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CELLvo Matrix Manufacturing Improvement

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Final Project Report

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Executive Summary

StemBioSys seeks to streamline their CELLvo matrix manufacturing process in order to decrease the cost of production. The main objectives of this project are to design, build, and test a system that StemBioSys can utilize to complete the decellularization process more quickly, with greater efficiency, and with lower production costs. The following report covers the features of our complete design and its three subsystems that combine to accomplish the individual tasks of the decellularization process. The primary functions of the three subsystems are as follows: the flask organizer holds 6 flasks together throughout the process, the guide positions the flask organizer at the desired angle for aspiration steps, and the liquid network dispenses reagents into the flasks for the washing steps. Following the design overview is an evaluation of the final design through the scope of project requirements and constraints, as well as the associated tests used in assessing the overall system's performance.

The design constraints of sterility, maintaining the integrity of the CELLvo Matrix, remaining within the provided budget, and the available space for the final process were all satisfied through our design solution. The sterility constraint is satisfied with the use of one of the recommended materials in Section 2.6. The integrity of the CELLvo Matrix was confirmed by checking that the matrix was not scratched or damaged during the aspiration or dispensing steps. Our final design was able to complete multiple runs of the decellularization process in a biosafety cabinet (BSC) without any damage to the subsystems or flasks, thus meeting the design constraint for the available space. Our budget was to remain under $1,200, and the AvENGRs spent roughly a total of $223 to satisfy this constraint.

Our final design is held to many project requirements that will be detailed in the following sections. The requirement that our solution is to be optimized for T150 cell culture flasks is met through the three subsystems' abilities to specifically fit T150 flasks. The liquid network subsystem is able to satisfy the requirement of effectively washing away all detergent without damaging the matrices, which contributes to the production of a uniform project. Additionally, the liquid network has multiple frames and bases that allow for the device to be versatile and portable. Moreover, the flask organizer and guide subsystems are designed so they can easily be reproduced and sterilized, and the quantity produced can be scaled to accommodate the various sizes of manufacturing runs. Since our final solution did not alter the foundation of the decellularization process, we maintain the assumption that our subsystems are capable of producing a uniform product, can be integrated into the current process, can be easily adopted by staff and are compatible with OSHA codes and prevailing industry standards. In addition, the process must be performed in a biosafety cabinet and should be able to accommodate any other processes conducted simultaneously, which were both satisfied during prototype testing. Lastly, the implementation of our three subsystems decreases the total time required to complete the decellularization process, satisfying this requirement. Our overall project requirement to reduce Full-Time Equivalents (FTEs) was also satisfied, as a reduction in time decreases the necessary time for one person to work.

Overall, the AvENGRs have created a successful, working prototype to streamline the CELLvo matrix manufacturing process. Since all of our requirements and constraints were met, there are no changes needed to achieve remaining goals, but there is always room for the improvement of our working prototype. As a result of the COVID-19 pandemic, the project remains unfinished in terms of producing several polished copies of each subsystem. Therefore, the AvENGRs have provided manufacturing recommendations should StemBioSys choose to further the development of our solution (Section 2.6 and Appendix 5.4).

1. Introduction

CELLvo Matrix, a cell-derived matrix produced by StemBioSys, is an ideal cell culture substrate that recreates the cell's native microenvironment. The problem StemBioSys has identified involves streamlining the CELLvo manufacturing process in order to decrease cost by minimizing Full-Time Equivalents (FTEs) without sacrificing productivity. FTEs are the hours worked by one employee on a full-time basis. The goal of the solution is to optimize the manufacturing procedure through the design and implementation of devices, not to replace steps in the process or change overall lab organization. An overview of the current process can be found in Appendix 5.3.

The schedule objectives were to perform initial tests of our new device by December 6, 2019; to test a functional prototype by March 6, 2020; and to provide the final deliverables by May 6, 2020. Our project objective is to reduce FTEs without sacrificing productivity or replacing steps in the current process. Our functional requirements include that the modified process shall effectively wash away all detergent without damaging the CELLvo Matrix, which can be verified through the use of a microscope. In addition, the modified process shall be optimized for the size T150 cell culture flask, and the solution should be scalable to work with various sizes of manufacturing runs, as well as versatile and portable to work in different rooms of the facility. In terms of non-functional requirements, the final design shall be reproducible and produce a uniform product that consumers can trust to work, may decrease the total time required to complete the decellularization process, shall be safe, and all tools or devices used in the process shall be able to be sterilized. Uniformity is verified by ensuring the matrix is not damaged when looking under a microscope. In terms of interface requirements, the solution should be able to be integrated into the current process, shall result in the same product or substantially equivalent, should be intuitive and easily adopted by staff with no preexisting knowledge of our solution, should be compatible with prevailing industry standards and may allow the biosafety cabinet to be used for other processes at all times.

The main constraints that were considered throughout the design process pertained to sterility, space available for the process, and maintaining the integrity of the CELLvo Matrix. Firstly, the process must be performed in a sterile environment, specifically within a biosafety cabinet or a clean bench. For any device that is created to streamline the process, each component must be able to be sterilized before being placed in the biosafety cabinet. When working with cell culture flasks, it is also imperative that the vented screw caps remain dry, as any liquid entering the filter would compromise the sterility. The devices being developed are also constrained to fit within the dimensions of a biosafety cabinet or a clean bench. To ensure the integrity of the matrix is not disrupted, the devices must not scrape the surface of the cell culture flask at any point in the decellularization process. In addition, the project cost must remain within a $1200 budget.

This design is governed primarily by the standard operating procedures of StemBioSys. These standards and protocols serve as a reference for laboratory safety guidelines and proper cell culture and sterile techniques. In addition, our devices and process must conform to OSHA

safety standards found in Appendix 5.5, such as:

- The Eye and Face Protection standard (29 CFR 1910.133)
- The Hand Protection standard (29 CFR 1910.138)
- The General Requirements for All Machines (29 CFR 1910.212)

The design is divided into three subsystems that tackle the three main issues we had identified last semester: organization, aspiration, and dispensing. The flask organizer is capable of holding six T150 flasks at one time so that they remain organized in the biosafety cabinet and to make the flasks easily accessible to aspirate from and dispense into. Organizing the biosafety cabinet contributes to satisfying the non-functional requirement to decrease the overall time required to complete the decellularization process, as well as the overall project objective to reduce FTEs. The compact design of the flask organizer allows six flasks to fit securely instead of having technicians pick up each flask one at a time. The guide was designed with the intent to increase consistency during the aspiration steps of the process. The guide features an angle that draws the liquid to the back of the flask, making it easier for technicians to aspirate more quickly. In addition, the guide allows the user to have a free hand instead of holding and positioning each flask individually, decreasing the chance of the user accidentally disrupting the matrix while aspirating. The last subsystem is the liquid network, which was designed with the capability to measure and dispense the required amount of liquid for each reagent. This feature contributes to ensuring the uniformity of the product and to the functional requirement of effectively washing away all detergent. Moreover, the liquid network can range from the use of one to three frames, making it versatile, portable, and scalable to the size of the manufacturing run. The chosen dimensions and material of each subsystem address the sterility and size constraints, as well as the safety and interface requirements.

The AvENGRs also developed assumptions related to the project scope in order to satisfy project requirements and the overall project objective. For example, we assumed the lab technicians utilizing the new devices are familiar with standard sterile techniques and will have access to the appropriate tools and industry standard cell culture materials. This assumption is based on StemBioSys being a small biotechnology company with a laboratory environment that is equipped with standard cell culture equipment and staff that routinely run experiments and manufacture cell-derived research products. In addition, per StemBioSys' standard operating procedures, the current processes being utilized result in some degree of variability in fluid volumes added and removed. Therefore, we are assuming variability within 10% is acceptable. Since our final solution did not alter the foundation of the decellularization process, we also maintain the assumption that our subsystems are capable of producing a uniform product. Lastly, we assumed we would have access to a biosafety cabinet for testing the prototype, similar to the environment at StemBioSys.

2. Overview of the Final Design

Our choice to design three separate but compatible subsystems was supported by our evaluation of existing cell culture technology, the current cell culture techniques and equipment utilized by StemBioSys, and the company's desire to streamline their current process. The MultiFlo FX from Biotek is an automated multi-mode reagent dispenser that StemBioSys uses when working with microplates [1]. This system is able to alternate between aspirating reagents from and dispensing new reagents into the wells of 6 to 384-well microplates and does so without damaging the 2D or 3D cell structures within the wells. The BioStack Microplate Stacker is a separate device that is compatible with the MultiFlo FX and organizes stacks of well plates as it transfers one plate at a time (Figure 1).

Figure 1. BioTek's MultiFlo FX (left) and BioStack Microplate Stacker (right) shown operating on well plates [1]

Although the MultiFlo FX and BioStack are only compatible with well plates, we drew a lot of inspiration for our designs from these devices. The flask organizer subsystem will act in a similar way to the BioStack by keeping stacks of cell culture flasks organized throughout the decellularization process. One aspect of the MultiFlo FX that played into our design of the liquid network was the use of separate dispensing manifolds for different reagents so they do not mix at any point. Aspirating and dispensing manifolds for the MultiFlo FX are also kept as separate entities, which further influenced our decision to create three separate subsystems rather than one unified system: one for organizing (flask organizer), one for aspirating (guide), and one for dispensing (liquid network).

The following sections provide descriptions of our complete system and each of its three subsystems, references to the applicable project requirements and constraints, and explanations of the changes that have been made since the submission of the preliminary design report (PDR). We have also provided a brief summary of the construction methods and equipment used to make the final prototypes of each subsystem. Because we were not able to produce several copies of the flask organizer and guide or construct additional frames for the liquid network as we had hoped, we have also provided recommendations for manufacturing larger quantities of each subsystem.

2.1 Complete System

As previously stated, our final design is composed of three individual subsystems, the flask organizer, guide, and liquid network, that come together to fulfill the primary function of streamlining StemBioSys' decellularization process. After placing six flasks into a flask organizer, the user moves the flasks as a unit from the guide, where aspiration occurs, to the liquid network, where reagents are dispensed. This movement between the two subsystems continues as the user progresses through the steps of the decellularization process. A more detailed description of how the complete system should be set up and operated is provided in Appendix 5.1.

The majority of our project requirements and constraints apply to the complete system and therefore had to be considered for both the design and construction of the individual subsystems and for the design of our solution as a whole (i.e. how the subsystems come together to complete the process). More specifically, we designed our complete system with the following constraints in mind: available space for the process, sterility, maintaining the integrity of the matrix, and remaining within the $1200 budget. In addition, the following requirements were considered: the design solution is intuitive, scalable, versatile, portable, reproducible, compatible with prevailing industry standards, can be integrated into the current process while resulting in the same product, and can be used to satisfy the overall project objective of reducing FTEs; the use of the complete system will also decrease the total time required to complete the process while maintaining a safe environment, all devices should be able to be sterilized, and there should be room in the biosafety cabinet to perform other processes.

The AvENGRs have made several changes to our overall design since the submission of the PDR. First, we have adjusted our sterility and available space requirements related to the use of a biosafety cabinet to also include the use of a clean bench, per Dr. Block's recommendation. A clean bench is similar to a biosafety cabinet in that it supplies the work surface with filtered air to prevent contamination. However, a clean bench does not have the front glass panel that serves to also protect the lab technician and the surrounding environment from aerosols leaving the work surface. We have also chosen to narrow our focus and optimize our design for only the T150 cell culture flasks. Therefore, we have removed the original design requirement of, "The current process shall be optimized for the size T150 cell culture flask, but should be optimized for the size T75 as well." A flask organizer that fits the dimensions of the T75 flasks can be produced if desired by scaling down the current CAD file.

Another major change to our overall design was removing the aspirator adapter from our list of subsystems. This device was intended to allow the user to aspirate from multiple flasks simultaneously. After running preliminary tests in the biosafety cabinet, we noticed that the anticipated size of the aspirator adapter and the action required for inserting the aspirator tips into the flasks would not be ergonomic. In addition, after further evaluation we determined that the time it would take to line the adapter up with the flask organizer might offset the time savings that would be observed from its use. This subsystem has been replaced by the "guide" which has gone through a series of design changes itself. As a result of these changes, instead of aspirating six

flasks at once, the user will aspirate from individual flasks that are contained in the flask organizer and placed on the guide.

