



AUTOMATIC INTRAMYOCARDIAL STEM CELL INJECTION DEVICE

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Abstract

Treating heart failure by injecting mesenchymal stem cells (MSC) into the myocardium via an injection device and a needle-tipped catheter is a novel approach that can improve quality of life. The procedure is currently performed manually with 10 - 14 sequential injections of a 0.5 mL MSC aliquot solution over a 30 or 60 second duration. Unfortunately, manual injections result in an uncontrolled flow rate, inconsistent cell delivery, and operator fatigue. This can limit cell retention and induce cell reflux, damage, or clumping. An automatic injection device is thus required to improve stem cell delivery and enhance the clinical success of intramyocardial MSC injections. Existing automatic injection devices are insufficient since they can not display intramyocardial MSC injection forces and are not approved or tailored for this procedure. As a result, a novel automatic injection device was fabricated that integrates with the procedural syringe-catheter system, limits operator intervention, provides two controlled injection rates of 30 and 60 seconds, maintains standard cell viability, constantly displays injection forces, and alerts the operator if catheter obstruction is imminent (illumination of a red LED when 2.40 N is exceeded). Validation testing was performed and the results confirmed that MSC viability did not decrease by more than 5% from the initial viability, the force feedback system was accurate and consistent, and the device delivered 0.5 mL of solution in 30 and 60 second intervals. This demonstrates that the device has the potential to enhance procedural success.

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Introduction

Motivation

Cardiovascular disease is the leading cause of death in the United States. There were 696,962 deaths in 2020 alone and this number continues to increase every year [1]. Heart failure affects around 5 million people per year, killing over 250,000 of those affected [2]. This high mortality rate is due to cardiac tissue having a very limited regenerating capacity compared to other tissues in the body. Following a myocardial infarction (heart attack), regions of cardiac muscle get replaced by scar tissue. This causes the heart to experience more stress because the scar tissue can not contract to distribute blood throughout the body. In order for the heart to still pump and supply blood to the body, the healthy cardiac tissue must work much harder, eventually leading to heart failure [3]. Despite current clinical interventions, the mortality rate of heart failure is still 20% within one year of diagnosis and 80% within eight years of diagnosis [4], demonstrating the importance of a new high efficacy novel approach.

The risk of heart failure increases with age and people over 65 years old are the most susceptible. Men have a higher chance of experiencing heart failure than women and the ethnicity that is most at risk is African-Americans. There is also an increased risk of heart failure based on genetics and family history, demonstrating that if there is a history of heart disease, other members within the family are more likely to experience similar problems. Other risk factors include diabetes, obesity, and high blood pressure as well as living a sedentary lifestyle or drug and alcohol abuse [5].

Mesenchymal stem cell (MSC) delivery treatment is a novel approach that has been developed to aid in repairing damaged heart tissue following myocardial infarction. MSC therapy is currently in clinical trials and has been proven to be useful in improving the function of the heart. MSCs had a positive safety profile meaning that they caused limited safety issues with people in various clinical trials. Additionally, autologous and allogeneic MSCs showed improvements in left ventricle function, stroke volume and myocardial mass and a decrease in adverse remodeling. Allogeneic MSCs, particularly, had even more benefits and resulted in major improvements in quality of life [4]. More information about the procedure, doses, etc., can be found in the “Intramyocardial Stem Cell Injection Procedure” section of the report.

An automatic intramyocardial stem cell injection device will provide a more accurate and efficient way to deliver MSCs into the heart, specifically the myocardium, as this device would inject stem cells at a controlled rate. This accurate rate regulation can improve the chances of regenerating the dead cardiac tissue, improving its function, through stem cell modification as it will improve cell retention during injections [6]. The automatic delivery system will help renew the tissue through the injection of healthy MSCs that can assist with pro-angiogenic and immune-modulatory properties, improving heart function. Integrating a feedback system into the device that measures the force of the stem cell injections into the myocardium throughout each procedure will improve clinical efficacy, as it will assist the clinician in determining the medium the MSCs are being injected into (body cavity, healthy tissue, or ideally, the diseased/scarred

tissue) and if there is a potential for catheter back-up. Currently, there is not an automatic injection device on the market targeted toward assisting in MSC delivery to the myocardium, demonstrating the importance and impact this high efficacy injector can have on intramyocardial stem cell injection procedures. The automated stem cell injector device will improve procedural accuracy by controlling the delivery of the stem cells which can lead to clinical success and improved patient recovery and survival, making this device a critical component of improving the treatment of myocardial infarction and heart failure.

Problem Statement

Treating heart failure by injecting mesenchymal stem cells (MSC) into the myocardium via an injection device and a needle-tipped catheter is a novel approach that can improve quality of life. Unfortunately, the procedure is currently performed manually (10 - 14 sequential injections), so MSCs are delivered into the myocardium at an uncontrolled flow rate with susceptibility to rapid injections that reduce cell retention in the myocardium and induce cell reflux and damage [7]. While slower injection rates improve cell retention, they incite operator hand fatigue, resulting in inconsistent cell delivery which can cause therapeutically ineffective MSC clumping, cell reflux, lower cell retention and even cell damage/death. As a result, a novel automatic injection device is required to improve stem cell delivery and cardiac repair, by integrating with the procedural syringe and catheter, limiting operator intervention, and providing a slow, controlled, and adjustable injection rate is needed to maximize cell retention and enhance clinical success.

Background

Current Cardiovascular Disease Treatment

The current treatments for heart failure include left ventricular assist devices (LVAD), medications such as beta blockers and heart transplants, but they do not assist in full recovery of the heart function. A left ventricular assist device (LVAD) mechanically unloads the portion of the heart in addition to neurohormonal blockade heart failure medications which can improve the patient's likelihood of recovery [8]. Beta blockers are able to cause neurohormonal alterations, specifically they can work by blocking the effects of epinephrine, which can help slow down the heart [9]. Another current treatment is a heart transplant, but this is a very invasive procedure, and there is a critical organ shortage not allowing everyone eligible to receive a heart transplant [10]. The survival rate after receiving a heart transplant decreases below 85% after just one year. Heart transplants are typically considered last if other treatments are not working, but due to the risks associated, the limited number of donors and the complications that can follow the procedure, it is not the best treatment [11]. Nanotechnology drug delivery systems have also been used to treat cardiovascular diseases. This type of treatment has the ability to enhance bioavailability by prolonging blood residence, deliver a wide range of payloads, and sustain release of therapeutic agents over time [12]. Unfortunately, although these current cardiovascular

disease treatments can help lower the mortality rate from heart failure, they are not long-term solutions and thus do not increase life expectancy by an impactful amount.

Mesenchymal Stem Cells

Mesenchymal stem cells are multipotent progenitor cells with pro-angiogenic and immune-modulatory properties [13]. They can be isolated from multiple sources, such as bone marrow, adipose, and cardiac tissue [13]. These stem cells are one of the most frequently used cell types for regenerative medicine due to their controlled differentiation capabilities. MSCs have been seen to treat many different pathologies including neurological disorders, cardiac ischemia, diabetes, and bone and cartilage diseases. MSCs have been successful therapeutically due to their ability to divide and differentiate into a variety of cell types, enabling the growth of specific cells in regions where they were lost or damaged as a result of disease [14]. Through inflammatory regulation, anti-fibrosis, neovascularization and differentiation into cardiomyocyte-like cells, MSCs are able to help heal cardiovascular diseases [15]. The delivery of MSCs into the myocardium as a mechanism for tissue regeneration and thus heart failure recovery is a novel approach with the potential to provide a more effective and long-term solution compared to current treatments.

Intramyocardial Stem Cell Injection Procedure

Assessment of the patient is required to ensure they are eligible before the procedure occurs. If the patient has heart failure after a myocardial infarction and they are receiving stable, guideline-directed medical therapy with a left ventricular ejection fraction between 20 and 40%, they are eligible for the procedure [6]. To perform the intramyocardial stem cell injection procedure, a 60 mL bone marrow aspiration is initially taken from the patient prior to injection and the MSCs are isolated for further use. The intramyocardial injection procedure begins with an introducer sheath placed into the femoral artery followed by a left ventriculogram that combines with a 17-segment polar map of the myocardial wall motion and thickness from the patient's echocardiogram in order to provide a patient-specific road-map. This road-map helps operators move the catheter around to specific injection points. Operators target myocardial segments where wall thickness is greater than 6 mm which indicates a peri-infarct zone. After the route to the target tissue is identified, a needle-tipped catheter is inserted into the myocardium region of interest. Surgeons ensure that the catheter needle is properly inserted and engaged with the myocardium by observing appropriate, stable fixation of the catheter tip via x-ray fluoroscopy and through visualization of a transient arc adjacent to the endocardial surface when a bolus of radio-contrast is injected. The catheter is either connected directly to the syringe containing the MSC solution that will be injected, or it is connected to medical grade tubing, which is directly connected to the procedural syringe. The target dose of stem cells per procedure is about 200 million. After appropriate catheter insertion is confirmed, bone marrow-derived MSCs are delivered into the myocardial tissue, typically through a Helix™ transendocardial injection catheter and a Morph® deflectable guiding catheter. Each injection transports 0.5 mL of

MSC solution and a typical procedure requires 10 to 14 injections (use a new syringe for each injection) [6]. These procedures normally display a MSC injection force value range of 0.14 N to 2.40 N, where 2.40 N corresponds to the force characteristic of catheter back-up [19]. Each injection targets a rate of 30 seconds or 60 seconds followed by a 60 second maximum dwell time between each MSC injection into the myocardium. However, all injections are performed manually, making them susceptible to rapid injections (less than 30 seconds) that lead to the reflux of cells through the needle tract and cell damage. While slower injection rates improve cell retention, they insight operator discomfort and hand cramping, reducing the efficacy of the delivery [6]. In both cases, the success of the treatment is severely restrained as the chances of complications occurring during the procedure is high which limits the potential of this novel treatment.

Flow Rate Impact

Unfortunately, the current injection devices used to perform this stem cell delivery are manually controlled and are unable to regulate cell flow rate through the catheter and into the myocardium. As a result, this procedure is susceptible to rapid injections (less than 30 seconds) that lead to the reflux of cells through the needle tract and cell damage. The cells can also be damaged during fast injections due to the possibility of a fluid shear force of more than 6.23×10^{-6} N being applied onto the cells [16]. The calculation of this shear force can be seen in Equation 1 below [17]. The cells could also end up off-target and not within the targeted myocardium tissue, overall reducing the cell retention. Slower injection rates can insight operator discomfort and hand cramping, reducing the efficacy of the delivery. Cell clumping can also occur with too slow of an injection rate, which can cause catheter obstruction and issues with the cells being delivered to the myocardium. Rate inconsistency through the injections can lead to rejection of the cells within the heart.

$$\begin{aligned}
 [1] \text{ Catheter Radius} &= 0.115 \text{ cm} = 0.00115 \text{ m} \\
 \text{Catheter Area} &= \pi * (0.115)^2 = 0.0415 \text{ cm}^2 = 4.15 \times 10^{-6} \text{ m}^2 \\
 \text{Apoptosis Inducing Fluid Shear Stress} &= 1.5 \text{ Pa} = \text{N/m}^2 \\
 \text{Required Force to Induce Apoptosis} &= \text{Shear Stress} * \text{Area} = 1.5 * 4.15 \times 10^{-6} = \mathbf{6.23 \times 10^{-6} \text{ N}}
 \end{aligned}$$

The myocardial stiffness can also affect the pressure being applied to the syringe during the procedure. The average myocardial stiffness for healthy individuals is 6.08 ± 1.06 kPa whereas 12.68 ± 2.91 kPa is the myocardium stiffness associated with an individual who experienced a myocardial infarction. The greater the stiffness, the more force required to inject the MSCs into the myocardium, which can slow down the rate at which the stem cells are

injected. The flow rate at which the stem cells are injected into the myocardium can have a great impact on the outcome of the procedure and can affect the retention of stem cells [18].

Existing Devices and Current Methods

There are currently products on the market that involve similar concepts to the automated stem cell injector, but no device has all of the components that will be included in the final design for this device.

One type of device that is a competitor to the injector is an automated syringe pump. These systems are used by anesthesiologists to inject a controlled volume of anesthesia into the patient over time. The Baxter Infus OR Syringe Pump ABC 4100, is an infusion pump device that is compatible with 1, 3, 5, 10, 20, 30, 60 and 140 mL syringe sizes. To operate the device, the syringe is front loaded and locked into place. The anesthesiologist would then enter a flow rate and the type of drug being used, clicking start to initiate the injection. The advantage to the Baxter device is that it is able to sense syringe plunger force and movement and has a system to detect and monitor delivery accuracy as well as alarms with audio and LED lights [19]. The disadvantages and limitations include that this system is not tailored to eject fluid at the rate that is needed for intramyocardial stem cell delivery and has not been proven to maintain cell viability for MSCs. See Figure 1 below for this Baxter Syringe Pump along with an additional Baxter Syringe Pump.



Figure 1: The “Baxter Infus OR Syringe Pump ABC 4100” that is sold for ~\$3000 using the trade-in program (left image) [19] and the “Baxter Infus O.R. Syringe Pump Refurbished” that is sold for ~\$4000 (right image) [20].

Another competing device is the apparatus used to inject IV fluids into patients. This device is able to transfer fluids from a bag directly into the person through connection tubing at a programmed rate. There is also another similar device called the Contract Delivery System from ACIST Medical Group that is used for angiographic procedures to deliver an iopamidol injection. The advantages to this device include a controlled flow rate system that is compatible

with catheters and has been used in procedures involving cardiology and radiology [21]. The disadvantages, however, is that this device is not tailored to eject the 0.5 mL volume of stem cells from a 1 mL syringe for 10-14 injections over a period of 30-60 seconds. In addition, similar to the Baxter device, the IV machines were not proven to be compatible with stem cells. This product can be seen in Figure 2 below.

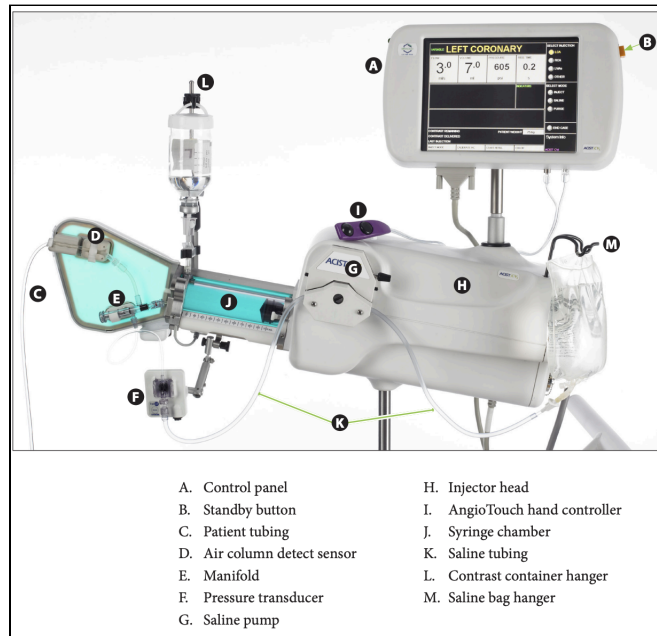


Figure 2: Diagram of the important user components in the Acist Contrast Delivery System a [21].

Lastly, there was a device in preclinical trials in 2017 that was fabricated to deliver cells. This product was called an “automated injection device for intradermal delivery of a cell-based therapy”. The current status of the product is unknown and the device is not currently patented or on the market. The limitation to this device is that it delivers solutions directly into a patient via the hypodermic needle and has not been proven to be compatible with the connection tubing / catheter system that is needed for the injections. Although it may be easy to operate by pushing the trigger button, the disadvantage is that the flow rate of the solution is not tailored for the 30 and 60 second injection times for 0.5 mL of solution in the 1 mL syringe which is needed for intramyocardial stem cell injection procedure. The device can be seen in Figure 3 below.

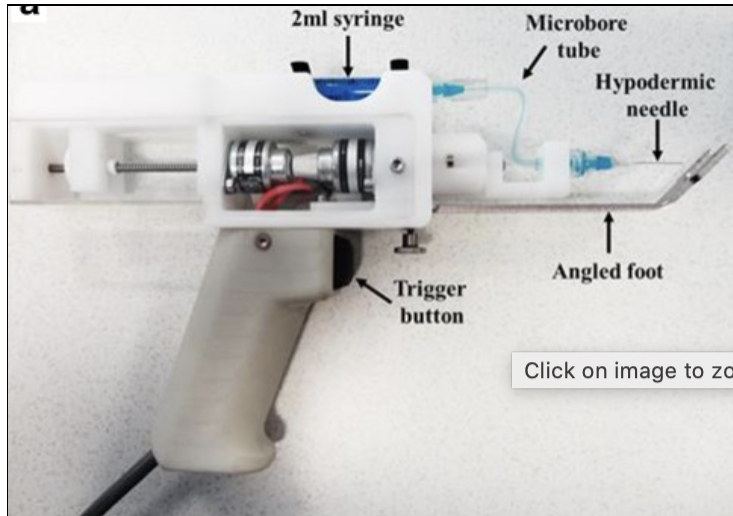


Figure 3: Automated injector device that was in preclinical development in 2017 [22].

Although these infusion pump systems and injection devices have many positives when it comes to injecting a solution into a patient, they have not been proven to work well with stem cell delivery and in conjunction with the catheter system that goes into the myocardium. These features are important because MSC viability must be maintained for the intramyocardial stem cell injection procedures to be successful and the MSC aliquot must be able to transport through the catheter and into the heart. Additionally, the devices on the market do not contain force detection systems that provide accurate algorithms for determining the pressure needed for injecting cells into the myocardium.

The JP2019069165A patent (see Figure 4 below) involves a system which has an automatic injector device that uses cassettes to hold the injectate. This is an automatic device that could be engaged by the push of a button via its superior end. However, this apparatus is not extremely similar to the cardiac repair injector device that will be fabricated since it does not have syringe or catheter compatibility, it does not have a controlled injection rate, and does not have a pressure-detecting system that can trigger visual feedback [23].

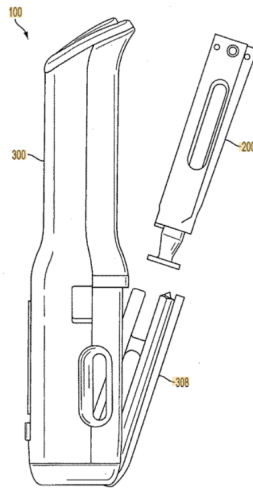


Figure 4: The cassette automatic injector device [23].

Standards and Regulations

The fabrication of the automatic injector and the product itself should adhere to various standards and regulations in order to allow for the development of a successful product that interacts accurately and safely with operators and patients and can be commercialized. The Code of Federal Regulations (CFR) Title 21 Part 820 provides quality system regulations, including the requirements related to the methods used in designing, manufacturing, packaging, labeling, storing, installing, and servicing of medical devices intended for human use [24]. As the injector will be used in conjunction with syringes, medical grade tubing, and catheters that interact directly with patients, it should follow and meet the quality guidelines outlined in the CFR. This will allow good standard operating procedures to be followed in relation to the fabrication and commercialization of the product. The CFR Title 21 Part 870 identifies a Percutaneous Transluminal Coronary Angioplasty Catheter used for the treatment of acute myocardial infarction as a Class II medical device (includes all components associated and utilized in conjunction with the catheter) and specifies the procedure required for this class [25]. As the injector will be used for treating heart failure induced by myocardial infarction and provides an intermittent risk to patients (controls MSCs injection into the myocardium), the injector can also be categorized as a Class II medical device and thus needs to adhere to the procedures outlined by this CFR. Current injection devices and syringe pumps used for drug delivery are identified as Class II medical devices [26]. This further supports the classification of the injector as a Class II device that must adhere to the process provided in CFR Title 21 Part 870, as MSC delivery will require considerations consistent with drug delivery.

The CFR Title 21 Part 3.2 categorizes the injector device as a combination product, due to its interaction with a medical device (syringe) and biological product (MSCs) in order to achieve its intended therapeutic effect. This standard provides the procedure for properly

identifying the designated agency component and preparing it for premarket review and regulation [27]. If the injector device is pursued as a combination product rather than focusing on its design individually, this CFR will have to be followed in order to allow adequate agency designation and to meet premarket review requirements. The CFR Title 21 Part 610 provides the performance, testing, and safety requirements, labeling standards, and sterility expectations for biological products, such as MSCs [28]. As the injector will interact with a syringe that contains MSCs, it is important to have familiarity with the MSCs themselves and the performance and safety requirements they must adhere to.

Client Information

The team's client is Dr. Amish Raval, a faculty member in the Division of Cardiovascular Medicine within the Department of Medicine at the University of Wisconsin-Madison. He also holds an affiliate appointment with the Department of Biomedical Engineering in the College of Engineering. Dr. Raval specializes in interventional cardiology, specifically working on reducing the effects of coronary artery disease and valvular heart disease. He performs different procedures including heart catheterizations to correct valvular and congenital defects and cardiomyopathies, coronary angiography, coronary revascularization and related coronary and pulmonary procedures. Clinical trials are currently being performed for this procedure with Dr. Raval as one of the operators. Dr. Eric Schmuck works closely with Dr. Amish Raval, acting as an additional client for the design. He is an associate Scientist and Director of Translational Research for the Dr. Amish Raval Laboratory.

Design Specifications

The client seeks a product that is capable of treating heart failure by automatically injecting MSCs into the myocardium via an injection device and a needle-tipped catheter. The client requests a device that will electronically inject mesenchymal stem cells into the myocardium and have less than 5% cell death relative to post-thawing cell viability, as this matches current procedures. The injector must be compatible with the standard catheters, medical grade tubing, and 1 mL procedural syringes currently used in practice. The stem cell injection flow rate must be adjustable, consisting of a 16.7 $\mu\text{L}/\text{sec}$ rate and a 8.33 $\mu\text{L}/\text{sec}$ rate capable of introducing the required 0.5 mL stem cell solution into the myocardium [6]. Each injection rate must be able to be performed within one second of the target time (30 seconds and 60 seconds). The volume of solution delivered from the syringe after the completion of either of these injection rates must be within 5% of the 0.5 mL volume of solution inserted into the syringe, with this error margin taking into account the inherent and inevitable dead space present in the distal tip of each syringe (see the *25 Gauge Injection Results* section below). Force values above a threshold of 2.40 N, as read from the syringe plunger, must cause the device to visually alert the user that the catheter or syringe is clogged with solution or exhibiting aliquot back flow [29]. The applied force needs to be displayed throughout the entire procedure with less than 20% error for force application less than 1.00 N and less than 15% error for force values greater than

or equal to 1.00 N. The client has a budget of \$3000 and a manufacturing cost of \$500 [30]. A complete outline of the product design specifications can be found in the Section 1 of the Appendix.

Preliminary Designs

Overview

As discussed in the *Design Specifications* section above, the automatic injection device needs to consist of a force sensor, a visual feedback system, an automation apparatus to inject MSCs into the medical grade tubing and catheter system and thus the myocardium, and a syringe insertion and locking mechanism. The force sensor and feedback system are standardized across each injector design idea. As a result, the three designs considered by the team vary only in their base, syringe insertion mechanism, and controlled injection automation.

FSR 400 Series Round Force Sensing Resistor

The FSR 400 Series Round Force Sensing Resistor (FSR) will be used as the force sensor within the injection device that detects the force applied between the injector and the syringe plunger (see Figure 5 below). It is a 47.24 mm long sensor with a 0.55 mm nominal thickness, making the FSR a relatively small sensor that should not interfere with the overall functionality of the injector. The active zone (detects force application) is about 12.70 mm in diameter, allowing it to adequately receive the entire force applied by the standard 1 mL clinical syringes (plunger diameter is ~ 4.78 mm [31]). The FSR sensor has an actuation force of 0.10 N, maintaining this sensitivity from 0.00 N to 10.00 N [32]. This demonstrates that the sensor will provide an accurate and precise force detection reading throughout each injection, as the anticipated force range during a typical intramyocardial stem cell injection procedure is 0.14 N to 2.40 N, with forces expected to reach no larger than 3.00 N (see the *Intramyocardial Stem Cell Injection Procedure* section above) [29]. The FSR's resistance changes with the force applied, decreasing in resistance with increasing pressure, providing a mechanism to determine force application when the FSR is used in conjunction with a circuit and microcontroller. The FSR will have a resistance of approximately 100 k Ω at 0.14 N of applied force and about 3 k Ω at 2.4 N of force. As seen in Figure 5 below, two wires connect from the active zone to two metal prongs. These prongs allow the FSR sensor to connect with electrical wires in order to carry the resistance generated by force application to the active zone into a circuit [32]. As a result, the FSR sensor can integrate with the feedback system and its Arduino microcontroller and Arduino IDE program (see the *Four Signal Feedback System* section below), allowing force detection to take place throughout the injection in adherence with the design specifications.

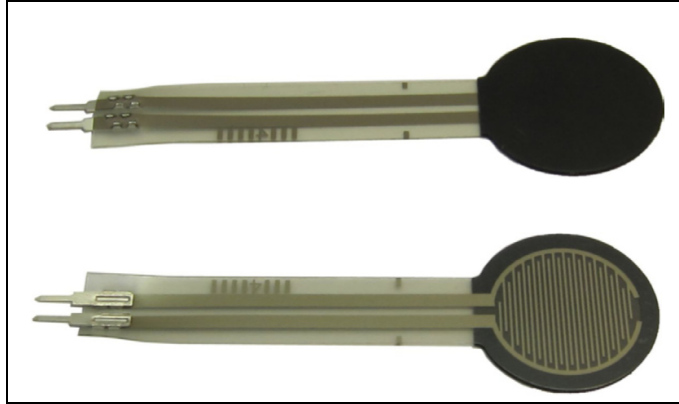


Figure 5: FSR 400 Force Sensing Resistor highlighting its 12.70 mm active zone (patterned circle) and its two prong connectors that allow breadboard and circuit integration [32].

Four Signal Feedback System

The injector's visual feedback system consists of a light emitting diode (LED) signal that alerts the operator of potential catheter backup and a digital display that constantly outputs the force value applied to the system throughout each injection, promoting procedural success. This system consists of a voltage divider circuit (see Figure 6 below) that connects to an Arduino Uno microcontroller. The FSR Sensor acts as the first resistor in the voltage divider circuit, allowing its variable resistance to alter the voltage output sent to the microcontroller. A 10 k Ω resistor is the second resistor in the voltage divider circuit and a 5 V voltage source powers the system. The expected force range that is applied to the FSR and its corresponding resistance values will result in a voltage input to the microcontroller ranging from 0.45 V to 3.85 V. This is well within the 4.88 mV resolution of the Arduino microcontroller [33], so it will accurately detect all voltage inputs from the circuit. The voltage output from the maximum and minimum anticipated force inputs to the FSR sensor are provided in Section 2.1 of the Appendix.

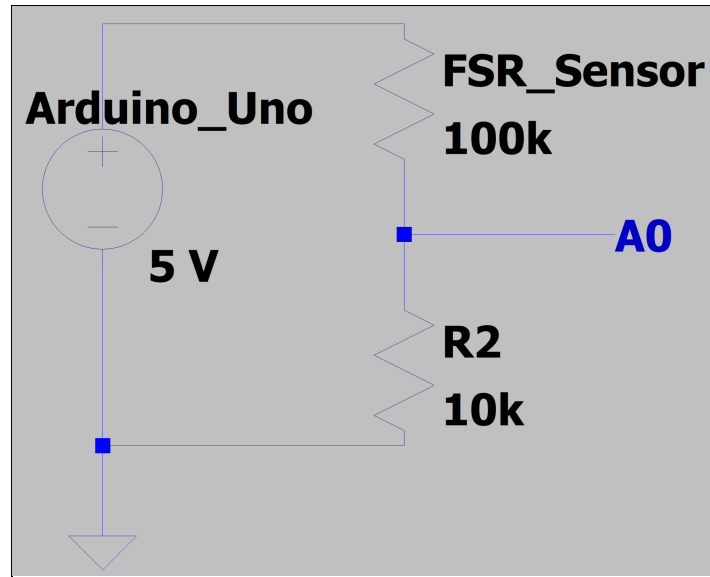


Figure 6: Voltage Divider Circuit containing the FSR sensor, a 5 V DC voltage source, and an output going into A0 of the Arduino Uno microcontroller.

The Arduino IDE software program is used in conjunction with the Arduino Uno microcontroller to receive the voltage input from the voltage divider. The calculations used to establish the force applied by the syringe plunger will be based on an experimentally determined standard calibration curve for the FSR sensor voltage divider circuit (see the *Methods* section below). Using this algorithm, the Arduino IDE program will identify the force value applied throughout each injection and constantly display it on an LED Serial 7-Segment Digital Display (see Figure 7 below) for easy operator visibility [34]. This will allow the operator to ensure that a proper injection is taking place within the desired tissue and without catheter blockage. To support this injection administration, the feedback system will also consist of a red LED signal that illuminates when the 2.40 N threshold is reached or exceeded, demonstrating that catheter obstruction is imminent [19]. Arduino IDE code to control the feedback system will be developed (see Section 2.2 of the Appendix). During each injection, this system will enable proper catheter insertion and placement as well as it will help promote successful MSC delivery by accurately depicting the injection force throughout each procedure. As a result, the visual feedback system will increase the efficacy of the intramyocardial stem cell injection procedures, enhancing clinical success and thus improving quality of life.

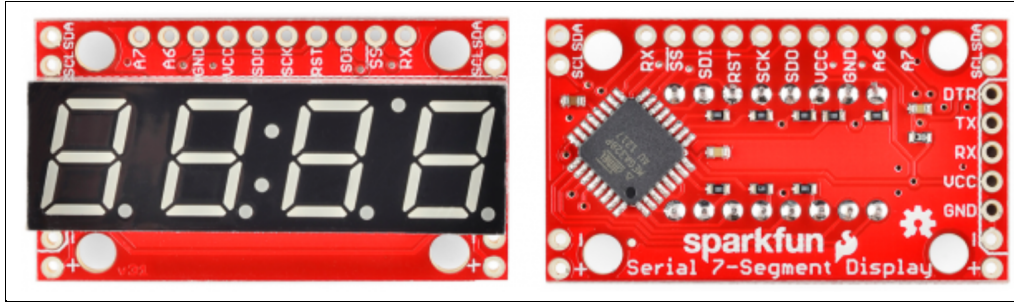


Figure 7: Top (left) and Bottom (right) view of the LED Serial 7-Segment Digital Display that is used in conjunction with the Arduino Uno microcontroller and FSR sensor voltage divider circuit. This display constantly outputs the force applied by the injector to the syringe plunger throughout each injection [34].

Design Ideas

Cellicopter: Propeller Controlled Injections

The Cellicopter design has a $20 \times 10 \times 5$ cm base and consists of a speed control DC motor, an Ultimaker PLA force application rod, and syringe clamp molds (see Figure 8 below). The Ultimaker PLA injector base is a rectangular structure that contains all injector components. The controlled injection automation will be established by a Brushless Speed Control DC Motor that will insert inside the base. Using a 0 - 10 VDC voltage input via a microcontroller, the motor's revolution speed can be precisely regulated [35]. An Ultimaker PLA force application rod will be attached to the rotating segment of the motor on its superior end and will extend transversely across the injector base in order to align with the syringe plunger (demonstrated in Figure 8 below). The DC motor will slowly rotate from left to right (as depicted in Figure 8), moving the force rod toward the syringe plunger, pushing it into the syringe at a controlled manner, allowing for an injection to take place. The syringe plunger will be completely compressed, injecting the entire 0.5 mL aliquot solution when the motor reaches a half revolution. This rotational force application mechanism will provide enough force to completely compress the syringe plunger while requiring only a minimal rotation (half revolution), delivering 0.5 mL of solution without requiring excessive use of the motor. The limited revolutions the motor will perform throughout its expected three year life cycle promotes its ability to be used repeatedly without experiencing substantial cyclic fatigue that will reduce its accuracy and precision over time. The FSR sensor will be attached to the end of the force rod that comes into contact with the syringe plunger, allowing for a constant force value to be detected throughout each injection [32]. The revolution rate administration provided by the voltage input will allow for a precise rotation of the motor and thus force rod, enabling a controlled force application on the syringe plunger that will correlate to performing injections at a rate of about 30 and 60 seconds. The motor can also rotate from right to left (based on the orientation in Figure 8), moving the force rod away from the syringe and its plunger. This will be critical when another injection needs to be performed, demonstrating the benefit of this rotational

system. However, as the motor only needs to perform a half revolution to complete a single 0.5 mL injection of MSC aliquot solution, the motor requires a very high degree of rotational control. Turning the motor's rotating segment by only a half revolution over the required 30 and 60 second injection times is very minimal and necessitates a large amount of precision. Consequently, this reduces the system's ability to successfully perform 30 and 60 second injections with a high degree of accuracy due to the rotational rate control limitations provided by the motor [35]. Two Ultimaker PLA syringe clamp molds built into the base will secure each 1 mL syringe in place within the injector, receiving all force provided during an injection without allowing any syringe displacement. These clamps are attached to the injector base to enable a stable connection to the injector and they are designed to tightly secure each procedural 1 mL syringe in place. Syringes have to be manually inserted into and removed from the syringe molds before and after each injection (10 - 14 injections per procedure). Within this design, the medical grade tubing and catheter will easily connect to the distal end of the syringe without experiencing any hindrance, allowing for proper MSC injection through the tubing-catheter system and into the myocardium.

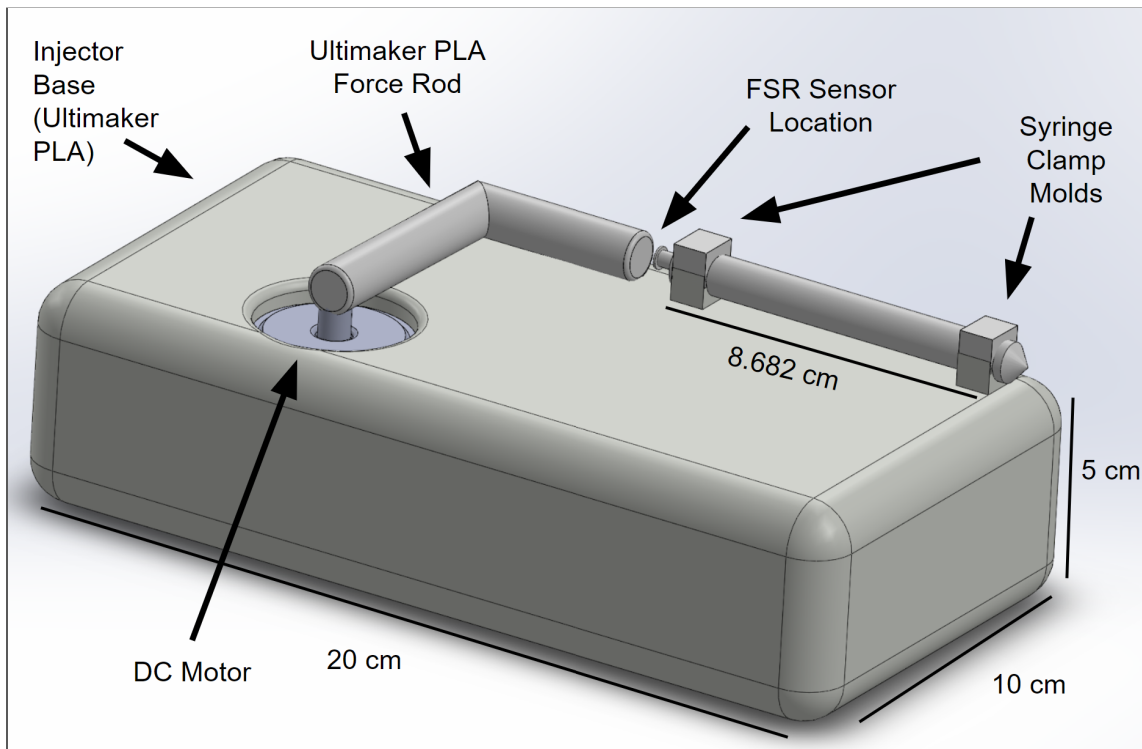


Figure 8: Cellicopter SolidWorks design, highlighting the Brushless Speed Control DC Motor, force application rod, FSR sensor placement, and syringe clamp molds. The injector base is 20 × 10 × 5 cm.

