DEVICE FOR AUTOMATED SELECTION AND PLACEMENT OF CELL CLUSTERS WITHIN BIOFABRICATED TISSUE CONSTRUCTS

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By

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Device for automated selection and placement of cell clusters within biofabricated tissue constructs

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Abstract

Organ transplant shortage is a worldwide issue, even within the United States. Bioprinting seeks to remedy this issue via creating organs *de novo*, but technological limitations have barred the field from bearing practical applications. Previous literature within the field has shown that cell clusters act as a useful ink analogue within bioprinting. However, further experiments on cell cluster viability are complicated by their complexity. This technical project seeks to combat limitations on experimental design with automation. The solution proposed is a micromanipulator paired with a computational framework allowing for computer control. The physical framework is composed of linear actuators in a 3-axis configuration anchored to a stationary frame. These actuators control the orientation of a mounted aspirator tool relative to a secured plate holder. The computational framework employs G-code and allows for manual control over the position of the linear actuators. The interface between these two frameworks is an Arduino Uno connected to motor drivers. The aim is to reduce the work of an individual researcher and allow for the acceleration of bioprinting research. The device produced by this project exceeds precision specifications when applying manual inputs. When paired with a functional aspirator tool, this device has the potential to greatly improve bioprinting research while leaving room for future incorporation of degrees of automation.

Keywords: Bioprinting, Micromanipulation, Microstepping

Introduction

Organ transplants can be a critical component in an individual's recovery from chronic disease; however, treatment remains unavailable even in the developed world. Patients remaining on an organ waiting list suffer a greatly increased mortality rate relative to the rest of the population. It is estimated that on average, 17 individuals die every day while waiting for a life-saving donation (*Organ Donation Statistics*, 2021).

The chief limiting factor to organ donation is supply. Even in successful cases, patients typically find themselves requiring immunosuppressant drugs for the remainder of their lives. Immunocompromised results in previously ill patients becoming further susceptible to disease.

Instead of sourcing preexisting organs for transplant, bioprinting seeks to combat the enumerated issues plaguing organ transplantation by manufacturing *de novo* tissues and organs. However, the field is still emerging and requires a laundry list of technological innovations in order to address the underlying issues of organ scarcity. Under the advisory of assistant professor of biomedical and chemical engineering Christopher Highley, a team consisting of Timothy Luu, Matthew Runyan, and Garrett McQuain created an automated micromanipulator that can aspirate and place cell clusters within bioprinted constructs. This seeks to further the field of bioprinting by creating new research tools and methods to increase throughput and repeatability.

An experiment conducted by Jakab et al. (2004) explored cell cluster spheroids as a viable ink for use in bioprinting. In order to verify this, researchers analyzed cell growth in a 10-cluster ring formation as shown in Figure 1. In order to further investigate the capabilities of cell spheroids as a viable bioink, cultures must be further assembled and analyzed. Barriers to this process have prevented the procedure from becoming commonplace. Chief among these is the work one researcher must undertake in experimental design. Placing individual cell spheroids with a diameter of just a few hundred micrometers precisely is tedious, and this must be accomplished 10 times for each trial simply to recreate the work done by Jakab. Furthermore, though the work of Jakab was groundbreaking, the human body is not a simple collection of rings. A small increase to the complexity of experimental design over numerous repeated trials represents a force multiplier on the work of a research team. Thus, our capstone group, Timothy Luu, Garrett McQuain, and Matthew Runyan, under the advisory of Christopher Highley of the Department of Biomedical Engineering sought to develop a mechanism by which we would be able to automate cell placement in order to further bioprinting research.