2.2 Flask Organizer

The primary function of the flask organizer (Figure 2) is to hold six flasks compactly to allow for easy movement around the workspace and to position the flasks for the aspirating and dispensing steps. To use this subsystem, place the flask organizer flat on the worksurface, orient a stack of six flasks so the matrix is facing to your right, then slide the flasks into the organizer at an angle with the caps facing forward. The main constraint that applies to the design of the flask organizer is that it must be able to be sterilized. To conform to this constraint, 3D printed flask organizers may be sterilized with 70% ethanol, ultraviolet light, or ethylene oxide, or should be printed using a filament that can be autoclaved. Another requirement that specifically applied to the flask organizer was that the design is optimized for the size T150 cell culture flask. This was achieved by basing our original dimensions for the flask organizer on the dimensions of a stack of six T150 cell culture flasks provided by StemBioSys. A gamma-prototype of the flask organizer was 3D printed in the spring semester, and no adjustments have been made to the overall design of this subsystem. However, in regard to the material used for this subsystem, we have moved away from our recommendation of using polypropylene and have provided new suggestions for materials in Section 2.6 and Appendix 5.4 that should help to improve the ease of manufacturing.

Figure 2. Flask Organizer

2.3 Guide

The guide subsystem can be used to position the flasks at the proper angle for the aspiration steps. To use this subsystem, simply slide the flask organizer on the guide until it hits the back support (Figure 3). No additional requirements or constraints other than those listed for the complete system in Section 2.1 applied directly to the design of the guide. Now looking specifically at design changes for the guide subsystem, the angle of the guide began at 30° and was adjusted to the final angle of 25° to increase comfort during the use of our beta-prototype and the final round of testing. The beta-prototype of the guide was also constructed without the additional arms that were used in the alpha-prototype to navigate the aspirator tip into the appropriate position. We removed this component of the guide as we discovered the arms restricted the motion of the aspirator tip and caused limited visibility of the inside of the flask. The use of the arms to

guide the aspirator tip also increased the amount of time it took to place the tip into the flask, which works against our main goal of reducing the time to complete the decellularization process. The remaining wedge component of the guide holds the flasks at a comfortable and steady angle for aspiration instead of the lab technician having to hold the flask organizer with one hand and aspirate with the other.

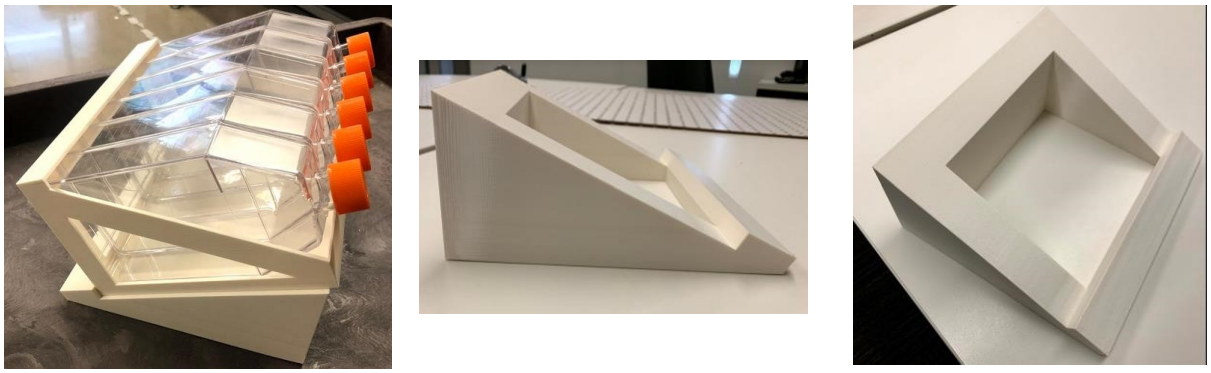


Figure 3. Guide

2.4 Liquid Network

The liquid network (Figure 4) is designed to eliminate the use of a pipette controller by allowing the user to automatically measure and dispense the proper amount of liquid (phosphate buffered saline, deionized water, or detergent) into each flask. The liquid network is composed of a frame and a dispensing mechanism, which consists of the reagent bottle, tubing and adapted shot dispenser (Appendix 5.2). To use the liquid network, place the six flasks contained in the flask organizer directly under the dispensing mechanism that is attached to the reagent bottle and tubing. To dispense, center the dispensing mechanism above one flask and press down to release the pre-measured liquid, then repeat for the remaining five flasks. The requirements that applied directly to the design of the liquid network subsystem included effectively washing away all detergent without damaging the matrix, creating a system that is versatile and portable, and producing a uniform product.

The primary change made to the liquid network since the PDR is that it is no longer designed to dispense into six flasks simultaneously. Instead, liquid will still be dispensed into flasks one at a time while they are held in the flask organizer. This change was made in an effort to reduce the amount of space that is taken up by the liquid network in the BSC or clean bench. An assumption for the acceptable level of liquid volume variability (<10%) has also been added since the PDR to conform to StemBioSys' standard operating procedures. Adjustments to the dimensions of the liquid network were made between the construction of the alpha-prototype and the design of the beta-prototype in order to increase stability. A remaining design change that was not able to be addressed due to the change to remote learning was adapting the dispensing mechanism to accommodate 15ml. One potential solution to this is to add a set amount of hydrophobic material to the dispensing mechanism container, reducing the possible liquid volume from 30 to 15ml.

Figure 4. Liquid Network

2.5 Construction Methods and Equipment Used

Each of our three subsystems were constructed using equipment provided in the Trinity University Makerspace. The gamma-prototype of the flask organizer and beta-prototype of the guide were printed using a Stratasys Fortus 250 3D printer and ABS plastic filament. These prototypes and the final Fusion 360 CAD files are included in our final deliverables to StemBioSys. The alpha-prototype of the liquid network was constructed using various wood-working tools/machines in the Makerspace. A list of materials and construction steps for this alpha-prototype are provided in Appendix 5.2. Due to the COVID-19 pandemic, we were not able to construct and test a beta-prototype in March as planned. A detailed list of suggested materials and construction steps for the beta-prototype are provided in Appendix 5.2, along with recommended tests for evaluating this updated prototype.

2.6 Manufacturer Recommendations

A summary of the quotes we received from custom 3D printing companies such as Stratasys and Forecast 3D is included in Appendix 5.4. If StemBioSys decides to proceed with manufacturing a larger quantity of the flask organizer and guide subsystems, the information provided in Table 5.4.1 can help decide which route is in the best interest of the company. For a manufacturing run of 200 flasks, roughly 34 flask organizers and one to three guides would be utilized. Tables 5.4.2 and 5.4.3 display preliminary cost estimates for an order of 34 flask organizers and 3 guides from each manufacturing company.

The material selection should consider the various environments each subsystem will be exposed to and the tasks they are being used to complete. The flask organizer and guide will be utilized in a BSC or a clean bench, and they will not be exposed to any extreme weather conditions, large forces/pressures, or extreme temperatures. The preferred method for sterilizing each subsystem should also be considered when choosing a material. All materials listed may be

sterilized using 70% ethanol, ultraviolet light, or ethylene oxide gas. However, only ULTEM 9085 has a high enough thermal resistance to be autoclaved. In terms of structure, the difference between sparse and solid is how the object is printed. Both sparse and solid printing may appear solid from afar, but sparsely printed objects contain honeycomb-like structures. The majority of our quotes use the process of fused deposition modeling (FDM), where the part is printed layer-by-layer by heating and extruding a thermoplastic filament. This technology is reliable and is able to produce strong and stable parts with accurate dimensions. PolyJet technology is more often used for final products that have more complex features and require greater accuracy. As for the beta-prototype of the liquid network, the material we recommend is stainless steel because it is more durable than wood and can be sterilized, satisfying our safety and sterility requirements. Final manufacturer recommendations can be found in Section 4 of this report.

3. Design Evaluation

Design Constraints

3.1 Space available for the process

The complete system is constrained to fit within the dimensions of a biosafety cabinet or a clean bench.

Associated Test: Successfully Perform Process Inside of a BSC

The AvENGRs tested whether or not the decellularization process can be completed successfully inside of a biosafety cabinet with the constructed subsystem prototypes.

Objectives

The goal of this test was to ensure that the three subsystems physically fit, operate successfully, and do not present any ergonomic issues inside of the biosafety cabinet.

Features Evaluated

This test examined the ergonomics and functionality of all three subsystem prototypes used in the decellularization process within the size constraint of the biosafety cabinet.

Test Scope

Key Conditions: Mimic the biosafety cabinet conditions used in the StemBioSys manufacturing facility, such as the 10” opening at the front. Although the flasks did not contain CELLvo matrices or the normal solutions, every necessary step of the decellularization process was tested inside of the biosafety cabinet using the constructed prototypes of each subsystem.

Test Plan

Materials: flask organizer gamma-prototype, guide beta-prototype, liquid network alpha-prototype, aspirator pump and tip, six flasks, water, and camera

Tools, Techniques, Skills:

- Aspirator pump + tip: tool used to extract liquid by producing a vacuum
- Aspiration technique: insert tip into flask to withdraw all liquid
- Dispensing technique: insert tube connected to liquid network into respective flask to dispense liquid
- Alignment of flask organizer & guide and flask organizer & liquid network

Assumptions:

- The AvENGRs are handling the liquids and flasks as if they were completing a real decellularization process at StemBioSys.

Data Collection:

- For 6 flasks, the AvENGRs performed the decellularization process (dispensing and aspirating) using all three subsystem prototypes.
    - Data Recorded: qualitative data regarding the comfort and ergonomics assessed by the AvENGRs during the process. Any signs of discomfort, problems, damage, or inconvenience were recorded when performing the stages of the decellularization process.
    - Trials: 4

Acceptance Criteria

The test will be deemed successful if no problems arise and a run through of the decellularization process can be completed. Possible problems include damaged components, broken flasks, or the incapability to perform a certain step of the decellularization process.

Test Results

All four trials of the decellularization process were performed successfully and smoothly in the biosafety cabinet. All three subsystems physically fit and were operated successfully within the biosafety cabinet.

Evaluation

We were able to satisfy the acceptance criteria by completing mock runs of the decellularization process with no problems or damaged flasks or subsystems. As a result, our solution is successful within the given biosafety cabinet constraint and should therefore also fit within the dimensions of a clean bench.

3.2 Sterility

The process must be performed in a sterile environment, specifically within the biosafety cabinet or a clean bench. Devices created to streamline the process must be able to be sterilized before being placed in the biosafety cabinet.

Evaluation

This constraint was evaluated using results from the previous test as it ensured the subsystems fit within the dimensions of a biosafety cabinet and would not hinder the performance of the process. In terms of sterilizing each device before being placed in the biosafety cabinet, we factored in this constraint when choosing materials for the final prototypes. As discussed in Section 2.6, the flask organizer and guide would ideally be manufactured using ABS plastic 3-D printer filament, and the liquid network would be manufactured using stainless steel. Stainless steel can be sterilized using standard practices for achieving an acceptable level of sterility for cell culture, such as spraying with 70% ethanol or exposing the device to UV light or ethylene oxide gas. Since the devices fit in the biosafety cabinet and can be sterilized, this constraint is satisfied.

3.3 Maintaining the integrity of the CELLvo Matrix

To ensure the integrity of the matrix is not disrupted, the device must not scrape the surface of the cell culture flask at any point in the decellularization process.

Associated Test: Compatibility of Flask Organizer and Guide

The AvENGRs tested the compatibility of the flask organizer and guide by verifying that all liquid falls into the back left corner and can be easily aspirated without disrupting the matrix.