Cellringe Pump: Thread Regulated Injections

The Cellringe Pump design consists of a $20 \times 10 \times 5$ cm base that includes a stepper motor, a threaded rod injection regulator, an Ultimaker PLA force application block, and syringe clamp molds (see Figure 9 below). The Ultimaker PLA injector base is a rectangular structure that contains all injector components. A NEMA-17 stepper motor will control the automated injection and will be housed in the $5 \times 8 \times 5$ cm gray compartment, depicted in Figure 8 below. This motor performs 200 steps per revolution (1.8° turn per step), is compatible with a maximum current of 350 mA, can be controlled by a 0 - 12 V rated voltage, and is driven by an Arduino Uno microcontroller [36]. This step rate and Arduino Uno microcontroller control will allow for a very precise and controlled rotation of the stepper motor that correlates to a delivery of 0.5 mL of MSC solution (within the required 5% error margin) across 30 and 60 second rates via a threaded bolt-force application block system. The compartment this motor is stored within contains an opening on its dorsal side in order to allow for the motor to interface with the microcontroller. The rotation shaft of the motor will connect to a threaded rod that penetrates through a nut sitting within the Ultimaker PLA force application block. The movement of the motor will rotate the threaded rod, driving the block toward the syringe plunger in a very controlled manner, applying a specified force to the syringe that will result in delivering the MSCs. The force block will completely compress the syringe plunger, injecting the 0.5 mL MSC solution into the medical grade tubing and catheter. The smaller the spacing between threads, the greater the force application and injection rate control, enabling the threaded bolt to be tailored to the precision and accuracy required for each injection and procedure and to the stepper motor's specifications. The FSR sensor will be attached to the force application block at the location that comes into contact with the syringe plunger, allowing for a constant force value to be detected throughout each injection [32]. The Arduino Uno motor revolution control and the threaded rod force application system allows for accuracy and precision when compressing the syringe plunger. This will result in the injector applying 30 and 60 second injection rates with a high degree of efficacy, delivering the 0.5 mL MSC aliquot solution within the required 5% error margin. This threaded bolt system is analogous to the force application mechanism of typical syringe pumps (see the *Existing Devices and Current Methods* section above), further demonstrating that this system will effectively and successfully perform the 30 and 60 second injections that are needed for therapeutically impactful intramyocardial MSC injection procedures. The motor can also be rotated in the opposite direction, causing the threaded rod to drive the force block away from the syringe and its plunger. This will be critical when another injection needs to be performed. Two additional unthreaded rods are located on either side of the threaded rod and are built into the stepper motor storage compartment, penetrating through the force application block to provide stability within the syringe compression system. This injector contains the same 1 mL fitted syringe clamp molds as the *Cellicopter: Propeller Controlled Injections* section above). This device will thus be compatible with the required 1 mL procedural syringes. Syringes have to be manually inserted into and removed from the syringe molds before and after each injection (10 - 14 injections per procedure). Within

this design, the medical grade tubing and catheter will easily connect to the distal end of the syringe without experiencing any hindrance, allowing for proper MSC injection through the tubing-catheter system and into the myocardium.

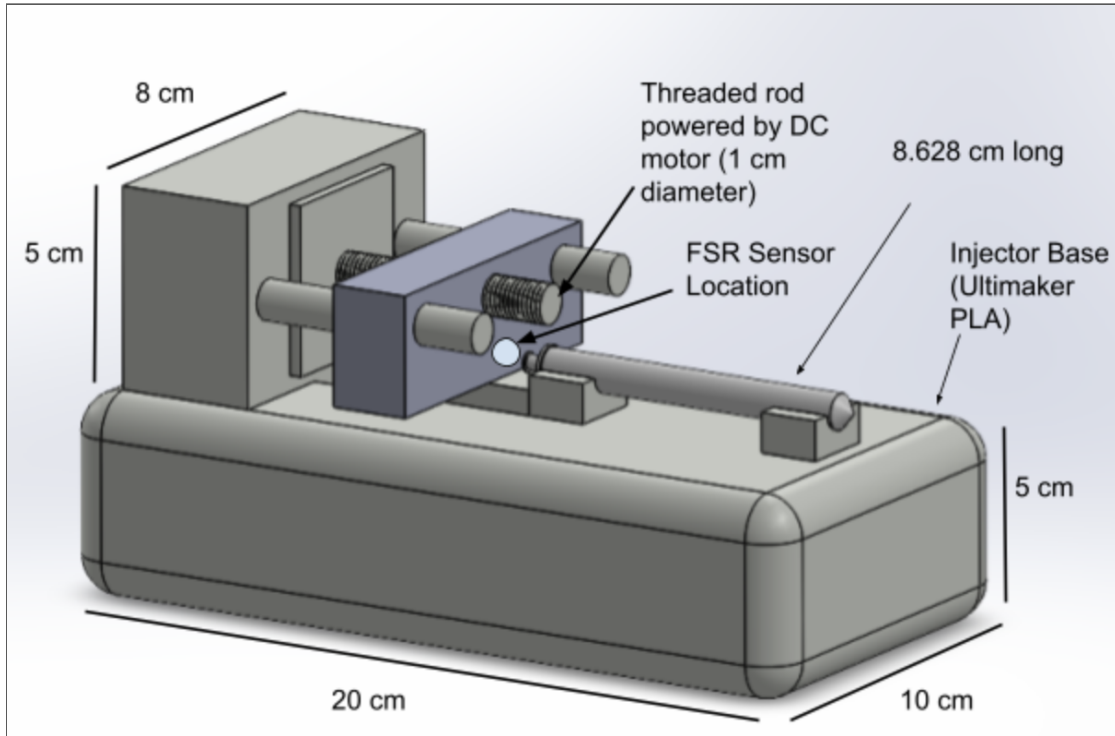


Figure 9: Cellringe Pump Sketch, highlighting the threaded force application control rod, DC Motor force application control, and the FSR sensor location. The injector base is $20 \times 10 \times 5$ cm.

Cellvolver: Fully Automatic Dual Motor Injections

The Cellvolver is an F-shaped injector that stands vertically and contains two DC motors, a rotating syringe wheel, a threaded rod injection regulator, and an Ultimaker PLA force application block (see Figure 10 below). The Ultimaker PLA injector base is an F-shaped structure that contains all design components. It has a height of 20 cm, total length of 10 cm, and depth of 6 cm. Similar to the Cellringe Pump concept (see the *Cellringe Pump: Thread Regulated Injections* section above), a NEMA-17 DC stepper motor will control the automated injection. It will be stored in the upper arm of the F-shaped base, depicted in Figure 9 below. This motor performs 200 steps per revolution (1.8° turn per step) and is driven by an Arduino Uno microcontroller [36]. This step rate and Arduino Uno microcontroller control will allow for a precise and controlled rotation of the stepper motor. The rotation shaft of the motor will connect to a threaded rod that penetrates through a nut sitting within an Ultimaker PLA force application block. This block is aligned with a hole in the lower extension of the F-shaped base that contains medical grade tubing linked to the catheter. A syringe is in the injection position when its proximal end is aligned with the force application block and its distal end protrudes

through the hole within the lower leg (see Figure 10 below). The movement of the motor will rotate the threaded rod, driving the block down toward the syringe plunger of the syringe in the injection position in a very controlled manner, applying a specified force that will result in a MSC injection. The force block will completely compress the syringe plunger, delivering the 0.5 mL MSC solution into the myocardium. The FSR sensor will be attached to the force application block at the location that comes into contact with the syringe plunger, allowing for a constant force value to be detected throughout each injection. The Arduino Uno motor revolution regulation and the threaded rod force application system allows for accuracy and precision when compressing the syringe plunger, enabling controlled 30 and 60 second injections to be performed and thus therapeutically successful procedures. The motor can also be rotated in the opposite direction (relative to the rotation required for downward block movement), the threaded rod will drive the force block in the superior direction, moving away from the syringe and its plunger. The injections are performed vertically, delivering MSCs into the catheter system located immediately beneath (inferior) the distal end of the syringe, as this downward transport of MSCs promotes their delivery to the myocardium relative to a horizontal injection. This vertical mechanism is also more susceptible to rapid injections that can lead to MSC damage, reflux, or limited cell retention. As this design contains an intricate and complex injection system due to its fully automated procedure (see below), an easier MSC delivery is advantageous and outweighs the cost of rapid injections.

Unique to this design is that it will be fully automatic after the desired amount of syringes (10 - 14) are loaded into the system. This automation will occur through the use of a rotatable wheel that attaches to the bottom arm in the F-shaped base. The center of the wheel connects to the middle of this extension and has a 5.5 cm diameter. The wheel consists of 14 syringe insertion sites, each designed to lock a single 1 mL syringe in place. A Brushless Speed Control DC Motor located within the lower leg will rotate the syringe wheel at a controlled rate. Using a 0 - 10 VDC voltage input via a microcontroller, the motor's revolution speed can be precisely regulated [35], rotating syringes into their injection position when required. The accuracy of the DC motor should allow each syringe to rotate into the correct injection position without substantial alignment error. The vertical injections and their easier MSC delivery will also help limit any injection error associated with slight misalignments between the syringe and force application block. Following the manual insertion of the desired amount of 1 mL syringes into the wheel (10 - 14 syringes), a single button click will begin the automation. A syringe will be automatically rotated into its injection position and its distal end will be subsequently twisted into the medical grade tubing, ensuring the syringe and tubing are connected for MSC transmission. Following the complete compression of the syringe plunger and thus the injection of 0.5 mL of the MSC aliquot solution, the force application block will autonomously move in the superior direction, away from the syringe, and the active syringe will be rotated out of the medical grade tubing. The wheel is now ready to perform a rotation to the next syringe. After 15 seconds of dwell time following the conclusion of the previous injection, this fully automatic sequence will repeat until all syringes have performed injections.

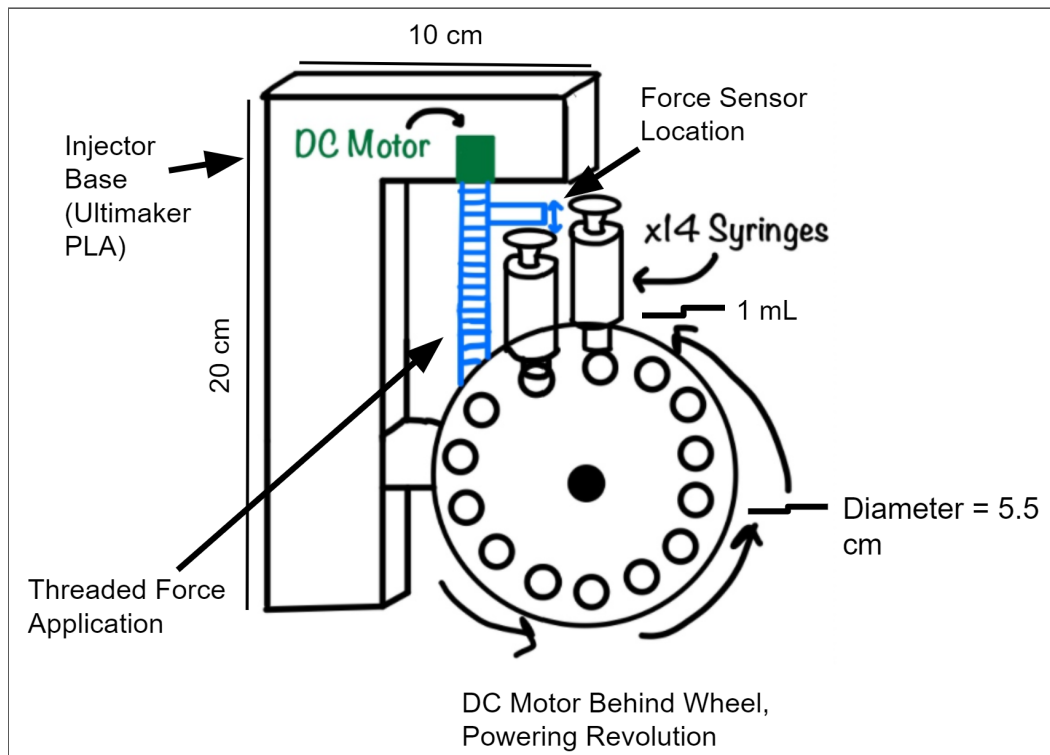


Figure 10: Cellvolver Sketch, highlighting automatic syringe rotation system, two DC motors, and its threaded force application mechanism.

Preliminary Design Evaluation

Design Evaluations

All three preliminary design ideas were placed into a design matrix consisting of carefully weighed criteria that aided in the finalization of the design that will be fabricated (see Table 1 below). As discussed in the *Design Ideas* section above, the FSR force sensor (as described in the *FSR 400 Series Round Force Sensing Resistor* section) is applied in a similar manner for all three designs. It is attached to the force application component in each design at the location where it comes into direct contact with the syringe plunger. As a result, this force sensor and thus the visual feedback system will behave in a very similar manner across all three concepts, so it will not be considered within the design matrix evaluations. All designs function as explained within the *Design Ideas* section above and they consist of components that are either similar to or the same as those addressed in the *Materials* section below.

Table 1: Stem Cell Injector Design Matrix. Consists of seven design criteria to evaluate each design. The Cellyringe Pump design won as the best choice with a total of 92/100, while the Cellicopter concept scored 83/100, and the Cellvolver design scored 68/100.

Automated Injection System Base Designs							
Design Criteria	Weight	Cellicopter: Propeller Controlled Injections		Cellyringe Pump: Threaded Regulated Injection		Cellvolver: Fully Automatic Dual Motor Injections	
Ease of Operation	25	4/5	20	4/5	20	5/5	25
Efficacy	20	4/5	16	5/5	20	3/5	12
Feasibility	20	4/5	16	5/5	20	3/5	12
Safety	10	5/5	10	5/5	10	3/5	6
Cost	10	3/5	6	5/5	10	2/5	4
Portability and Maneuverability	10	5/5	10	4/5	8	3/5	6
Durability	5	5/5	5	4/5	4	3/5	3
Total (100)	100	Sum	83	Sum	92	Sum	68

Winner	Tie
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To ensure proper scoring and evaluation of the three design ideas, each criterion was carefully and conscientiously selected and weighed based on its importance to the overall

injector design and the design specifications (see the *Design Specifications* section above and Section 1 of the Appendix). Ease of Operation was given the highest weight (25) compared to the other criteria since the main goal of the injection device is to provide an easier MSC injection mechanism that helps limit operator intervention. The injector's stem cell delivery should require minimal operator participation. The designs were thus graded on how much easier and more efficient their entire process of stem cell delivery into the myocardium is compared to performing the injection procedure manually as well as between each design idea. Efficacy is weighted highly (20) due to the importance accurate injection rates have on the success of each procedure and in increasing efficiency and mitigating safety concerns. The designs were rated on how effective and accurate they will be in performing 10 to 14 injections at the designated flow rate of 0.5 mL for 30 or 60 seconds. Feasibility is also weighted highly (20) due to the time constraint imposed on the team. The injector needs to be fabricated in a single semester. More complex designs that are less focused on the main purpose of the injection device are thus susceptible to lower scoring, as these designs were scored based on overall simplicity and thus their ability to be fabricated successfully within the semester timeline. Safety has a lower rating (10) which is rationalized by the designs' interactions with the mesenchymal stem cells. As all materials used to hold and deliver the MSCs within each design are fully biocompatible with the stem cells, the Safety score was graded based on any additional factors that will pose a threat to the patient, operator, or the MSCs themselves, such as bubble formation within the MSC solution (see the *Ethical Considerations of Design* section below). Cost, along with Portability and Maneuverability, were both weighted on the lower end of the scale (10). The Cost grades were based on how expensive the fabrication and production of the device would be. This weight is lower because the manufactured cost goal is under \$500, while the overall budget for the project is \$3000. Both values are much higher than the anticipated cost for each design making this criteria less important than some of the others. Portability and Maneuverability is based on how easy the design will be to set up prior to each procedure, move around during each procedure, transport to different operating rooms in between procedures, and deliver to storage units. All designs are fairly small compared to the operating space, justifying the lower weight of the criteria. Durability has the lowest value (5) due to the low amounts of force that will be encountered by the devices' components and thus the limited amount of fatigue and failure that is expected throughout the injector's shelf-life. All moving parts and their vulnerability to damage are taken into account in this score.

Ease of Operation was scored highest in the Cellvolver (5), as the automated syringe loading feature allows for the least amount of work required from the operator due to its fully automatic system following syringe insertion into the wheel. This design takes away all loading and unloading of the 10 to 14 syringes, allowing a single click of a button to run the automation. The Cellicopter and Cellringe Pump both offer easy loading and operation of the device, reflected by their equally high scores (4). The Cellringe pump scored highest in Efficacy (5) and Feasibility (5) as the simple threaded rod design offers a precise and accurate force to be applied to the syringe plunger during every injection. The Cellvolver, although having the same threaded

rod push block design, scored the lowest in both categories (3) because any mistakes in the fabrication of the complicated revolving syringe design would result in misconnections and failed injections. The more complex system associated with the full automation also makes it the most difficult to fabricate. The safety of both the Cellicopter and Cellringe pumped scored highest (5) due to the connections between the syringe and medical grade tubing being controlled and monitored by the operator. This will allow for adequate connections to be formed between the syringe and tubing, minimizing any injection risk associated with these connections, such as bubble formation. This can not be said for the Cellvolver, as the automated syringe loading system allows for susceptibility of bubbles to form in the tubing when connecting the syringe and catheter (see the *Ethical Considerations of Design* section for more details on the threat of bubbles in the tubing). This resulted in the Cellvolver having the lowest score in the safety category (3). Cost for the Cellringe Pump scored the highest (5) as the anticipated materials required to fabricate the device are at a much lower cost than that of the Cellicopter and Cellvolver (3 and 2, respectively), primarily due to the difference in motors used. The Cellvolver will be the most expensive as a result of it containing the most components and two motors. While all designs are assumed to cost under the \$500 manufacturing target goal, a lower manufacturing cost allows for more future additions to be made to the device. The Cellicopter scored highest in Portability and Maneuverability (5) due to its lightweight base and associated motor system providing minimal shifting of weight during movement of the device. All components are locked into place within this design, including the motor. The Cellringe Pump scored lower (4) due to the movement of the push block causing concern for a displacement in weight of the device, while the Cellvolver scored the lowest (3) due to the syringe holder needing to be moved carefully to avoid spilling of any syringes. It is also the bulkiest design and thus presents the greatest difficulty in transport. Durability was also scored highest with the Cellicopter (5) as there are little moving pieces that would account for deterioration of the device mechanics, minimizing its susceptibility to cyclic fatigue. The Cellringe Pump and Cellvolver scored lower (4 and 3, respectively) due to the increase in number of moving parts raising the probability of deterioration or malfunction of the device mechanics. The threaded system in both of these designs presents a higher risk of fatigue and error over repeated use.

Final Design

The design with the highest score in the Design Matrix was the Cellringe Pump, scoring a 92 overall grade. This design was followed by the Cellicopter with a score of 83 and the Cellvolver with a score of 68. The Cellringe Pump design scored highest in Efficacy, Feasibility, Safety, and Cost, validating its score and ability to successfully meet all of the design specifications. Although it did not score the highest in the heaviest weighted category, Ease of Operation, this design still incorporates a simple syringe loading system that allows for safe and efficient exchange of syringes into the device. The Cellringe Pump will consist of an injector base that supports the force application block system and syringe, a feedback system, and a stepper motor controlled threaded bolt force application system. The injector will provide spots

for the three force application control buttons, feedback system digital display, and threshold LED to sit within, enabling the operator to easily access and see all three components throughout each injection. A syringe clamp mold will permanently connect to the superior side of the injector base, locking 1 mL syringes in place during each injection. The stepper motor storage compartment will also sit on the superior side of the injector base, housing the stepper motor. This motor will connect to a threaded bolt that sits within a force application block, rotating the bolt in a controlled manner, causing the force application block to be driven toward or away from the syringe plunger of the 1 mL syringe locked within the syringe clamp. To provide stability for this force application block, a support block will be built into the injector base, with support rods that provide structural support to the force application block, preventing it from rotating and assisting with its force application movement. The feedback system will consist of an FSR sensor to detect the force applied by the injector to the syringe plunger throughout each procedure, LED digital display that constantly outputs the applied force during each injection, and LED that will illuminate when the 2.40 N threshold is reached or exceeded. The FSR sensor will permanently attach to the force application block in the location where the block contacts the syringe plunger and connects to the circuitry of the feedback system, enabling the digital display to accurately depict all applied force and to identify when the LED should illuminate. The injector base will be hollow in order to allow all circuitry (feedback system and stepper motor electronics) to be stored within it. Arduino Uno microcontrollers will control the stepper motor and feedback system. Each component of the injector base and support system will be developed in SolidWorks and 3D printed through the UW Makerspace. Following fabrication, the resulting components will be assembled together along with all necessary circuitry in order to form the injector prototype. All designed circuitry and base designs will revolve around ease of use and accuracy to maximize procedural efficiency and efficacy. The *Materials* section outlines the materials that will be purchased and used throughout the course of fabrication. All fabrication and testing steps that need to be completed can be found in the *Methods* and *Testing* sections below.

Fabrication/Development Process

Materials

Overview

The force sensor based visual feedback system consists of an FSR 400 Series Round Force Sensing Resistor [32], an Arduino Uno microcontroller [33], Arduino IDE [37], an LED Serial 7-Segment Digital Display [34], and standard circuitry wires, resistors, an LED, and an adhesive breadboard [38]. See Section 3.1, Section 3.2, and Section 3.3 of the Appendix for the technical data sheets of the FSR sensor, Arduino Uno microcontroller, and LED Serial 7-Segment Digital Display. The injector base, stepper motor storage compartment, support block (includes support rods), force application block, and syringe clamp were fabricated with

Ultimaker PLA via 3D printing (see Section 3.4 of the Appendix for the technical data sheet of the PLA) [39]. The automated injection is controlled by a NEMA-17 stepper motor [36], an Arduino Uno microcontroller [33], Arduino IDE [37], a fully threaded zinc coated aluminum bolt [40], and three tactile buttons [41]. See Section 3.5 and Section 3.6 of the Appendix for the technical data sheets of the stepper motor and threaded bolt. A nylon hex nut (see Section 3.7 of the Appendix for the technical data sheet of the nut) [42] is embedded within the Ultimaker PLA force application block to allow for the threaded rod to control its movement and thus syringe plunger compression. Standard 1 mL clinical syringes, medical grade tubing, and therapeutic catheters are compatible with the Cellringe Pump, but as they are not part of the fabrication process and the actual injector design, their material composition will not be incorporated in the discussion.

Force Sensor

The FSR sensor is only 47.24 mm long and 0.55 mm thick, making it a small sensor that will not interfere with injector functionality [32]. Typical 1 mL standard syringes used within the intramyocardial MSC injection procedure have a 4.78 mm plunger diameter [31], making the FSR sensor a great fit for the injector force sensor as its 12.70 mm diameter active zone will allow for the entire force applied by standard syringes to be adequately received throughout each procedure. This force reception will be read with a high degree of accuracy as the FSR sensor has an actuation force of 0.10 N across a 0.00 N to 10.00 N range [32], which is well within the expected 0.14 N to 3.00 N injector force range during a typical intramyocardial stem cell injection procedure (see the *Intramyocardial Stem Cell Injection Procedure* section above) [29]. This high sensitivity force sensor allows force values to be detected during each injection with a high degree of efficacy, a critical component that will impact the success of each procedure. The ability for the FSR sensor to contribute to the visual feedback system via its integration with standard circuitry, breadboards, and an Arduino Uno microcontroller further promotes its use as the injector force sensor [32]. Alternative force sensors, such as a TFX292X-040B-0025-L compact compression load cell or a MS5611 Barometric Sensor, require additional circuitry components in addition to just typical electrical wires to integrate with the Arduino Uno microcontroller and they do not have large enough active zones to receive the entire load applied by the 1 mL syringe plungers. The FSR sensor is also from the BME 201 kit that the team received last year, so they did not result in any additional cost to the design, while the load cell and barometric sensor cost 42.40 and 10.93 dollars, respectively [43,44]. As a result, the FSR sensor is the most cost effective choice and it can properly detect the entire force applied by the injector to the 1 mL syringes during each injection due to its 12.70 mm diameter active zone, confirming that this sensor is the appropriate force identification device.

Microcontroller and Software

The Arduino Uno microcontroller has a very high sensitivity to voltage change with a resolution of 4.88 mV [33]. This allows the microcontroller to pick-up very slight changes in

voltage input, with one ADC value representing every 4.88 mV input into the board. This resolution allows for accurate microcontroller ADC value designations based on the voltage input from the voltage divider circuit, that is used to establish force. This demonstrates the effectiveness of using the Arduino Uno as the feedback system's microcontroller. This microcontroller is also compatible with the NEMA-17 stepper motor, connecting to it via an adafruit Motor Shield v2.3. This allows the microcontroller to easily drive and accurately control the revolution rate of the motor through Arduino code and thus the force application (to the syringe plunger) and injection rates [45, 36]. The Arduino Uno has a length of 68.6 mm, a width of 53.4 mm, and a weight of 25 g, so it is a small microcontroller that fits within the $27.50 \times 10.00 \times 7.50$ cm injector base, is easy to transport, and does not create a burden during procedures and storage [33]. One of the main benefits of using the Arduino Uno microcontroller is the Arduino IDE software program that is coupled with it. This program takes the precise and accurate voltage readings from the microcontroller and utilizes algorithms to perform calculations that convert these inputs into force values based on a standard calibration curve (see the *Methods* section below). These values are then displayed in real-time to the operator throughout the injections via an LED Serial 7-Segment Digital Display that integrates with the software and microcontroller, assisting in the success of the procedure. Code also illuminates an LED signal based on the force value that is being output by the program, enabling the visual feedback system to function properly. The IDE software program contains additional code that regulates the stepper motor via the microcontroller, controlling its step rate and rotation velocity and thus the application of force to the syringe plunger [37]. As a result, this Arduino Uno and Arduino IDE system controls and maintains the success of the injector. See Section 2.2 and Section 4.1 of the Appendix for the feedback system and stepper motor Arduino IDE code, respectively.

Visual Feedback Display and Circuitry

The circuit jumper wires, 10 k Ω resistor, and adhesive breadboard are used in combination to form a circuit that integrates the FSR sensor with the Arduino Uno microcontroller, allowing for a visual feedback system to be fabricated with the use of a red LED and an LED Serial 7-Segment Digital Display. The jumper wires are used to connect the FSR sensor to the breadboard, power and ground the circuit, connect the voltage output from the voltage divider topology to the microcontroller, link the digital display and the microcontroller, and connect the microcontroller to the red LED. These connections allow for the circuit and visual feedback system to function. The 10 k Ω resistor acts as the second resistor in the voltage divider circuit since it results in the voltage divider having a voltage output ranging from 0.45 V to 3.85 V based on the expected force range that is applied to the FSR and its corresponding resistance values (100 k Ω and 3 k Ω respectively). This allows for a large enough voltage output range for proper Arduino Uno microcontroller detection and response. The calculations for this voltage output can be found in Section 2 of the Appendix. The Sparkfun resistors were also used due to their greater lead thickness compared to typical resistors, promoting their durability and

resistance to cyclic fatigue, as well as its low $\pm 5\%$ tolerance that will reduce error [46]. The Sparkfun adhesive breadboard was specifically chosen as they are relatively small ($83.5 \times 54.5 \times 8.5$ mm) and can strongly adhere to the bottom of the injector base [47]. The small size minimizes the interference of the breadboard with the overall injector functionality and MSC injection procedure as well as enables it to fit within the $27.50 \times 10.00 \times 7.50$ cm injector base. The sturdy connection to the injector will prevent any displacement of the breadboard throughout each injection, limiting the susceptibility to loose connections and thus feedback system error. The LED Serial 7-Segment Digital Display outputs the force provided to the FSR sensor throughout each injection on a $41 \text{ mm} \times 23 \text{ mm}$ screen. This allows the operator to easily track the force being applied by the injector throughout each procedure, assisting in clinical success. It is compatible with the Arduino Uno microcontroller, contains a baud rate of 9600, and can provide two decimal places when displaying force values. As a result, the display can easily integrate with the microcontroller-Arduino IDE system, displaying a highly accurate force output in real-time (as the force is applied to the FSR sensor) [34]. While there are additional digital displays that can connect with an Arduino Uno microcontroller, such as a common cathode 7-Segment display, they require additional components in order to properly connect with the microcontroller and they consist of complex interfaces, increasing their susceptibility to error during display system fabrication [48]. As a result, the LED Serial 7-Segment Digital Display is the most cost effective option as well as it provides an accurate system that can integrate with the Arduino Uno system with minimal error, demonstrating that it is the correct display device. The red LED is used to provide the visual feedback for the operator and is controlled by the Arduino Uno microcontroller and its IDE software. These components are all from the BME 201 kit that the team received last year, so they did not result in any additional cost to the design, promoting their use for the feedback system.

Ultimaker PLA Injector Components

Ultimaker PLA contains a detailed surface quality and supports the creation of high-resolution parts, enabling the injector base, stepper motor storage compartment, support block (includes support rods), force application block, and syringe clamp mold to be fabricated with a high degree of detail, complexity, and accuracy. This allows these features to interact together successfully and correctly, producing controlled injection rates that meet the 30 and 60 second requirements. The high quality generation of the syringe clamp mold enables it to properly interact with the 1 mL syringes, preventing any movement or displacement of the syringe during force application and the injection. The Ultimaker PLA has excellent ultimate strength (49.5 MPa), is highly resistant to deformation over time and cyclic fatigue, and has very high stiffness (2,346.5 MPa) [39]. This allows for a durable and strong injector base, stepper motor storage, support block, force application block, and syringe clamp that will be able to withstand all repeated forces applied to the injector throughout each procedure. As the syringe clamp is permanently attached to the injector base, it should not experience failure at its connection site as this attachment will be reliable and permanent, rather than exchangeable,

integrating the clamp into the base to make one cohesive syringe insertion complex. The clamp will not display the weakness and susceptibility to failure that a replaceable and exchangeable mold would display as it is fully and permanently attached to the injector base rather than just clipped or temporarily locked into the base. Testing confirmed the durability and strength of the syringe clamp as seen in the *Syringe Clamp Connection and Locking Testing* and *Syringe Clamp Connection and Locking Results* sections below. Although forces may be applied throughout the injector as a result of operator interaction with the device, the main point of force reception will be where the force application block comes into contact with the syringe plunger. In order to confirm that the force application block will be able to withstand the expected maximum force of 3.00 N (see the *FSR 400 Series Round Force Sensing Resistor* section above), the stress generated on this block (cross-sectional area of contact $6.362 \times 10^{-5} \text{ m}^2$) at the maximum applied force was calculated using Equation 2 below [49].

$$[2] \sigma = F / A$$

Under the maximum applied force of 3.00 N, the force application block will experience 0.047 MPa of stress based on its geometry, which is much less than its 49.5 MPa ultimate strength. As a result, the force application block will be able to withstand all applied forces during each injection, demonstrating its success as a force application block. As a worst case scenario, Equation 2 above was used to establish the stress generated on the block if 10.00 N of force (maximum force detected by the FSR sensor) is applied to the block. This force correlates to a stress of only 0.16 MPa, which is also much less than the 49.5 MPa ultimate strength. This durability demonstrates that the Ultimaker PLA model will last for at least three years, meeting the shelf-life client requirement. These mechanical properties will also allow the stepper motor storage, force application block, and syringe clamps to act as stable and reliable holders for the motor, threaded rod, and syringes, respectively. While Tough Ultimaker PLA and Formlabs' Clear Resin have comparable ultimate strengths to Ultimaker PLA (37 MPa and 38 MPa respectively), the Tough Ultimaker PLA has a stiffness of only 1,820 MPa and both materials are not resistant to deformation over time and cyclic fatigue [50, 51]. Ultimaker PLA has thus the optimal mechanical properties to be able to withstand the cyclic nature of each injection procedure, solidifying this material as an adequate choice to use throughout the injector that will allow for successful injections and enable it to meet the three year life specification.

Injector Force Application Materials

A NEMA-17 stepper motor is used to control the automated injection due to its compatibility with the high resolution Arduino Uno microcontroller and the very precise and controlled rotation it can provide as a result of its 200 steps per revolution and 0 - 12 V rated voltage revolution rate dependence [36]. The accuracy this motor can provide will allow it to regulate the force application and thus injection rates with a high degree of efficacy, allowing the 30 and 60 second injection rate design requirements to be met. A zinc plated aluminum fully threaded bolt was chosen to control the force application of the block to the syringe plunger as a

result of its durability, corrosion and fatigue resistance, and strength relative to typical aluminum, steel, and stainless steel bolts (do not contain a zinc coat), which have limited corrosion resistance, strength, and cyclic durability. The aluminum threaded bolt also has a much greater tensile strength and Young's Modulus compared to nylon bolts [40, 52]. These are critical features for generating consistent and reliable injections, displaying the zinc coated aluminum bolt's ability to perform accurate and successful injection procedures. A nylon hex nut was chosen to mediate the threaded bolt's rotation and ultimately the force application as it contains higher strength and durability compared to typical metal fasteners, corrosion resistance, decreased wear on mating parts, and good bearing properties [53]. The bolt and nut are also lightweight and the nut is nonconductive, increasing the portability of the injector and eliminating any concerns associated with the circuitry from the feedback system or stepper motor interacting with the threaded rod system [42]. The properties characteristic of the zinc plated aluminum bolt and nylon hex nut will allow the threaded rod system to resist fatigue from cyclic loading and provide a better connection between the bolt and hex nut, increasing the accuracy and precision of the force application system over a greater amount of uses and thus decreasing its susceptibility to cyclic stress compared to traditional metal bolts and hex nuts. A 5 mm to 6.35 mm coupler is used to securely connect the bolt to the stepper motor [54], enabling the stepper motor to control the rotation rate of the bolt with a high degree of accuracy. Three 12 mm tactile buttons control the stepper motor via the Arduino Uno microcontroller. Standard jumper wires (see the *Visual Feedback Display and Circuitry* section above) connect the tactile buttons to the microcontroller and a simple click of the button results in either a 30 second or 60 second injection or a reset of the injection (in case an issue arises during the procedure). These buttons integrate with the Arduino Uno microcontroller and are $12.00 \times 12.00 \times 8.50$ mm, so they easily fit within the injector base and do not disrupt the system [41].

The full list of materials used for the injector, the budget, and all calculations related to mechanical properties can be found in Section 3 of the Appendix.

