Designing a bioreactor to test mechanical properties of decellularized heart valves

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***Abstract*—Aortic valvular disease is a severe ailment that causes great suffering and many deaths worldwide. The exponentially increasing prevalence of the disease has accelerated the demand of a new functioning valve prosthesis. A new approach is to decellularize homograft heart valves, which has demonstrated promising results in clinical trials. The Lung Bioengineering and Regeneration Lab in Lund has developed a new method to decellularize human heart valves with containing cardiac tissue, yet questions remain as to how they function *in vivo*.**

The aim of this project is to design and build a customized bioreactor in order to test the mechanical function of the decellularized heart valves with cardiac tissue. The design and methods used sought to create a bioreactor that allowed testing of valves with various sizes in a nontoxic environment which mimics the flow dynamics of a working heart. Most parts in the bioreactor were 3D-printed at Xlab in Lund with PETG filament.

The project resulted in a bioreactor which enabled testing of decellularized valves with different diameters during static and pulsatile flow. The bioreactor creates the opportunity to investigate the working valve in real time. The methods used in this project are indicative, but not conclusive. Future efforts should focus on using a different manufacturing process and should accurately measure pressures up- and downstream of the valve to validate it's function.

I. Introduction

Valvular heart diseases (VHDs) are an increasing cause of death worldwide, and has grown exponentially in prevalence for both pediatric and geriatric patients in recent years. VHDs will result in dysfunctional valves which will either start to leak (regurgitation) or get too narrow and stiff (stenosis). The most common afflict in Sweden is aortic valve stenosis (AVS), with more than 85 000 persons over the age of 65 suffering from the disease [31]. Untreated severe AVS is a serious condition, leading to a survival rate of 50 % to live another 2 years [9]. Moreover, AVS is a leading risk factor to other heart diseases such as heart failure and angina pectoris. Symptoms associated with AVS can eventually culminate in a decrease in life quality, particularly by interfering with physical activities due to the loss of heart function [27]. There is no cure for late AVS and for that reason, the most frequently used treatment is to replace the valve with a prosthesis. The procedure is lifesaving and will in most cases improve the blood flow and reduce complications linked to AVS [8]. However, today's prosthesis come with several drawbacks that will affect the patients daily life.

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A. Physiology and Composition

The valves in the heart prevent backflow of blood, and will thus create a difference in pressure between each chamber. The heart has four valves: the mitral- and biscupid valves which separate the atrias from the chambers, and the pulmonary- and aortic valves which separate the chambers from the great arteries [14]. All valves have 3 leaflets except the mitral valve, containing just 2 leaflets, which open and close the passages between the cavities of the heart. In order to withstand the challenging mechanical environment in a pumping heart, the valves need to be resistant against stresses and strains. Therefore, the outer layer consists of valvular endothelial cells which support the hemodynamic forces [7]. The scaffold of the valve is divided into 3 layers with different compositions. The fibrosa layer, located on the artery side of the pulmonary- and aortic valve, is mostly composed of collagen fibre that will provide tensile stiffness [16]. The inside of the valve is filled with proteoglycans and collagen, named the spongiosa layer. The connective tissue in this layer enables the valve to be compressed [2]. Located in between the the extracellular scaffold is the valvular interstitial cells, helping the ECM to maintain its normal function. These cells are responsible for degradation and synthesis of new ECM, which are essential attributes that the valve needs to withstand the mechanical stresses [28]. The last ECM-layer, ventricularis, will be found on the ventricular side of the pulmonary- and aortic valve. The elastin in this layer provides elastic properties allowing the valve to be extended [29].

