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Cell Factory

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Abstract

This project is aimed to refine current cell culturing practices being used for 3T3 fibroblast preparation for Rochester Institute of Technology Biomedical Engineering Department's labs. Current practices have not been optimized for the investment of time and materials required. We have developed an automated cell culturing device that reduces consumables and cost associated with subculturing.. This device will assist the lab instructor by increasing consistency in passaging liquid volumes and the throughput of multiple plates. Through refining and standardizing the process to maintain and grow 3T3 fibroblasts through use a pressure driven automation system, consistency in results will be increased while time, and material consumption will be reduced.

Background

Cell culturing, by definition, is the process of removing cells from a plant or animal body and growing the cells in a different environment, typically in a laboratory setting. This is a common practice used in many fields including biomedical scientists, cell biologists, and clinicians among many others. With such an impact in many fields, cell culturing is a major factor in developing vaccines, as the ability to grow a large quantity of cells is imperative. Large quantities of cells created by cell culturing allows scientist to study different alternatives relatively quickly, without having to grow a new set of cells every time a new test is done. Cell culturing is also crucial in cancer research in testing different cancer cells will forms of radiation and chemicals [1].

Figure 1 depicts the general steps taken in any cell culturing process. Every cell culturing process is different depending on the type of cells and what the desired outcome is, generally the steps are the same.

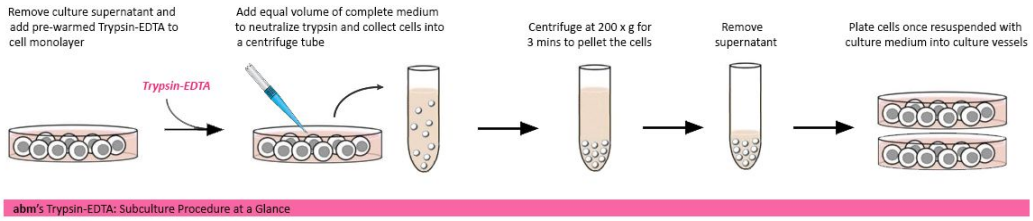

Figure 1. Cell Culturing Overview [1]

The typical cell culturing process used across the nation are not optimized to reduce time and materials, nor has automation been implemented into any practice. The cost of micropipette tips and serological pipettes is high compared to the amount needed per passage. This high cost alongside the environmental impact of disposing high volumes of plastic waste is a major issue.

The Biomedical Engineering Department of Rochester Institute of Technology curriculum includes many lab classes that use 3T3 fibroblast cells (3T3-L1). The department has the responsibility of culturing the cells so that they are ready for the lab classes. The current lab practices used by Biomedical Engineering professors is very inefficient and wastes a lot of materials which is in turn extremely costly to the department. These costs accumulate over time and the funds take away other opportunities for the students in the department.

Description of Design

The product design was based around the customer and engineering requirements (Table 1 and 2, respectively) provided in the project readiness package submitted with the project concept by the project customer, Dr. Jennifer Bailey. Rather than looking to reinvent the entire cell culturing process, the project was aimed to determine where assistance could be given to the user that would increase time efficiency and repeatability while reducing excessive use of supplies. To determine where in the process alteration could be made, several steps were assumed to be constants throughout that have a smaller margin of improvement. These included the time between subculturing, seeding new flasks (initially and following subculturing), and intermediate cell culturing steps (warming media, PBS, trypsin; waiting for trypsin to lift the cells). By setting these as a constant and assuming that the overall framework of the process is similar, a majority of the project's customer requirements (Table 1, CR# 1, 2, 3, 4, 7) would be met. The remaining customer requirements focused on the ease of use, safety for user, and efficiency of the process.

Seeing that efficiency was one of the remaining requirements to be met, it was determined that the part of the process led to the most waste of time and products was subculturing a plate. Through the process of subculturing, multiple pipets are required for the process to remain sterile and all liquid handling has a factor of human error associated with it due to technique and use of the micropipette. Upon talks with both the project customer and Dr. Vinay Abhyankar, the project automated cell culturing device that could assist the user in subculturing a plate. It was determined that automation would be limited to liquid addition and removal steps in the subculturing process.