Methods

The injector base fabrication process relied on using Ultimaker PLA in conjunction with the NEMA-17 stepper motor and zinc plated aluminum threaded bolt (see the *Materials* section above for more information on the described materials). The different features within the injector are on the centimeter scale with some detail that is required on the millimeter scale. Therefore, the design and fabrication method must be able to account for this precision and complexity. The SolidWorks Computer Aided Design (CAD) software allows for the development of detailed and complex designs and provides a SimulationXpress Analysis Wizard extension that determines the location of the weakest points and thus where failure is imminent. This software was used to ensure that the entirety of the final design will be able to withstand the maximum expected force of 3.00 N, which includes critical points such as the force application block and syringe clamp mold. The outer base, force application block, and force block outer rods were all designed using the CAD software SolidWorks and fabricated out of the Ultimaker PLA material that is created

through the Ultimaker 3D printers at the University of Wisconsin-Madison MakerSpace [39]. 3D printing through the UW-MakerSpace was easily accessible and allowed for the creation of all the necessary parts needed to fabricate the prototype through the use of the CAD created part files. All CAD created components of the base were compiled into an assembly with each other to confirm correct dimensioning and to take into account tolerances applied to the 3D printed products. Each 3D printed piece serves an essential function, requiring the resolution, precision, and accuracy associated with SolidWorks and all parts were created so they can easily assemble together with all base features. The outer base was designed to hold and conceal the NEMA-17 Stepper Motor, 1.0 mL syringe, and all required circuitry systems implemented throughout the injection device. The NEMA-17 Stepper Motor holder was designed to house the stepper motor and allow the connecting wires to flow to the circuitry underneath the entire base. A 1.0 mL syringe mold was designed to hold the syringe firmly in place during injections and allow the syringe to be easily replaceable in between injections. The zinc plated aluminum threaded bolt was connected with a coupler to fit over the stepper motor in a way that allows for a complete transition of power from the motor to the threaded rod, and from the threaded to the force application block. To enable proper fitting of the bolt within the force application system and between the support block and coupler, a drop saw was used to accurately and precisely cut the bolt down to a length of 3.50 cm. The force application block is prevented from spinning and thus promotes horizontal motion by the use of the 3D printed cylinder rods that are easily detachable to allow for easy adjustments to be made to the positioning of the force application block. The FSR sensor was adhesively applied to the force application block and positioned so there is minimal interaction interference between the FSR and syringe plunger. Wiring systems coming from the FSR to Arduino Microcontroller feed down into the designed bay of the outer base to limit contact exposure between the circuitry and the operator. All wires attached to the FSR are long enough to allow free movement of the FSR that is attached to the push block. The exact fabrication protocols for the development of the FSR feedback system, NEMA-17 Stepper Motor circuitry, and overall device can be found in Section 4 of the Appendix and all associated SolidWorks part files and drawings can be found in Section 5 of the Appendix

To provide an equation to accurately and reliably establish the injector force applied to the syringe plunger throughout each injection, a calibration curve was created based on the FSR feedback system testing force and voltage data. Using a line of best fit that takes into account noise, an equation was identified that correlates voltage (V) to weight (N) and thus allows the force to be determined based on each voltage input to the Arduino Uno microcontroller. The purpose of the calibration curve is primarily to derive the linear trendline which can then be used to calculate the unknown applied force during the injection. This equation will need to be implemented into the feedback system Arduino IDE code in order to allow the force to be constantly output to the operator throughout each procedure. This will assist in the accuracy of each injection, mitigating MSC reflux and catheter obstruction as well as establishing catheter tissue location, and thus promote the efficacy of the intraoperative stem cell injection procedure. The independent variable (x) in the line of best fit equation represents the applied force and the

dependent variable (y) describes the output voltage. A 6th degree polynomial (Figure 11). was created to calculate the force values for voltage readings less than or equal to 3.05 V. A 4th degree polynomial can be seen in Section 6.1 in the Appendix, this calibration curve was used for voltage values greater than 3.05 V. The 4th degree polynomial was less accurate for voltage values less than 3.05, so the 6th degree calibration curve was created in order to have more accuracy. The curves were created dependent on residuals, the correlation coefficients and the distribution of residuals. The complete FSR calibration curve testing protocol can be seen in Section 6.1 of the Appendix. Both of these calibration curves are used within the FSR feedback system Arduino Code.

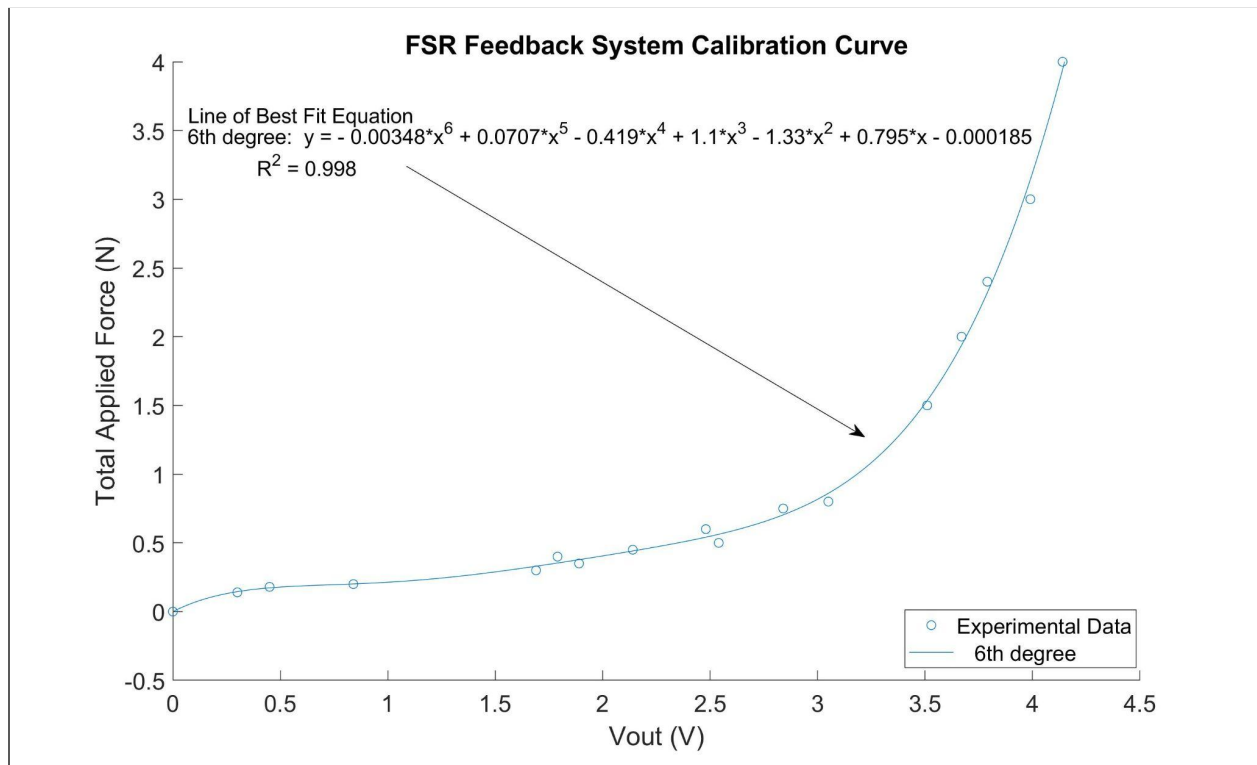


Figure 11: Calibration curve for the FSR feedback system under various loading enabling applied force calculation.

A calibration curve and equation was also created to implement the correct speed and travel distance into the Arduino code controlling the Stepper Motor. To create an equation for the distance traveled per step number applied within the code, a plot was created to differentiate the step size number versus the distance traveled by the nylon nut up the threaded rod. Taking the average of each step size versus distance traveled created the equation as seen in Equation 3 below that was used to calculate the step size needed to have the device fully travel the length of a 0.5 mL filled 1.0 mL syringe.

$$[3] \text{ Distance Traveled} = (\text{Step Size Number}) * 0.0064 \text{ mm}$$

This equation works for any step size number and will allow for accurate and reliable injection travel distance. This equation can then be correlated with the speed setting of the motor within the code to have the device. The speed equation used to determine the speed number coded in the Arduino program for the 30 and 60 second injections was determined by testing known speeds to find the time it took to travel a known distance. The Stepper Motor rotating times were hand timed using a stopwatch and plotted against the same distance each test to determine the mm/sec equation. The plot can be seen below in Figure 12, as the logarithmic equation can be solved to find the necessary step speed number for the velocities of the push block during the 30 and 60 second injections. The equation developed through the line of best fit can be seen below in Equation 4. This equation was used to find the motor speed implemented in the Arduino when the 30 and 60 second velocity requirements were determined. The code for controlling the Stepper Motor can be found in Section 4.1 of the Appendix.

[4] Velocity (mm/sec) = $-0.825 + 0.38 \cdot \ln(\text{Motor Speed})$

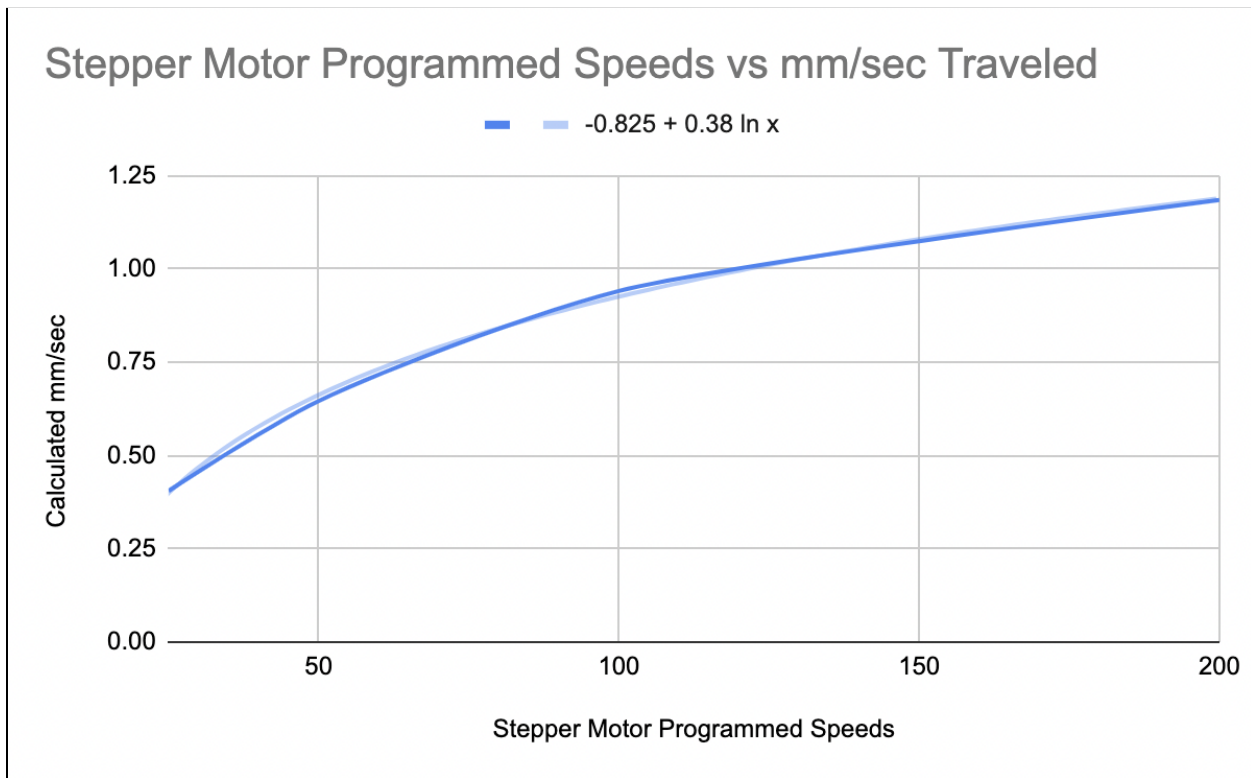


Figure 12: The plotted Stepper Motor programmed speeds versus the velocity of the nylon nut during testing. A line of best fit was applied to generate the implemented equation used in the Arduino coding.

The circuitry of the FSR feedback system and Stepper Motor were assembled and designed to serve all the requirements necessary to enable the automatic syringe injection system to perform the injections and display the force at the same time. The Stepper Motor utilizes the Arduino Uno Microcontroller (see *Microcontroller and Software*) and integrates a Motor Shield and buttons to allow for easy fabrication and testing of the injection rate components.

Final Prototype

The prototype injection device was designed based on the Product Design Specifications and client requirements in order to maximize efficiency, accuracy, and reliability during each myocardium stem cell injection. The 1 mL Syringe Mold secures the placement of the loaded syringe during injections, while also allowing the operator to easily remove and replace the syringe in between injections. The 3 buttons are utilized to control the device through connections to the Arduino and Motor Shield, which thus power the NEMA-17 Stepper Motor with the buttons' respective commands. Pressing either the 30 or 60 second button will initiate the start of the injection. Pressing the same button that was pressed to start the injection will pause the device for the duration of however long the button is held. The Force Application Block returns to its' starting position either after a successful 30 or 60 second injection, or if the reset button is pressed while the device is still injecting. The use of 3 buttons minimizes user control to maximize the device's ease of operation.

During each injection, the FSR attached to the Force Application Block will send a voltage to the Arduino that is then calculated and displayed on the LED Digital Display. This allows the operator to be able to constantly monitor the amount of force being applied to the syringe and have an idea of when there is anything wrong with the procedure. The LED Threshold Light will illuminate red when the FSR picks up a force above 2.40 N. This will alert the user when the catheter is predicted to be experiencing clogging or back flow. The device does not automatically shut off when this value is reached due to the uncertainty of whether or not there is obstruction significant enough to need device intervening stoppage. The light serves as a warning to the user, who can easily stop or pause the device to further assess the situation without needing to reset the procedure whenever the 2.40 N threshold is reached. Figure 13 and Figure 14 below display these features.

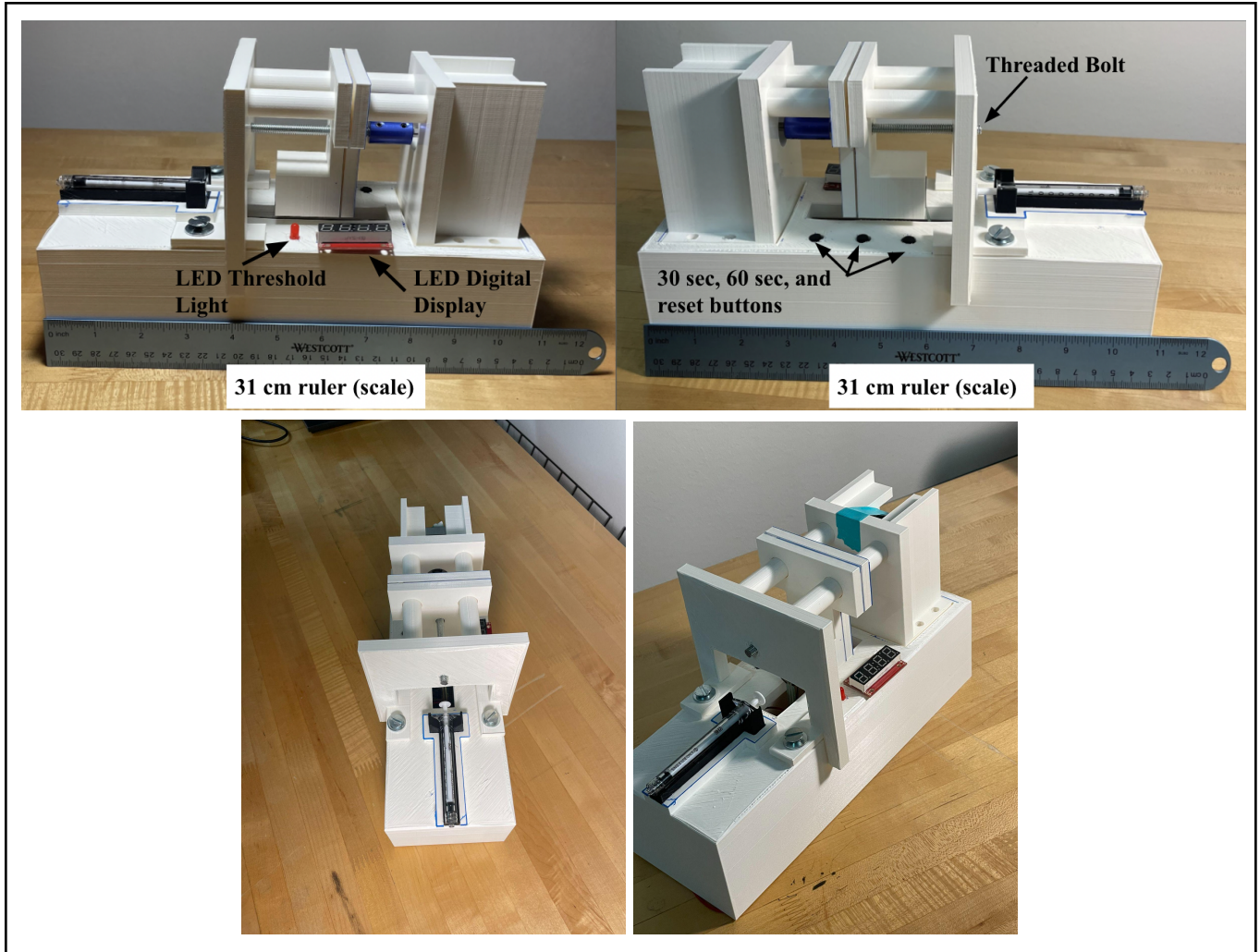


Figure 13: Right end (top left), Left end (top right), overhead (bottom left), and isometric (bottom right) views of the final prototype with labeled features and 31 cm ruler for scale.

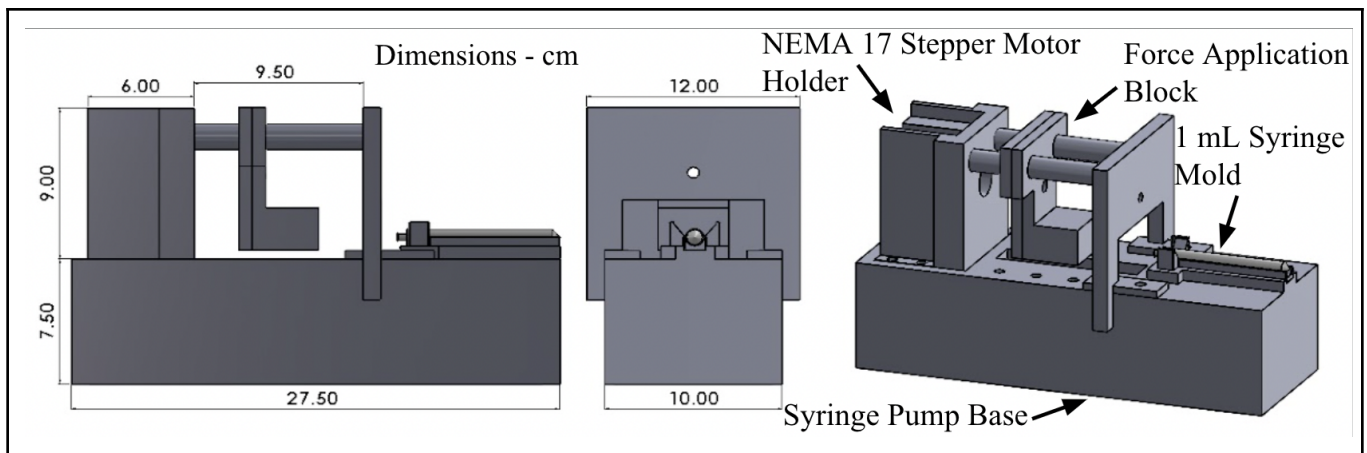


Figure 14: Dimensional depictions of the final prototype as seen in Solidworks with labeled components.

The Syringe Pump Base was designed to hold all components together and house the circuitry underneath. This allows for easier assembly and disassembly of the prototype. When the prototype is put together, the pieces are held firmly in place by 6.25 mm bolts. This allows for successful injections to be performed and the device to be taken completely apart if needed. The base components of the prototype are all made of Ultimaker PLA through the 3D printers at the University of Wisconsin-Madison MakerSpace Lab (see *Materials: Ultimaker PLA Injector Components*, and *Methods* sections). Further figures and dimensions of the device can be found in Section 5 of the Appendix.

Testing

Feedback System Force Detection Accuracy Testing

Testing of the feedback system was completed to ensure the FSR was correctly detecting the force being applied. This testing was completed by choosing a specified force value within the corresponding typical injector force application range of 0.14 N - 4.00 N. The required amount of water was then added to an empty water bottle based on the force value chosen. The water bottle was then placed on the FSR and the force and voltage value was read from the spiral monitor. After recording the values, the percent error was calculated to determine if the force value output by the feedback system was within a 20% error margin of the actual applied force (calculated by the weight of the bottle for all applied forces) for values below 1.00 N and an error margin of 15% for all forces greater than or equal to 1.00 N. Force values less than 1.00 N during a typical injection correspond to an error margin of 20% rather than 15% as very small changes (less than ± 0.1 N) can correlate to errors greater than 15% while these same force variations for values greater than or equal to 1.00 N correspond to an error less than 15%. Using the percent error equation seen in Equation 5 below [55], for the lowest expected force of 0.14 N, if the feedback system detects a force of 0.17 N, the error between the two values is $\sim 21.4\%$. The error sensitivity is much larger than in force values greater than or equal to 1.00 N although the variance is similar. As a result, the error margin is 20% for values less than 1.00 N in order to accommodate the discrepancy in error over the same magnitude difference across all force values (0.14 N - 3.00 N), enabling the acceptable feedback system-applied force variation to remain consistent throughout the injection force range. The 20% error margin was tailored to mimic force discrepancies consistent with the 15% error margin for values greater than or equal to 1.00 N, as the magnitude error associated with this 15% margin is small enough to still enable proper identification of each injection's susceptibility to catheter obstruction and the success of each procedure. While 2.40 N represents the potential initiation of catheter obstruction, once the onset of obstruction takes place, the force will significantly increase, exceeding the 0.36 N error associated with the current 15% error margin [29], demonstrating that this error can correctly determine catheter obstruction risk. If the feedback system provides force values within the 20% and 15% error margins, the system will thus be capable of providing accurate force outputs required for the operator to adequately determine the efficacy of each injection.

$$[5] \text{ Percent Error} = (|V_{\text{theoretical}} - V_{\text{experimental}}| / V_{\text{theoretical}}) * 100$$

Lower force values (0.14 N - 1.00 N) represent the most common forces exhibited on the syringe plunger throughout each injection [13]. To account for this force dominance and ensure that the device was very accurate over this range, more tests were performed on these lower force values (5 tests/N) relative to the higher force values (3 tests/N). The complete protocol can be found in Section 6.2 of the Appendix below. This testing procedure made sure the device met the required error margins as specified in the *design specifications* section above. The results should show that the device is able to meet these standards and forces applied are within the correct error margin.

FSR Feedback System Testing

Testing of the FSR feedback system was performed to ensure that the system and display were working properly. The feedback system needs to accurately display the force being applied throughout the procedure with less than 5% difference and light the red LED if the force applied is greater than 2.40 N, as specified by the design criteria. To test this system, different amounts of water in increments of 50 - 200 mL was added to a water bottle. The water bottle was then placed on the FSR sensor and the values were read and recorded from both the serial monitor and the LED digital display. After recording the values, the percent difference was calculated between the value read from the serial monitor and the value read from the LED digital display. A force greater than 2.40 N was applied to the sensor to ensure that the red LED would light up. The complete protocol can be seen in Section 6.3 of the Appendix. The design specifications required the force on the LED digital display to be within 5% of the force on the serial monitor as well as a force of 2.40 N or greater would light up the red LED. This testing should be able to conclude that both of these specifications were met and the FSR feedback system is functioning properly.

Syringe Clamp Connection and Locking Testing

Testing of the 1 mL Syringe Clamp Mold was performed to ensure the stem cell loaded syringe can withstand the forces provided by the injection device when undergoing 30 and 60 second injections. This test also ensures that the syringe can be easily removed and replaced in between injections, as timeliness of the injection procedure is crucial to success. Using the 1.0 mL Syringe Mold, a 1.0 mL syringe, and medical grade tubing, the syringe was exposed to forces that are normally higher than that of an injection with the device. Applying a 5.0 N force to the syringe plunger and ensuring it stays in place will validate the design, as it is predicted the syringe will not see above 5.0 N of force when being used in the injection device. Following each successful application of 5.0 N of force, the syringe will be tested on removability from the mold. The syringe should easily slide out of the mold without sticking, scraping, or having any unnecessary force applied in order to remove the syringe. Results of this testing procedure

provides validation for use of the 1.0 mL Syringe Mold in the injection device during clinical procedures. The complete Syringe Clamp Connection and Locking Testing protocol can be found in Section 6.4 of the Appendix.

Injection Rate Testing

Testing of the injection rates was completed to ensure the device will completely empty the syringe in the correct time frame. The device and incorporated Stepper Motor must cause the nylon nut on the threaded rod to travel the required distance needed to empty out 0.5 mL of liquid from the syringe in both 30 and 60 seconds. The device needs to finish injections within 5% of 30 or 60 seconds, while also traveling within 5% of the required distance of 30.5 mm that would completely empty a 1 mL syringe loaded with 0.5 mL of solution. To begin, the nylon nut was placed in the center of the threaded rod with this starting point being marked with tape. The circuit controlling the Stepper Motor was then powered on. Both the 30 and 60 second injection rates were recorded using a stopwatch, being sure to start and stop the stopwatch as soon as the motor starts and finishes spinning. After each test, the recorded time on the stopwatch was compared to the expected time of 30 or 60 seconds. During the time the motor was spinning, the nylon nut was held in place by the user to prevent it from spinning, thus allowing it to travel up the threaded rod. After each test, the distance the nylon nut traveled up the threaded rod was recorded and compared to the value of 30.5 mm. The percent errors and standard deviations in each testing group was calculated to validate the injection rate times and nylon nut distance traveled. The overall results of this testing should ensure that the injection rate components of the device were ready to be moved forward with and incorporated into the final prototype. The complete injection rate testing protocol can be found in Section 6.5 of the Appendix.

Cell Viability Testing

Cell viability testing was completed to ensure there was not more than 5% cell death from starting viability after completing 30 and 60 second automatic injections, as specified by the design criteria. Pig MSCs were thawed and prepped to get a 40 mL of solution that was used throughout the entire testing process. A 1 mL syringe with a 25 gauge needle attached was loaded with 0.5 mL of the solution and placed in the syringe holder on the device, the button to start the 30 second injection was then pressed. The solution was collected in a small vial in order to test viability after injection. This was repeated five times total for 30 second automatic injections as well as five times total for 60 second automatic injections. Manual injections were also performed in order to compare the automatic injection vs manual injection cell viability. Manual injections were performed three times at 30 seconds and an additional three times at 60 seconds, collecting the solution in a small vial after each injection. Rapid injection (less than one second) was also performed three times. Using trypan blue, cell suspension, and a hemocytometer, cell viability was measured and recorded. The cell viability was then compared to the starting viability to determine if there was any cell death from the injection. The cell viability testing should be able to conclude if there is less than 5% cell death during the 30 and

60 second automatic injections. The complete cell viability protocol can be found in Section 6.6 of the Appendix.

Overall Device Testing

25 Gauge Injection Testing

Once the final prototype was assembled and confirmed to work, finalizing tests were performed to ensure all components of the device worked during each injection rate. To further validate the design, the 1.0 mL syringe was loaded with 0.5 mL of water and placed into the syringe mold. The 1.0 mL syringe had an attached 25 gauge needle to allow the water to be collected into the collecting dish and mimic the connection tubing and catheter forces. After each 30 and 60 second injection, the water emptied from the syringe was collected, weighed, and compared to the expected value. Along with testing how well the device empties the syringe, the injection times and force ranges were also recorded. The injection times were then compared to the expected injection times of 30 and 60 seconds, while the force ranges were recorded and compared with each test to determine the repeatability of each injection's force production. Similar to the *Injection Rate Testing*, the times for each injection are expected to be within 5% of the expected value. The force values were observed from the LED Display, with the smallest and largest observed values being recorded. It is anticipated that the volume of water delivered from the syringe will be within 5% of the required 0.5 mL value, as the injector is expected to be accurate and this 5% error margin accounts for water that is inherently and inevitably present in the dead space of the needle and distal tip of the syringe following each injection.

Clinical Simulation Testing

The Clinical Simulation Testing procedure is set up the same way as the *25 Gauge Injection Testing*. The 1.0 mL syringe loaded with 0.5 mL of water was hooked up to the provided connection tubing and catheter system to mimic the conditions the syringe will go through during stem cell injection procedures. The 30 and 60 second injection times were recorded and compared to their original values to ensure the times were within 5% error margin of the expected time. The volume of fluid ejected from the syringe was not recorded, as most of the fluid will be within the connection tubing and catheter after the injection. Visually observing the syringe being completely emptied will validate that the device can perform the stem cell injection procedure using the appropriate tools. The force ranges were also recorded again to determine the repeatability of each injection's force production.

Catheter Backup Testing

Testing that force value that is observed by the FSR Feedback System within the device when the catheter is backed up was tested to determine what values correlate to an obstructed catheter. Each injection rate was performed once, where the 1.0 mL syringe loaded with 0.5 mL

of water was hooked up to the medical grade tubing and catheter system. This catheter system was entirely filled with solution prior to the attachment of the syringe to the medical grade tubing in order to enable proper flow of solution through the system during each injection and to mimic the procedural setting utilized during clinical trials. After proper system set-up and connection of the syringe to the catheter system, the distal end of the catheter was then blocked with a finger applying force and preventing any liquid from escaping. The only recorded values for this test were the force range values, peak force, and if the 2.40 N threshold value was exceeded, causing the LED threshold light to signal to the operator the threshold value was passed. This test will provide data establishing the force values that correspond to an obstructed catheter and whether or not the 2.40 N threshold correctly indicates catheter obstruction susceptibility. This test will also determine the strength of the Stepper Motor and whether or not the injection device is powerful enough to reach the 2.40 N threshold.

Results

Feedback System Force Detection Accuracy

The Feedback System Force Detection Accuracy testing (see the *Feedback System Force Detection Accuracy Testing* section above) was used to establish the force identification efficacy of the feedback system. As the sixth-degree polynomial line of best fit equation and 4th-degree polynomial line of best fit equation from the system's calibration curves (Figure 11 above and Section 6.1 of the Appendix respectively) was utilized to calculate the force applied by the injector to the syringe plunger, this test also verified the accuracy and reliability of the calibration curves. Forces across the typical injector range (0.14 N - 3.00 N [29]) were tested following the force detection testing protocol described in the *Feedback System Force Detection Accuracy Testing* section above and in Section 6.2 of the Appendix. To determine if the feedback system met the required error margin specifications (see the *Design Specifications* section above), the percent error between the force detected by the feedback system and the actual applied force was calculated using Equation 5 above [55].

Establishing the discrepancy between the force displayed by the feedback system and the actual applied force via percent error determines if the injector accurately detects force and thus properly assists the operator throughout each injection. As a result, calculating the percent error between the feedback system and the applied force identified the efficacy of the feedback system throughout the intramyocardial MSC injection procedure, including when catheter obstruction is imminent or present. The feedback system's percent difference across the injector force range and thus its accuracy can be seen in Figure 15 below.

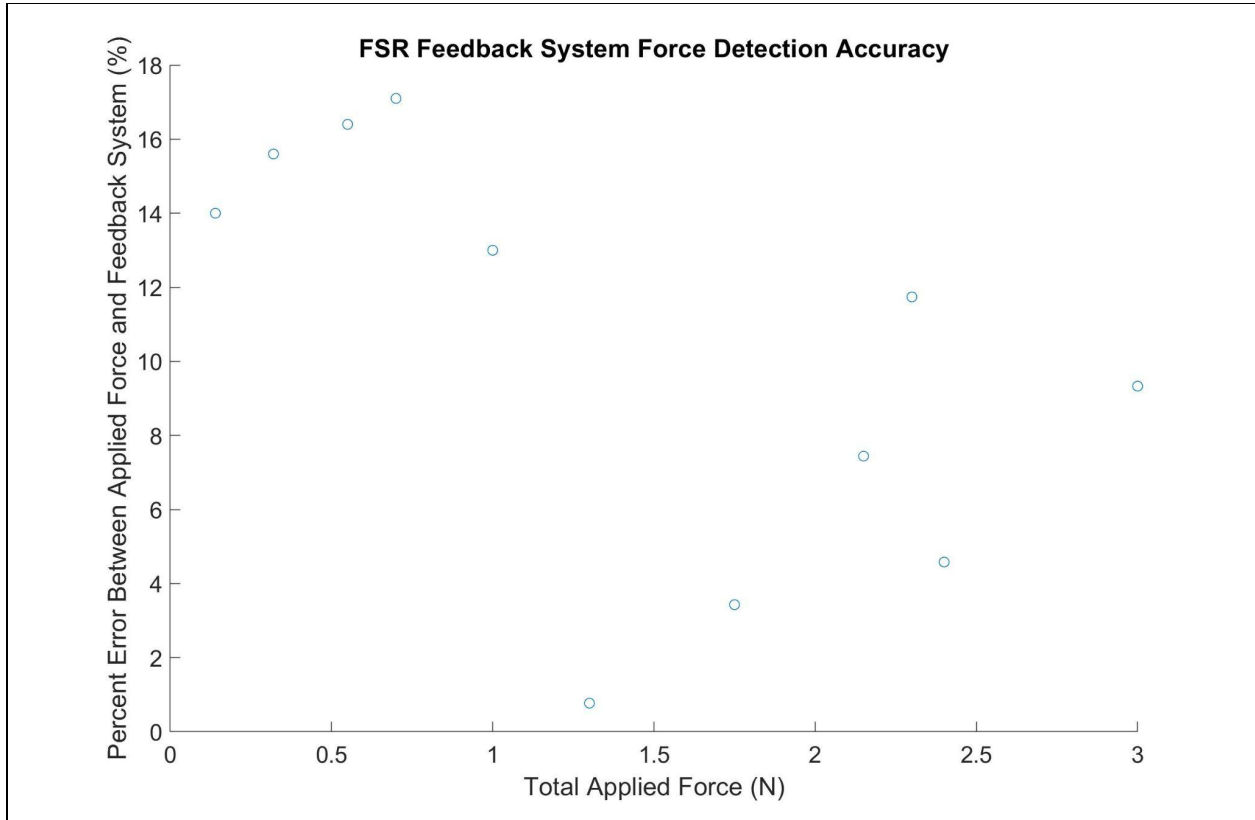


Figure 15: Feedback system’s force output error margin relative to their respective applied forces over the typical 0.14 N - 3.00 N [29] injector force range.

As seen in Figure 15 above, all output force values less than 1.00 N were accurate within the required 20% error margin, averaging an error of $15.78 \pm 1.33\%$. All detected force values at or above 1.00 N adhered to the 15% error margin specification, with an average error of $7.18 \pm 4.50\%$. All force values output by the feedback system over the expected 0.14 N - 3.00 N injector force range met their required error margin, demonstrating the injector’s ability to accurately display force values throughout each procedure and thus its high force detection efficacy. Confirming the success of the injector at lower force values (0.14 N - 1.00 N) was pertinent as they represent the most common forces exhibited on the syringe plunger throughout each injection [13]. To account for this force dominance and ensure that the device was very accurate over this range, a relatively large amount of tests were performed on the lower force values (5 tests/N). The 20% error margin represents the proper force magnitude difference between the feedback system’s output and the actual applied force for values less than 1.00 N to enable accurate and proper injection success identification (see the *Feedback System Force Detection Accuracy Testing* section above). As all of the percent errors for force values less than 1.00 N were less than this 20% error margin ($15.78 \pm 1.33\%$), the feedback system is accurate, enabling the operator to adequately determine the efficacy of each injection and thus demonstrating that the feedback system will properly assist each injection.

While it is important to correctly detect the force applied to the injector throughout each injection, it is critical that the feedback system can establish when the 2.40 N threshold is reached and catheter obstruction is imminent. The feedback system needs to identify when this value is reached with a high degree of accuracy in order to motivate the operator to monitor the injection and stop it if catheter obstruction is present (determined by operator). If the injection continues while the catheter is obstructed, the cells will build-up in the catheter and the increasing pressure within the catheter will induce cell damage and lysing, drastically reducing the efficacy of the intramyocardial MSC injection procedure [56]. As seen in Figure 15 above, there is only a 4.58% difference between the feedback system and actual applied force when 2.40 N of force is delivered to the FSR sensor, confirming the accuracy associated with detecting catheter obstruction susceptibility. All force accuracy detection data can be found in Section 7.1 of the Appendix below.