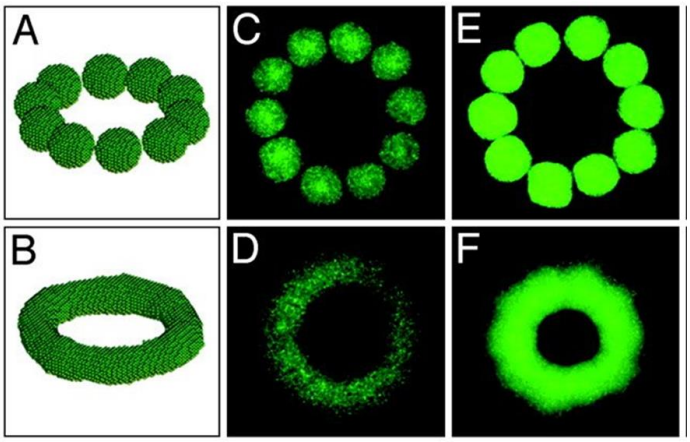

Fig. 1. Central Experiment of Jakab et al.. Experiment conducted by Jakab et al. (2004). The upper row (A,C,E) show cluster placement pre-culture, while the bottom row (B,D,F) show post-culture formations.

Results

The outcome of our project was a low-cost micromanipulator controlled manually via open-source code. Designs for this project were inspired by the work of Xu et al., who proposed a similar low-cost micromanipulation solution to bioprinting. The final design more closely resembles a modified 3D printer in its conception. The device is made to be tailorable to fit future needs and functions while still accomplishing

its goal as a micromanipulator. The design can be divided into three frameworks: physical, electrical, and computational.

Physical

The basic physical framework of the device is a mounted aspiration device that is capable of moving in 3 dimensions relative to a stage. The device, illustrated in Figure 2, consists of 3 linear actuators that control positioning of the aspirator in the X, Y, and Z planes. The X and Y-axes actuators form one table unit and are anchored to the device's frame. A plate is mounted to a stage in the center of the actuator set. The aspirator is mounted to a cantilever attached to the Z-axis actuator, which is mounted to the frame. This decision was made in order to minimize motion of the aspirator, as the part was deemed to be more easily disrupted by excess movement. Thus, while the aspirator remains stationary in the X and Y-axes, the action of the table unit causes the aspirator to move relative to the plate.

The aspirator device, pictured in Figure 3, consists of a pulled glass capillary with an inner diameter of 0.68 mm connected to a soft plastic tube with an inner diameter of 1 mm. Suction force is generated by the aspirator via the action of a 1mL syringe, which is connected to the other end of the soft plastic tubing. The syringe is mounted to a fourth, unpictured, linear actuator to be placed alongside the device rather than mounted upon it. The aspirator tool is housed within the holding box mounted at the end of the cantilever and secured by O-rings(*Thomas Scientific - Lab Supplies, Lab Equipment, Lab Chemicals, & Lab Safety*, n.d.).

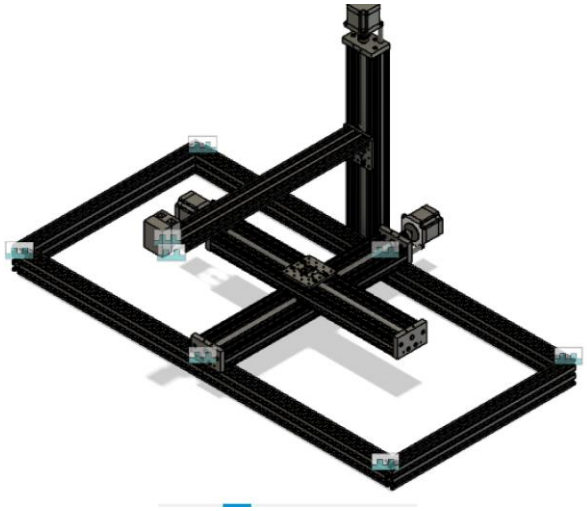

Fig. 2. Images of our Autodesk 3D model for the entire assembly. The final design for the micromanipulator created in this project. The X and Y positioning of a stage via linear actuators, while the aspirator tool is mounted on a cantilever attached to a separate, vertical linear actuator.

Electrical

The electrical framework for our device consists of a power supply, four TB 6600 stepper motor drivers, and an Arduino Uno. The power supply is an AC to DC current converter, which supplies 36V DC and can be powered by any standard wall outlet. The power supply provides DC current to the high voltage section of each of the four TB 6600 drivers, which each power their respective stepper motor. The signal section of each driver is connected to the Arduino Uno, with separate wires for the ENA+, DIR+, and PUL+ sections being connected to their respective pin on the Arduino board, according to our Universal G-Code setup. The ENA-, DIR-, and PUL- sections for each TB 6600 driver are all connected to the GRD (ground) pin on the Arduino board. A full visualization of the electrical setup is shown below in Figure 4.

