Automated Decellularization of Heart Valves

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***Abstract*—For those living today with severely malfunctioning heart valves, replacement is the only available treatment. Patients are today typically offered either mechanical valves, xenograft or allograft. Decellularized heart valves have the potential of becoming one of the standard alternatives for such heart valve transplants. Decellularized heart valves offer a reduced risk of calcification and immunogenicity compared to allograft, xenograft or mechanical valves and enables the valve to grow with the patient. The processes of decellularization is however labor intensive and time consuming, which severely limits access to decellularized transplants for patients. Automating this process would lower the cost and increase the reproducability of decellularized transplants.**

This paper will investigate in-depth how a decellularization device could be constructed, considering Computer Aided Designs, physical prototyping and software controlling the electronics. The device could also include decellularization programs for skin grafts and aortae. The final product will be made to reduce the labor of decellularizing tissue to the a press of a button, thus contributing to increasing availability of decellularized organs.

I. Introduction

Recipients of heart valve transplants are today typically offered either mechanical valves or cryopreserved human or porcine heart valves. All three transplantation methods entail serious medical complications, as the patients require a lifetime of anti-thrombotic and immunosuppressing medication and often have to undergo reoperation. [1] In order to increase the patient's quality of life a new generation of heart valve transplants is emerging; Decellularized heart valves have shown great promise in clinical trials, allowing the transplant to grow with the patient and reducing the risk for calcification and immunogenicity. [1] Decellularized tissue is stripped of cellular and genetic material, leaving only the extracellular matrix (ECM). [2] Once transplanted, the recipients own cells populate the scaffold, greatly reducing the risk of triggering a foreign body response. [1] The process of decellularization is however still time consuming and expensive, which today is the prime limiting factor for accessibility. Automating the process reduces the time and labor to produce a decellularized heart valve, thus lowering the cost and increasing reproducability.

Automated decellularization could be performed for other organs, skin grafts and aortae especially, as they are of similar size and require only immersion-based decellularization techniques. Decellularization of skin grafts, aortae and heart valves would be the three programs run by the Automated Decellularization Unit (ADU) for the future continuation of this project.

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A. Goal

The aim of this project is to design a prototype for a device that will conduct the entire procedure of decellularization of heart valves autonomously with minimal need for surveillance from lab personnel. The device has the working title Automated Decellularization Unit (ADU).

B. Agenda

This report will present and discuss the Computer Aided Design (CAD), chosen components and the software conducting the procedure. Further, examples of how following prototype iterations may be designed will be discussed.

C. Demarcations

This paper will focus on the technical aspects of constructing a device used for decellularization. It will include the methods by which the parts product could be constructed and manufactured and look further ahead on forming the next iteration of the prototype. The details regarding chemical and medical aspects of decellularizing tissue and transplanting organs will only be briefly discussed. Decellularization of tissues and organs that require perfusion based techniques would require more functions than explored in this project and will thus also not be covered in the report.

D. Theory

Executive summary of decellularization process

The protocol consists of the homograft being submerged in different solutions in a certain order, while subjected to a vacuum and motion from a shaker table, for a specified time depending on the solution. [5] The vacuum is essential to release air bubbles from within the tissue and allow more contact between tissue and solution. The latter is also the reason for the mechanical agitation. In between each solution the tissue container is filled with new DI water five times, under the same vacuum and motion, to wash the tissue and container of any residue of the previous solution. When the protocol is finished, the homograft is stored in antibacterial and antifungal solutions to later be cryogenically preserved.

E. CAD

Computer Aided Design is the use of computers to aid in the drafting, creation, modification and simulation of design. CAD for mechanical drafts uses vector-based graphics to convey information of things such as dimensioning, materials, tolerances and aesthetics. In this project, all CAD sketches were drawn using *SolidWorks2020.*

F. Solvent welding

To create a joint between parts of thermoplastics, a solvent can be inserted along their border of contact to dissolve and later join the parts. In the case of acrylic polymers, acetone can act as a solvent, which upon evaporation leaves a welded seam between the previously disjointed acrylic parts. [4]

G. Solenoid valves

Solenoids are electromagnetically operated valves, in their most basic form consisting of a magnetic metal plunger surrounded by an isolated electrical coil. When current is run through the coil a magnetic field is induced, directed parallel to the direction of the plunger, causing it to move. The plunger is also connected to a spring, meant to reverse its motion once current stops flowing through the coil. The valve therefore has two settings, open and closed. In this report, the valves discussed are closed while in neutral and opened with current flow. [6]

II. Method

A. Manual Decellularization Protocol

The decellularization protocol can be described using the following list of key steps. The list was used to define essential functions of the ADU.

