	N-Channel MOSFET 60V 30A	COM-10213	Sparkfun	$0.86	$10.32
2	ProtoBoard - Wombat (PTH)	PRT-08619	Sparkfun	$9.95	$19.90
1	Reed Switch - Insulated	COM-10601	Sparkfun	$1.95	$1.95
6	Relay SPDT Sealed - 5A	COM-00100	Sparkfun	$1.76	$10.56
4	Toggle Switch	COM-09276	Sparkfun	$1.95	$7.80
4	Slide Pot - Medium (10k Linear Taper)	COM-11621	Sparkfun	$2.50	$10.00
5	LED - Basic Green 3mm	COM-09650	Sparkfun	$0.32	$1.60
1	Arduino Mega 2560 R3	DEV-11061	Sparkfun	$58.95	$58.95
2	20% Cozir CO2 Sensor	GC-0006	CO2Meter.com	$209.00	$418.00
1	2 Pin - Double Ended - Precision Lighting GPH793T5L	AU-LGPH793T5L	1000Bulbs.com	$14.47	$14.47
1	Atlantic Ultraviolet 10-0054C	AU-B100054C	1000Bulbs.com	$95.00	$95.00
1	Peltier-Fan Assembly	926-1070-ND	Digikey	$179.55	$179.55
4	Kapton® (Polyimide Film) Insulated Flexible Heaters	KH-104/5-P	Omega	$36.00	$144.00
1	Conductive Epoxy	473-1089-ND	Digikey	$52.99	$52.99
1	Swiftech MCP355 3/8in Water Pump	20721	xoxide.com	$74.99	$74.99
1	NC Gas Solenoid valve 1/4" NPT - CO2	21996	ASCO Valves	$57.00	$57.00
1	Centrifugal Blower Motor 1000 CFM	T9FB187594	Global Industrial	$282.51	$282.51
1	Cigar Oasis II XL	OA2-1300	Cigar Oasis	$199.00	$199.00
10	Temperature Sensor	SEN-10988	Sparkfun	$1.35	$13.50
2	logic level translators	BOB-08745	Sparkfun	$1.95	$3.90
1	Break Away Headers	PRT-00116	Sparkfun	$1.50	$1.50
1	Heat Shrink Kit	PRT-09353	Sparkfun	$7.95	$7.95
4	1/4", 4-40, 10 Pack Phillips Head Screws	PRT-10453	Sparkfun	$1.50	$6.00
2	Nylon Stand offs, 10 Pack	PRT-10927	Sparkfun	$2.95	$5.90
6	1/8"x1/8"x1/2" Block, N42 Magnet	B228	K&J Magnetics, Inc.	$0.52	$3.12
1	12V 6A converter	285-1825-ND	Digikey	25.08	$25.08
1	5V 0.8A regulator	NOPB-ND	Digikey	1.49	$1.49
2	100' 22 gauge wire solid	2781215	Radioshack	$8.54	$17.08
1	Extension Cord, 12 gauge, 25 feet	34083	Miners	$27.99	$27.99
1	12 gauge winged quick connects	34926	Miners	$2.59	$2.59
2	12V coil/125VAC 15A Relay	2750031	Radioshack	$4.49	$8.98
3	90' 22 gauge wire solid	2781221	Radioshack	$8.49	$25.47
1	two prong connectors	2761388	Radioshack	$3.59	$3.59
3	Toggle switch	314999	Miners	$2.99	$8.97
1	10 gauge ring crimp connectors	34544	Miners	$3.59	$3.59
1	Single plug 20A	3201639	Miners	$7.99	$7.99
1	plug cover white	3220787	Miners	$0.59	$0.59
1	thermal grease	2801098	Radioshack	$7.19	$7.19

1	Metal Stand off 4 pack	2760195	Radioshack	$1.79	$1.79
1	Desoldering braid	6402090	Radioshack	$4.04	$4.04
1	protoboard	2760158	Radioshack	$3.14	$3.14
1	solder	6400005	Radioshack	$5.39	$5.39
2	two prong connectors 4 pack	2761388	Radioshack	$3.60	$7.20
2	butt connectors 22-18 gauge 10pack	34558	Miners	$2.99	$5.98
1	10 gauge ring crimp connectors	34548	Miners	$3.59	$3.59
1	butt connectors 22-18 gauge 100pack	34559	Miners	$14.99	$14.99
15	16 gauge wire red	89748	Miners	$0.39	$5.85
15	16 gauge wire blue	83183	Miners	$0.39	$5.85
1	Ring crimp connectors 22-18 gauge	34537	Miners	$3.59	$3.59
1	3' uv lamp		Home Depot	$23.86	$23.86
2	Fuses		Miners	$4.66	$9.32
2	8 circuit terminal block		Home Depot	$7.74	$15.48
2	Female to female jumper wires	2760144	Radioshack	$5.39	$10.78
Hardware					
3	Plexiglass 48x48x.125"		eplastics	$49.02	$147.06
1	6061 Aluminum Strap (1" Wide x 1/8" thick) 3'		OnlineMetals.com	$1.86	$1.86
1	6061 Aluminum Strap (1" Wide x 1/8" thick) 6'		OnlineMetals.com	$3.06	$3.06
1	6061 Aluminum Strap (1" Wide x 1/8" thick) 8'		OnlineMetals.com	$3.86	$3.86
3	6061 Aluminum Square Tube 8' (1" Wide x 1/8" Thick)		OnlineMetals.com	$16.53	$49.59
11	6061 Aluminum Angle 8' (3/4" Wide x 1/8" Thick)		OnlineMetals.com	$5.63	$61.93
2	6061 Aluminum Sheet 24x48" (1/16" Thick)		OnlineMetals.com	$45.75	$91.50
7	6061 Aluminum Sheet 36x48" (1/16" Thick)		OnlineMetals.com	$65.44	$458.08
2	24x30x3" Standard Volume HEPA Filter	HC240300X4A7	HEPAfilters.com	$211.85	$423.70
1	Scott-Foam Prefilter		HEPAfilters.com	$25.00	$25.00
4	Static Control Caster Wheels	2358T63	McMaster-Carr	$8.08	$32.32
1	10 pack eye hooks	9489T17	McMaster-Carr	$2.99	$2.99
2	S hooks	6043T5	McMaster-Carr	$3.75	$7.50
1	10' PVC tubing	5231K196	McMaster-Carr	$7.20	$7.20
1	Hose Clamp	5388K14	McMaster-Carr	$5.87	$5.87
8	Barbed Hose Straight for 3/8" Tube ID x 1/4 Male Pipe Size	44555K133	McMaster-Carr	$2.17	$17.36
2	Unversal Hose Socket 1/4" NPTF Female, 1/4 Coupling Size	53475K21	McMaster-Carr	$9.30	$18.60
5	18-8 Stainless Steel Round Head Phillips Machine Screw	91773A827	McMaster-Carr	$6.90	$34.50
5	18-8 Stainless Steel Machine Screw Hex Nut	91841A195	McMaster-Carr	$4.30	$21.50
4	Gasket Tape 1/2" wide 0.02" thick 50 ft long	9477K21	McMaster-Carr	$41.08	$164.32
1	3/8 90 degree female x male elbow	4429K152	McMaster-Carr	$9.08	$9.08
1	1/4 90 degree female x male elbow	4429K151	McMaster-Carr	$9.08	$9.08
2	3/8 Pipe Locknut	4429K123	McMaster-Carr	$2.00	$4.00
4	1/4 Pipe Locknut	4429K122	McMaster-Carr	$1.53	$6.12
1	8 feet 1/8" thick 1/2" Width Silicon Rubber	2614T18	McMaster-Carr	$1.50	$1.50