FSR Feedback System Results

As outlined in the FSR Feedback System Testing Protocol, this test compares the force output value outputted on the scoreboard display with the force printed by the serial monitor. The test also confirms that the LED light turns on when the force exceeds 2.4 N. In order to interpret the results, the average and standard deviation of the 15 force output values (from the three sets of five second samples where each measurement was taken one second apart) for both the display and serial monitor was calculated. This was repeated for each of the five aliquots of water. Next, the percent difference between the scoreboard display and the serial monitor was calculated using the formula in Equation 6 below [57].

$[6] (SM_{avg} - Display_{avg} / (((SM_{avg} + Display_{avg})/2))) * 100 = \text{Percent Difference}$ <p style="text-align: center;">SM = Serial Monitor Display = Scoreboard</p>

The results of the testing concluded that the FSR Feedback System is functioning accurately because there is less than 5% difference between the scoreboard display output and the serial monitor. The average percent difference between the two was 0.137% so this means that for the most part, the serial monitor and scoreboard display will be similar to each other. This result means that the surgeon can rely on the scoreboard display to accurately output the approximate force that is being applied on the syringe which corresponds to how much the cells are experiencing. In addition, the results confirmed that the LED light does turn on and stay on when the force exceeds 2.4 N. The raw data and explanation for how the mean, standard deviation and percent error were calculated can be found in Appendix Section 7.2.

Table 2: Results for the difference between the readings on the scoreboard display and the serial monitor output.

	Scoreboard Display Average (N)	Serial Monitor Average (N)	Percent Difference (%)	Was the LED on?
Aliquot of water #1	0.213 ± 0.0140	0.213 ± 0.0145	0%	No
Aliquot of water #2	0.679 ± 0.00743	0.681 ± 0.00743	0.387%	No
Aliquot of water #3	1.21 ± 0.00737	1.21 ± 0.00862	0.166%	No
Aliquot of water #4	1.93 ± 0.00594	1.93 ± 0.00594	0.104%	No
Aliquot of water #5	2.48 ± 0.00676	2.48 ± 0.00704	0.0283%	Yes
Average Percent Difference: 0.137%				

Syringe Clamp Connection and Locking Results

The Syringe Clamp Connection and Locking tests confirmed that the syringe was able to be easily swapped as well as lock and stay in place when force is applied. The force testing was passed because the syringe clamp was able to securely hold the syringe in place when any force above 5 N was applied. In addition, the average time for swapping the syringes with the syringe clamp was 13.50 ± 1.74 seconds. This result is less than the 60 second maximum that is required for surgeons to be able to change the syringes during the course of the surgery. Even with the standard deviation, the time for swapping the syringes is much less than 60 seconds. Of note, despite the standard deviation demonstrating that the syringe exchange may take up to 15.24 seconds, for all five trials performed, the exchange time was never greater than 14.78 seconds, so the optimal 15 second dwell time was obtained for each procedure. The syringe clamp successfully provides an easily exchangeable mechanism that is sturdy enough to support well above the maximum amount of force that it may experience during the course of the procedure. All syringe clamp testing data can be found in Section 7.3 of the Appendix below.

Injection Rate Results

The Injection Rate testing proved that the motor was set at an accurate setting for both the 30 second and 60 second injection rates and moved the appropriate distance to deliver 0.5 mL of solution. The average injection times for the 30 second and 60 second groups were recorded along with the distance the motor was able to “travel” during each injection. Although the motor itself does not move, the distance that the motor can displace an object via the rotation of the bolt is important since it will drive the force application block forward at a certain distance, compressing the syringe plunger over this distance as well. The plunger must travel 30.50 mm in order to deliver the full 0.5 mL solution. To establish the accuracy of the stepper motor, Equation 5 above was used to calculate the percent error between each injection time and their desired target time as well as the distance “traveled” by the motor relative to the required 30.5 mm distance. The results can also be seen in Table 3 below.

Table 3: Results of the Injection Rate Testing performed for the two rates (30 seconds and 60 seconds). Both the injection time and the distance traveled was evaluated.

		Mean	Standard Deviation	Percent Error (%)
30 Second Rate	Injection Time	30.32 seconds	± 0.14 seconds	1.067%
	Distance Traveled	30.48 mm	± 0.23 mm	0.06557%
60 Second Rate	Injection Time	60.80 seconds	± 0.84 seconds	1.333%
	Distance Traveled	30.49 mm	± 0.15 mm	0.03279%

As seen in Table 3 above, the 30 second injection rate had an average time of 30.32 ± 0.01 seconds, representing a percent error of 1.067%. For this rate, the distance traveled was 30.48 ± 0.23 mm, corresponding to a percent error of 0.06557%. For the 60 second rate sample, the mean speed measured was 60.80 ± 0.84 seconds and the average distance traveled was 30.49 ± 0.15 . The percent error for the injection duration and travel distance was 1.333% and 0.03279%, respectively. The raw data and explanation for how the mean, standard deviation and percent error were calculated can be found in Appendix Section 7.4.

Cell Viability Results

Cell viability testing using Trypan Blue staining was performed as outlined in the *Cell Viability Testing* section above to determine if cell viability was reduced by more than 5% following each injection of 0.5 mL of MSC aliquot solution relative to the initial viability

immediately after MSC thawing. The cell viability testing followed the standard procedure utilized by Dr. Raval during clinical trials [13], so the results from these tests confirm how well the injector will maintain MSC viability during intramyocardial MSC injection procedures and thus its ability to perform successful injections without reducing therapeutic potential. The cell viability results for 30 second and 60 second automatic and manual injections can be seen in Figure 16 below. The rectangles represent the interquartile range and the whiskers display the full range of MSC viability for each rate and injection modality (manual and automatic).

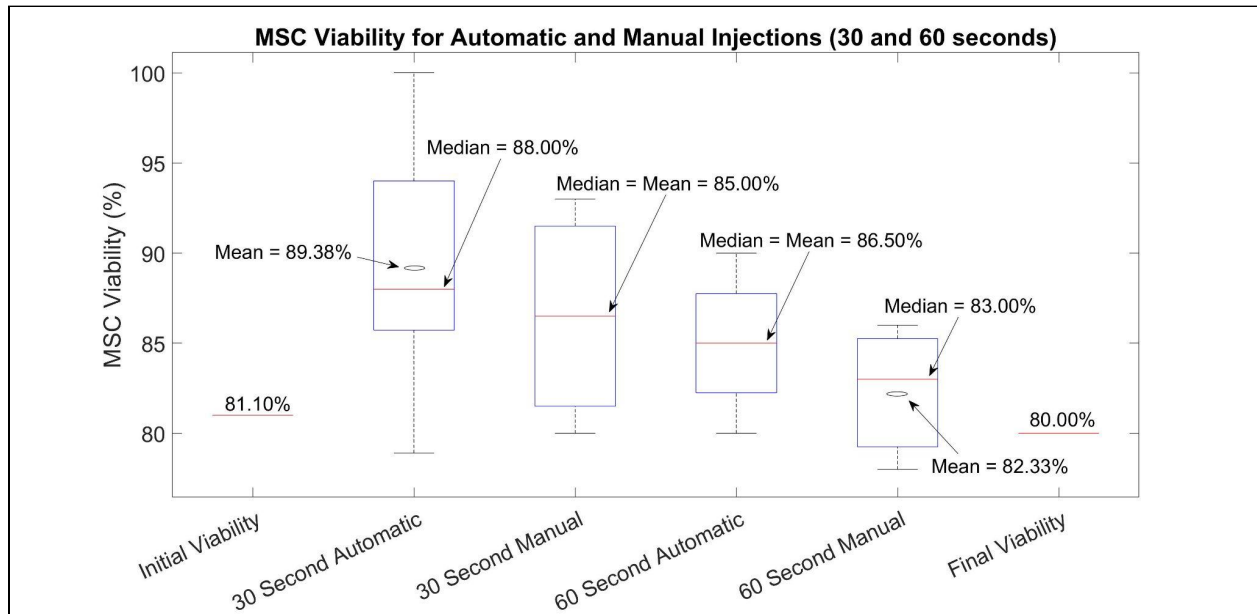


Figure 16: Boxplot comparing the viability of MSCs following automatic and manual 30 and 60 second injections to each other and to the starting and final MSC viability. The rectangles represent the interquartile range, the whiskers display the full range of MSC viability, the red lines locate the median, and the circles identify the mean (if it is not the same as the median) for each rate and injection modality (manual and automatic).

As seen in Figure 16 above, the initial MSC viability after thawing was 81.10% and the final viability at the conclusion of the testing was 80.00%. A Student paired t-test was performed on these viabilities, yielding a p-value of ~ 0.634 . Using a significance value of $p = 0.05$, it can not be concluded that the 80.0% end viability has a statistically significant difference relative to the starting 81.10% viability. As a result, the cells did not experience any substantial death during the course of the testing due to sitting out in typical laboratory benchtop conditions, so the results collected throughout the experiment do not need to be normalized prior to comparison and all the data for each injection rate and delivery modality can be statistically analyzed. For the automatic injections, the average cell viability was $89.38 \pm 7.64\%$ and $85 \pm 3.81\%$ for the 30 second and 60 second injection rates respectively. Out of these two groups, the 30 second injection rate had the lowest cell viability value for all five trials, 78.90%, which is not a 5% reduction in cell viability relative to the initial 81.10% starting viability. This confirms that the

automatic injections do not reduce cell viability by greater than 5% during each MSC delivery and thus the injections meet the MSC viability design specifications.

After confirming the efficacy of each automatic injection in terms of cell viability, the automatic injections were compared to the 30 second and 60 second manual injections in order to determine how the automatic MSC delivery compares to current manual procedures. The average cell viability for the 30 second manual injections was $86.50 \pm 6.02\%$ while the average viability was $82.33 \pm 4.04\%$ for the 60 second manual injections group. To compare the four groups, an ANOVA was performed with each injection rate and delivery modality corresponding to a separate group. The ANOVA p-value was 0.41, verifying that there is not a statistically significant difference between the four groups when comparing their MSC viabilities following each injection (significance value of 0.05). Student paired t-tests were also performed on all groups, further establishing that MSC viability is not significantly different between any pair of injection groups (see Section 7.5 of the Appendix for these additional comparisons). This confirms that the automatic injections are able to maintain the cell viability seen in typical intramyocardial MSC injection procedures, demonstrating that the automatic injections can be implemented clinically without reducing the therapeutic potential of each injection. All of the cell viability data, calculations, and statistical analyses can be found in Section 7.5 of the Appendix.

Rapid injections were also performed during the cell viability testing, with an average injection rate of 0.748 ± 0.400 seconds and an average MSC viability of $86.25 \pm 6.65\%$ following each injection. Similar to the 30 and 60 second injections, an ANOVA was performed to compare the cell viability between the 30 second, 60 second, and rapid injections with each injection rate and delivery modality (automatic and manual) corresponding to separate groups. Interestingly, while one of the rapid injection trials had the lowest cell viability relative to all other groups and trials (77.0%), this viability still did not exceed a 5% reduction in cell viability relative to the initial 81.10% that is not acceptable based on the design specifications. Consequently, the ANOVA p-value was 0.59, demonstrating that there is not a statistically significant difference between the five groups when comparing their MSC viabilities following each injection (significance value of 0.05). Student paired t-tests were performed on all groups, confirming that MSC viability is not significantly different between any pair of injection groups, including the rapid injection group. The data and analysis related to the rapid injection cell viability is in Section 7.5 of the Appendix. The implications of these results are described in the *Discussion of Results* section below.

Throughout the MSC viability testing, the force value range for the 30 second and 60 second injections and both manual and automatic delivery mechanisms were collected. The variation across each set of maximum and minimum force values that were consistently cycled between during each injection was calculated in order to determine the force application consistency across each injection and thus the stability of the cell flow rate through the catheter system and into the myocardium. The more consistent the slow flow rate at the desired 30 and 60 second injection rates compared to inconsistent rates containing rapid injection intervals during

the 30 and 60 seconds, the greater the cell retention at the localized site of interest within the myocardium and thus the greater the therapeutic impact [58]. Only the values that were part of the constant cycling of force experienced throughout each procedure are included in this analysis without taking into account the peak force experienced at the end of each injection, as this force is a result of the syringe plunger experiencing applied pressure directly after being fully compressed. Thus, the 0.5 mL of solution was delivered prior to the peak force being applied, so this force does not correlate to injecting any MSCs and is excluded from analysis. The average maximum and minimum force values that were consistently cycled between throughout each injection can be seen in Table 4 below.

Table 4: 30 second and 60 second injection maximum and minimum force values, including their variation, for both automatic and manual injections.

Injection Type	Average Minimum Force (N)	Average Maximum Force (N)	Variance Across Maximum and Minimum Values (N)
30 Second Automatic	0.194	0.264	± 0.00528
30 Second Manual	0.438	0.475	± 0.0310
60 Second Automatic	0.230	0.260	± 0.00435
60 Second Manual	0.333	0.493	± 0.00573

The variance across the maximum and minimum values (within the consistent cycle of values seen during the injection without including the peak force experienced at the end of each injection) for each 30 second automatic and manual injection as well as each 60 second automatic and manual injection was ± 0.00528 N, ± 0.0310 N, ± 0.00435 N, and ± 0.00573 N, respectively. This demonstrates that the variance across the manual injections is the highest relative to their respective automatic injection rates as well as that the manual injections have the two highest variances overall. As a result, the automatic injections have a greater force and MSC flow rate consistency throughout their injections relative to their respective manual injections. The p-value between the two 30 second injection groups (automatic and manual) from a Student paired t-test is 0.0193. With a significance level of 0.05, the variance between these two rates is significantly different, further supporting the greater force and thus MSC flow rate consistency within the 30 second automatic injections relative to the 30 second manual injections. This increased MSC delivery rate stability provided by the automatic injections compared to the manual injections will enable greater cell retention at the site of interest within the myocardium, which could improve the therapeutic potential of each injection. All of the cell viability force data, calculations, and statistical analyses can be found in Section 7.5 of the Appendix.

Overall Device Results

25 Gauge Injection Results

Upon final assembly of the device, testing was completed to ensure the device was functioning properly and meeting the design criteria. The system was set up as if it were going to be used in a procedure except for a 2.22 cm 25 gauge needle was used to mimic the medical grade tubing-catheter system. This needle was selected as it has a similar radius relative to the procedural catheter system and thus generates forces that correspond to the values experienced when utilizing the clinical catheter system. The 25 gauge needle was used for the first round of testing to check for volume injection levels because all of the fluid immediately comes out of the needle with the exception of minimal fluid left within the dead space. The device was able to inject approximately 0.5 mL of solution in the allotted 30 or 60 second intervals with an average error of less than 5%. The volume injection values did display some variation from the desired 0.5 mL solution amount because there is dead space present in the distal tip of the syringe and in the needle. There is inherent dead space at the distal tip of every syringe and within each needle that can not be avoided, even during manual injections. As a result, the volume delivered will be reduced and the entire 0.5 mL of solution will not be delivered during each injection. The expected injected volume was approximately 0.5 mL, but due to the dead space, not all of the fluid was accounted for after each injection. To account for the dead space, the delivery volume needs to be within 5% of 0.5 mL, corresponding to a maximum dead space of ~0.025 mL throughout the entire syringe-needle system. The mean of the injected volume was established and then a percent error was calculated between the injector's delivery volume and the desired 0.5 mL injection amount, establishing the accuracy of each injection. The force that was applied by the system to inject the fluid did not exceed 2.40 N, demonstrating that there was not any backup or obstruction experienced during the tests. The injection time, injection volume, and force ranges, as well as the serial plotter were measured and can be seen in Table 5 below and Section 7.6 of the Appendix respectively. A box plot displaying the percentage difference between the injection times and delivery volumes to their respective target values can also be seen in Section 7.6 of the Appendix.

Table 5: Results of the Overall Device Testing performed for the two rates (30 seconds and 60 seconds) with a 2.22 cm, 25 gauge needle. The injection times, volumes, and force ranges were evaluated.

		Mean	Standard Deviation	Percent Error (%)
30 Second Rate	Injection Time (seconds)	30.6	± 0.121 seconds	1.88 ± 0.410
	Volume Injected (mL)	0.486	± 0.0134	3.60 ± 1.67
	Force Range (N)	0.164 - 0.498	-	-
60 Second Rate	Injection Time (seconds)	60.2	± 0.150 seconds	0.260 ± 0.250
	Volume Injected (mL)	0.480	0	4.00
	Force Range (N)	0.156 - 0.462	-	-

Clinical Simulation Results

The assembled device was then tested while connected to the catheter and connection tubing system to simulate the clinical use of the device. The same tests were performed as with the 25 gauge needle except the ejected volume was not collected because there would be an inaccurate amount due to bubbles in the tubing systems and dead space. Simply verifying if all of the solution was out of the syringes was all that was done to ensure enough of the fluid was injected. These tests were done to ensure that the force values when the syringe was connected to the rest of the system were still within range, that the device was able to exert enough force to push the fluid through the thin connection tubing and catheter system, and that the times for the ejections were still accurate. The results confirmed that there was less than 5% error with the ejection times and that the force range was still well under 2.4 N indicating that there was no catheter backup. The 30 and 60 second serial plotter results with the force values over time can be seen in Section 7.7 of the Appendix. It should be noted that although the serial plot was taken for each trial, the exact force values cannot be interpreted for them so the force value ranges in the table below were obtained by looking at the display. This is because the serial monitor and serial printer can not be on at the same time.

Table 6: Results of the Overall Device Testing performed for the two injection rates (30 seconds and 60 seconds) with the connection tubing and catheter system utilized during clinical trials.

The injection times, volumes, and force ranges were evaluated.

		Mean	Standard Deviation	Percent Error (%)	Was the syringe empty at the end of the injection?
30 Second Speed	Injection Time	30.6 seconds	± 0.176 seconds	2.04 ± 0.58	Yes, for all trials
	Reverse Time	23.6 seconds	± 0.359 seconds	-	
	Force Range	$(0.236 \pm 0.049) - (0.484 \pm 0.043)$ N		-	
60 Second Speed	Injection Time	60.3 seconds	± 0.187 seconds	0.55 ± 0.31	Yes, for all trials
	Reverse Time	24.5 seconds	± 0.224 seconds	-	
	Force Range	$(0.168 \pm 0.038) - (0.448 \pm 0.040)$ N		-	

Catheter Backup Results

Following the Catheter Backup Testing Protocol discussed in the *Catheter Backup Testing* section above, the force values corresponding to the onset of catheter obstruction were tested to confirm the accuracy of the 2.40 N catheter backup literature value. However, the 30 second and 60 second injection rates were both only tested once with a catheter obstruction indicator force value of 1.51 N and 1.81 N respectively. Only one trial was performed for each rate as it was determined that the force value associated with catheter back-up for each rate during this set-up was not characteristic of the forces that will be experienced during a typical clinical MSC injection into the myocardium. The 12.68 ± 2.91 kPa myocardium stiffness associated with an individual who experienced a myocardial infarction [18] will increase the force required to inject the MSCs into the myocardium relative to the current testing that was performed with the medical grading tubing-catheter system delivering the solution into a petri dish. This will correlate to a higher force value indicating the onset of catheter obstruction during clinical procedures compared to the blockage force values picked up during testing. As a result, the force values identified during the catheter backup testing do not correlate to the catheter obstruction force values that will be picked up during a clinical intramyocardial MSC injection procedure, preventing these tests from providing information that correctly identifies catheter obstruction susceptibility. One test was performed for each rate and then stopped due to the inability for these results to provide any clinical relevance for the injector.

Discussion

Discussion of Results

Feedback System Force Detection Accuracy Testing was performed in order to establish the force identification efficacy of the feedback system using a line of best fit equation from the calibration curve that was developed. This testing confirmed that all output force values over the typical injector force range were accurate within the required 20% error margin in accordance with the team's design specifications. All output force values less than 1.00 N were accurate within the required 20% error margin, averaging an error of $15.78 \pm 1.33\%$ and all force values above 1.00 N adhered to the 15% error margin specification, with an average error of $7.18 \pm 4.50\%$. Meeting this client requirement is important because the device will be used by surgeons to see approximately how much force they are applying to the cells. It will also inform them of what type of tissue they are injecting into because healthy heart tissue will require a different amount of force than diseased tissue. Having an accurate force detection system is therefore going to be able to provide the surgeon with feedback for how the injections are going and indicate if there are any issues and this is something that no other devices on the market are capable of doing. FSR Feedback System Testing was then performed in order to compare the force output value displayed with the force printed by the serial monitor along with LED illumination when 2.4 N is exceeded. The results from this test successfully validated that the LED turned on when the FSR received at least 2.40 N of force and that the FSR Feedback System is functioning accurately due to the less than 5% difference between the scoreboard display output and the serial monitor with a 0.14% difference on average. These results are important because they show that the feedback system is thus able to accurately receive and display the force throughout each injection and alert the user when catheter obstruction is imminent, helping the operator to perform successful injections.

Next, Syringe Clamp Connection and Locking tests were performed to confirm that the syringe was able to be easily swapped as well as lock and stay in place when force is applied. In compliance with design specifications, the results from this testing validated that the syringe clamp is stable enough to support well above the maximum amount of force that it may experience during the course of the procedure. This is important because the syringe must stay in place throughout the course of the injections while also being easily swappable so that the surgeon can deliver the cells in a timely manner. Injection Rate testing was performed to ensure that the device is capable of providing the 30 second and 60 second injection rates requested by the client and also moved the appropriate distance to deliver 0.5 mL of solution. The 30 second injection rate resulted in a percent error of 1.08% for the injection rate timing and a 0.0721% error for distance traveled. The 60 second injection rate resulted in a percent error of 1.33% for the injection rate timing and a 0.0328% error for distance traveled. These percent errors are well within the desired 15% error margin. These results exemplify the accuracy of the injection device in accordance with design specifications. This is important because one of the main goals of the project was to be able to accurately deliver cells in a controlled manner at the two specified rates

that the client uses in clinical trials. Although the best rate for this therapy has not been determined, the 30 and 60 second rates have been proven to work well with this therapy so it was important to be able to inject the cells at these rates.

Following assembly of the entire injector, MSC viability testing using Trypan Blue staining was performed to determine if cell viability was reduced by more than 5% following 30 second, 60 second, and rapid manual injection. This verified that each automatic injection rate kept MSC viability within 5% of initial cell survival, in congruence with typical intramyocardial injections. This confirms that the automatic device did not have a lower cell viability than the manual injections and thus does not reduce the therapeutic impact of the current procedure. This is important because having a device with similar cell viability compared to the manual injections means that the device can deliver the cells in a way that will allow for similar procedural success (or potentially more success) than if the injections were done manually. Although the results showed that there is not a statistically significant difference between the four groups when comparing their MSC viabilities following each injection, automatic injections are expected to aid in improved cell retention in comparison to manual. Manual injections had inconsistent force values across their injections with force ranges that did not display a steady pattern throughout each injection as some manual injections had high force values early within the injection and then lower force applications at later times, other injections started out with low force application values and then the force application peaked at the end. As a result, there is less consistency across the manual injections in terms of syringe plunger force application compared to the consistency seen in the automatic injections which all displayed about the same force range value across each set of trials for each respective injection rate (minimal variation seen in Table 4 above). As automatic injections have a greater force and flow rate consistency at the desired, slow 30 second and 60 second rates throughout their injections relative to manual injections, their MSC delivery should enable greater localization at the myocardium site of interest, which can enable greater cell retention in the myocardium that will improve the therapeutic impact [58]. Additional bovine steak and *ex vivo* porcine heart testing will need to be performed to confirm the cell retention differences between the automatic and manual injections and thus the greater clinical success the automatic injections can promote.

Additional testing was performed with the fully assembled device to ensure that it was functioning properly along with meeting the design criteria. 25 Gauge Injection Testing was performed using a 25 gauge needle to mimic the connection between the tubing and the catheter system and validate the volume ejected. The device was successfully able to eject approximately 0.5 mL of fluid with an average error of less than 5% with the consideration that dead space is present in the needle. This is important because the device needs to be able to inject all of the fluid so that the stem cells will all be delivered in full within the preferred time (either 30 or 60 seconds). Finally, clinical simulation testing and catheter backup testing was performed to confirm the overall design functionality which was important because the device must be able to still operate when connected to the connection tubing/catheter system because that is what will be used when delivering the stem cells into the myocardium with patients. The force ranges for

the clinical simulation testing also confirmed that the force value ranges for injecting the cells stayed well below the 2.4 N indicating that there is no backup in the system and too much force is not being applied on the cells. Catheter Backup testing was performed with the 30 and 60 second injection rates resulting in catheter obstruction force value of 1.51 N and 1.81 N respectively. During clinical simulation testing the entire device was assembled and connected to the catheter via tubing and the same tests were performed as the 25 gauge needle testing without collection of the ejected volume. The results confirmed that there was less than 5% error with the ejection times and that the force range was still well under 2.4 N. However, the force values identified during the catheter backup testing cannot properly simulate the catheter obstruction force values during a clinical intramyocardial MSC injection procedure. Thus Catheter Backup testing results cannot provide information that correctly identifies catheter obstruction susceptibility. See *Future Work* below for plans to complete *ex vivo* catheter obstruction testing. In conclusion, the device was able to successfully pass all of the testing requirements thus no integral changes need to be made to the device at this time.

Ethical Considerations of Design

One area of ethical considerations the team needs to be taken into account is safety. The team needs to ensure both operator and patient safety. In terms of operator safety, if the system were exposed to liquid or malfunctions the team needs to ensure that there would be no harm to the operator from the moving parts or from the electrical components. Regarding patient safety, the risk of bubble formation in the catheter tubing must be reduced as much as possible. Air bubbles in the arteries due to catheter removal or insertion can lead to lethal coronary air embolism [59]. The team chose the manual design instead of a fully automated design to reduce this risk. Additionally, the device must ensure that two flow rates, a 30 second rate and a 60 second rate, reliably and consistently inject 0.5 mL of stem cell solution into the myocardium with dwell time in between. If the injection rate is too fast it can lead to the reflux of cells through the needle tract and cell damage. Furthermore the device must be capable of notifying the user of a potential clog in the catheter tubing. If the user encounters a clog and the injection device does not notify the user, it could potentially lead to damage of the system or an improper volume of stem cells injected into the myocardium. The team also needs to ensure that the system is sustainable and holds up to its everyday use and when deciding on materials to construct the injection device, the impact that each material will have on the environment must also be taken into consideration.

Another important ethical consideration is the ethics of animal testing. The device will first be tested in the animal cath lab for porcine procedures before it is used during human procedures. In all animal testing it is essential that research has a sufficient purpose to justify the use of animals. Humane consideration for the well-being of the animals must be incorporated into the design and procedure and all animal testing must be approved prior by the Institutional Animal Care and Use Committee (IACUC). During a procedure, animals must remain under anesthesia for the entirety of the procedure and aseptic technique must be used whenever

possible [60]. All personnel involved in the procedure must be properly trained and receive instruction in the care, maintenance, and handling of the species being studied. Procedures where the research animal is anesthetized and is euthanized before regaining consciousness are generally acceptable. Animals also should not be subjected to successive survival surgical procedures, except as required by the nature of the research or surgery. Multiple surgeries on the same animal must receive approval from the IACUC [61]. Additionally, animals that were raised in a laboratory must not be released into the wild because it is likely they cannot survive. Euthanasia must be performed in a humane manner to ensure immediate death, ensuring that no animal is discarded until its death is verified. Euthanasia is often accomplished by injection of a death-inducing drug that stops brain function and is not painful to the animal [62].

Ethical concerns in relation to stem cells for use in both the procedure and for the team's testing is also relevant. Concerns for clinical application of MSCs are unwanted differentiation of the transplanted MSCs and their potential to suppress anti-tumor immune response and generate new blood vessels that may promote tumor growth and metastasis. In the case of injection into the myocardium it is desired that the MSCs differentiate into cardiac muscle cells, unwanted differentiation or tumor formation could lead to a serious safety issue. Another major concern with injecting MSCs into the body is the possibility for an inflammatory response. MSCs can adopt a pro-inflammatory phenotype and produce neutrophils and activate T-cells invoking an inflammatory response [63]. Chronic inflammation can lead to further health complications. On the other hand, if stem cell-based interventions live up to their therapeutic potential, it will be important to ensure equitable access to these treatments. As cell therapies are cost and labor intensive, the automatic intramyocardial MSC injection procedures will presumably be costly [64]. As a result, an equitable distribution of this new therapy is a significant consideration for clinical usage. Non-infectious porcine derived MSCs will be used in testing that were derived from euthanized pigs, one must ensure that these cells were obtained in an ethical manner.

Sources of Error

Possible sources of errors may be variability of force detection with the FSR 400 Force Sensing Resistor, count-to-count variability during stem cell viability testing, injection time variability, volume injection value errors, and 3D printing errors. During overall device testing with the 25 gauge needle the volume ejection values measured were not extremely precise due to the dead space present in the needle and where the needle connects to the syringe. The expected delivery volume was 0.5 mL but typically only 0.49 mL - 0.48 mL of solution was measured demonstrating that there is 0.01 mL - 0.03 mL of dead space that accounts for this error. The team thus took this into consideration when establishing the error margin of 5% for injection volume. Possible sources of error in stem cell viability testing could be due to count-to-count variability. Count-to-count variability of a single sample by an experienced cell biologist is commonly 10% or more and when comparing variation of single-sample counts across multiple scientists, counting variability can exceed 20% [65]. Other sources of error may include injection time outside the specified range of 60 seconds \pm 0.5 seconds and 30 seconds \pm

0.5 seconds, and volume dispensed must be within 0.5% of 0.5 mL. During 25 Gauge Injection Testing error in the volume dispensed could be due to the inaccuracy of the scales used to weigh the water ejected, misconsideration of the dead space present, and inaccurate uptake of the water by hand. There will also be errors associated with each component that is fabricated from 3D printed Ultimaker PLA. The Ultimaker S5 3D printer that was used to print each Ultimaker PLA component contains a tolerance, resulting in slight variations in precision and accuracy during each 3D print [66]. This difference across each 3D printed part will result in dimensional errors across components that may hinder the cohesiveness and mating of the injector. However, the dimensional difference between parts should be minimal, so the overall functionality of the injector should not be impacted to any significant degree, especially since the connections within the force application threaded system will not be impacted.

Conclusion

Overview

The client, Dr. Amish Raval, requests an automatic injection device for use during stem cell delivery procedures to the myocardium. The device must integrate with 1 mL procedural syringes, medical grade tubing and clinical catheter. Its aim is to limit operator intervention and provide a 60 and 30 second injection rate. The current procedure is susceptible to rapid injections that lead to the reflux of cells through the needle tract and cell damage. Thus it is imperative that the injector maintain the desired injection rates as well as maximize cell viability throughout the entirety of the procedure. The initial designs consisted of the Cellicopter: Propeller Controlled Injections, the Cellringe Pump: Threaded Force Application Injection, and the Cellvolver: Fully Automatic Dual Motor Injections. The Cellringe Pump won the design matrix due to its efficacy, feasibility, and cost. This design consists of a base that includes a stepper motor, a threaded rod injection regulator, an Ultimaker PLA force application block, and adjustable syringe clamp molds. To achieve accurate injections, the injector consists of a stepper motor that autonomously rotates a fully threaded metal bolt to drive a force application block toward the procedural syringe, injecting 0.5 mL of MSC solution into the catheter tubing and into the myocardium at a 30 or 60 second injection rate. A syringe clamp mold within the injector base fastens the required 1 mL syringe in place, preventing any displacement during each injection. Following each successful injection, the force application block moves back to the starting position, allowing syringe removal and insertion to take place within a maximum 60 second dwell time between injections. The injector feedback system is controlled by an Arduino Uno microcontroller and detects force via an FSR force sensor attached to the force application block, receiving and displaying the force applied by the injector to the syringe throughout each injection on the scoreboard. An LED built into the feedback circuit signals when the 2.40 N threshold is reached to indicate potential catheter obstruction.

Injection testing verified that each injection rate delivered the complete 0.5 mL of solution within the required time. A calibration curve was developed for the FSR sensor based on

initial reliability testing and subsequent force detection testing confirmed that all output force values over the typical injector force range were accurate within the required 20% error margin for values less than 1.00 N and 15% for values greater than or equal to 1.00 N.

Threshold value testing validated that the LED turned on when the FSR received at least 2.40 N of force. Following assembly of the entire injector, MSC viability testing verified that each injection rate kept MSC viability within 5% of initial cell survival, in congruence with typical intramyocardial injections. Overall design functionality was confirmed with a clinical MSC injection procedure simulation that demonstrated 0.5 mL of MSCs were properly delivered with both injection rates and that the FSR feedback system correctly displayed force values and alerted the user when threshold was exceeded.

The design specification testing results validate the efficacy of the injection device, confirming its reliability and accuracy. The injector provides the required slow, consistent injection rates, maintaining cell viability and maximizing cell retention. Its automatic MSC delivery at the required flow rates and dwell time limits operator intervention, meeting design specifications and promoting its clinical applicability. The feedback system accurately receives and displays the force throughout each injection, correctly alerting the operator when catheter obstruction is imminent. As a result, it meets all feedback system requirements, providing a simplistic interface that assists the operator with each injection, increasing MSC injection procedural success. With the injection device, the user is no longer subject to hand fatigue and the susceptibility to rapid injections is removed, promoting procedural efficacy. This device is thus a critical component of improving the treatment of myocardial infarction and mitigating cardiac arrest risk.

Future Work

Moving forward, the team plans to continue testing the feedback system in different situations testing the device's accuracy against different viscosities. Additionally, to establish force ranges associated with different myocardium locations: diseased or scarred tissue, healthy tissue, and body cavity the team plans to perform bovine steak and *ex vivo* heart injection testing. A bovine steak model of different densities and properties can be used to determine what is most replicable to healthy tissue and what is the threshold difference between healthy and unhealthy tissue. Other testing to be performed in the future is *ex vivo* catheter obstruction testing to simulate the catheter obstruction force values during a clinical intramyocardial MSC injection procedure as this was not achieved during Catheter Backup testing. Additionally, during the procedure there will still be user interaction with the device to change out the 1 mL syringes which may result in bubble formation in the tubing. To achieve the goal of no bubble formation actions may include getting FDA approval to alter the protocol from using 10 to 14 aliquots in 1 mL syringes to using a single aliquot in a 5 mL or 10 mL syringe. This would limit the risk of bubble formation and make the entire process easier for the medical professional. To make this device easier to incorporate in a clinical setting with the least amount of wires and to take up the least amount of space it would also be ideal to work on transitioning the injector device into

battery operation. Additionally, to keep the hMSCs suspended in solution and not clumping at either end of the syringe the team will look into the addition of an agitation apparatus. This addition could also help reduce the risk of catheter obstruction due to cells clumping and improve the overall procedural success. The final validation of the device's functionality in a clinical setting could be confirmed by *in vivo* testing ensuring that the feedback system can respond to catheter obstruction as well as correctly function to dispense 0.5 mL of hMSC solution into the catheter and into the myocardium at the 60 and 30 specified rates.

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Appendix

Section 1: Product Design Specifications

Product Design Specification

Automatic Intraoperative Stem Cell Injection Device

Team Heartthrob
Lab 307
May 4th, 2022

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Function:

Treating heart failure by injecting stem cells into the myocardium via an injection device and a needle-tipped catheter is a novel approach that can save lives and significantly improve quality of life. Unfortunately, the current injection devices used to perform this stem cell delivery are manually controlled and are unable to regulate cell injection and flow rate through the catheter and into the myocardium. To improve stem cell delivery and cardiac repair, an automatic injection device that integrates with 1 mL procedural syringes, medical grade tubing and clinical catheter is desired. This device will limit operator intervention and provide a slow, controlled, and adjustable injection rate to maximize cell retention and enhance clinical success. The device will operate at two different injection rates, 30 seconds and 60 seconds, and complete 10 - 14 injections, each containing 0.5 mL of stem cell solution, per procedure. The injector should also maintain cell viability throughout the procedure. This automatic injector will result in less labor-intensive intramyocardial stem cell injection procedures, enhancing the accuracy, efficiency, and efficacy of each operation.

