Automatic Intramyocardial Stem Cell Injection Device

Preliminary Report

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BME 301

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Lab Section 307

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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 save lives and improve quality of life through cardiac tissue repair. Currently, the 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. Although automatic syringe pumps containing various injection rates are available, these systems are not tailored for MSC myocardium injections and do not incorporate a force sensing feedback system to aid in each injection. As a result, an automatic injection device that properly integrates with the procedural syringes (1 - 20 mL), medical grade tubing, and clinical catheter characteristic of the myocardium injection procedure will drastically improve MSC delivery and thus cardiac repair. The injector will limit operator intervention and provide a slow, controlled, and adjustable injection rate to maximize cell retention. It will be fabricated via 3D printing with Ultimaker PLA and will include a visual feedback system based on an FSR 400 series force sensing resistor and an Arduino Uno microcontroller. The automated injections will be controlled by a stepper motor. The efficacy of the injector will be established through accuracy and reliability tests that focus on the injector's liquid volume dispensing, injection rate timing, and force sensing system. This injector will improve the efficacy of current intramyocardial stem cell injection procedures, enhancing clinical success and thus survival rate.

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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 [4]. 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 differentiate into cardiomyocytes following localization in the myocardium, 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 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, clinical success, and patient recovery and survival, making this

device a critical component of improving the treatment of myocardial infarction and heart failure.

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 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 [7]. 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) [7] and the “Baxter Infus O.R. Syringe Pump Refurbished” that is sold for ~$4000 (right image) [8].

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 [9]. This product can be seen in Figure 2 below.

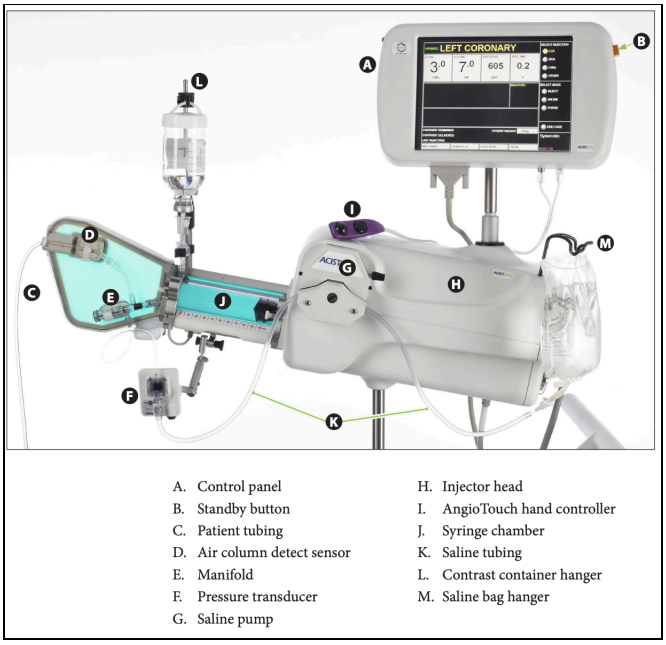


Figure 2: Diagram of the important user components in the Acist Contrast Delivery System a [9].

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 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 [10]. The device can be seen in Figure 3 below.

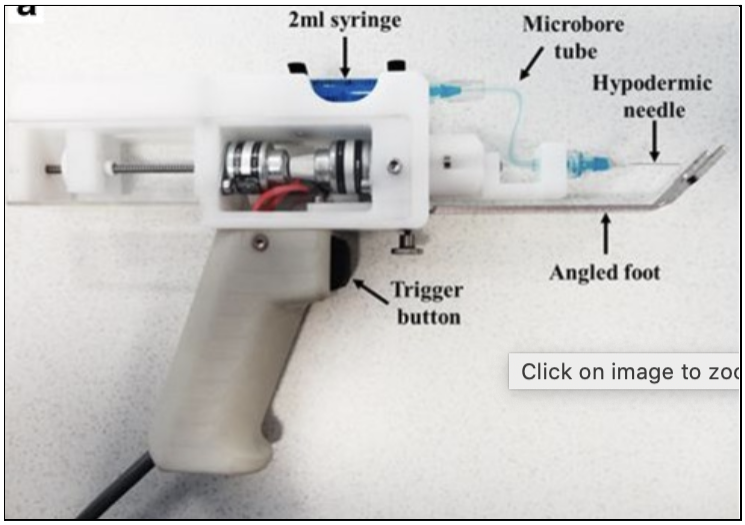


Figure 3: Automated injector device that was in preclinical development in 2017 [10].

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 [11].

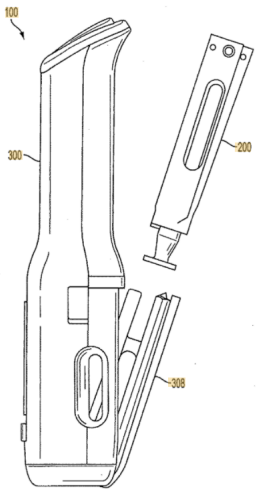


Figure 4: The cassette automatic injector device [11].

Problem Statement

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 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 [12]. While slower injection rates improve cell retention, they insight operator discomfort and hand cramping, reducing the efficacy of the delivery. In both cases, the success of the treatment is severely restrained, limiting the potential of this novel treatment. To improve stem cell delivery and cardiac repair, an automatic injection device that integrates with the procedural syringe and

catheter, limits operator intervention, and provides 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 [13]. 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 [14]. 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 [15]. Nanotechnology has 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 [16]. 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 [17]. They can be isolated from multiple sources, such as bone marrow, adipose, and cardiac tissue [17]. 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. As heart failure is characterized by a loss of cardiomyocytes, the differentiation of MSCs into cardiomyocytes and their subsequent proliferation within cardiac tissue will promote the regeneration and recovery of these cells in the myocardium following heart failure. MSCs can thus aid in the recovery of myocardium tissue [18]. 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

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. Operators target myocardial segments where wall thickness is greater than 6 mm. 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 15 second 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, limiting the potential of this novel treatment.

Sterilization Techniques

The requirement for sterility within the operating room and during the procedure will result in the device being cleaned with bleach or CaviWipes and kept within a sterile bag throughout each procedure [20]. These bags are sterilized with ethylene oxide [21]. Prior to and following each procedure, the device may be exposed to ethylene oxide sterilization [20]. These sterilization techniques require the entire injector to be capable of withstanding sodium hypochlorite (bleach) [22], isopropanol (Cavi Wipes) [23], and ethylene oxide. As the injector needs to be sterile, but will not be in contact with internal regions of the body, it should have a sterility assurance level (SAL) of 10^{-3} [24]. A sterility assurance level is the probability of a single viable microorganism occurring on a product after sterilization, deeming that unit non-sterile. It is an estimate of the lethality of the entire sterilization process on a compound. Following the sterilization of the entire injector, it should contain a probability of 10^{-3} or less that

a unit within the device still remains non-sterile. The injector will not experience heat-based sterilization, namely autoclaving, eliminating the need for the device to have a maximum melting point. 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, however, sterile bags (ethylene oxide) are the only sterilization technique they will be exposed to.

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 [25]. 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 and specifies the procedure required for this class [26]. 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 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 Food and Drug Administration (FDA) outlines the testing and sterilization specifications for combination injection products within “Technical Considerations for Injectors Intended for Use with Drugs and Biological Products”. This guidance recommends that sterile injection devices have a sterility assurance level (SAL) of 10^{-6} unless they are only intended for intact skin contact, resulting in a SAL suggestion of 10^{-3} [24]. The specific SAL requirements of the injector is discussed in the *Sterilization Techniques* section above. 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.

The International Organization for Standardization (ISO) provides requirements for the development, validation, and routine control of an ethylene oxide sterilization process for medical devices in health care facility settings in standard 11135:2014 [29]. ISO 11137 discusses medical device sterilization by addressing the environment of the product, personnel and their hygiene, packaging and storage of the device, and sterilization doses required to inactivate microbiological contaminants [30]. The injector requires sterilization before, during, and after each intramyocardial stem cell injection procedure, specifically ethylene oxide sterilization, so it will need to follow these two ISOs in order for proper and effective sterilization techniques to be performed on the device.

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. 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. The injector must be compatible with the standard catheters, medical grade tubing, and procedural syringes (1 - 20 mL) currently used in practice. The device must also be fabricated out of materials that are capable of being sterilized with standard cleaning techniques, namely bleach, CaviWipes, and ethylene oxide, and can function properly for at least three years under this exposure [20]. A sterility assurance level of 10^{-3} will be mandated whenever the device is sterilized [23]. 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 [6]. Force values above a threshold of 2.4 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 [19]. The client has a budget of $3000 and a manufacturing cost of $500 [31]. 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. Each design is composed of materials that can withstand Bleach, CaviWipe, and ethylene oxide gas sterilization.

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 standard syringes (plunger diameter less than 12.07 mm [32]). The FSR sensor has an actuation force of 0.1 N, maintaining this sensitivity from 0 N to 10 N [33]. This demonstrates that the sensor will provide an accurate and precise force detection reading throughout each injection, as the expected 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 and force alteration to take place at increments greater than 0.10 N (see the *Intramyocardial Stem Cell Injection Procedure* section above) [19]. 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 kΩ at 0.14 N of applied force and about 3 kΩ 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 [33]. 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.