1	3/8" Universal Hose Socket	53475K23	McMaster-Carr	$10.18	$10.18
1	3/8" Brass Ball Valve Male x Female	47865K42	McMaster-Carr	$9.67	$9.67
2	Air Hose Plug, 3/8" Hose ID, 1/4 Coupling Size	1077T26	McMaster-Carr	$2.58	$5.16
2	Brass Hex Nipple, 3/8"	5485K23	McMaster-Carr	$3.28	$6.56
1	2 1/2" square tube, 1 ft length, aluminum	88875K791	McMaster-Carr	$12.72	$12.72
1	10 pack, square tube caps	9565K92	McMaster-Carr	$7.80	$7.80
1	12 feet, silicon rubber 3/32" thick x 1/2" Width	2614T39	McMaster-Carr	$1.21	$1.21
1	1/4" through wall pipe fitting	8682T22	McMaster-Carr	$10.25	$10.25
2	3/8" through wall pipe fitting	8682T23	McMaster-Carr	$13.62	$27.24
1	10 pack, 3/4" Hose coupling	5345K11	McMaster-Carr	$5.74	$5.74
1	10 pack, 1" Hose coupling	5345K12	McMaster-Carr	$5.95	$5.95
1	2" 1/4" brass pipe, threaded	4568K133	McMaster-Carr	$2.17	$2.17
3	3/8" Hose ID, 1/4" coupling size, Air Hose Plug	1077T26	McMaster-Carr	$2.58	$7.74
7	PEM nuts 10-32 for 0.06" minimum	95117A466	McMaster-Carr	8.07	$56.49
2	1/4" pipe coupling	50785K92	McMaster-Carr	1.5	$3.00
1	10-32 philips head x 3/8" packs of 100	91773A827	McMaster-Carr	6.9	$6.90
1	2-56 philips head x 3/8" packs of 100	91773A079	McMaster-Carr	4.33	$4.33
1	2-56 Nuts packs of 100	91841A003	McMaster-Carr	2.53	$2.53
1	Funnel	1479T83	McMaster-Carr	2.43	$2.43
1	3/8" pipe threaded 5"	4568K159	McMaster-Carr	4.92	$4.92
1	CO2 Pressure Regulator		Airgas	$67.00	$67.00
1	5lb Aluminum CO2 Tank		Airgas	$12.50	$12.50
1	20 Gal Nitrogen Tank		Airgas	$13.00	$13.00
1	Nitrogen Regulator		Airgas	$135.00	$135.00
1	CO2 Gas - 5lb		Airgas	$95.00	$95.00
1	Nitrogen - 20 Gallons		Airgas	$79.19	$79.19
10	2"x2"x.125 Alum. Sq. Tube 24'		McCarthy Steel	$5.85	$58.50
2.205	1/8" x 1" Alum. Flat Bar 12'		McCarthy Steel	$5.10	$11.25
2.9	1/8" x 2"x2" Alum. Angle 25'		McCarthy Steel	$5.10	$14.79
0.888	1/2"x1/2"x1/32" Alum Channel 16'		McCarthy Steel	$5.10	$4.53
1	Air Hose, 3/8" Male Fitting, 3 Feet		Contractors Mainenance	$17.20	$17.20
1	Air Hose, 1/4" Male Fitting, 3 Feet		Contractors Mainenance	$17.25	$17.25
2	1/4" NPT Male x Male Couplings	13096	Ace Hardware	$2.99	$5.98
1	90' 22 gauge wire solid	2781221	Radioshack	$8.49	$8.49
2	Lock Washer #10	30699326013	Home Depot	$1.18	$2.36
1	Washer #10		Home Depot	$1.18	$1.18
2	1" corner brace 20 pack		Home Depot	$7.48	$14.96
2	1.5" corner brace 4 pack		Home Depot	$2.67	$5.34
9	3/4" corner brace 4 pack		Home Depot	$1.97	$17.73
5	1' rope 1/4" white		Home Depot	$0.22	$1.10
1	10-32 Hex nuts	H140024	Miners	$3.59	$3.59

2	philips 10-32x1/2"	H92196	Miners	$4.79	$9.58
1	Sheet Metal Screw #4x3/8"		Home Depot	$1.18	$1.18
2	Aluminum Bar 1/8"x3/4"x4'	5117973	Miners	$6.99	$13.98
2	Handles	5384136	Miners	$2.99	$5.98
1	Superglue	13003	Miners	$2.59	$2.59
1	Distilled Water 1 gallon	9215419	Miners	$1.79	$1.79
1	Thread Locker	18615	Miners	$3.99	$3.99
1	White silicon caulk	11315	Miners	$4.99	$4.99
Subtotal					$4,392.39
Taxes					$351.39
Total					$4,743.79

Figure 24 and Figure 25 show the initial estimated project timeline and the final project timeline which guided the timing of the design, build, and test processes for the project. Synchronizing the control system process with the assembly of the engineering hood effected the changes in the final timeline the most.

