Regenerative Medicine Minnesota Final Progress Report

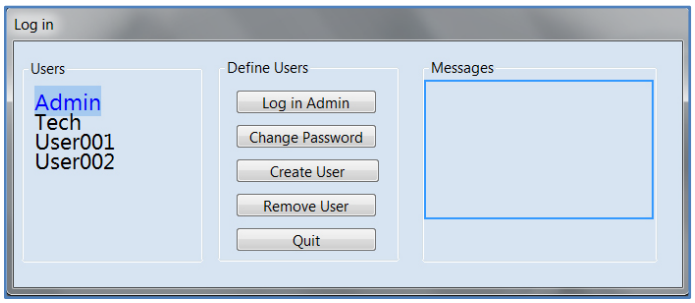
Grant Title: Regenerative Medicine Bioreactor Drive System Development
Grant Number: RMM-2016-BB-03
Requester: Kent Vilendrer
Project Timeline: June 1, 2016 – May 30, 2017 no cost extension to 2/28/2018

Brief description of project: This project's goal was to address the shortcomings of current tissue engineering bioreactors by producing a fully-automated, computer-controlled, and scalable bioreactor drive system (BDS) suitable for use in large-scale GMP manufacturing facilities. A key aspect of the project is that the BDS is designed to be compatible with a large range of customer-provided bioreactor chambers. The dynamic flow generated by the BDS can be used for cardiovascular, orthopedic or other engineered tissue applications that require oscillatory flow.

Where did this project take place? ST3 Development Corporation, Minnetonka, MN.

People impacted by project and where they are from: The first group impacted by the project is the Cardiovascular Tissue Engineering research group led by Dr. Robert Tranquillo at the University of Minnesota. Drs. Zeeshan Syedain and Robert Tranquillo were the project's collaborators and the beneficiaries of receiving the first bioreactor drive system developed through this project.

What was the outcome of the project? A modular and scalable bioreactor drive system was developed with GMP-compliant StimWorks™ software that meets 21CFR part 11 requirements to protect electronic records and provide audit trail documentation. Figure 1 shows the login options available that allow access to different capabilities and actions in the software and the user level permissions matrix.



trol Parameters No

Figure 1: Login options (left) and user level permissions matrix (right).

The BDS is comprised of the following hardware: BDS linear drive with up to 4 pump cartridges, control box, and laptop computer (Figure 2). Additional components include: cables, power supply (100-240 VAC that powers the BDS control box and linear drive), and optional transducers. The BDS linear drive contains a single linear motor with a moving magnet design supported by a flexural assembly that provide an infinite fatigue life. The position of the linear motor is very precisely controlled by a linear displacement transducer that is embedded in the linear drive system. The motor shafts can be connected with up to 4 pump cartridges per linear drive system, two on each side of the linear drive box as shown in Figure 2. The pump cartridges have bellows and flow inlet and outlet ports that convert the motor's linear motion into volumetric output. The pump cartridges are then connected to the customer's bioreactor chamber flow loop using the inlet and outlet ports. One to four pump cartridges can be connected to a single linear drive

system so that multiple samples can be run under identical conditions to increase tissue production. Multiple linear drive systems can be controlled simultaneously by the same laptop so that engineered tissue manufacturing can be easily scaled up as needed. The pump cartridges and all parts in contact with tissue/medium and flow loop are disposable and independently designed for each sample to prevent cross-contamination between samples and allow harvesting of individual samples during culture.