B. Clinical Applications

There are two different main types of heart valve replacements that is clinically used: mechanical heart valves (MHVs) and biological heart valves (BHVs). MHVs are today's golden standard for patients under the age of 55 due to its lifelong durability. The leaflets are comprised of a non-biological material, and surrounded by a knitted ring which enables the valve to be sewn onto the right place in the heart. The materials used in the leaflets need to have the right mechanical properties in order to withstand the high pressures during millions of heartbeats. In addition, the harsh environment of the body requires a material with good biocompatibility in order to decrease risk of infections and other immunological concerns. The most frequently used materials to manufacture MHVs are pyrolytic carbon, titanium and cobalt [12]. Recent studies have shown that mechanical valves bring a 50% lower risk to get prosthetic valve endocarditis compared with BHVs. Prosthetic valve endocarditis is a severe infection on the valve linked

to high mortality and despite this, the most common valve prosthesis used in the clinics are the BHVs although the risk of infection increases [11]. The reason why MHVs aren't used to a wider extent is due to the elevated risk of calcification and valve thrombosis. Both conditions will cause the valve opening to become narrow, leading to a reduction of blood flow. Therefore, all patients with MHVs will get a lifelong treatment with blood thinning medicine in order to reduce the adhesion of calcium and platelets on the valve. This can be problematic for patients with comorbidities, or patients older than 65 due to a higher risk of bleeding [5]. Moreover will reoperations be needed for younger growing patients, since the MHVs eventually will become too small.

BHVs are usually xenografts from bovine or porcine, but can also be homografts sourced from human cadavers. Since the valves are composed of a biological material, the risk of getting valve thrombosis and calcification are much lower due to its exceptional hemodynamic properties compared with MHVs. The valves are pretreated with detergents to suppress the immunological reaction from the host's immune system [23]. BHVs have different mechanical properties compared with MHVs and the native valve since the chemically treated ECM is unable to repair itself, making it prone to mechanical degeneration [17]. This will affect the expected durability of the BHVs, which in most cases will last for 10-15 years. By this reason it's not an appropriate solution to use BHVs in younger patients, due to an elevated risk of needing reoperations of the valve prosthesis.

C. Decellularized Heart Valves

A new upcoming approach regarding valve prosthesis is to use decellularized heart valves (DHVs) from human cadavers. The cells will be removed, leaving a composition of connective tissue to be left in the valve. Recent European studies have demonstrated promising clinical outcomes, which surpassed today's BHVs. Since the valve doesn't contain any cells, it will carry less immunological concerns. Moreover, it was shown that the degradation rate was slower for DHVs, leading to a lower risk of retransplantation [6]. In contrast to MHVs, the DHVs are composed of naturally occurring connective tissue which are less accessible for thrombosis and calcification. However, the human valves used in the trial didn't contain cardiac tissue, which are preferred by many surgeons worldwide, including them in Scandinavia.

D. Bioreactors

The harsh environment of the heart sets high demands on valve prosthesis. This will result in difficulties testing new valves *in vivo* due to the high risk of complications. It is thus necessary to evaluate the abilities of the valves to work in a condition similar to a normal heart. Bioreactors are a fundamental tool used for physiological and chemical *in vitro* testing of new valve prostheses. There are different types of bioreactors out on the market, but the essential components of any bioreactor are somewhat similar. A bioreactor has a pump in order to mimic the cardiac output. The most commonly used are pneumatic air pumps which moves a diaphragm, and peristaltic pumps which pushes liquid through rotational motion [4], [3]. A bioreactor system should also include a valve holder to help keep the valve in the right place, a reservoir with cell culture media, a capacitance chamber to store and release energy every pulse cycle and a resistance [4]. This will create an environment comparable with a pumping heart, which will give scientists crucial information regarding the mechanical and biological properties of the heart valve prosthesis.

E. Objective

A new decellularization method for DHVs with cardiac tissue has been developed in the Lung Bioengineering and Regeneration Lab in Lund. This method will allow more surgeons to work with the material they are used to, which is an important requirement in order to establish DHVs in the clinics. A crucial step forward is to test the ability on whether the valve with cardiac tissue can withstand the high pressures in the heart or not. The aim of this project is therefore to design a customized bioreactor that can test the mechanical function of the DHVs during static and cyclic pressures, mimicking the mechanical environment in a pumping heart. The DHVs used in this work is demonstrated in Fig. 1. With the help of two pressure gauge sensors, one upstream and one downstream from the valve, information regarding the different pressures could be collected.