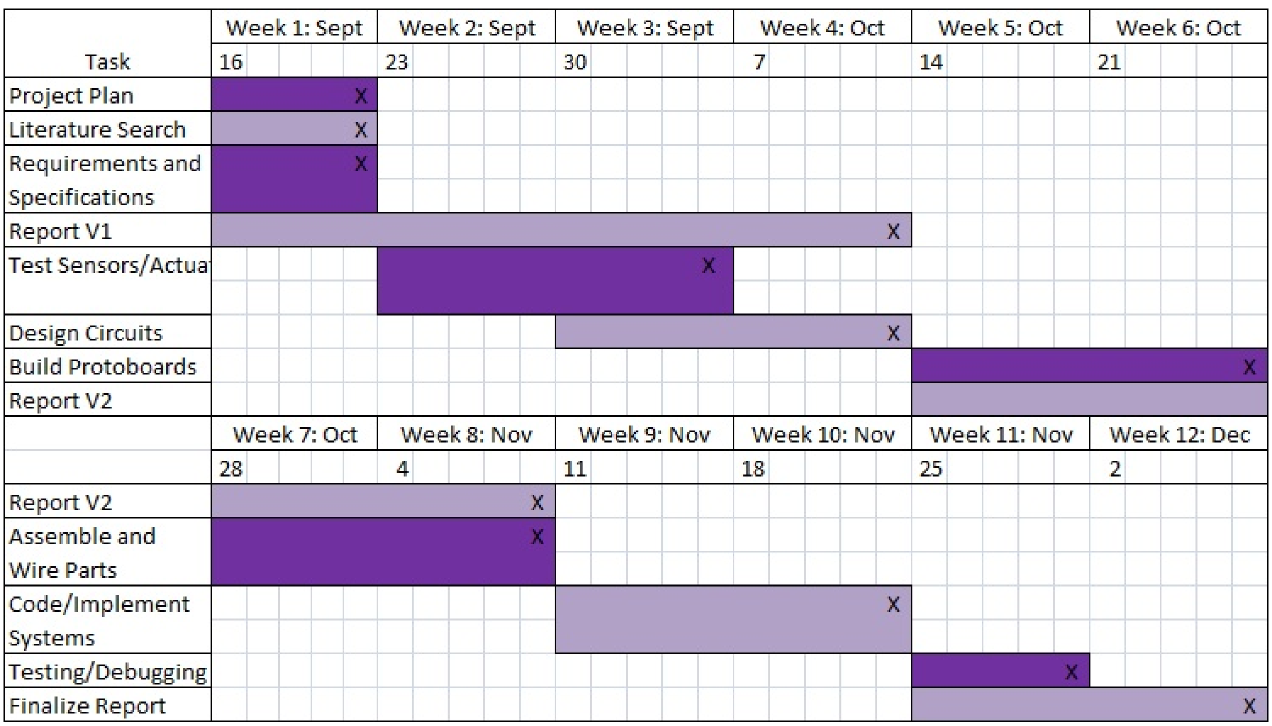

Figure 24: Initial Control System Gantt Chart

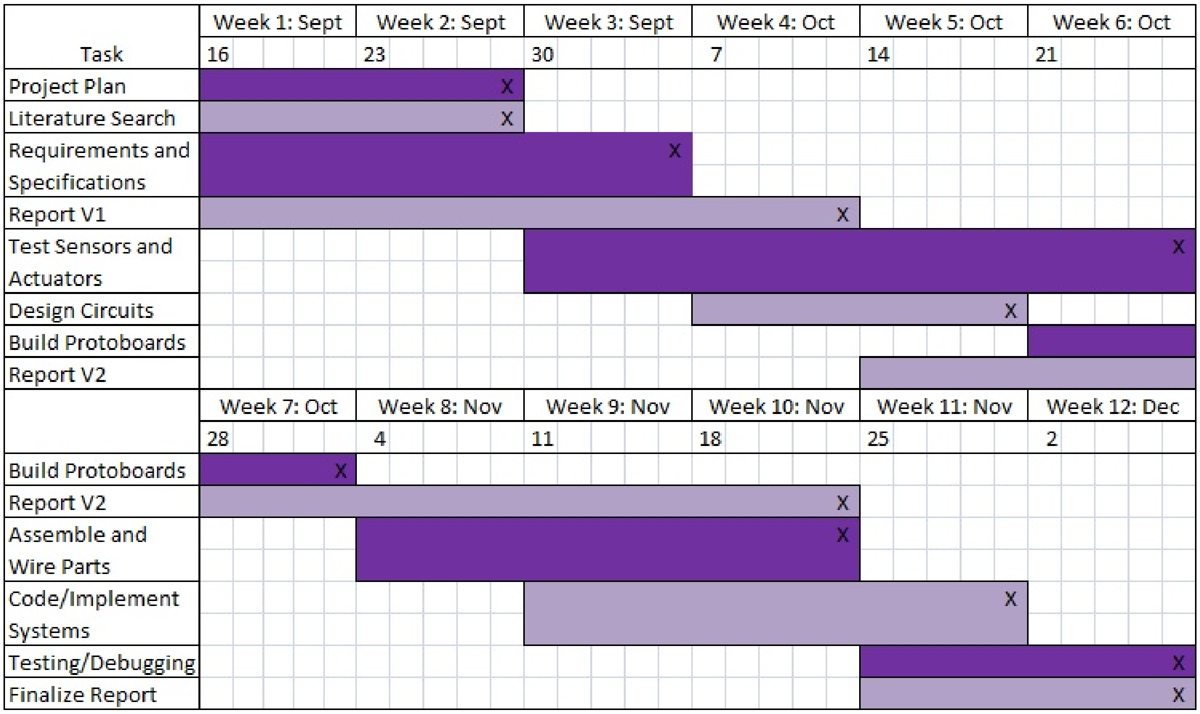

Figure 25: Final Control System Gantt Chart

If Manufactured on a Commercial Basis:

Hospital labs would purchase the finalized, fully functional tissue engineering hood. A fixed cost of $11,700 accounts for labor. If manufactured on a larger scale, the optimistic cost per unit of the hood would be around $4,000. Using a sales price per unit of $7,000, four units would need to be sold to break even. Table 6 summarizes the costs and Figure 26 illustrates the break even analysis of the tissue engineering hood.

Table 6: Summary of Costs

Fixed Cost	$11,700.00
Optimistic Cost/unit	$4,000.00
Sales Price/Unit	$7,000.00

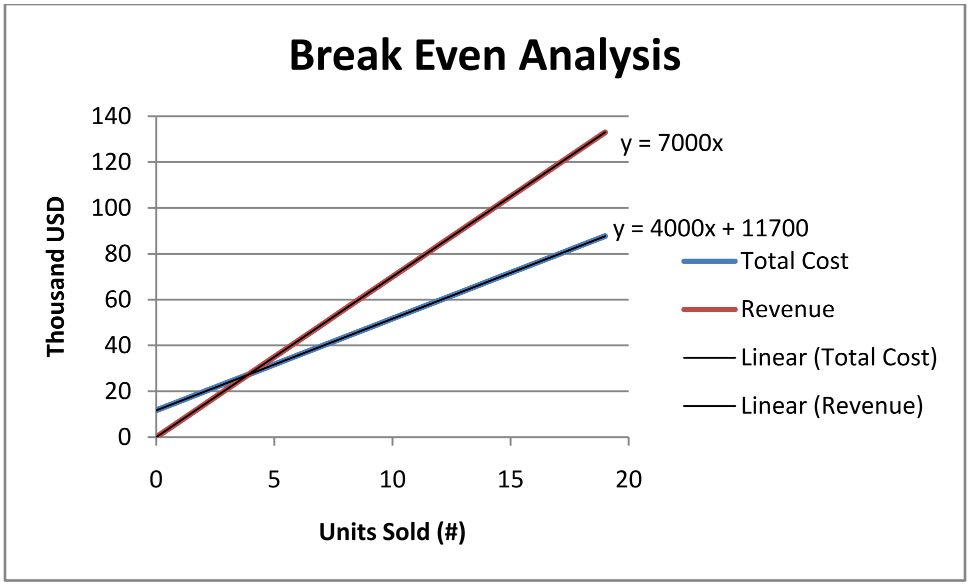

Figure 26: Break Even Analysis

One hundred tissue engineering hood sales would lead to a profit of around $300,000.

Environmental:

Environmental impacts of the engineering hood include use of natural resources such as carbon dioxide and water, in addition to wasted scrap metal, fiberglass, wire, and silicon. Fabricating circuit boards also contributes to unhealthy chemical waste byproducts. However, recycling leftover scrap metal from manufacturing and end-of-life electronic boards and metals reduce the overall environmental impact. Additionally, health risks may arise from biohazards due to tissue waste when using the hood. Proper disposal of biohazardous wastes protects the water supply and all who use and drink from it.

Furthermore, powering the device uses resources derived from coal, petroleum, or natural gas which have negative effects on the environment by increasing green house emissions. To make powering the device more environmentally friendly it would require a sustainable power grid provided by wind or solar power.

Manufacturability:

Difficulties may arise while manufacturing an airtight hood to prevent leakage of carbon dioxide, humidity, or heat. Insulating the hood increases labor costs while using printed circuit boards and creating digital control buses would decrease some of the labor costs.

Sustainability:

Carbon dioxide tanks and the humidifier water tank are reusable. However, the leakage of carbon dioxide, humidity, and heat from the hood greatly affect the amount of power used to maintain an ideal tissue environment while wasting resources. Loss of heat to surroundings especially affects the amount of power the hood draws. Insulating the tissue engineering hood helps reduce these losses. Additionally, optimizing code can improve control of the hood's systems to reduce the amount of power consumed while maintaining its environment.

Ethical:

The tissue engineering hood supports research using stem cells, a highly debated topic in the United States. However, induced pluripotent stem cells, which are adult differentiated cells reverted back to their undifferentiated state, reduce this controversy. However, the success of tissue engineering may lead people to neglect their personal health if body parts like the liver were considered easily replaceable.

Health and Safety:

Biohazard precautions which maintain a sterile environment increase the safety of using the tissue engineering hood in addition to a user guide that directs proper usage of the hood protects the user from electric shock.

Social and Political:

Tissue engineering hoods benefit research and development of manufacturable tissues and organs. Developments could help alleviate the disparity between the supply and demand of organs. Patients normally waiting years for organ donors would receive medical attention faster. Medical companies stand to profit by meeting the organ demand. However, they may risk lawsuits due to tissue rejection or organ failure in patients, although using cells of the patient should reduce this risk. Also, the risk of only the wealthy affording tissue engineered products could create a sense of inequality among people. However, the increased engineered organ supply would help stop the organ black market.

Development:

The development of this project included tissue engineering knowledge, component integration, coding innovation, power system design, and cell testing.

Literature Search:

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Appendix B: Cell Viability Assessment Protocol

Cell Viability Assessment of Tissue Engineering Hood

Goal: Compare the viability of cells housed in the Tissue Engineering Hood over a period of time to cells exposed to an uncontrolled environment and the standard of a cell culture incubator.

Equipment:

- 3 – T75 Flasks of 3T3 Fibroblast cells, approx. 20% confluent
- 1 – Inverted microscope w/ Camera
- Gloves
- 70% IPA
- Paper Towels
- Pipet-Aid
- 10 mL glass pipets
- 50 mL DCF-PBS
- 10 mL Trypsin
- 40 mL Media
- 3 – Hemocytometer
- Micropipet and tips

Protocol:

1. Receive 3 T75 flasks with containing 3T3 Fibroblasts that have been thawed and suspended in media and have had time to adhere to the flask. The flasks will be in the incubator.