The general process for the removal end of the system was aimed to be modeled after the vacuum system already used in cell culturing. Without adding any external components to the biosafety cabinet, the liquid addition subsystem of the device could be hooked up to the air-in line. By attaching the air-in line to a closed container, this would create a pressurized vessel that, when opened to atmospheric pressure, would cause the liquid held by the container to flow. To control the pressure of the air-in line, a pressure regulator was added in between in the air-in valve and the pressure vessels. The air-in line in most biosafety cabinet (BSCs) is not a closely regulated number and can fall within a range of approximately 40 psi. By keeping the pressure in the vessel at a constant pressure, a constant liquid flow could be achieved when the container is opened to atmospheric pressure. To release liquids from the pressure vessel in consistent, precise volumes, it was determined that a valve could be used. With the valve innately in the off state (closed), liquid could be retained within the vessel, and then could be opened to release water. To control the valve, a microcontroller was salvaged from an Anet 3D-printer. The microcontroller can determine a set time for the valve to be opened to dispense liquid. Individual switches were used for each of the three liquids used in subculturing, PBS, trypsin, and media. These switches were then connected to the valves of the

three pressure vessel containers. Programming through Arduino allowed to for each switch to, when engaged, open its correlated valve for a preset time.

Since a microcontroller was already being used to open and close the liquid addition valves, stepper motors from the salvaged 3D-printer were used to further automate the liquid removal system. For the liquid removal process, the microcontroller was programmed that when a switch connected to the stepper motors was engaged, the stepper motors were used to move a pipet over into the petri dish placed on the platform. Once the pipet tip is placed into the liquid, expended liquid would be pulled from the petri dish and emptied into a waste container. To house both of these systems an aluminum beam extrusion kit was used to build our frame; this style of beam is modular and allows for making quick alterations to the design (Figure 2). The overall footprint of the design only takes up a 9”x13” space in the BSC.

The final automation device (Figure 3) is housed on the described frame with the three pressurized vessels for growth media, trypsin, and PBS for liquid addition, and one vacuum waste container for the liquid removal container attached to the 3D-printer stepper motor system. The system is powered by a 120 V wall outlet, the liquid addition system is run by the standard BSC air-in line, and the vacuum system is run by the standard BSC vacuum line. The user simply fills each container with its respective liquid, powers the system and attaches the air-in and vacuum lines. Once connected, the user is able to follow a procedure for any number of dishes. All setup, operation, and tear down instructions are included with the device. The overall device cost was approximately $200 to build. It is estimated that each subculture use of the device will save approximately $0.94. Assuming two subcultures per week, one plate maintained for a year could have savings of approximately $97.34.

Table 1. Customer requirements for the cell factory project.

CR#	Requirements	Importance
1	Maintain proper culture conditions (sterility, temperature, humidity, and CO2)	1
2	Maintain pH levels of media	1
3	Maintain growth of cells for 3 weeks	9
4	Allow for viewing of cells during culture	3
5	Easy media changes	3
6	Safe for student population	3
7	Easy ability for collection of culture or culture products	1
8	More efficient than current practice	1

Table 2. Engineering requirements for the cell factory project.

ER#	Importance	Description	Ideal Value	Units of Measurement	Tolerance	CR Map
1	1	Media is Contained	Yes	[-]	N/A	1
2	1	Maintains Media pH	7.4	[mol/L]	± .15	2
3	3	Fits on Microscope During Culture	Yes	[-]	N/A	4
4	9	Maintains Cells in Culture	>= 3	[wk.]	N/A	3
5	9	Cell Growth Minimally Impacted	Standard T-flask	[%]	±10	1,3,7
6	1	Assembly Time	< 60	[min]	N/A	8
7	1	Disassembly Time	< 30	[min]	N/A	7
8	3	Time to Change Media	< 1	[min]	N/A	5
9	3	Pinch Hazard	No	[-]	N/A	6
10	3	Spill Hazard	No	[-]	N/A	6
11	9	Reduction in Subculturing and Maintenance Time	50	[%]	N/A	8
12	9	Reduction in Subculturing and Maintenance Consumables	50	[%]	N/A	8
13	3	Cell Confluency upon Passaging	65	[%]	± 5	8