- The homograft is placed in a transparent plastic container with a screw on lid. The lid prevents liquid spilling but does not form a tight seal which allows the air inside to escape when vacuum is applied. The tissue is first stored and washed in DI water.

- The tissue container is placed in a metal bucket with a rubber-coated lid connected to the house air pump. This set-up is sufficient to lower the air pressure inside to approximately 0.25 atmospheres.

- The entire vacuum chamber is placed on a shaker table to keep the solution in motion allowing for more reactive contact with the tissue. The tissue container is kept in place on the floor of the chamber by styrofoam bricks that expand and squeeze the container in place when air pressure is reduced.

- All solutions are manually prepared in a laminar flow hood with autoclaved beakers to ensure sterility.

Automating this process would require many more mechanisms. A list of functions and component requirements for the device:

- Tissue container:
  When automating this process the container must have inlets and an outlets with connecting tubes so solutions can flow into and out of the container. Since the tubing is washed with DI water in between each step, there is no considerable risk that a solution will be contaminated by passing through the same tube as the previous solution. Therefore no more than a single solution inlet is required. Tubing could instead be branched in a manifold structure from each container to the main inlet tube.
  The detachable lid could be used to fixate the inlet and connecting tube. Still important is that this lid allows the air inside to escape when vacuum is applied. Finally, to ensure sterility and simplify the transport and storage of tissue, this containers may have to be disposable and purposed for one time use.

- Vacuum Chamber:
  The chamber must allow for solution to flow in and out of the tissue container without compromising the vacuum. It could also be helpful if the chamber is transparent, granting the user some visual monitoring.
  Just like the chamber used in the manual process, the chamber must be easily opened and sealed shut and the container inside must be mounted in such a way to prevent it from moving relative to the chamber.
  Assuming all clinics and labs have a central vacuum system the device itself will not have to be equipped with a vacuum pump.

- Shaker table:
  The protocol does not specify any amplitude or frequency of shaking, the motion is simply meant to keep the liquid from being stationary relative to the tissue. The final design of this device will include a built-in shaker table, but since such tables are commonplace the coming iterations of prototypes will most likely feature a purchased existing shaker table. Future iterations, and perhaps even the final product, could feature a shaking mechanism manufactured by an external actor, made specifically for the ADU.

- Solution containers:
  All solutions must be prepared in correct volumes and concentrations before starting the process. Solutions would in an automated setting be kept in separate containers on standby to later enter the tissue container. Ensuring the sterility of these containers and their connecting tubes is essential to the purpose of this device.
  The containers themselves could be detachable and equipped with spring-loaded ball valves at the bottom end that open once they are mounted in place.
  To circumvent the need for additional pumps driving the flow of solution, the solution containers could be mounted above the tissue container, allowing gravity to create the appropriate pressure gradient.

- Valves and pressure:
  With constant pressure from above due to gravity, the flow of solution would only have to be controlled by valves that open and close at certain times. As these

valves would be part of the hermetic seal, they would have to open and close in a particular order.

B. Computer Aided Design

With the above stated requirements an initial conceptual sketch (Figure 1) was drawn with a transparent tissue container (1), a lid with solution and vacuum nozzles (2), an outlet for waste solution (3), all contained in a box (5) acting as a protective shell with a partially transparent lid (6). The shaking mechanism was imagined to consist of a rotating plate (4) to which the tissue container is connected off-center.
To circumvent the need for hydraulic pumps when moving solutions into the tissue container, the solution containers were to be mounted on a rack (7) above the box, allowing gravity to create the pressure gradient over the valve. This also allows for minimizing the device's footprint on the work bench, which in discussion with lab personnel often was mentioned, as work bench space is limited and valuable. This concept of a vertical design was kept in mind throughout the entire design process.

