Development of a physiologic ex vivo vessel perfusion system.

Mitchell J. Buller

University of Louisville

Follow this and additional works at: https://ir.library.louisville.edu/etd

Recommended Citation

https://doi.org/10.18297/etd/177

This Master's Thesis is brought to you for free and open access by ThinkIR: The University of Louisville's Institutional Repository. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of ThinkIR: The University of Louisville's Institutional Repository. This title appears here courtesy of the author, who has retained all other copyrights. For more information, please contact thinkir@louisville.edu.
DEVELOPMENT OF A PHYSIOLOGIC *EX VIVO* VESSEL PERFUSION SYSTEM

By

Mitchell J. Buller, B.S.
University of Louisville, 2012

A Thesis
Submitted to the Faculty of the
Speed School of Engineering of the University of Louisville
as Partial Fulfillment of the Requirements
For the Professional Degree of

MASTER OF ENGINEERING

Department of Bioengineering
University of Louisville
Louisville, Kentucky

July 12, 2013
AN EX VIVO PERFUSION SYSTEM FOR BOVINE CAROTID ARTERIES

By

Mitchell J. Buller, B.S.
University of Louisville, 2012

A Thesis Approved on

_____________________________________
(DATE)

By the following Thesis Committee

_____________________________________
Steven C. Koenig, PhD, Thesis Director

_____________________________________
Guruprasad A. Giridharan, PhD – Thesis Advisor

_____________________________________
Stuart J. Williams, PhD – Thesis Advisor

_____________________________________
Mark S. Slaughter, MD – Thesis Advisor

_____________________________________
Kevin G. Soucy, PhD – Thesis Advisor
DEDICATION

This thesis is dedicated to my parents,

Mr. James M. Buller

and

Mrs. Ann C. Buller
ACKNOWLEDGMENTS

I would like to acknowledge and thank Dr. Mark Slaughter and Dr. Steven Koenig for allowing me the opportunity to join their research group in the spring of 2012. I would like to thank Michael Sobieski for teaching me more than I could have ever imagined in my one co-op semester with their group, affirming my decision to attend medical school at the University of Louisville and setting me up with a uniquely challenging and clinically relevant topic for this Master's thesis. I would also like to thank the entire research group for their support in sending me on multiple trips to present their research at international medical and engineering conferences, from which I gained a plethora of experience and knowledge. My entire thesis advising team deserves praise for their efforts in guiding my thesis to completion, especially Dr. Kevin Soucy, as this thesis would not have been possible without his daily tutelage and motivation. In addition, I would like to recognize all the members of the CII staff for their help with my thesis work, particularly Cary Woolard, Mary Anne Hauck, and Mickey Ising. Outside of the lab, I wish to express my most sincere thanks to my friends and family for keeping me sane throughout the trials and tribulations of this five year journey, especially my parents, without whose unwavering support I would be hopelessly lost. Last, but certainly not least, I would like to thank all the Louisville Cardinal Athletics teams for providing me with the most exhilarating school year possible in which to be a Louisville Cardinal. Go Cards.
ABSTRACT

Introduction: Over time, continuous flow ventricular assist devices (VADs) have become the primary form of implanted mechanical circulatory support (MCS) due to their smaller size, higher energy efficiency, longer durability, and fewer LVAD-related complications when compared to pulsatile flow VADs. However, continuous and pulsatile flows may elicit different cellular and tissue response, particularly in the arterial vasculature, which could have a profound impact on the future operation of MCS devices. Therefore, a unique ex vivo perfusion system integrated with a mock adult circulatory system was designed to study the impact of VAD-generated flow patterns on vascular function.

Methods: The benefits of a mock circulatory loop and an ex vivo perfusion system were combined by designing and integrating a vessel perfusion chamber to an adult-sized mock circulatory loop as a parallel flow branch distal to VAD outflow. Testing was conducted using a mock over several physiologic conditions (normal, heart failure, and hypertension) and at various levels of VAD flow. The system was integrated into an incubator to allow for control of pH and temperature in future studies and fitted with a vessel for feasibility testing. Data was collected using a custom Labview program and analyzed using the HEART program, an automated beat-to-beat cardiovascular analysis program based in Matlab.
**Results:** The chamber was successfully fabricated and installed in the mock circulatory system, allowing for perfusion and longitudinal stretching of bovine carotid arteries. The waveforms and values for pressures and flows created in the mock loop were similar to physiologic values under each tested condition. Under normal hemodynamic conditions (CO = 4.5 L/min, MAP = 91 mmHg) perfusion chamber flow was 0.51 L/min, while under HF conditions (CO = 3.3 L/min, MAP = 81 mmHg) it was reduced to 0.18 L/min, which are representative of in vivo carotid artery hemodynamics. Due to physiologic preloads and afterloads, VAD performance was as would be expected in clinical application. The system was found to be sufficient for future testing with bovine carotid arteries and extended perfusion times (>24 hours).

**Conclusions:** This study resulted in an ex vivo vessel perfusion system that can successfully expose bovine carotid arteries to physiologic and VAD-specific hemodynamic waveforms. The ability to combine the mock ventricle with clinically implanted VADs makes this system both unique and clinically relevant for studying the effects of continuous versus pulsatile flow on the peripheral vasculature.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPROVAL PAGE</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>A. Heart Failure</td>
<td>1</td>
</tr>
<tr>
<td>B. Treatment Options</td>
<td>2</td>
</tr>
<tr>
<td>C. Effects of VADs</td>
<td>5</td>
</tr>
<tr>
<td>D. Mock Circulatory Loops</td>
<td>7</td>
</tr>
<tr>
<td>E. <em>Ex vivo</em> Perfusion Systems</td>
<td>9</td>
</tr>
<tr>
<td>F. Purpose of the Research</td>
<td>11</td>
</tr>
<tr>
<td>II. MATERIALS AND METHODS</td>
<td>14</td>
</tr>
<tr>
<td>A. <em>Ex vivo</em> Vessel Perfusion Chamber</td>
<td>14</td>
</tr>
<tr>
<td>B. Mock Circulatory Loop</td>
<td>15</td>
</tr>
<tr>
<td>C. Incubator Integration</td>
<td>18</td>
</tr>
<tr>
<td>D. Testing</td>
<td>19</td>
</tr>
<tr>
<td>E. Data Collection and Analysis</td>
<td>22</td>
</tr>
<tr>
<td>III. RESULTS</td>
<td>24</td>
</tr>
<tr>
<td>A. Bench Top Testing Results</td>
<td>24</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 1. Target blood pressures for hemodynamic states\textsuperscript{1-5}. Values without literature references were extrapolated from known values in other conditions......................................................................................................................... 20

Table 2. Carotid artery pressures and flows in bench top testing versus target values. Values were obtained from figures due to influence of valve ringing in data analysis. Bolded values fall within 10% sensor error range of target values........................................................................................................................................ 30

Table 3. Carotid artery pressures and flows in incubator testing versus target values. Values were obtained from figures due to influence of valve ringing in data analysis. Bolded values fall within 10% sensor error range of target values........................................................................................................................................ 33

Table 4. Pulse pressures in pulsatile synchronous mode of VAD operation..... 37

Table 5. Carotid artery pressures and flows in vessel testing versus target values. Values were obtained from figures due to influence of valve ringing in data analysis. Bolded values fall within 10% sensor error range of target values........................................................................................................................................ 40
LIST OF FIGURES

**Figure 1.** Trends in heart transplantations in the U.S........................................ 3

**Figure 2.** Prototype of vessel perfusion chamber........................................... 15

**Figure 3.** Block diagram of modified mock circulatory loop with components: A. pneumatic ventricle, B. VAD, C. proximal compliance, D. vessel chamber, E. distal compliance, and F. volume reservoir................................................................. 16

**Figure 4.** Complete setup of modified mock circulatory loop........................... 17

**Figure 5.** Screenshot of HVAD pulsatility controller....................................... 18

**Figure 6.** Incubator testing setup....................................................................... 19

**Figure 7.** Bovine carotid artery integrated into vessel perfusion chamber........... 22

**Figure 8.** Baseline pressures........................................................................... 25

**Figure 9.** Pressures in heart failure condition.................................................. 25

**Figure 10.** Pressures in heart failure condition with high VAD flow............... 26

**Figure 11.** Flows in heart failure condition with high VAD flow...................... 27

**Figure 12.** Pressures in hypertensive condition............................................. 28

**Figure 13.** Pressures for hypertensive condition with high VAD flow........... 29

**Figure 14.** Flows for hypertensive condition with high VAD flow................. 29

**Figure 15.** Pressures in baseline condition...................................................... 30

**Figure 16.** Pressures in heart failure condition............................................... 30

**Figure 17.** Pressures in hypertensive condition............................................. 31
Figure 18. Pulse pressures (mmHg) in incubator testing with increasing CF VAD support................................................................. 32