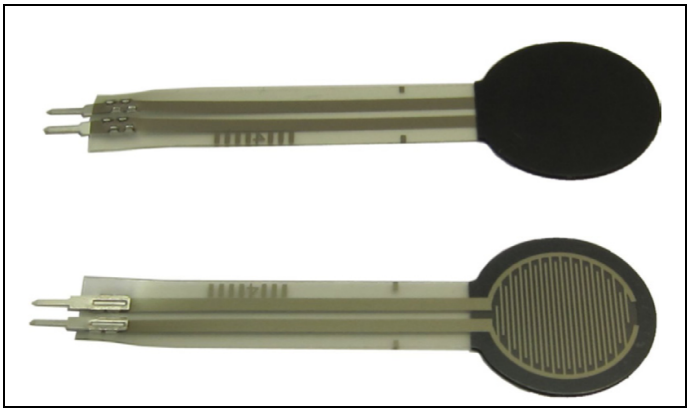


Figure 5: FSR 400 Force Sensing Resistor highlighting its 12.70 mm active zone (patterned circle) and its two prong connectors that allow breadboard and circuit integration [33].

Four Signal Feedback System

The injector's visual feedback system will consist of four signals that assist in catheter placement and alert the operator of potential catheter backup. This system will consist of a voltage divider circuit (see Figure 6 below) that connects to an Arduino Uno microcontroller. The FSR Sensor will act as the first resistor in the voltage divider circuit, allowing its variable resistance to alter the voltage output sent to the microcontroller. A 2.2 kΩ resistor will be the second resistor in the voltage divider circuit and a 5 V voltage source will power 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 0.11 V to 2.12 V. This is well within the 4.88 mV resolution of the Arduino microcontroller [34], 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 of the Appendix.

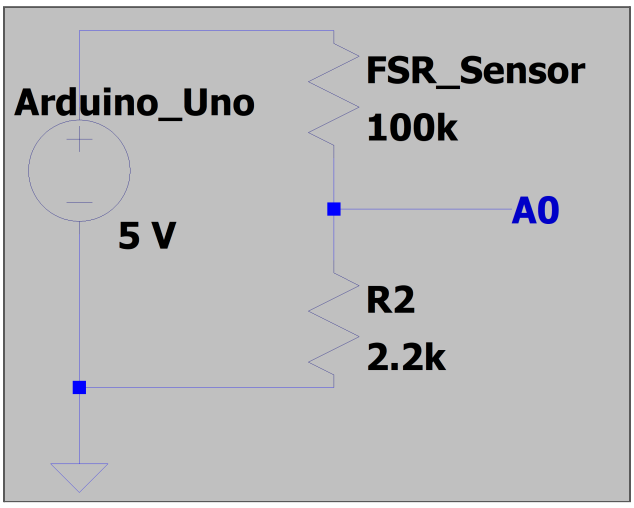


Figure 6: Voltage Divider Circuit containing the FSR sensor, a 5 V DC voltage source, and an output going into A0 of the Arduino Uno microcontroller.

The Arduino IDE software program will be 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 curve for the FSR sensor voltage divider circuit. Using this algorithm, the Arduino IDE interface will constantly display the force value applied throughout each injection on the IDE interface for easy operator visibility. 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 four visual Light emitting diode (LED) signals. A blue LED light will signify that the catheter is injecting the MSC aliquot solution into air or a body cavity, corresponding to a detected force value less than 0.6 N, an orange LED light indicates the transmission of injectate into healthy myocardium tissue, a green LED light resembles the dispensing of the cells into diseased or scarred myocardium tissue, and a red LED light demonstrates that the 2.4 N threshold has been exceeded and the catheter may be backed up [19]. Arduino IDE code will be written to control the visual feedback. During each injection, this system will allow proper catheter insertion and placement as well as it will help promote successful MSC delivery. 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.

Design Ideas

Cellicopter: Propeller Controlled Injections

The Cellicopter design has a 20 × 10 × 5 cm base and consists of a speed control DC motor, an Ultimaker PLA force application rod, and adjustable syringe clamp molds (see Figure 7 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 7 below). The DC motor will slowly rotate from left to right (as depicted in Figure 7), 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. 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. 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 7), moving the force rod away from the syringe and its plunger. This will be critical when another injection needs to be performed. Ultimaker PLA syringe clamp molds will secure each syringe in place within the injector, receiving all force provided during an injection without allowing any syringe displacement. These clamps will be exchangeable and come in different sizes, with each different mold designed to secure a syringe within the required 1 - 20 mL range. There will be 1, 3, 5, 10, and 20 mL molds. This allows for any syringe ranging from 1 mL to 20 mL to be capable with the design. Syringes have to be manually inserted into and removed from the injector before and after injections. Within this design, the medical grade tubing and catheter will be able to 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.

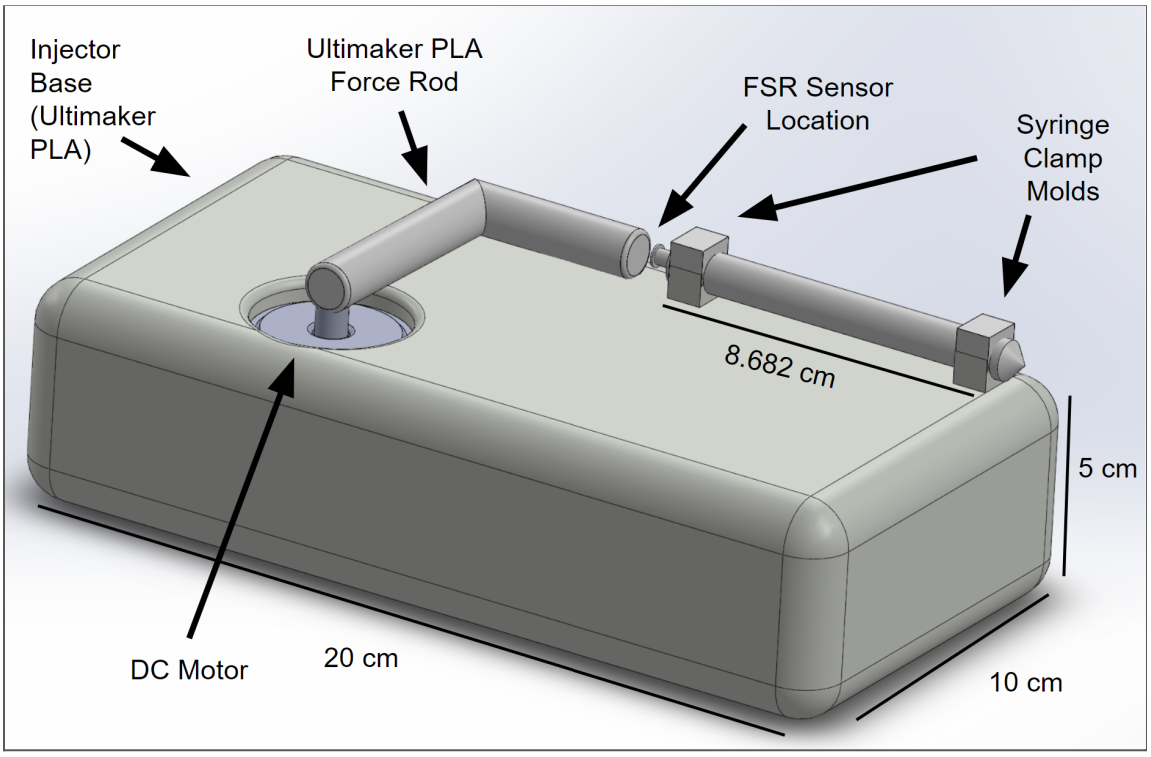


Figure 7: Cellicopter SolidWorks design, highlighting the Brushless Speed Control DC Motor, force application rod, FSR sensor placement, and syringe clamp molds. The injector base is 20 × 10 × 5 cm.

Cellringe Pump: Thread Regulated Injections

The Cellringe Pump design consists of a 20 × 10 × 5 cm base that includes a stepper motor, a threaded rod injection regulator, an Ultimaker PLA force application block, and adjustable syringe clamp molds (see Figure 8 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 × 8 × 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. 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 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 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 injections rates with a high degree of efficacy. 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 exchangeable and multi-sized syringe clamp molds as the Cellicopter design (see the *Cellicopter: Propeller Controlled Injections* section above). This device will thus be compatible with syringes ranging from 1 mL to 20 mL in volume. Syringes have to be manually inserted into and removed from the injector before and after injections. Within this design, the medical grade tubing and catheter will be able to 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.

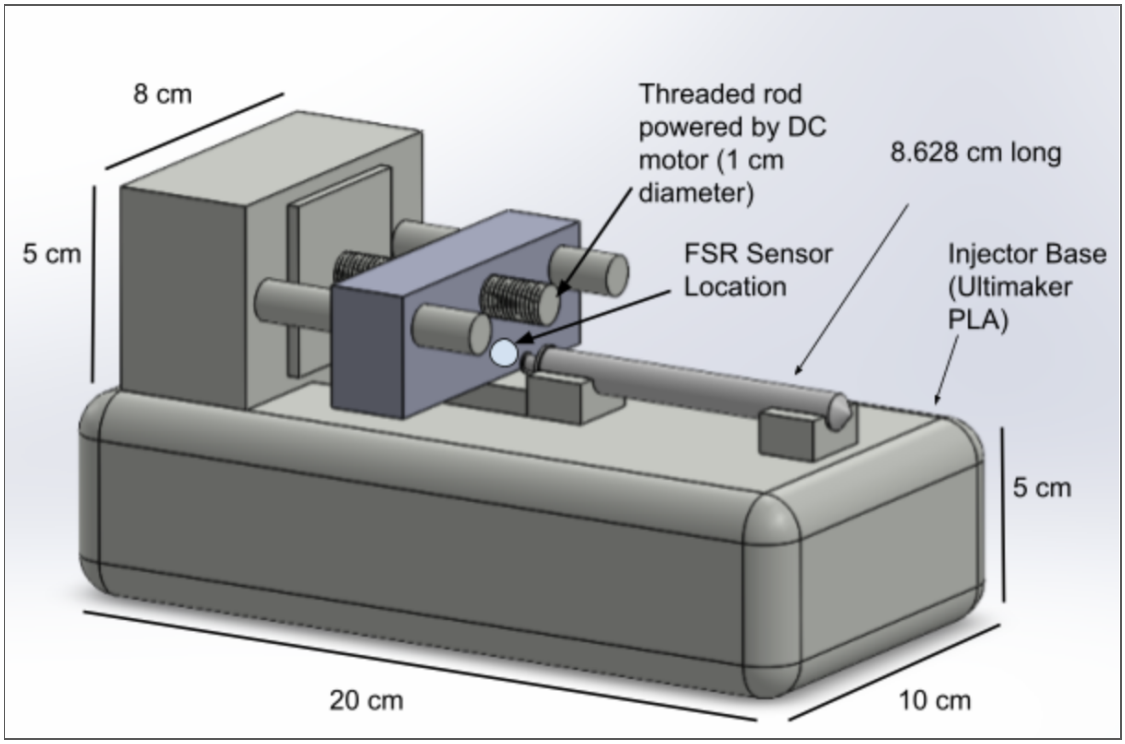


Figure 8: Cellringe Pump Sketch, highlighting the threaded force application control rod, DC Motor force application control, and the FSR sensor location. The injector base is 20 × 10 × 5 cm.