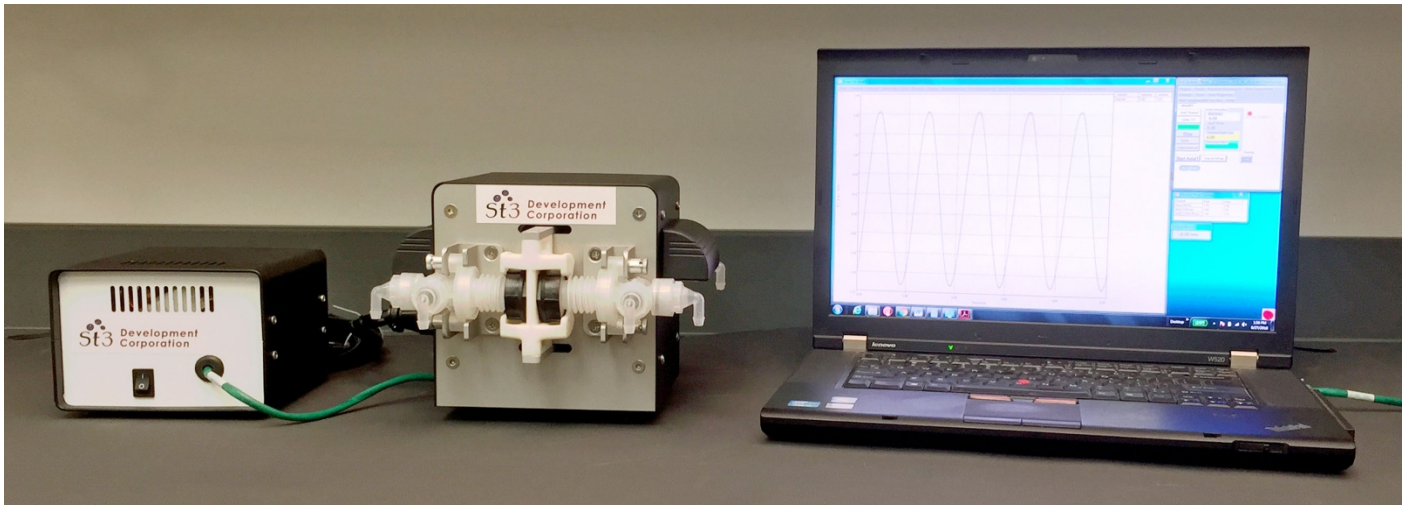


Figure 2: Main Components of the Bioreactor Drive System (BDS): control box (left), linear drive system with pump cartridges (middle), laptop computer (right).

A one-way check valve inside the pump cartridge assembly prevents backflow and creates a forward moving oscillatory flow through the engineered tissue. The check valve can be removed if desired. A mean flow pump such as a peristaltic or centrifugal style pump can be connected to the pump cartridge and the software to create superimposed flow and high flow rates, depending on the application requirements. Several transducers such as pressure, flow rate and strain transducers can also be fully integrated in the system and serve as feedback control channels depending on the application. The prototype system is equipped with one pressure transducer to be used with any of the pump cartridges, and one laser micrometer that measures strain and can be rotated between flow loops to measure sample radial strain. The pressure transducer is disposable and is obtained sterile so it can be assembled under sterile conditions. The laser micrometer is external and does not contact the cell culture media or the sample.

The oscillatory flow stimulation parameters are programmed into the StimWorks™ software (Figure 3). Operators can control and configure individual linear drive systems and track data for each sample. Patient ID tracking helps identify each sample manufactured by the BDS and log activity history enables quality control and data integrity. Users can enter the desired number of cycles (experiment duration) as well as limits for different measurements such as pressure. A variety of standard waveforms are available for selection such as sinusoidal, triangle, square and ramp, combinations thereof, as well as completely customizable waveforms that can be imported from an excel spreadsheet into the software. Conditional branching allows the definition and input of if/then commands that can be used in the tissue growth phase, for quality control purposes, and/or as production release criteria. For example, an if/then command can be set that if pressure exceeds a certain value, the waveform amplitude should be reduced by a specified amount. System alerts to the operators can also be set up so that users receive an email and/or text message when pre-specified events occur during manufacturing. These automation and programmability capabilities are essential components of successfully manufacturing engineered tissues that often require stimulation adjustments during culture to produce patent tissues.