Objectives

The goal of this test was to verify the compatibility of the flask organizer and the guide by performing the aspiration step of the decellularization process with the flask organizer resting on the guide and confirming that all liquid falls to the back left corner. With the liquid resting in one place, it will be easier for the user to aspirate. The guide should help navigate the aspirator tip into the appropriate position every time, which will also reduce the probability of hitting the matrix. This test was the first step in syncing all of the subsystems together and can be used to determine if we satisfied our requirement to not disrupt the integrity of the matrix.

Features Evaluated

This test examined the compatibility of the flask organizer and guide by performing the aspiration step of the decellularization process using both devices.

Test Scope

Key conditions for this test included: performing the process in a biosafety cabinet, the angle of the guide (25°), and the vacuum settings for the aspirator pump (high).

Test Plan

Materials: flask organizer gamma-prototype, guide beta-prototype, aspirator pump and tip, six flasks, camera

Tools, Techniques, Skills:

- Aspirator pump + tip: tool used to extract liquid by producing a vacuum
- Aspiration technique: insert tip into flask to withdraw all liquid

Assumptions:

- The AvENGRs assumed one side of the flasks contained CELLvo matrices.

Data Collection:

- Six flasks containing 30 mL of water were placed in the organizer and positioned at the optimal angle by the guide.
- Each AvENGR was recorded aspirating 30 mL from the six flasks.
    - Data recorded: Film
        - Analyzed film to see whether or not the aspirator tip touched the side with the CELLvo matrix and if the liquid fell to the back left corner of the flasks
    - Trials: 4

Acceptance Criteria

The test can be deemed successful if the liquid fell into the back left corner of the flasks, and if the side containing the matrix was not damaged.

Test Results

Table 1. Results from compatibility of flask organizer and guide test

Trial	Was the matrix scratched during aspiration?	Did the liquid fall in the back left corner?
1	No	No
2	No	No
3	No	No
4	No	No

Table 1 displays the results from testing the compatibility of the flask organizer and guide subsystems during the aspiration stage. In terms of compatibility, half of the acceptance criteria was met in that the matrix was not scratched during aspiration, but the liquid did not fall specifically in the back left corner.

Evaluation

After evaluating the film, the AvENGRs can confirm that the matrix was not scratched during aspiration; therefore, this test ensures that the matrix would not be disrupted with the use of these two subsystems, satisfying this requirement. Even though the liquid did not fall in the back left corner, we did not have any issues aspirating all of the liquid, therefore the guide and flask organizer are still deemed compatible.

Design Changes

Nonetheless, we can easily adjust the guide to direct the liquid to the back left corner by simply raising the right side. The guide was easy to maneuver in the cabinet and provided greater comfort for the aspiration step, but once the user reached the third or fourth flask, it was somewhat hard to tell how much liquid was left by simply looking through the sides of the organizer. While this issue does not hinder the functionality of the subsystems, it could be addressed when designing a gamma-prototype of the guide and could also potentially be resolved by elevating one side.

3.4 Remain within a $1200 budget

The AvENGRs were tasked with remaining within a $1200 budget as allocated by Trinity University's Engineering Science Department.

Evaluation

The AvENGRs satisfied this constraint by spending roughly $223 of the $1200 budget.

Project Requirements

3.5 Effectively wash away all detergent without damaging the matrix

It is essential to the decellularization process that all detergent is removed from the matrix before consumer use, therefore the design solution must be able to effectively accomplish this task. Our top-functional requirement is to effectively wash away all detergent without damaging the matrix,

which coincides with the above constraint to maintain the integrity of the matrix. StemBioSys follows their own protocol that consists of verification through a microscope to determine if the detergent is completely washed away and no damage is present; however, we did not have access to live matrices to conduct a similar test. Instead, we focused on testing the dispensing mechanism of the liquid network to ensure that this subsystem could dispense the proper amount to theoretically wash away all detergent without disrupting the matrix.

Associated Test: Dispensing Mechanism of Liquid Network

The AvENGRs tested the dispensing mechanism of the liquid network subsystem by verifying that the correct volume of liquid was dispensed into each flask and ensuring that it did not hit the side of the flask containing the matrix.

Objectives

The goal of this test was to evaluate the dispensing mechanism for the liquid network subsystem. This test determines if the liquid network fulfills the functional requirement of washing away all detergent without damaging the matrix.

Features Evaluated

This test evaluated the functionality of the liquid network during the dispensing/washing steps of the decellularization process.

Test Scope

Key conditions for this test included: dispensing 30 mL of liquid within the accepted 10% error.

Test Plan

Materials: liquid network alpha-prototype, six flasks, graduated cylinder, camera

Tools, Techniques, Skills:

- Dispensing technique: insert tube connected to liquid network into respective flask to dispense liquid
- Absolute and percent difference calculations

Assumptions:

- The AvENGRs assumed that water is an adequate model for the three reagents used in the decellularization process

Data Collection:

- Six flasks were secured in the flask organizer and placed under the liquid network, where 30 mL of liquid was dispensed into each flask
    - Data recorded: film
    - Trials: 4 trials of 6 flasks with 30 mL per flask
- Each AvENGR operated the liquid network, dispensed 30 mL of water per flask, then measured the volume of liquid that was collected in each flask by pouring into the graduated cylinder and reading the value at the bottom of the meniscus
    - Data recorded: volume of liquid dispensed into flasks [mL]
    - Trials: 4 trials of 6 flasks with 30 mL per flask

Acceptance Criteria

If the matrix was not disrupted during this process and the appropriate amount of liquid was dispensed within the 10% range, then the results can be considered acceptable and the functionality of the liquid network can be approved.

Test Results

Table 2. Results from 30 mL dispensing mechanism of liquid network

Trial	Flask Number and Percent Difference from 30mL											
	1	**%**	**2**	**%**	**3**	**%**	**4**	**%**	**5**	**%**	**6**	**%**
1	31.0	3.33	31.0	3.33	33.5	11.7	32.0	6.67	34.0	13.3	33.0	10.0
2	31.0	3.33	31.5	5.00	30.0	0.00	31.0	3.33	30.0	0.00	30.0	0.00
3	30.0	0.00	30.0	0.00	30.0	0.00	31.0	3.33	31.0	3.33	30.0	0.00
4	30.0	0.00	30.5	1.67	31.0	3.33	30.5	1.67	30.0	0.00	31.0	3.33

The film recorded was evaluated to ensure the liquid did not directly hit the side of the flask containing the matrix. After reviewing the film, we confirmed that the matrix was not penetrated by the liquid during the dispensing process. In addition, Table 2 displays that the liquid network dispensed the same amount of liquid required according to StemBioSys' standard operating procedures within a 10% error, so we can conclude that this will suffice to wash away all detergent.

Evaluation

As a result, the liquid network fulfills the functional requirement of washing away all detergent without damaging the matrix.

3.6 Optimized for the size T150 cell culture flask

The T150 flask size was selected by our sponsor for the design solution due to its frequent use in StemBioSys' matrix manufacturing processes.

Evaluation

Since the flask organizer was created with the size of a T150 flask as its foundation, we did not find it necessary to test this requirement. As a result, this requirement was satisfied during the design and construction phases of the flask organizer.

3.7 Scalable to work with various sizes of manufacturing runs

Manufacturing runs can vary from a minimum of 40 to 400 flasks, so the solution should be able to accommodate any size run.

Evaluation

We did not test this requirement because we designed our flask organizer to be easily reproducible so that the lab technician could use as many of this subsystem as needed to fulfill the given manufacturing run. Moreover, you would only have the number of flask organizers that you need

for a specific size run in the cabinet; therefore, the design is scalable to accommodate run sizes starting from six flasks to however many flasks fit in the available organizers. In addition, we designed the liquid network to use a 3L bottle, which provides the user with reagents for a maximum run with minimal replacements. Since the bottle is designed for larger runs, the volume of reagents added can easily be scaled down to accommodate smaller runs, which would also require fewer replacements. As a result, our solution is scalable to work with various sizes of manufacturing runs, and the requirement is satisfied.

3.8 Versatile and portable

This requirement allows the modified process to be performed in different rooms of the facility given that the room has a biosafety cabinet or clean bench.

Evaluation

We did not test this requirement because its fulfillment can be inferred from the design of the subsystems. We designed the liquid network to consist of three frames that attach to two sets of bases, but the user can decide to use one, two, or all three frames depending on the size of the run or the available space in the biosafety cabinet, ultimately depicting the versatility of our design. In addition, we constructed our devices on the 2nd floor of CSI and performed tests in two different workspaces on the 4th floor, which demonstrated that our solution is portable. As a result, our solution satisfies the requirement to be versatile and portable.

3.9.1 Reproducible

The solution must be reproducible so manufacturing runs can occur simultaneously across multiple biosafety cabinets.

Evaluation

We did not conduct tests to confirm the reproducibility of our solution because that requirement was satisfied during the design and construction of our devices. We designed the flask organizer and guide using Fusion 360 so that it would be easier for our sponsor to reproduce these subsystems through a manufacturer. In addition, we researched possible manufacturers that were able to print our designs, as discussed in Section 2.6. We designed the beta-prototype of the liquid network to be constructed using stainless steel and have created a step-by-step construction manual on how we would have produced it had we had the opportunity (Appendix 5.2.2). Since the liquid network should be tested before the design is finalized, we did not obtain any quotes, but we did research possible steel manufacturers that could reproduce the liquid network design. Therefore, the requirement that our solution must be reproducible is satisfied.

3.9.2 Produce a uniform product

The solution must produce a uniform product as StemBioSys must uphold their reputation and satisfy consumer needs. In terms of producing a uniform product, we tested the consistency, accuracy and uniformity of the liquid dispensing system.

Associated Test: Dispensing Mechanism of Liquid Network

The AvENGRs tested the dispensing mechanism of the liquid network subsystem by verifying that the correct volume of liquid was consistently dispensed into each flask.

Objectives

The goal of this test was to evaluate the dispensing mechanism for the liquid network subsystem. This test confirmed whether or not the liquid network fulfills the non-functional requirement to produce a uniform product by consistently and uniformly dispensing 30 mL of liquid into each flask with an accepted variability of 10%.

Features Evaluated

This test evaluated the functionality and uniformity of the liquid network during the dispensing/washing steps of the decellularization process.

Test Scope

Key conditions for this test included: dispensing 30 mL of liquid within the accepted 10% error.

Test Plan

Materials: liquid network alpha-prototype, six flasks, graduated cylinder or beaker, camera

Tools, Techniques, Skills:

- Dispensing technique: insert tube connected to liquid network into respective flask to dispense liquid
- Absolute and percent difference calculations

Assumptions:

- The AvENGRs assumed that water is an adequate model for the three reagents used in the decellularization process

Data Collection:

- Six flasks were secured in the flask organizer and placed under the liquid network, where 30 mL of liquid was dispensed into each flask
- Each AvENGR operated the liquid network, dispensed 30 mL of water per flask, then measured the volume of liquid that was collected in each flask by pouring into graduated cylinder and recording the value at the bottom of the meniscus
    - Data recorded: volume of liquid dispensed into flasks [mL]
    - Trials: 4 trials of 6 flasks with 30 mL per flask

Acceptance Criteria

If the volume of liquid dispensed into each of the six flasks was within 10% of the intended 30 mL, then the results can be considered acceptable and the functionality of the liquid network can be approved.

Test Results

Table 2 from Section 3.5 displays the liquid network dispensing mechanism test results. The data clearly represents the consistency, accuracy and uniformity that the liquid network exhibits. Only two of the twenty-four trials resulted in an unacceptable value above a 10% error, and of the two, both errors were just slightly over 10%. These two trials could be outliers resulting from either human error by incorrectly reading the graduated cylinder or from leftover water droplets

increasing the volume measurements. Of the entire prototype test, 91.25% of trials met the 10% error threshold. Therefore, the liquid network fulfills the non-functional requirement to produce a uniform product by consistently and uniformly dispensing 30 mL of liquid into each flask with an accepted variability of 10%.

Evaluation

We confirmed that the liquid network can dispense 30mL of liquid into each flask with an accepted variability of 10%, increasing the uniformity of the product. Since our acceptance criteria was met, we deem our results reliable and the requirement to produce a uniform product is satisfied. Moreover, we did not alter the foundation of the decellularization process, and are under the assumption that the introduction of our devices did not alter the final product. Since we did not have access to live matrices to confirm this assumption, we are relying on StemBioSys to confirm the uniformity of the product as the company already has verification procedures in place.