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 9 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 9 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. 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.

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, all designed to lock a specific syringe size in place. As a result, there will be multiple wheels available, each containing different sized syringe insertion sites to accommodate the required 1 - 20 mL syringe size range. 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. Following the insertion of the desired amount of correctly sized syringes into the wheel, a single button click will begin the automation. A syringe will be rotated into its injection position and its distal end will be 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 be moved 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 automation sequence will repeat until all syringes have performed injections.

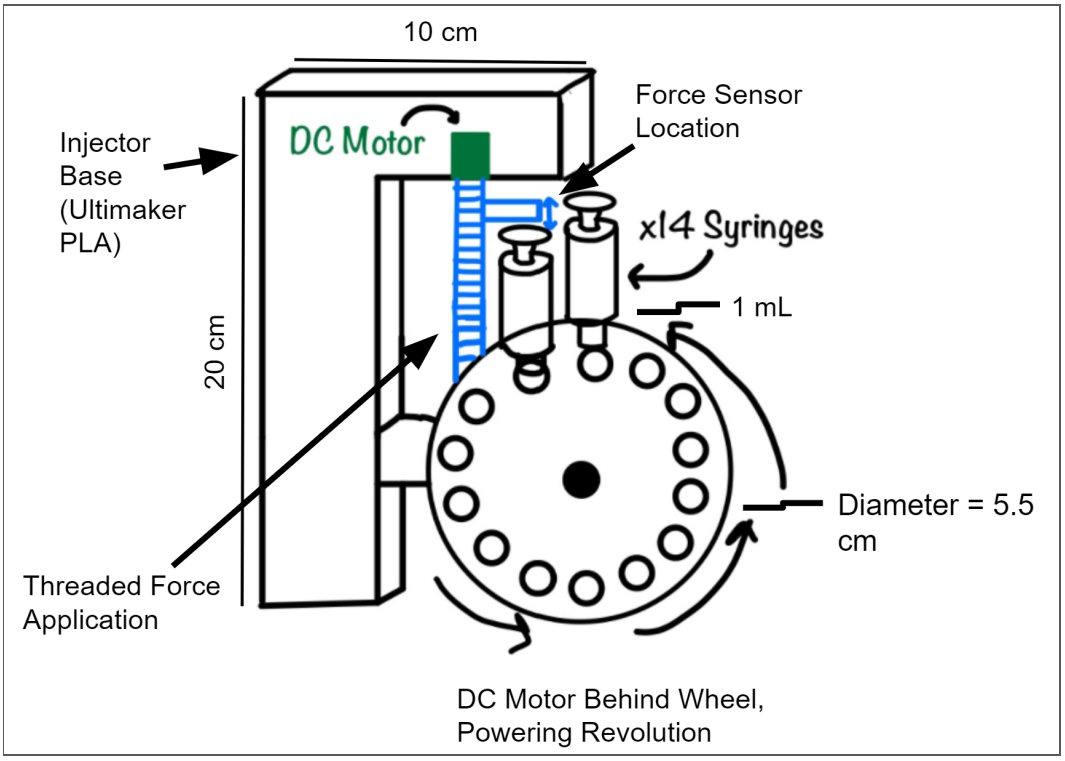


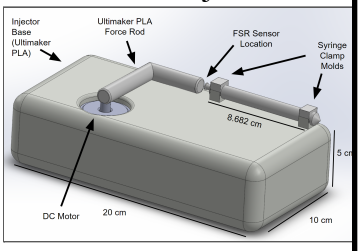
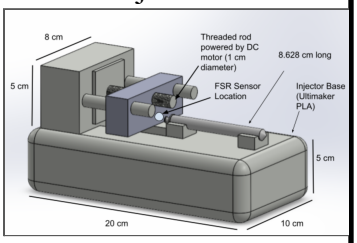
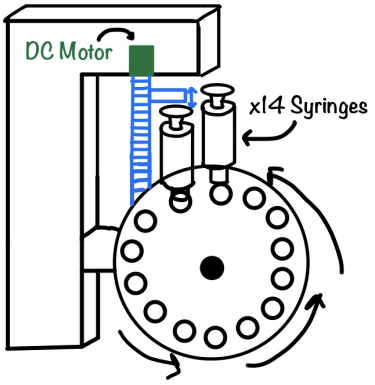
Figure 9: Cellvolver Sketch, highlighting automatic syringe rotation system, two DC motors, and its threaded force application mechanism.

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 		Cellringe 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

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.

Proposed 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. More details on the specifications of the design can be found in the *Cellringe Pump: Thread Regulated Injections* section above. The Cellringe Pump will be fabricated using carefully formulated procedures to quickly and effectively construct the device in order to prepare for testing. The *Materials* section outlines the things that will be purchased and used throughout the course of fabrication. All testing and pre-fabrication steps that need to be completed can be found in the *Methods* and *Pre-Fabrication Testing* sections below.

Fabrication/Development Process

Materials

Overview

The force sensor based visual feedback system will consist of an FSR 400 Series Round Force Sensing Resistor [33], an Arduino Uno microcontroller [34], Arduino IDE [37], and standard circuitry wires, resistors, LEDs, and adhesive breadboards [38]. See Section 3.1 and Section 3.2 of the Appendix for the technical data sheets of the FSR sensor and the Arduino Uno microcontroller. The injector base, stepper motor storage compartment (includes force application block support rods), force application block, and syringe clamp molds will be fabricated with Ultimaker PLA (see Section 3.3 of the Appendix for the technical data sheet of the PLA) [39]. The automated injection will be controlled by an NEMA-17 stepper motor [36], an Arduino Uno microcontroller [34], Arduino IDE [37], and a fully threaded nylon bolt [40]. See Section 3.4 and Section 3.5 of the Appendix for the technical data sheets of the stepper motor and threaded bolt. A nylon hex nut (see Section 3.6 of the Appendix for the technical data sheet of the nut) [41] will be embedded within the Ultimaker PLA force application block to allow for the threaded rod to control its movement and thus syringe plunger compression. Standard clinical syringes (1 - 20 mL), 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 [33]. Typical standard syringes used within the intramyocardial stem cell injection procedure have plunger diameters ranging from 9 mm to 12.07 mm [32], 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 [33], 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) [19]. This high sensitivity force sensor will allow for 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 as well as its ability to withstand bleach, CaviWipes, and ethylene oxide sterilization further promotes its use as the injector force sensor [33].

Microcontroller and Software

The Arduino Uno microcontroller has a very high sensitivity to voltage change with a resolution of 4.88 mV [34]. This will allow 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 will allow for accurate microcontroller ADC value designations based on the voltage input from the voltage divider circuit, that can be 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, allowing it to easily drive and accurately control the revolution rate of the stepper motor and thus the force application (to the syringe plunger) and injection rates [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 will be easy to transport and will not create a burden during procedures and storage [34]. One of the main benefits of using the Arduino Uno microcontroller is the Arduino IDE software program that is coupled with it. This program can take the precise and accurate voltage readings from the microcontroller and perform calculations to convert these inputs into force values based on a standard curve. These values can then be displayed in real-time to the operator throughout the injections, assisting in the success of the procedure. Code will also be used to turn on 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 can also be coded to enable the microcontroller to input a specified voltage value to the stepper motor, controlling its rotation 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.

Visual Feedback Circuitry

The circuit jumper wires, 2.2 kΩ resistor, and adhesive breadboards 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 blue, green, orange, and red LEDs. 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, and connect the microcontroller to the four LEDs. These connections allow for the circuit and visual feedback system to function. The 2.2 kΩ resistor acts as the second resistor in the voltage divider circuit since it will result in the voltage divider having a voltage output ranging from 0.11 V to 2.12 V based on the expected force range that is

applied to the FSR and its corresponding resistance values (100 kΩ and 3 kΩ respectively). This allows for a large enough voltage output 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 its durability and resistance to cyclic fatigue, as well as its low ± 5% tolerance that will reduce error [42]. The Sparkfun adhesive breadboards were specifically chosen as they are relatively small (83.5 × 54.5 × 8.5 mm) and can strongly adhere to the side of the injector base [43]. The small size will minimize the interference of the breadboard with the overall injector functionality and MSC injection procedure and the sturdy connection to the injector will decrease the distance the jumper wires have to travel, limiting the susceptibility to loose connections and thus feedback system error. The LEDs are used to provide the visual feedback for the operator and are controlled by the Arduino Uno microcontroller and its IDE software. These materials were also chosen for the feedback system as they are all able to withstand the sterilization techniques the injector will be exposed to [38]. 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, allowing the injector base, stepper motor storage compartment (includes force application block support rods), force application block, and syringe clamp molds to be fabricated with a high degree of detail, complexity, and accuracy. This will allow these features to interact together successfully and correctly, allowing for controlled injection rates that meet the 30 and 60 second requirements. The high quality generation of the syringe clamp molds will allow them to properly interact with each other and the desired syringe, 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, force application block, and syringe clamps that will be able to withstand all repeated forces applied to the injector throughout each procedure. 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, the stress generated on this block (cross-sectional area of contact 6.362 x 10^{-5} m^2) at the maximum applied force was calculated using Equation 1 below [44].