Figure 19. Pressures in asynchronous VAD operation at 2900 +/- 1100 rpm; cycle time 0.8 seconds................................................................. 34

Figure 20. Synchronous co-pulsation at 3200 +/- 800 rpm; cycle time 0.795 seconds........................................................................................................ 35

Figure 21. Synchronous counter-pulsation at 3200 +/- 800 rpm; cycle time 0.795 seconds........................................................................................................ 35

Figure 22. Pressures at full VAD support in Heart Failure condition.............. 37

Figure 23. Pressures in normal condition during vessel testing..................... 38

Figure 24. Pressures in heart failure condition during vessel testing............. 39

Figure 25. Change in CdAP with increasing VAD flow in heart failure condition........................................................................................................ 39

Figure 26. Fluid shear stresses as a function of flow for each hemodynamic condition in initial testing................................................................. 42

Figure 27. Carotid artery waveform in vivo measured via MRI..................... 47

Figure 28. Overlay of obtained CdAF waveform versus estimated polynomial best fit of literature waveform of CdAF................................................................. 47

Figure 29. Segment of CdAF waveform in vessel testing during asynchronous VAD pulsation at 3200 +/- 800 rpm; cycle time 3.2 seconds............... 48

Figure 30. Vessel length adjustment system with components: A. rotating knob, B. traveling screw element, C. fixed plunger, D. sliding plunger............... 50
A. Heart Failure

An estimated 5.1 million Americans over the age of 20 are currently living with heart failure, while over 200,000 of that population have refractory end-stage heart failure with a mortality rate of 70% - 90%. That number is expected to increase by 25% by the year 2030. Cardiovascular disease is also costly, as it is projected to incur $358 billion in medical expenses in 2015, with that number climbing to $818.1 billion by 2030. Congestive heart failure (CHF) alone accounts for $32.4 billion and $77.7 billion of those figures, respectively, or approximately 9% of the total cost. These numbers indicate that CHF is a disease that warrants continued research and attention.

CHF occurs when blood backs up in other areas of the body due to ineffective pumping of the heart. This ineffectiveness can result from systolic failure, when the heart is too weak to eject a sufficient volume of blood, or diastolic failure, when the cardiac muscle becomes stiff and does not fill with blood easily. Typically, CHF results from a combination of these deficiencies and thus prohibits the heart from delivering sufficient amounts of oxygen-rich blood to the body due to decreased pressures and compliances throughout the cardiovascular system.

As CHF progresses, the body initiates a multitude of compensatory mechanisms in varying degrees. The Frank-Starling mechanism allows the heart to change its
force of contraction and therefore stroke volume in response to venous return\(^9\). In CHF, this mechanism can have potentially adverse effects, leading to ventricular dilation and increased heartrate. Several neurohumoral responses are also initiated, such as the activation of the sympathetic nerves, the renin-angiotensin system of the kidneys, and increased release of antidiuretic hormone and atrial natriuretic peptide. These mechanisms result in increased fluid retention and edema, leading to arterial vasoconstriction, venous constriction, and increased blood pressure.

B. **Treatment Options**

The most effective long-term remedy for CHF is a heart transplant. Unfortunately, less than 2,400 such procedures are performed in the United States each year due to a widespread shortage of available donor organs (Figure 1). The number of annual heart transplants has been consistent for over 20 years, even in spite of the increasing HF diagnoses. For the remaining end-stage HF patients who are unable to receive a transplant due to lack of donor organs, age, or other comorbidities, additional treatment methods must be utilized.
For patients suffering from New York Heart Association (NYHA) Class I and Class II heart failure, proper medical management is the first step in effectively managing its symptoms. Two common classes of medications prescribed in the treatment of heart failure are inotropes and beta-blockers. Inotropes are used to alter the force of cardiac muscle contraction by either weakening the contractions (negative inotropes) or strengthening them (positive inotropes)\textsuperscript{10}. Examples include epinephrine, norepinephrine, and dobutamine. Beta-blockers reduce the workload on the heart and dilate your blood vessels, causing your heart to beat slower and with less force\textsuperscript{1}. These medications are often best used in combination and supplemented with diuretics and ACE inhibitors.

When patients enter the NYHA Class III and IV stages of heart failure and proper medical management is not enough to combat their severe limitations in activity, mechanical circulatory support (MCS) must be considered. The original form of MCS was the cardiopulmonary bypass (CPB) machine, in which a series of
external roller pumps takes over the heart’s blood-pumping function while the blood is simultaneously oxygenated extracorporeally. Fully implantable devices known as ventricular assist devices (VADs) were later developed. VADs provided more prolonged periods of MCS outside of the hospital.

The first generation of VADs were introduced in the late 1970s and consisted of bulky, pneumatically driven pumps, such as the HeartMate XVE (Thoratec Corp., Pleasanton, CA) and the Novacor LVAD (Novacor Corp., Oakland, CA), that produced pulsatile flow (PF) patterns. Like most VADs, these pumps were designed to supplement the function of a single ventricle, typically the left. Despite the restrictive size and associated risks with early generation PF VADs, clinical management of HF with these devices was largely successful and established VADs as an effective option for late-stage HF patients.

This second generation of VADs, led by the HeartMate II (Thoratec Corp., Pleasanton, CA) and the HVAD (HeartWare, Inc., Framingham, MA), gained widespread clinical acceptance after the turn of the century due to their smaller pump size and driver size, increased reliability, and improved efficiency. Improvements on this technology have allowed for even smaller and less invasive devices to be developed. Still other devices in development include the third generation of VADs, such as the HeartMate III (Thoratec Corp., Pleasanton, CA), that are CF but have the capability to produce PF through various algorithms currently under development.
C. Effects of VADs

As previously mentioned, CF VADs have become the primary form of implanted MCS for several reasons. First, their smaller size allows them to be more easily implanted in the thoracic cavity. CF VADs have also demonstrated higher energy efficiency, longer durability, and fewer post-surgical LVAD-related complications when compared to their PF counterparts\textsuperscript{11}. However, the effects of continuous versus pulsatile flow on the body continue to be studied and could have a profound impact on future MCS device technologies.

The effects of flow patterns can be seen on various levels of bodily organization, from the cellular level, to the vascular level, and finally to the level of organ systems and their interactions. On a cellular level, pulsatile flow has been shown to reduce endothelial damage compared to nonpulsatile flow\textsuperscript{12} and possibly suppress the release of potentially harmful cytokines\textsuperscript{13, 14}. Increased pulse pressure from pulsatile flow in baroreceptor units has been shown to decrease systemic vascular resistance\textsuperscript{15} and the frequency-dependent component of these baroreceptors can be altered during different hemodynamic conditions\textsuperscript{16}. Studies also show that the endothelial release of nitric oxide, a key cellular component in cardiovascular function, in the peripheral vasculature is enhanced by pulsatile flow\textsuperscript{17}.

On a vascular level, the effects of critical shear stresses can be seen through the activation and manipulation of several different biological mechanisms within the
vasculature, which are induced by two types of mechanical stretch in the vessels\textsuperscript{18, 19}. These changes can have long term effects on endothelial-mediated vascular adaptive mechanisms\textsuperscript{20}. Moreover, nonpulsatile flow in a total artificial heart experiment has been shown to cause narrowing and rarefaction in the microcirculatory network, causing dystrophy in samples from the lungs, liver, and kidneys\textsuperscript{21}.

Those microcirculatory effects can lead to changes in end-organ behavior stemming from the varying flow patterns. In the kidneys, short-term results seem to favor the use of pulsatile flow\textsuperscript{22}, while long-term continuous flow obtains better renal perfusion than short-term usage and is not technically inferior to pulsatile flow\textsuperscript{23}. Studies conducted on the liver show that pulsatile flow sustains total hepatic blood flow more effectively than continuous flow, due to the specific preservation of hepatic arterial and portal venous blood flow (attributed to prevention of hepatic arterial vasoconstriction)\textsuperscript{24}. Similar impact was reported in the neurological system, in that lower vascular resistance and higher flow rates were associated with pulsatile versus nonpulsatile flow\textsuperscript{23, 25}. Studies conducted on the gastrointestinal (GI) system are concerning, where data has shown that continuous flow is significantly more likely to cause GI bleeding than pulsatile flow, with a near ten-fold incidence difference in some cases\textsuperscript{26}.