2. Leave one flask in the incubator. Get a new pair of gloves and bring two flasks to room 331. Clean one flask with IPA and place into Tissue Engineering Hood. Place the other flask on the table, in the clear labeled area, marked biohazard, cleaned with IPA.

Testing

3. Every 2 hours, examine each flask under the inverted microscope and take a picture. Be sure to clean the stage and flask before examining.

4. Repeat until for a total duration of 48 hours. This can be amended based on results. End this phase of testing when cells kept on the bench top appear to all be dead.

Trypan Blue Assesment

5. Bring all flasks back to room 328. On bench top, repeat the steps 12 through 19 for each flask.

6. Aspirate of media, save for later.

7. Rinse with DCF-PBS.

8. Add 3 mL of trypsin, and wait for cells to detach.

9. Deactivate with 6 mL of media that was set aside and mix cell solution with pipet.

10. Remove 150 µL of cell solution and place in microcentrifuge tube.

11. Add 50 µL of Trypan Blue to the centrifuge tube. Cap tightly and mix by flicking.

12. Remove 10 µL and inject them into a hemocytometer. Place Hemocytometer on cleaned inverted microscope.

13. Count the number of cells, the number live cells (clear), and the number of dead (blue) in each of the square regions. Record these numbers as well as images of each area.

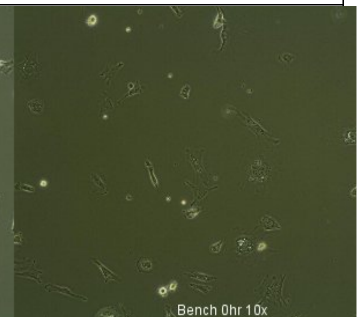
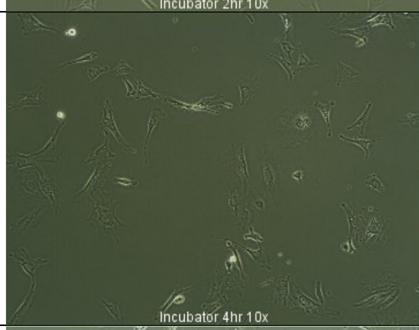
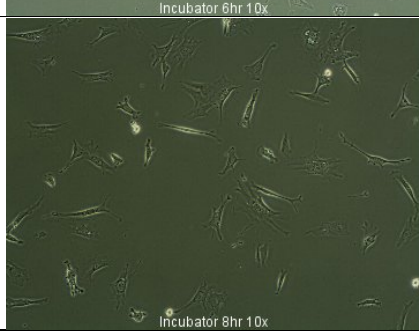
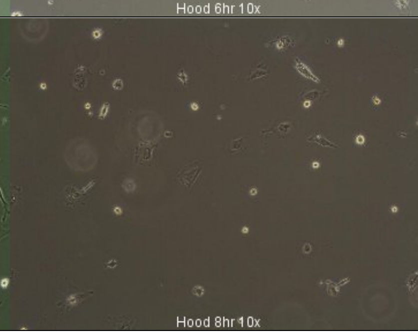
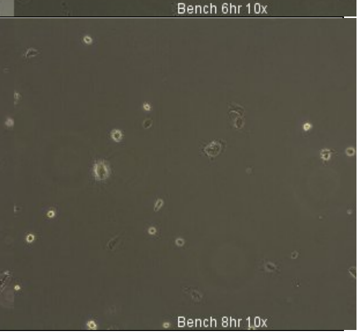
Clean up

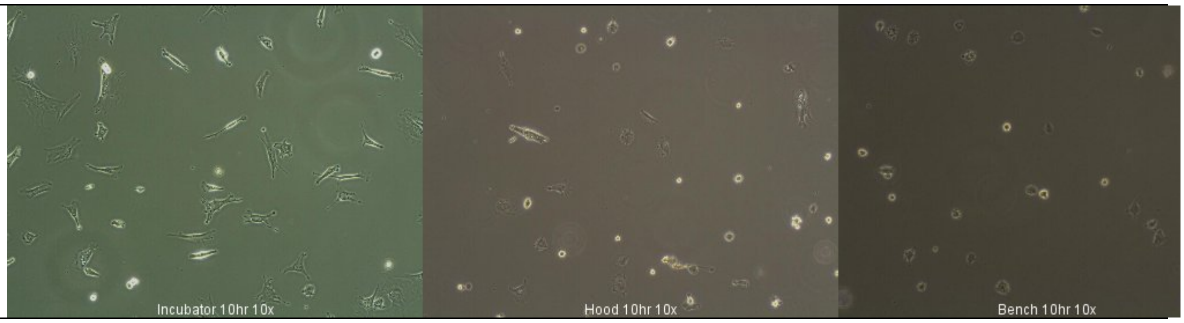
14. Discard anything that touched cells in the Biohazard waste. This being, all pipet tips, flasks, hemocytometers, microcentrifuge tubes, and centrifuge tube.

15. Clean all bench top work areas with IPA as well as the microscope stage.

16. Throw all other trash in the garbage. No liquids should go down the sink.

APPENDIX C: CELL VIABILITY PICTURES

Time Point	Image of Cells Under Microscope (Incubator/Hood/Bench)
0 hr	  
2 hr	  
4 hr	  
6 hr	  
8 hr	  