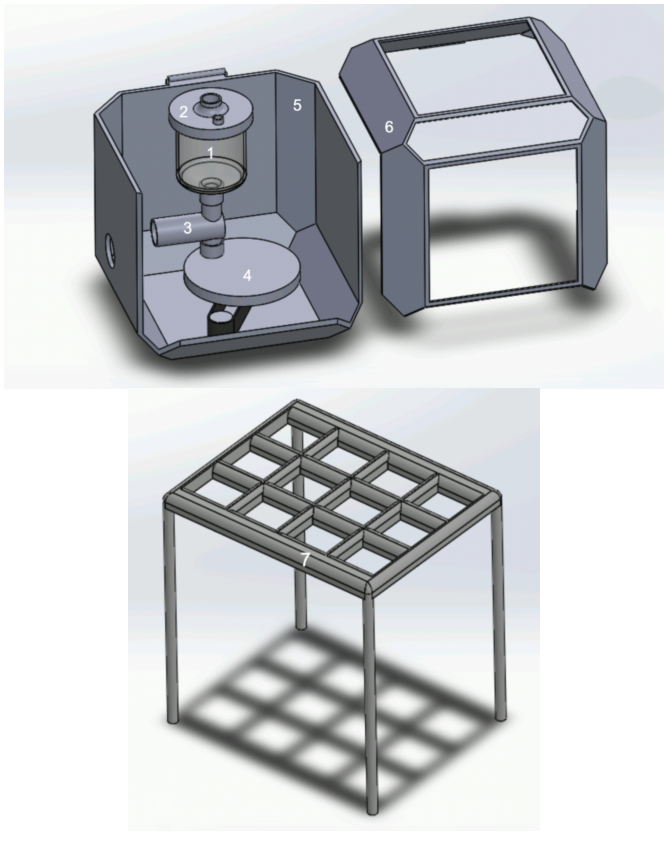

Figure 1. Initial concept sketch

C. Components and manufacturing

To create a working physical prototype of this device, some mentioned components can be purchased, some can be manufactured and some can be temporarily replaced:

- Vacuum chamber:
  For this project, a box was manufactured using thin sheets of transparent acrylic. Acrylic was used because as it was the most suitable and affordable material available that still fit the requirements of stiffness and joinability. The sheets, 3 mm thick, were cut into duplicates of three different pieces using an Epilog Zing 24 laser cutter. Figure 2 shows the dimensions of the pieces, where the grooves seen in the bottom and right piece were etched onto the surface to mark their locations for milling. Using a ShaperTool Origin the grooves were milled to half the thickness of the sheet and the pieces were fit together, see figure 2. This was done simply to test the efficacy of the cutting and milling procedures, as well as the geometries of the pieces.
  To test the efficacy of solvent welding two pieces of scrap acrylic were solvent welded together. In this case, acetone was inserted by syringe along the border between the edge of one piece and a milled groove at the center of the other, see figure 9. The strength of the weld was tested in an ad hoc setting, where two filled coffee cups were placed on either side of the weld and (successfully) lifting the entire piece, see figure 10.

- Solenoid valves:
  The flow of liquid from solution to tissue container needs only bimodal regulation; On and off. Because of this, the flow can be controlled using a simple two-port solenoid valve that opens and closes with the electrical current run through it. Affordable valves that can be regulated with small currents and maintain the hermetic seal are widely available for purchase. It must also be ensured that the metal parts of the solenoids won't chemically react with any of the solutions they are thought to come into contact with. [9]

- Vinyl tubing:
  High-flex vinyl tubing is ubiquitously available at affordable prices [10] and would make excellent connections. Due to their ductility they can be fit tightly inside the connector. As these are thought to remain in place in the device, their connections can also be reinforced with some elastic lining such as silicone glue.

- Connectors:
  The T-, Y, and cross-connectors, see figure 11, at the center of the manifold tubing structure, see figure 5 can be 3D-printed using ABS plastic. This material is inexpensive, strong, non-reactive to chemicals in this protocol and shrinks slightly after prolonged exposure to air, causing it to tighten its connection around the outer face of the vinyl tube over time.