Client requirements:

- Injector must be compatible with the standard catheters, medical grade tubing, and procedural syringes currently used in practice
- The injector must be able to integrate with standard 1 mL syringes, securely locking each syringe in place during the intramyocardial injection procedure
- Non-infectious porcine derived mesenchymal stem cells (MSCs) will be used for cell viability testing (less than 5% cell death relative to post-thawing cell viability) and procedural injections
- The injector will consist of two different injection rates (30 and 60 seconds) that deliver 0.5 mL of stem cell solution per injection
- Less than 20% error for force application less than 1.00 N and less than 15% error for force values greater than or equal to 1.00 N

- Less than 5% volume lost due to dead space in the needle
- Injection automation should begin and end with a single start/stop button click and be electrically controlled
- Visual feedback must be provided to the operator when catheter back-up is detected (force threshold value)
- The project's budget is \$3000
- The injector should cost no more than \$500 to manufacture

Design requirements:

1. Physical and Operational Characteristics

a. Performance requirements:

The automatic injector device must be electronically controlled and capable of injecting MSCs into the myocardium. The stem cell injection flow rate must be adjustable, consisting of a 30 second rate and a 60 second rate capable of introducing the required 0.5 mL stem cell solution into the myocardium [1]. The 30 second rate correlates to an injection of 16.7 $\mu\text{L}/\text{sec}$ while the 60 second rate represents a delivery of 8.33 $\mu\text{L}/\text{sec}$ when transmitting 0.5 mL of solution. These two dispensary rates will be produced in 1 mL standard syringes integrated within the injector. In standardized 1 mL syringes, a 30 second flow rate corresponds to the syringe plunger traveling 1 mm/sec while it travels 0.5 mm/sec for a 60 second flow rate. The flow rate must be controlled within the device and capable of repeatability throughout the procedure. Syringes 1 mL in volume must be securely locked into the device, receiving the force provided by the injector without exhibiting displacement. The syringes must also be easily replaceable within the device, allowing for rapid reloading of stem cell loaded syringes. The syringes within the device will connect to medical grade tubing that is integrated with the procedural catheter, allowing the catheter to move without hindrance or obstruction. During a typical intramyocardial injection procedure, the injector will perform 10 - 14 injections, each consisting of 30 second or 60 second injection rates followed by a maximum 60 second dwell time [1]. Any blockage or backflow within the syringe or catheter must be monitored. Force values above a threshold of 2.40 N, as read from the syringe plunger, must cause the device to visually alert the user that the catheter or syringe is clogged with solution or exhibiting aliquot back flow [2]. A red LED light will illuminate in order to demonstrate that the 2.40 N threshold has been reached or exceeded and the catheter may be backed up [2].

b. Safety:

The International Organization for Standardization (ISO) describes medical device risks that must be considered while the device of interest is undergoing its intended use in standard 14971. All risk factors must be mitigated in order to decrease the potential for accidental injury or injection caused by the device [3]. ISO 60601 states that any electric components within the medical device should present no

potential risks to either the healthcare professional or the patient [4]. The device must be fabricated in a manner that effectively covers all wired components and is free of any sharp items in order to ensure the well-being of the user and patient. All electrical components within the device will be properly labeled. Each item in contact with the MSCs can not present biological hazards or chemical altering materials that may change the biological components of the individual stem cells per ISO 10993 [5]. This also requires that the materials in contact with the stem cells are mechanically stable, capable of enduring any applied force from the injector without breaking, preventing potential contact with the MSCs. Each component within the device must be able to perform each injection without exhibiting deformation. Instructions for the device will be provided and labels will be printed on the device to prevent any confusion and mitigate risk of endangerment to the user. These labels and instructions will also ensure the device is not damaged by misuse. Liability will not be charged if damage is a result of misuse. The injector can not be toxic, carcinogenic, and harmful upon touch.

c. Accuracy and Reliability:

Provided the contents within the syringe are of identical fluid properties to each other, all recorded injection rates must be within a 2% difference from the average to prove precision of the automated system and its hardware components. A 2% difference allows for miniscule errors when considering dead space in the catheter and any used tubing. Dispensary times for each rate (30 seconds or 60 seconds) must be within 0.5 seconds of the target goal to ensure the device accurately dispenses the given quantity of cells at the desired rate. This error margin will account for inaccurate syringe loading. The volume of fluid dispensed from the syringe after the completion of either injection rate (30 seconds or 60 seconds) must be within 5% of the 0.5 mL volume of fluid inserted in the syringe. The force sensor (includes electronics for monitoring) used to detect the syringe's injection force must provide readings that are within 20% of the actual applied force for forces greater less than 1.00 N and within a 15% error margin for applied forces greater than or equal to 1.00 N. All forces read from the device will come from a standardized curve generated via testing and will consist of the average of five different trials, each following the same applied force sequence. The aforementioned accuracy requirements must be met for each injection performed throughout the device's typical life cycle (see *Life in Service* below).

The MSC viability will be measured following thawing of the frozen cells and before use in the injection device. Typically, MSC viability is ~94.2% after thawing [7]. To ensure successful treatment, the cell viability of MSCs following each injection can not be more than 5% lower than the viability found after thawing (~94.2%).

d. Life in Service:

The injector must be operable and maintain accurate injection rates for at least three years, aligning with current injection devices and pumps [6]. Any stepper DC/AC motors applied to the dispensing system will allow accurate and precise injection rates

until after 10,000 hours of operative use. As a result, the injector will be able to perform 1.2 million injections when using the 30 second rate and 600,000 injections using the 60 second rate before motor replacement or repair is required [8]. If the device uses an exterior power supply, such as an outlet, it will be capable of functioning all day, performing procedures whenever required. The device must be small enough to allow for easy transport to different locations within the hospital or different buildings without need for mechanical assistance (see the *Size* and *Weight* sections below). If the injection device is powered by a rechargeable lithium-ion battery, it must be able to perform automated injections for at least 20 consecutive minutes, as this is the maximum amount of time required per procedure, correlating to 14 stem cell injections at 60 seconds each and a maximum 60 second dwell time in between each injection. See the *Shelf Life* section below for more details about the lifespan of rechargeable lithium-ion batteries. There are no restrictions on the power supply for the injector, so it can use a battery or exterior power supply, such as an outlet or computer, during procedures.

e. *Shelf Life:*

When the injector is not in use, it will be kept in an airtight sterile bag and stored within the clinical operating room used for intramyocardial stem cell injection procedures or in a storage room with environmental conditions that can be considered the same as the operating room. As a result, while in storage, the device will experience typical clinical operating room conditions; room temperature (20 °C to 25 °C), low and stable humidity (30% - 50% relative humidity) [9], and average atmospheric pressure (101.35 kPa) [10]. The sterile bag that the injector will be sealed in during storage is sterilized with ethylene oxide, so all components of the injector (including electronics) must consist of materials that can withstand ethylene oxide [10]. In this storage environment and within the sterile bag, the entire injector should be able to maintain functionality and efficacy for at least three years, consistent with current injection devices [6]. As a result, all components must be minimally affected by corrosion and should not experience any deterioration during the injector's typical life cycle. Rechargeable lithium-ion batteries have a shelf-life of about five years (~2000 charge cycles) [11], so these batteries should not require replacement during the typical timetable of the injector if they are used within the design. The circuitry components, such as the wires, within the injector have a shelf-life of 50 - 70 years [20]. There should not be deterioration or loss of functionality concern with any component of the injector that is in storage throughout its typical usage cycle.

f. *Operating Environment:*

The injector device will be used for intramyocardial stem cell injections that are performed in typical clinical operating rooms. As a result, the injector base, its automatic and electrical components, and the feedback system will be exposed to room temperature (20 °C to 25 °C) and low and stable humidity (30% - 50% relative humidity) [9]. The average atmospheric pressure that the injector will experience is 101.35 kPa [10]. As the injector will rest on a table, a patient bed, or

within a clinician's hand, it will be exposed to the dust particles and dirt contained on these surfaces and within the air.

Shock loading, vibration, and noise level are considered negligible. As the device is used for clinical injections, medical professionals will be the primary handlers of the injector. When not in use, the device will be kept within a sterile bag and either left in the clinical operating room or stored in a storage unit within the same or a similar room. As a result, the aforementioned operating conditions apply to the injector during storage and idle time.

g. Ergonomics:

The injector is used in conjunction with a syringe, medical grade tubing, and an intravenous catheter to inject MSCs through the syringe-catheter system at a controlled and adjustable rate. The injector should not be used for any other purpose or in conjunction with other devices. It should be properly connected to the syringe (see *Performance Requirements* section above) and positioned for the procedure following the insertion of the catheter into the target tissue and the connection of the injecting syringe to the medical grade tubing and catheter. The device should be kept within a sterile bag when not in use and throughout each intramyocardial stem cell injection procedure.

The injector base should not experience a stress value greater than 49.5 MPa [14] and the injector should be stopped by the operator when it applies a force greater than 2.40 N (indicated by a visual feedback signal) to the syringe [2]. The electrical components within the injector should not be modified or altered unless errors result during injector operation. Outside of blood, the injector should not be exposed to liquids. The injector should not be dropped from a height greater than one meter [15] and sharps should be treated with care when they are used in conjunction with the device.

h. Size:

The device should not take up more space than $45 \times 20 \times 20$ cm in order to fit within the desired operating table or drape location. A typical cardiac operating room ranges from 121.92 - 198.12 m² and contains large equipment such as anesthesia machines, a stretcher, monitors, sterilization equipment, and a surgical table [16]. The device will potentially rest on the cathtable where there is space for it or be positioned on drapes in the patient's leg region. The device should also be easily transportable, especially since the device may need to be repositioned during a procedure. Additionally, all components of the device should be easily accessible for maintenance and sterilization.

i. *Weight:*

The device should be lightweight, ideally less than 3.00 kg, in order to make it easily transportable and comparable to other devices that are currently on the market such as the Baxter device (2.81 kg) [17]. A proper weight will ensure that the user does not incur a hand cramp if holding the device during the 10 - 14 cycles of 30 - 60 second injections.

j. *Materials:*

The force sensor based visual feedback system consists of an FSR 400 Series Round Force Sensing Resistor [18], an Arduino Uno microcontroller [19], Arduino IDE [20], an LED Serial 7-Segment Digital Display [21], and standard circuitry wires, resistors, an LED, and an adhesive breadboard [22]. The injector base, stepper motor storage compartment, support block (includes support rods), force application block, and syringe clamp were fabricated with Ultimaker PLA via 3D printing [23]. The automated injection is controlled by a NEMA-17 stepper motor [24], an Arduino Uno microcontroller [19], Arduino IDE [20], a fully threaded zinc coated aluminum bolt [25], and three tactile buttons [26]. A nylon hex nut [27] is embedded within the Ultimaker PLA force application block to allow for the threaded rod to control its movement and thus syringe plunger compression. Standard 1 mL clinical syringes, medical grade tubing, and therapeutic catheters are compatible with the Cellringe Pump, but as they are not part of the fabrication process and the actual injector design, their material composition will not be incorporated in the discussion.

k. *Aesthetics, Appearance, and Finish:*

The device should be able to produce visual feedback that is obvious and easy to interpret. It will notify the user that the threshold syringe pushing force has been reached and that there may be a clog within the catheter that will result in product damage. The operator should understand that they must slow the injection or stop the injection in order to ensure maximum cell viability, which can not decrease more than 5% from the starting viability. Other aesthetics associated with the device are not important.

2. Production Characteristics

a. *Quantity:*

One unit that is compatible with 1 mL syringes is needed.

b. *Target Product Cost:*

The device should be manufactured at a cost of no more than \$500 [28]. Although there are not any automated injector devices used for the delivery of MSCs and thus there is

not a direct price comparison that can be adequately made, there are similar devices that can be evaluated. For example, Baxter's Infus OR Syringe Pumps are used by anesthesiologists to deliver anesthesia from large syringes at a specified controlled rate. These products tend to sell for ~\$3000 - \$4000 [29]. See the *Competition* Section below for more information on the cost of similar devices.

3. Miscellaneous

a. *Standards and Specifications:*

The Code of Federal Regulations (CFR) Title 21 Part 820 provides quality system regulations, including the requirements related to the methods used in designing, manufacturing, packaging, labeling, storing, installing, and servicing of medical devices intended for human use [30]. As the injector will be used in conjunction with syringes and catheters that interact directly with patients, it should follow and meet the quality guidelines outlined in the CFR. This will allow good standard operating procedures to be followed in relation to the fabrication and commercialization of the product. The CFR Title 21 Part 870 identifies a Percutaneous Transluminal Coronary Angioplasty Catheter used for the treatment of acute myocardial infarction as a Class II medical device and specifies the procedure required for this class [31]. As the injector will be used for treating heart failure induced by myocardial infarction and provides an intermittent risk to patients (controls MSCs injection into the myocardium), the injector can also be categorized as a Class II medical device and thus needs to adhere to the procedures outlined by this CFR.

The CFR Title 21 Part 3.2 categorizes the injector device as a combination product, due to its interaction with a medical device (syringe) and biological product (MSCs) in order to achieve its intended therapeutic effect. This standard provides the procedure for identifying the designated agency component and preparing it for premarket review and regulation [32]. If the injector device is pursued as a combination product rather than focusing on its design individually, this CFR will have to be followed in order to allow proper agency designation and to meet premarket review requirements. The CFR Title 21 Part 610 provides the performance, testing, and safety requirements, labeling standards, and sterility expectations for biological products, such as MSCs [33]. As the injector will interact with a syringe that contains MSCs, it is important to have familiarity with the MSCs themselves and the performance and safety requirements they must adhere to.

b. *Customer:*

The customers that would be using this product are cardiac surgeons, specifically, Dr. Amish Raval (the client). This product will be used during Dr. Raval's intramyocardial

stem cell injection clinical trials and then during clinical procedures when stem cell injection therapy is approved for clinical use. Surgeons would like to be able to have less manual labor and thus fatigue (hand-cramping or freezing up) when performing these procedures and the injector devices. As a result, having a simple device that can be programmed to inject the cells at a certain injection rate over a specified amount of time with a single click of a button (turns the device on and off) would be very beneficial for the surgeons. This product should provide a precise way to inject the cells over a steady rate and stop at certain force differentials so that surgery proceeds successfully.

c. Patient-related concerns:

The device needs to be sterile as it will be in the operating room and on the table next to the patient or on a drape in the patient's leg region. Due to the fact that this automated injection system will be used on sterile products that are going into the human body, the product must be sterilized during and in between surgeries as the device will be non-disposable. The device supports the injection of MSCs into the heart so the patient has a high risk of infection or reaction to the cells. As a result, it is important to consider the proper sterilization approaches prior to surgery.

d. Competition:

There are currently products on the market that involve some similar concepts to the automated stem cell injector, but no device has all of the components that will be included in the final design for this device.

One type of device that is a competitor to the injector is an automated syringe pump. These systems are used by anesthesiologists to inject a controlled volume of anesthesia into the patient over time. The Baxter Infus OR Syringe Pump ABC 4100, is an infusion pump device that is compatible with 1, 3, 5, 10, 20, 30, 60 and 140 mL syringe sizes. To operate the device, the syringe is front loaded and locked into place. The anesthesiologist would then enter a flow rate and the type of drug being used, clicking start to initiate the injection. The Baxter device is able to sense syringe plunger force and movement and has a system to detect and monitor delivery accuracy as well as alarms with audio and LED lights. See Figure 1 below for this Baxter Syringe Pump along with an additional Baxter Syringe Pump.



Figure 1: The “Baxter Infus OR Syringe Pump ABC 4100” which can be obtained for ~\$3000 using the trade-in program (left image) [20]. The “Baxter Infus O.R. Syringe Pump Refurbished” that is sold for ~\$4000 (right image) [29].

Another competing device is the apparatus used to inject IV fluids into patients. This device is able to transfer fluids from a bag directly into the person through connection tubing at a programmed rate. There is also another similar device called the Contract Delivery System from ACIST Medical Group that is used for angiographic procedures to deliver an iopamidol injection. This device has a controlled flow rate system, is compatible with catheters, and has been used in procedures involving cardiology and radiology. This product can be seen in Figure 2 below.

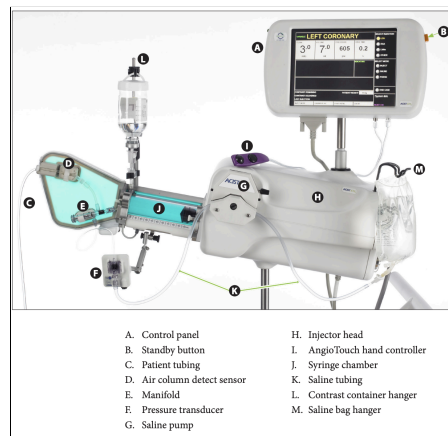


Figure 2: Diagram of the important user components in the Acist Contrast Delivery System a [34].

Lastly, there was a device in preclinical trials in 2017 that was fabricated to deliver cells. This product was called an “automated injection device for intradermal delivery of a cell-based therapy”. The current status of the product is unknown and the device is not currently patented or on the market. The device delivers a solution directly into a patient via its hypodermic needle. Therefore, this product is not compatible with catheters and would not apply the proper force required to inject cells through a catheter into the heart. The device can be seen in Figure 3 below.

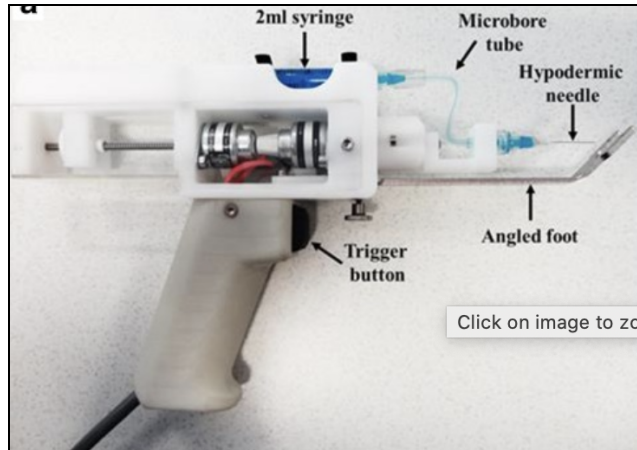


Figure 3: Automated injector device that was in preclinical development in 2017 [35].

Although these infusion pump systems and injection devices have many positives when it comes to injecting a solution into a patient, they have not been proven to work well with stem cell delivery and in conjunction with the catheter system that goes into the myocardium. These things are important because MSC viability must be maintained for the intramyocardial stem cell injection procedures to be successful and the MSC aliquot must be able to transport through the catheter and into the heart. Additionally, the devices on the market do not contain force detection systems that provide accurate algorithms for determining the pressure needed for injecting cells into the myocardium.

The JP2019069165A patent (see Figure 4 below) involves a system which has an automatic injector device that uses cassettes to hold the injectate. This is an automatic device that could be engaged by the push of a button via its superior end. However, this apparatus is not extremely similar to the cardiac repair injector device that will be fabricated since it does not have syringe or catheter compatibility, it does not have a controlled injection rate, and does not have a pressure-detecting system that can trigger visual feedback.

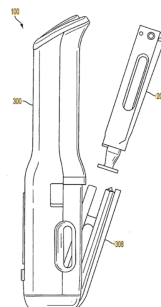


Figure 4: The cassette automatic injector device [36].

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Section 2: Feedback System Circuitry and Code

Section 2.1: Voltage Divider Circuit Calculation and Simulation

Voltage Divider Circuit:

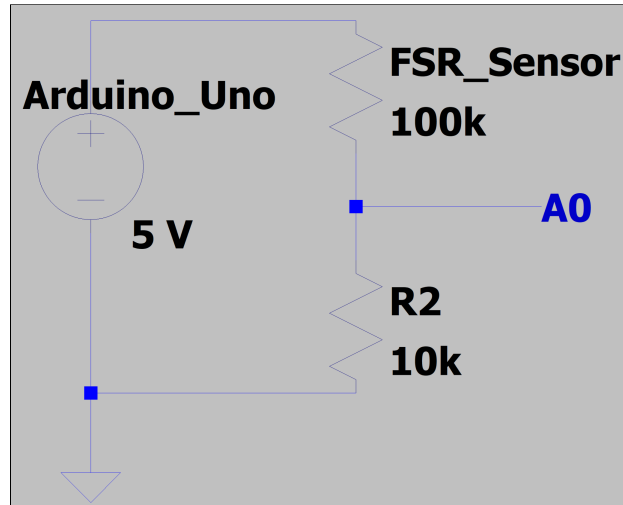


Figure 1: Voltage Divider Circuit containing the FSR sensor, a 5 V DC voltage source, and an output going into A0 of the Arduino Uno microcontroller.

Calculation:

Based on the circuit from Figure 1 above, the voltage divider equation (see equation 1 below [1]) was used to calculate the voltage output from the voltage divider topology using an FSR resistance value of 100 k Ω and 3 k Ω , corresponding to the FSR resistance at 0.14 N of applied force and at 2.4 N of force, respectively [2].

$$[1] V_{out} = (R_2 / (R_1 + R_2)) * V_{in}$$

$$R_1 = \text{FSR Sensor (100 k}\Omega \text{ or 3 k}\Omega)$$

$$R_2 = 10 \text{ k}\Omega$$

$$V_{in} = 5 \text{ V}$$

$$R_1 = 100 \text{ k}\Omega$$

$$V_{in} * R_2 = 5 * 10.0 = 50 \text{ V} * \text{k}\Omega$$

$$V_{out} = 50 / (10 + 100) = 0.45459 \text{ V} \approx 0.45 \text{ V}$$

$$R_1 = 3 \text{ k}\Omega$$

$$V_{in} * R_2 = 5 * 10.0 = 50 \text{ V} * \text{k}\Omega$$

$$V_{out} = 50 / (10 + 3) = 3.846 \text{ V} \approx 3.85 \text{ V}$$

LTspice Simulation:

To confirm these calculations, an LTSpice Simulation (Figures 2 and 3 below) was run utilizing the same exact voltage divider circuit from Figure 1 above. The simulation was performed using the FSR sensor value of 100 k Ω and 3 k Ω .

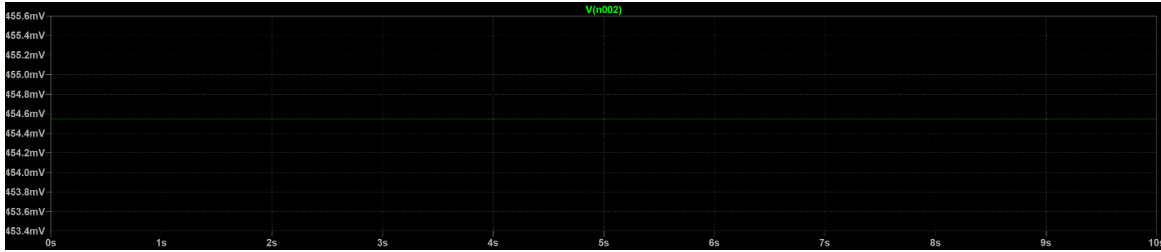


Figure 2: LTspice simulation of the V_{out} from a voltage divider circuit containing a $100\text{ k}\Omega$ resistor for R_1 and a $10\text{ k}\Omega$ resistor for R_2 . The voltage output is about 0.454 V which can be estimated as 0.45 V .

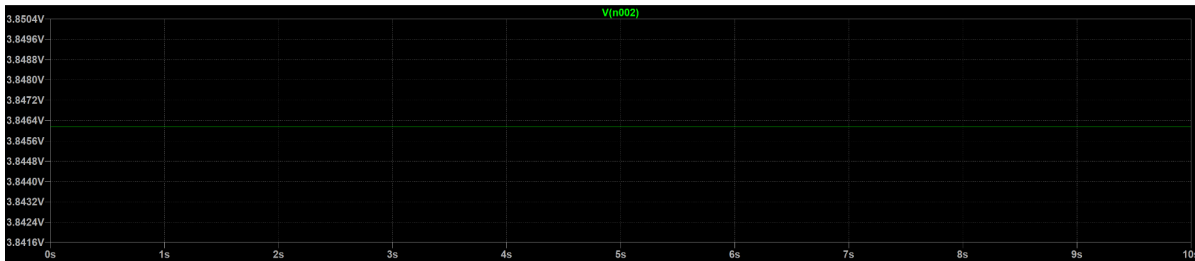


Figure 3: LTspice simulation of the V_{out} from a voltage divider circuit containing a $3\text{ k}\Omega$ resistor for R_1 and a $10\text{ k}\Omega$ resistor for R_2 . The voltage output is about 3.846 V which can be estimated as 3.85 V .

As the LTSpice simulations display a 0.45 V and 3.85 V voltage output when the FSR sensor has a resistance of $100\text{ k}\Omega$ and $3\text{ k}\Omega$, respectively, it supports the $0.45\text{ V} - 3.85\text{ V}$ voltage divider output range predicted by the calculations, providing confidence that these voltage values are correct. As these FSR resistance values correlate to the minimum anticipated force that will be applied to the FSR sensor throughout each injection and the threshold value, the voltage divider circuit should provide voltage inputs to the Arduino Uno microcontroller that are well within the 4.88 mV resolution of the Arduino microcontroller [3] throughout each injection. As a result, the microcontroller will be able to accurately detect all voltage inputs from the circuit, allowing for accurate force values and visual feedback signals (LEDs) to be presented to the operator, promoting the efficacy of each intramyocardial MSC injection procedure.

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Section 2.2: Feedback System Code

```
/*  
  
Circuit:  
Arduino ----- Serial 7-Segment  
5V ----- VCC  
GND ----- GND  
8 ----- SS  
11 ----- SDI  
13 ----- SCK  
*/  
  
#include <SPI.h> // Include the Arduino SPI library  
  
// Define the SS pin  
// This is the only pin we can move around to any available  
// digital pin.  
const int ssPin = 8;  
char tempString[10]; // Will be used with sprintf to create strings  
  
int ledRed = 10; // pin that Orange LED is attached to  
int analogPin = A0; // pin that the sensor is attached to  
float force = 0; // initializing the force variable  
double power_series = 2.17; //power series exponent for the FSR curve  
  
void setup()  
{  
  
  Serial.begin(9600); // initialize serial communication at 9600 bits per second:  
  
  // ----- SPI initialization  
  pinMode(ssPin, OUTPUT); // Set the SS pin as an output  
  digitalWrite(ssPin, HIGH); // Set the SS pin HIGH  
  SPI.begin(); // Begin SPI hardware  
  SPI.setClockDivider(SPI_CLOCK_DIV64); // Slow down SPI clock  
  // -----  
  
  // Clear the display, and then turn on all segments and decimals  
  clearDisplaySPI(); // Clears display, resets cursor  
  
  // Custom function to send four bytes via SPI  
  // The SPI.transfer function only allows sending of a single  
  // byte at a time.  
  s7sSendStringSPI("-HI-");  
}
```

```

setDecimalsSPI(0b111111); // Turn on all decimals, colon, apos

// Flash brightness values at the beginning
setBrightnessSPI(0); // Lowest brightness
delay(1500);
setBrightnessSPI(255); // High brightness
delay(1500);

// Clear the display before jumping into loop
clearDisplaySPI();
}

void loop()
{

// read the input on analog pin 0:
int sensorValue = analogRead(A0);
float voltage = sensorValue * (5.0/1023.0); // initialize a variable that solves for the
voltage value that corresponds to the read ADC value from pin A0
Serial.print("FSR Voltage Output = "); // Prints "FSR Voltage Output = " to the serial
monitor
Serial.println(voltage); // Prints the voltage value read from the A0 pin to the serial
monitor

if (voltage <= 3.05) {
force = (0.0707 * pow(voltage, 5)) - (0.00348 * pow(voltage, 6)) - (0.419 * pow(voltage,
4)) + (1.10 * pow(voltage, 3)) - (1.33 * pow(voltage,2)) + (0.795 * voltage) - 0.000185; //
will use the flipped 6th-degree polynomial curve when equal to or below a voltage value
of 3.05 V
}

else {
force = (0.19 * pow(voltage, 4)) - (1.5 * pow(voltage, 3)) + (3.89 * pow(voltage,2)) -
(2.97 * voltage) - 0.0000245; // will use a 4th-degree polynomial curve for voltage
values above 3.05 V
}

Serial.print("Force = "); // Prints "Force = " to the serial monitor

Serial.println(force); // Displays the force value on a monitor

if (force < 2.4) {
digitalWrite(ledRed, LOW); // should keep the light off if the force is below 2.4 N
}

else { // should be 2.4 and up

```

```

digitalWrite(ledRed, HIGH);
Serial.println("Stop"); // turns light on and prints stop on serial monitor when above 2.4
N
}

```

```

// Delay the code for 10 millisecond to allow for the Vout reading to be picked up by the
user

```

```

// Magical sprintf creates a string for us to send to the s7s.
// The %4d option creates a 4-digit integer.

```

```

//int forceInt = force * 100;
String forceString = String(force, 4);

```

```

sprintf(tempString, "%4d", force);

```

```

// This will output the tempString to the S7S
s7sSendStringSPI(forceString);

```

```

// Print the decimal at the proper spot
if (force < 10000)
    setDecimalsSPI(0b00000010); // Sets digit 3 decimal on
else
    setDecimalsSPI(0b00000100);

```

```

delay(1000); // This will make the display update at 100Hz.*/
}

```

```

// This custom function works somewhat like a serial.print.
// You can send it an array of chars (string) and it'll print
// the first 4 characters in the array.

```

```

void s7sSendStringSPI(String toSend)

```

```

{
    digitalWrite(ssPin, LOW);
    for (int i=0; i<4; i++)
    {
        SPI.transfer(toSend[i]);
    }
    digitalWrite(ssPin, HIGH);
}

```

```

// Send the clear display command (0x76)
// This will clear the display and reset the cursor

```

```

void clearDisplaySPI()
{
    digitalWrite(ssPin, LOW);
}

```



```

SPI.transfer(0x76); // Clear display command
digitalWrite(ssPin, HIGH);
}

// Set the displays brightness. Should receive byte with the value
// to set the brightness to
// dimmest----->brightest
// 0-----127-----255
void setBrightnessSPI(byte value)
{
  digitalWrite(ssPin, LOW);
  SPI.transfer(0x7A); // Set brightness command byte
  SPI.transfer(value); // brightness data byte
  digitalWrite(ssPin, HIGH);
}

// Turn on any, none, or all of the decimals.
// The six lowest bits in the decimals parameter sets a decimal
// (or colon, or apostrophe) on or off. A 1 indicates on, 0 off.
// [MSB] (X)(X)(Apos)(Colon)(Digit 4)(Digit 3)(Digit2)(Digit1)
void setDecimalsSPI(byte decimals)
{
  digitalWrite(ssPin, LOW);
  SPI.transfer(0x77);
  SPI.transfer(decimals);
  digitalWrite(ssPin, HIGH);
}

```

Section 3: Material Information and Calculations

Section 3.1: FSR 400 Series Round Force Sensing Resistor Technical Data Sheet

Interlink Electronics FSR 400 series is part of the single zone Force Sensing Resistor family. Force Sensing Resistors (FSRs) are robust polymer thick film (PTF) devices that exhibit a decrease in resistance with increase in force applied to the surface of the sensor. This force sensitivity is optimized for use in human touch control of electronic devices such as automotive electronics, medical systems, and in industrial and robotics applications. The standard 402 sensor is a round sensor 18.28 mm in diameter. Custom sensors can be manufactured in sizes ranging from 5 mm to over 600 mm. Female connector and short tail versions can also be ordered.

Table 1: FSR 400 Series Sensor Characteristics [1].

Feature	Condition	Value*	Notes
Actuation Force		0.1 Newtons	
Force Sensitivity Range		0.1 - 10.0 ² Newtons	
Force Repeatability³	(Single part)	± 2%	
Force Resolution³		continuous	
Force Repeatability³	(Part to Part)	±6%	
Non-Actuated Resistance		10M W	
Size		18.28mm diameter	
Thickness Range		0.2 - 1.25 mm	
Stand-Off Resistance		>10M ohms	Unloaded, unbent
Switch Travel	(Typical)	0.05 mm	Depends on design
Hysteresis³		+10%	$(R_{F+} - R_{F-})/R_{F+}$.
Device Rise Time		<3 microseconds	measured w/steel ball
Long Term Drift		<5% per log ₁₀ (time)	35 days test, 1kg load
Temp Operating Range	(Recommended)	-30 - +70 °C	
Number of Actuations	(Life time)	10 Million tested	Without failure

*Specifications are derived from measurements taken at 1000 grams, and are given as one standard deviation / mean, unless otherwise noted.

1. Max Actuation force can be modified in custom sensors
2. Force Range can be increased in custom sensors. Interlink Electronics have designed and manufactured sensors with operating force larger than 50 kg

3. Force sensitivity depends on mechanics, and resolution depends on measurement electronics.

References:

[1] I. Electronics, “FSR 400 Data Sheet Figure 1 -Typical Force Curve Industry Segments Interlink Electronics -Sensor Technologies FSR 400 Series Round Force Sensing Resistor,” 2021. Accessed: Feb. 15, 2022. [Online]. Available: <https://cdn.sparkfun.com/datasheets/Sensors/ForceFlex/2010-10-26-DataSheet-FSR400-Layout2.pdf>.

Section 3.2: Arduino Uno Rev 3 Technical Data Sheet

Arduino Uno is a microcontroller board based on the ATmega328P. It has 14 digital input/output pins (six can be used as PWM outputs), six analog inputs, a 16 MHz ceramic resonator (CSTCE16M0V53-R0), a USB connection, a power jack, an ICSP header and a reset button.

Table 1: Arduino Uno Rev 3 Technical Specifications [1].

MICROCONTROLLER	ATmega328P
OPERATING VOLTAGE	5V
INPUT VOLTAGE (RECOMMENDED)	7-12V
INPUT VOLTAGE (LIMIT)	6-20V
DIGITAL I/O PINS	14 (of which 6 provide PWM output)
PWM DIGITAL I/O PINS	6
ANALOG INPUT PINS	6
DC CURRENT PER I/O PIN	20 mA
DC CURRENT FOR 3.3V PIN	50 mA
FLASH MEMORY	32 KB (ATmega328P) of which 0.5 KB used by bootloader
SRAM	2 KB (ATmega328P)
EEPROM	1 KB (ATmega328P)
CLOCK SPEED	16 MHz
LED_BUILTIN	13

References:

- [1] A. Staff, "Arduino Uno Rev3," Arduino.cc, 2019.
<https://store.arduino.cc/usa/arduino-uno-rev3> (accessed Feb. 25, 2021).

Section 3.3: LED Serial 7-Segment Digital Display Technical Data Sheet

The Serial 7-Segment Display is an easy-to-use 4-digit display that is controlled using a serial interface and only requires a pin to control the LEDs. Using either a serial, I²C, or SPI interface, all digits, decimal points, the colon, and the apostrophe can be controlled.