Among many others, these findings have driven a growing movement by researchers to determine whether pulsatility is truly needed in the human cardiovascular system and, if so, to what degree it is needed. While studies are
being conducted to investigate the need for this pulsatility\textsuperscript{27}, the VAD industry and engineers are preemptively developing PF algorithms for current and next-generation CF VADs\textsuperscript{28}. In order to test the biologic responses to these highly-specialized VAD flow patterns with adequate scientific rigor and accuracy, specialized flow simulation systems must be developed.

D. Mock Circulatory Loops

A common method of mimicking \textit{in vivo} flow conditions for the study of various flow patterns and/or MCS devices is the mock circulatory loop. These loops begin with a source of flow, typically a mock ventricle driven pneumatically or with a piston\textsuperscript{29}. Compliance chambers for arterial and/or venous compliance adjustment are typically used\textsuperscript{30}, along with some sort of adjustable resistance to account for systemic and pulmonary vascular resistance\textsuperscript{31}. Connections are made via polypropylene or other plastic tubing. While the components seem basic, mock circulatory loops can be modified via any number of components or configurations to study many different circulatory scenarios.

Many mock loop systems are created in order to be able to adapt to test the various types of MCS devices\textsuperscript{32-37}. Other systems are more complex and specified, such as those used to study a pediatric circulation\textsuperscript{38,39} or the associated Norwood procedure\textsuperscript{40} and Fontan circulation\textsuperscript{41}. Still others are used to develop and verify the aforementioned pulsatile flow patterns produced by continuous flow VADs\textsuperscript{42-44}. 
This diversity and adjustability is one of the main advantages of mock circulatory systems. Not only can these systems be set up to mimic various types of circulation, but the compliance, resistance, preload, afterload, and other included parameters can be adjusted to create a multitude of different cardiovascular conditions, such as hypertension or heart failure. Devices ranging from VADs\textsuperscript{33}, to TAHs\textsuperscript{43}, to IABPs\textsuperscript{36} can be included, as well as other prostheses\textsuperscript{37}. Fluids of varying viscosities, such as water, glycerol, or blood can be utilized to achieve desired viscosities. Mock circulatory loops enable the user to instantaneously change hemodynamic conditions and spare the lives of animals that would typically be experimented on, while also being more cost efficient and reproducible than said animal experiments.

The biggest disadvantage of these systems is that they are only models, and assumptions must inherently be made and accounted for. However, systems with increased complexity are able to account for more variables and therefore can provide highly accurate models of natural hemodynamic conditions.

One such system is the standard mock loop setup used by investigators at the Cardiovascular Innovation Institute (CII). The standard setup consists of a silicone left ventricle pneumatically driven by an external driver, aorta, arterial resistance and compliance, and venous reservoir. These elements are connected using plastic tubing connectors and flexible $\frac{3}{4}$” silicone tubing. Ventricular pressure, heart rate, resistances, and compliances can be adjusted to reproduce hemodynamic pressure and flow waveforms of the physiology of an adult human
in several distinct conditions: normal, heart failure, and hypertension. Aortic and LVAD flows are measured using in-line and clamp Transonic Flow Probes (Transonic Systems, Ithica, NY), respectively. Aortic and left ventricular pressures are measured using single-tipped Millar pressure catheters (Millar Instruments, TX). One of two pneumatic ventricle drivers can be used (LB Engineering, Germany; Thoratec Corp., Pleasanton, CA). These drivers enable the modification and adjustment of key parameters such as ventricle systolic and diastolic time periods and pressure, vacuum, and motor percentages.

E. Ex vivo Perfusion Systems

*Ex vivo* perfusion systems are another tool with a multitude of uses. As the name implies, these systems involve the perfusion of blood or another nutrient-rich substance throughout a group of cells, tissue, or an organ in an environment outside the body. These systems come in a wide range of sizes, from smaller systems for the perfusion of cell cultures to large configurations for the perfusion of entire organs.

Two transplanted organs that are often perfused are the lungs and kidneys. Lungs can be perfused for 4-6 hours prior to transplant\(^{45}\), often normothermically\(^{46, 47}\) and in combination with static cold preservation to remedy the effects of warm ischemia\(^{48}\). This *ex vivo* lung perfusion has proven to be an effective way to help separate ‘good’ lungs from a previously rejected pool to assist with the shortage of donor lungs\(^{49}\). Specific interest lies in the area of
donation after circulatory death\textsuperscript{46, 50}. In terms of kidneys, simple systems of
normothermic perfusion immediately prior to transplant have been effective after
prolonged periods of static cold storage\textsuperscript{51}. Because of this success, perfusion of
kidneys with various substances has been studied\textsuperscript{52, 53}. Other \textit{ex \textit{vivo}} perfusion
systems have involved liver REFs, cirrhotic tissue REF, mouse placentas REF, and
even bone REF, among others\textsuperscript{54-61}.

It is not surprising that \textit{ex \textit{vivo}} perfusion systems for cardiovascular tissues are
extensively used in scientific research. Specifically, researchers have developed
various systems for the perfusion of hearts. As with other organs, the primary
focus is preparing hearts for transplant\textsuperscript{62, 63}. Some systems have utilized animal
hearts in order to develop tools to assess the viability of hearts for transplant\textsuperscript{64-}
\textsuperscript{67}. While different parameters for evaluating the potential hearts are being
determined\textsuperscript{68, 69}, the effects of various parameters on the peripheral vasculature
are also being examined.

Like all \textit{ex \textit{vivo}} perfusion systems, these vascular perfusion systems can also vary
widely in size, beginning with smaller microfluidic systems used for the perfusion
of vascular endothelial cell cultures. These types of systems are useful for
identifying the effects of pulse waves on a cellular level and use similar
components to mock circulatory loops, such as pulsatile blood pumps and flow
meters\textsuperscript{70}. Unfortunately, these systems can typically only study a specific cell
type, as opposed to the effects of flow on an entire vessel or organ.
More advanced systems attempt to mimic vessels by culturing vascular cells in flexible rubber tubing\textsuperscript{71,72}. These systems are able to moderate shear stress levels\textsuperscript{72} and even mimic disease states\textsuperscript{71}, but are unable to generate physiologic waveforms or waveforms associated with pathologic conditions. Additional systems are also able to produce pulsatile flow patterns, but only incorporate static cell cultures and have issues with retrograde flow and shear stress and strain measurements\textsuperscript{73,74}.

Similar to static culture protocol, \textit{ex vivo} perfusion of blood vessels requires them to be cultured at physiologic temperature and pH, and with appropriate culture medium with antibiotic and antifungal agents, with the added parameter of being exposed to various flow patterns. Systems are typically driven via peristaltic pumps\textsuperscript{75-78} in order to mimic various pulsatile flow waveforms. They typically utilize precise pressure, flow, and gas exchange control to measure a variety of endpoints, such as fluid mechanical forces\textsuperscript{75,76} and biological marker expression\textsuperscript{78-82}. Some even have unique abilities like the capacity for longitudinal stretch\textsuperscript{77,79} or the measurement of vessel diameter\textsuperscript{80,83}. Like mock circulatory loops, these systems are highly adaptable and can be modified to support a variety of research needs.

\textbf{F. Purpose of the Research}

Although the effects of mechanical stimulation to the vasculature have been extensively studied, the response to CF VAD-generated continuous flow and
pulsatile flow profiles has not been examined. This project combines the advantages of a mock circulatory loop with those of an \textit{ex vivo} perfusion by attaching a vessel within the mock loop and exposing it to those unique flow profiles. By mimicking the complexity of the physiologic \textit{in vivo} environment by using the combination of a pneumatic ventricle and VAD in series, this system is capable of providing valuable information on the effects of continuous and pulsatile flow on the peripheral vasculature.

The \textit{ex vivo} perfusion system in this project will be able to accept a bovine carotid artery in order to accurately mimic \textit{in vivo} conditions. The chamber will be able to be integrated into a mock adult circulatory flow loop, and the system will include design features for temperature control, pH regulation, media exchange, and sterilization.

The research performed in this thesis project accomplishes several aims. An \textit{ex vivo} perfusion chamber is designed and integrated into the mock loop to prove feasibility of the system. Physiologic, pathologic, and mechanically-altered hemodynamics are created. Finally, the mock circulation system is reduced and re-designed for tissue culture considerations. In addition, improvements to the perfusion chamber are presented, which are ready for fabrication and implementation with future studies.

The vessel chamber for the artery is watertight, with cannulas at each end to attach the vessel and three ports for fluid exchange, ventilation, and proper gas
exchange. The system is adjustable in length to allow for proper vessel stretching and is completely sterilizable.