3.10 Decrease the total time required to complete the decellularization process

The final design solution may decrease the total time required to complete the decellularization process.

Associated Test: Time Savings with Flask Organizer

The AvENGRs performed and timed the aspiration step of the decellularization process with and without the incorporation of the flask organizer and computed the time difference.

Objectives

The goal of this test was to determine the time savings from the addition of a single subsystem, the flask organizer, and to demonstrate that we can save StemBioSys time even without modification to other subsystems. A decrease in aspiration time meets the non-functional requirement of reducing the total time it takes to complete the process.

Features Evaluated

This test examined the functionality of the flask organizer in that the organizer can accommodate six flasks to complete the decellularization process.

Test Scope

Key conditions for this test included: vacuum settings for the aspirator pump (high), variability in fluid volumes within 10% error, and having four people perform the process in the biosafety cabinet.

Test Plan

Materials: flask organizer gamma-prototype, stopwatch, aspirator pump and tip, six flasks, camera

Tools, Techniques, Skills:

- Aspirator pump + tip: tool used to extract liquid by producing a vacuum
- Aspiration technique: insert tip into flask aimed at back left corner to withdraw all liquid
- Absolute and percent difference calculations

Assumptions:

- The AvENGRs assumed that the time discrepancies between individual users are not relevant.

Data Collection:

- The AvENGRs recorded how long it takes to aspirate six flasks secured in the flask organizer using a single aspirator tip.
    - Data Recorded: time it takes to aspirate [seconds]
    - Trials: 4
- The AvENGRs recorded how long it takes to aspirate six flasks individually using a single aspirator tip.
    - Data Recorded: time it takes to aspirate [seconds]
    - Trials: 4
- Compared experimental values using absolute and percent difference calculations.
- Conducted t-test to determine if there was a significant difference in the times

Acceptance Criteria

If the time it took to complete the aspiration step of the decellularization process using the flask organizer was significantly less ($p < 0.05$ from t-test) than the time it took to complete the process without the organizer, then the results can be deemed acceptable, and the design requirement has been satisfied.

Test Results

Table 3. Results for aspiration with and without the flask organizer

Trial	With Flask Organizer [s]	Without Flask Organizer [s]	Percent Difference [%]	Absolute Difference [s]
1	34.38	50.98	32.56	16.60
2	29.77	44.58	33.22	14.81
3	27.08	59.55	54.53	32.47
4	28.40	45.93	38.17	17.53
Average	29.91	50.26	40.49	20.35

According to Table 3, the use of the flask organizer during the aspiration stage of the process decreased the time by approximately 41%. While we did not create an acceptance criterion for the percent or absolute difference in times, typically 25% is considered to be a considerable difference, therefore, the use of the flask organizer substantially reduced the aspiration time.

Table 4. t-Test for aspiration results with flask organizer

	Without Organizer	With Organizer
Mean	50.26	29.91
Variance	45.94	10.10
Observations	4	4

Hypothesized Mean Difference	Degree of Freedom	t Stat	P (t≤T) two-tail	t Critical two-tail
0.00	3.00	4.99	**0.02**	3.18

Table 4 displays our results from the paired two sample t-test we conducted using an alpha value of 0.05. Our null hypothesis was that there would be no difference in aspiration times with or without the flask organizer, hence the hypothesized mean difference of zero.

Evaluation

According to Table 4, our p-value for a two-tailed test is 0.02, which is less than the alpha value of 0.05, therefore we have met our acceptance criteria of achieving a p-value of less than 0.05. As a result, there is a large decrease in aspiration time using the flask organizer compared to aspirating the flasks individually. In addition, our t-value is 4.99, which is larger than the critical two-tailed t value of 3.18, suggesting that we reject the null hypothesis. Since the p-value is less than alpha and the t-value is greater than the critical t-value, we can fully reject the null hypothesis and confirm that the use of the flask organizer significantly impacts the aspiration time. As a result, implementation of the flask organizer meets the non-functional requirement to reduce the total time required to complete the decellularization process.

Associated Test: Time Savings with Flask Organizer + Guide + Liquid Network

The AvENGRs performed and timed one round of aspirating and dispensing with and without the incorporation of the flask organizer, guide, and liquid network and computed the time difference.

Objectives

The goal of this test was to determine the time savings from the three proposed subsystems and demonstrate that we can save StemBioSys time. A decrease in time to complete one round of aspirating and dispensing meets the non-functional requirement of reducing the total time it takes to complete the process.

Features Evaluated

This test examined the functionality and compatibility of the flask organizer, guide, and liquid network. We examined whether or not the organizer can accommodate six flasks, if the guide can consistently guide the pipette tips to the desired position without damaging the matrix, and if the

liquid network can consistently dispense the proper amount of liquid without damaging the matrix to complete the decellularization process.

Test Scope

Key conditions for this test included: vacuum settings for the aspirator pump (high), variability in fluid volumes within 10% error, and four people performing the process in the biosafety cabinet.

Test Plan

Materials: flask organizer gamma-prototype, guide beta-prototype, liquid network alpha-prototype, stopwatch, aspirator pump and tip, six flasks, camera

Tools, Techniques, Skills:

- Aspirator pump + tip: tool used to extract liquid by producing a vacuum
- Aspiration technique: insert tip into flask to withdraw all liquid
- Dispensing technique: insert tube connected to liquid network into respective flask to dispense liquid
- Absolute and percent difference calculations

Assumptions:

- The AvENGRs assumed that the time discrepancies between individual users are not relevant.

Data Collection:

- Recorded how long it takes to complete one round of aspirating and dispensing for six flasks secured in the flask organizer using the guide, liquid network, and a single aspirator tip.
    - Data Recorded: time it takes to aspirate and dispense liquid [seconds]
    - Trials: 4
- Recorded how long it takes to complete one round of aspirating and dispensing for six flasks individually using a single aspirator tip.
    - Data Recorded: time it takes to aspirate and dispense manually [seconds]
    - Trials: 4
- Compared experimental values using absolute and percent difference calculations.
- Conducted t-test to determine if there is a significant difference in the times

Acceptance Criteria

If the time it took to complete the aspiration and dispensing steps of the decellularization process using the flask organizer, guide, and liquid network was significantly less ($p < 0.05$ from t-test) than the time it took to complete the process without any devices, then the results can be deemed acceptable, and the design requirement is satisfied.

Test Results

Table 5. Results from aspirating and dispensing with all subsystems

Trial	With Subsystems [s]	Without Subsystems [s]	Percent difference [%]	Absolute difference [s]
1	73.63	129.64	43.20	56.01
2	68.72	167.90	59.07	99.18
3	70.65	173.68	59.32	103.03
4	68.72	132.43	48.11	63.71
Average:	70.43	150.91	53.33	80.48

Table 5 provides results from completing one round of aspirating and dispensing, displaying a 53.33% time savings using all three subsystems. This translates into an absolute difference of 1 minute and 21 seconds. We have over doubled our 25% threshold, resulting in considerable time savings.

Table 6. t-Test aspiration results with flask organizer and guide

	Without Subsystems	With Subsystems
Mean	150.91	70.43
Variance	533.69	5.38
Observations	4	4

Hypothesized Mean Difference	Degree of Freedom	t Stat	P (t≤T) two-tail	t Critical two-tail
0.00	3.00	6.69	**0.01**	3.18

Evaluation

According to Table 6, this test achieved a p-value of 0.01, which satisfies our acceptance criteria of 0.05. This p-value was calculated using a paired two sample t-test at an alpha value of 0.05. In addition, our t-value was 6.69, which is much higher than 3.18, suggesting to reject the null hypothesis of no difference between the two tests. Therefore, the use of all three subsystems has satisfied the non-functional requirement to decrease the total time required to complete the decellularization process.

3.11 Process will be safe to complete

The incorporation of our three subsystems into StemBioSys' current decellularization process should not present any additional safety risks for the users.

Evaluation

We did not make a specific prototype test to evaluate the safety of our design because every test we performed served as an indicator of whether or not our devices were safe for use in a laboratory environment. Our subsystems were also designed to conform to existing safety standards and protocols provided by OSHA and StemBioSys. All actions required for using the complete design solution were performed by each AvENGR and did not present any safety issues. Therefore, the use of the subsystem prototypes meets the requirement of the process being safe to complete.

3.12 All tools or new devices used in the process shall be able to be sterilized

Any devices created shall be able to be sterilized before being placed in the biosafety cabinet to reduce any possibility of contamination and to follow standard cell culture protocols.

Evaluation

This requirement coincides with our sterility constraint, which was met through performing the procedure in a biosafety cabinet discussed in Section 3.1 and through the selection of materials for the final prototype.

3.13 Integratable into the current process and result in the same product or substantially equivalent

The design solution must be able to be integrated without significantly altering the current steps of the decellularization process, found in Appendix 5.3, so that the product is substantially equivalent to that of the current process.

Evaluation

This requirement was met during the construction and design phases of our solution as each subsystem was created to maintain the foundation of StemBioSys' decellularization process according to their standard operating procedures. Since we did not alter any core steps from StemBioSys' procedure, we worked under the assumption that the integration of our solution will result in the same product or substantially equivalent. As a result, these two interface requirements are satisfied.

3.14 Intuitive and easily adopted by staff with no pre-existing knowledge of our solution

The solution should not be too complicated that the lab technicians cannot determine how to assemble it or implement the subsystems in the current process.

Evaluation

This requirement was not tested because we had planned to deliver our final prototypes to the StemBioSys manufacturing facility to test, but due to the recent COVID-19 situation, we were not given this opportunity. Nonetheless, with the use of our assembly instructions (Appendix 5.2), our SOP (Appendix 5.1), and the lab technician's current knowledge of the process, we are certain the

staff will be able to intuitively and easily adopt our devices into the current process. As a result, these interface requirements are satisfied.

3.15 Compatible with prevailing industry standards

The design solution should be compatible with prevailing industry standards so StemBioSys can continue to produce their product and satisfy customer needs.

Evaluation

Prevailing industry standards include the grade of the tissue culture flask, the ability to be sterilized, and the ability to fit in a biosafety cabinet or clean bench. We have already satisfied the sterility and biosafety cabinet constraints in Sections 3.2 and 3.1, respectively, therefore there was no need to test them again. In terms of the grade of the tissue culture flask, the flasks were provided to us by our sponsor and are compatible with prevailing industry standards. In addition, we designed our solution using StemBioSys' standard operating procedures as our foundation, which were developed by the company to already meet industry standards. As a result, this requirement is satisfied.

3.16 Allow biosafety cabinet to be used for other processes at all times

The design solution cannot populate the entirety of the BSC and should allow for other processes to be performed simultaneously.

Evaluation

This requirement was satisfied during our testing to determine if the decellularization process could successfully be completed within the constraints of the biosafety cabinet seen in Section 3.1. The cabinet we used for testing was housing other experiments and equipment used by the Chemistry Dept., and we noted in our test results that the biosafety cabinet did feel slightly cramped as a result. There were a lot of large and unnecessary items in the biosafety cabinet we had access to, so the ergonomics would be less of an issue in a clean cabinet. Nonetheless, we were able to satisfy the acceptance criteria by completing runs of the decellularization process with no problems or damaged components or subsystems. As a result, our solution can complete the decellularization process successfully despite other processes being conducted at the same time, satisfying this requirement.

3.17 Overall Project Objective

Reduce Full-Time Equivalents

Evaluation

The overall project objective was tested using test plans from Section 3.10. For the complete decellularization process, liquid will be aspirated from each flask a total of 6 times. Using the data in Table 3 to extrapolate for a manufacturing run of 204 flasks, the total time required for aspiration with the flask organizer is 1 hour and 42 minutes and without is 2 hours and 51 minutes. In this case, the use of the flask organizer decreases total aspiration time by approximately 1 hour and 9 minutes. Concerning FTEs, this decrease in time would reduce the cost of having one lab

technician perform the process by about one hour's worth of pay. The amount of time spent performing the process could be further reduced by having two lab technicians working simultaneously, and this should equate to approximately the same cost in terms of FTEs. Overall, this test satisfies the project objective to reduce Full-Time Equivalents.