- Shaker table:
  The shaker table used for the manual protocol [13] basically consists of an electrical engine connected to three rotating cylinders by a drive band. The tabletop is connected off-center on these cylinders, causing it to move along a circular path when the engine is active. As this technology is standardized and widely available, coming iterations of this prototype would not require its

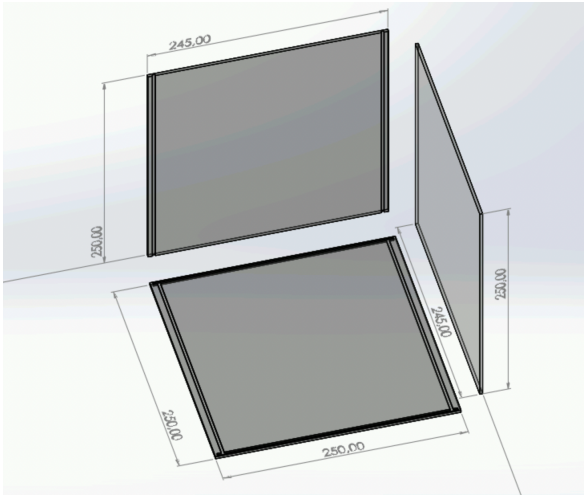

Figure 2. Acrylic pieces for laser cutting and milling

in-house design and manufacture.

D. Software Development

A Java program was written to demonstrate how the device would be controlled. The Java software of the ADU controls the buttons, the shaker table, the DI valve, the solution container valves, the tissue container valve and the vacuum pump. Solenoid valves are used for all tubing connections, since they can easily be electromagnetically controlled using electrical circuits and software. [6] The ADU software will be monitoring when all the solution and chemicals are to enter the tissue container during the process and will enable the unit to work autonomously for 24 hours.

III. Results

A. Final concept design

The final concept design, see figure 3, consists of a transparent vacuum chamber (1) mounted on a shaker table (2), a compartment for tubing and electrical wiring (3), solution containers (4) and a waste bin (5). The lids on the vacuum chamber, solution containers and the waste bin are in this sketch elevated to show that these parts are detachable. The container lids also feature holes to allow for air flow into the container when the solution is released below. The yellow lining between chamber and its lid represents a soft rubber surface meant to reinforce the hermetic seal.

With this device the user would place the tissue in the vacuum chamber, fill the large solution container with DI water and the smaller containers with each solution. When started the program will apply a vacuum, initiate the shaking motion and release each solution in order and at the end of each cycle, empty the solution into the waste bin. To keep the design as simple as possible, the device relies on an external vacuum pump, which is assumed to be available in most lab settings. As each solution flows into and out of the container, the vacuum must be released and reapplied, since the inlet and outlet valves are part of the hermetic seal.

A cross sectional view, see figures 4 and 5 show how all tubes from DI water and solution containers are connected in a manifold using T-, Y-, cross-connections (6) that all gather into a main tube (7) leading to the tissue container. This tube is closely coupled and sealed with the lid of the vacuum chamber (8), and dimensioned to connect to the fixed connection on the tissue container inside the chamber (9). Finally, when a part of the cycle is finished, the solution is emptied into the waste bin by way of the bottom outlet (10). Underneath each container will be a solenoid valve, controlled by the computer.

The homografts this machine are thought to treat are small enough to become entirely submerged in a filled 250 ml container with a 100 mm diameter. As the DI water container has a volume of 3 liters, the size of the tissue container and height of the chamber are not drawn to scale, but exaggerated to show more detail of their design.

With a cubic vacuum chamber dimensioned at 250x250x250 mm and a waste bin with approximately the same footprint area, this final concept design would take up circa 500x250 mm of workbench space. The size of the waste bin can easily be increased if needed by providing it more space under the device. If manufactured using standard thermoplastics (Polyethylene, polypropylene, ABS, acrylic) the entire device would, including shaker table, weigh less than 20 kg when empty. With all tubing and electrical wiring contained inside a compartment, this device would have no considerably fragile parts easily damaged by routine work.