10 hr	

Appendix D: Tissue Engineering Hood Pictures

Figure 27: Completely Assembled Tissue Engineering Hood

Figure 28: Assembled Control Panel

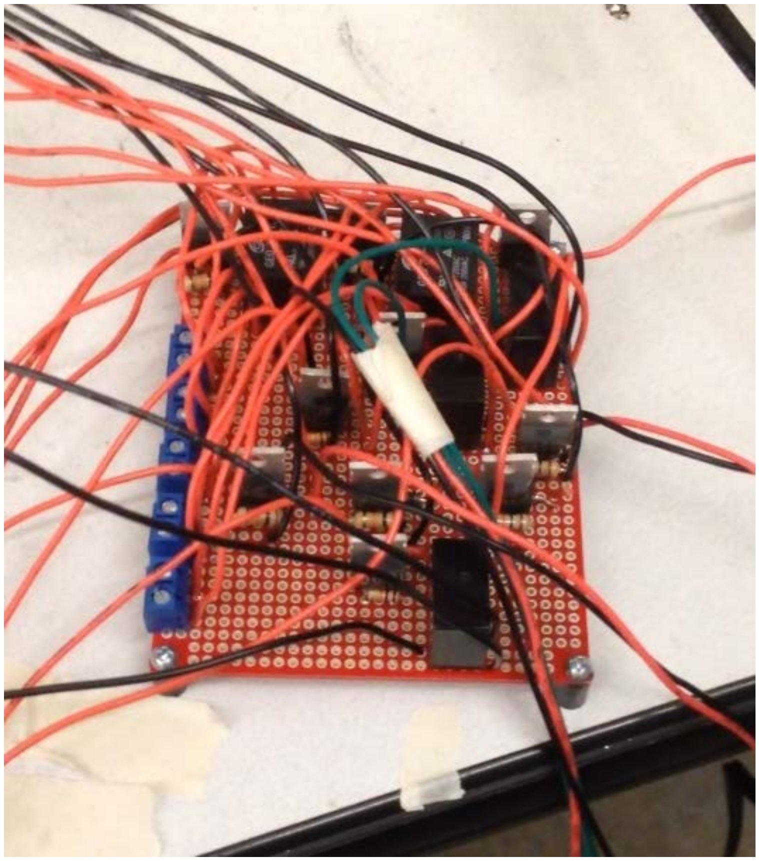


Figure 29: MOSFET and Relay Prototype Board

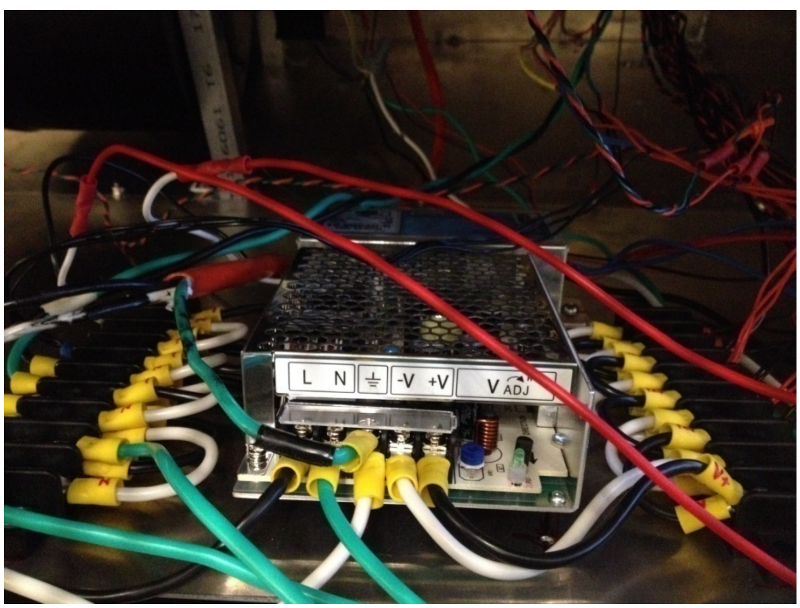


Figure 30: Power Converter

APPENDIX E: BILL OF MATERIALS

Table 7: Bill of Materials

Bill of Materials					
Quantitiy	Description	Product #	Supplier	Unit Price	Subtotal
Electronics					
2	Humidity sensor - HH10D	SEN-10239	Sparkfun	$9.95	$19.90
12	N-Channel MOSFET 60V 30A	COM-10213	Sparkfun	$0.86	$10.32
2	ProtoBoard - Wombat (PTH)	PRT-08619	Sparkfun	$9.95	$19.90
1	Reed Switch - Insulated	COM-10601	Sparkfun	$1.95	$1.95
6	Relay SPDT Sealed - 5A	COM-00100	Sparkfun	$1.76	$10.56
4	Toggle Switch	COM-09276	Sparkfun	$1.95	$7.80
4	Slide Pot - Medium (10k Linear Taper)	COM-11621	Sparkfun	$2.50	$10.00
5	LED - Basic Green 3mm	COM-09650	Sparkfun	$0.32	$1.60
1	Arduino Mega 2560 R3	DEV-11061	Sparkfun	$58.95	$58.95
2	20% Cozir CO2 Sensor	GC-0006	CO2Meter.com	$209.00	$418.00
1	2 Pin - Double Ended - Precision Lighting GPH793T5L	AU-LGPH793T5L	1000Bulbs.com	$14.47	$14.47
1	Atlantic Ultraviolet 10-0054C	AU-B100054C	1000Bulbs.com	$95.00	$95.00
1	Peltier-Fan Assembly	926-1070-ND	Digikey	$179.55	$179.55
4	Kapton® (Polyimide Film) Insulated Flexible Heaters	KH-104/5-P	Omega	$36.00	$144.00
1	Conductive Epoxy	473-1089-ND	Digikey	$52.99	$52.99
1	Swiftech MCP355 3/8in Water Pump	20721	xoxide.com	$74.99	$74.99
1	NC Gas Solenoid valve 1/4" NPT - CO2	21996	ASCO Valves	$57.00	$57.00
1	Centrifugal Blower Motor 1000 CFM	T9FB187594	Global Industrial	$282.51	$282.51
1	Cigar Oasis II XL	OA2-1300	Cigar Oasis	$199.00	$199.00
10	Temperature Sensor	SEN-10988	Sparkfun	$1.35	$13.50
2	logic level translators	BOB-08745	Sparkfun	$1.95	$3.90
1	Break Away Headers	PRT-00116	Sparkfun	$1.50	$1.50
1	Heat Shrink Kit	PRT-09353	Sparkfun	$7.95	$7.95
4	1/4", 4-40, 10 Pack Phillips Head Screws	PRT-10453	Sparkfun	$1.50	$6.00
2	Nylon Stand offs, 10 Pack	PRT-10927	Sparkfun	$2.95	$5.90
6	1/8"x1/8"x1/2" Block, N42 Magnet	B228	K&J Magnetics, Inc.	$0.52	$3.12
1	12V 6A converter	285-1825-ND	Digikey	25.08	$25.08
1	5V 0.8A regulator	NOPB-ND	Digikey	1.49	$1.49
2	100' 22 gauge wire solid	2781215	Radioshack	$8.54	$17.08
1	Extension Cord, 12 gauge, 25 feet	34083	Miners	$27.99	$27.99
1	12 gauge winged quick connects	34926	Miners	$2.59	$2.59
2	12V coil/125VAC 15A Relay	2750031	Radioshack	$4.49	$8.98
3	90' 22 gauge wire solid	2781221	Radioshack	$8.49	$25.47
1	two prong connectors	2761388	Radioshack	$3.59	$3.59
3	Toggle switch	314999	Miners	$2.99	$8.97

1	10 gauge ring crimp connectors	34544	Miners	$3.59	$3.59
1	Single plug 20A	3201639	Miners	$7.99	$7.99
1	plug cover white	3220787	Miners	$0.59	$0.59
1	thermal grease	2801098	Radioshack	$7.19	$7.19
1	Metal Stand off 4 pack	2760195	Radioshack	$1.79	$1.79
1	Desoldering braid	6402090	Radioshack	$4.04	$4.04
1	protoboard	2760158	Radioshack	$3.14	$3.14
1	solder	6400005	Radioshack	$5.39	$5.39
2	two prong connectors 4 pack	2761388	Radioshack	$3.60	$7.20
2	butt connectors 22-18 gauge 10pack	34558	Miners	$2.99	$5.98
1	10 gauge ring crimp connectors	34548	Miners	$3.59	$3.59
1	butt connectors 22-18 gauge 100pack	34559	Miners	$14.99	$14.99
15	16 gauge wire red	89748	Miners	$0.39	$5.85
15	16 gauge wire blue	83183	Miners	$0.39	$5.85
1	Ring crimp connectors 22-18 gauge	34537	Miners	$3.59	$3.59
1	3' uv lamp		Home Depot	$23.86	$23.86
2	Fuses		Miners	$4.66	$9.32
2	8 circuit terminal block		Home Depot	$7.74	$15.48
2	Female to female jumper wires	2760144	Radioshack	$5.39	$10.78
Hardware					
3	Plexiglass 48x48x.125"		eplastics	$49.02	$147.06
1	6061 Aluminum Strap (1" Wide x 1/8" thick) 3'		OnlineMetals.com	$1.86	$1.86
1	6061 Aluminum Strap (1" Wide x 1/8" thick) 6'		OnlineMetals.com	$3.06	$3.06
1	6061 Aluminum Strap (1" Wide x 1/8" thick) 8'		OnlineMetals.com	$3.86	$3.86
3	6061 Aluminum Square Tube 8' (1" Wide x 1/8" Thick)		OnlineMetals.com	$16.53	$49.59
11	6061 Aluminum Angle 8' (3/4" Wide x 1/8" Thick)		OnlineMetals.com	$5.63	$61.93
2	6061 Aluminum Sheet 24x48" (1/16" Thick)		OnlineMetals.com	$45.75	$91.50
7	6061 Aluminum Sheet 36x48" (1/16" Thick)		OnlineMetals.com	$65.44	$458.08
2	24x30x3" Standard Volume HEPA Filter	HC240300X4A 7	HEPAfilters.com	$211.85	$423.70
1	Scott-Foam Prefilter		HEPAfilters.com	$25.00	$25.00
4	Static Control Caster Wheels	2358T63	McMaster-Carr	$8.08	$32.32
1	10 pack eye hooks	9489T17	McMaster-Carr	$2.99	$2.99
2	S hooks	6043T5	McMaster-Carr	$3.75	$7.50
1	10' PVC tubing	5231K196	McMaster-Carr	$7.20	$7.20
1	Hose Clamp	5388K14	McMaster-Carr	$5.87	$5.87
8	Barbed Hose Straight for 3/8" Tube ID x 1/4 Male Pipe Size	44555K133	McMaster-Carr	$2.17	$17.36
2	Unversal Hose Socket 1/4" NPTF Female, 1/4 Coupling Size	53475K21	McMaster-Carr	$9.30	$18.60
5	18-8 Stainless Steel Round Head Phillips Machine Screw	91773A827	McMaster-Carr	$6.90	$34.50
5	18-8 Stainless Steel Machine Screw Hex Nut	91841A195	McMaster-Carr	$4.30	$21.50
4	Gasket Tape 1/2" wide 0.02" thick 50 ft long	9477K21	McMaster-Carr	$41.08	$164.32
1	3/8 90 degree female x male elbow	4429K152	McMaster-Carr	$9.08	$9.08