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

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 result of this durability, 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. Ultimaker PLA is also able to withstand bleach, CaviWipes, and ethylene oxide gas sterilization [45], solidifying this material as an adequate choice to use throughout the injector that will allow for successful injections.

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 nylon fully threaded bolt and hex nut were chosen to control the force application of the block to the syringe plunger as a result of their ability to withstand bleach, CaviWipes, and ethylene oxide gas sterilization. Typical aluminum, steel, or stainless steel bolts and hex nuts are not resistant to these sterilization techniques and are thus not compatible with the injector [45]. As these two components are composed of nylon plastic, they are lightweight and nonconductive. This increases the portability of the injector and eliminates any concerns associated with the circuitry from the feedback system or stepper motor interacting with the threaded rod system. Using nylon also provides the bolt and hex nut with higher strength and durability compared to typical metal fasteners, corrosion resistance, decreased wear on mating parts, and good bearing properties [40, 41, 46]. This 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 metal bolts and hex nuts.

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

Methods

A majority of the outer base fabrication process will rely on using Ultimaker PLA in conjunction with the NEMA-17 stepper motor and nylon threaded bolt (see the *Materials* section above for more information on the described materials). The outer base, push block, and push block outer rods will all be designed using the CAD software SolidWorks and made of the Ultimaker PLA material that is created through the 3D printer machines at the University of Wisconsin-Madison MakerSpace Lab. All CAD created pieces of the base will be compiled into an assembly with each other to confirm correct dimensioning and any tolerances applied to the

3D printed products. The outer base will be designed to hold and conceal the NEMA-17 stepper motor and all required wiring systems implemented throughout the injection device. The nylon threaded bolt will be fabricated to fit over the stepper motor in a way that allows for a complete transition of power from the motor to the threaded screw, and from the threaded screw to the push block. The FSR sensor will be adhesively applied to the push block and positioned so there is minimal interaction interference between the FSR and syringe plunger. Wiring systems coming from the FSR to Arduino Microcontroller will 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 will be long enough to allow free movement of the FSR that is attached to the push block.

Testing

Pre-Fabrication Testing

Prior to fabrication of the injection device, there are tests that need to be made in order to effectively integrate the force sensing system. Such tests include calibration of the FSR and recording the force applied when injecting into various tissues while using the designated flow rates. This will be done prior to fabrication because these tasks are essential to the coding and circuitry involved with the Arduino Uno microcontroller.

Calibration of the FSR with the applied circuitry will include multiple tests of known applied forces being distributed across the active zone of the FSR. Because the expected range of force values does not surpass 3.0 N, the calibration can be performed with small enough increments that will maximize the accuracy of the FSR. Starting from 0.0 N and going to 3.0 N, 0.25 N increments will be added to the FSR using an MTS machine. The voltage values recorded from the Arduino Uno microcontroller at each increment will be recorded. Following 5 separate cycles of 0.25 N increments, the voltage values at each increment will be averaged and applied to an equation deriving the force that is sensed from the FSR.

Force applied while the catheter is experiencing healthy tissue injection, unhealthy tissue injection, blockage, or no tissue will be recorded and used for implementation of the feedback system installed in conjunction with the force sensing system. These 4 separate values will establish the thresholds for each light that corresponds to what the catheter is experiencing. These tests will be performed by placing the FSR on the syringe plunger and using the calibrated code and circuit to measure the force applied to the syringe plunger by the tester.. Tests for blockage can be performed by pinching the catheter or medical grade tubing to replicate any backup of the stem cells and pushing against the syringe plunger. Tests for injection with an absence of tissue can be performed by placing the catheter into liquid that replicates the fluidic properties of interstitial fluid near the myocardium and testing the force provided when moving the syringe plunger. Tests for healthy and unhealthy myocardium tissue can be performed by using bovine steak of different density and properties to determine what is most replicable to healthy tissue and what is the threshold difference between healthy and unhealthy tissue. Every

test will be performed a minimum of 5 times, with the average of each test resulting in the determination of the threshold values for the force feedback system.

Post-Fabrication Testing

Following fabrication of the injection device, various tests will be performed to ensure the device provides accurate and reliable results regarding liquid volume dispensed, flow rate timing, and the force sensing system installed. This will allow the team to find any errors in the prototype's mechanics and circuitry, giving clear evidence of where the error occurred. Practice clinical trial tests will also be performed to distinguish if the injection device can perform the desired stem cell injection procedure safely and efficiently.

Testing of the volume of solution dispensed following the conclusion of a 30 or 60 second injection will determine injector reliability. Before the desired clinical syringe is loaded with solution, a weigh boat containing 0.5 mL of water will have its total weight recorded in grams using a calibrated scale. The scale should be zeroed when it only contains the weigh boat prior to the addition of water. The syringe will then be loaded with this 0.5 mL water solution and connected to the medical grade tubing and catheter system. Following complete connection, the syringe will be inserted into the injector. The injector will undergo the volume dispensing test when the desired injection rate (30 or 60 seconds) is selected and the delivery of water begins. The injectate will be released into the weight boat that was used during the weight measuring step. After the system halts following the completion of the injection, the weigh boat and water complex will be weighed again. This value will be compared to the weight recorded prior to syringe loading. This procedure will be performed using both the 30 second and 60 second injection rates, with each rate being tested three times, for a total of six tests. During each test, the pre-measured volumes of water must be within 0.05 g of the 0.5 mL target (0.45 - 0.55 g). The compared weights of water prior to syringe loading and following injection need to be within 0.05 g of each other to ensure the injection device fully and reliably dispenses the entire 0.5 mL solution from the loaded syringe. Although this initial testing will be performed using water, the end goal is to follow the same procedure, but with the aliquot solution that will be delivered into the myocardium during an actual procedure. The number of trials for each injection rate will be limited by the aliquot solution available. The flow rate timing of the device must be accurate to mitigate all safety and efficiency concerns that arise from a rate that is too fast or slow. This measured distance of the syringe plunger will also affect the validity of the programmed timing system as inaccurate measurements will change the desired speed of the device's injection apparatus. Using a syringe loaded with 0.5 mL of water, the injection device and a stopwatch, both the 30 second and 60 second injection rates will be tested for statistical accuracy of the programmed timing system. Once the 0.5 mL loaded syringes are placed in the device, either the 30 second or 60 second injection rate button will be selected. The stopwatch will be started simultaneously with perceived contact between the syringe plunger and push block. The stopwatch will then be stopped once the device stops all movement turning threaded rod and affected push block. Both the 30 second and 60 second injection rates will be tested 3

times to confirm validity. Results of 0.5 seconds within the target injection rate provide enough statistical accuracy to assume the device's injection rate characteristics are safe enough to be used in clinical procedure settings.

Accurate force readings from the FSR-Arduino Uno program readings will be tested, along with the integrated feedback system provided by the four different colored LEDs. Known forces in Newtons will be applied to the FSR that is attached to the push block. Readings of Newtons from the Arduino IDE program that has been calibrated with the FSR will be compared to the known applied force from an MTS machine. 0.5 N increments will be applied, going from 0.0 to 2.5 N to the FSR setup. At each 0.5 N increment, the force will be measured from the serial monitor of the Arduino program and compared to the known applied force. Force values within 5% of the known applied force will be accepted as an accurate reading. Five cycles will be performed from 0.0 to 2.5 N to ensure validity of the force readings. The lighted feedback system will also be tested using known applied forces from the MTS machine. During the five cycles of 0.5 N increments, every threshold displaying light's range of forces will be measured during the time the light is on. These range of forces will be compared with the expected range of values determined through prior research and testing of healthy and unhealthy tissues. Range thresholds that are within 5% of the expected threshold value will be considered accurate and allow the user to trust the injecting device to provide accurate and reliable feedback of the force applied to the syringe plunger.

Discussion

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 [47]. 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 a 15 second 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 testing must approved prior by the Institutional Animal Care and Use Comittee (IACUC). During a procedure, animals must remain under anesthesia for the entirety of the procedure and aseptic technique must be used whenever possible [48]. 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 [49]. 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 [50].

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 [51]. 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 [52]. 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, inconclusive stem cell viability testing results, incorrect dwell time, improper injection time, incorrect volume dispensed, and flaws in using bovine steak as a model for the myocardium. The injection device must be able to provide accurate force feedback within 5% consistently and light up the correct LED so that the user knows where the catheter is injecting into the myocardium. Additionally, it is possible that the team may encounter difficulties during

MSC viability testing. The cell viability testing performed by the previous team yielded inconclusive results as there was no significant difference in viability when cells were subjected to shear forces compared to when they were only subject to the pressure of the injection. The previous team also found that viability increased after injection which is not possible. Possible sources of error 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% [53]. Other sources of error may include a dwell time outside the specified range of 15 seconds ± 0.5 seconds, injection time outside the specified range of 60 seconds ± 0.5 seconds and 30 seconds ± 0.5 seconds, and volume dispensed must be within 0.5% of 0.5 mL. Finally, using bovine steak as a model for the myocardium is an imperfect model thus could lead to error in correctly identifying the force value that corresponds to obstruction of the catheter in the myocardium. There will also be errors associated with each component that is fabricated from 3D printed Ultimaker PLA. The Ultimaker S5 3D printer that will be used to print each Ultimaker PLA component contains a tolerance, resulting in slight variations in precision and accuracy during each 3D print [54]. 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

Summary

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 the procedural syringes (1 - 20 mL), 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. The motor will power the rotation of the threaded rod which will drive the block towards the syringe plunger to dispense the stem cell solution into the catheter tubing. The FSR will be attached to the force application block and the Arduino IDE interface will constantly display the force value applied. Finally, a feedback system consisting of

a blue LED light to signify the catheter is located in air or a body cavity, a green LED light to indicate healthy myocardium tissue, an orange LED to signify scarred or diseased myocardium tissue, and a red LED to indicate that the force threshold has been exceeded and the catheter may be backed up will be incorporated.