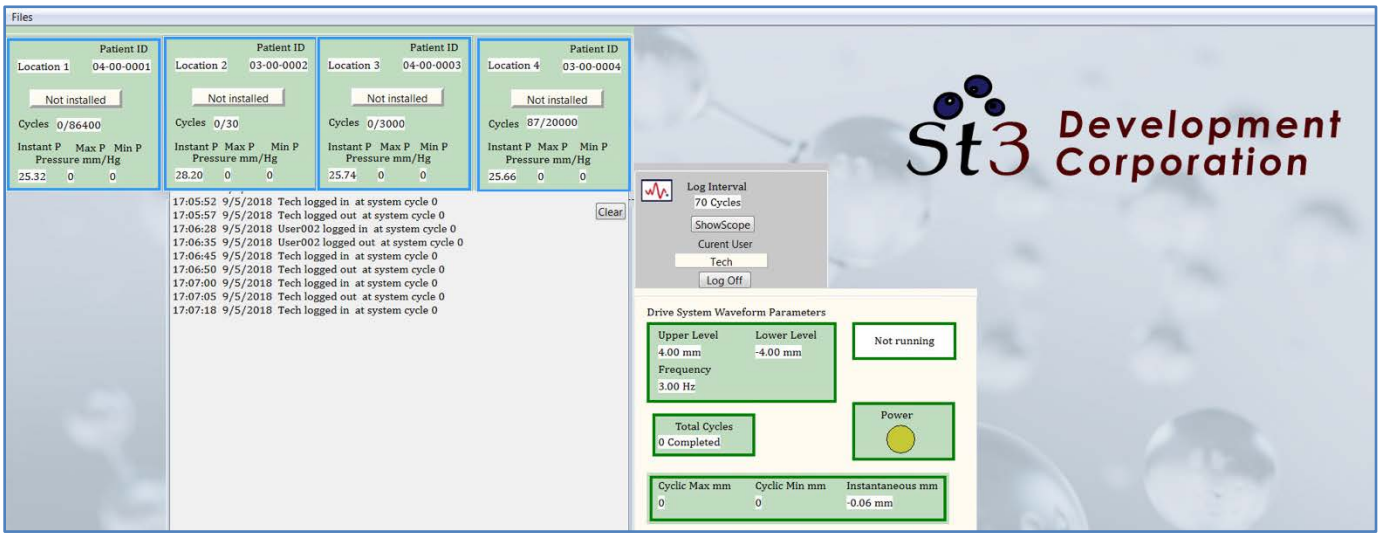


Figure 3: Operator Panel Identification and Drive System Waveform Parameters and Status.

Product design requirements were captured at the start of the project from our collaborator, ST3 Development engineers who have been designing bioreactors for over 20 years and our regenerative medicine consultant. A product validation plan was also developed, and our prototype system was used for validation testing. Finite element analysis was performed to assess the motor flexure durability, and it was found that stresses in the flexures were within limits for infinite fatigue life. The BDS performed as expected in terms of electrical testing (motor inductance, resistance, and time constant, processor output voltage, and analog output), force constants at different displacements, displacement vs. current, motor stiffness, volumetric flow output and pressure range.

Most engineered tissue applications employ low frequency waveforms for tissue stimulation, typically up to 5 Hz, with some exceptions such as vocal cord tissue culture. While the linear motor of the BDS has exceptional dynamic performance (up to hundreds of Hz), its performance in the system is determined by the frame stiffness of the linear drive system and is rated to 10 Hz for the BDS which covers the vast majority of engineered tissue applications. BDS performance testing assessed the dynamic performance of the actuator with one vs. four pump cartridges installed. Having four pump cartridges installed increases resistance and is therefore worst case scenario for motor performance. The graphs in Figure 4 show the displacement and corresponding flow rate output when four pump cartridges are installed over the range of the frequency rating. Higher displacement and stroke volume can be achieved at lower frequencies, and higher flow rates (stroke volume multiplied by frequency) are achieved at higher frequencies. As a reminder, constant flow pumps can be added to the system, as previously mentioned, to create superimposed flow and higher flow rates if desired.

System ergonomics and incubator compatibility were also part of the design requirements. The BDS can be placed either inside or outside a cell culture incubator. The linear drive system's small form factor (approximately 6"x6"x6") and low power (~10 Watts) motor allow for the placement of several systems within a standard cell culture incubator without incubator overheating.