1	1/4 90 degree female x male elbow	4429K151	McMaster-Carr	$9.08	$9.08
2	3/8 Pipe Locknut	4429K123	McMaster-Carr	$2.00	$4.00
4	1/4 Pipe Locknut	4429K122	McMaster-Carr	$1.53	$6.12
1	8 feet 1/8" thick 1/2" Width Silicon Rubber	2614T18	McMaster-Carr	$1.50	$1.50
1	3/8" Universal Hose Socket	53475K23	McMaster-Carr	$10.18	$10.18
1	3/8" Brass Ball Valve Male x Female	47865K42	McMaster-Carr	$9.67	$9.67
2	Air Hose Plug, 3/8" Hose ID, 1/4 Coupling Size	1077T26	McMaster-Carr	$2.58	$5.16
2	Brass Hex Nipple, 3/8"	5485K23	McMaster-Carr	$3.28	$6.56
1	2 1/2" square tube, 1 ft length, aluminum	88875K791	McMaster-Carr	$12.72	$12.72
1	10 pack, square tube caps	9565K92	McMaster-Carr	$7.80	$7.80
1	12 feet, silicon rubber 3/32" thick x 1/2" Width	2614T39	McMaster-Carr	$1.21	$1.21
1	1/4" through wall pipe fitting	8682T22	McMaster-Carr	$10.25	$10.25
2	3/8" through wall pipe fitting	8682T23	McMaster-Carr	$13.62	$27.24
1	10 pack, 3/4" Hose coupling	5345K11	McMaster-Carr	$5.74	$5.74
1	10 pack, 1" Hose coupling	5345K12	McMaster-Carr	$5.95	$5.95
1	2" 1/4" brass pipe, threaded	4568K133	McMaster-Carr	$2.17	$2.17
3	3/8" Hose ID, 1/4" coupling size, Air Hose Plug	1077T26	McMaster-Carr	$2.58	$7.74
7	PEM nuts 10-32 for 0.06" minimum	95117A466	McMaster-Carr	8.07	$56.49
2	1/4" pipe coupling	50785K92	McMaster-Carr	1.5	$3.00
1	10-32 philips head x 3/8" packs of 100	91773A827	McMaster-Carr	6.9	$6.90
1	2-56 philips head x 3/8" packs of 100	91773A079	McMaster-Carr	4.33	$4.33
1	2-56 Nuts packs of 100	91841A003	McMaster-Carr	2.53	$2.53
1	Funnel	1479T83	McMaster-Carr	2.43	$2.43
1	3/8" pipe threaded 5"	4568K159	McMaster-Carr	4.92	$4.92
1	CO2 Pressure Regulator		Airgas	$67.00	$67.00
1	5lb Aluminum CO2 Tank		Airgas	$12.50	$12.50
1	20 Gal Nitrogen Tank		Airgas	$13.00	$13.00
1	Nitrogen Regulator		Airgas	$135.00	$135.00
1	CO2 Gas - 5lb		Airgas	$95.00	$95.00
1	Nitrogen - 20 Gallons		Airgas	$79.19	$79.19
10	2"x2"x.125 Alum. Sq. Tube 24'		McCarthy Steel	$5.85	$58.50
2.205	1/8" x 1" Alum. Flat Bar 12'		McCarthy Steel	$5.10	$11.25
2.9	1/8" x 2"x2" Alum. Angle 25'		McCarthy Steel	$5.10	$14.79
0.888	1/2"x1/2"x1/32" Alum Channel 16'		McCarthy Steel	$5.10	$4.53
1	Air Hose, 3/8" Male Fitting, 3 Feet		Contractors Mainenance	$17.20	$17.20
1	Air Hose, 1/4" Male Fitting, 3 Feet		Contractors Mainenance	$17.25	$17.25
2	1/4" NPT Male x Male Couplings	13096	Ace Hardware	$2.99	$5.98
1	90' 22 gauge wire solid	2781221	Radioshack	$8.49	$8.49
2	Lock Washer #10	30699326013	Home Depot	$1.18	$2.36
1	Washer #10		Home Depot	$1.18	$1.18
2	1" corner brace 20 pack		Home Depot	$7.48	$14.96

2	1.5" corner brace 4 pack		Home Depot	$2.67	$5.34
9	3/4" corner brace 4 pack		Home Depot	$1.97	$17.73
5	1' rope 1/4" white		Home Depot	$0.22	$1.10
1	10-32 Hex nuts	H140024	Miners	$3.59	$3.59
2	philips 10-32x1/2"	H92196	Miners	$4.79	$9.58
1	Sheet Metal Screw #4x3/8"		Home Depot	$1.18	$1.18
2	Aluminum Bar 1/8"x3/4"x4'	5117973	Miners	$6.99	$13.98
2	Handles	5384136	Miners	$2.99	$5.98
1	Superglue	13003	Miners	$2.59	$2.59
1	Distilled Water 1 gallon	9215419	Miners	$1.79	$1.79
1	Thread Locker	18615	Miners	$3.99	$3.99
1	White silicon caulk	11315	Miners	$4.99	$4.99
Subtotal					$4,392.39
Taxes					$351.39
Total					$4,743.79