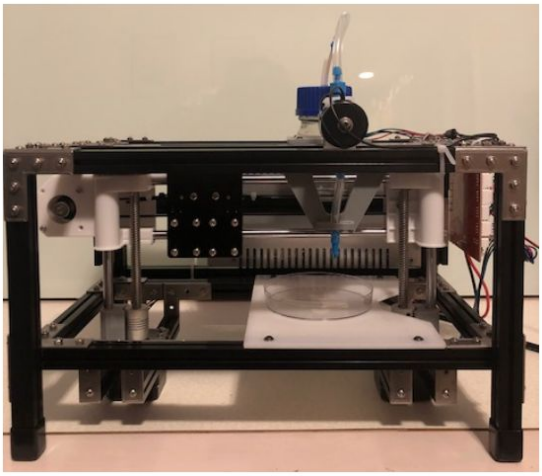

Figure 2. Automation device frame and removal hardware.

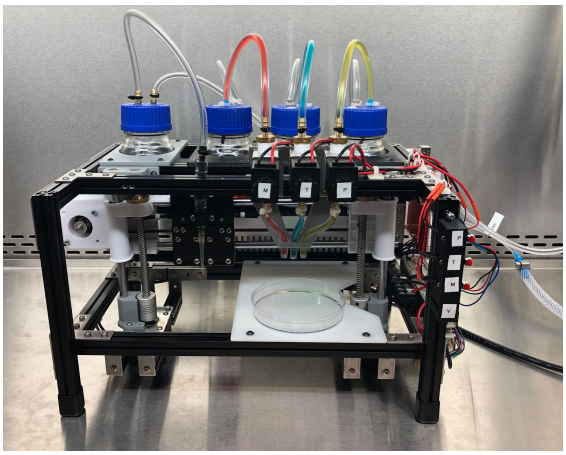

Figure 3. Final automation device design.

Supporting Feasibility Evidence

Cell Culturing Testing

The purpose of the cell culture testing was to see how different seeding densities affect the doubling time of the 3T3-L1 cell line. This was done producing and analyzing growth curves for three different seeding densities in triplicate. The seeding densities that were tested were 3,000 cells per centimeter squared, 6,000 cells per centimeter squared, and 9,000 cells per centimeter squared. The growth curve was developed by seeding 8-wells of each density in a 24-well plate. This was to make sure all the conditions were constant for each density; the media was changed on the fourth day of testing for each plate. The first and second plate were seeded with cells on passage number 13, and the third plate was seeded with cells on passage number 14. To produce the growth curve, each day for eight days a well was randomly chosen from each density and the number of cells were counted. The cells were counted using a hemocytometer and fluorescent imaging.The cell counts were made into growth curves to analyze the doubling time and the growing tendencies of the cell line. The results can be seen in Figure 4.In order to find the doubling times, this formula in Eq. 1 was used to obtain the doubling time (time variable) [2].

$$cell\ count\ (t) = cell\ count_i \cdot e^{growth\ rate \cdot time} \quad (1)$$

The doubling time results can be seen in Table 3. This data was used to understand different cell densities and to understand the cell line. The results show that all doubling times fall within or around the approximate doubling

time, then cell doubling time acts independent of seeding density. Seeding density was not altered by the process parameters; all liquid amounts were held constant between each seeding density (i.e. 3000, 6000, and 9000 cells/cm2 all had same amount of media). The data from Plate 3 was removed from overall doubling time calculations. It was determined with the project customer that the trend of the graph was not representative of a typical growth curve and should be removed from the results.

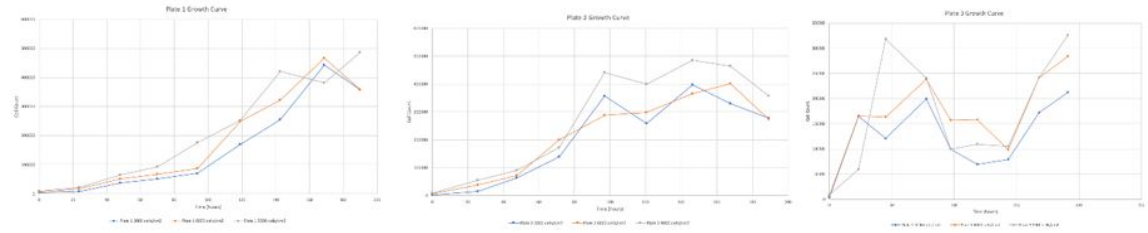

Figure 4. The graphs show the growth curves from the three plates in order. Cell number on the y-axis and time in hours on the x-axis. The blue line is the 3,000 cells/cm2, orange line is the 6,000 cells/cm2, and the gray line is the 9,000 cells/cm2.