For the complete decellularization process, there are 5 dispensing steps and 6 aspirating steps. Using the data in Table 5 to extrapolate for a manufacturing run of 204 flasks, the total time required when using the three subsystems is 3 hours and 37 minutes and without is 7 hours and 35 minutes. In this case, the use of the three subsystems decreases the overall time for the decellularization process by approximately 3 hours and 58 minutes. Concerning FTEs, this reduces the cost of having one lab technician perform the process by nearly 4 hour's worth of pay. If StemBioSys manufactures one run of 204 flasks per week, the total time savings during a year would be 222 hours and 8 minutes, for a total of $4871.38 in labor savings if only one person performed each run. For approximately the same cost in FTEs, the amount of time spent performing the process could be dramatically reduced by having two lab technicians working simultaneously. Therefore, the time savings may allow StemBioSys to redeploy employees to other company priorities. Overall, the use of all three subsystems is highly successful, satisfies the overall project objective to reduce Full-Time Equivalents, and also meets the non-functional requirement of reducing the total time it takes to complete the process.

4. Conclusions

Of the twelve project requirements, four constraints, and the overall project objective, all have been successfully met by the AvENGR's completed design. Three of the four constraints were met through the satisfaction of some of our requirements, as the sterility, performance in the biosafety cabinet, and integrity of the matrix constraints coincide with our most essential requirements. The last constraint is cost, which the AvENGRs only spent roughly $223 of our total $1200, ultimately satisfying our final constraint.

The more crucial requirements that we stated "shall" be accomplished included that the process effectively wash away all detergent without damaging the CELLvo Matrix and be optimized for the size T150 cell culture flask, and that the final design be reproducible, produce a uniform product, be safe to use and be able to be sterilized. The design of the liquid network satisfies the requirements to wash away detergent without damaging the matrix and contributes to the production of a uniform product. Furthermore, the flask organizer was designed specifically for the size T150 flasks, satisfying the size requirement. The reproducibility, ability to be sterilized and safety requirements for our devices were satisfied during the design and construction of each device. The flask organizer and guide were designed with Fusion 360, allowing us to research potential manufacturers for reproduction and receive quotes for various types of materials that can be 3D printed and sterilized.

Once the above requirements were satisfied, we focused on requirements that we stated "should" be accomplished, including the scalability, versatility, and portability of our final design, the ability for it to be integrated into the current process and easily adopted by staff, and the final

design's compatibility with industry standards. The reproducibility of the flask organizer and guide and the size of the bottle for the liquid network allow the solution to be scaled to work with various sizes of manufacturing runs depending on how many copies of flask organizers and guides are available. The construction of each device contributes to its versatility and portability. Moreover, the liquid network can vary from one to three frames depending on the size of run or space available in the biosafety cabinet. In addition, prototype tests were conducted in two different biosafety cabinets, showcasing the portability of our solution. Each device was designed with the intent to streamline the manufacturing process, not alter its foundation. Therefore, the solution was designed to be integrated into the current process. In addition, since the current process is compatible with prevailing industry standards, and we did not alter the groundwork of the process, our devices remain compatible with industry standards. Lastly, we provided StemBioSys with an updated standard operating procedure that includes the set-up and use of our devices, so lab technicians can refer to this document to confirm the intended uses of our devices.

The last set of requirements stated that we "may" accomplish them, including reduction in total completion time and simultaneous use of the biosafety cabinet for other processes. All of the time savings tests produced results varying from a 39% to 53% time reduction, satisfying this requirement. In addition, all prototype tests were conducted in biosafety cabinets that were filled with other items, and we were still able to complete the decellularization process. Therefore, the implementation of our devices allowed for processes to be conducted simultaneously in the biosafety cabinet.

Our overall project objective to reduce Full-Time Equivalents was also satisfied as a reduction in total time decreases the time required for one person to complete the manufacturing process. In addition, the implementation of our devices allows one or two people to complete the entire process in the biosafety cabinet for approximately the same cost in terms of FTEs.

Overall, the AvENGRs have created a successful, working prototype to streamline the CELLvo matrix manufacturing process. Since all of our requirements were achieved, there are no changes needed to reach any remaining goals, but there is always room for improvement in terms of our working prototype.

4.1 Next Steps and Recommendations

If a gamma-prototype of the guide were to be produced, we recommend increasing the height of the right side of the guide to draw liquid specifically into the back left corner, allowing for greater consistency during aspiration. In terms of next steps for manufacturing the flask organizer and guide, the AvENGRs recommend consulting with Xometry for the production of 34 flask organizers made of ABS plastic for a total of $8,840. For the production of three guides made of ASA plastic, our recommendation is to purchase from Stratasys for a total of $1,178. Per StemBioSys' profit margin obtained from their confidential "Cost of Goods" document, approximately 88 flasks would need to be manufactured and sold to break even with the cost to produce 34 flask organizers and 3 guides. These recommendations are based on minimizing cost and satisfying the requirement that all equipment is sterilizable. When used in the CELLvo matrix

manufacturing process, the two items will not be exposed to any extreme temperatures or have large forces applied to them. Therefore, after discussion and consideration of cost, materials, and requirement satisfaction, the AvENGRs have recommended the route that we believe to be in the best interest of StemBioSys. However, it should be noted that the manufacturing companies listed in Appendix 5.4 offer a much wider variety of materials than what is reflected in this report based on the quotes we were able to receive.

In addition, the AvENGRs have provided a detailed construction manual and test plan for the beta-prototype of the liquid network should StemBioSys choose to continue development of our design. The AvENGRs recommend building and testing a beta-prototype given the instructions provided in Appendix 5.2.2, and if it is deemed successful, producing a CAD model. If a CAD model is generated, we recommend receiving quotes from all three companies listed in Appendix 5.4 and proceeding with a selection process based on cost and requirement satisfaction. The other, less likely option would be to pitch the project to a senior design team at a university. This option could be advantageous in that unforeseen pitfalls could be discovered and improved, but disadvantageous in that the time frame could range from many months to over a year. Until a decision about how to move forward with the liquid network subsystem is reached, we recommend also looking into utilizing a commercially available product similar to those also listed in Appendix 5.4.

4.2 Potential Pitfalls and Alternatives

One limitation of our testing is that we did not evaluate the time it takes to put flasks into the flask organizer. In our experience, this time is negligible and will not impact the overall time savings. If StemBioSys finds that this is not the case, they may consider performing additional tests to ensure that substantial time savings are still achieved relative to performing the process without the flask organizer subsystem. Another limitation of our testing is that the overall time savings were extrapolated from a smaller test size (number of flasks used for a run); however, we see no reason why this approach is not valid for larger manufacturing runs. A potential pitfall in our final prototype arises should StemBioSys need to change their flask manufacturer, where the exact dimensions may not be identical to the flasks currently used. However, we anticipate that only the flask organizer would need to be redesigned in the provided CAD file to modify the dimensions to fit the height, width, and length of the new flasks.

Specific pitfalls that may arise from the liquid network beta-prototype include a lack of stability and imbalanced weight distribution, which could result in a collapse from the weight of the filled bottle. Increasing the stability of the liquid network could be achieved by fixing a large flat stand to the biosafety cabinet that the liquid network frames would attach to. Another potential pitfall to be aware of is if there is failed liquid flow from the reagent bottle into the flasks due to air bubbles forming in the liquid dispensing mechanism and tubing. Alternatively, if air bubbles arise when bottles are changed, a separate waste bottle may be necessary to prime the line. Similar to the time issues related to the flask organizer, unforeseen additional time required to place new

bottles of reagents in the liquid network frame may arise. If this occurs, additional timed tests will need to be performed to determine if this adds a substantial amount of time to the complete process.

The AvENGRs would like to thank Dr. Travis Block, Principal Scientist of StemBioSys, for all of his time, feedback, encouragement, and patience with our team throughout this project.

5. Appendices

5.1 AvENGRs Standard Operating Procedure (SOP)

Bone Marrow Stromal Cell-Derived ECM

1. **Purpose**
   The purpose of this procedure is to provide instructions for making bone marrow stromal cell-derived ECM starting with cell extraction on day 17.

2. **Scope**
   This protocol has been developed for the consistent production of bone marrow stromal cell-derived ECM on standard cell culture treated polystyrene.
   The following procedure is adapted from StemBioSys' SOP-01-0002 rev D and is designed to be used for T150 cell culture flasks in combination with the complete AvENGRs manufacturing system.

   Definitions

Acronym	**Expanded Term**
SOP	standard operating procedure
ECM	extracellular matrix
MSDS	material safety data sheets
PPE	personal protective equipment
L	liter
BSC	biosafety cabinet
PBS	phosphate buffered saline
ml	milliliter

3. **References**
   SOP-01-0000, SOP-01-0001, SOP-01-0002, SOP-01-0005, SOP-01-0009

4. **Responsibilities**
   The Process Manager is responsible for overseeing the processes outlined in this protocol and that all criteria are met.
   Trained personnel are responsible for following the protocol, reading MSDS prior to processing, and wearing all required PPE.

5. **Forms/Templates To Be Used**
   BR-01-0002

6. Specific Procedure (Continued from Day 14/15 Induction in SOP-01-0002 rev D)

DAY 16 - Reagent Prep

6.92 PPE (SOP-01-0000)

6.93 Autoclave required number of 3L reagent bottles depending on total number of flasks in the manufacturing run (see Table 5.1.1 for examples)

6.94 Biosafety cabinet prep (SOP-01-0001)

6.95 In the BSC, fill the necessary number of 3L bottles with PBS (SOP-01-0005), deionized water, or extraction buffer (SOP-01-0009)

6.96 Label the bottles including the solution name, lot #, method of sterilization, and date

6.97 Store PBS and extraction buffer at 4°C and store deionized water at room temperature

DAY 17/18 - Cell Extraction

6.98 PPE (SOP-01-0000)

6.99 Biosafety cabinet prep (SOP-01-0001)

6.100 Place 3L bottles of 1X PBS (SOP-01-0005) and extraction buffer (SOP-01-0009) in the water bath at 37°C +/-0.5 for 30-45 minutes

6.101 Remove bottles from the water bath, dry with a paper towel, and spray with 70% ethanol before placing one of each reagent inside the BSC or clean bench

6.102 Sterilization of Three Subsystems
Spray flask organizer, guide, and liquid network (frames and dispensing mechanisms) with 70% ethanol before placing inside the BSC or clean bench
Connect liquid network tubing and dispensing mechanism to each reagent bottle
Invert each reagent bottle and secure in corresponding liquid network frame (replace reagents as needed throughout the remainder of the process by transferring tubing and dispensing mechanism to a new bottle)

6.103 Load six T150 cell culture flasks into each flask organizer (approx. 7 flask organizers can be in the BSC or clean bench at a time)

6.104 Place flask organizer on guide during each aspiration step

6.105 Aspirate the old medium from each individual flask

6.106 Orient flask organizer on its back so flasks are in the upright position and place flask organizer under the liquid network

6.107 To wash: Guide the liquid network dispensing mechanism containing 1X PBS to hover over the center of the flask opening

6.108 Carefully press down on the mechanism while positioned over the middle of the flask opening until the appropriate amount of solution has been dispensed and repeat for the remaining five flasks in the flask organizer (T150=30+/-2ml)

6.109 Orient the flask organizer on its side so the PBS covers the matrix, then place flask organizer on guide and aspirate the PBS from each flask

6.110 Add extraction buffer using liquid network as described previously (T150=15+/-2ml)

6.111 Orient flask organizer on its side so the extraction buffer covers the matrix and incubate for 7-10 minutes at room temperature

6.112 Place flask organizer on guide and aspirate the extraction buffer
6.113 Wash with 1X PBS and aspirate (T150=30+/-5ml) as described previously
6.114 Wash with 1X PBS and aspirate (T150=30+/-5 mL) as described previously
6.115 Wash with deionized water and aspirate (T150=30+/-5 mL) as described previously
6.116 Air dry under sterile conditions and aspirate excess water (T150 flasks can dry inside the BSC, approximately 2-3 days)

DAY 21 - Packaging T150 Flasks

6.117 Cap the T150 flasks
6.118 Wipe the external surface of flasks with a sterile gauze and 70% ethanol
6.119 Label the flasks with the batch number and expiration date (BM10XX.X.XXXX)
6.120 Store at 2-8°C

Table 5.1.1. Number of Flask Organizers and 3L reagent bottles needed for three sizes of manufacturing runs

Number of Flasks/Size of Run	42	204	402
Number of Flask Organizers	7	34	67
Total PBS [L]	3.78	18.36	36.18
Number of Bottles PBS	2	7	13
Total Detergent [L]	0.63	3.06	6.03
Number of Bottles Detergent	1	2	3
Total DI Water [L]	1.26	6.12	12.06
Number of Bottles DI Water	1	3	5

*Typically, the manufacturing procedure is performed in batches of 200 to 400, with 40 cell culture flasks in any particular biosafety cabinet at a time. These numbers have been adjusted slightly to account for the flask organizer holding 6 flasks at a time.