Once the chamber was completed, the mock circulatory loop was modified for integration of the perfusion system in order to demonstrate that physiologic flows and pressures can be achieved. First, the chamber was integrated with only the mock ventricle to demonstrate normal physiologic pressures and flows. The CF VAD (HVAD, HeartWare Corp., Framingham, MA) was then added to demonstrate typical flows of the VAD in conjunction with the native ventricle. Finally, the pulsatile flow algorithm was implemented and data was collected. The mock loop system was then transferred to an incubator to achieve physiologic temperature and pH, as described in the following section.
A. *Ex vivo* Vessel Perfusion Chamber

The *ex vivo* vessel perfusion chamber was created using a 100 mL syringe of inner diameter 1.4 inches. The tip was first cut off to create a hollow tube of length 4.8 inches. Three holes were drilled in the side of the tube: two to allow for fluid recirculation and one to vent. Luer lock ports were then fixed in each hole and capped. The handles of two syringe plungers were then removed so that only the rubber end was intact. A hole was drilled in each plunger to allow for the insertion of a ¼” to 3/16” plastic tubing connector. One end of each connector was connected externally to ¼” silicone tubing, while the end on the inside of the chamber was connected to the vessel. Because no actual vessels were used in this study, a piece of 3/16” tubing was used to simulate the vessel for the preliminary design. In hemodynamic verification studies, a section of penrose drain was used, as it better mimicked the elasticity of a natural vessel. The prototype can be seen in **Figure 2**.
For the purpose of this study, several modifications were made to the standard mock circulatory system to allow for testing of the carotid artery. The *ex vivo* vessel perfusion chamber with the mock carotid artery, as described in the next section, was placed in a looped branch after the proximal compliance chamber. While a connection of the branch between the arterial and venous sides was considered, as this would be more physiologically accurate, the resulting system would likely require an additional compliance chamber and fluid reservoir, adding to the overall complexity of the system. A second compliance chamber was added to represent the additional compliance of the carotid artery branch. The remainder of the system remained physically unchanged. An additional clamp Transonic Flow Probe (Transonic Systems, Ithica, NY) was placed just proximal to the vessel chamber to measure carotid artery pressure and an additional
single-tipped Millar pressure catheter (Millar Instruments, TX) was placed just distal to the chamber to measure carotid artery flow. A block diagram of the system can be seen in **Figure 3**, as well as the complete setup in **Figure 4**.

**Figure 3.** Block diagram of modified mock circulatory loop with components: A. pneumatic ventricle, B. VAD, C. proximal compliance, D. vessel chamber, E. distal compliance, and F. volume reservoir.
One of the key advantages of this *ex vivo* arterial perfusion system is the ability to integrate various MCS devices into the circulation, including several types of LVADs and counter-pulsation devices. For the purposes of this study, the LVAD used was the centrifugal flow HVAD by HeartWare (Miami Lakes, FL). For the first portion of the experiment, the standard controller was used to provide continuous flow to the system.

However, the second portion of the experiment involved the use of a pulsatile flow algorithm for the pump. For this purpose, a controller was programmed by engineers at HeartWare under the direction of the investigators to enable LVAD pump speed modulation (*Figure 5*). The program enabled the user to alter the degree of pulsatility by changing three parameters: the change in RPM (amplitude), the time period, and the time interval between pulses. This

*Figure 4.* Complete setup of modified mock circulatory loop
controller allowed for the creation of pulsatile waveforms from the continuous flow HVAD to test the carotid artery under various flow conditions.

![Screenshot of HVAD pulsatility controller](image)

**Figure 5.** Screenshot of HVAD pulsatility controller

C. **Incubator Integration**

For additional testing, the mock circulatory system was integrated into the incubator (Isotemp, Fischer Scientific) in order to prepare the system for testing under physiologic pH and temperature with actual carotid arteries. The lack of space in the interior made some slight modifications necessary, including the shortening of tubing lengths and the fixation of the fluid reservoir at a fixed height. A new fluid reservoir was also fabricated from a shallow plastic bin to allow it to be placed on the top shelf of the incubator. The complete setup can be seen in **Figure 6** and a full schematic is attached in **Appendix A**. Once
integrated, data sets were collected at the same physiologic conditions as the initial testing to demonstrate similar performance of the system in its new configuration.

![Incubator testing setup](image)

**Figure 6.** Incubator testing setup

D. **Testing**

After the creation of the vessel chamber, testing was conducted using the aforementioned mock circulatory system. First, data was collected under normal physiologic conditions without the inclusion of the HVAD. This data was
collected at varying levels of pressure and flow in an attempt to match target values and waveforms (Table 1). The HVAD was then included and data sets were collected for heart failure and hypertension conditions at the following levels of VAD flow: no flow, low flow, medium flow, and high flow. Low flow was defined as 2000 rpm. High flow was defined as 3200 rpm according to the high rpm setting used clinically by physicians associated with the CII. Medium flow was then taken as the midway point between low and high flow, 2600 rpm. The heart failure condition was created by increasing the compliance to reduce pulse pressures and decreasing the drive pressure and increasing the preload to simulate a dilated left ventricle and increase left ventricular end-diastolic pressure. The hypertensive condition was created by increasing resistance and decreasing compliance to simulate stiffening of the arteries.

Table 1. Target blood pressures for hemodynamic states\textsuperscript{1-5}. Values without literature references were extrapolated from known values in other conditions.

<table>
<thead>
<tr>
<th>Hemodynamic Condition</th>
<th>AoP (mmHg)</th>
<th>CdAP (mmHg)</th>
<th>CdAF (L/min)</th>
<th>AoF (L/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
<td>Pulse</td>
<td>Systolic</td>
</tr>
<tr>
<td>Normal</td>
<td>120</td>
<td>76</td>
<td>44</td>
<td>100</td>
</tr>
<tr>
<td>Heart Failure</td>
<td>97</td>
<td>60</td>
<td>37</td>
<td>81</td>
</tr>
<tr>
<td>Hypertension</td>
<td>&gt;140</td>
<td>&gt;90</td>
<td>&gt;50</td>
<td>&gt;117</td>
</tr>
</tbody>
</table>

In the second round of testing, after integration into the incubator, additional levels of flow were added. A ‘full flow’ VAD condition set at 3800 rpm was added in an attempt to significantly reduce any remaining pulse pressure and completely unload the ventricle. Prior to the administration of pulsatile flow
algorithms, a modified heart failure condition was created to match the exact parameters used in previous work\textsuperscript{84}. Pulsatile flow algorithms at 2900 +/- 1100 rpm and 3200 +/- 800 rpm were then added. As detailed previously\textsuperscript{85}, the first condition allowed for the maximum rpm modulation, while the second condition provided full unloading of the ventricle. These two conditions were run both asynchronously at periods of 0.4, 0.5, 0.8, and 1.6 seconds and synchronously. In order for this to be accomplished, the cardiac cycle on the ventricle driver was set at 0.8 seconds and at 0.79 seconds on the VAD controller. This allowed the VAD to drift from phases of co-pulsation to counter-pulsation over a two minute data set. Co-pulsation occurs when the VAD produces flow at the same time the ventricle is contracting, whereas counter-pulsation occurs when the VAD produces flow when the ventricle is filling. The difference can be observed in the offset between the pulses of the aortic and left ventricular pressures. In co-pulsation, a zero degree offset exists between the pulses, whereas a 180 degree offset exists in counter-pulsation.