Table 3. The doubling time results for the Cell Culture Testing.

	Doubling Time (hours)		
Seeding Density (cells/cm2)	3000	6000	9000
Plate 1	22.07	30.85	22.73
Plate 2	15.85	24.47	23.67
Average	18.96	27.66	23.20

Liquid Handling Testing

The initial liquid testing took place through a single stream system (wall hook-up to pressurized vessel to valve) shown in Figure 5. These tests were to serve as a proof of concept for time to liquid dispensed prior to connecting all three pressurized vessels. The final liquid testing was done on the final automation device which is shown in Figure 3. For this initial testing, it was done by priming the line until the liquid level reached 80 mL. The liquid used was water for the initial testing. The valve was then opened for 0.5 seconds when the switch was pressed; this was conducted until the vessel was emptied. The mass of the liquid in the petri dish was recorded following dispensing liquid. The results are shown in Figure 6. The results show there is no difference in the amount of water dispensed with the changing of the head space, this is show because the results are in a straight line with no variation. The liquid dispensing unit is able to dispense a specific amount of liquid based off a unit of time which how the final automotive device functions.

Our final liquid handling testing was performed across the entire three valve system. As previously performed, the line was primed until liquid level reached 80 mL. The valve was then opened for 0.5 seconds when the switch was pressed; this was conducted until the vessel was emptied. The mass of the liquid in the petri dish was recorded following dispensing liquid. This testing focused on worst case scenario situation: one container being emptied while two were held empty. The results can be seen below for each of the three valves; PBS in Figure 7, trypsin in Figure 8, and media in Figure 9.

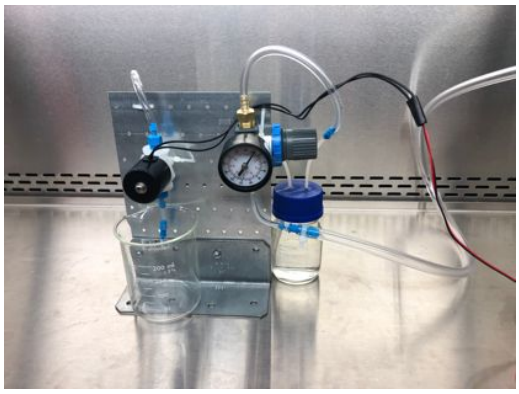

Figure 5. Original single stream pressure vessel model.

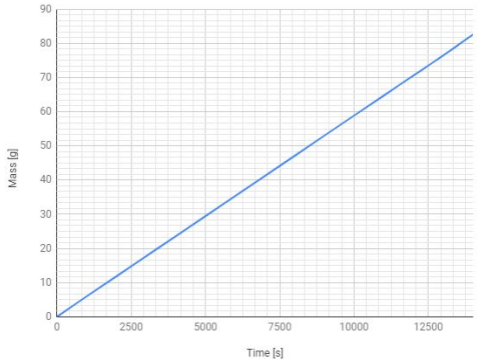

Figure 6. The graph shows Liquid Handling Test results. Time in seconds is on the x-axis and mass in grams is on the y-axis.

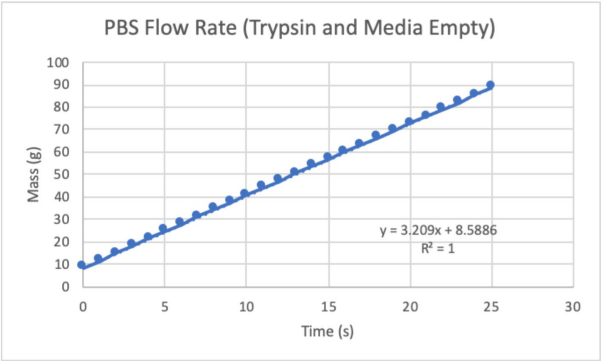

Figure 7. The graph shows Liquid Handling Test results for PBS. Time in seconds is on the x-axis and mass in grams is on the y-axis.