5.2 Liquid Network Fabrication and Testing Instructions

This appendix begins with a list of materials and instructions for building the existing alpha-prototype of the liquid network. This is followed by an updated materials list, instructions for constructing a beta-prototype, and a new test plan.

5.2.1 Alpha Prototype Materials and Construction Process

Materials

Frame (Table 5.2.1.1)

- 4 pieces of wood for legs (1”x1”x25”)
- 4 blocks of wood for bottleneck holder supports (2.5”x4”x2”)
- 1 piece of wood for the bottleneck holder (4”x4”x1”)
- 4 stabilizing pieces of wood to connect legs at the bottom and top on either side of the frame (1”x1”x5”)
- 1 piece of wood (back panel) to connect the two sides of the frame at the top (9.5”x0.5”x4”)
- Screws:
    - ¼” head 2” shaft
    - ¼” head 1” shaft

Dispensing Mechanism

- 1L autoclavable bottle
- Shot dispenser
- Latex Tubing: 3/4 I.D. x 1/8 W - 1 O.D. (10 inches in length)

Necessary Tools

- Tape measure
- Drill & drill bits
- Vertical Bandsaw

Construction

1. Cut a 2.4” diameter circle out of the center of the 4”x4”x1” bottleneck holder (Table 5.2.1.2: Step 1)
2. Cut a 3.4”x1.5” rectangle through the 2.4” diameter circle (from step 1) starting at one end of the 4”x4” block to allow room for the movement of the tube (Table 5.2.1.2: Step 2)
3. Cut a 1.25”x11/16”x1” rectangle from a corner of each bottleneck holder support (2.5”x4”x2”) so that the four pieces align to make a cut-out rectangle (Table 5.2.1.2: Step 3)
4. Drill each leg into the outer corners of the bottleneck holder supports (opposite from the rectangle cut in step 3) using four 2” screws for a total of four base pieces (Table 5.2.1.2: Step 4)
5. Screw the four stabilizing pieces (1”x1”x5”) using eight 2” screws to connect the bases (Table 5.2.1.2: Step 5)

a. Looking at the front of the liquid network:
    i. (two 2” screws) Screw one piece directly underneath the bottleneck holder supports on the left side in between the front and back left legs to stabilize them
    ii. (two 2” screws) Screw one piece at the very bottom of the legs in between the front and back left legs for reinforcement
    iii. (two 2” screws) Screw one piece directly underneath the bottle holder supports on the right side in between the front and back right legs to stabilize them
    iv. (two 2” screws) Screw one piece at the very bottom of the legs in between the front and back right legs for reinforcement
6. For further reinforcement, screw back panel (9.5”x0.5”x4”) at the top between the two back legs for stability using two 2” screws (Table 5.2.1.2: Step 6)
7. Place the bottleneck holder (Step 2) in the space created by the four notches cut out from step 3 (Table 5.2.1.2: Step 7)
8. Fill 1L bottle with solution
9. Insert one end of the 10 inch tubing into the 1L bottle
10. Attach shot dispenser on loose end of tubing
11. Flip the bottle upside down and slide tubing through the 1.5” gap in the bottleneck holder at the top of the frame
12. Place the bottle on the bottleneck holder

Links for Materials and Necessary Tools

- Screws:
    - ¼” head 2” shaft
    - https://www.acehardware.com/departments/hardware/screws-and-anchors/wood-screws/5327143?x429=true&gclid=EAIaIQobChMIj7L10PTt6AIVltJkCh3zUQbVEAkYAyABEgJY2PD_BwE&gclsrc=aw.ds
    - ¼” head 1” shaft
    - https://www.homedepot.com/p/Everbilt-8-x-1-in-Phillips-Flat-Head-Zinc-Plated-Wood-Screw-100-Pack-801822/204275495
- Shot dispenser
    - https://www.amazon.com/Wyndham-House-4-Bottle-Liquor-Dispenser/dp/B00BI02YEQ/ref=sr_1_38?keywords=liquor+dispenser&qid=1580936288&sr=8-38
    - https://www.amazon.com/Replacement-Nozzle-Dispenser-Liquor-Revolving/dp/B07X1R859Q/ref=sr_1_49?crid=32HMQFHRMO02V&keywords=liquor%2Bdispenser&qid=1580930276&sprefix=liquor%2B%2Caps%2C190&sr=8-49&th=1
- Latex Tubing: 3/4 I.D. x 1/8 W - 1 O.D. (10 inches in length)
    - http://www.latex-tubing.com/d034018-012.html

Table 5.2.1.1. Alpha-prototype Frame Materials

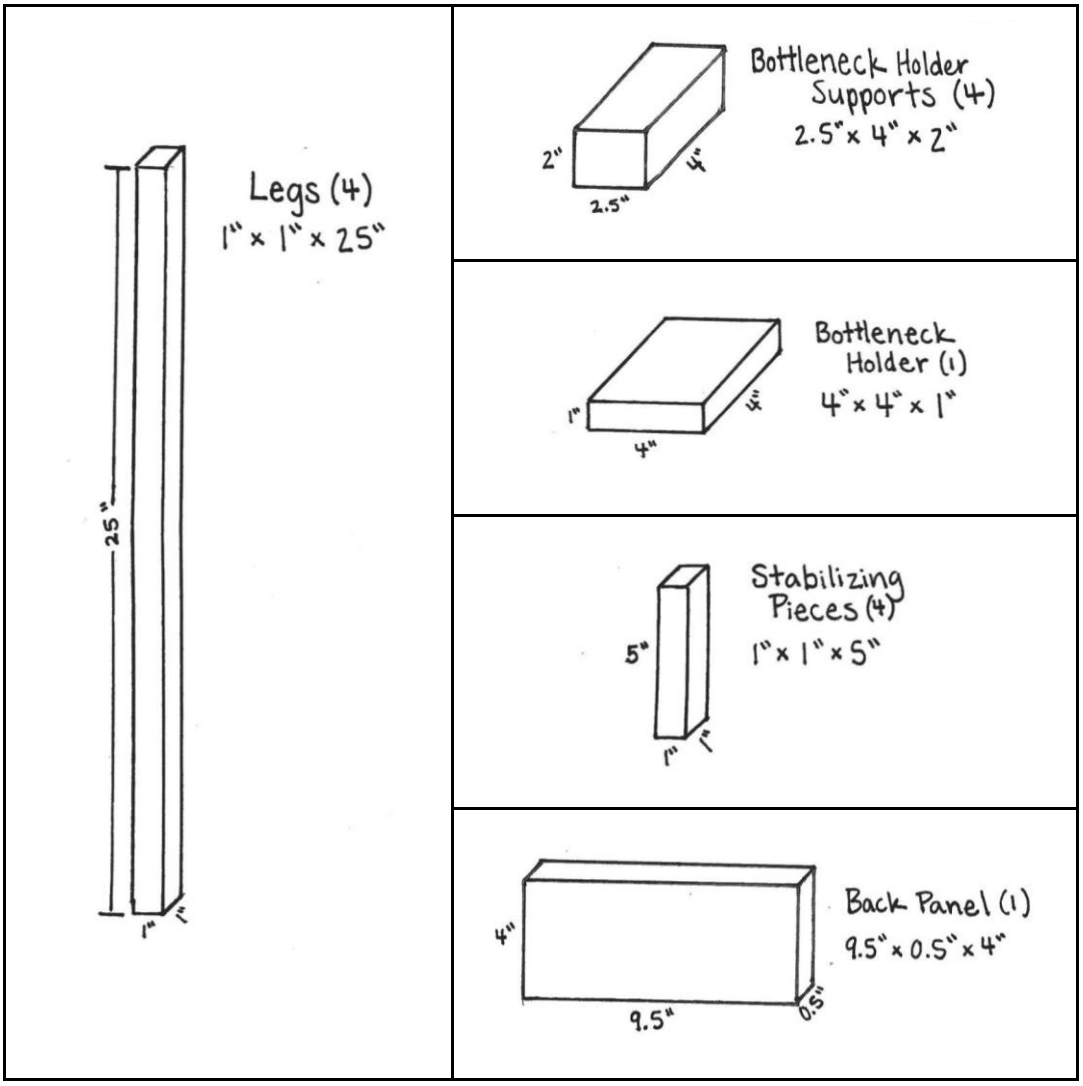
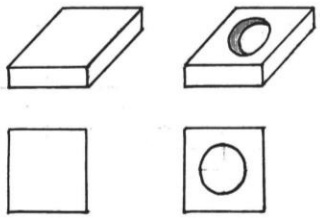
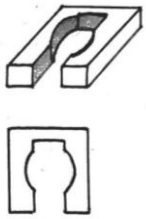
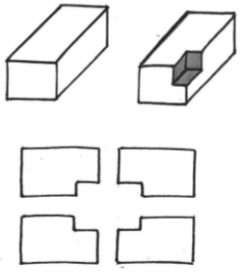
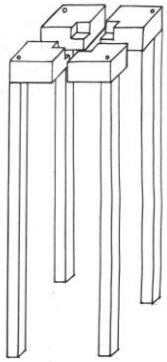
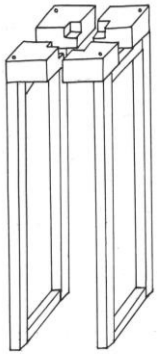
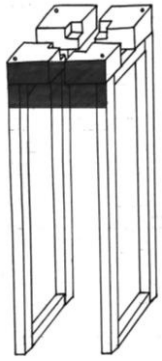
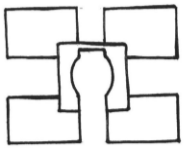


Table 5.2.1.2. Alpha-prototype Frame Construction

Step 1 	**Step 2** 
Step 3 	**Step 4** 
Step 5 	**Step 6** 
Step 7 	

5.2.2 Beta Prototype Materials, Construction Process, and Test Plan

Materials

Frame (Table 5.2.2.1)

- 12 pieces of stainless steel for legs (1”x1”x28”)
- 3 stainless steel slabs for bottleneck holders (9.5”x7”x2”)
- 3 flat pieces of stainless steel for the top plates (9.5”x7”x0.5”)
- 3 latches
- 4 flat pieces of stainless steel for base (9”x2.5”x0.5”)
- 12 stabilizing pieces of stainless steel to connect legs at the bottom and top on either side (1”x1”x5”)
- 3 pieces of stainless steel (back panels) to connect the two sides of the frames at the top (9.5”x0.5”x4”)
- Screws ¼” head 2” shaft

Dispensing Mechanism

- 3 3L autoclavable bottles (H:12 in, D:6 in)
- Shot dispenser
- Latex Tubing: 3/4 I.D. x 1/8 W - 1 O.D. (5 inches in length)

Necessary Tools

- Tape measure
- Drill and Cobalt M-35 Drill Bit Set
- Vertical Bandsaw

For Welding

- Welding mask
- MIG torch (for thicker pieces)
- TIG welder (for thinner pieces)
- Argon-carbon dioxide shielding gas
- Filler metal that matches the base metal
- Wire brush
- Acetone
- Clamps or vices

Construction

To make one system...