In the final round of testing, a bovine carotid artery (1/8” diameter) was incorporated into the system (Figure 7) in the vessel perfusion chamber to prove feasibility of the system. No vessel viability testing was conducted, but similar conditions as in the first two testing sets were replicated. Specifically, baseline normal and heart failure conditions were established, followed by several data sets of continuous and pulsatile VAD flow.
E. Data Collection and Analysis

Data was collected using a custom Labview program designed by investigators at the CII. This program allows for the display of up to 9 pressures and flows and the acquisition of data sets of any size. In this study, the parameters recorded were left ventricular pressure, aortic pressure, carotid artery pressure, aortic flow, VAD flow, and carotid artery flow. Data sets of 15 or 30 seconds in length were acquired during bench top testing and for the basic hemodynamic and CF VAD conditions in incubator testing. For the asynchronous pulsatile VAD flow modulation data sets, data sets ranging from 12 – 48 seconds were taken in order to record 15 ’beats’ of the VAD. For synchronous VAD data sets, data sets of two minutes were taken in order to record periods of both co-pulsation and counter-pulsation. Prior to the commencement of each data set, the circulatory loop was run for 20 – 30 seconds to achieve a steady state for each condition. The data was analyzed using the HEART program, an automated beat-to-beat cardiovascular analysis package using Matlab, along with additional custom.
Matlab programs developed for the analysis of pulsatile VAD waveforms\textsuperscript{85}. This beat analysis allowed for the calibration of data channels, the calculation of various peaks, averages, and pulses, and the creation of data plots. Four second plots of pressure and flow waveforms were plotted to provide a snapshot of the data sets.
CHAPTER III – RESULTS

A. Bench Top Testing Results

Initial testing of the *ex vivo* arterial perfusion system was successful in replicating physiologic and pathologic (heart failure and hypertension) waveforms for various pressures and flows. Target pressure and flow parameters are listed in Table 1. Resistance, compliance, and ventricle driver settings were first adjusted to create a baseline condition (no VAD) representing normal cardiovascular parameters (Figure 8). It should be noted that large spikes in pressure and flow will appear due to ringing of the mechanical aortic and mitral valves. Baseline aortic flow (AoF) was kept at approximately 4.0 L/min to represent a normal cardiac output. Normal average pressures of 95 mmHg for AoP and 86 mmHg for CdAP were also obtained. The baseline condition was followed by data sets of low, medium, and high VAD flow to verify proper functionality of the VAD and perfusion system setup (Appendix A).
Next, a heart failure condition was created. At the onset of the heart failure condition, left ventricular end-diastolic pressure rose (Figure 9). This resulted in an overall decrease in pressures. Mean AoF decreased to 3.3 L/min, while mean CdAF decreased to 0.18 L/min.
Similar to the hypertensive condition, pressures and flows increased with increasing VAD support until the ventricle became fully unloaded (Figure 10). However, AoF dropped below zero due to the elimination of flow through the aortic valve with increased ventricular unloading along with some slight retrograde flow due to the mock loop setup (Figure 11).

**Figure 10.** Pressures in heart failure condition with high VAD flow
After an additional baseline condition was created to restore normal function, a hypertensive condition was created (Figure 12). This resulted in higher pressures, including higher pulse pressures for the aorta and carotid artery, caused \textit{in vivo} by the stiffening of the vascular walls. Due to the increased resistance, mean AoF was decreased to approximately 3 L/min and mean CdAF to 0.12 L/min, values that were slightly below literature references (Table 2).

\textbf{Figure 11.} Flows in heart failure condition with high VAD flow
Figure 12. Pressures in hypertensive condition

Again, data sets of low, medium, and high VAD flow were taken to simulate VAD function in a hypertensive condition. Pressures increased, likely due to the increased flow through the vessels (Figure 13), as the major hemodynamic effect of VAD support is increased pressure and flow\(^8\). Mean AoF decreased with increasing VADF as more of the flow bypassed the aortic valve and went through the VAD and the ventricle became increasingly unloaded (Figure 14).
In terms of the behavior of the carotid artery branch, the perfusion system was able to attain pressure and flow values near the target values. While the values for the maximum and average carotid flows and pressures were able to be

**Figure 13.** Pressures for hypertensive condition with high VAD flow

**Figure 14.** Flows for hypertensive condition with high VAD flow
obtained (Table 2), waveform morphology for the CdAF differed slightly from published figures\textsuperscript{4, 88, 89}.

**Table 2.** Carotid artery pressures and flows in bench top testing versus target values. Values were obtained from figures due to influence of valve ringing in data analysis. Bolded values fall within 10% sensor error range of target values.

<table>
<thead>
<tr>
<th>Hemodynamic Condition</th>
<th>Target Values</th>
<th>Obtained Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CdAP (mmHg)</td>
<td>CdAF (L/min)</td>
</tr>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td>Normal</td>
<td>100</td>
<td>65</td>
</tr>
<tr>
<td>Heart Failure</td>
<td>81</td>
<td>51</td>
</tr>
<tr>
<td>Hypertension</td>
<td>&gt; 117</td>
<td>&gt; 77</td>
</tr>
</tbody>
</table>

**B. Incubator Testing Results**

Once the *ex vivo* arterial perfusion system was transferred from the bench top to the incubator, the first goal of testing was to reestablish proper pressure and flow waveforms for baseline, heart failure, and hypertensive hemodynamic conditions (Figures 15-17).
Figure 15. Pressures in baseline condition

Figure 16. Pressures in heart failure condition
After each of these basic conditions were established, four levels of VAD support were tested under each condition. The three levels used in the initial testing were replicated along with the addition of the aforementioned ‘full VAD’ condition, in which the VAD was set to 3800 rpm in order to fully unload the ventricle and reduce or eliminate any pulse pressures. As expected and observed in the initial testing, the pulse pressures were reduced as the level of CF VAD support increased to full ventricular unloading (Figure 18). However, they still remained slightly higher than the pulse pressures that have been observed in clinical VAD patients, which can drop as low as 3 – 5 mmHg, although pulse pressures are often kept between 10 – 20 mmHg in optimal VAD use.87

Figure 17. Pressures in hypertensive condition
Figure 18. Pulse pressures (mmHg) in incubator testing with increasing CF VAD support

While carotid artery pressures were similar to those from the initial testing, a calibration error led to negative readings for the carotid artery flows. While the flows were negative, they still showed similar amplitude and waveform morphology as those in previous testing. The error was remedied for the final round of testing and proper flows were achieved.

Table 3. Carotid artery pressures and flows in incubator testing versus target values. Values were obtained from figures due to influence of valve ringing in data analysis. Bolded values fall within 10% sensor error range of target values.
As one of the primary advantages of this system is its ability to create a multitude of VAD-specific waveforms, a laptop fitted with a VAD pulsatility program was then connected to the system as the VAD controller. Prior to the VAD pulsation data sets, an altered heart failure state was created to match the state established by Ising in previous research\textsuperscript{85}. As detailed in the previous section, the VAD was pulsed asynchronously and synchronously at varying time periods and levels of rpm modulation. In the asynchronous mode, unique, yet inconsistent waveforms can be produced when the asynchronous timing produces high degrees of variability in pulse pressure (\textbf{Figure 19}). In the synchronous mode, the VAD support drifted from periods of co-pulsation (\textbf{Figure 20}) to counter-pulsation (\textbf{Figure 21}).

\textbf{Figure 19.} Pressures in asynchronous VAD operation at 3200 +/- 800; cycle time 3.2 seconds
Figure 20. Synchronous co-pulsation at 3200 +/- 800 rpm; cycle time 0.795 seconds

Figure 21. Synchronous counter-pulsation at 3200 +/- 800 rpm; cycle time 0.795 seconds

In the clinical context, the ultimate goal of these algorithms is to increase pulsatility in the peripheral vasculature, which can be measured by the
calculation of AoP and CdAP pulse pressures. In the experimental context, these tests were performed to demonstrate that the \textit{ex vivo} system could create complicated VAD-specific hemodynamic waveforms in the presence of a mock left ventricle and with the potential to support artery perfusion. The results in the incubator setup show that, particularly in the synchronous co-pulsation mode of operation, as the 180 degree offset of the pulses in counter-pulsation mode produces destructive interference and results in a near continuous signal. The co-pulsation synchronous mode is able to show near-physiologic aortic pulse pressures, while the counter-pulsation synchronous mode exhibits low aortic pulse pressures. The asynchronous mode of pulsatile VAD flow delivers a variable pulse pressure from beat to beat, which is still greater than pulse pressures provided on full CF VAD support. These compare to a pulse pressure of approximately 10 mmHg at a continuous VAD output of 3200 rpm (Figure 22). It should be noted that the low pulse pressure during asynchronous pulsation at 3200 +/- 800 rpm with a pulse width of 0.4 seconds is due to the timing of the pulses, which happened to occur in exact counter-pulsation to the pulses of the ventricle, minimizing pulse pressure as in synchronous counter-pulsation mode.
**Figure 22.** Pressures at full VAD support in Heart Failure condition

**Table 4.** Pulse pressures in pulsatile mode of VAD operation during incubator testing

<table>
<thead>
<tr>
<th>Mode</th>
<th>rpm</th>
<th>Δt</th>
<th>AoP Pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asynchronous</strong></td>
<td>2900 ± 1100</td>
<td>0.4</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>2900 ± 1100</td>
<td>0.5</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>2900 ± 1100</td>
<td>0.8</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>2900 ± 1100</td>
<td>1.6</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>3200 ± 800</td>
<td>0.4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>3200 ± 800</td>
<td>0.5</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>3200 ± 800</td>
<td>0.8</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>3200 ± 800</td>
<td>1.6</td>
<td>85</td>
</tr>
<tr>
<td><strong>Sync Co-pulse</strong></td>
<td>2900 ± 1100</td>
<td>0.3975</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>3200 ± 800</td>
<td>0.3975</td>
<td>33</td>
</tr>
<tr>
<td><strong>Sync Counter-pulse</strong></td>
<td>2900 ± 1100</td>
<td>0.3975</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3200 ± 800</td>
<td>0.3975</td>
<td>9</td>
</tr>
</tbody>
</table>
C. Vessel Testing Results

Baseline normal (Figure 23) and heart failure (Figure 24) conditions were again established, followed by CF VAD conditions at low, medium, high, and full VAD flow. Trends in flow and pressure in these conditions were normal and matched previous testing results (Figure 25).