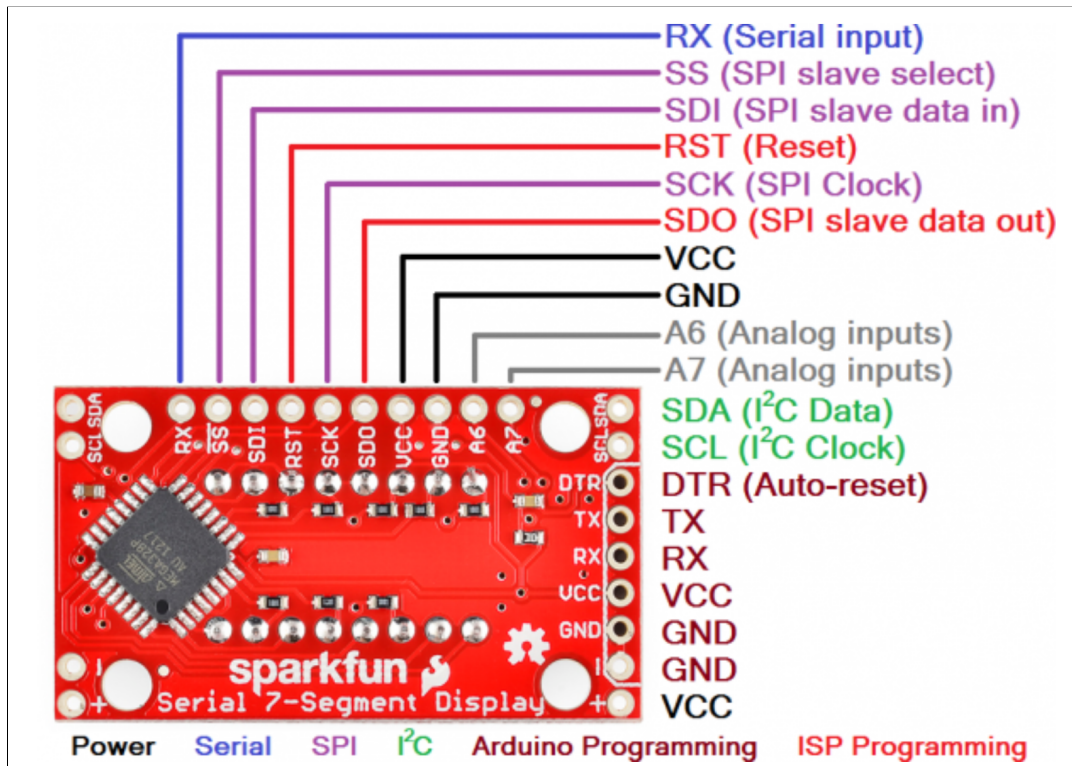


Figure 1: Pin-out schematic for the LED Serial 7-Segment Digital Display [1].

References:

- [1] J. Blom, "Using the Serial 7-Segment Display," learn.sparkfun.com, 2022.
<https://learn.sparkfun.com/tutorials/using-the-serial-7-segment-display/introduction>

Section 3.4: Ultimaker PLA Technical Data Sheet

PLA for Ultimaker (FFF)

\$0.08 / g

Ultimaker PLA filament provides a no-hassle 3D printing experience thanks to its reliability and good surface quality. The PLA is made from organic and renewable sources. It's safe, easy to print with, and it serves a wide range of applications for both novice and advanced users.

Key Features: Good ultimate strength and surface quality, easy to work with at high print speeds, user-friendly for both home and office environments, and PLA allows the creation of high-resolution parts.

Table 1: Material and Mechanical Properties of Ultimaker PLA [1].

Mechanical properties*	Injection molding		3D printing	
	Typical value	Test method	Typical value	Test method
Tensile modulus	-	-	2,346.5 MPa	ISO 527 (1 mm/min)
Tensile stress at yield	-	-	49.5 MPa	ISO 527 (50 mm/min)
Tensile stress at break	-	-	45.6 MPa	ISO 527 (50 mm/min)
Elongation at yield	-	-	3.3%	ISO 527 (50 mm/min)
Elongation at break	-	-	5.2%	ISO 527 (50 mm/min)
Flexural strength	-	-	103 MPa	ISO 178
Flexural modulus	-	-	3,150 MPa	ISO 178
Izod impact strength, notched (at 23 °C)	-	-	5.1 kJ/m ²	ISO 180
Charpy impact strength (at 23 °C)	-	-	-	
Hardness	-	-	83 (Shore D)	Durometer

* Properties reported here are average of a typical batch. The 3D printed test specimens were printed in the XY plane, using the normal quality profile in Ultimaker Cura 2.1, an Ultimaker 2+, a 0.4 mm nozzle, 90% infill, 210 °C nozzle temperature, and 60 °C. The values are the average of five white and five black specimens for the tensile, flexural, and impact tests. The Shore hardness D was measured in a 7-mm-thick square printed using the normal quality profile in Ultimaker Cura 2.5, an Ultimaker 3, a 0.4 mm print core, and 100% infill. The electrical properties were measured on a 54-mm-diameter disk with 3 mm thickness printed in the XY plane, using the fine quality profile (0.1 mm layer height) in Ultimaker Cura 3.2.1, an Ultimaker 3, a 0.4 mm print core, and 100% infill. Ultimaker is constantly working on extending the TDS data.

References:

[1] Ultimaker, "Technical Data Sheet - PLA," Nov. 2018. Accessed: Dec. 02, 2021. [Online]. Available: <https://ultimaker.com/materials/pla>.

Section 3.5: NEMA-17 Stepper Motor Technical Data Sheet

A stepper motor to satisfy all your robotics needs! This 4-wire bipolar stepper has 1.8° per step for smooth motion and a nice holding torque. The motor was specified to have a max current of 350 mA so that it could be driven easily with an Adafruit motor shield for Arduino (or other motor driver) and a wall adapter or lead-acid battery. Some nice details include a ready-to-go cable and a machined drive shaft.

Table 1: NEMA-17 Stepper Motor Technical Specifications [1].

Item	Specifications
Step Angle	1.8°
Step Angle Accuracy	±5% (full step, no load)
Resistance Accuracy	±10%
Inductance Accuracy	±20%
Temperatru Rise	80°CMax. (rated current,2 phase on)
Ambient Temperatuar	-20°C~+50°C
Insulation Resistance	100M?Min.,500VDC
Dielectric Strength	500VAC/ for one minute
Shaft Radial Play	0.02Max. (450 g-load)
Shaft Axial Play	0.08Max. (450 g-load)
Max. radial force	28N (20mm foom the flange)
Max.axial force	10N

References:

- [1] A. Industries, “Stepper Motor - NEMA-17 Size - 200 steps/rev, 12V 350mA,” www.adafruit.com, 2022.
https://www.adafruit.com/product/324?gclid=Cj0KCCQiAjc2QBhDgARIsAMc3SqQi_8rpHenYK-iXt9KMn2Y-33zOxCpePZHou_C26oTArivpTp67CgQaArgPEALw_wcB (accessed Feb. 21, 2022).

Section 3.6: Grainger Zinc Coated Aluminum Fully Threaded Bolt Technical Data Sheet

Zinc coated aluminum fully threaded rods and studs are nonmagnetic and corrosion resistant with a clear finish. They have male threads running their entire length for full engagement of female-threaded nuts. Weighing less than steel, they are commonly used when a lightweight fastener is needed, such as with electronics or aircraft parts.

Table 1: Grainger Zinc Coated Aluminum Fully Threaded Bolt Technical Specifications [1].

Item	Fully Threaded Rod	Thread Direction	Right Hand
System of Measurement	Inch	Threaded Rod Thread Type	UNC
Threaded Rod Material	Aluminum	Min. Tensile Strength	42,000 psi
Thread Size	1/4"-20	Rockwell Hardness	Not Rated
Length	3 ft	Thread Class	1A

References:

- [1] W. W. Grainger, Inc, "Fully Threaded Rod, Aluminum, 1/4"-20, 3 Ft Length," Grainger, 2022.
<https://www.grainger.com/product/GRAINGER-APPROVED-Fully-Threaded-Rod-10P782>

Section 3.7: Grainger Nylon Hex Nut Technical Data Sheet

The nylon hex nut has a temperature range from -40° to 85 °C. These lightweight, strong nylon nuts are for use with nylon machine screws in applications that require vibration, abrasion, and corrosion resistance. They also provide electrical insulation and meet IFI standards. These hex nuts are free-spinning, non-locking, and have right-hand thread direction for use with nylon male threaded parts.

Table 1: Grainger Nylon Hex Nut Technical Specifications [1].

Item	Hex Nut	Fastener Thread Direction	Right Hand
Nut Style	Machine Screw Nut	Dia./Thread Size	1/4"-20
System of Measurement	Inch	Fastener Thread Type	UNC (Coarse)
Basic Material	Nylon	Width Across Flats	27/64 in
Fastener Finish	Black Nylon coat	Nut Height	15/64 in

References:

- [1] W. W. Grainger, "Hex Nut, Machine Screw Nut, Nylon, Not Graded, Black Nylon coat, 1/4"-20 Dia./Thread Size," Grainger, 2022.
<https://www.grainger.com/product/GRAINGER-APPROVED-Hex-Nut-4AGF9> (accessed Feb. 26, 2022).

Section 3.8: Maximum Stress Calculations

Using Equation 1 below, the maximum applied stress for the force application block in the location where the block comes into contact with the syringe plunger was calculated using the maximum expected applied force during injection (3.00 N) and the smallest plunger cross-sectional contact area ($6.362 \times 10^{-5} \text{ m}^2$) [1].

$$[1] \sigma = F / A$$

Force Application Block:

$$\text{Cross-Sectional Area} = \pi * (0.0045 \text{ m})^2 = 6.362 \times 10^{-5} \text{ m}^2$$

$$\text{Maximum Force} = 3.00 \text{ N}$$

$$\text{Stress} = 3.00 \text{ N} / 6.362 \times 10^{-5} \text{ m}^2 = 47157.02 \text{ Pa} = 0.047 \text{ MPa}$$

$$\text{Cross-Sectional Area} = \pi * (0.0045 \text{ m})^2 = 6.362 \times 10^{-5} \text{ m}^2$$

$$\text{Worst Case Force} = 10.00 \text{ N}$$

$$\text{Stress} = 10.00 \text{ N} / 6.362 \times 10^{-5} \text{ m}^2 = 157190.07 \text{ Pa} = 0.16 \text{ MPa}$$

References:

[1] F. P. Beer, E. R. Johnston Jr., J. T. DeWolf, and D. F. Mazurek, Mechanics of Materials, 8th Edition. McGraw Hill Education, 2020.

Section 3.9: Materials List and Budget Table

Table 1: Itemized list of components used throughout fabrication and their purchasing information.

Item	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	Link
Component 1								
Hex Nut	¼" - 20 Nylon Black	Grainger	4AGF9	03/15	1	3.20	3.20	
Component 2								
Threaded Rod	Nylon 6/6 ¼" - 20 x 2 ft	Grainger	2KA63	03/15	1	14.06	14.06	
Component 3								
Stepper Motor	NEMA-17 size - 200 steps/rev, 12 V 350 mA	adafruit	324	03/15	1	14.00	14.00	
Component 4								
Aluminum Flex Shaft Coupler	5 mm to 5 mm	adafruit	1175	03/15	1	4.95	4.95	
Component 5								
Potentiometer	Panel Mount 10k	adafruit	562	03/15	1	0.95	0.95	
Component 6								
SN754410 Quad Half H-Bridge 1A Motor Driver IC	H-Bridge Microcontroller for NEMA-17 Stepper Motor	Banana Robotics	BR010086	03/15	2	1.75	3.50	
Component 7								
¼ in x 36 in Zinc Plated Threaded Rod	Threaded rod allows for motion of the nylon nut to move in a horizontal direction	UW Makerspace	6717	03/23	1	2.00	2.00	
Component 8								
Dasani Water Bottle	Force Application Device for FSR Calibration Curve Testing	Dasani Water	N/A	03/26	1	1.50	1.50	
Component 9								
Adafruit Motor/Stepper/Servo Shield for Arduino v2 Kit - v2.3	Intermediate connection between Arduino Uno and NEMA-17 Stepper Motor	adafruit	1438	03/29	1	19.95	19.95	
Component 10								
5 mm to ¼ inch Aluminum Alloy Coupling Set Screw	Allows for connection between the ¼ inch threaded rod and NEMA-17 Stepper Motor	uxcell	L25XD14	04/01	1	6.49	6.49	
Component 11								
Ultimaker PLA 3D printed Syringe clamp	Syringe clamp mold that tightly secures and locks the procedural 1 mL syringes in place,	Ultimaker and UW Makerspace	N/A	04/05	1	0.64	0.64	

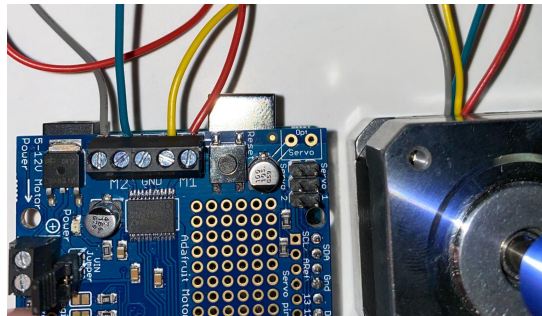
	preventing any displacement throughout each procedure								
Component 12									
Ultimaker PLA 3D printed Motor Holder, Cylinder Rods and Force Application Block	Motor holder houses the NEMA-17 Stepper Motor, Cylinder Rods prevent rotation and enable horizontal motion of the Force Application Block, which pushes against the syringe	Ultimaker and UW Makerspace	N/A	04/08	1		20.41	20.41	
Component 13									
Ultimaker PLA 3D printed Syringe Base and Force Application Block	The Syringe Base will hold all other components as well as house all circuitry underneath the base. The updated Force Application Block will better hold the nylon nut and push against the syringe	Ultimaker and UW Makerspace	N/A	04/13	1		54.56	54.56	
Component 14									
12V 2A Power Supply Adapter	12V supply to the Arduino will better power the motor and create enough torque to supply the MSC's through the catheter	SANSUN	FBA-12000 01P	04/18	1		7.99	7.99	
Component 15									
Ultimaker PLA 3D printed Syringe Rods	Reprinted syringe rods	Ultimaker and UW Makerspace	N/A	04/22	1		9.44	9.44	
TOTAL:									\$163.64

Section 4: Fabrication Protocols

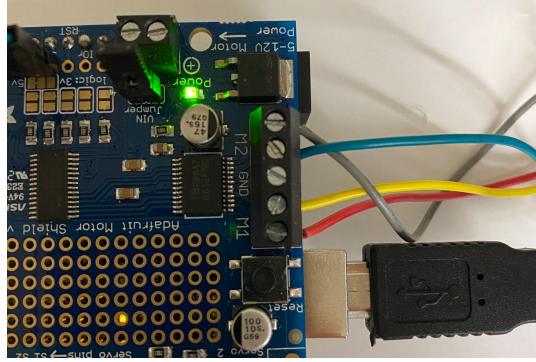
Section 4.1: NEMA-17 Stepper Motor Circuit and General Fabrication

The NEMA-17 Stepper Motor is the main component that controls the injection rates within the device. The Stepper Motor and subsequent software coding will allow the injections to be programmed at 30 and 60 seconds, along with other features controlled by the buttons. The following protocol provides step-by-step instructions for how to assemble the Stepper Motor and incorporate it into the rest of the device.

1. Obtain a NEMA-17 Stepper Motor, breadboard, Arduino Uno Microcontroller, adafruit Motor Shield v2.3, 5 mm to 6.35 mm (¼ inch) coupler, 3.50 cm long 6.35 mm (¼ inch) threaded rod, 3 breadboard compatible buttons, 3 10k Ohm resistors, 15 connecting wires, 5/64 inch hexagonal screwdriver and small flathead screwdriver.
2. Connect the Stepper Motor with the Arduino Uno and Motor Shield
 - a. Connect the adafruit Motor Shield with the Arduino Uno Microcontroller by soldering the connecting pins onto the Motor Shield and placing the pins in the compatible compartments within the Arduino.
 - b. Connect the NEMA-17 Stepper Motor to the adafruit Motor Shield v2.3 by placing the wires in the M1 and M2 compartments with orientation as shown.



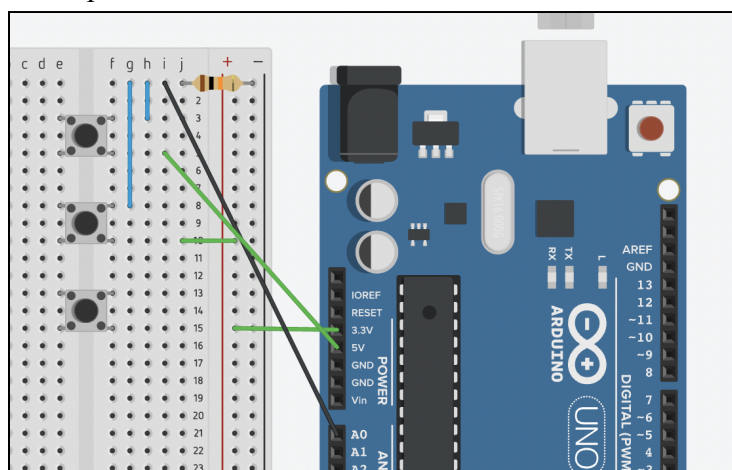
- c. Ensure the power supply from the Arduino is being transferred to the Stepper Motor by completing the jumper circuit with the jumper attachment provided with the Stepper Motor. This will be evident if the Motor Shield LED lights up upon providing the Arduino with power.



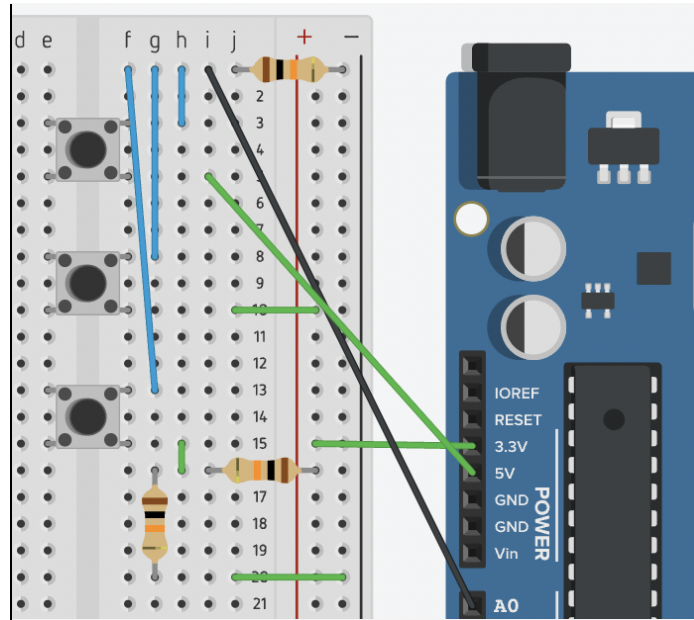
3. Assemble circuitry to control the Stepper Motor with 3 buttons
 - a. Each button will be used to either start or stop the motor by providing different voltage values to the Arduino Uno. Start by soldering 2 wires to each button, ensuring the wires are either diagonal or next to each other. Connect the buttons to the breadboard.



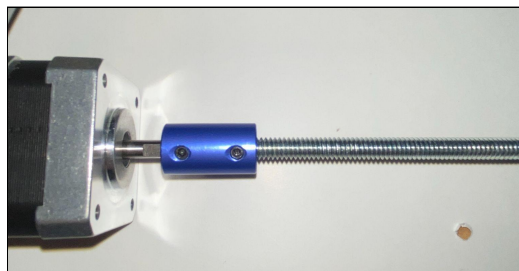
- b. Using power from the Arduino, connect 5V to button 1 and 3.3V to the positive rail. From the positive rail, connect 3.3V to button 2. Connect the adjacent legs of the buttons to ground (using a 10k Ohm resistor) and the A0 pin of the Arduino.



- c. Using power from the 3.3V of the Arduino, create a voltage divider circuit so that 1.65V of power is applied to button 3 (use 2 10k Ohm resistors). Connect the adjacent leg of the button to ground and A0 as well.



- d. Ensure the buttons and Arduino work by reading the ADC values off the serial monitor of the power being supplied to the A0 pin. When pressing each button, button 1 should read as 1023, button 2 should read as 512/513, and button 3 should read as 256 in the serial monitor.
4. Connect the threaded rod to the Stepper Motor
 - a. Place the 5 mm hole on the coupler onto the Stepper Motor cylinder. Use a small screwdriver to secure the coupler in place.
 - b. Place the 6.35 mm diameter threaded rod into the 6.35 mm side of the coupler that is attached to the Stepper Motor. Use the same screwdriver to secure the threaded rod in place. Ensure the rod is as vertical as possible.



5. Implement code into the Arduino software that allows each button to control different movements of the Stepper Motor (speed, step count, direction, etc.).

Arduino IDE Code

```
#include <Adafruit_MotorShield.h>
```

```
// Create the motor shield object with the default I2C address
```

```

Adafruit_MotorShield AFMS = Adafruit_MotorShield();
// Or, create it with a different I2C address (say for stacking)
// Adafruit_MotorShield AFMS = Adafruit_MotorShield(0x61);

// Connect a stepper motor with 200 steps per revolution (1.8 degree)
// to motor port #2 (M3 and M4)
Adafruit_StepperMotor *myMotor = AFMS.getStepper(200, 1);

void setup() {
  // put your setup code here, to run once:
  Serial.begin(9600);
  AFMS.begin();
}

void loop() {
  // put your main code here, to run repeatedly:
  int Repos = 1;
  int sensorValue = analogRead(A0);
  Serial.println(sensorValue);
  if (sensorValue > 1000) {
    myMotor->setSpeed(120); //SPEED FOR 30 SECONDS
    for (int i = 0; i <= 4656; i++) { //STEP COUNT FOR MM TRAVELING
      sensorValue = analogRead(A0);
      Serial.println(sensorValue);
      myMotor->step(1, BACKWARD, SINGLE);
      while (sensorValue > 1000) {
        delay(1);
        sensorValue = analogRead(A0);
      }
      if (sensorValue < 300 && sensorValue > 200) {
        delay(2000);
        myMotor->setSpeed(200);
        myMotor->step(i, FORWARD, SINGLE);
        Repos = 0;
        break;
      }
    }
  }
  if (Repos == 1) {
    delay(2000);
    myMotor->setSpeed(200);
    myMotor->step(4656, FORWARD, SINGLE); //STEP COUNT FOR MM RESET
  }
}

```



```

} else if (sensorValue < 800 && sensorValue > 500) {
myMotor->setSpeed(34); //STEP SPEED FOR 60 SECONDS
for (int i = 0; i <= 4656; i++) { //STEP COUNT FOR MM TRAVEL
sensorValue = analogRead(A0);
Serial.println(sensorValue);
myMotor->step(1, BACKWARD, SINGLE);
while (sensorValue < 800 && sensorValue > 500) {
delay(1);
sensorValue = analogRead(A0);
}
if (sensorValue < 300 && sensorValue > 200) {
delay(2000);
myMotor->setSpeed(200);
myMotor->step(i, FORWARD, SINGLE);
Repos = 0;
break;
}

}
if (Repos == 1) {
delay(2000);
myMotor->setSpeed(200);
myMotor->step(4656, FORWARD, SINGLE); //STEP COUNT FOR MM RESET
}

} else if (sensorValue < 300 && sensorValue > 200) {
myMotor->setSpeed(15);
for (int i = 0; i <= 4656; i++) { //STEP COUNT FOR MM TRAVEL
sensorValue = analogRead(A0);
Serial.println(sensorValue);
//myMotor->step(1, BACKWARD, SINGLE);
myMotor->step(1, FORWARD, SINGLE);

if (sensorValue < 300 && sensorValue > 200) {
break;
}

}

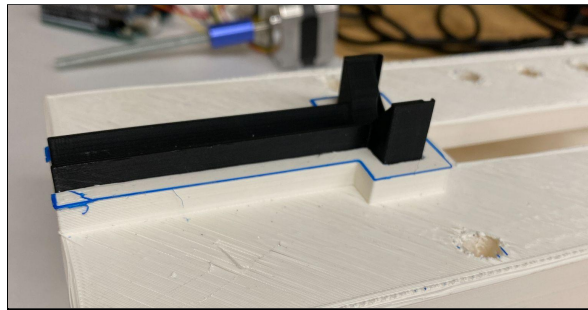
}
}
}

```

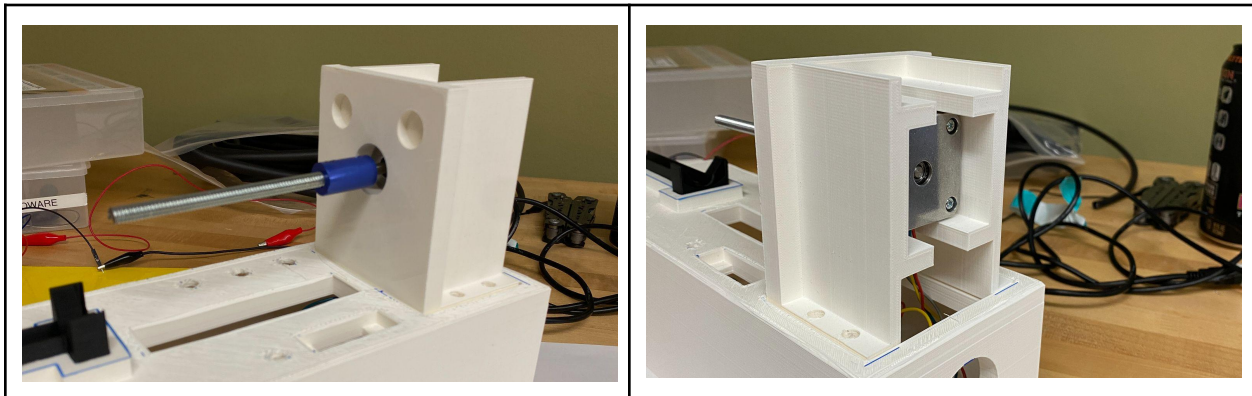
Section 4.2: Cellringe Pump Fabrication

The final Cellringe Pump prototype was assembled by following this protocol in order to assemble all components into one functioning device. The following protocol provides step-by-step instructions for how to assemble the feedback system, FSR sensor, force application threaded system, the Stepper Motor, all 3D printed parts, and circuitry components into the Cellringe Pump.

1. Obtain the FSR Feedback System Circuit, NEMA-17 Stepper Motor Circuit, Cellringe Base, Motor Holder, Cylinder Rods, Force Application Block, 1 mL Syringe Mold, 3 nylon nuts (6.35 mm), and 2 bolts (6.35 mm).
2. Place the 1 mL Syringe Holder into the syringe holder compartment

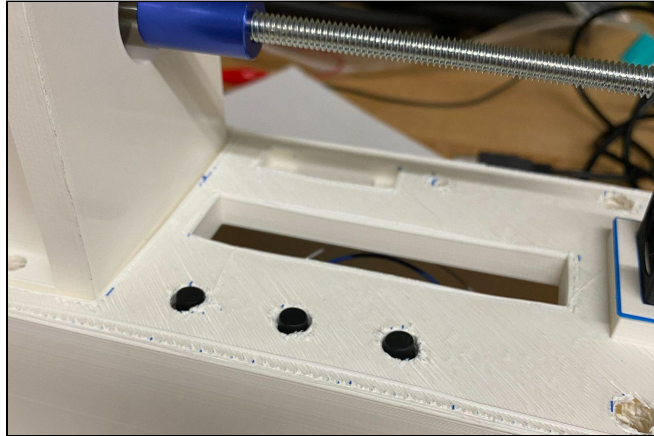


3. Assemble NEMA-17 Stepper Motor Circuit into Cellringe Base
 - a. With the circuitry system for both the Feedback System and Stepper Motor Circuit placed underneath the base, feed the motor through the large square hole on the top of the base.
 - b. Place the motor into the Motor Holder. The Motor Holder can now be placed onto the cut out in the base.

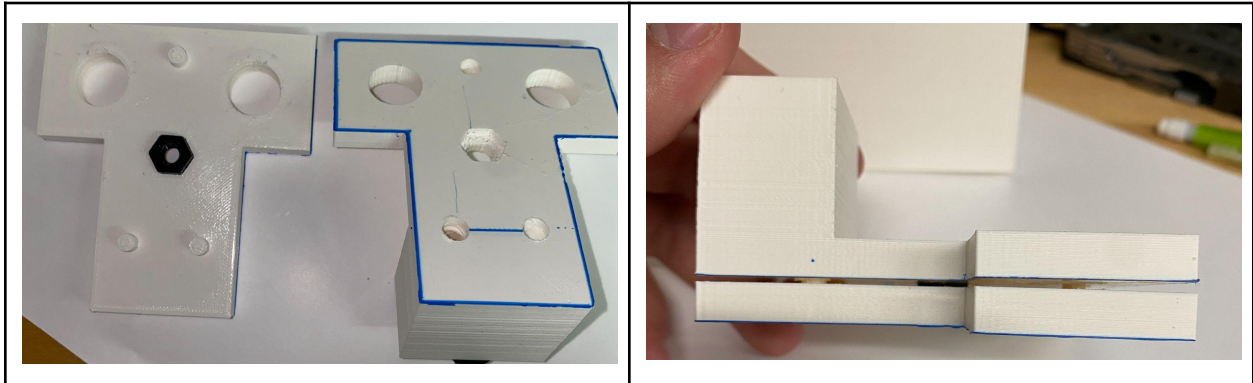


- c. Push the soldered buttons into the correct holes within the top of the base, with the 30 second button closest to the Motor Holder, 60 second button in

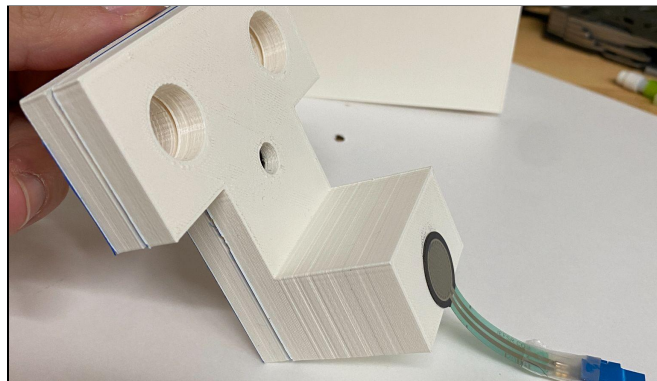
the middle, and the stop/reset button closest to the Cylinder Rod attachment.



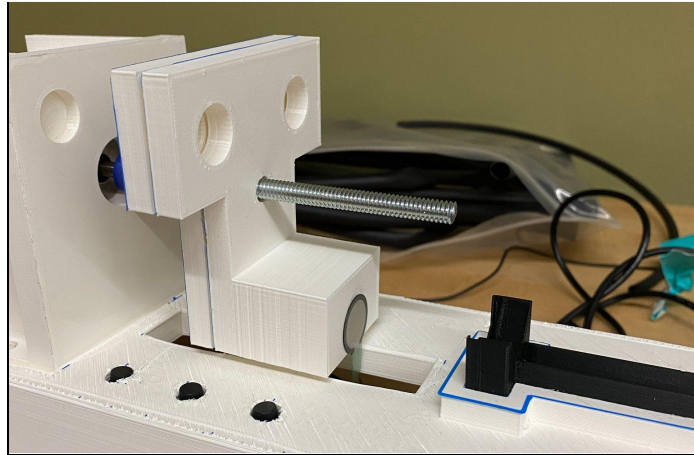
4. Attach the Force Application Block
 - a. Place the 6.35 mm nylon nut into the cut out in one piece of the Force Application Block. Take the other conjoining piece and press them together, holding the nylon nut in place in the center of the 2 Force Application block pieces.



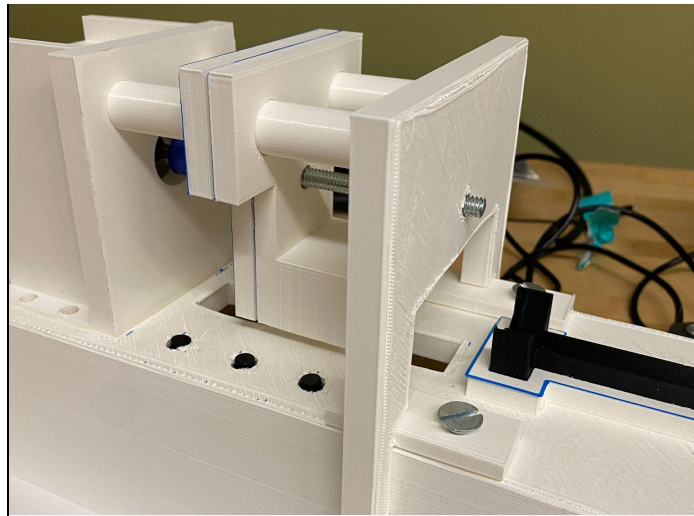
- b. Detach the FSR from the FSR circuit. Use the adhesive on the back side of the FSR to apply the FSR onto the face of the longer side of the FSR.



- c. Spin the Force Application block onto the 6.35 mm threaded rod until it is positioned over the rectangular hole in the center of the base. Reattach the FSR to the FSR circuit.



- d. Place the cylinder rods through the 2 holes in the Force Application Block. Further push the cylinder rods into the shallow holes in the Motor Holder. Use 2 of the 6.35 nylon nuts and bolts to secure the cylinder rods into the base.



5. Integrate the Feedback System's LED threshold light and LED digital display.
 - a. Bring the wires for the LED digital display from underneath the base up through the small rectangular hole designated for the LED digital display.
 - b. Attach the wires in the correct pins of the LED digital display.
 - c. Apply an adhesive to hold the LED digital display in place



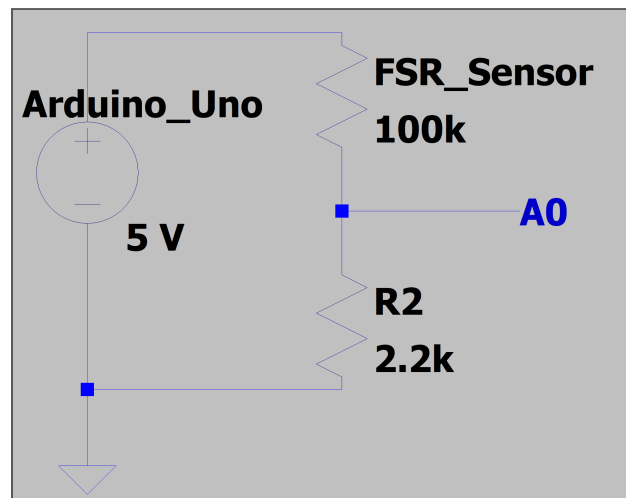
- d. Repeat steps a-b with the LED threshold light and corresponding wires.
- e. Set the LED threshold light firmly in place in the designated hole.



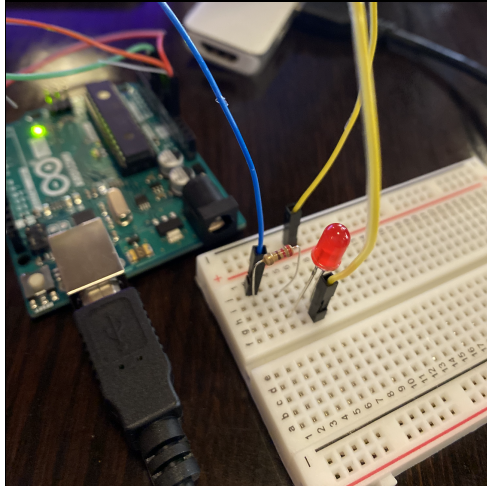
Section 4.3: FSR Feedback System Circuit and General Fabrication

There are two components to the feedback system. The first is the red LED light that will turn on once the force is above the 2.4 N of force that identifies potential catheter obstruction and the second is the display scoreboard that will show the surgeon how much force they are applying. The following protocol provides step-by-step instructions for how to assemble the Feedback System and incorporate it into the rest of the device.