1. Carve a 2.4” diameter circle out of the center of the bottleneck holder (9.5”x7”x2”) (Table 5.2.2.2.: Step 1)
2. Carve a 1.5”x6”x2” rectangle going through the circle from step one, starting from the front edge of the bottleneck holder (9.5”x7”x2”) (Table 5.2.2.2.: Step 2)
3. Carve a 1”x1”x2” rectangle from each corner of the bottleneck holder (9.5”x7”x2”) (Table 5.2.2.2.: Step 3)

4. Carve a 6” diameter circle from the center of the top plate (9.5”x7”x0.5”) (Table 5.2.2.2.: Step 4)
5. Carve a 6” rectangle starting from the front edge of the top plate until it meets the circle (Table 5.2.2.2.: Step 5)
6. Attach a latch across the opening created in step 5 using two screws (Table 5.2.2.2.: Step 6)
7. Weld each leg into the four corners of the top plate (Table 5.2.2.2.: Step 7)
8. Weld the four stabilizing pieces (1”x1”x5”) to connect the legs (Table 5.2.2.2.: Step 8)
    a. Weld one piece 23” from the bottom of the legs on the left side in between the front and back left legs to stabilize them
    b. Weld one piece 1” from the bottom of the legs on the left side in between the front and back left legs for reinforcement
    c. Weld one piece 23” from the bottom of the legs on the right side in between the front and back right legs to stabilize them
    d. Weld one piece 1” from the bottom of the legs on the right side in between the front and back right legs for reinforcement
9. For further reinforcement, screw back panel (9.5”x0.5”x4”) at the top between the two back legs for stability using two 2” screws (Table 5.2.2.2.: Step 9)
10. Weld the final piece from step three so the bottom of the bottleneck holder is 18” from the bottom of the legs (Table 5.2.2.2.: Step 10)
11. Cut two 1”x1”x0.5” squares 7” apart from each other (measuring from the outer edges) and 0.17” from the right edge of the 9”x2.5”x0.5” base pieces so that the legs of the frame can slide in (Table 5.2.2.2.: Step 11)
    a. Repeat 0.17” from the left edge
12. Fill 3L bottle with solution
13. Insert one end of the 5 inch tubing into the 3L bottle
14. Attach shot dispenser on loose end of tubing
15. Flip the bottle upside down and slide tubing through the 1.5” opening of the bottleneck holder at the middle of the frame
16. Place the bottle on the bottleneck holder
17. Latch the bottle in place at the top

Repeat steps #1 through 17 **two** times for a total of three frames

Links for Materials and Necessary Tools

- Latches
    - https://www.newegg.com/p/1FZ-0040-000T5
- 3L autoclavable bottles (H:12 in, D:6 in)
    - https://www.amazon.com/Borosilicate-Storage-Bottles-Culture-Autoclavable/dp/B07668GB6T/ref=sr_1_2?keywords=3%2Bliter%2Bmedia%2Bbottle&qid=1583184728&sr=8-2&th=1
- Necessary Tools Section
    - https://www.wikihow.com/Weld-Stainless-Steel

- Stainless Steel Drilling
    - https://www.grainger.com/know-how/industry/metalworking/kh-which-drill-bit-does-the-job

Table 5.2.2.1. Beta-prototype Frame Materials

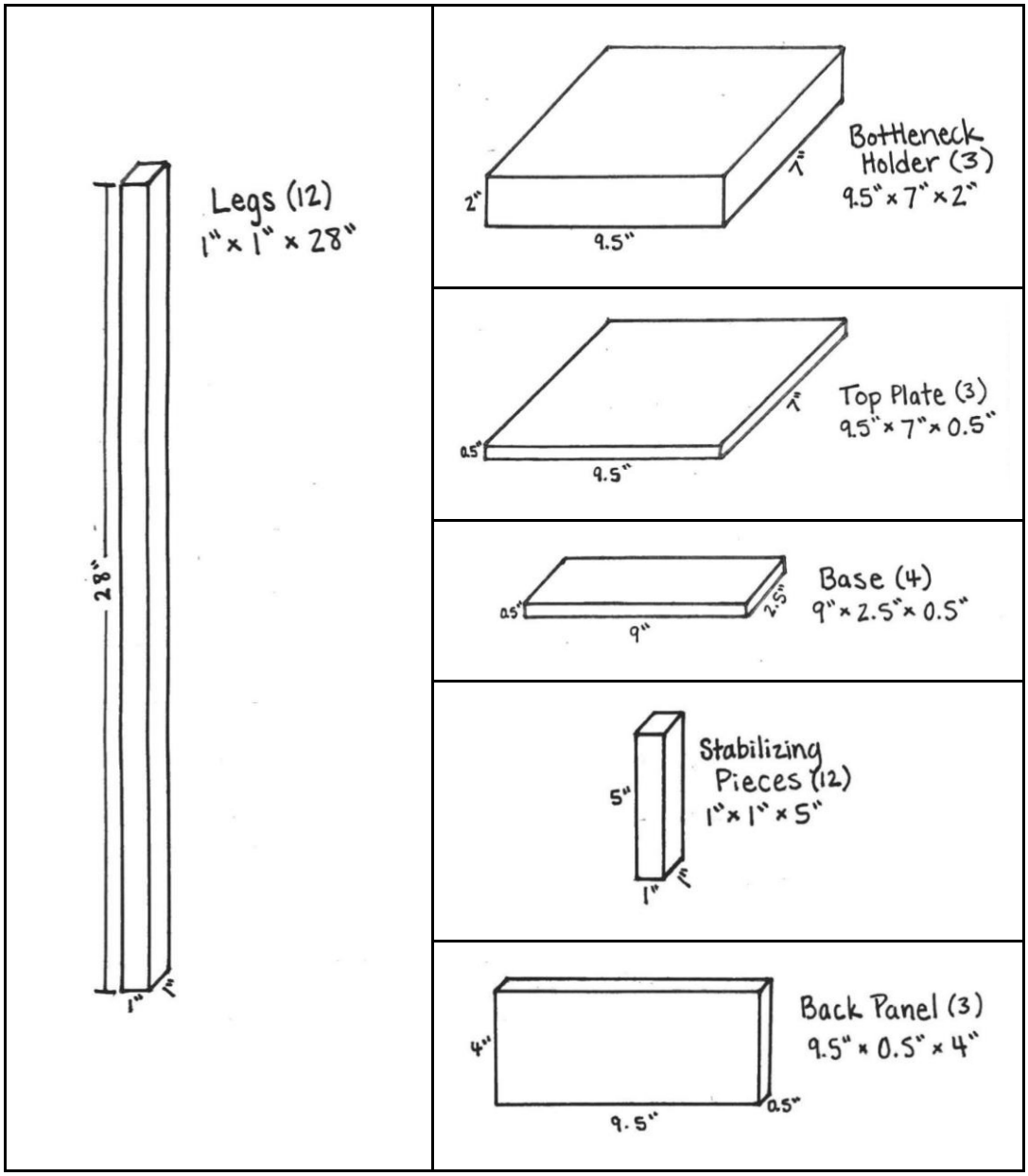
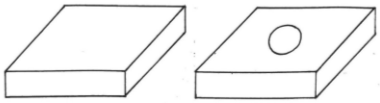
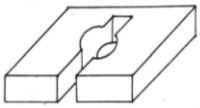
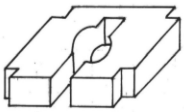
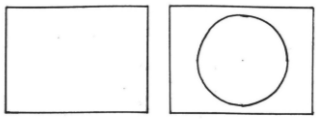
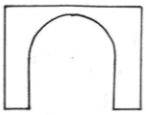
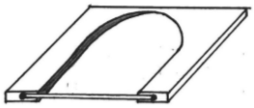
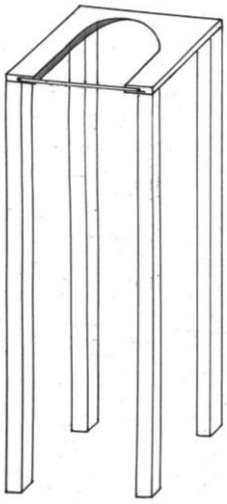
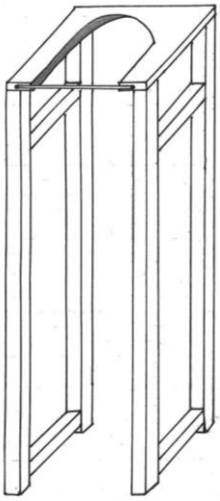
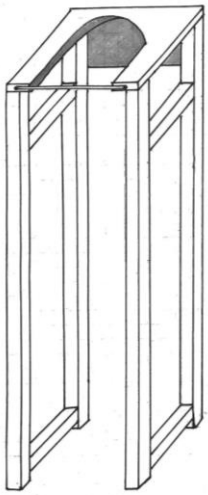
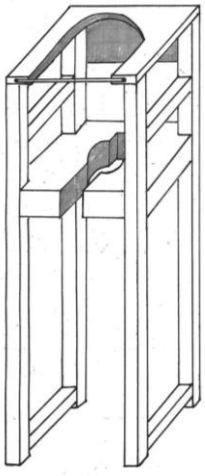
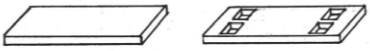
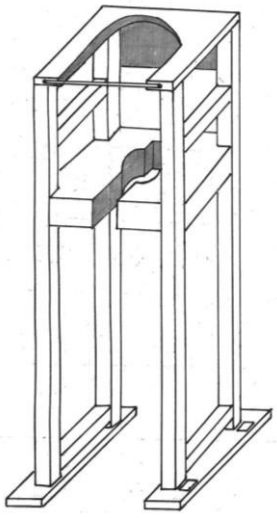


Table 5.2.2.2. Beta-prototype Frame Construction

Step 1 	**Step 2** 	**Step 3** 
Step 4 	**Step 5** 	**Step 6** 
Step 7 	**Step 8** 	**Step 9** 
Step 10 	**Step 11** 	**Final** 

Testing

Test #1 Overview: Dispensing Mechanism of Liquid Network

This test will evaluate the dispensing mechanism of the liquid network subsystem by verifying all of the detergent is washed away, that the matrix is not disrupted, and that the subsystem produces a uniform product.

Objectives

The goal of this test is to evaluate the dispensing mechanism for the liquid network subsystem. This test determines if the liquid network fulfills the functional requirement of washing away all detergent without damaging the matrix. This test will also fulfill the non-functional requirement that the final design shall produce a uniform product, as well as the interface requirement that the solution shall result in the same product or substantially equivalent.

Features Evaluated

This test evaluates the functionality of the liquid network during the dispensing/washing steps of the decellularization process.

Test Scope

Key conditions for this test include: dispensing 30 mL of liquid within the accepted 10% error.

Test Plan

Materials: liquid network beta-prototype, six flasks, flask organizer, microscope, aspirator pump and tip

Tools, Techniques, Skills:

- Dispensing technique: insert tube connected to liquid network into respective flask to dispense liquid
- Aspirator pump + tip: tool used to extract liquid by producing a vacuum
- Aspiration technique: insert tip into flask to withdraw all liquid
- Microscope: operating a standard microscope

Assumptions:

- The AvENGRs assume that StemBioSys will perform this test using materials that are consistent with the current decellularization process.

Data Collection:

- Secure six flasks in the flask organizer and place under the liquid network
- Dispense 30 mL of liquid into each flask and aspirate once complete
- Place each flask underneath a microscope to look for damage and determine if the cell matrix was disrupted
    - Data Recorded: description of matrix structure (damaged vs intact)
    - Trials: 4 trials of 6 flasks

Acceptance Criteria

If the matrix is not disrupted during this process and all of the detergent was washed away, then the results can be considered acceptable and the functionality of the liquid network can be approved. If the matrix is consistently undisturbed after each trial, the liquid network would have fulfilled the non-functional/interface requirements to produce a uniform product.