Figure 23. Pressures in normal condition during vessel testing
Once those conditions were replicated, data sets of pulsatile VAD flow were taken both asynchronously and synchronously, with both counter-pulsation and co-pulsation being achieved. Pulse pressures in the VAD pulsatility data sets were similar to those previously recorded (Table 4), while all carotid artery flows
and pressures matched their target values (Table 5). All other data from the vessel testing can be found in Appendix C.

**Table 5.** Carotid artery pressures and flows in vessel testing versus target values. Values were obtained from figures due to influence of valve ringing in data analysis. Bolded values fall within 10% sensor error range of target values.

<table>
<thead>
<tr>
<th>Hemodynamic Condition</th>
<th>Target Values</th>
<th>Obtained Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CdAP (mmHg)</td>
<td>CdAF (L/min)</td>
</tr>
<tr>
<td></td>
<td>Systolic</td>
<td>Diastolic</td>
</tr>
<tr>
<td>Normal</td>
<td>100</td>
<td>65</td>
</tr>
<tr>
<td>Heart Failure</td>
<td>81</td>
<td>51</td>
</tr>
</tbody>
</table>

D. Shear Stress Calculations

As previously mentioned, shear stresses in vessels have profound effects on a wide variety of vascular response mechanisms. Therefore, shear stresses were calculated from the obtained volumetric flow measurements, per the methods utilized by Estrada et al. First, the kinematic viscosity of the fluid solution used was calculated using equation (1):

\[ \nu = \frac{\mu}{\rho} \quad (1) \]

Normosol-R, a sterile, nonpyrogenic isotonic solution of balanced electrolytes in water, was used as the fluid solution in order to provide the slight increase in viscosity over water needed for HVAD operation. Its density was assumed to be 1,010 kg/m³, between the densities of water and blood plasma. Likewise, the
dynamic viscosity was assumed to be 1.10 cP to lie between those of water and blood plasma. Because of their low dynamic viscosities, water and plasma are assumed to be Newtonian fluids whose viscosity is not dependent upon flow rate. Therefore, Normosol-R is also assumed to be a Newtonian fluid. To characterize the flow, the dimensionless Reynolds number was then obtained using equation (2):

$$R = \frac{QD_H}{\nu A} \quad (2)$$

The volumetric flow rate, $Q$, was attained from the CdAF flow sensor. The hydrodynamic diameter, $D_H$, for a cylindrical tube is simply the diameter of the tube, and $A$ is the cross-sectional area. The calculation resulted in a value of 1,074, indicating laminar flow. Next, to evaluate the ratio of pulsatile flow frequency to viscous effect, the Wormersly Number was calculated using equation (3):

$$\alpha = R_H \sqrt{\frac{2\pi f}{\nu}} \quad (3)$$

The pulsatile frequency, $f$, was taken as 1.167 Hz for the heart rate of 70 bpm used in most data sets. Similarly to the hydrodynamic diameter, the hydrodynamic radius, $R_H$, is merely the radius of the tube. A value of 8.238 was obtained, indicating that the pulse frequency is sufficiently low that a parabolic
velocity profile develops during each cycle and is a good approximation to Poiseuille Flow\textsuperscript{91}. Because of this, the shear stress could be estimated using equation (4):

\[ \tau = \frac{\alpha}{\sqrt{2}} \frac{6Q\mu}{2r^3} \quad (4)^{91} \]

The radius of the carotid artery channel is represented here as \( r \). Using this equation, the shear stresses for each hemodynamic condition were calculated and plotted using Matlab (Figure 26).

**Figure 26.** Fluid shear stresses as a function of flow for each hemodynamic condition in initial testing

These shear stresses are slightly higher (~10 dynes/cm\(^2\)) than those in previous literature that closely match clinical values\textsuperscript{91}. However, they do follow a similar trend where the maximum shear stresses for the normal and heart failure
conditions are approximately 5-10 dynes/cm$^2$ higher than in heart failure.

Average values are not presented, as they are skewed due to the influence of the mechanical valve ringing.
A. Limitations

While the system has major advantages over others in literature, it is not without its limitations. First and foremost, there are inherent limitations associated with mock circulatory systems. The mechanical valves cause a ringing effect on the AoP and CdAP, while also having some effect on the LVP waveform. If these effects were considered to be detrimental, the inclusion of tissue valves in the system may remedy some of these effects.

The choice of the incubator for tissue preservation severely constrains the space for the system. However, the majority of the components were able to fit inside. The only exception is the volume reservoir, which required some slight modifications. In the end, the data shows that this limitation was overcome, as the pressure and flow waveforms from the bench top testing were matched in the incubator testing. Expansion of the current system would be difficult given the space constraints of the incubator.

Finally, two limitations specific to the perfusion chamber are vessel loading and throughput. The two unconstrained plungers could cause the vessel to twist and tear in loading, so a more sophisticated loading system is desirable. Also, the single vessel system provides low throughput, although a multiple vessel system is not feasible due to greatly increased complexity to the system and the space constraints previously mentioned.
B. Discussion

Due to limitations of the system, there are some unique features in the data that require explanation. In some instances, the left ventricular pressure drops below zero during diastole. This suction can occur clinically in patients with mitral valve stenosis\textsuperscript{106}. As a mechanical valve is rigid, it can behave similarly to a stenosed valve and produce similar suction, depending on the drive pressure of the ventricle. This behavior can also explain the LVP spike in some conditions, as the pressure builds up inside the ventricle momentarily between the time the mitral and aortic valves open. Finally, the ringing caused by the mechanical aortic valve is visible in the pressure waveforms for both AoP and CdAP. While this makes the calculation of pulse pressures more difficult, it otherwise has no effect on the overall results.

While most of the data agrees with known clinical and literature values, some discrepancies do exist. Some pulse pressures and flows are slightly below literature values, which can likely be attributed to operator error. These values can be difficult to attain in real time and are not generated until the full analysis is complete. However, after several rounds of testing and modification, all carotid artery flows and pressures generated fell into their target ranges (\textbf{Table 5}), indicating that the system is indeed capable of generating the necessary pressures and flows for the next phase of vessel testing.

Another interesting observation from the results is the behavior of the pulse pressures with increasing VAD flow. The pulse pressures increase slightly with
the introduction of low VAD flow then proceed to drop with increasing VAD flow to below their original levels. An explanation for this trend is that the low VAD flow brings the cardiac output back to a normal level, providing the left ventricle with enough pressure to generate a normal pulse pressure in the arteries. However, as the VAD speed increases and the ventricle is unloaded, this pulse pressure slowly dissipates to levels below the initial value.

One more noteworthy finding from the results is the morphology of the CdAF waveform. While peak and mean CdAF values matched those found in literature, as previously discussed, the resulting waveform was visibly less complex than the referenced waveform (Figure 27). The reasoning behind this is the limited complexity of the system. In the end, the available combinations of resistors and compliance chambers were unable to perfectly recreate the measured waveform and the wave reflections exhibited in a true physiologic circulation. This is most clearly observed when the best fit estimations of both literature and obtained CdAF waveforms were plotted (Figure 28). However, with additional testing in the pulsatile VAD, a more accurate waveform may be created due to the ability of the VAD pulsatility to create more complex waveforms (Figure 29).
Figure 27. Carotid artery waveform *in vivo* measured via MRI\textsuperscript{88}

Figure 28. Overlay of obtained CdAF waveform versus estimated polynomial best fit of literature waveform of CdAF
In conclusion, the benefits of the system certainly outweigh its limitations. The hybrid system integrates an *ex vivo* perfusion chamber into a mock circulatory loop driven by a combination of a mock ventricle and VAD in series. This allows the system to expose any type of vessel to physiologic, pathologic, and VAD-specific flows and pressures, a feat that is unprecedented in the current literature. With the help of future testing, this system will allow for the collection of valuable data on the vascular response to a wide variety of VAD-specific flow conditions.