The methods and materials used in this report will be based on the design requirements to obtain a functioning bioreactor. All prototypes, sketches and the complete bioreactor system will be presented in results. The limitations and strengths regarding the bioreactor will then be analyzed in discussions.

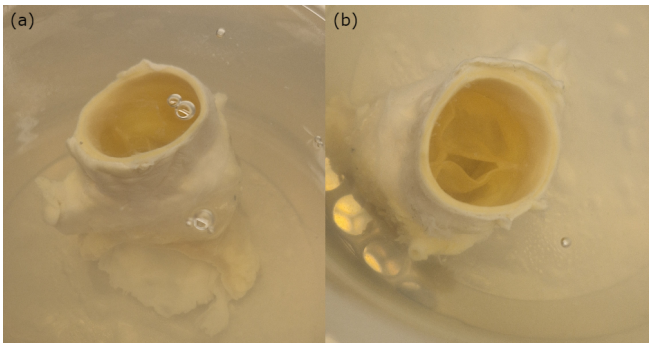

Fig. 1. Pictures of the decellularized heart valve used during this project. Pictures (a) and (b) are taken from two different angles.

II. Materials and Methods

A. Design Requirements and Process

The development of the bioreactor system was treated and approached as a design project. Therefore, some core design concepts were applied to establish desirable characteristics. Early on in the project, a list of needs that the system would have to meet were identified. This helped to create some requirements from which the design specifications were defined. These specifications are listed below. The design should...

1) ...take inspiration from previously established methods to test mechanical and bioprosthetic valves.
2) ...have good mimicry of the flow conditions of a working heart, and by that subjecting the valves to mechanical forces including compression and expansion, as well as hydrodynamic forces like shear stress and pressure.
3) ...control the pulsatile flow of media, allowing for multiple cardiac outputs to be tested.
4) ...allow for easy mounting and remounting of different sized valves.
5) ...ensure that valves open and close cyclically due to measurable pressures.
6) ...use materials that are non-toxic, easily sterilized and are biocompatible with the valves.
7) ...yield replicable results.

Additionally, a major emphasis was placed on being able to monitor and film the valve as the system was running. This feature would help to determine proper flow and provide additional information of the mechanical properties of the valve.

As mentioned in design requirement number 2, good flow mimicry is essential to accurately evaluate the valve dynamics. To accommodate this in the design of the system, inspiration was drawn from the 2-element Windkessel model. Windkessel models are a way of modeling vascular flow by using parameters such as resistance, compliance and inertance [24]. In terms of the heart, it can be modelled as a current source generating aortic pressure through contraction and relaxation. Another two important components are compliance due to flex in the arterial walls as well as flow resistance due to vasoconstriction. To approximate the flow dynamics of a working heart, the design of the system would have to incorporate all of these parameters.

Another considerable design challenge was to design a valve holder which makes room for the extra cardiac tissue. Most prior bioreactor designs seem to not make such accommodations. Instead, they only utilize the small section containing the three semi-lunar leaflets which are usually sutured to a mount and then the mount is clamped shut to a holder. In this case of this work, it was important to incorporate the extra tissue.

B. Materials and System Overview

The system developed (Fig. 2) consists of 5 notable components and is based on designs found in various prior literature [13], [15], [18], [21], [24], [26], [30]. A valve holder (Fig. 2a), two pressure sensors (Fig. 2b), a compliance chamber (Fig. 2c), a fluid reservoir (Fig. 2d) and a peristaltic pump (Fig. 2e, "Fisher Scientific DP2000"). The valve holder, compliance chamber and fluid reservoir were all custom designed in CAD and then 3D-printed.