Test #2 Overview: Time Savings with Flask Organizer + Guide + Liquid Network

This test will include the performance and timing of one round of aspirating and dispensing by one person with and without the incorporation of the flask organizer, guide, and liquid network. The time difference between performing the process with and without the subsystems will be computed. This test can be repeated with two people performing the dispensing and aspirating steps concurrently.

Objectives

The goal of this test is to determine the time savings from the three proposed subsystems and demonstrate that we can save StemBioSys time. A decrease in time for a given number of people to complete the process satisfies the overall project objective to reduce Full-Time Equivalents, as well as meets the non-functional requirement of reducing the total time it takes to complete the process.

Features Evaluated

This test evaluates the functionality of the flask organizer, guide, and liquid network. This test examines whether or not the organizer can accommodate six flasks, if the guide can assist with aspirating without damaging the matrix, and if the liquid network can consistently dispense the proper amount of liquid without damaging the matrix to complete the decellularization process.

Test Scope

Key conditions for this test include: vacuum settings for the aspirator pump (high), variability in fluid volumes within 10% error, and one to two people performing the process in the biosafety cabinet.

Test Plan

Materials: flask organizer gamma-prototype, guide beta-prototype, liquid network beta-prototype, stopwatch, aspirator pump and tip, and six flasks

Tools, Techniques, Skills:

- Aspirator pump + tip: tool used to extract liquid by producing a vacuum
- Aspiration technique: insert tip into flask to withdraw all liquid
- Dispensing technique: insert tube connected to liquid network into respective flask to dispense liquid
- Absolute and percent difference calculations and statistics

Assumptions:

- The AvENGRs assume that the time discrepancies between individual users are not relevant.

Data Collection:

- Record how long it takes one person to complete one round of aspirating and dispensing for six flasks secured in the flask organizer using the guide, liquid network, and a single aspirator tip.
    - Data Recorded: time it takes to aspirate and dispense liquid [seconds]
    - Trials: 4

- Record how long it takes one person to complete one round of aspirating and dispensing for six flasks individually using a single aspirator tip.
    - Data Recorded: time it takes to aspirate and dispense manually [seconds]
    - Trials: 4
- Record how long it takes two people to complete one round of aspirating and dispensing concurrently for six flasks secured in the flask organizer using the guide, liquid network, and a single aspirator tip.
    - Data Recorded: time it takes to aspirate and dispense liquid [seconds]
    - Trials: 4
- Record how long it takes two people to complete one round of aspirating and dispensing concurrently for six flasks individually using a single aspirator tip.
    - Data Recorded: time it takes to aspirate and dispense manually [seconds]
    - Trials: 4
- Compare experimental values using absolute and percent difference calculations.
    - One person: with vs without subsystems
    - Two people: with vs without subsystems
    - One person with subsystems vs two people with subsystems
- Conduct t-test to determine if there is a significant difference in the times

Acceptance Criteria

If the time it took to complete the aspiration and dispensing steps of the decellularization process using the flask organizer, guide, and liquid network was significantly less ($p < 0.05$ from t-test) than the time it took to complete the process without any devices, then the results can be deemed acceptable, and the design requirement is satisfied. If the time is reduced for two people using the subsystems compared to one person, then the design requirement would also be satisfied.

Test #3 Overview: Compatibility of Three Subsystems

This test will evaluate the compatibility and ergonomics of the three subsystems during the decellularization process.

Objectives

The goal of this test is to ensure that there are no consistency issues among the subsystems and between the subsystems and the flasks.

Features Evaluated

This test evaluates the compatibility of the flask organizer, guide, and liquid network subsystems. The operator will observe how compatible each subsystem is with one another by primarily focusing on how the liquid network and guide perform individually with the flask organizer for the dispensing and aspirating steps, respectively.

Test Scope

Key conditions include: focusing on the individual instances when the subsystems interact, and therefore only focusing on the performance and consistency during those times. These interactions included:

- Flask Organizer + Liquid Network (dispensing liquids)

- Flask Organizer + Guide (oriented correctly for aspiration)

Test Plan

Materials: flask organizer gamma-prototype, liquid network beta-prototype, guide beta-prototype, aspirator pump and tip, six flasks

Tools, Techniques, Skills:

- Aspirator pump + tip: tool used to extract liquid by producing a vacuum
- Aspiration technique: insert tip into flask to withdraw all liquid
- Alignment of the flask organizer with the other subsystems

Assumptions:

- The AvENGRs assume that liquids and flasks will be treated as if they are in a real decellularization environment at StemBioSys.

Data Collection:

- For six flasks inside of the flask organizer, perform the action of aligning each of the subsystems and checking for consistency
    - Data Recorded: If any user observes an incorrect alignment or any noticeable flaws in the dimensions.
    - Trials: 4

Acceptance Criteria

The subsystem prototypes can be considered compatible if no alignment errors were observed. If any were, changes must be made and retested by each group member before the subsystems can be deemed compatible. Alignment errors may consist of undesired bending, morphing, or misalignment of predetermined subsystem orientations.

Test #4 Overview: Assembly and Disassembly Time of Liquid Network

This test will evaluate the versatility, mobility, and time cost of the liquid network during the assembly and disassembly of the subsystem. The operator may choose to assemble one, two, or three frames. This aspect of the liquid network highlights the versatility of the design and the mobility of the individual frames.

Objectives

The goal of this test is to evaluate how long it takes to assemble and disassemble the liquid network in the biosafety cabinet. The assembly and disassembly time can be added to the time measurement for performing the process with all three subsystems to determine if there is still substantial time savings when compared to performing the process with the use of no subsystems.

Features Evaluated

This test evaluates the versatility and mobility of the liquid network. The operator will measure the time it takes to assemble and disassemble the liquid network in the biosafety cabinet. This includes securing the frames in the bases, attaching the tubing and shot dispensers, and inserting the bottle into the frame.

Test Scope

Key conditions include: the use of a standard 6ft biosafety cabinet

Test Plan

Materials: liquid network beta-prototype, biosafety cabinet, stopwatch

Tools, Techniques, Skills:

- Liquid network assembly: steps for the assembly and disassembly of the liquid network subsystem are provided in the AvENGR's SOP

Assumptions:

- The AvENGRs assume that the time discrepancies between individual users are not relevant.

Data Collection:

- Perform the action of assembling and disassembling the liquid network in the biosafety cabinet using one, two, or three frames.
    - Data Recorded: Record how long it takes for the assembly and disassembly process.
    - Trials: 4

Acceptance Criteria

The time required for the assembly and disassembly of the liquid network will be considered acceptable if there is still substantial time savings once this is subtracted from the total time savings achieved with the use of all three subsystems. This can be evaluated by performing a t-test to determine if the measured times are significantly different ($p<0.05$).

5.3 Overview of Current Decellularization Process

1. Uncap cell culture flasks (T150's)
2. Aspirate media
3. Wash once with PBS (Phosphate-Buffered Saline)
4. Add detergent
5. Incubate for 7 minutes
6. Aspirate detergent
7. Wash twice with PBS (Aspirating between each wash)
8. Wash with deionized water
9. Aspirate deionized water
10. Air Dry (Usually takes 2 days occupying the BSC)
11. Recap flasks
12. Package for customer

5.4 Manufacturing Results

Table 5.4.1. Manufacturing Research for Flask Organizer and Guide

Component	Company	Material	Process	Cost per Individual Item
Flask Organizer	Forecast 3D	ULTEM 9085	Fused Deposition Modeling	$391
Flask Organizer	Stratasys	ASA (Solid 10)	Fused Deposition Modeling	$792
Flask Organizer	Stratasys	ASA (Sparse 10)	Fused Deposition Modeling	$627
Flask Organizer	Stratasys	Digital ABS	PolyJet	$2,282
Flask Organizer	Xometry	ABS	Fused Deposition Modeling	$260
Guide	Forecast 3D	ULTEM 9085	Fused Deposition Modeling	$1,438
Guide	Stratasys	ASA (Solid 10)	Fused Deposition Modeling	$1,158
Guide	Stratasys	ASA (Sparse 10)	Fused Deposition Modeling	$393

Table 5.4.2. Cost Estimates - Flask Organizer

Company	Quantity	Material	Unit Cost	Cost
Forecast 3D	34	ULTEM 9085	$391	$13,294
Stratasys	34	ASA (Solid 10)	$792	$26,928
Stratasys	34	ASA (Sparse 10)	$627	$21,318
Stratasys	34	Digital ABS	$2,282	$77,588
Xometry	34	ABS	$260	$8,840

Table 5.4.3. Cost Estimates - Guide

Company	Quantity	Material	Unit Cost	Cost
Forecast 3D	3	ULTEM 9085	$1,438	$4,314
Stratasys	3	ASA (Solid 10)	$1,158	$3,474
Stratasys	3	ASA (Sparse 10)	$393	$1,178

ABS Plastic

ABS Plastic is very sturdy, inexpensive, and holds up well with external impacts. This is a good material for finishing a recently developed prototype due to its relatively low cost and reliability.

ASA Plastic

ASA Plastic is an amorphous thermoplastic designed to serve as an alternative to ABS. Therefore, it is structurally similar to ABS, still relatively inexpensive, and also holds up well with external impacts. The only differences are its improved weather and UV radiation resistance, better chemical resistance, and higher long-term heat resistance.

ULTEM 9085

This strong, lightweight and flame-retardant thermoplastic is one of the superior mechanically performing 3D printing materials, as it is primarily utilized in the aerospace and automotive industries. This material's high strength to weight ratio and high thermal and chemical resistance makes it a promising, yet a relatively more expensive choice.

Manufacturer Recommendations for Liquid Network

a) PROTOLABS https://lp.protolabs.com/3d-dmls-production?utm_source=google&utm_medium=cpc&utm_campaign=us-3dp-dmls&utm_term=%2Bsteel%20%2B3d%20%2Bprinting&gclid=EAIaIQobChMIipaW85mM6QIVYR6tBh15LQyJEAAYASAAEgJoWfD_BwE
b) Markforged https://markforged.com/metal-x/?mfa=sga-na-tof-metalaudience&adg=80188519409&kw=metal%203d%20printer&device=c&gclid=EAIaIQobChMIipaW85mM6QIVYR6tBh15LQyJEAAYAyAAEgKTEPD_BwE
c) GPI Prototype & Manufacturing Services https://gpiprototype.com/metal-3d-printing

Recommendations for Commercially Available Products

a) Fisherbrand Autoclavable Bottletop Dispensers https://www.fishersci.com/shop/products/fisherbrand-autoclavable-bottletop-dispensers-6/p-4753023
b) DOSE IT Laboratory Peristaltic Pump - Integra Biosciences https://www.integra-biosciences.com/united-states/en/peristaltic-pumps/dose-it

5.5 OSHA Standards

1910.133 - Eye and face protection: https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.133

1910.138 - Hand Protection: https://www.osha.gov/laws-regs/regulations/standardnumber/1910/1910.138

1910.212 - General Requirements for all Machines: https://www.osha.gov/enforcement/directives/std-01-12-009

6. Bibliography (APA)

[1] BioTek. (2019). MultiFlo FX multi-mode dispenser. Retrieved from https://www.biotek.com/products/liquid-handling-multi-mode-washer-dispensers/multiflo-fx-multi-mode-dispenser/?Product_Interest_Source=PPC%20-%20Adwords&source=PPC%20-%20Adwords&Lead_Campaign_Source=7011O00000389Mh&Campaign_Source=7011O00000389Mh&gclid=CjwKCAjwlovtBRBrEiwAG3XJ-4c906t0y7oJ448QfweV5IPQovVDFms0DWPBna_4qDSOrBKXJgQeZBoCZIcQAvD_BwE

[2] UNITED STATES DEPARTMENT OF LABOR. (n.d.). Retrieved May 5, 2020, from https://www.osha.gov/

SIGNATURES		
Project Name: CELLvo Matrix Manufacturing Improvement		
The undersigned have reviewed and approved the final version of this document.		
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Team Adviser: T. Jackson Block, PhD	04/30/2020	5/6/20