Future Work

Moving forward, the team will work to create an effective prototype. The team plans to 3D print the injector base using Ultimaker PLA because it is able to withstand the appropriate sterilization techniques. Followed by fabrication of the multi-light force feedback system and desired injection automation. The team plans to begin with the FSR 400 Force Sensing Resistor and if it does not meet requirements purchase a new sensor. The team plans to begin force gauge testing using the previous team's force values as a starting point and use bovine steak injections to obtain force values that correspond to forces in the myocardium. Additionally, the team plans to use MSCs to test stem cell viability under different forces to ensure that there is no more than 5% cell death from starting viability using trypan blue staining. Other tests to be performed will ensure that the prototype dispenses the appropriate amount of liquid accurately each time (0.5 mL) achieving the desired flow rate, flow rate timing of the device, and force readings from the FSR-Arduino Uno program readings along with the integrated feedback system provided by the 4 different colored LEDs. While this is a good first step towards creating an automatic stem cell delivery injection device, there still will be user interaction with the device to change out the 1 mL syringes which could 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.

References

[1] Centers for Disease Control and Prevention, “Leading Causes of Death,” *Centers for Disease Control and Prevention*, 2019. https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm.

[2] G. F. Tomaselli and D. P. Zipes, “What Causes Sudden Death in Heart Failure?,” *Circulation Research*, vol. 95, no. 8, pp. 754–763, Oct. 2004, doi: 10.1161/01.res.0000145047.14691.db.

[3] J. Duan, I. Pillai, Y. Lu, J. Huang, and R. Fritz, “Cardiac Repair and Regeneration Research Unit | Heart and Vascular Services at UCLA,” *Uclahealth.org*, 2019. https://www.uclahealth.org/heart/cardiac-repair-regeneration.

[4]M. Rheault-Henry, I. White, D. Grover, and R. Atoui, “Stem cell therapy for heart failure: Medical breakthrough, or dead end?,” *World Journal of Stem Cells*, vol. 13, no. 4, pp. 236–259, Apr. 2021, doi: 10.4252/wjsc.v13.i4.236.

[5] “Heart Failure: Risk Factors | University Health Care System,” *www.universityhealth.org*. https://www.universityhealth.org/heart-failure/risk-factors/.

[6] A. N. Raval et al., “Point of care, Bone Marrow Mononuclear Cell Therapy in Ischemic Heart Failure Patients Personalized for Cell potency: 12-month Feasibility Results from CardiAMP Heart Failure roll-in Cohort,” International Journal of Cardiology, vol. 326, pp. 131–138, Mar. 2021, doi: 10.1016/j.ijcard.2020.10.043.

[7]“Baxter Infus or Syringe Pump ABC 4100 Trade in Program.” Wilburn Medical Equipment and Supplies, https://wilburnmedicalusa.com/baxter-infus-or-syringe-pump-abc-4100-trade-in-program/

[8] “Infus O.R. Syringe Pump Refurbished,” *4MD Medical Solutions*. https://www.4mdmedical.com/index.php/catalog/product/view/id/253457/s/infusor-syringe-pump-refurbished?CAWELAID=120141310000056386&%3BCAGPSPN=pla&%3Bgclid=CjwKCAiAo4OQBhBBEiwA5KWu_8f3uyspUWEZJ9eX8-duXozZ5qbgAjz8DOYtmfDnjrIz8zi4LdJ5RxoCcrIQAvD_BwE (accessed Mar. 01, 2022).

[9] “User’s Guide ACIST | CVi® Contrast Delivery System,” Acist Medical Group, Aug. 2019. Accessed: Feb. 07, 2022. [Online]. Available: http://acist.com/wp-content/uploads/2019/09/901418-00301-Users-Guide-CVi-MU-English-USA.pdf.

[10] Leoni, G., Lyness, A., Ginty, P., Schutte, R., Pillai, G., Sharma, G., Kemp, P., Mount, N., & Sharpe, M. (2017). Preclinical development of an automated injection device for intradermal delivery of a cell-based therapy. Drug delivery and translational research, 7(5), 695–708. https://doi.org/10.1007/s13346-017-0418-z

[11] P. Mounce et al., “Medicament cassette, automatic injector, and automatic injector system,” May 09, 2019.

[12] M. H. Amer, F. R. A. J. Rose, L. J. White, and K. M. Shakesheff, “A Detailed Assessment of Varying Ejection Rate on Delivery Efficiency of
Mesenchymal Stem Cells Using Narrow-Bore Needles,” Stem Cells Translational Medicine, vol. 5, no. 3, pp. 366–378, Mar. 2016, doi: 10.5966/sctm.2015-0208.

[13] Lenneman AJ, Birks EJ. Treatment strategies for myocardial recovery in heart failure. Curr Treat Options Cardiovasc Med. 2014 Mar;16(3):287. doi: 10.1007/s11936-013-0287-9. PMID: 24492922.

[14] R. N. Doughty, S. MacMahon, and N. Sharpe, “Beta-blockers in heart failure: Promising or proved?,” Journal of the American College of Cardiology, vol. 23, no. 3, pp. 814–821, Mar. 1994, doi: 10.1016/0735-1097(94)90773-0.

[15] M. J. Everly, “Cardiac transplantation in the United States: an analysis of the UNOS registry,” Clinical Transplants, pp. 35–43, 2008, [Online]. Available: https://pubmed.ncbi.nlm.nih.gov/19708444/.

[16] J. H. Park, D. Dehaini, J. Zhou, M. Holay, R. H. Fang, and L. Zhang, “Biomimetic nanoparticle technology for cardiovascular disease detection and treatment,” Nanoscale Horizons, vol. 5, no. 1, pp. 25–42, 2020, doi: 10.1039/c9nh00291j.

[17] E. G. Schmuck et al., “Intravenous Followed by X-ray Fused with MRI-Guided Transendocardial Mesenchymal Stem Cell Injection Improves Contractility Reserve in a Swine Model of Myocardial Infarction,” Journal of Cardiovascular Translational Research, vol. 8, no. 7, pp. 438–448, Oct. 2015, doi: 10.1007/s12265-015-9654-0.

[18] A. Hmadcha, A. Martin-Montalvo, B. R. Gauthier, B. Soria, and V. Capilla-Gonzalez, “Therapeutic Potential of Mesenchymal Stem Cells for Cancer Therapy,” Frontiers in Bioengineering and Biotechnology, vol. 8, no. 43, Feb. 2020, doi: 10.3389/fbioe.2020.00043.

[19]A. Vo, M. Doumit, and G. Rockwell, “The Biomechanics and Optimization of the Needle-Syringe System for Injecting Triamcinolone Acetonide into Keloids,” Journal of Medical Engineering, vol. 2016, 2016, doi: 10.1155/2016/5162394.

[20] C. for Disease Control and Prevention, “Sterilization,” Centers for Disease Control and Prevention, 2019. https://www.cdc.gov/infectioncontrol/guidelines/disinfection/sterilization/index.html (accessed Feb. 08, 2022).

[21] W.-P. Nasco, “Whirl-Pak® Sterile Sampling Bags (Nasco),” www.weberscientific.com, 2022. https://www.weberscientific.com/whirl-pak-sterile-sampling-bags-nasco-2 (accessed Feb. 08, 2022).

[22] C. Institute, “Sodium Hypochlorite,” The Chlorine Institute, 2022. https://www.chlorineinstitute.org/stewardship/sodium-hypochlorite/#:~:text=Sodium%20hypochlorite%20(NaOCl)%20is%20a (accessed Feb. 08, 2022).

[23] D. Disposables, “CaviWipes Disinfectant Wipes,” Discount Disposables | Infection Control Products, 2022. https://www.infectioncontrolproducts.com/products/caviwipes-disinfectant-wipes#:~:text=Cavi%2DWipes%20Active%20Ingredients%3A%20Isopropanol (accessed Feb. 08, 2022).

[24] F. and D. Administration, “Guidance for Industry and FDA Staff: Technical Considerations for Pen, Jet, and Related Injectors Intended for Use with Drugs and Biological Products,” Food and Drug Administration, 10903 New Hampshire Avenue, WO-32 Hub 5129 Silver Spring, MD 20993, Jun. 2013. Accessed: Feb. 08, 2022. [Online]. Available: https://www.fda.gov/files/about%20fda/published/Technical-Considerations-for-Pen--Jet--and-Related-Injectors-Intended-for-Use-with-Drugs-and-Biological-Products.pdf.

[25] Food and Drug Administration, “CFR - Code of Federal Regulations Title 21,” Fda.gov, 2019. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=820 (accessed Feb. 09, 2021)

[26] F. and D. Administration, “CFR - Code of Federal Regulations Title 21 Part 870,” www.accessdata.fda.gov, Jan. 06, 2022. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=870.5100 (accessed Feb. 08, 2022).