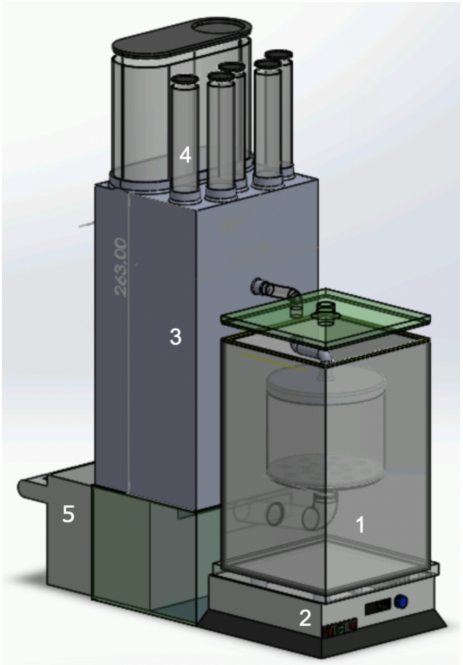

Figure 3. Final concept sketch

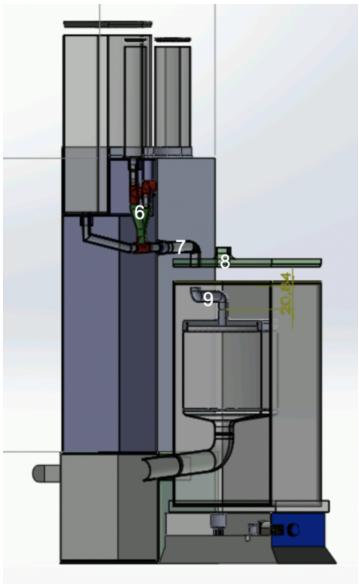

Figure 4. A cross-sectional view

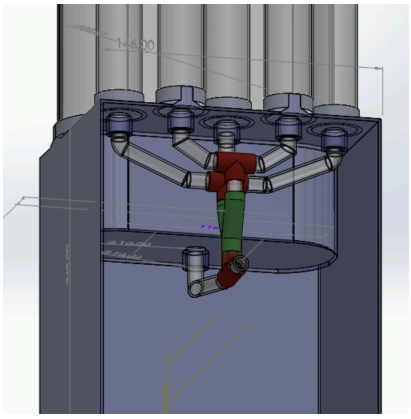

Figure 5. Manifold tube connection

B. Software

The ADU of today is constructed to decellularize heart valves. It would not require much effort to include skin grafts and aorta as separate programs of the device. The decellularization protocol differs however the solution containers could hold any given substance and the software could be adjusted to fit the decellularization protocol of these organs. The user would then turn the ADU on using the switch and then be asked to choose decellularization program, see 6. If pressing the heart valve button the user would enter decellularization of heart valve menu, see figure 8

C. First software version

The first software version was constructed aiming for a slim and simple design with three buttons. An on/off-switch, a start button and a stop button, see figure 7. The switch would turn the power on/off for the device. The start button would initialize the three day program that would work uninterruptedly for three days without any need for monitoring. The program could be stopped safely by pressing the stop button.

Stopping the program by pressing the stop button triggers a series of events, stopping the machine safely without damaging the tissue. The stop program forces the shaking table to stop, the tissue container to empty and the vacuum chamber to reset to normal air pressure. After pressing the stop button the user will be free to remove the heart valve from the device or restart the program, continuing the decellularization process. The machine will remain switched on after pressing the stop button.

D. Latest software version

The latest software version included a couple of new functionalities and improvements, see figure 8. First of all a sterilization program, where peracetic acid is used to sterilize the equipment. Peracetic acid is a widely used chemical to sterilize medical equipment [3].

The sterilization program consists of two steps, flushing all parts with peracetic acid and the DI water respectively. First, the user is instructed to fill all 6 containers with 250 ml of peracetic acid and press the sterilize button. The sterilization program will for each container in turn order allow peracetic acid to run through the tubing and fill the tissue container. When the tissue container is full the tissue container valve opens disposing the peracetic acid into the waste container. Second the user is instructed to fill all 6 containers with 250 ml DI water and press sterilize once again to flush all parts from residues of the peracetic acid, completing the sterilization procedure.

All containers and tubing could be removed and cleaned manually or be replaced. After replacement sterilizing using the program is recommended.

The latest software and design is equipped with four buttons, one for each day, replacing the start button, see 8. The change was made due to that the solutions used for decellularization preferably are prepared the same day. This would require monitoring of the process once per day, however lab personnel would most likely be present in the lab every day of decellularization either way. With the design the user would only need to prepare the solutions for day 1, hit the day 1 button and return the coming days of the program performing the same procedure.