**C. Design Improvements**

The first and simplest design improvement that could be addressed is incorporating a thicker plastic tube for the vessel perfusion chamber. For the
current prototype, a 100 mL syringe was used, which has a wall thickness of just under 1/16”. This presented an issue when the three screw-in Luer lock ports were inserted into the tubing for fluid exchange and ventilation. The minimal wall thickness required the addition of multiple epoxies and sealants to provide enough material for the ports to screw into. A potential solution would be to use plastic PVC pipe of the same inner diameter, but with an increased thickness of at least 1/8”. This would allow the ports to be screwed in to the tubing and create a watertight seal without the use of epoxies and sealants that could potentially have adverse effects on the biocompatibility of the chamber. The placement of these ports could also be adjusted so that the two ports for perfusate circulation are located farther apart to prevent the creation of microcurrents during fluid recirculation.

The main functional flaw with the vessel perfusion chamber prototype was the difficulty in attaching the vessel to the two unconstrained and free moving plungers, then inserting the system into the tube. The possibility existed for the vessel to become twisted or tear, prompting the need for a design that provides a more rigid structure for the cannulated vessel and plungers during reloading into the tube. An additional desired feature was the ability to adjust the length of the vessel inside the chamber. Thus, a screw system similar to that used in a syringe pump (Figure 30) was designed. While one plunger remains stationary, the other can be adjusted simply by turning the knob at the end of the threaded rod. O-rings and proper seals should be used to ensure the chamber remained
watertight and the entire system was removable from the tube to allow for proper vessel loading.

Figure 30. Vessel length adjustment system with components: A. rotating knob, B. traveling screw element, C. fixed plunger, D. sliding plunger

Another design improvement would be to address one of the main constraints of the incubator setup: the inability to properly adjust the height of the fluid reservoir. This controls the preload and has a direct effect on the left ventricular end-diastolic pressure. The lack of adjustability is due to the reservoir being contained on the top shelf of the incubator, which is set at a maximum height due to the height of the two compliance chambers below. However, a vacant space exists below the top shelf and above the mock ventricle to the side of the compliance chambers. A potential solution to allow some degree of preload adjustment is to create a secondary shelf that attaches to the top incubator shelf. The shelf would be half the width of the main incubator shelves so as not to interfere with the compliance chambers, and would be suspended from the upper shelf by four metal rods that would rise up through the holes in the metal
shelf. The height of the shelf would be adjustable by knobs at the end of each rod, thus allowing for the maximal degree of preload adjustability.

D. Future Testing

This *ex vivo* vessel perfusion system lays the foundation for a tremendous amount of testing on bovine carotid arteries utilizing complex hemodynamic and VAD-specific waveforms. These proposed studies will test the hypothesis that diminished pulsatility produced by CF VAD adversely alters vascular function and remodeling, which may contribute to the observed clinical complications, as well as limit the potential for myocardial recovery. If substantiated experimentally in these studies, flow modulation by varying pump speed (rpm) may provide a viable solution. To test this hypothesis, two specific aims will be completed. The first specific aim is to quantify the vascular function of carotid arteries and aorta from healthy and heart failure (HF) bovine models in a mock circulation system with *ex vivo* perfusion for 72 hours and compare aortic gene expression to human HF patients. These experiments will utilize the *ex vivo* vessel perfusion system to demonstrate that *ex vivo* bovine carotid arteries and aortas remain viable for up to 72 hours with simulated physiologic flows. Tissue viability will be measured using hematoxylin and eosin (H&E) staining and methyl thiazole tetrazolium (MTT) assays. Arterial contractility, endothelial function, and mechanical properties will be quantified for normal and HF animals. This will be accomplished by using flow-mediated dilation techniques, examining pressure-
diameter-thickness relationships, performing calculations of compliance, using vascular tension assays, and comparing clinical gene expression.

Finally, the second specific aim will test the hypothesis that increased vascular pulsatility by flow modulation of rotary blood pumps can restore vascular phenotype, structure, and function using the *ex vivo* perfusion system. This aim will measure arterial responses to CF VAD flow modulation, including vasoconstriction, endothelial function, and arterial remodeling via methods previously described in the first two specific aims. Testing will again be performed at three VAD speeds in addition to various levels of flow modulation using the aforementioned VAD pulsatility program.

The long-term objective of this testing is to continue to improve HF patient outcomes and quality of life by developing safe and effective mechanical support strategies for ventricular unloading as well as peripheral targets of the integrated HF condition. While much testing still needs to be performed, the creation of the *ex vivo* vessel perfusion system is the first step in the process. The system’s ability to create hemodynamically complex, VAD-specific waveforms via the combination of the mock ventricle and VAD in series in three different hemodynamic conditions, while still being small enough to fit in the incubator lays a foundation for years of clinically-relevant future experimentation, specifically on the influence of pulsatile versus continuous VAD-generated flow on the arteries.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
<th>Value 3</th>
<th>Value 4</th>
<th>Value 5</th>
<th>Value 6</th>
<th>Value 7</th>
<th>Value 8</th>
<th>Value 9</th>
<th>Value 10</th>
<th>Value 11</th>
<th>Value 12</th>
<th>Value 13</th>
<th>Value 14</th>
<th>Value 15</th>
<th>Value 16</th>
<th>Value 17</th>
<th>Value 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>AoFpulse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AoFavg</td>
<td>114.17</td>
<td>0.25812</td>
<td>77.101</td>
<td>3.366</td>
<td>-0.51935</td>
<td>5.611</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVDFavg</td>
<td>7.4475</td>
<td>1.5198</td>
<td>178.34</td>
<td>-0.19499</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AoFpkpos</td>
<td>114.11</td>
<td>118.38</td>
<td>1.605</td>
<td>87.737</td>
<td>16.644</td>
<td>-0.39182</td>
<td>0.9545</td>
<td>-0.52897</td>
<td>-0.56645</td>
<td>72.457</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVPmax</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AoPavg</td>
<td></td>
<td>-5.3294</td>
<td>-0.18927</td>
<td>-2876.9</td>
<td>2304.6</td>
<td>-0.37358</td>
<td>4.8064</td>
<td>6.4349</td>
<td>1679.4</td>
<td>3993.9</td>
<td>185.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF, med VAD</td>
<td></td>
<td>8.0182</td>
<td>17.496</td>
<td>5.7103</td>
<td>15.098</td>
<td>5966.8</td>
<td>5.9888</td>
<td>93.902</td>
<td>-0.75059</td>
<td>112.85</td>
<td>0.057059</td>
<td>9.7863</td>
<td>3.8431</td>
<td>167.48</td>
<td>102.62</td>
<td>0.35194</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HF, hi VAD</td>
<td></td>
<td>7.8019</td>
<td>7.5544</td>
<td>0</td>
<td>-0.24062</td>
<td>0.006</td>
<td>3.3013</td>
<td>7019.2</td>
<td>158.05</td>
<td>0.085447</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline lo VAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>full VAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline HF, no VAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline HF, lo VAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline HF, hi VAD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**APPENDIX B – INCUBATOR TESTING DATA**
## APPENDIX C – VESSEL TESTING DATA