NEMA 23 High torque stepper motors drive the XYZ stages of the device, while a NEMA 11 stepper motor drives the aspirator stage. The input voltage for both the NEMA 23 and 11 stepper motors is 24-48V DC, and our AC-DC converter supplies all four of these motors with 36V. However, the rated current for the NEMA 23 stepper motors is 3A DC, while the rated current for the NEMA 11 stepper motor is 2A DC. The TB6600 drivers in our setup can output anywhere from 0.5 - 4 amps, based on their dip switch setting. The dip switch settings for each of our TB 6600 drivers was configured to supply the correct current, based on which stepper it is powering.

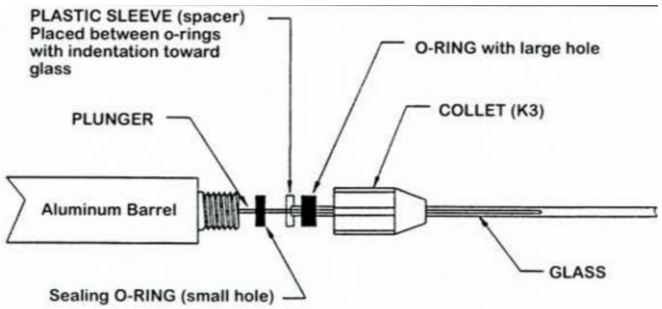

Fig. 3. Images of the aspiration tool we used as well as some of the parts we fitted it with. The aspiration tool applied in our resulting device. Suction is generated by motion of the attached linear actuator away from the actuator's tip much like a typical syringe.

Computational

G-code is the most popular programming language for computer numerical control (CNC). CNC facilitated by G-code is the most widely used method for automation of machine tools. G-code commands are passed to the microcontroller in order to control which stepper motors move, how fast they move, and the path they move on.

Control Software (G-Code Sender)

Universal G-code sender is an open-source program that is used to send G-code commands and files to the Arduino microcontroller. This program is simple but provides a means for users to input G-code commands to the GRBL controller.

Microcontroller Firmware (GRBL)

GRBL is a G-code firmware is a program written in optimized C which interprets and compiles G-code sent from the control software. In this regard the GRBL is the brains of our machine. GRBL firmware reads G-code commands and containing files and converts them into actual electrical pulses sent to the motors, thus providing motion across the tree axis. The firmware accepts commands in standard G-code form and can

use outputs of CAD designs as commands, making this firmware appropriate for future directions of this machine.

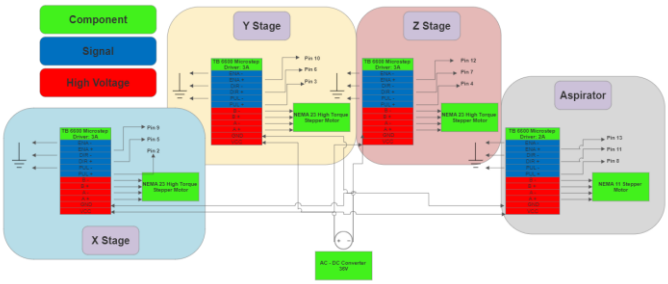

Fig. 4. The Electrical setup of our device is shown Our 36V power supply provides power to the high voltage section of each of our four drivers. The signal section of each of our drivers is connected to the Arduino Uno.