The entire system was designed with regard to biocompatibility, and thus deliberately made from materials which are minimally toxic and sterilizable. 3D-printed parts were printed with PETG, which has been demonstrated to be non-cytotoxic and sterilizable by conventional methods such as ethanol oxide [25]. It is also relatively cheap and mechanically strong. The different parts were connected by silicone tubing (Witeg), which is a material widely used in clinical applications due its inert nature and hydrophobic surface [33]. O-rings used to watertight threaded parts and secure the valve were made of nitrile rubber, a material which has been shown to be non-toxic in biocompatibility tests [20].

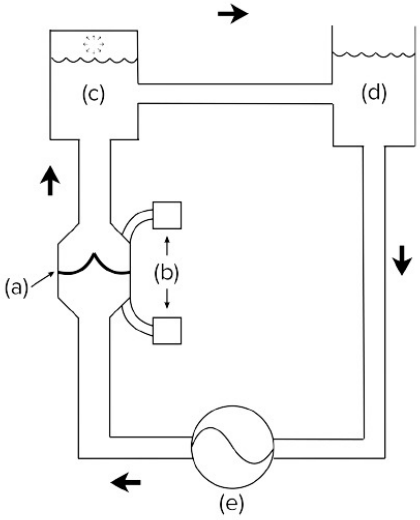

Fig. 2. Sketch of the initial design idea of the entire bioreactor system. Components shown are the valve holder section (a), pressure sensors (b), compliance chamber (c), fluid reservoir (d) and a peristaltic pump (e).

C. Peristaltic Pump

The goal of any bioreactor system should be to produce a pulsating flow which imitates *in vivo* conditions, meaning pressures of about 120 mmHg systolic and 80 mmHg diastolic for aortic circulation, heart rates between 60 and 100 bpm as well as a stroke volume between 50-60 mL [10]. Aiming to achieve such values, previously made bioreactors have utilized many different types of mechanisms such as peristaltic, centrifugal, or pneumatic pumps [4]. In some cases this is also coupled with programmable waveform generators to recreate the cardiac physiological flow [13].

In the case of this work a peristaltic pump was used to generate a pulsating flow, thus emulating the cardiac cycle of a working heart. A peristaltic pump is a simple way to produce relevant parameters such as stroke rate and stroke volume and is also programmable, allowing testing of different pulsatile environments according to design requirement number 3.

D. Custom Valve Holder and Compliance Chamber

From the peristaltic pump fluid flows to the valve holder section. Numerous valve holder design have been proposed in prior related literature, and most involve the valve being sutured to a valve holder [13], [15], [26]. However, to satisfy design requirement number 4, it is desirable to avoid suturing at all if possible. Furthermore, the valves that would be tested contained additional cardiac muscle, and a lot of thought went into how to accommodate for this in the valve holder design.

The valve holder design can be seen in Fig. 3. The main function of the section is to secure the valve in its place while subjecting it to physiological flow dynamics. The bottom section of the valve holder (Fig. 3c) features a cone-shaped structure onto which the extra cardiac tissue, which connects to the valve itself, is positioned. To fasten the tissue, an o-ring (Fig. 3b) is placed on top. Once all the parts are put together the o-ring will apply a small amount of pressure onto the tissue which guarantees that the valve will stay in place during the simulated cardiac cycles. The top section (Fig. 3a) is then carefully slid on top. Finally, a threaded ring (Fig. 3d) completes the section by both tightening the valve into its place as well as water tightening the connection between the sections with the use of additional o-rings.

Both the bottom section and the top section (Fig. 3a & 3c) were made in multiple sizes to fit valves with an internal diameter between 19-25mm, thus accomplishing design requirement number 4.