<table>
<thead>
<tr>
<th></th>
<th>002</th>
<th>003</th>
<th>004</th>
<th>005</th>
<th>006</th>
<th>007</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>modified HF</td>
<td>HF, lo VAD</td>
<td>HF, med VAD</td>
<td>HF, hi VAD</td>
<td>HF, full VAD</td>
</tr>
<tr>
<td>LVHR</td>
<td>74.672</td>
<td>74.703</td>
<td>74.734</td>
<td>74.679</td>
<td>74.691</td>
<td>74.686</td>
</tr>
<tr>
<td>LVSV</td>
<td>69.233</td>
<td>53.301</td>
<td>27.455</td>
<td>4.9203</td>
<td>0.0061392</td>
<td>0.00061392</td>
</tr>
<tr>
<td>LVCO</td>
<td>5.1697</td>
<td>3.9817</td>
<td>2.0518</td>
<td>0.36745</td>
<td>0.460E-05</td>
<td>0.460E-05</td>
</tr>
<tr>
<td>LVPbd</td>
<td>16.463</td>
<td>11.776</td>
<td>38.08</td>
<td>36.034</td>
<td>34.246</td>
<td>31.908</td>
</tr>
<tr>
<td>LVPpksys</td>
<td>146.81</td>
<td>119.7</td>
<td>134.66</td>
<td>131.52</td>
<td>128.52</td>
<td>121.56</td>
</tr>
<tr>
<td>LVpdpdPdt</td>
<td>4048.9</td>
<td>3422.7</td>
<td>9829</td>
<td>9401.2</td>
<td>9617.7</td>
<td>9269.1</td>
</tr>
<tr>
<td>LVpndPdt</td>
<td>-3743.1</td>
<td>-3203.3</td>
<td>-9492.7</td>
<td>-9356.2</td>
<td>-9498.6</td>
<td>-9490.6</td>
</tr>
<tr>
<td>LVEd</td>
<td>0.092439</td>
<td>0.45639</td>
<td>0.1236</td>
<td>1.7087</td>
<td>207.14</td>
<td>NaN</td>
</tr>
<tr>
<td>LVEW</td>
<td>7470.4</td>
<td>3964.8</td>
<td>2130.8</td>
<td>428.35</td>
<td>-0.17921</td>
<td>-0.92132</td>
</tr>
<tr>
<td>AoPavg</td>
<td>92.155</td>
<td>75.991</td>
<td>81.262</td>
<td>94.963</td>
<td>118.49</td>
<td>148.5</td>
</tr>
<tr>
<td>AoPpulse</td>
<td>96.043</td>
<td>83.169</td>
<td>72.974</td>
<td>32.207</td>
<td>18.026</td>
<td>16.662</td>
</tr>
<tr>
<td>AoPsys</td>
<td>167.11</td>
<td>141.32</td>
<td>134.99</td>
<td>115.84</td>
<td>127.69</td>
<td>157.06</td>
</tr>
<tr>
<td>AoPdia</td>
<td>71.065</td>
<td>58.152</td>
<td>62.013</td>
<td>83.634</td>
<td>109.66</td>
<td>140.4</td>
</tr>
<tr>
<td>AoFavg</td>
<td>4.8139</td>
<td>3.6885</td>
<td>1.7599</td>
<td>0.056954</td>
<td>-0.32597</td>
<td>-0.43026</td>
</tr>
<tr>
<td>AoFpulse</td>
<td>23.405</td>
<td>17.111</td>
<td>11.851</td>
<td>3.4949</td>
<td>0.48393</td>
<td>0.37386</td>
</tr>
<tr>
<td>AoFpapos</td>
<td>18.415</td>
<td>12.608</td>
<td>7.8923</td>
<td>2.0376</td>
<td>-0.05578</td>
<td>-0.22685</td>
</tr>
<tr>
<td>AoFpkaneg</td>
<td>-4.9897</td>
<td>-4.5032</td>
<td>-3.9592</td>
<td>-1.4573</td>
<td>-0.53971</td>
<td>-0.60071</td>
</tr>
<tr>
<td>TPRc</td>
<td>1531.7</td>
<td>1648.8</td>
<td>3697.9</td>
<td>1.39E+05</td>
<td>-29162</td>
<td>-27712</td>
</tr>
<tr>
<td>CdAPavg</td>
<td>86.329</td>
<td>71.252</td>
<td>75.811</td>
<td>89.237</td>
<td>111.39</td>
<td>139.63</td>
</tr>
<tr>
<td>CdAPmax</td>
<td>149.79</td>
<td>123.35</td>
<td>111.99</td>
<td>104.23</td>
<td>119.24</td>
<td>146.58</td>
</tr>
<tr>
<td>CdAPmin</td>
<td>52.244</td>
<td>41.108</td>
<td>53.612</td>
<td>80.144</td>
<td>103.77</td>
<td>132.88</td>
</tr>
<tr>
<td>LVpavg</td>
<td>57.095</td>
<td>51.451</td>
<td>50.462</td>
<td>50.782</td>
<td>47.435</td>
<td>42.231</td>
</tr>
<tr>
<td>LVPmax</td>
<td>146.81</td>
<td>119.7</td>
<td>127.17</td>
<td>131.52</td>
<td>128.52</td>
<td>121.56</td>
</tr>
<tr>
<td>CdAFavg</td>
<td>0.4558</td>
<td>0.29041</td>
<td>0.32644</td>
<td>0.34437</td>
<td>0.33255</td>
<td>0.46865</td>
</tr>
<tr>
<td>CdAFpapos</td>
<td>1.045</td>
<td>0.67858</td>
<td>0.79536</td>
<td>0.57406</td>
<td>0.46783</td>
<td>0.65522</td>
</tr>
<tr>
<td>CdAFpkaneg</td>
<td>0.079613</td>
<td>0.02929</td>
<td>0.072997</td>
<td>0.18039</td>
<td>0.20823</td>
<td>0.30817</td>
</tr>
<tr>
<td>CdAFpulse</td>
<td>0.96536</td>
<td>0.64929</td>
<td>0.72237</td>
<td>0.39366</td>
<td>0.2596</td>
<td>0.34705</td>
</tr>
</tbody>
</table>
1. High blood pressure (hypertension). Mayo Clinic. 2010


42. Herreros J, Berjano EJ, Sales-Nebot L, Mas P, Calvo I, Mastrobuoni S, Merce S. A new method of providing pulsatile flow in a centrifugal pump:


44. Farrar DJ, Bourque K, Dague CP, Cotter CJ, Poirier VL. Design features, developmental status, and experimental results with the heartmate iii centrifugal left ventricular assist system with a magnetically levitated rotor. *Asaio J*. 2007;53:310


61


105. Lab MV. Whole blood viscosity vs. Serum and plasma viscosity. 2013;2013

CHAPTEVI – CURRICULUM VITAE

Mitchell J. Buller
Graduate Student
Department of Bioengineering
University of Louisville
Research Assistant
Cardiovascular Innovation Institute
Louisville, Kentucky, USA
859-760-7050
mjbull01@louisville.edu

Education

Future (Fall 2013) University of Louisville
School of Medicine
Class of 2017

2012 – Present University of Louisville
J.B. Speed School of Engineering
Master of Engineering
Department of Bioengineering
With High Honors

2008 – 2012 University of Louisville
J.B. Speed School of Engineering
Bachelor of Science
Department of Bioengineering
With High Honors

Research Experience

2012 – Present Cardiovascular Innovation Institute
Mentors: Mark Slaughter, MD; Steven Koenig, PhD; Guruprasad Giridharan, PhD; Kevin Soucy, PhD; Michael Sobieski, RN, CCP
- Investigated effects of novel mechanical circulatory support devices in bovine and mock flow loop models
- Trained in clinical and animal blood and tissue harvesting and histological and pathological assessment
- Trained in instrumentation, data acquisition and analysis, and developing experimental protocols for acute and chronic device testing
- Trained in-vitro hemolysis testing for FDA IDE and 510K approvals

**Work Experience**

2010 – 2011
Ethicon Endo-Surgery
Cincinnati, OH
Co-op Directors: Kevin Doll, Tony Nguyen, Catherine Corbett

- Data analysis using Minitab and Excel software
- Mechanical testing for compressive, tensile, and torsional loads
- Tolerance stack-ups
- Predictive and applied failure analysis
- Professional written communication via engineering studies

**Peer Reviewed Conference Presentations**

Slaughter, MS; Sobieski, MA; **Buller, MJ**; McCants, K; Burkes, E. *Single Center Experience with Formation of Ring Thrombus on Inlet Bearing with Axial Flow Left Ventricular Assist Device*. 58<sup>th</sup> Annual ASAIO Conference, San Francisco, CA, 2012.

**Buller, MJ**; Sobieski, MA; Soucy, KG; Giridharan, GA; Koenig, SC; Slaughter, MS. *Investigation of myocardial recovery in a chronic ischemic heart failure bovine model*. 39<sup>th</sup> Annual ESAO Conference, Rostock, Germany, 2012.

Soucy, KG; Ansert, AC; Holley, KP; Spalding, CT; Benzingher, CJ; **Buller, MJ**; Giridharan, GA; Koenig, SC; Sobieski, MA; Slaughter, MS. *Feasibility of Ventricular-Arterial Cannula for Aortic Valve Bypass*. 2012 BMES Annual Meeting, Atlanta, GA.
Honors and Awards

1. Dean’s List and Dean’s Scholar
2. Pi Kappa Alpha Member of the Year, 2012
3. Tau Beta Pi Engineering Honor Society

Campus Involvement and Community Service

1. Pi Kappa Alpha fraternity
2. U of L Dance Marathon
3. U of L Relay for Life
4. Alternative Spring Break
5. Ken-ducky Derby
6. Prevent Child Abuse America
7. Make A Wish Foundation
8. Big Brothers Big Sisters of Kentuckiana
9. WHAS Crusade for Children