Appendix F: Microcontroller Code

```
//Tissue Engineering Control Program for the ATMEGA 2560
//By Malcolm Lapera and Sophie Schneider
//Last Revised Date: 12/4/13
// Declare Libraries Here
#include <Wire.h>
#include <FreqCount.h>
#include <LiquidCrystal.h>
#include <SoftwareSerial.h>
#include <Average.h>
#include <Math.h>
#include <Timer.h>

// ******Pinouts*****
LiquidCrystal lcd(12, 11, 22, 23, 24, 25);     //LCD Pinouts
int potPin[] = {A9, A10, A11, A12};          //Slide Pot Pinouts
//Heater Pinout
int tempPin[] = {A0, A1, A2, A6, A7, A8};  //Temp Sensors
int heaterCTRL = 33;                        //Heater Relay
int heatTog = 2;                            //Heater Toggle
int heatLED = 38;                           //Heater LED
//Humidifier Pinout
int humSel = 28;           //Humidity Sensor Relay
int humCTRL = 26;          //Humidifier Relay
int humTog = 43;           //Humidity Toggle
int humLED = 39;           //Humidity LED
//CO2 Pinout
int CO2CTRL = 34;          //CO2 Solenoid Relay
int CO2Tog = 44;           //CO2 Toggle
int CO2LED = 40;           //CO2 LED
//H2O Heater Pinout
int tempH2OPin = A4;          //Temp Sensor for H2O
int H2OCTRL1 = 30;           //Peltier Hot
int H2OCTRL2 = 31;           //Peltier Cold
int H2OTog = 45;             //Water Coolant Toggle
int H2OLED = 41;             //Water Coolant LED
int H2OFan = 29;             //Peltier Fan
int H2OPump = 32;              //Water Pump
//Blower Pinout
int ReedSW = 36;             //Reed Switch
int blowCTRL = 35;           //Blower Control Relay
//Converter Pinout
int convertLED = 37;          //ON/OFF LED
// ****************** Global Variables:*********************
```

```
float potSensor[4]; // temperature, humidity, CO2, H2O
float potCur[4];    //array for checking change in slide pots
float potPrev[4];
int action[4];
int hum_pot = 0;         //Global int variable for humidity slide pot
int temp_pot = 0;        //Global int variable for temperature slide pot
int water_pot = 0;       //Global int variable for water temp slide pot
// Flags
int Pump_Flag = 0; int Heat_Flag = 0; int Hum_Flag = 0; int CO2_Flag = 0; int H2O_Flag = 0;  //
Flags for controlling actuators
//Global Temp Variables
int tempInC[6]; // stores temp sensor values (in C)
//Global Humidity Sensor Variables
int sens;
int offset;
int enHum=0;
//Global CO2 Variables
SoftwareSerial mySerial(50, 14); //Rx,Tx
String val= ""; //holds the string of the value
double co2 =0; // holds the actual value
double multiplier = 10;
uint8_t buffer[25];
uint8_t ind =0;
float co2percent = 0.0;    //Global CO2 value
//Global H20 Variables
int tempInH2OC = 0;       //Global Water Temp value
int hoodTemp = 0;         //Global Hood Temp
int RHAVG = 0;            //Global Humidity average
int RH0 = 0;              //Global Humidity one
int RH1 = 0;              //Global Humidity two

//Counterrr
unsigned long count1=0;     //Counter for delaying humidity sensors
unsigned long count2=0;

// Any One-Time Operations Here
void setup(){
  //Pin declarations
  pinMode(heaterCTRL, OUTPUT); pinMode(heatTog, INPUT_PULLUP); pinMode(heatLED,
OUTPUT);
  pinMode(humSel, OUTPUT); pinMode(humCTRL, OUTPUT); pinMode(humTog,
INPUT_PULLUP);
  pinMode(humLED, OUTPUT); pinMode(CO2CTRL, OUTPUT); pinMode(CO2Tog,
INPUT_PULLUP);
```

```
  pinMode(CO2LED, OUTPUT); pinMode(H2OCTRL1, OUTPUT); pinMode(H2OCTRL2, OUTPUT);
  pinMode(H2OFan, OUTPUT); pinMode(H2OTog, INPUT_PULLUP); pinMode(H2OLED, OUTPUT);
  pinMode(ReedSW, INPUT_PULLUP); pinMode(blowCTRL, OUTPUT); pinMode(H2OPump,
OUTPUT);
  pinMode(convertLED, OUTPUT);

  Serial.begin(9600);  //Serial Monitor Setup
  lcd.begin(16,2);     //LCD Setup
  //Temp Setup
  initSet_Temp();
  //Hum Setup
  Wire.begin();
  FreqCount.begin(1000);
  sens   =  i2cRead2bytes(81, 10); //Calibration for hum sensor
  offset =  i2cRead2bytes(81, 12); //Calibration for hum sensor
  initSet_Hum();
  //CO2 Setup
  mySerial.begin(9600); //Start Serial connection with CO2 Sensor
  initSet_CO2();
  //H20 Temp Setup
  initSet_H2O();
  digitalWrite(convertLED, HIGH);
  actuator_low();
  //Initial LCD Display
  lcd.clear();
  lcd.print("Tissue Engr Hood");
  lcd.setCursor(0,1);
  lcd.print("Initialized");
}

// MAsterMind Function
void loop(){
  count1++;               //Increment humidity sensor counter
  sensor_read();          //read sensors
  Standard_Display();     //displays sensor values
  check_pots();           //displays change in slide pots for setting values
  check_toggles();        //checks if toggles have been switched
  update_actuators();     //Updates actuators based on sensors and if toggles are on
  if(count1 > 6){
    count1 = 0;
  }
}
```

```
//************** Initialization Functions ***************

//Initializes Previous/Current Pot Values
void initSet_Temp(){
  int temp = analogRead(potPin[0]);
  potSensor[0] = int(temp*30/1024+20);
  potPrev[0] = int(potSensor[0]);
  potCur[0] = int(potSensor[0]);
}

//Sets Intial Desired Humidity Level
void initSet_Hum(){
  int hum = analogRead(potPin[1]);
  potSensor[1] = int(hum*29/1020 + 70);
  potPrev[1] = int(potSensor[1]);
  potCur[1] = int(potSensor[1]);
}

//Sets Initial Desired CO2 Level
void initSet_CO2(){
  int CO_2 = analogRead(potPin[2]);
  potSensor[2] = (CO_2*0.0096 + 0.2);
  potPrev[2] = int(potSensor[2]*10);
  potCur[2] = int(potSensor[2]*10);
}

//Sets Initial Desired H2O Level
void initSet_H2O(){
  int temp2 = analogRead(potPin[3]);
  potSensor[3] = int(temp2*0.04505387+4);
  potPrev[3] = int(potSensor[3]);
  potCur[3] = int(potSensor[3]);
}

//Set Actuators Low
void actuator_low(){
  digitalWrite(heaterCTRL, LOW);
  digitalWrite(humCTRL, LOW);
  digitalWrite(CO2CTRL, LOW);
  digitalWrite(H2OCTRL1, LOW);
  digitalWrite(H2OCTRL2, LOW);
  digitalWrite(H2OFan, LOW);
  digitalWrite(H2OPump, LOW);
}
```

```
//*************************** Heater Functions *******************************//
// Returns Laminar Flow Hood Temperature
int Get_Temp(){
  int tempV[6]; // raw temp sensor values
  int i=0;
  for(i=0; i<6; i++){
     tempV[i] =  analogRead(tempPin[i]);
     tempInC[i] = ((tempV[i]*(4.8685)-500)/10)+5;
//**************************************************************************
*********************************
     delay(50);
  }
  hoodTemp = mean(tempInC, 6);
  return hoodTemp;
}

// Sets Temperature Via Pot Sensor Value
int Set_Temp(){
  int temp = analogRead(potPin[0]);
  potSensor[0] = int(temp*30/1020+20);
  potPrev[0] = int(potCur[0]);
  potCur[0] = int(potSensor[0]);
  temp_pot = int(potSensor[0]);
  return potSensor[0];
}

// Displays Potentiometer Set Temperature
void Display_Update_Temp(){
    lcd.clear();
    lcd.print("Set Temp:");
    lcd.setCursor(0,1);
    lcd.print(temp_pot);
    lcd.print(" C");
}

// Heater Control
void Adjust_Temp(){
   if(hoodTemp < temp_pot){
     digitalWrite(heaterCTRL, HIGH);
   }
   else{
   digitalWrite(heaterCTRL, LOW);
   }
```

```
}


//************************** CO2 Functions
**********************************//

// Returns Average of Two CO2 Sensor Readings
float Get_CO2(){
  //Cozir sensors ship from the factory in streaming mode
//So we read incoming bytes into a buffer until we get '0x0A' which is the ASCII value for new-
line
  float CO2_1;
  //float CO2_2;

  while(buffer[ind-1] != 0x0A)
  {
    if(mySerial.available())
    {
      buffer[ind] = mySerial.read();
      ind++;
    }
  }
  CO2_1 = report(); //Once we get the '0x0A' we will report what is in the buffer
  return CO2_1;
}

// Returns CO2 Sensor Reading in Percent
float report()
{
//Cycle through the buffer and send out each byte including the final linefeed