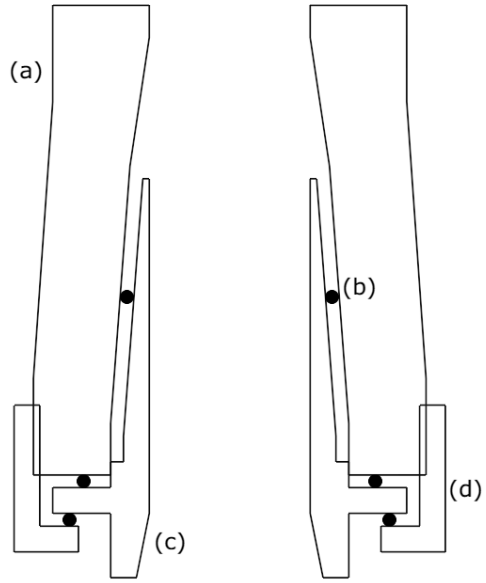

Fig. 3. 2D schematic of the aortic valve holder, consisting of an 'upper' part (a), nitrile rubber o-rings (b), conical holder (c) and a threaded ring (d).

From the valve holder section, the fluid travels to a compliance chamber. The compliance chambers purpose is to emulate the elasticity found in arteries in an otherwise rigid system, thus echoing the arterial compliance found in the Windkessel model. To achieve this, the chamber was designed as a closed reservoir made to contain a specific amount of fluid while the rest would be filled with air. As the fluid level rises during systole, the air trapped in the chamber is compressed causing pressure to build up and thereby storing energy. As systole ends and fluid circulation is stopped, the air begins to decompress and thus releases the stored energy back into the system. This causes the fluid level to be pushed back down, thereby generating a small amount of back-flow which closes the valve.

In our final design, the compliance chamber was incorporated with the valve holder section. The entire setup is depicted in Fig. 4 and the compliance chamber in Fig. 4c. Other key design features include tube connections (Fig. 4a), allowing media to flow in and out of the chamber. On top (Fig. 4b), a piece of transparent plexi-glass was incorporated into the design which allowed clear sight of the valve. The different parts (Fig. 4d-f) were connected by threads and water sealed by nitrile rubber o-rings.

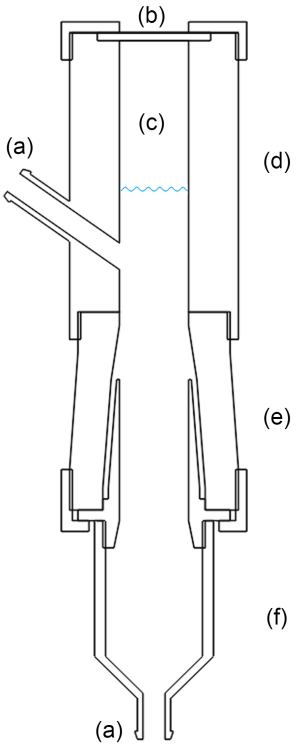

Fig. 4. 2D schematic of the entire configuration. Elements noted are: tube connections (a), plexi-glass top (b), compliance chamber (c), top part (d), valve holder section (e) and bottom part (f).

The entire configuration should also incorporate pressure sensors in close proximity to the valve. Two sensors would have been used (one upstream and one downstream of the valve) and were accounted for in the design to measure transvalvular pressures and fulfill design requirement number 5. Unfortunately however, due to extended delivery times, the sensors did not arrive in time for us to utilize them.

E. Reservoir

From the compliance chamber the fluid travels to a reservoir. The reservoir serves as a place for the circulating fluid to gather before it is passively supplied to the pump via gravity. It also functions as a place where potential air bubbles can escape.

F. System Testing

All parts that would come into contact with the valve, either directly or indirectly, were first sterilized with 70% ethanol and placed inside a cell culture cabinet. The valve was carefully mounted onto the valve holder section, and then all sections

were connected and sealed with the use of threads and o-rings. Each component was then attached to the silicone tubing before mounting the valve holder section/compliance chamber to a lab stand. De-ionized water was used as the circulating fluid and supplied to the system via the reservoir. The total estimated volume of fluid used was 1000 mL. The pump was then run on static flow to fill all parts of the system with fluid and to ensure most air bubbles could escape. Following about 30 seconds of static flow, the cyclic flow testing begun. Different settings on the peristaltic pump were tried, however the most useful setting was found to be the 'Volume Dispense Mode' which allowed for precise control of the stroke volume. Various stroke volumes were tested (5, 10, 15, 20 and 25 mL) and allowed to run for a 2 minute period. Pictures and videos documenting the valve functioning were recorded using a phone camera.