[27] F. and D. Administration, “CFR - Code of Federal Regulations Title 21 3.2,” Code of Federal Regulations, Feb. 07, 2022. https://www.ecfr.gov/current/title-21/chapter-I/subchapter-A/part-3/subpart-A/section-3.2 (accessed Feb. 08, 2022).

[28] F. and D. Administration, “CFR - Code of Federal Regulations Title 21 Part 610,” U.S. Food and Drug Administration, Jan. 06, 2022. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=610 (accessed Feb. 08, 2022).

[29] ISO, “ANSI/AAMI/ISO 11135:2014 - Sterilization of Health Care Products - Ethylene Oxide - Requirements for development, Validation and Routine Control of a Sterilization Process for Medical Devices,” International Organization for Standardization, 2014. Accessed: Feb. 08, 2022. [Online]. Available: https://webstore.ansi.org/standards/aami/ansiaamiiso111352014.

[30] ISO, “ANSI/AAMI/ISO 11137 - Sterilization of Health Care Products Package,” International Organization for Standardization, 2017. Accessed: Feb. 08, 2022. [Online]. Available: https://webstore.ansi.org/standards/aami/ansiaamiiso11137sterilization.

[31] Dr. A. Raval, “Manufacturing cost of final automatic injection system product,” Jan. 2022

[32] C. Inc., “BD Plastic Syringe | Maximum & Minimum Flow Rates,” Chemyx Inc, Dec. 21, 2017. https://www.chemyx.com/uncategorized/bd-plastic-syringe-minimum-maximum-flow-rates/ (accessed Feb. 15, 2022).

[33] 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.

[34] A. Staff, “Arduino Uno Rev3,” Arduino.cc, 2019. https://store.arduino.cc/usa/arduino-uno-rev3 (accessed Feb. 25, 2021).

[35] O. Motor, “Speed Control Motors - Variable Speed Motors,” Orientalmotor, 2020. https://www.orientalmotor.com/speed-control-motors/index.html (accessed Feb. 15, 2022).

[36] A. Industries, “Stepper Motor - NEMA-17 Size - 200 steps/rev, 12V 350mA,” www.adafruit.com, 2022. https://www.adafruit.com/product/324?gclid=Cj0KCQiAjc2QBhDgARIsAMc3SqQi_8rpHenYK-iXt9KMn2Y-33zOxCpePZHOu_C26oTAriVpTp67CgQaArgPEALw_wcB (accessed Feb. 21, 2022).

[37] Arduino, “Arduino - H,” www.arduino.cc, 2022. https://www.arduino.cc/en/math/h.

[38] S. Electronics, “UW-Madison BME Supply List (BME 310 and BME 201) - SparkFun Electronics,” www.sparkfun.com, 2021. https://www.sparkfun.com/wish_lists/122199.

[39] Ultimaker, “Technical Data Sheet - PLA,” Nov. 2018. Accessed: Dec. 02, 2021. [Online]. Available: https://ultimaker.com/materials/pla.

[40] W. W. G. Inc., “Fully Threaded Rod, Nylon, 1/4"-20, 2 Ft Length, Off-White,” Grainger, 2022. https://www.grainger.com/product/GRAINGER-APPROVED-Fully-Threaded-Rod-2KA63.

[41] 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).

[42] S. Electronics, “Resistor Kit - 1/4W (500 total) - COM-10969 - SparkFun Electronics,” www.sparkfun.com, 2021. https://www.sparkfun.com/products/10969.

[43] S. Electronics, “Breadboard - Self-Adhesive (White) - PRT-12002 - SparkFun Electronics,” www.sparkfun.com, 2021. https://www.sparkfun.com/products/12002.

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

[45] I. O. for Standardization, ISO 9001:2015 - Plastics Compatibility with Sterilization Methods | ISM. ISM, 2022.

[46] C. Plastics, “Nylon Plastic | Strong, Stiff, Bearing & Wear Material | Curbell Plastics,” Curbellplastics.com, 2022. https://www.curbellplastics.com/Research-Solutions/Materials/Nylon#:~:text=Nylon%20is%20a%20strong%2C%20stiff (accessed Feb. 27, 2022).

[47] S. H. Mun, D. A. An, H. J. Choi, T. H. Kim, J. W. Pin, and D. C. Ko, “Lethal coronary air embolism caused by the removal of a double-lumen hemodialysis catheter: a case report,” Korean Journal of Anesthesiology, vol. 69, no. 3, pp. 296–300, Jun. 2016, doi: 10.4097/kjae.2016.69.3.296.

[48] M. Ghasemi and A. R. Dehpour, “Ethical considerations in animal studies,” Journal of medical ethics and history of medicine, vol. 2, p. 12, 2009, [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3714121/.

[49] American Psychological Association, “Guidelines for Ethical Conduct in the Care and Use of Animals,” https://www.apa.org, 2012.

[50] “Euthanasia,” American Veterinary Medical Association, 2007. https://www.avma.org/resources/pet-owners/petcare/euthanasia.

[51] V. Volarevic et al., “Ethical and Safety Issues of Stem Cell-Based Therapy,” International Journal of Medical Sciences, vol. 15, no. 1, pp. 36–45, Jan. 2018, doi: 10.7150/ijms.21666.

[52] J. Lowenthal and J. Sugarman, “Ethics and Policy Issues for Stem Cell Research and Pulmonary Medicine,” Chest, vol. 147, no. 3, pp. 824–834, Mar. 2015, doi: 10.1378/chest.14-1696.

[53] “Accuracy and Precision With the Countess II FL Automated Cell Counter - US,” www.thermofisher.com. https://www.thermofisher.com/us/en/home/life-science/cell-analysis/cell-analysis-learning-center/cell-analysis-resource-library/cell-analysis-application-notes/accuracy-precision-countess-ii-fl-automated-cell-counter.html#:~:text=When%20manually%20counting%20cells%20using (accessed Feb. 27, 2022).

[54] Ultimaker, “Ultimaker S5: Reliability at Scale,” ultimaker.com, 2022. https://www.ultimaker.com/3d-printers/ultimaker-s5 (accessed Feb. 28, 2022).

Appendix

Section 1: Product Design Specifications

Product Design Specification

Automatic Intraoperative Stem Cell Injection Device

Team Heartthrob
Lab 307
March 2nd, 2022

Team Members:	Parker Esswein	Team Leader
	Macy Frank	Communicator
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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 the procedural syringes (1 - 20 mL), 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 syringes ranging from 1 mL - 20 mL, securely locking each syringe in place during the intramyocardial injection procedure
- Materials must be capable of being sterilized with standard cleaning techniques, namely bleach, CaviWipes, and ethylene oxide
- The entire injector must be able to fit inside a sterile bag during storage and each 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
- 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 µL/sec while the 60 second rate represents a delivery of 8.33 µL/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 ranging from 1 - 20 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 15 second dwell time [1]. Any blockage or backflow within the syringe or catheter must be monitored. Force values above a threshold of 2.4 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 blue LED light will signify that the catheter is injecting the MSC aliquot solution into air or a body cavity, corresponding to a detected force value less than 0.6 N, an orange LED light indicates the transmission of injectate into healthy myocardium tissue, a green LED light resembles the dispensing of the cells into diseased or scarred myocardium tissue, and a red LED light demonstrates that the 2.4 N threshold has been 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.

Sterilization of the device is mandatory and will be performed during and in between every procedure. A sterility assurance level of 10^{-3} will be mandated whenever the device is sterilized [6]. See the *Standards and Specifications* section for more information about the sterility standards.

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 0.5% 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 5% of the actual applied force and be able to output the same force reading three times in a row for the same force application. 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 15 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 mannequin will experience typical clinical operating room conditions; room temperature (20 ^{o}C to 25 ^{o}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) [12], 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 [13]. All electrical and circuitry components will be coated with plastic insulations and covered by compounds that are able to withstand ethylene oxide. 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 ^{o}C to 25 ^{o}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.

The requirement for sterility within the operating room and during the procedure will result in the device being cleaned with bleach or CaviWipes and kept within a sterile bag throughout each procedure [14]. These bags are sterilized with ethylene oxide [11]. Prior to and following each procedure, the device may be exposed to ethylene oxide sterilization [14]. These sterilization techniques require the entire injector to be capable of withstanding sodium hypochlorite (bleach) [15], isopropanol (Cavi Wipes) [16], and ethylene oxide. The injector will not experience heat-based sterilization, namely autoclaving, eliminating the need for the device to have a maximum melting point.

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, however, sterile bags (ethylene oxide) are the only sterilization technique they will be exposed to.

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 [17] and the injector should be stopped by the operator when it applies a force greater than 2.4 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 [18] 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 × 20 × 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 m^2 and contains large equipment such as anesthesia machines, a stretcher, monitors, sterilization equipment, and a surgical table [19]. 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) [20]. 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 device will be used in a sterile operating room thus it is imperative that the materials are able to be sterilized with bleach, CaviWipes, and ethylene oxide. It can not be made of metals that are corrosive to bleach such as copper or stainless steel. It must be made of materials that are porous enough to allow for ethylene oxide gas to penetrate the material as ethylene oxide sterilization penetrates well through porous materials. It can not be made of plastic that may deform at 60 ℃ [21]. Ethylene oxide sterilization is compatible with nearly every polymer [22]. As a result, a 3D printable plastic material such as Ultimaker PLA would be compatible with the sterilization methods required [17].