The latest version of the ADU is equipped with an emergency break. This button is to be pressed in case of an accident, e.g. where the machine might break or harm the user. The emergency break will bring the machine to stop immediately and shut down the power supply. The shaker table will stop and all valves will close. The emergency break, not included in figure 8, will be positioned low on the side of machine clearly visible for the user.

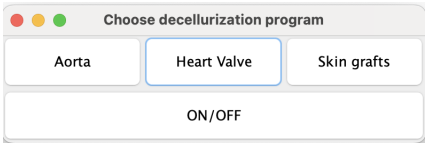

Figure 6. Java Window displaying top menu where the user can choose tissue to decellularize.

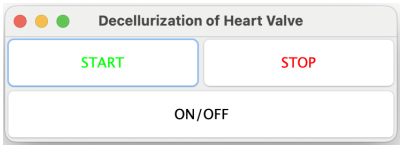

Figure 7. Java Window displaying the first version of the menu for decellularizing heart valves.

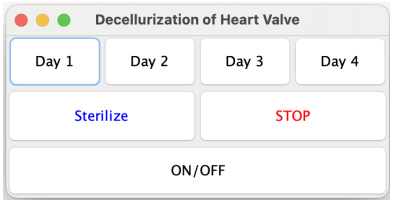

Figure 8. Java Window displaying the latest version of the menu for decellularizing heart valves.

IV. Discussion

A. Software

The ADU program demonstrates how the software would perform in the next step when continuing this project further into becoming a physical product. The future hardware would in the first stages of development be controlled using a Raspberry Pi, since a Raspberry Pi is easily modified and has all necessary functions. Java is an efficient programming language for Raspberry Pi purposes [12].

B. Concept sketches

The initial concept sketch has several flaws. The main design differences between the first and final design are the location of the shaking mechanism and vacuum connection. In the initial design, the vacuum was thought to be applied directly on the tissue container, which would place many manufacturing requirements on a component later decided to be disposable.
The shaker mechanism was moved outside the entire box after deciding the shaking mechanism would not be manufactured in-house for the coming iterations of this prototype.

C. Vacuum Chamber

Many things can be done to create an even stronger weld between the acrylic walls. The surfaces welded in this trial were milled and sawed off by hand, creating rough contact surfaces. The grooves in these pieces of scrap acrylic were also made as practice using the ShaperTool, making their boundaries unnecessarily irregular. With a smooth, laser cut surface and a milled groove made to fit perfectly, the contact surface between the pieces and would increase and decrease the number of air pockets inside the weld. The borders of the weld can be reinforced by some elastic material, such as silicone glue, that expands into irregularities and pockets when pressure is reduced.
To create a working vacuum chamber many things must be improved on the current design. First of all, the walls must be thicker than the current 3 mm to decrease deflection and withstand the pressure gradient. Thicker plates can be purchased, but at considerable cost [7]. Before making such a purchase, thicker walls can be manufactured by stacking multiple 3 mm plates and welding them with acetone.
The rectangular shape of the walls might also not be optimal. To maximise air evacuation and minimize material deflection and distribute stresses uniformly, the optimal shape of a chamber is a sphere, followed by a cylinder with domed ends [8]. However for the purposes of this project, with the relatively small pressure gradient required (0.25 atm), the difficulty in manufacturing a spherical or cylindrical chamber could override its utility.
The main difficulty in creating a stable vacuum is expected to be manufacturing the seam between lid and nozzle, and between nozzle and pump. For the manual decellularization process, the connection between nozzle and pump consists of a fastened steel wire reinforced vinyl tube, see figure 12. For coming iterations of the prototype this solution is assumed to suffice. To manufacture a nozzle, a hole could be drilled in the lid and welded with an acrylic pipe. The same difficulties as welding edges could arise here, making it essential that the inside surface of the drilled hole is smooth and fits the pipe perfectly.

D. Solenoid valves

Before purchasing solenoid valves [9], the program can be test-run by replacing the valves with another bimodal component coupled to a switch, such as a diode. Having the program turn the switches on and off in the right order and duration is essential to the automated unit and vacuum chamber. As these solenoids are built for hydraulic purposes, it would also be important to test how well they form a hermetic seal. This could be done by initially purchasing only one solenoid and connecting it to the existing vacuum chamber for the manual protocol. Further, it would be desirable for the valves to be small and require small electrical currents to operate.