III. Results

The bioreactor and the fluid reservoir were manufactured using 3D-printers available at X-lab (Original Prusa i3 MK3S+) using PETG filament based on CAD designs supplied by the authors. The entire system setup is shown in Fig. 5. The CAD designs and their 3D-printed counterparts of the valve holder section and compliance chamber are shown in isolation in Fig. 6.

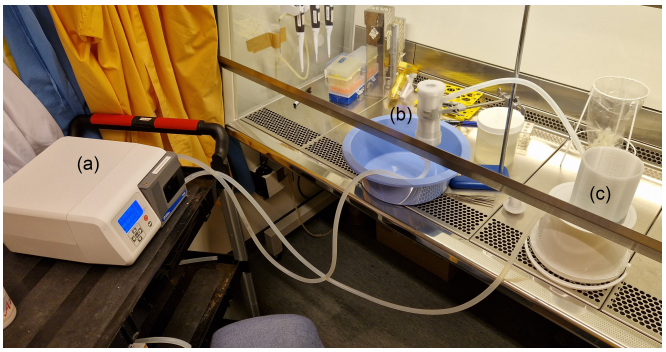

Fig. 5. Setup of the entire testing environment. Key elements shown are the peristaltic pump (a), the valve holder section and compliance chamber (b) and the fluid reservoir (c).

Prior to valve testing, the system was tested for leakage. Performance was satisfactory with minimal leakage after a few small adjustments were made.

One valve was tested and mounted using the 21mm internal diameter valve holder. The design provided a snug fit and the valve was well held in place when exposed to both static and cyclic flow. Pictures presented in Fig. 7 are taken from testing with 5 mL stroke volume. Video footage of the valve shown in Fig. 7 is also presented, see supplementary materials (Heading VII).

IV. Discussion

A. Design and System Testing

The system fulfills most of the design requirements previously presented. The design allows for multiple sized valves to be mounted due to the valve holder section being manufactured

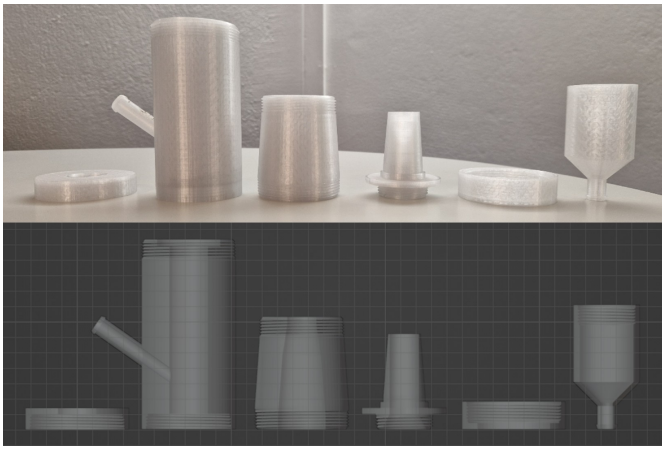

Fig. 6. The final 3D-print of the valve holder section and compliance chamber (top) based on CAD designs (bottom).