Resolution Calculation

The resolution requirement set by our advisor was 10 microns, meaning that our device should be able to position itself in any dimension with a step size of 10 microns or less. The NEMA 23 high torque stepper motors used in our setup have a step angle of 1.8 degrees, which translates to 200 steps per revolution. The pitch of the lead screw that our motors drive is 2 millimeters. Using Equation 1, the resolution of our device without microstepping was calculated to be 10 microns. While this is an impressive resolution that is within our advisor's requirements, it is possible to vastly increase the resolution of our device through the use of microstepping. The TB 6600 drivers used in our setup can be modified to provide 2, 4, 8, 16, or 32 microsteps per step, depending on the dip switch setting. When using the highest microstep setting of 32, the maximum theoretical resolution of our device was calculated to be 0.3125 microns or thirty two times the original resolution without the use of microstepping.

$$Resolution\ (um) = \frac{1}{(\#\ of\ steps\ per\ revolution) \times (\frac{1\ Revolution}{Lead\ Screw\ Pitch\ (mm)}) \times (\frac{1\ mm}{1000\ um})} \quad [1]$$

Validation

Validating our device's movement was a unique challenge due to the incredibly small scale associated with its movements. To work around the task of having to physically measure a distance on the scale of microns, a representative measuring distance of 10 millimeters was selected. This distance was selected because it was large enough to easily measure and visualize. A microstep setting of 16 was chosen for our validation tests. Using Equation 1, the size of each microstep using this setting was calculated to be 0.625 microns. With this resolution size, the number of microsteps needed for our device to move itself 10 millimeters is 16,000 microsteps. To validate our device, we programmed our universal g-code sender to move our device 16,000 microsteps in a particular direction from its zero position and repeated this test multiple times for each axis of movement. Our device moved to the 10 mm mark in each of our tests; this was further validated through the use of the visualizer tool of our g - code sender. This tool shows the readout of where the computer thinks our device is located, relative to its zero position. As shown below in Figure 5, our device moved exactly 10 millimeters in the specified direction during each of our validation tests.

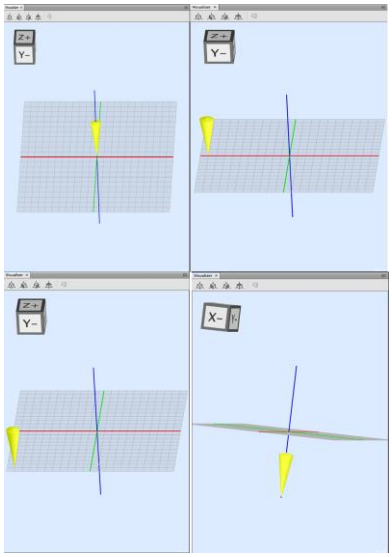

Fig. 5. Validation Tests. Images of the G-code interpretation of the positioning of the aspirator tool. Top Left: Initial position centered at the origin (0,0,0); Top Right: X-axis test (10,0,0); Bottom Left: Combined X-Y-axis test (10,10,0); Bottom Right: Z-axis test (0,0,10).

Discussion

Initially, the intent of this project was to create a device capable of automatically aspirating and placing cell clusters in a 3-dimensional space. This device would have applications in bioprinting research, as it would remove human elements from experimental design. Typically, a researcher would manually place individual cell clusters approximately 500 microns in diameter within a polymer gel. In an experiment conducted by Jakab et al. (2003), each trial consisted of 10 precisely placed clusters each, as shown in Figure 1. Manual placement of these clusters poses severe limitations. The sample size of such an experiment is constrained by the time a researcher can dedicate to experimental design preparation. Any marginal increase to the complexity of cell cluster patterns represents a force multiplier on the work a researcher would have to dedicate to experimental design given repeat trials.

Our initial wiring setup called for the use of an Arduino CNC Motor Shield, combined with BIQU A4988 stepper motor driver modules. This is a setup that is very commonly used with Arduino - stepper motor setups, however, the large power requirements of our NEMA 23 high torque stepper motors (24-48VDC, 3A), proved to be too much for our initial Arduino setup to handle without overheating. To remedy this problem, we modified our wiring setup to replace the CNC Motor Shield and driver modules with TB 6600 microstep drivers. With this modification, our device can handle the large power requirements of our stepper motors without overheating.