/*
each packet in the stream looks like "Z 00400 z 00360"
'Z' lets us know its a co2 reading. the first number is the filtered value and the number after the
'z' is the raw value.
We are really only interested in the filtered value
*/
  for(int i=0; i < ind+1; i++)
  {
    if(buffer[i] == 'z') //once we hit the 'z' we can stop
    break;
    if((buffer[i] != 0x5A)&&(buffer[i] != 0x20)) //ignore 'Z' and white space
      {
        val += buffer[i]-48; //because we break at 'z' the only bytes getting added are the numbers
        // we subtract 48 to get to the actual numerical value
```

```
      // example the character '9' has an ASCII value of 57. [57-48=9]
     }
 }
 co2 = (multiplier * val.toInt()); //now we multiply the value by a factor specific ot the sensor.
see the Cozir software guide
 co2percent = co2/10000;
 ind=0; //Reset the buffer index to overwrite the previous packet
 val=""; //Reset the value string
 return co2percent;
}

// Sets Desired CO2 Level
float Set_CO2(){
 int CO_2 = analogRead(potPin[2]);
 potSensor[2] = (CO_2*0.0096 + 0.2);
 potPrev[2] = int(potCur[2]);
 potCur[2] = int(potSensor[2]*10);
 potSensor[2] = int(potSensor[2]*10);
 potSensor[2] = float(potSensor[2]/10);
 return potSensor[2];
}

//Updates display with slide pot value
void Display_Update_CO2(){
 lcd.clear();
 lcd.print("Set CO2 Percent:");
 lcd.setCursor(0,1);
 lcd.print(potSensor[2]);
 lcd.print("%");
}

//Controls solenoid to adjust CO2 level
void Adjust_CO2(){
 if(co2percent < potSensor[2]){
    digitalWrite(CO2CTRL, HIGH);
 }
   else digitalWrite(CO2CTRL, LOW);
}

//*************************** H2O Pump Functions
*****************************//
//Reads Temperature sensor on water tank
int Get_H2O(){
 int tempV; // raw temp sensor values
```

```
  tempV =  analogRead(tempH2OPin);
  tempInH2OC = ((tempV*(4.8685)-500)/10)+6;
//********************************************************************************
*******  return tempInH2OC;
}

//Reads slide pot for setting water tank temperature
int Set_H2O(){
  int temp2 = analogRead(potPin[3]);
  potSensor[3] = int(temp2*0.04505387+4);
  potPrev[3] = int(potCur[3]);
  potCur[3] = int(potSensor[3]);
  water_pot = int(potSensor[3]);
  return potSensor[3];
}

//Updates display with slide pot value for water tank
void Display_Update_H2O(){
    lcd.clear();
    lcd.print("Set H2O Temp:");
    lcd.setCursor(0,1);
    lcd.print(water_pot);
    lcd.print(" C");
}

//Adjusts peltier to control water tank temperature and controls water pump
void Adjust_H2O(){
  if(tempInH2OC < water_pot){
    digitalWrite(H2OCTRL1, HIGH);
    digitalWrite(H2OCTRL2, LOW);
    digitalWrite(H2OFan, HIGH);
  }
  else if (tempInH2OC > water_pot){
    digitalWrite(H2OCTRL1, LOW);
    digitalWrite(H2OCTRL2, HIGH);
    digitalWrite(H2OFan, HIGH);
  }
  else if (tempInH2OC == water_pot){
    if (Pump_Flag == 0){
      digitalWrite(H2OPump, HIGH);
      Pump_Flag = 1;
    }
  }
  else{
```

```
    digitalWrite(H2OCTRL1, LOW);
    digitalWrite(H2OCTRL2, LOW);
    digitalWrite(H2OFan, LOW);
    digitalWrite(H2OPump, LOW);
  }
}

//************************** Humidity Functions *****************************//
// Function for reading from humidity sensors
int i2cRead2bytes(int deviceaddress, byte address)  {
 // SET ADDRESS
 Wire.beginTransmission(deviceaddress);
 Wire.write(address); // address for sensitivity
 Wire.endTransmission();
 // REQUEST RETURN VALUE
 Wire.requestFrom(deviceaddress, 2);
 // COLLECT RETURN VALUE
 int rv = 0;
 for (int c = 0; c < 2; c++ )
 if (Wire.available()) rv = rv * 256 + Wire.read();
 return rv;
}

//Read Humidity Sensors and Return Average
float Get_Hum(){
if(count1 > 5){
  digitalWrite(humSel, LOW);
  delay(200);
  if (FreqCount.available()) {
    unsigned long count = FreqCount.read();
    RH0 =  (offset-count)*sens/4096;
  }
  digitalWrite(humSel, HIGH);
  delay(200);
  if (FreqCount.available()) {
    unsigned long count1 = FreqCount.read();
    RH1 =  (offset-count1)*sens/4096;
  }
    RHAVG = ((RH0+RH1)/2)-4;
    return RHAVG;
}
}

//Reads humidity value from slide pot
```

```
int Set_Hum(){
  int hum = analogRead(potPin[1]);
  potSensor[1] = hum*0.0785 + 20;
  potPrev[1] = int(potCur[1]);
  potCur[1] = int(potSensor[1]);
  hum_pot = int(potSensor[1]);
  return potSensor[1];
}

//Updates display with humidity value from slide pot
void Display_Update_Hum(){
  lcd.clear();
  lcd.print("Set Humidity:");
  lcd.setCursor(0,1);
  lcd.print(hum_pot);
  lcd.print("%");
}

//Adjusts Humidity by turining on humidifier
void Adjust_Hum(){
  if(RHAVG < potSensor[1]){
     digitalWrite(humCTRL, HIGH);
  }
  else {digitalWrite(humCTRL, LOW);}
}

//**************************** Blower Control ****************************//
//Adjusts blower output depending on position of door
void Adjust_Blower(){
   int ReedVal = digitalRead(ReedSW);
   if(ReedVal == HIGH){
     digitalWrite(blowCTRL, LOW);
   }
   else if(ReedVal == LOW){
     digitalWrite(blowCTRL, HIGH);
   }
   else digitalWrite(blowCTRL, LOW);
}

//**************************** Display
*****************************************//
//Displays sensor values
void Standard_Display(){
  lcd.clear();
```

```
 lcd.print("Temp:");
 lcd.print(hoodTemp);
 lcd.print("C ");
 lcd.print("Hum:");
 lcd.print(RHAVG);
 lcd.print("%");
 lcd.setCursor(0,1);
 lcd.print("CO2:");
 lcd.print(co2percent);
 lcd.print("%");
 lcd.print("H2O:");
 lcd.print(tempInH2OC);
 lcd.print("C");
}


//*************************** Functions for MasterMind ********************//
//Reads Sensor values and Slide pots
void sensor_read(){
 Get_Temp();
 Get_Hum();
 Get_CO2();
 Get_H2O();
 Set_Temp();
 Set_Hum();
 Set_CO2();
 Set_H2O();
}

//Checks if toggles have been flipped
void check_toggles(){
 int heatOnOff = digitalRead(heatTog);
 int humOnOff = digitalRead(humTog);
 int CO2OnOff = digitalRead(CO2Tog);
 int H2OOnOff = digitalRead(H2OTog);

 if(heatOnOff == LOW){
  digitalWrite(heatLED, HIGH);
  Heat_Flag = 1;
 }
 else if(heatOnOff == HIGH) {
  digitalWrite(heatLED, LOW);
  digitalWrite(heaterCTRL, LOW);
  Heat_Flag = 0;
```

```
  }
  if(humOnOff == LOW){
    digitalWrite(humLED, HIGH);
    Hum_Flag = 1;
  }
  else if(humOnOff == HIGH){
    digitalWrite(humLED, LOW);
    digitalWrite(humCTRL, LOW);
    Hum_Flag = 0;
  }
  if(CO2OnOff == LOW){
    digitalWrite(CO2LED, HIGH);
    CO2_Flag = 1;
  }
  else if(CO2OnOff == HIGH){
    digitalWrite(CO2LED, LOW);
    digitalWrite(CO2CTRL, LOW);
    CO2_Flag = 0;
  }
  if(H2OOnOff == LOW){
    digitalWrite(H2OLED, HIGH);
    H2O_Flag = 1;
  }
  else if(H2OOnOff == HIGH){
   digitalWrite(H2OLED, LOW);
   digitalWrite(H2OCTRL1, LOW);
   digitalWrite(H2OCTRL2, LOW);
   digitalWrite(H2OFan, LOW);
   digitalWrite(H2OPump, LOW);
   H2O_Flag = 0;
   Pump_Flag = 0;
  }
}

//Checks if slide pots have changed
void check_pots(){
    int i;
  for(i=0; i<4; i++){
    if((potCur[i] != potPrev[i])) action[i] = 1;
    else action[i] = 0;
  }

  if(action[0] == 1){
    Display_Update_Temp();
```

```
    delay(100);
  }
  if(action[1] == 1){
    Display_Update_Hum();
    delay(100);
  }
  if(action[2] == 1){
   Display_Update_CO2();
   delay(100);
  }
  if(action[3] == 1){
   Display_Update_H2O();
   delay(100);
  }
}

//Updates actuators based on slide pot values and sensor values
void update_actuators(){
  //heater
  if(Heat_Flag == 1){
    Adjust_Temp();
  }
  //Humidity
  if(Hum_Flag == 1){
    Adjust_Hum();
  }
  //CO2
  if(CO2_Flag == 1){
    Adjust_CO2();
  }
  //Water Pump
  if(H2O_Flag == 1){
    Adjust_H2O();
  }
  //Blower
  Adjust_Blower();

}
```