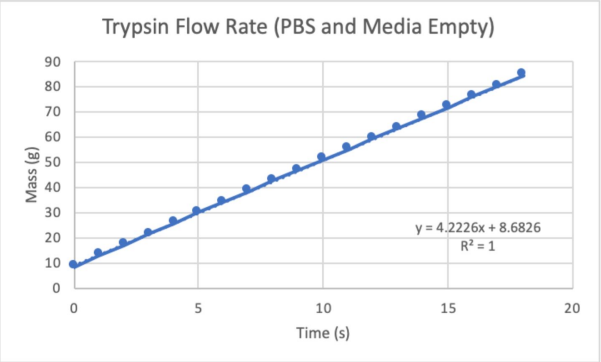

Figure 8. The graph shows Liquid Handling Test results for trypsin. Time in seconds is on the x-axis and mass in grams is on the y-axis.

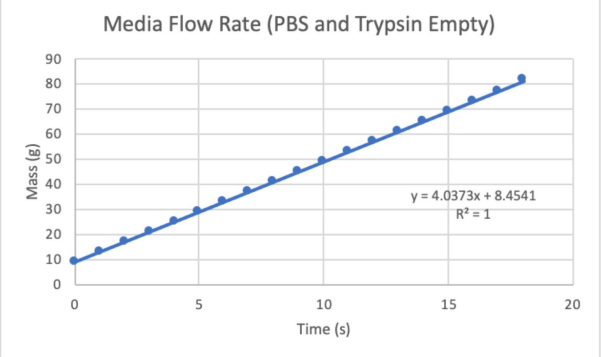

Figure 9. The graph shows Liquid Handling Test results for media. Time in seconds is on the x-axis and mass in grams is on the y-axis.

Results, conclusion, and recommendations

Testing Results

The final results of the cell culture tests indicate that the doubling time of the 3T3-L1 cells is independent of initial seeding density, as seen in Table 3. This affords future users of the device the confidence that by assuming a doubling time of 28 hours (roughly the max average), a desired final cell concentration can be approximated based on time in culture and initial seeding density. Due to the nature of culturing cells for student-lab use, it is safest to assume max doubling time in order to ensure that the minimum cell concentration is reached.

The final results of the liquid handling test prove that the amount of liquid dispensed from the pressurized vessel is directly related to the amount of time that the valve is open, as shown in Figure 7, Figure 8, and Figure 9. There is also negligible loss of dispensing rate as the headspace in the vessel increases. This indicates that the liquid in the vessel is uniformly dispensed regardless of headspace volume. These results ensure that the user of the device will know exactly how much liquid volume they are dispensing at all times.

Conclusion

The testing results thoroughly indicate that the device will perform its chief functions. The user of the device will still interact with the cultured cells at critical stages, but liquid handling functions can be operated entirely by the device. The device will save time and resources previously spent on liquid handling for cell culture, as well as accurately and consistently dispense liquid volumes.

Recommendations

For future iterations of this project, possible areas of improvement could be with increasing the user selections available through the device. A possibility for this would be to have include a screen that prompts the user with questions regarding the size of the vessel that they are subculturing and intended seeding densities into a new tray. From this, the selection could change the delays written into the code (allowing for a different volume of liquid to be dispensed) and be flashed on to the micro controller. This would add additional steps to the scope of the

project to completly automate all steps of the subcultuirng process. The device also has further areas of optimization and removal of user interaction by adding further programming to have the liquid removal system pickup and remove pipette tips as part of the program. While this iteration the device has been aimed primarily to optimize time to perform actions and the device's footprint within the biosafety cabinet, additional attention could be given to ensuring that all electrical components are properly isolated from both the user and any contact to liquids.

References

[1] "Cell Culture - An Introduction." ***Cell Culture - Introduction | ABM Inc.***, www.abmgood.com/marketing/knowledge_base/cell_culture_introduction.php.

[2] ***QUANTIFYING CELL DIVISION***, www.tiem.utk.edu/~gross/bioed/webmodules/celldivision.html.

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