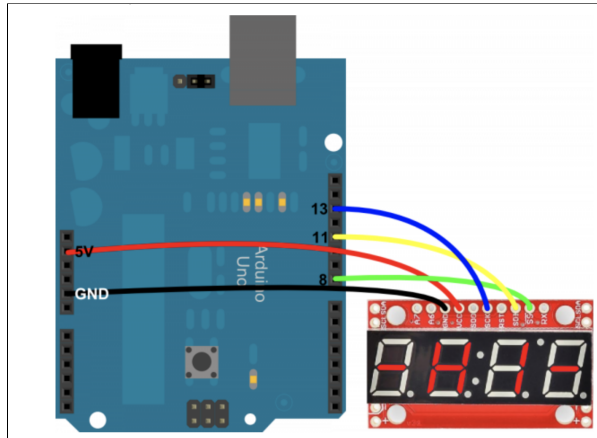
1. Obtain a breadboard, display scoreboard, Force Sensor, 200 Ohm resistor, 10kOhm resistor, red LED light, Arduino microcontroller, power supply for the Arduino and many wires.
2. Assemble the voltage divider circuit with the FSR using a 10 kOhm resistor.
 - a. The schematic shown below depicts the wiring that should be set up to obtain the voltage divider with the exception that the 2.2 kOhm resistor in the figure should be a 10 kOhm resistor.



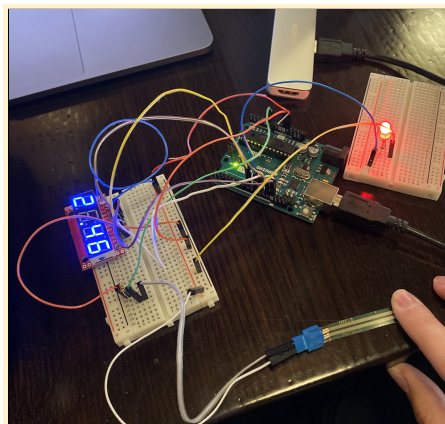
- b. Connect the 5V power from the Arduino to one side of the FSR and the other side of the FSR to the 10 kOhm resistor with room for a wire to go in between and connect to A0 of the Arduino to provide a Vout.
 - c. Connect the other leg of the 10 kOhm resistor to the ground which can be supplied by the Arduino as well.
3. Add the LED component of the circuit
 - a. Connect a red LED to a digital pin (digital pin 10 will work for the code below) where there will be a write out for whether the light should be on or off by giving it a HIGH output or LOW output
 - b. Use a 200 Ohm resistor before the red LED to prevent the LED from burning out.
 - c. Ground the other side of the red LED
 - d. The LED part of the circuit will look like the image below where the yellow wire is connected to the ground and the blue wire is connected to pin 10 of the Arduino



4. Add the Display component of the circuit
 - a. Connect the display to the breadboard and add the pins in the proper location as seen in the image below:



5. Make sure your breadboard looks as follows and begin the “FSR Feedback System Testing”



Section 5: SolidWorks Drawings

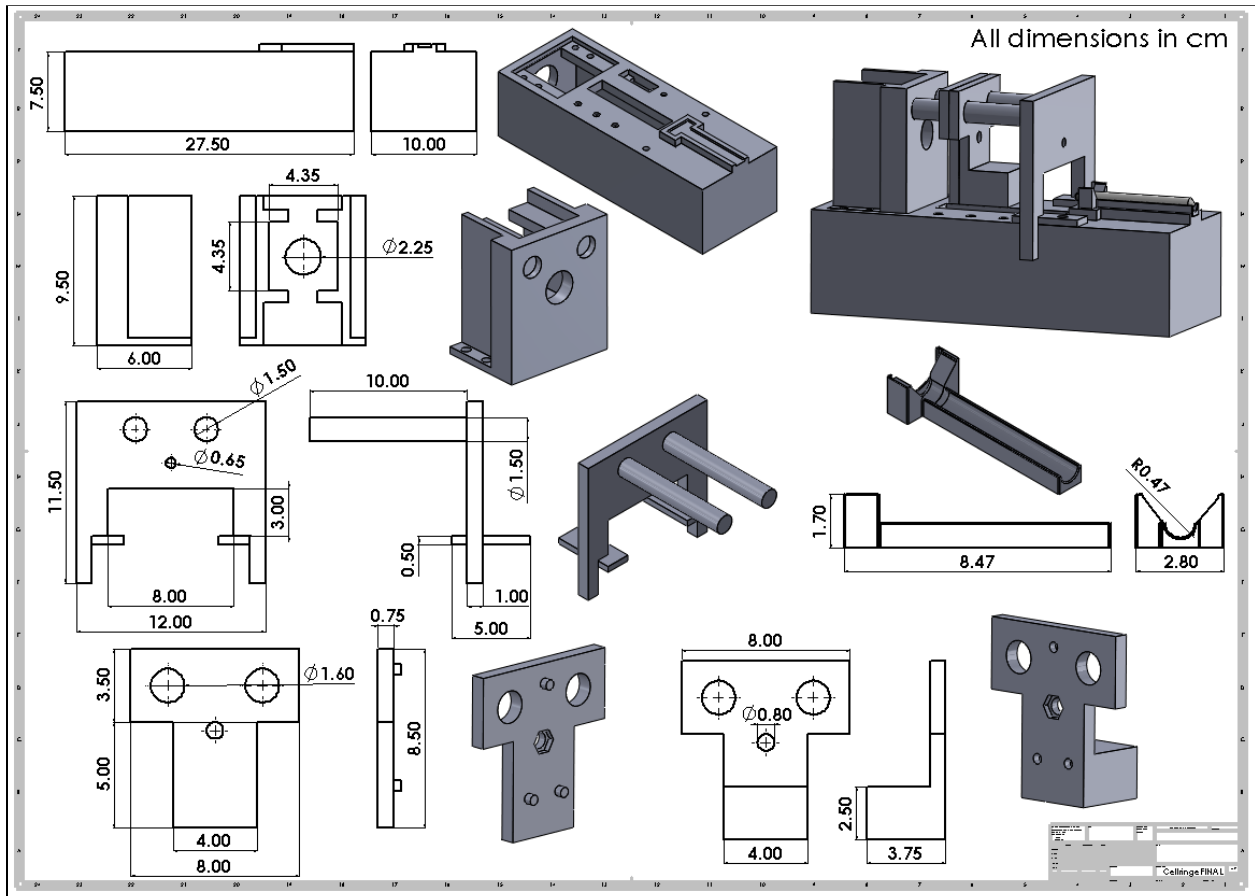


Figure 1: SolidWorks designs of individual components. Each piece is displayed and dimensioned, with the full assembly reference seen in the upper right corner. Individual pieces starting from the top to bottom: Cellringe Base, Motor Holder, Cylinder Rods (left), 1 mL Syringe Holder (right), Force Application Block pt. 1 (bottom left), and Force Application Block pt. 2 (bottom right).

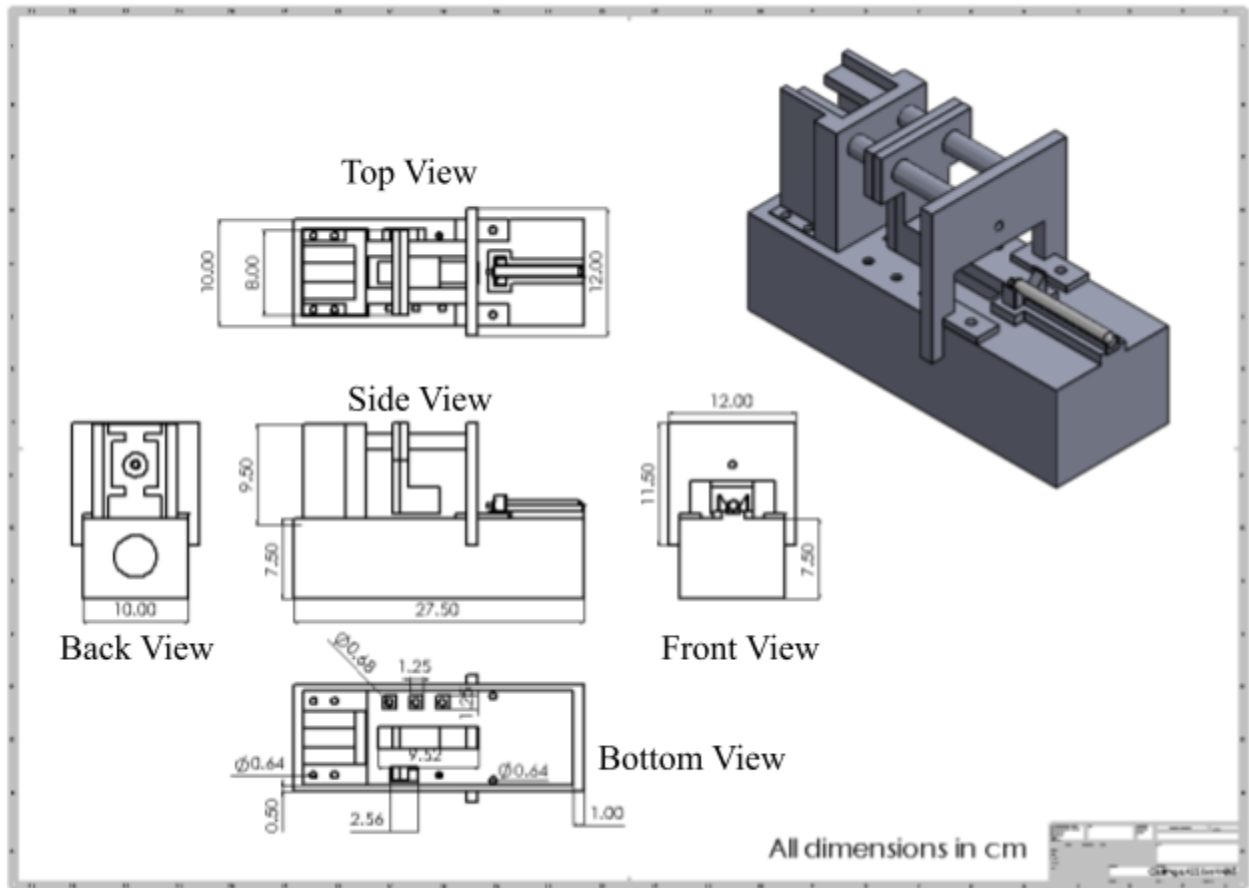


Figure 2: SolidWorks dimensions and reference drawings of the entire assembly.

Section 6: Testing Protocols

Section 6.1: FSR Calibration Curve Testing Protocol

To provide an equation to accurately and reliably establish the injector force applied to the syringe plunger throughout each injection, a calibration curve was created based on the FSR feedback system testing force and voltage data. Using a line of best fit that takes into account noise, an equation was identified that correlates voltage (V) to weight (N) and thus allows the force to be determined based on each voltage input to the Arduino Uno microcontroller. The purpose of the calibration curve is primarily to derive the linear trendline which can then be used to calculate the unknown applied force during the injection. The team will use the linear trendline from the FSR calibration curve to calculate the force being applied to the sensor by measuring the output voltage. The independent variable (x) in the line of best fit equation represents the applied force and the dependent variable (y) describes the output voltage.

1. Prior to testing, a digital caliper battery case (includes battery inside), 500 mL water bottle, scale (in grams), and various sized micropipettes and graduated cylinders will need to be obtained.
2. Open the “FSR_Feedback_System_Vout” within Arduino IDE on the computer that will be used for testing.
3. After the feedback system is set-up, attach the Arduino Uno microcontroller to the testing computer via the Arduino Uno microcontroller USB cord.
4. Upload the Arduino IDE code and open the serial monitor of the Arduino IDE program. Keep the serial monitor open for the entirety of the testing.
 - a. Verify that no error messages pop-up when the upload button was selected in the software to run the code
 - b. Verify that the serial monitor is displaying voltage and that it is 0.0 V, to confirm that the code is running properly
5. Place the digital caliper battery case (including battery) on top of the active zone of the FSR sensor with the rounded, smallest diameter end of the battery in contact with the FSR sensor active zone.
 - a. **Ensure that the battery is only in contact with the active zone of the FSR sensor throughout the testing. If the battery moves and is only partially in contact with the active zone of the FSR sensor, the results will be inaccurate**
6. Weigh an empty 500 mL water bottle on the scale (keep the cap on the water bottle during this measurement) . Record this value so it can be referenced later when determining the proper water amounts that need to be added to the water bottle in order to apply a specified force to the FSR sensor.
7. Choose a force value to test within the 0.14 N - 4.00 N force range that corresponds to the typical injector force application range.

8. Remove the cap off of the water bottle and add the required amount of water to it based on the selected force application value. Use micropipettes and graduated cylinders for this addition.
 - a. Use Newton's Second Law ($F = ma$) [1] to calculate the required weight to add to the water bottle. Keep units in mind when performing these calculations as the mass used for this equation is in kilograms rather than grams
 - i. 1 mL = 1 g
 - ii. 1 mL = 1000 μ L
 - iii. Take into account the weight of the water bottle when calculating the amount of water that needs to be added
9. Put the cap back on the water bottle and use the scale to measure the new weight of the bottle.
 - a. Verify that the weight of the water bottle correlates to the desired force and thus that the correct amount of water has been added to the water bottle
10. Ensure that the cap is tightly secured onto the bottle. Balance the water bottle on the battery case with the bottle cap in contact with the flat end (contains the largest surface area) of the digital caliper battery case.
 - a. Make sure the water bottle is only in contact with the digital caliper battery case
 - b. **Ensure that the battery is only in contact with the active zone of the FSR. If the battery is only partially in contact with the active zone of the FSR sensor, the force detection results will be inaccurate**
11. When the water bottle is properly balancing on the FSR sensor via the battery case without any support, view the voltage value output by the system as displayed on the Arduino IDE serial monitor.
12. Record the voltage value that is settled on or that is most consistently displayed (if the value is constantly shifting between multiple values) by the serial monitor. This may take a couple of seconds.
13. Repeat steps 7-12, three times each for each new force value applied.
 - a. Calculate the average voltage applied between the three trials for each weight used.
 - b. Calculate the standard deviation for each weight used as well.
14. Calculate the average applied pressure using Equation 1 below [1].

[1] Applied Pressure = $N/m^2 = \text{Total Applied Force} / \text{Area}$

- a. The area is equal to the area of the battery which was applying the pressure to the sensor, this area is calculated as seen in Equation 2 below.

[2] Area = $(6/1000)^2 * \pi = 113.10 \text{ mm}^2 = 1.13 * 10^{-4} \text{ m}^2$
--

15. Create a graph of the Total Force (N) vs Vout (V) and generate a line of best fit that will have the highest R^2 value, an equal distribution of residuals about the line of best fit, and a Gaussian distribution of residuals about 0.

16. This calibration curve line of best fit equation can then be rearranged and used to calculate the applied force based on the voltage value the Arduino is reading.

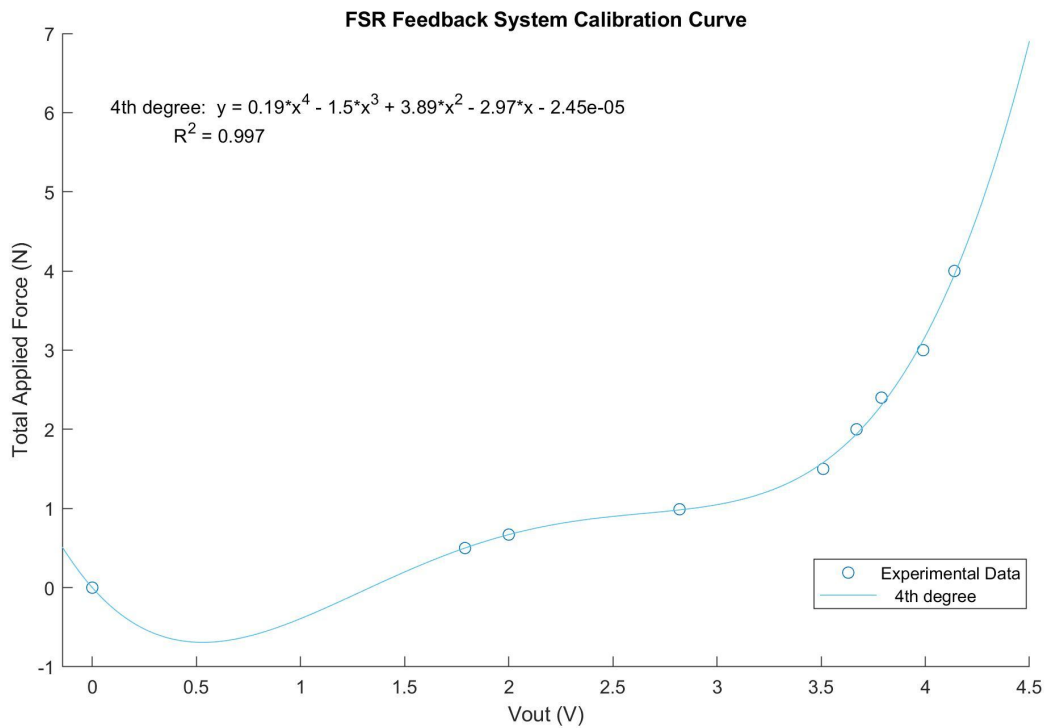


Figure 1: 4th Degree calibration curve FSR feedback system under various loading enabling applied force calculation.

References

[1] F. P. Beer, E. R. Johnston Jr., J. T. DeWolf, and D. F. Mazurek, Mechanics of Materials, 8th Edition. McGraw Hill Education, 2020.

Section 6.2: Feedback System Force Detection Accuracy Testing Protocol

The feedback system consists of a voltage divider circuit, FSR force sensor, Arduino Uno microcontroller, red LED, and an Arduino Uno compatible scoreboard. The LED and scoreboard are not used in this test (see the FSR Feedback System Testing protocol for their testing). The feedback system is fabricated as described in the FSR Feedback System Circuit Fabrication protocol. The following provides a step-by-step protocol to test the accuracy of the feedback system and if it is within the required error margin relative to the actual applied force to the FSR sensor (20% for values less than 1.00 N and 15% for values greater than or equal to 1.00 N). This testing protocol is used prior to incorporation of the FSR and feedback system into the injector base in order to confirm that the force detection system contains the required accuracy. Read through the protocol before performing the steps.

1. Prior to testing, a digital caliper battery case (includes battery inside), 500 mL water bottle, scale (in grams), and various sized micropipettes and graduated cylinders will need to be obtained.
2. Open the “Modified_FSR_Feedback_System_Force_Output” within Arduino IDE on the computer that will be used for testing.
3. After the feedback system is set-up, attach the Arduino Uno microcontroller to the testing computer via the Arduino Uno microcontroller USB cord.
4. Upload the Arduino IDE code and open the serial monitor of the Arduino IDE program. Keep the serial monitor open for the entirety of the testing.
 - a. Verify that no error messages pop-up when the upload button was selected in the software to run the code
 - b. Verify that the serial monitor is displaying voltage and force values and that they are 0.0 V and 0.0 N, respectively, to confirm that the code is running properly
5. Place the digital caliper battery case (including battery) on top of the active zone of the FSR sensor with the rounded, smallest diameter end of the battery in contact with the FSR sensor active zone.
 - a. **Ensure that the battery is only in contact with the active zone of the FSR sensor throughout the testing. If the battery moves and is only partially in contact with the active zone of the FSR sensor, the results will be inaccurate**
6. Weigh an empty 500 mL water bottle on the scale (keep the cap on the water bottle during this measurement) . Record this value so it can be referenced later when determining the proper water amounts that need to be added to the water bottle in order to apply a specified force to the FSR sensor.
7. Choose a force value to test within the 0.14 N - 4.00 N force range that corresponds to the typical injector force application range.
8. Remove the cap off of the water bottle and add the required amount of water to it based on the selected force application value. Use micropipettes and graduated cylinders for this addition.

- a. Use Newton's Second Law ($F = ma$) [1] to calculate the required weight to add to the water bottle. Keep units in mind when performing these calculations as the mass used for this equation is in kilograms rather than grams
 - i. 1 mL = 1 g
 - ii. 1 mL = 1000 μ L
 - iii. Take into account the weight of the water bottle when calculating the amount of water that needs to be added
 9. Put the cap back on the water bottle and use the scale to measure the new weight of the bottle.
 - a. Verify that the weight of the water bottle correlates to the desired force and thus that the correct amount of water has been added to the water bottle
 10. Ensure that the cap is tightly secured onto the bottle. Balance the water bottle on the battery case with the bottle cap in contact with the flat end (contains the largest surface area) of the digital caliper battery case.
 - a. Make sure the water bottle is only in contact with the digital caliper battery case
 - b. **Ensure that the battery is only in contact with the active zone of the FSR. If the battery is only partially in contact with the active zone of the FSR sensor, the force detection results will be inaccurate**
 11. When the water bottle is properly balancing on the FSR sensor via the battery case without any support, view the force and voltage value output by the system as displayed on the Arduino IDE serial monitor.
 12. Record the force and voltage value that is settled on or that is most consistently displayed (if the value is constantly shifting between multiple values) by the serial monitor. This may take a couple of seconds.
 - a. Verify that the force value output by the feedback system is within a 20% error margin of the actual applied force as calculated by the weight of the bottle for all applied forces below 1.00 N and an error margin of 15% for all forces greater than or equal to 1.00 N
 - i. Calculate the error margin using Equation 1 below, with the theoretical value corresponding to the calculated weight [2]
- [1] Percent Error = $(|V_{\text{theoretical}} - V_{\text{experimental}}| / V_{\text{theoretical}}) * 100$
13. Repeat steps 7 - 12 at least four more times, selecting a new force value each time this step sequence is repeated.
 - a. For each new force value, verify that the force value output by the feedback system is within a 20% error margin of the actual applied force as calculated by the weight of the bottle for all applied forces below 1.00 N and an error margin of 15% for all forces greater than or equal to 1.00 N
 - i. Calculate the error margin using Equation 1 below, with the theoretical value corresponding to the calculated weight [2]

$$[1] \text{ Percent Error} = (|V_{\text{theoretical}} - V_{\text{experimental}}| / V_{\text{theoretical}}) * 100$$

If any of the steps can not be completed as outlined above or the verification steps fail, reevaluate the design and determine how the feedback circuit can be modified in order to allow for the force detection accuracy tests to pass. If there is an error within the test protocol or a modification is agreed upon by the team, a deviation can be made within the protocol in order to allow a verification step to pass or a step to be completed.

References

- [1] Britannica, "Newton's Laws of Motion - Newton's Second law: $F = ma$," Encyclopedia Britannica, 2022.
<https://www.britannica.com/science/Newtons-laws-of-motion/Newtons-second-law-F-ma>
- [2] Dr. A. M. Helmenstine, "How to Calculate Percent Error," ThoughtCo, Nov. 02, 2020.
<https://www.thoughtco.com/how-to-calculate-percent-error-609584> (accessed Dec. 03, 2021).

Section 6.3: FSR Feedback System Testing Protocol

This testing procedure aims at establishing whether the feedback system and display are working properly. The following protocol includes a step-by-step procedure for how to conduct the testing. The tests include applying constant forces and recording what the values are to see if they compare to the serial monitor output as well as applying a force that exceeds that 2.4 N of force to see if the red light turns on.

1. Assemble the circuit which was outlined in the “FSR Feedback System Circuit and General Fabrication” protocol document
2. Follow a similar procedure as done with the “FSR Calibration Curve Testing Protocol” where you begin by obtaining a digital caliper battery case (includes battery inside), 500 mL water bottle, scale (in grams), and various sized micropipettes and graduated cylinders for testing and set the caliper battery case on top of the FSR while it lays on a flat surface.
3. Instead of looking for the accuracy for the force that is being applied to the FSR as is done in the “FSR Calibration Curve Testing Protocol”, the accuracy of the display in comparison to the serial monitor values will be compared
4. Slowly add water in increments of 50 - 200 mL at a time. The exact amount of water does not matter because all that needs to be confirmed is that the force values on the display should be similar to the force value on the serial monitor.
5. Record the value from the serial monitor corresponding to the force applied to the FSR by the water as well as record the value that is output on the scoreboard display over a period of five seconds where the delay time in the code is one second
6. Calculate the percent difference between the display and the serial monitor and ensure there is less than 5% difference
7. Check that the LED lights up if the force value is at or above 2.4 N (this is also when the serial monitor will display a line that says “Stop”)
8. Repeat steps 4 - 8 with five different aliquots of water, doing three trials per aliquot.

After testing is finished, a conclusion can be made about whether or not the display shows accurate force values in comparison to the serial monitor where the voltage values are converted into force values and outputted. A conclusion can also be made about where the red LED warning light works to alert the surgeon that they have exceeded the 2.4 N of force and there may be potential catheter blockage.

Section 6.4: Syringe Clamp Connection and Locking Testing Protocol

The syringe clamp is a 3D printed material composed of Ultimaker PLA [1]. The following provides a step-by-step protocol to test if the syringe clamp mold enables an easy removal and insertion of the required 1 mL syringe (less than 20 seconds) and if it creates a firm and secure connection, ensuring that user and design requirements are met. This testing protocol is used prior to incorporation of the syringe clamp into the injector base in order to confirm that the mold contains the correct connection system. Read through the protocol before performing the steps.

1. Place the syringe clamp mold on a flat surface, preferably a table, with its entire inferior side (does not contain the semi-circle cut-out) sitting flush with the smooth, flat surface.
 - a. The direction and orientation of the syringe clamp does not matter for this test as long as it is in an appropriate position for efficient syringe insertion, force application, and removal
2. Prior to inserting the 1 mL syringe into the clamp, properly connect it to the procedural medical grade tubing (fasten it into place, ensuring a tight connection is present) and then connect the medical grade tubing to the needle-tipped catheter.
 - a. Connections should be made and tightened following the current clinical process
3. Pull the syringe plunger out as far as it can go without popping out of the proximal end of the 1 mL syringe.
4. With this syringe-tubing-catheter complex kept intact, place the 1 mL syringe into the syringe clamp, with the widest diameter portion of the syringe located on the proximal end of the syringe, inserting into the slit on the proximal end of the clamp. The body and distal end of the syringe should be sitting in the semi-circle mold.
 - a. Verify that the syringe's widest diameter segment fits within the mold's slot
 - b. Verify that the entire body of the syringe sits within the semi-circle mold
 - c. Verify that the syringe rests completely horizontal within the syringe clamp mold without requiring intervention
5. Lay the medical grade tubing-catheter complex connected to the syringe on the table in a location just distal of the syringe and the clamp as well as in a position and orientation that mimics clinical injections (the catheter sits flat initially and then eventually rotates in the superior direction relative to the flat surface). The system can be held in place in order to replicate the clinical orientation if required
 - a. Verify that the syringe's widest diameter segment still sits within the mold's slot
 - b. Verify that the entire body of the syringe still rests within the semi-circle mold
 - c. Verify that the syringe still sits horizontally in the syringe clamp mold without requiring intervention
6. Secure the syringe clamp mold in place on the flat surface with either hands or a temporary adhesive that can be easily removed following the end of the procedure.

- a. If an adhesive is used, ensure that there are not any residues left on the syringe clamp that will disrupt its ability to connect to the injector base following the removal of the adherent material
 - b. The syringe clamp should be tightly secured to the surface, preventing any movement of the clamp during the following steps of the protocol**
7. With the syringe clamp secured in place, apply a minimum of 5.00 N of force to the syringe plunger until it completely inserts into the plunger. The force application should be applied horizontally in the axial direction of the syringe plunger's circular end to simulate the injector's force application to the syringe plunger.
 - a. Verify that the clamp and syringe are able to withstand this force without breaking or cracking
 - b. Verify that the syringe does not experience any displacement (horizontal, vertical, rotational, etc.) throughout this force application
8. After full compression of the syringe plunger, continue applying at least 5.00 N of force to the syringe plunger for 10 seconds (can use a stopwatch)
 - a. Verify that the clamp and syringe are able to withstand this force without breaking or cracking
 - b. Verify that the syringe does not experience any displacement (horizontal, vertical, rotational, etc.) throughout this force application

Read the rest of the steps prior to performing them as they need to be performed sequentially in a timely manner.

9. Following the completion of the compression testing and verification that all steps passed, remove the syringe from the syringe clamp mold. As soon as the syringe removal process is initiated (when the syringe is first touched), another individual needs to begin a stopwatch.
 - a. Verify that the syringe can be removed from the clamp without catching on the clamp or becoming stuck
10. After the syringe is completely removed from the clamp, disconnect the medical grade tubing-catheter complex from the syringe and place both the medical grade tubing and syringe on the surface next to the syringe clamp mold.
11. Pick up either the same 1 mL syringe that was just removed from the syringe clamp mold or a different 1 mL syringe and properly connect it to the procedural medical grade tubing (fasten it into place, ensuring a tight connection is present).
 - a. Connections should be made and tightened following the current clinical process
12. Pull the syringe plunger out as far as it can go without popping out of the proximal end of the 1 mL syringe.
13. With this syringe-tubing-catheter complex kept intact, place the 1 mL syringe into the syringe clamp, with the widest diameter portion of the syringe located on the proximal end of the syringe, inserting into the slit on the proximal end of the clamp. The body and distal end of the syringe should be sitting in the semi-circle mold. Stop the stopwatch

when the syringe insertion is complete and it is properly located within the syringe clamp mold.

- a. Verify that the syringe's widest diameter segment fits within the mold's slot
- b. Verify that the entire body of the syringe sits within the semi-circle mold
- c. Verify that the syringe rests completely horizontal within the syringe clamp mold without requiring intervention
- d. Ensure that the syringe removal and insertion process took less than 20 seconds

If any of the steps can not be completed as outlined above or the verification steps fail, reevaluate the design and determine how the syringe clamp mold can be modified in order to allow for the connection and locking tests to pass. If there is an error within the test protocol or a modification is agreed upon by the team, a deviation can be made within the protocol in order to allow a verification step to pass or a step to be completed.

References

- [1] Ultimaker, "Technical Data Sheet - PLA," Nov. 2018. Accessed: Dec. 02, 2021. [Online]. Available: <https://ultimaker.com/materials/pla>.

Section 6.5: Injection Rate Testing Protocol

The following provides a step-by-step protocol to test if the stepper motor consistently provides a 30 and 60 second injection rate while also traveling the appropriate distance to fully eject 0.5 mL of solution. This protocol is used to evaluate the accuracy and consistency of the two injection rates the device provides. Read through the protocol before performing the steps.

1. Power on the device
2. Measure the distance between the force application block and the end of the syringe that is set to 0.5 mL in mm
3. Set up a timer and press start and at the same time press the 60 second flow rate button
4. Stop the timer when the device stops
5. Record the time
6. Record the distance that the force application block traveled in mm
7. Press the reset button and record the time it takes for the device to fully reset back to the original position
8. Repeat five times and calculate the average injection time to ensure that each trial is within 5% error
 - a. Calculate the error margin using Equation 1 below, with the theoretical value corresponding to the required 60 second injection time [1]

$$[1] \text{ Percent Error} = (|V_{\text{theoretical}} - V_{\text{experimental}}| / V_{\text{theoretical}}) * 100$$

9. Perform one more trial and press the pause button multiple times throughout the injection to ensure that the injection comes to a full stop whenever the pause button is pressed
10. Follow the previous steps using the 30 second injection rate
11. Repeat five times calculate the average injection time to ensure that it is within 2.5% error
 - a. Calculate the error margin using Equation 1 below, with the theoretical value corresponding to the required 30 second injection times [1]

$$[1] \text{ Percent Error} = (|V_{\text{theoretical}} - V_{\text{experimental}}| / V_{\text{theoretical}}) * 100$$

References

- [1] Dr. A. M. Helmenstine, "How to Calculate Percent Error," ThoughtCo, Nov. 02, 2020.
<https://www.thoughtco.com/how-to-calculate-percent-error-609584> (accessed Dec. 03, 2021).

Section 6.6: Cell Viability Testing Protocol

The cell viability testing protocol is outlined in this document. The protocol includes thawing the cells, using trypan blue and conducting tests with the automatic device at 30 and 60 seconds, manually with the 30 and 60 second rates, and a rapid test.

1. Start with five vials full of pig MSCs that are at 5 million cells / dose and remove them from the liquid N₂
 2. Pig MSCs are more robust than other cell types so the sterility shouldn't matter as much but use gloves throughout the course of the experiments if directly dealing with the cells and spray down hands using 70% EtOH if working in the hood.
 3. Thaw the cells in a 37 degrees C water bath while continuously mixing them by hand.
 4. In the hood, obtain two 20 mL empty and sterile vials and add 2 mL of MCDB 131 media with FBS for the cells to begin with (Note: Cells are frozen in DMSO media so we use different media to wash it off)
 5. Add two of the cell vials (about 1 mL of solution) to one of the media-containing vials and three of the cell vials to the other media-containing vials.
 6. After the cells are added, fill the vials with media until there is 7mL of total solution for each of the 2 vials
 7. Place both vials on opposite ends of a centrifuge machine and set on 1000 RCF for 5 minutes
 8. Aspirate the supernatant using a vacuum in the hood and combine the 2 vials with cells adding 1 mL of MCDB 131 media and resuspend so they can be counted via Trypan Blue staining
 9. In a 1.5-2 mL tube, add trypan blue and cell suspension according to the appropriate dilution (Note: dilution may need to be adjusted depending on if cells were cultured in a smaller flask, how many flasks are accounted for in each tube, or if cell count may be low).
 10. Using a hemacytometer, count cells in all four quadrants.
 - a. Count the initial number of cells that are alive (clear looking) and dead (blue)
 11. To determine the total cell count: cell count x dilution factor x 2500 = cell count
 12. Add media to the rest of the cells and get 40mL of solution
 13. Load up the syringe with 0.5 mL
 14. Test at 30 seconds with machine x5
 15. Test at 60 seconds with machine x5
 16. Test at 30 seconds with manual x3
 17. Test at 60 seconds with manual x3
 18. Test at rapid rate x3
 19. Record the cell viability after each testing using protocol in steps 9 and 10.
 20. Repeat steps 9 and 10 after all testing is finished to get the end cell viability
-

Section 7: Testing Results and Data Analysis

Section 7.1: Feedback System Force Detection Accuracy Results

Calculations:

$$\begin{aligned}
 &1 \text{ mL} = 1 \text{ g} \\
 &1 \text{ mL} = 1000 \text{ } \mu\text{L} \\
 &[1] \text{ Weight of Water Bottle} = 9.440 \text{ g} \\
 &\text{Total Weight} = 9.440 \text{ g} + \text{mL} \\
 &\text{Force} = (\text{Total Weight} / 1000) * 9.81
 \end{aligned}$$

$$\begin{aligned}
 [2] \text{ \% Difference} &= (|\text{Actual Value} - \text{Theoretical Value}| / \text{Theoretical}) * 100 \\
 \text{Theoretical Value} &= \text{Estimated Force}
 \end{aligned}$$

Table 1: Feedback system's force output error margin relative to their respective applied forces over the typical 0.14 N - 3.00 N injector force range.

Unknown sample of water	Force of the bottle + water (N)	Measured Force of the bottle + water using FSR and Arduino (N)	% Difference between Force Values in Column 2 and 3 Using Estimate as Theoretical
1	0.14	0.16	14%
2	0.32	0.27	15.6%
3	0.55	0.64	16.4%
4	0.70	0.82	17.1%
5	1.00	1.15	13%
6	1.30	1.31	0.77%
7	1.75	1.82	3.43%
8	2.15	1.99	7.44%
9	2.30	2.03	11.74%
10	2.40	2.29	4.58%
11	3.00	2.72	9.33%

Scatter Plot Matlab Code

```

%% BME 301 - FSR Feedback System Force Detection Results
% Author - Parker Esswein
%%

```

x = [0.14 0.32 0.55 0.70 1.00 1.30 1.75 2.15 2.30 2.40 3.00] % Applied Voltage Values
y = [14.00 15.60 16.40 17.10 13.0 0.77 3.43 7.44 11.74 4.58 9.33] % Corresponding error
provided by the feedback system in detecting the force value

```
figure (1);  
hold on;  
bar(x, y)  
title('FSR Feedback System Force Detection Accuracy')  
xlabel('Total Applied Force (N)')  
ylabel('Percent Error Between Applied Force and Feedback System (%)')  
hold off;  
%%  
lforce = [14.00 15.60 16.40 17.10];  
mean(lforce)  
std(lforce)  
hforce = [13.0 0.77 3.43 7.44 11.74 4.58 9.33];  
mean(hforce)  
std(hforce)
```

References

- [1] F. P. Beer, E. R. Johnston Jr., J. T. DeWolf, and D. F. Mazurek, *Mechanics of Materials*, 8th Edition. McGraw Hill Education, 2020.
- [2] Dr. A. M. Helmenstine, "How to Calculate Percent Error," ThoughtCo, Nov. 02, 2020. <https://www.thoughtco.com/how-to-calculate-percent-error-609584> (accessed Dec. 03, 2021).