The initially proposed solution to the enumerated issues with bioprinting research was to be an automatic micromanipulator. Limitations of time and resources necessitated a limiting of the project's scope to designing and building a framework device and computational model that could be built upon by later groups. The design specifications of the original device were retained. The device is capable of resolutions well under the 10 micron ideal decided upon during design specification. However, multiple constraints prevent the device from fully utilizing the most fine resolution scale. First, there is simply limited use for such specificity in positioning. The targets for this device will be on average 500 microns in diameter, and will be aspirated by a 680 micron capillary. Practically speaking, successful aspiration will hinge upon precision of placement

on the scale of tens of microns rather than tenths. Additionally, inherent errors of small percentages have the potential to compound over hundreds if not thousands of microsteps. Thus, without user supervision, as would be expected for an automated device, there exists the possibility that the device will eventually veer off course when operating at sub-micrometer scales. Though mechanical constraints bar the device from operating at its full potential of 0.3125 microns, the mechanical and computational frameworks developed over the course of this project have exceeded expectations.

Future Directions

The further implications of the technological frameworks devised by this project are numerous and adaptable. Constraints on this project were such that the aspirator tool is presently nonfunctioning. Attachment of this tool to a linear actuator would be necessary for the device to fully function. Primary directions for innovation to address the aforementioned issues inherent in bioprinting would be to incorporate layers of automation to the design. Presently the device operates on computer keyboard inputs, but future directions could specify a coordinate system and direct the device to aspirate a target at one location and place it at another. This process, while not being fully automated, would allow for the placement of cell clusters with high precision while reducing human workload. This is a stepping stone in service of the ideal level of automation, which would incorporate computer vision. A device that could differentiate between cell clusters via visual detection and select a specified one to move to a determined location minimizes human input. This ideal device would greatly reduce the work an individual researcher would have to do in experimental design. Compared to the methods employed by Jakab, which inspired this project, an automatic micromanipulator would be a major step up. A computer vision-integrated micromanipulator would not only be capable of assembling constructs at a higher degree of precision with fewer human input, but also allows for the creation of more complex assemblies and polycultures. This technology is applicable to all manner of cell culture avenues. These future endeavors would allow research methods into 3D cell growth as outlined in Jakab et al. (2004) to become more widespread and accelerate the research rate for bioprinting.

Materials and Methods

Materials

Linear Rails
Linear rails are strong and rigid aluminum shafts used to carry the load and support the linear movement of the machine.

Microcontroller Board
Arduino Uno Rev3 is used to control motion of the machine. The board has 16 output pins that are used as connections to the motor stepper drivers and connected in the orientation concurrent with specified firmware pin settings.

Stepper Motor
A stepper motor is an electric motor that uses pulses to divide its rotation into a certain number of steps.

Power Supply
The power supply converts AC current into DC current and in turn powers all the devices within the machine.
Glass Capillary
The glass capillaries used in this project are fine glass tubes with inner diameters of 0.68 mm, outer diameters of 1.2 mm, and lengths of 5”.

O-Rings
This project uses soft Viton O-rings with an inner diameter of 0.042”, an outer diameter of 0.142”, and a width of 0.05”.

Tubing
The tubing consists of a soft, flexible PVC plastic tubing with an inner diameter of 1mm and an outer diameter of 2mm. The tubing is cut to a length appropriate to allow for the full range of motion of the linear actuator without being excessive.

Syringe
A 1mL syringe is attached to a Luer-lock metal tip with an outer diameter of 0.042”.

Methods

In order to evaluate the function of the device, a test was performed moving the tool 10 mm in all directions. This translated to 16,000 steps, and the resulting computational interpretation is shown in Figure 5. This figure shows a computational construction of where the device is positioned within a coordinate system. Figure 5 Top Left shows the device at its original position, (0,0,0) as determined by the G-code. Figure 5 Top Right shows the device after receiving a command to move 16,000 steps in the positive X direction, and Figure 5 Bottom Left shows its positioning after a further 16,000 steps in the positive Y direction. Finally, Figure 5 Bottom Right shows the displacement from the origin after being given 16,000 steps in the positive Z direction. All of these movements translated to a physical displacement of 10 mm during each trial (N=3).

End Matter

Author Contributions and Notes

T.T.L designed research, M.T.R. coded, G.J.M. wired, G.J.M and M.T.R., and T.T.L. analyzed data; and T.T.L, G.J.M. and M.T.R. wrote the paper.
The authors declare no conflict of interest.

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Timothy Allen, Shannon Barker, and the rest of the teaching team
Our friends and family

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