E. Regulation

Before launching the ADU as a commercially available product, it must pass the approval of a notified body and live up to the requirements of GMP (Good Manufacturing Practices) protocol. As the device is sterile, non-invasive and active it could possibly be classified as IIa och IIb withing the bounds of European legislation. Since the unit is meant to treat homografts for transplantation, sustaining human life

and posing substantial risk for the recipient, an argument can be made for classifying the ADU as III.
GMP regulation for ATMP (Advanced Therapy Medicinal Products) follows a similar procedure, stating *"Compliance with good manufacturing practice (GMP) is mandatory for all medicinal products that have been granted a marketing authorization. Likewise, the manufacture of investigational medicinal products must be in accordance with GMP. Advanced therapy medicinal products that are administered to patients [...] must be manufactured under equivalent quality standards to the manufacturing of advanced therapy medicinal products with a marketing authorization."* [11]

F. Sterility

The maintenance of sterility in the ADU is imperative and can be done in several ways. The prototype's program as of now includes a routine specifically for sterilizing the internal surfaces of the unit. The disposable tissue containers will according to standard lab procedure have to be autoclaved before use. Along with routine washing and sterilization of the unit, regular sterility tests twice per year would have to be conducted by a ADU technician.

G. Ethics and sustainability

When conducting decellularization with future ADU prototypes the main ethical consideration will be similar to when conducting manual decellularizations, with regards to sourcing, management and disposing of donated tissue.
No imagined future components of the ADU will require any materials of shipping methods that are of considerable concern, with regards to both ethics and environmental impact. It will however still have to be made a priority that components and materials are sourced ethically from trustworthy manufacturers and long shipping lines are avoided if possible.
The main achievement of this project with regards to sustainable design is the gravity-driven flow made possible with the ADU's vertical design. This will considerably lower the unit's overall power consumption and reduce the number of components that must be ordered for the device's further development.

V. Conclusion

A device like the ADU would be of tremendous utility to the medical field, as it makes available the most promising new generation of organ transplants to clinics worldwide. This project was one of exploring the possible designs and functions of such a machine. Moving forward developing the ADU would require further specification of parts and functions, including further design improvements, issues in manufacturing parts and integrating purchased components.

VI. Acknowledgements

We want to thank Darcy Wagner, Ph.D, Principal Investigator at Lung Bioengineering and Regeneration, Lund Stem Cells Center and Group Leader for entrusting us with this project and her assistance with the product development throughout the entire process. Many times when we were stuck her guidance led us to the greatest discoveries in this project.

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This project was with the help of advisors, conducted by Brendan Carlson and Filip Nyberg, Bachelor's students of Biomedical Engineering at LTH. The entire process of learing the manual decellularisation protocol, conducting literature study and defining the ADU's function requirements was done jointly, as well as the writing of this report. The details of the software development was done by Nyberg and the sketches from the CAD-terminal was done by Carlson.

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APPENDIX

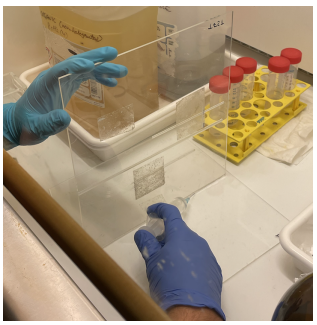

Figure 9. Syringe application of acetone

Figure 10. Ad hoc stress test of solvent weld

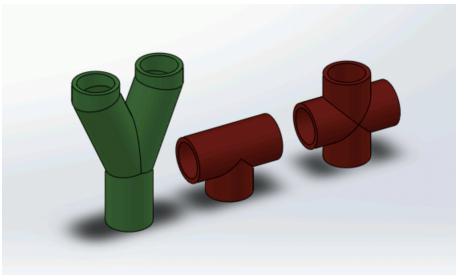

Figure 11. From left to right: Y- ,T- , and cross-connector

Figure 12. Connection between lid and air pump

Figure 13. Cut and milled acrylic box, with Filip for scale