Section 7.2: FSR Feedback System Testing Results

Table 1: Results for each of the three trials that were done over the course of five seconds where both the display and serial monitor force values were recorded

		Trial 1		Trial 2		Trial 3	
		Display	SM	Display	SM	Display	SM
Aliquot of water #1	1 sec	0.20	0.20	0.21	0.20	0.23	0.23
	2 sec	0.20	0.20	0.20	0.19	0.23	0.23
	3 sec	0.21	0.22	0.19	0.20	0.23	0.23
	4 sec	0.22	0.21	0.20	0.21	0.23	0.23
	5 sec	0.21	0.20	0.21	0.22	0.23	0.23
Aliquot of water #2	1 sec	0.67	0.67	0.68	0.68	0.69	0.69
	2 sec	0.67	0.67	0.69	0.69	0.68	0.69
	3 sec	0.67	0.67	0.69	0.69	0.67	0.68
	4 sec	0.67	0.68	0.68	0.69	0.68	0.68
	5 sec	0.68	0.68	0.68	0.68	0.68	0.68
Aliquot of water #3	1 sec	1.20	1.21	1.21	1.21	1.21	1.20
	2 sec	1.22	1.23	1.21	1.21	1.20	1.20
	3 sec	1.20	1.21	1.21	1.21	1.20	1.20
	4 sec	1.22	1.22	1.20	1.21	1.20	1.20
	5 sec	1.21	1.21	1.20	1.20	1.20	1.20
Aliquot of water #4	1 sec	1.93	1.93	1.93	1.93	1.93	1.93
	2 sec	1.93	1.93	1.92	1.93	1.93	1.93
	3 sec	1.94	1.94	1.93	1.93	1.93	1.94
	4 sec	1.93	1.94	1.93	1.93	1.94	1.94
	5 sec	1.93	1.93	1.92	1.92	1.94	1.94

Aliquot of water #5 (light was on)	1 sec	2.47	2.47	2.48	2.48	2.47	2.47
	2 sec	2.47	2.47	2.48	2.48	2.47	2.47
	3 sec	2.48	2.48	2.48	2.48	2.47	2.47
	4 sec	2.49	2.49	2.48	2.48	2.48	2.47
	5 sec	2.49	2.49	2.48	2.48	2.48	2.48

The mean and standard deviation of the display and serial monitor for the three trials (over five seconds) per each aliquot of water was taken (15 data points for each aliquot of water). The percent difference was calculated to indicate how much difference there was between the serial monitor and the display using the following equation [1]:

$$\left(\frac{|SM_{\text{avg}} - \text{Display}_{\text{avg}}|}{\left(\frac{SM_{\text{avg}} + \text{Display}_{\text{avg}}}{2} \right)} \right) * 100 = \text{Percent Difference}$$

SM = Serial Monitor
Display = Scoreboard

References:

[1] “Percent Difference Calculator - with Step-by-Step Guide,” Inch Calculator.

<https://www.inchcalculator.com/percent-difference-calculator/> (accessed May 03, 2022).

Section 7.3: Syringe Clamp Connection and Locking Results

Table 1: Syringe Clamp Connection and Locking force testing and exchange time results.

Trial	Force Testing Result	Exchange Time (seconds)
1	Pass	14.67
2	Pass	10.56
3	Pass	13.31
4	Pass	14.78
5	Pass	14.17

Section 7.4: Injection Rate Testing Results

30 Second Injection Rate Testing

Run 1

Injection time: 30.5 seconds

Traveled: 30.86 mm

Run 2

Injection time: 30.4 seconds

Traveled: 30.31 mm

Run 3

Injection time: 30.34 seconds

Traveled: 30.34 mm

Run 4

Injection time: 30.26 seconds

Traveled: 30.36 mm

Run 5

Injection time: 30.12 seconds

Traveled: 30.52 mm

Results:

Time: Mean = 30.324, standard deviation $s=0.1438$, percent error from 30 seconds: 1.08%

Distance Traveled: Mean = 30.478mm, standard deviation $s=0.2285$ mm, percent error from 30.5mm = 0.0721%

60 Second Injection Rate

Run 1

Injection time: 1 min 2 sec

Traveled: 30.25 mm

Run 2

Injection time: 1 min 1 sec

Traveled: 30.51 mm

Run 3

Injection time: 1 min 1 sec

Traveled: 30.49 mm

Run 4

Injection time: 1 min

Traveled: 30.55 mm

Run 5

Injection time: 1 min

Traveled: 30.65 mm

Results:

Time: Mean = 60.8, standard deviation $s = 0.83666$, percent error from 30 seconds: 1.333%

Distance Traveled: Mean = 30.49mm, standard deviation $s=0.1476$ mm, percent error from 30.5mm = 0.03281%

*Note: The following percent error equation was used for calculations.

$$[1] \text{ Percent Error} = (|V_{\text{theoretical}} - V_{\text{experimental}}| / V_{\text{theoretical}}) * 100$$

References:

[1] Dr. A. M. Helmenstine, "How to Calculate Percent Error," ThoughtCo, Nov. 02, 2020.

<https://www.thoughtco.com/how-to-calculate-percent-error-609584> (accessed Dec. 03, 2021).

Section 7.5: Cell Viability Testing Results and Analysis

Table 1: Cell Viability of Porcine-derived MSCs following automatic and manual injections at various rates. All injections were performed with a 25 gauge needle containing a length of 2.22 cm unless otherwise noted.

Injection Type	Trial Number	Injection Rate (Seconds)	Force Range (N)	Maximum Force (N)	Cell Viability (%)
Automatic	1	30 (20 gauge needle)	~ 0.16 N	0.29 N	88.0
Automatic	2	30 (20 gauge needle)	0.14 - 0.18 N	0.21 N	88.0
Automatic	3	30 (20 gauge needle)	0.16 - 0.18 N	0.29 N	78.9
Automatic	4	30 (20 gauge needle)	~ 0.19 N	0.33 N	100.0
Automatic	5	30	0.32 N - 0.61 N	1.43 N	92.0
Automatic	1	60	~ 0.14 N	0.17 N	85.0
Automatic	2	60	0.21 - 0.22 N	0.41 N	83.0
Automatic	3	60	0.21 - 0.22 N	0.67 N	90.0
Automatic	4	60	0.29 - 0.42 N	0.52 N	80.0
Automatic	5	60	~ 0.30 N	1.02 N	87.0
Manual	1	30	0.29 - 0.31 N	1.03 N	90.0
Manual	2	30	0.59 - 0.63 N	0.73 N	93.0
Manual	3	30	0.59 - 0.62 N	1.05 N	80.0
Manual	4	30	0.28 - 0.34 N	0.39 N	83.0
Manual	1	60	0.42 - 0.54 N	0.71 N	86.0
Manual	2	60	0.30 - 0.51 N	1.91 N	83.0
Manual	3	60	0.28 - 0.43 N	0.46 N	78.0

Manual (Rapid)	1	1.03	Initial Force ≈ 0.39 N	3.21 N	86.0
Manual (Rapid)	2	1.15	Initial Force ≈ 0.21 N	3.34 N	92.0
Manual (Rapid)	3	0.44	Initial Force ≈ 0.30 N	8.17 N	90.0
Manual (Rapid)	4	0.37	Initial Force ≈ 0.49 N	9.30 N	77.0

Table 2: One-way ANOVA table comparing the viability of MSCs following automatic and manual 30 and 60 second injections. The p-value when comparing all four groups as separate is 0.41, demonstrating that there is not a significant difference between the automatic and manual injections when using a significance value of $p = 0.05$.

ANOVA Table					
Source	SS	df	MS	F	Prob>F
Groups	102.961	3	34.3202	1.03	0.4113
Error	432.955	13	33.3042		
Total	535.915	16			

Table 3: Comparison of all four injection groups (automatic and manual) via pairs and each pair's respective p-value.

Group 1	Group 2	Lower Confidence Interval of the Difference	Estimated Difference in the Means	Upper Confidence Interval of the Difference	p-value (CType correction)
1	2	-6.334	4.380	15.093	0.637
1	3	-8.483	2.880	14.243	0.878
1	4	-5.323	7.047	19.417	0.376
2	3	-12.863	-1.500	9.863	0.979
2	4	-9.703	2.667	15.037	0.920
3	4	-8.770	4.167	17.104	0.782

Cell Viability Box Plot, ANOVA, and Comparison Matlab Code

```
%% BME 301 - Cell Viability ANOVA
```

```
% Author - Parker Esswein
```

```
%% Variable Initialization
```

```
A30 = [88.00 88.00 78.90 100.00 92.00]' % Cell Viability for the 30 second automatic injections
```

```
A60 = [85.00 83.00 90.00 80.00 87.00]' % Cell Viability for the 60 second automatic injections
```

```
M30 = [90.00 93.00 80.00 83.00 NaN]' % Cell Viability for the 30 second manual injections
```

```
M60 = [86.00 83.00 78.00 NaN NaN]' % Cell Viability for the 60 second manual injections
```

```
SV = [81.10 81.10 81.10 81.10 81.10]' % Cell Viability after Thawing
```

```
EV = [80.0 80.0 80.0 80.0 80.0]' % Final Cell Viability
```

```
%% Average and Std for each injection group
```

```
mean(A30)
```

```
std(A30)
```

```
mean(A60)
```

```
std(A60)
```

```
mean(M30(1:4,:))
```

```
std(M30(1:4,:))
```

```
mean(M60(1:3,:))
```

```
std(M60(1:3,:))
```

```
%% Box Plot Generation
```

```
name = ["Initial Viability", "30 Second Automatic Injections", "30 Second Manual Injections",  
"60 Second Automatic Injections", "60 Second Manual Injections", "Final Viability"];
```

```
figure(1)
```

```
boxplot([SV, A30, M30, A60, M60, EV], name)
```

```
ylabel('MSC Viability (%)')
```

```
title('Cell Viability of Porcine-derived MSCs Following Automatic and Manual Injections at  
Various Rates')
```

```
%% ANOVA between automatic and manual injection values
```

```
viability = [A30 A60 M30 M60]
```

```

name = ["30 Second Automatic Injections", "60 Second Automatic Injections", "30 Second
Manual Injections", "60 Second Manual Injections"];
figure(2)
hold on;
[p, tbl, stats] = anova1(viability, name)
hold off;
figure (5)
hold on;
[c,m,h,gnames] = multcompare(stats)
hold off;

```

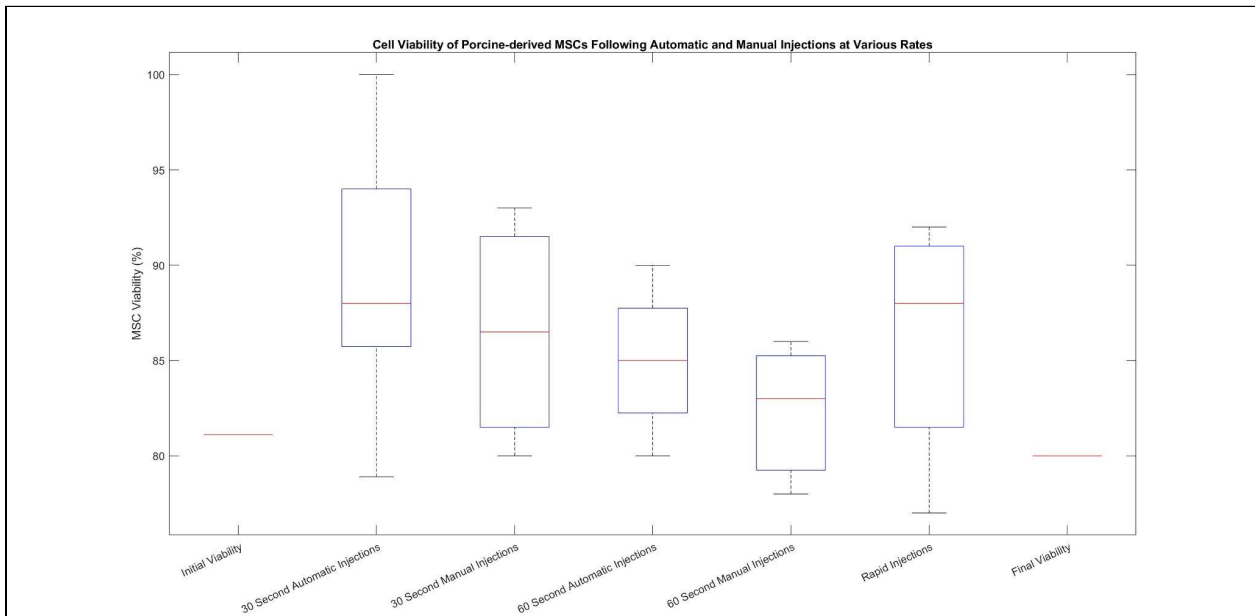


Figure 1: Boxplot comparing the viability of MSCs following automatic and manual 30 and 60 second injections as well as rapid injections to each other and to the starting and final MSC viability. The rectangles represent the interquartile range, the whiskers display the full range of MSC viability, and the red lines locate the median for each rate and injection modality (manual and automatic).

Table 4: One-way ANOVA table comparing the viability of MSCs following automatic and manual 30 and 60 second injections as well as rapid injections. The p-value when comparing all five groups as separate is 0.59, demonstrating that there is not a significant difference between the automatic and manual injections when using a significance value of $p = 0.05$.

ANOVA Table					
Source	SS	df	MS	F	Prob>F
Groups	102.981	4	25.7453	0.73	0.5857
Error	565.705	16	35.3565		
Total	668.686	20			

Table 5: Comparison of all five injection groups (automatic and manual) via pairs and each pair's respective p-value.

Group 1	Group 2	Lower Confidence Interval of the Difference	Estimated Difference in the Means	Upper Confidence Interval of the Difference	p-value (CType correction)
1.0000000000000000	2.0000000000000000	-7.141454155022577	4.3799999999999995	15.901454155022568	0.770507398690263
1.0000000000000000	3.0000000000000000	-9.340347543219583	2.8799999999999995	15.100347543219574	0.948273148098677
1.0000000000000000	4.0000000000000000	-6.257162649049764	7.046666666666667	20.350495982383098	0.504930748924941
1.0000000000000000	5.0000000000000000	-9.090347543219583	3.1299999999999995	15.350347543219574	0.931385677226005
2.0000000000000000	3.0000000000000000	-13.720347543219578	-1.5000000000000000	10.720347543219578	0.995302029608716
2.0000000000000000	4.0000000000000000	-10.637162649049760	2.6666666666666671	15.970495982383103	0.97072375334347
2.0000000000000000	5.0000000000000000	-13.470347543219578	-1.2500000000000000	10.970347543219578	0.997677655194277
3.0000000000000000	4.0000000000000000	-9.746811068698481	4.1666666666666671	18.080144402031824	0.886030466159960
3.0000000000000000	5.0000000000000000	-12.631377345138937	0.2500000000000000	13.131377345138937	0.999996820763444
4.0000000000000000	5.0000000000000000	-17.830144402031824	-3.9166666666666671	9.996811068698481	0.906398343857326

Cell Viability Box Plot, ANOVA, and Comparison including Rapid Injections Matlab Code

%% BME 301 - Cell Viability ANOVA with Rapid Injections

% Author - Parker Esswein

%% Variable Initialization

A30 = [88.00 88.00 78.90 100.00 92.00]' % Cell Viability for the 30 second automatic injections

A60 = [85.00 83.00 90.00 80.00 87.00]' % Cell Viability for the 60 second automatic injections

M30 = [90.00 93.00 80.00 83.00 NaN]' % Cell Viability for the 30 second manual injections

M60 = [86.00 83.00 78.00 NaN NaN]' % Cell Viability for the 60 second manual injections

RI = [86.00 92.00 90.00 77.00 NaN]' % Cell Viability for the 60 second manual injections

SV = [81.10 81.10 81.10 81.10 81.10]' % Cell Viability after Thawing

EV = [80.0 80.0 80.0 80.0 80.0]' % Final Cell Viability

%% Average and Std for each injection group

mean(A30)

std(A30)

mean(A60)

std(A60)

mean(M30(1:4,:))

std(M30(1:4,:))

mean(M60(1:3,:))

std(M60(1:3,:))

mean(RI(1:4,:))

std(RI(1:4,:))

%% Box Plot Generation

name = ["Initial Viability", "30 Second Automatic Injections", "30 Second Manual Injections", "60 Second Automatic Injections", "60 Second Manual Injections", "Rapid Injections", "Final Viability"];

figure(1)

boxplot([SV, A30, M30, A60, M60, RI, EV], name)

ylabel('MSC Viability (%)')

title('Cell Viability of Porcine-derived MSCs Following Automatic and Manual Injections at Various Rates')

%% ANOVA between automatic and manual injection values

viability = [A30 A60 M30 M60 RI]

```

name = ["30 Second Automatic Injections", "60 Second Automatic Injections", "30 Second
Manual Injections", "60 Second Manual Injections", "Rapid Injections"];
figure(2)
hold on;
[p, tbl, stats] = anova1(viability, name)
hold off;
figure (5)
hold on;
[c,m,h,gnames] = multcompare(stats)
hold off;

```

Cell Viability Force Variance Matlab Code

```

%% BME 301 - Cell Viability Force Variation
% Author - Parker Esswein
%% Minimum Force Values
FA30 = [0.16 0.14 0.16 0.19 0.32];
FM30 = [0.29 0.59 0.59 0.28];
FA60 = [0.14 0.21 0.21 0.29 0.30];
FM60 = [0.42 0.30 0.28];
%% Average of Minimum Force Values
mean(FA30)
std(FA30)
mean(FM30)
std(FM30)
mean(FA60)
std(FA60)
mean(FM60)
std(FM60)
%% Maximum Force Values
FA30 = [0.16 0.18 0.18 0.19 0.61];
FM30 = [0.31 0.63 0.62 0.34];
FA60 = [0.14 0.22 0.22 0.42 0.30];
FM60 = [0.54 0.51 0.43];
%% Average of Maximum Force Values
mean(FA30)
std(FA30)
mean(FM30)
std(FM30)
mean(FA60)
std(FA60)
mean(FM60)
std(FM60)
%% Variance Across Maximum and Minimum Force Values
TFA30 = [0.16 0.14 0.16 0.19 0.32];
TFM30 = [0.29 0.59 0.59 0.28];
TFA60 = [0.14 0.21 0.21 0.29 0.30];

```

```

TFM60 = [0.42 0.30 0.28];
var(TFA30)
var(TFM30)
var(TFA60)
var(TFM60)
%% ANOVA between automatic and manual injection values
TFA30 = [0.16 0.14 0.16 0.19 0.32]';
TFM30 = [0.29 0.59 0.59 0.28 NaN]';
TFA60 = [0.14 0.21 0.21 0.29 0.30]';
TFM60 = [0.42 0.30 0.28 NaN NaN]';
force = [TFA30 TFA60 TFM30 TFM60]
name = ["30 Second Automatic Injections", "60 Second Automatic Injections", "30 Second
Manual Injections", "60 Second Manual Injections"];
figure(2)
hold on;
[p, tbl, stats] = anova1(force, name)
hold off;
figure (5)
hold on;
[c,m,h,gnames] = multcompare(stats)
hold off;

```

Section 7.6: Overall Device - 25 Gauge Needle Testing Results

30 second Overall Testing

Trial 1:

Actual delivery time: 30.72 seconds

Force Value Ranges: 0.14-0.49

Volume ejected: 0.49 mL

Trial 2:

Actual delivery time: 30.61 seconds

Force Value Ranges: 0.14-0.54

Volume ejected: 0.49 mL

Trial 3:

Actual delivery time: 30.60 seconds

Force Value Ranges: 0.13-0.55

Volume ejected: 0.47 mL

Trial 4:

Actual delivery time: 30.40 seconds

Force Value Ranges: 0.18-0.41

Volume ejected: 0.47 mL

Trial 5:

Actual delivery time: 30.50 seconds

Force Value Ranges: 0.23-0.50

Volume ejected: 0.46 mL

60 second Overall Testing

Trial 1:

Actual delivery time: 60.40 seconds

Force Value Ranges: 0.23-0.40

Volume ejected: 0.47 mL

Trial 2:

Actual delivery time: 60.11 seconds

Force Value Ranges: 0.11-0.50

Volume ejected: 0.47 mL

Trial 3:

Actual delivery time: 60.30 seconds

Force Value Ranges: 0.18-0.45
Volume ejected: 0.47 mL

Trial 4:

Actual delivery time: 60.04 seconds
Force Value Ranges: 0.13-0.46
Volume ejected: 0.47 mL

Trial 5:

Actual delivery time: 60.12 seconds
Force Value Ranges: 0.13-0.50
Volume ejected: 0.47 mL

The mean of the ejection time, force ranges and volume ejected were calculated using the typical mean equation for a sample of five trials and the standard deviation was calculated using the standard deviation equation for samples rather than populations. The percent error was calculated to indicate how much error there was for the 30 and 60 second time settings respectively using the following equation:

$$[1] \text{ Percent Error} = (|V_{\text{theoretical}} - V_{\text{experimental}}| / V_{\text{theoretical}}) * 100$$

The serial plots were also obtained for this round of testing and can also be seen in the figures below.

Serial Plot for a 30 Second Injection: 25 Gauge Needle

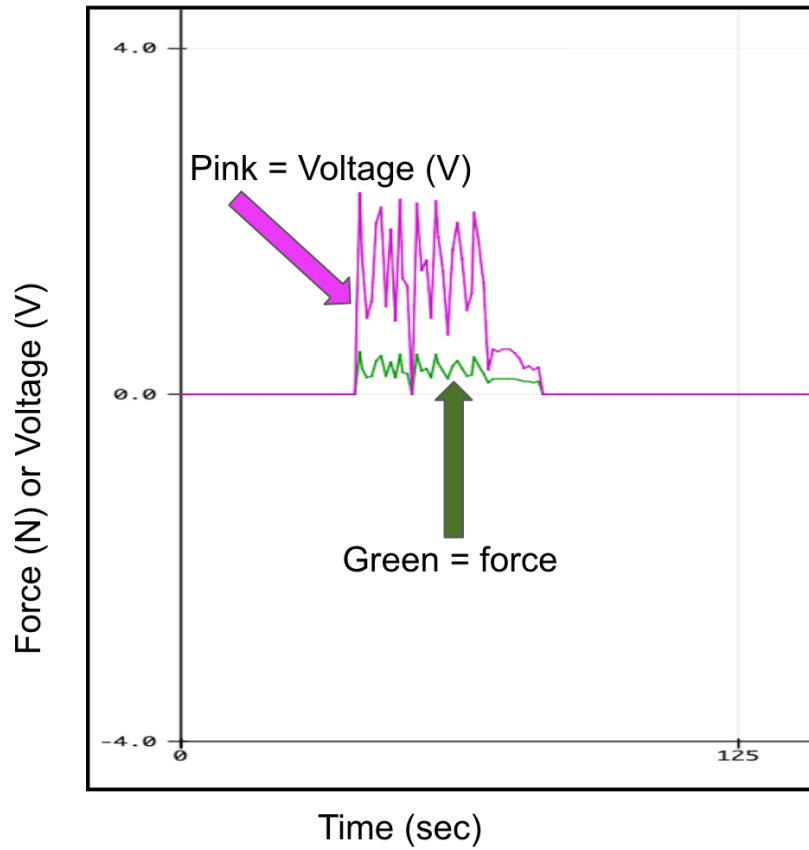


Figure 1: The serial plot with force values vs. time for the 25 gauge for one of the 30 second trials. The green line is the force values.

Serial Plot for a 60 Second Injection: 25 Gauge Needle

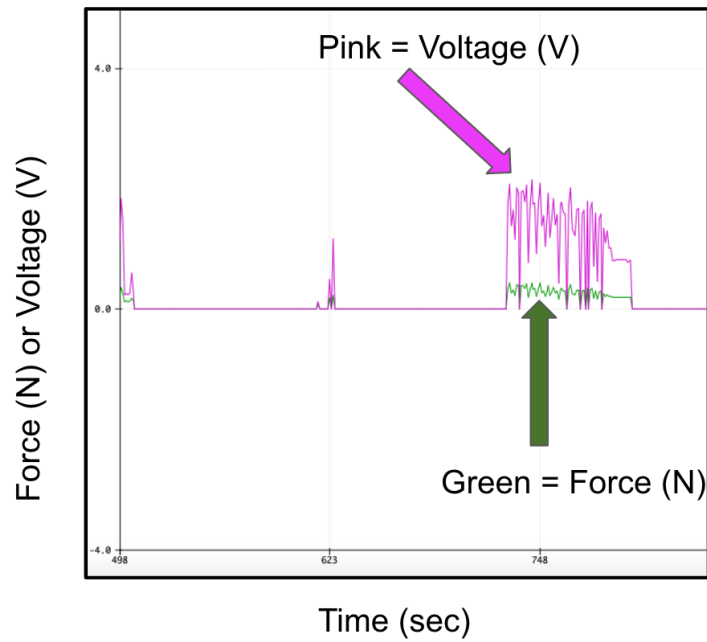


Figure 2: The serial plot with force values vs. time for the 25 gauge for one of the 60 second trials. The green line is the force values.

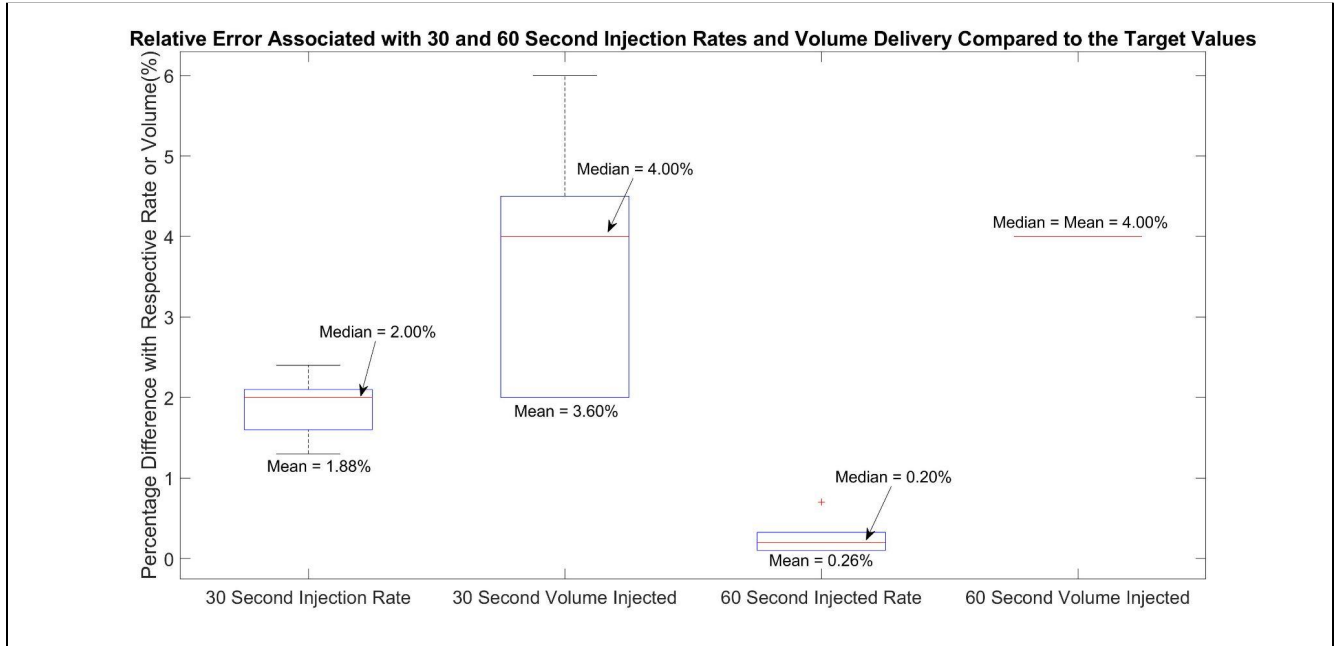


Figure 3: Boxplot displaying the percentage difference between the injection rates and delivery volumes to their respective 30 second, 60 second, and 0.5 mL target values. The rectangles represent the interquartile range, the whiskers display the full range of MSC viability, the red lines locate the median, and the plus signs resemble outliers for each rate and injection volume.

Injection Rate and Volume Analysis Matlab Code

```
%% BME 301 - Injection Testing Results
% Author - Parker Esswein
%%
A30t = [2.4 2.0 2.0 1.3 1.7]' % Percentage difference between injection time for the 30 second
automatic injection setting and 30 seconds
A30v = [2.0 2.0 4.0 4.0 6.0]' % Percentage difference between the corresponding volume
injected by the device over the injection time and the required 0.5 mL
A60t = [0.7 0.2 0.1 0.1 0.2]' % Percentage difference between injection time for the 60 second
automatic injection setting and 60 seconds
A60v = [4.0 4.0 4.0 4.0 4.0]' % Percentage difference between the corresponding percent area
volume injected by the device over the injection time and the required 0.5 mL

figure(1);
hold on;
name = ["30 Second Injection Rate", "30 Second Volume Injected", "60 Second Injected Rate",
"60 Second Volume Injected"];
figure(1)
boxplot([A30t, A30v, A60t, A60v], name)
ylabel('Percentage Difference with Respective Rate or Volume(%)')
title('Relative Error Associated with 30 and 60 Second Injection Rates and Volume Delivery
Compared to the Target Values')
hold off;
```

References:

- [1] Dr. A. M. Helmenstine, "How to Calculate Percent Error," ThoughtCo, Nov. 02, 2020.
<https://www.thoughtco.com/how-to-calculate-percent-error-609584> (accessed Dec. 03, 2021).

Section 7.7: Overall Device - Clinical Simulation Testing

Table 1: 30 Second Clinical Simulation Data for Overall Device Testing.

		Results	Percent Error (%)
Trial 1	Ejection Time	30.5 seconds	1.67
	Force Range	0.30-0.54 N	-
	Reverse time	24.15 seconds	-
Trial 2	Ejection Time	30.65 seconds	2.17
	Force Range	0.27-0.48 N	-
	Reverse time	23.25 seconds	-
Trial 3	Ejection Time	30.85 seconds	2.83
	Force Range	0.18-0.42 N	-
	Reverse time	23.30 seconds	-
Trial 4	Ejection Time	30.39 seconds	1.30
	Force Range	0.20-0.50 N	
	Reverse time	23.55 seconds	
Trial 5	Ejection Time	30.67 seconds	2.23
	Force Range	0.23-0.48 N	
	Reverse time	23.62 seconds	-

Table 2: 60 Second Clinical Simulation Data for Overall Device Testing.

		Results	Percent Error (%)
Trial 1	Ejection Time	60.42 seconds	0.70
	Force Range	0.13-0.41 N	-
	Reverse time	24.67 seconds	-
Trial 2	Ejection Time	60.45 seconds	0.75
	Force Range	0.17-0.42 N	-
	Reverse time	24.69 seconds	-
Trial 3	Ejection Time	60.01 seconds	0.017
	Force Range	0.15-0.50 N	-
	Reverse time	24.62 seconds	-
Trial 4	Ejection Time	60.45 seconds	0.75
	Force Range	0.16-0.48 N	-
	Reverse time	24.23 seconds	-
Trial 5	Ejection Time	60.32 seconds	0.53
	Force Range	0.23-0.43 N	-
	Reverse time	24.28 seconds	-

The mean of the ejection time, force ranges and reverse times were calculated using the typical mean equation for a sample of five trials and the standard deviation was calculated using the standard deviation equation for samples rather than populations. The percent error was calculated to indicate how much error there was for the 30 and 60 second time settings respectively using the following equation:

$$[1] \text{ Percent Error} = (|V_{\text{theoretical}} - V_{\text{experimental}}| / V_{\text{theoretical}}) * 100$$

The serial plots were also obtained for this round of testing and can also be seen in the figures below.

Serial Plot for a 30 Second Injection: Clinical Stimulation

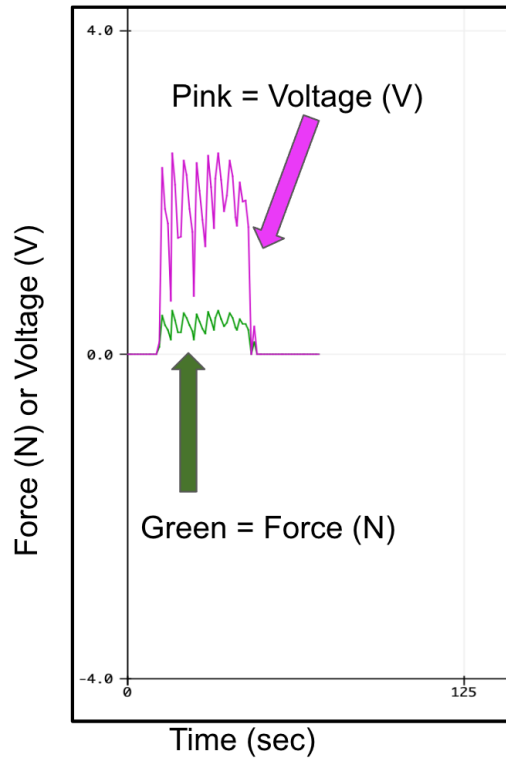


Figure 1: The serial plot with force values vs. time for the clinical simulation testing for one of the 30 second trials. The green line is the force values.

Serial Plot for a 60 Second Injection: Clinical Stimulation

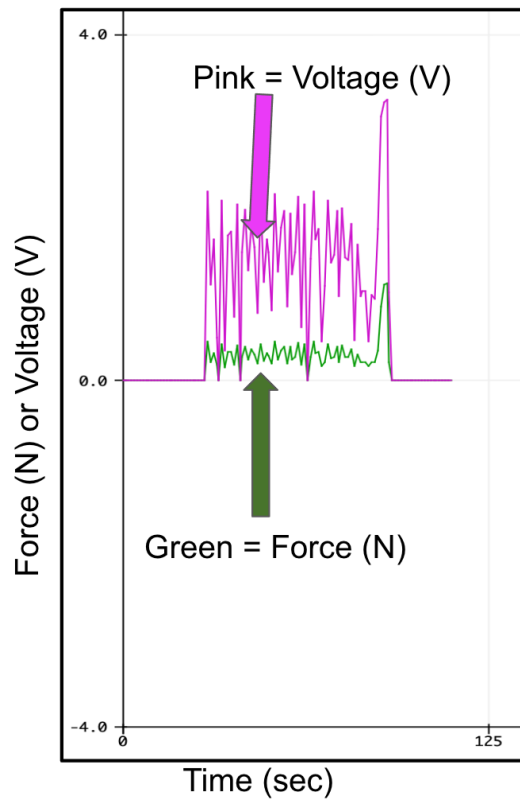


Figure 2: The serial plot with force values vs. time for the clinical simulation testing for one of the 60 second trials. The green line is the force values.

References:

- [1] Dr. A. M. Helmenstine, "How to Calculate Percent Error," ThoughtCo, Nov. 02, 2020.
<https://www.thoughtco.com/how-to-calculate-percent-error-609584> (accessed Dec. 03, 2021).