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 - 20 mL syringes is needed.

b. *Target Product Cost*:

The device should be manufactured at a cost of no more than $500 [23]. 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 [24]. 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 [25]. 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 [26]. 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 [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 proper agency designation and to meet premarket review requirements. The Food and Drug Administration (FDA) outlines the testing and sterilization specifications for combination injection products within "Technical Considerations for Injectors Intended for Use with Drugs and Biological Products". This guidance recommends that sterile injection devices have a sterility assurance level (SAL) of 10^{-6} unless they are only intended for intact skin contact, resulting in a SAL suggestion of 10^{-3} [6]. As the injector needs to be sterile, but will not be in contact with internal regions of the body, it should contain a probability of a non-sterile unit less than 10^{-3}. 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.

ISO 11135:2014 provides requirements for the development, validation, and routine control of an ethylene oxide sterilization process for medical devices in health care facility settings [29]. ISO 11137 considers medical device sterilization by addressing the environment of the product, personnel and their hygiene, packaging and storage of the device, and the sterilization doses required to inactivate microbiological contaminants [30]. The injector requires sterilization before, during, and after each intramyocardial stem cell injection procedure, specifically ethylene oxide sterilization, so it will need to follow these two ISOs in order for proper and effective sterilization techniques to be performed.

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.

Another patient risk of the procedure is embolisms. Due to the fact that the syringes have to be connected to connection tubing and then to the catheter, there is a risk of air bubbles being inserted into the system. Currently, the operation is done with 10 - 14 different one mL syringes, each containing 0.5 mL of solution, that need to be exchanged throughout the procedure. The 0.5 mL solution needs to be dispensed at a rate of at least 30 seconds so there is a better chance of the cells not being rejected by the body. Ideally, this device should lower the risk of the embolisms since it must be compatible with 5 mL and 10 mL syringes and can be programmed to inject the 0.5 mL MSC solution 10 - 14 times, eliminating the syringe exchange requirement during the procedure.

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.

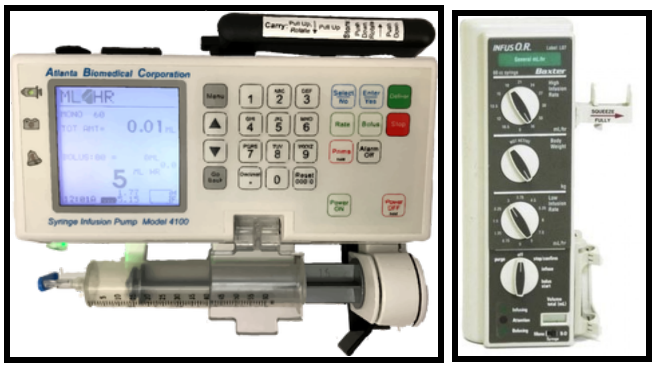

Figure 1: The "Baxter Infus OR Syringe Pump ABC 4100" which can be obtained for ~$3000 using the trade-in program (left image) [20]. The "Baxter Infus O.R. Syringe Pump Refurbished" that is sold for ~$4000 (right image) [24].

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.

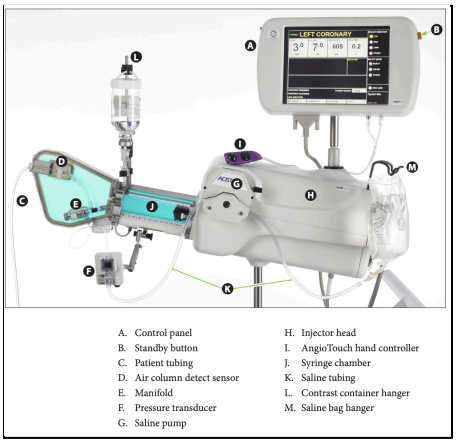

Figure 2: Diagram of the important user components in the Acist Contrast Delivery System a [31].

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 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.

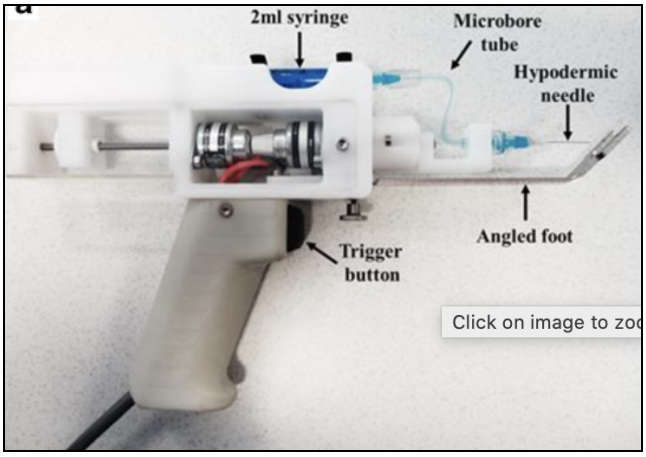

Figure 3: Automated injector device that was in preclinical development in 2017 [32].

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.

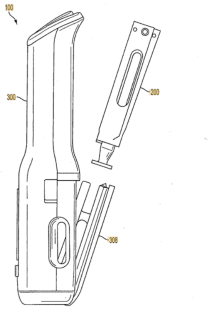

Figure 4: The cassette automatic injector device [33].

References:

[1] A. N. Raval et al., "Point of care, Bone Marrow Mononuclear Cell Therapy in Ischemic Heart Failure Patients Personalized for Cell potency: 12-month Feasibility Results from CardiAMP Heart Failure roll-in Cohort," International Journal of Cardiology, vol. 326, pp. 131–138, Mar. 2021, doi: 10.1016/j.ijcard.2020.10.043.

[2] A. Vo, M. Doumit, and G. Rockwell, "The Biomechanics and Optimization of the Needle-Syringe System for Injecting Triamcinolone Acetonide into Keloids," *Journal of Medical Engineering*, vol. 2016, 2016, doi: 10.1155/2016/5162394.

[3] 14:00-17:00, "ISO 14971:2019," *ISO*, Dec. 2019. https://www.iso.org/standard/72704.html.

[4] ISO - International Organization for Standardization, "IEC 60601-1-11:2015," *ISO*, 2015. https://www.iso.org/standard/65529.html.

[5] 14:00-17:00, "ISO 10993-1:2018," *ISO*, Oct. 2018. https://www.iso.org/standard/68936.html.

[6] F. and D. Administration, "Guidance for Industry and FDA Staff: Technical Considerations for Pen, Jet, and Related Injectors Intended for Use with Drugs and Biological Products," Food and Drug Administration, 10903 New Hampshire Avenue, WO-32 Hub 5129 Silver Spring, MD 20993, Jun. 2013. Accessed: Feb. 08, 2022. [Online]. Available: https://www.fda.gov/files/about%20fda/published/Technical-Considerations-for-Pen--Jet--and-Related-Injectors-Intended-for-Use-with-Drugs-and-Biological-Products.pdf.

[7] Oja S, Kaartinen T, Ahti M, Korhonen M, Laitinen A and Nystedt J (2019) The Utilization of Freezing Steps in Mesenchymal Stromal Cell (MSC) Manufacturing: Potential Impact on Quality and Cell Functionality Attributes. *Front. Immunol.* 10:1627. doi: 10.3389/fimmu.2019.01627

[8] "Brushless DC Motors | Low Prices, High Quality," *www.anaheimautomation.com*. https://www.anaheimautomation.com/marketing/brushless-dc/brushless-dc-motors.php .

[9] Primex, "Laboratory Temperature and Humidity Requirements & Compliance | Primex," www.primexinc.com, Dec. 22, 2020. https://www.primexinc.com/en/blog/laboratory-temperature-humidity-requirements.html (accessed Sep. 21, 2021).

[10] M. Bell, "What is the average air pressure at the surface of the Earth?," Columbia.edu, 2019. https://iridl.ldeo.columbia.edu/dochelp/QA/Basic/atmos_press.html (accessed Sep. 21, 2021).

[11] W.-P. Nasco, “Whirl-Pak® Sterile Sampling Bags (Nasco),” www.weberscientific.com, 2022. https://www.weberscientific.com/whirl-pak-sterile-sampling-bags-nasco-2 (accessed Feb. 08, 2022).

[12] A. Lerma, “Lithium-Ion Vs Lead Acid Battery Life,” www.fluxpower.com, Mar. 14, 2019. https://www.fluxpower.com/blog/lithium-ion-vs.-lead-acid-battery-life (accessed Feb. 09, 2022).

[13] H. Miles, “How Long Does Electrical Wiring Last in a Home?,” Home Inspection Insider, Jan. 08, 2022. https://homeinspectioninsider.com/how-long-electrical-wiring-last/#:~:text=The%20average%20life%20expectancy%20of (accessed Feb. 08, 2022).

[14] C. for Disease Control and Prevention, “Sterilization,” Centers for Disease Control and Prevention, 2019. https://www.cdc.gov/infectioncontrol/guidelines/disinfection/sterilization/index.html (accessed Feb. 08, 2022).

[15] C. Institute, “Sodium Hypochlorite,” The Chlorine Institute, 2022. https://www.chlorineinstitute.org/stewardship/sodium-hypochlorite/#:~:text=Sodium%20hypochlorite%20(NaOCl)%20is%20a (accessed Feb. 08, 2022).

[16] D. Disposables, “CaviWipes Disinfectant Wipes,” Discount Disposables | Infection Control Products, 2022. https://www.infectioncontrolproducts.com/products/caviwipes-disinfectant-wipes#:~:text=Cavi%2DWipes%20Active%20Ingredients%3A%20Isopropanol (accessed Feb. 08, 2022).

[17] Ultimaker, “Technical Data Sheet - PLA,” Nov. 2018. Accessed: Dec. 02, 2021. [Online]. Available: https://ultimaker.com/materials/pla.

[18] A. Schwirtz and H. Seeger, “Are Adrenaline Autoinjectors Fit for purpose? a Pilot Study of the Mechanical and Injection Performance Characteristics of a cartridge- versus a syringe-based Autoinjector,” Journal of Asthma and Allergy, vol. 3, p. 159, Nov. 2010, doi: 10.2147/jaa.s14419.