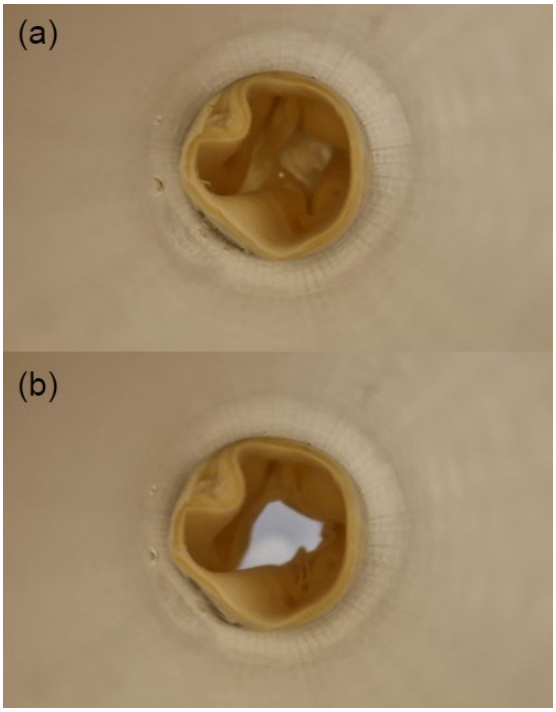

Fig. 7. Pictures of the valve functioning in the bioreactor. Noteworthy events are illustrated, such as the valve closing during diastole (a) and opening during systole (b).

in different internal diameter sizes. Additionally, since suturing was not utilized, mounting and remounting of the valve was particularly quick and easy. Another contributing factor making the system easy to operate was the fact that the entire valve holder section was removable. This meant that a valve could be detached and another mounted without disconnecting any of the tubing. The design also provided excellent view of the valve during testing, permitting the ability to document and monitor proper valve function. All of the above was achieved using materials which are biocompatible and sterilizable by conventional methods.

While the design features many positive aspects, one prominent drawback was that the internal diameter of the top part

(Fig. 4d) was too small. This resulted in folding of the downstream cardiac tissue, which can be seen in Fig. 7. The folding is without a doubt unfavorable for valve performance in the chamber, yet could easily be corrected by an increased diameter.

When it comes to valve performance, the system performed adequately. The valve was able to open and close periodically due to conditions created by the pump. Flow conditions tested ranged from 5-25 mL, which does not quite meet aortic physiological conditions. Instead, the flow environment created in the bioreactor is more aligned with pulmonary valve *in vivo* conditions. While it was possible to increase the stroke volume on the pump to mimic aortic conditions, that increase seemed to affect valve performance as it caused the valve to not open and close properly. As DHVs move closer to being implanted, flow testing conditions must increase without compromising function to represent aortic conditions. In the initial stages of testing however, the bioreactor performance is acceptable. Regarding achieved pressures it is hard to determine whether the presented bioreactor emulated *in vivo* conditions, since it could not be measured due to the absence of sensors. However, the nature of 3D-printing as a manufacturing process, which is discussed in detail in the next paragraph, suggests that the presented bioreactor did not fully achieve *in vivo* pressures.

B. Limitations and Future Improvements

The 'Volume Dispense Mode' on the peristaltic pump made it possible to vary the volume per revolution, representing the stroke volume of the heart, as well as the time between revolutions which represents diastole duration during the cardiac cycle. A normal resting heart should have a rate of approximately 60-80 beats per minute and in order to mimic that, the time for one cardiac cycle should take about 0.75-1 seconds. However, the minimum amount of time between revolutions that the peristaltic pump allowed for was 1 second. This will cause a mimicked heart rate for the bioreactor system that is too slow since the time for diastole is shorter *in vivo*. In addition, the time for the heart to contract in a healthy body is much shorter than the pump time per revolution. When it comes to stroke volume, *in vivo* conditions at rest are generally between 50-60 mL. The pump used could easily allow for these stroke volumes. In summary, using a different pump that could more accurately control the time for diastole and systole would be a potential improvement for future works in order to more accurately mimic the flow conditions of the heart.