System operation in a GMP-compliant facility requires complete documentation that outlines system use, troubleshooting, maintenance plans, calibration certificates, spare parts list, disposables list, etc. This information has been captured in the user manual provided with the BDS. Furthermore, there are no

lubricants in the BDS that could contaminate engineered tissues, and system surfaces are not reactive, additive or absorptive, per FDA regulations.

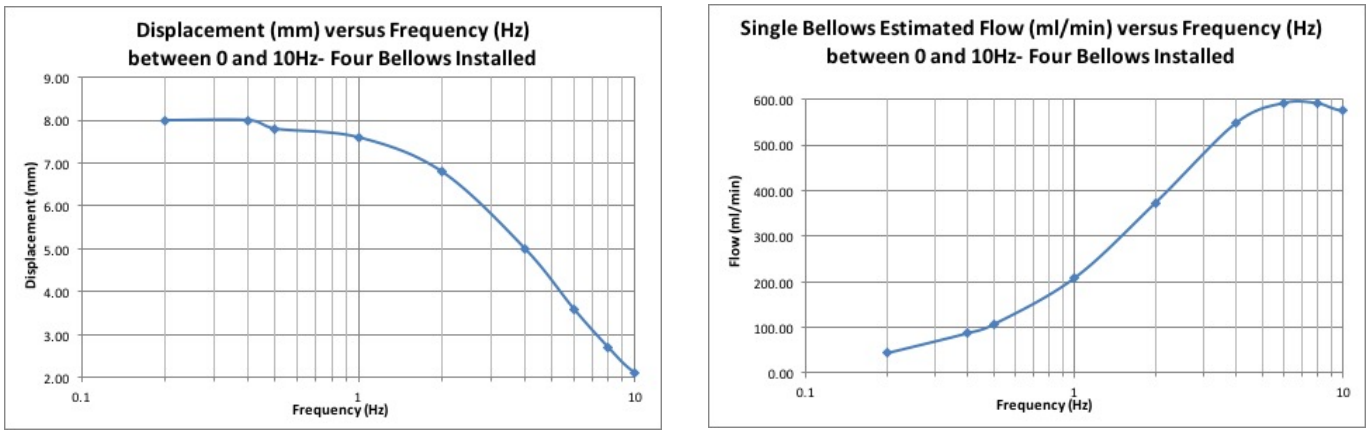


Figure 4: Bioreactor Drive System Dynamic Performance.

ST3 Development Corporation has joined the Advanced Regenerative Manufacturing Institute (ARMI) BioFabUSA consortium, with core funding from the Department of Defense, to form deeper connections in the regenerative medicine community, gather more information about the tissue manufacturing bioreactor requirements tissue developers have, and to more broadly disseminate information about the BDS and receive feedback. ARMI's mission is perfectly aligned with ST3's objectives: make practical the large-scale manufacturing of engineered tissues and tissue-related technologies, to benefit existing industries and grow new ones. ST3 presented the project results at a recent ARMI workshop hosted by the University of Minnesota (http://www.dmd.umn.edu/2018/ARMI-BioFab.html). The BDS is also available to the regenerative medicine community worldwide and has been added to ST3's product offerings (http://st3corp.com/products-overview.cfm).

Next steps include delivery of the prototype system to our collaborator at the University of Minnesota where the BDS will be used to culture tissue-engineered vascular grafts. Tissue outcomes will be compared against results previously obtained from homebuilt bioreactor drive systems, and feedback will be provided to ST3 regarding the system, its operation, capabilities and documentation that ST3 will utilize for continuation engineering and product improvements.

Responsible Spending:
Funds were spent according to the proposed budget outlined in the grant application to support hardware and software design, materials and supplies for the bioreactor drive system, and consulting services on user requirements, validation testing, and documentation. Actual spending exceeded the grant allocation by 23% to support software design, additional sensor (laser micrometer for strain monitoring) and hardware assembly. These additional costs were covered by ST3 Development Corporation.