[19] “Design distinctions for exam, procedure and operating rooms,” www.hfmmagazine.com, Oct. 08, 2019. https://www.hfmmagazine.com/articles/3764-design-distinctions-for-exam-procedure-and-operating-rooms#:~:text=While%20the%20minimum%20requirements%20for (accessed Feb. 06, 2022).

[20] “Baxter Infus or Syringe Pump ABC 4100 Trade in Program.” Wilburn Medical Equipment and Supplies, https://wilburnmedicalusa.com/baxter-infus-or-syringe-pump-abc-4100-trade-in-program/.

[21] “GUIDANCE DOCUMENT Compatibility of materials used for Sterile Barrier Systems with sterilisation processes,” Aug. 2017. Accessed: Feb. 08, 2022. [Online]. Available: https://sterilebarrier.org/wp-content/uploads/2019/12/Sterilisation-compatibility-August-2017.pdf.

[22] “Ethylene Oxide - an overview | ScienceDirect Topics,” www.sciencedirect.com, 2013. https://www.sciencedirect.com/topics/engineering/ethylene-oxide (accessed Feb. 11, 2022).

[23] Dr. A. Raval, “Manufacturing cost of final automatic injection system product,” Jan. 2022

[24] “Baxter Infus O.R. Syringe Pump Refurbished.” 4MD Medical Solutions, https://www.4mdmedical.com/index.php/catalog/product/view/id/253457/s/infusor-syringe-pump-refurbished?CAWELAID=120141310000056386&CAGPSPN=pla&gclid=CjwKCAiAo4OQBhBBEiwA5KWu_8f3uyspUWEZJ9eX8-duXozZ5qbgAjz8DOYtmfDnjrIz8zi4LdJ5RxoCcrIQAvD_BwE.

[25] Food and Drug Administration, “CFR - Code of Federal Regulations Title 21,” Fda.gov, 2019. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart=820 (accessed Feb. 09, 2021)

[26] F. and D. Administration, “CFR - Code of Federal Regulations Title 21 Part 870,” www.accessdata.fda.gov, Jan. 06, 2022. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=870.5100 (accessed Feb. 08, 2022).

[27] F. and D. Administration, “CFR - Code of Federal Regulations Title 21 3.2,” Code of Federal Regulations, Feb. 07, 2022. https://www.ecfr.gov/current/title-21/chapter-I/subchapter-A/part-3/subpart-A/section-3.2 (accessed Feb. 08, 2022).

[28] F. and D. Administration, “CFR - Code of Federal Regulations Title 21 Part 610,” U.S. Food and Drug Administration, Jan. 06, 2022. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=610 (accessed Feb. 08, 2022).

[29] ISO, “ANSI/AAMI/ISO 11135:2014 - Sterilization of Health Care Products - Ethylene Oxide - Requirements for development, Validation and Routine Control of a Sterilization Process for Medical Devices,” International Organization for Standardization, 2014. Accessed: Feb. 08, 2022. [Online]. Available: https://webstore.ansi.org/standards/aami/ansiaamiiso111352014.

[30] ISO, “ANSI/AAMI/ISO 11137 - Sterilization of Health Care Products Package,” International Organization for Standardization, 2017. Accessed: Feb. 08, 2022. [Online]. Available:

https://webstore.ansi.org/standards/aami/ansiaamiiso11137sterilization.

[31] Acist | CVI® Contrast Delivery System. http://acist.com/wp-content/uploads/2019/09/901418-00301-Users-Guide-CVi-MU-English-USA.pdf.

[32] Leoni, G., Lyness, A., Ginty, P., Schutte, R., Pillai, G., Sharma, G., Kemp, P., Mount, N., & Sharpe, M. (2017). Preclinical development of an automated injection device for intradermal delivery of a cell-based therapy. Drug delivery and translational research, 7(5), 695–708. https://doi.org/10.1007/s13346-017-0418-z

[33] P. Mounce *et al.*, "Medicament cassette, automatic injector, and automatic injector system," May 09, 2019.

Section 2: Voltage Divider Circuit Calculation and Simulation

Voltage Divider Circuit:

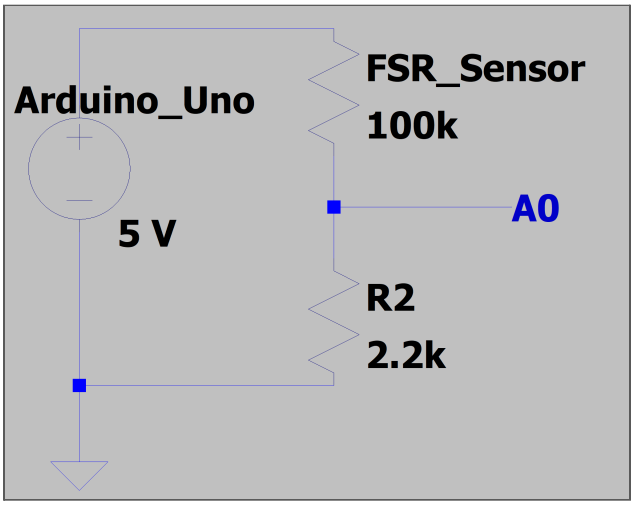

Figure 1: Voltage Divider Circuit containing the FSR sensor, a 5 V DC voltage source, and an output going into A0 of the Arduino Uno microcontroller.

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 kΩ and 3 kΩ, 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 = FSR Sensor (100 kΩ or 3 kΩ)
R_2 = 2.2 kΩ
V_{in} = 5 V

R_1 = 100 kΩ
$V_{in} * R_2 = 5 * 2.2 = 11$ V * kΩ
$V_{out} = 11 / (2.2 + 100) = 0.108$ V ≈ 0.11 V

R_1 = 3 kΩ
$V_{in} * R_2 = 5 * 2.2 = 11$ V * kΩ
$V_{out} = 11 / (2.2 + 3) = 2.115$ V ≈ 2.12 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 kΩ and 3 kΩ.

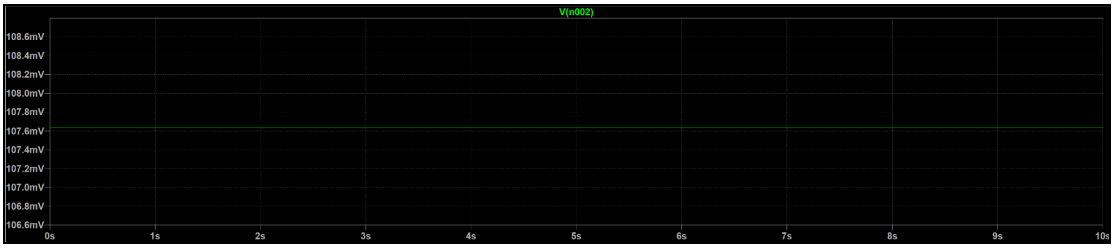

Figure 2: LTspice simulation of the V_{out} from a voltage divider circuit containing a 100 kΩ resistor for R_1 and a 2.2 kΩ resistor for R_2. The voltage output is about 0.108 V which can be estimated as 0.11 V.

Figure 3: LTspice simulation of the V_{out} from a voltage divider circuit containing a 3 kΩ resistor for R_1 and a 2.2 kΩ resistor for R_2. The voltage output is about 2.115 V which can be estimated as 2.12 V.

As the LTSpice simulations display a 0.11 V and 2.12 V voltage output when the FSR sensor has a resistance of 100 kΩ and 3 kΩ, respectively, it supports the 0.11 V - 2.12 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.

References:

[1] S. Staff, “Voltage Dividers - learn.sparkfun.com,” *Sparkfun.com*, 2019. https://learn.sparkfun.com/tutorials/voltage-dividers/all (accessed Feb. 21, 2022).

[2] 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.

[3] A. Staff, “Arduino Uno Rev3,” Arduino.cc, 2019. https://store.arduino.cc/usa/arduino-uno-rev3 (accessed Feb. 25, 2021).

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 Repeatability[3]	(Single part)	± 2%	
Force Resolution[3]		continuous	
Force Repeatability[3]	(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
Hysteresis[3]		+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: 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/m²	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 3.4: 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=Cj0KCQiAjc2QBhDgARIsAMc3SqQi_8rpHenYK-iXt9KMn2Y-33zOxCpePZHOu_C26oTAriVpTp67CgQaArgPEALw_wcB (accessed Feb. 21, 2022).

Section 3.5: Grainger Nylon Fully Threaded Bolt Technical Data Sheet

Nylon fully threaded rods and studs are nonmagnetic and nonconductive, making them ideal for use in electronics applications. Lightweight and corrosion resistant, they are often used instead of metal fasteners in light-duty furniture applications. Plastic fully threaded rods and studs are corrosion-resistant fasteners used to connect parts together that require extended reach or lengths. Lightweight and nonconductive, plastic fasteners provide higher strength and durability than metal fasteners, making them a good choice when connecting components in electronics applications.

Table 1: Grainger Nylon 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	Nylon	Meets/Exceeds	IFI
Threaded Rod Color	Off-White	Rockwell Hardness	Not Rated
Thread Size	1/4"-20	Temp. Range	-40 Degrees to 185 Degrees F
Length	2 ft	Thread Class	2A

References:

[1] W. W. G. Inc., “Fully Threaded Rod, Nylon, 1/4"-20, 2 Ft Length, Off-White,” Grainger, 2022. https://www.grainger.com/product/GRAINGER-APPROVED-Fully-Threaded-Rod-2KA63.

Section 3.6: 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 3.7: 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 (6.362×10^{-5} m^2) [1].

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

Force Application Block:

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

Maximum Force = 3.00 N

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

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.