When 3D printing, the object is built layer by layer of thin melted filament lines until it is finished. This means that there will be microscopic gaps between the lines due to the nature of the 3D printing process. Consequently, this creates an object surface that is not completely watertight, which affects the pressures that are achieved inside of the system. The first prototype printed in this work was found to leak water into the infill of the bioreactor walls. By that reason, the prototypes needed to be reprinted with other 3D printer settings. The working temperature was then altered causing neighbouring filament lines to melt together. The filament extrusion rate was increased and the the speed was decreased in order to accomplish better filament line precision and accuracy. In addition, the number of perimeters were increased in order to get multiple layers of filament on the object surface. The results when applying these settings formed prototypes with better watertight properties. However, it is difficult to design 3D printed objects which is completely resistant against infill leaking. This will also affect the compliance in the system, because if water is able to escape then air is most certainly also able to do so. A different manufacturing process would thus be an option worth considering for future studies.

A design attribute which could be added to the bioreactor is a variable resistor. The design in this paper relies purely on the inert resistance found within the system, yet a separate component which would provide a predetermined resistance to flow could improve function. Changing the resistance in the system would give the opportunity to mimic narrowed blood vessels. This would enable the option to simulate different patient groups with different blood pressures.

C. Comparison with Other Bioreactors

Quite a few bioreactor designs to test heart valve function have been developed and published in prior works. However, hardly any of them illustrate the actual design process which would promote advancement by enabling other researchers to further develop the ideas. This work on the other hand presents an in depth review of the design process, thus enabling future improvements in the field.

Previously developed bioreactors have varied substantially in regard to design and complexity, leading to different levels of performance and accuracy. Similarly, valve mounting methods as well as manufacturing methods often differ, and commonly lack the flexibility to adapt to multiple sizes of valves. While the presented bioreactor is designed specifically to accommodate DHVs with extra cardiac tissue, it does allow for numerous sizes to be tested.

D. Ethics and Sustainable Development

As mentioned previously, the materials used in the bioreactor needed to be sterilizable and nontoxic. Otherwise, the valve could be damaged or infected. This also opens the door to reuse the bioreactor, since it then can be disinfected after usage. PETG, which was the material used for all 3D printed parts, is a completely recyclable plastic that could be used as raw material in the making of new PETG. Producing new plastics have a negative environmental impact since it uses largely amounts of water and energy, but the negative effect could be reduced if the plastic is recycled correctly [32].

Some of today's MHVs are partly composed of a cobalt alloy. This metal brings environmental, human and ethical concerns. The cobalt mines produce pollution which will spread through precipitation to neighbouring lakes and villages. In addition, the biggest cobalt mines worldwide are located in the Democratic Republic of Congo, and these mines have high amounts of uranium. This has led to an elevated radioactivity level that has affected the mine workers and the residents in the surrounding area [22]. The indirect impact from MHVs could be reduced if DHVs reaches the clinics.

The increasing prevalence of valvular diseases have caused a shortage of suitable prosthetic valves, especially for certain patient groups. For instance, the ethical concerns with porcine heart valves in Islamic belief have accelerated the demand of other alternatives for Muslim patients [1]. In fact all bioprosthetic valves, including DHVs, could possibly lead to ethical issues due to their sourcing from human cadavers or animals.

V. Conclusions

This design project resulted in a custom bioreactor for decellularized heart valves with cardiac tissue. The bioreactor enabled the testing of valves with different diameters, during static and pulsatile flow conditions. While the methods used in this projects are indicative, many improvements can be made to support a more conclusive result. Future endeavors should focus on using a different manufacturing method rather than 3D-printing, and should implement sensors to measure up- and downstream pressures.

VI. Acknowledgments

We would like to extend out gratitude to our supervisors Nika Gvazava and Darcy Wagner who's contributions have been instrumental throughout this project. Their feedback and insightful suggestions have played a crucial role in shaping the direction and quality of this thesis.

Additionally we would like to thank the people over at X-lab for graciously allowing us to utilize their facilities as well as providing us with guidance and expertise.

VII. Supplementary Materials

Click here to view (YouTube link) or copy the link below: https://www.youtube.com/watch?v=gZejeHlgQr0.

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