

TRANSLATIONAL PREDICTIVE MODEL FOR HEART FAILURE RECOVERY IN LVAD PATIENTS RECEIVING STEM CELL THERAPY

By

Philemon Mikail

Copyright © Philemon Mikail 2016

A Thesis Submitted to the Faculty of the
THE GRADUATE INTERDISCIPLINARY PROGRAM IN
BIOMEDICAL ENGINEERING

In Partial Fulfillment of the Requirements For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

2016

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that an accurate acknowledgement of the source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the copyright holder.

Signed: Philemon Mikail

Approval by Thesis Director

This thesis has been approved on the date shown below:

Zain I. Khalpey

Associate Professor of Surgery

May 9, 2016

Date

Acknowledgments

Portions of this thesis, specifically portions of chapters 4 and 5 have been and will be used to write journal articles for publication.

To my committee members: Dr. Zain Khalpey, Dr. Marvin Slepian, Dr. Timothy Secomb, and Dr. Donald Uhlmann; thank you all for sharing your extensive knowledge and support with me throughout my Master's degree. Your guidance and feedback has helped bring this research thesis to fruition and I could not have completed it without you.

To the Artificial Heart Department at Banner University Medical Center: Dr. Rich Smith and Eddie Betterton: Thank you for allowing me to use the Donovan Mock Circulation System within your facility to familiarize myself with its functions. Also, thank you for allowing me to use the department's HVAD to run my experiments at Syncardia.

To the staff of Syncardia Systems, Inc. and Jessica Crosby; thank you for allowing me to use the Donovan Mock Tank and Total Artificial Heart within your facility. Portions of this research were dependent on your help and I greatly appreciate it. Also, Jessi, thank you for providing me your insights for my experiments and ensuring the DMCS was properly set up and functioning.

Employees of Amnio Technology, LLC: Thank you for providing data and information on the stem cell allograft and providing support throughout the study.

Dr. Khalpey: Thank you for providing me the opportunity to work in your lab and perform the patient study with you. It was probably the best decision I made during my graduate degree. You are a phenomenal example of a research physician. Your enthusiasm for advancing medical technologies and providing optimal patient care has been an inspiration. You have pushed me to perform the best research possible and I could not have completed it without you.

All the members of the Khalpey lab: It has been a pleasure working with all of you. Destiny Dicken; thank you for being a mediator between myself and all our affiliates. Also, thank you for aiding in writing our manuscript and providing your insights. Tia Pilikian; thank you for helping gather our patient data and aiding in writing our manuscript.

Phat Tran: Thank you for introducing me to lab techniques and triggering my interest in research during both my undergraduate and graduate degrees.

My parents: Thank you for your continual support, love, and care. I would have never gotten to this point without you and could never thank you enough.

Tanya Aksamentova: Thank you for always being there and supporting me throughout my undergraduate and graduate degrees. Also, thank you for helping me with Photoshop and providing your opinions on my writing.

Table of Contents

List of Figures	5
List of Tables	6
Abstract	6
Chapter 1- Heart Failure and the Current Healthcare environment	8
Background.....	8
Pharmacological Treatment of Heart Failure.....	11
ICD Implantation for Treatment of Heart Failure.....	14
Coronary Revascularization for Heart Failure.....	16
Heart Transplants	16
Mechanical Circulatory Support.....	27
Chapter 2- Using the Total Artificial Heart and Donovan Mock Circulation System as a Heart Failure Model	22
Chapter 3- Research Aims	25
Project Significance.....	25
Create a LV Failure Model for Varying Degrees of Heart Failure using the TAH and DMC tank in Conjunction with the Heartware Ventricular Assist Device	26
Correlate Pulsatility Waveform Amplitude to Varying Degrees of Heart Failure.....	26
Correlate Modelled Pulsatility and Patient Data to Determine Stages of Patient Recovery with Stem Cell Intervention.....	27
Chapter 4- Characterization of Pulsatility Index using the Donovan Mock Circulation System	29
Introduction.....	29
Methods.....	30
Results.....	31
Discussion.....	39
Chapter 5- Stem Cell Therapy for Cardiac Applications	42
Background of Mesenchymal Stem Cells.....	42
Preclinical Studies of MSCs.....	44
Clinical Studies of MSCs.....	45
Human Amniotic Allograft Liquid Matrix Can Provide a Bridge to Regeneration in Patients with Cardiac Mechanical Circulatory Support.....	46
Abstract.....	47

Introduction.....	48
Results.....	52
Discussion.....	60
Conclusion.....	64
Comparison of Patient Data to In Vitro Model.....	65
Methods.....	66
Chapter 6- Future studies of Mesenchymal Stem Cells and Liquid Matrix.....	72
References.....	74

List of Figures

Figure 1- LVAD types adapted from [26].....	20
Figure 2- Schematic of DMCS[30].....	23
Figure 3- Pressure-Volume Loops from DMCS Heart Failure Model[30].....	24
Figure 4- Image of TAH and DMC tank.....	31
Figure 5- Pulsatility using TAH and DMC Tank Under Varying Afterloads.....	32
Figure 6- Stroke Volume/ Pulsatility comparison from TAH/DMC Tank.....	34
Figure 7- Total Cardiac Output with Afterload Variation.....	35
Figure 8- Pulsatility under LV Vacuum Variation with TAH/DMC Tank.....	36
Figure 9- Pulsatility using TAH and DMC Tank Under Varying Preloads.....	37
Figure 10- LV DP/dt_{max} with VAD variation.....	38
Figure 11- AoP DP/dt_{max} with VAD variation.....	38
Figure 12- Cardiac Parameters Relationships (<i>Konhilas BME 511 slides, Contractile function of the Heart</i>).....	40
Figure 13- Progression of MSC bridge to regeneration.....	51
Figure 14- Flow Cytometry of KardiaFlow™ Flow Cells.....	52
Figure 15- Biological Function of KardiaFlow™ Flow Cells.....	54
Figure 16- Pulsatility of MSCs+LM versus Control Groups.....	57
Figure 17- Visual Representation of Pulsatility.....	71

Supplement Figure 1- Components of Amnion.....54

Supplement Figure 2- Molecular Function of Proteins in KardiaFlow™55

List of Tables

Table 1- True Pulsatility versus HVAD Calculation.....32

Table 2- Normalized Pulsatility for KardiaFlow™ and Control Groups.....58

Table 3- Ejection Fractions for KardiaFlow™ and Control Groups.....59

Table 4- Clinical Demographics.....60

Supplement Table 1- Concentration of cytokines and growth factors in in KardiaFlow™56

Supplement Table 2- Proteins in KardiaFlow™53

Abstract:

Introduction:

Heart failure remains a major public health problem, with recent estimates indicating that end-stage heart failure with two-year mortality rates of 70-80% affects over 60,000 patients in the US each year. Medical management can be used but success declines for patients with end stage heart failure. Although cardiac transplantation is optimal, less than 2500 cardiac transplants are performed annually due to the severely limited supply of donor organs. Mechanical circulatory support (MCS) devices are now routinely used to bridge patients with end-stage heart failure who become critically ill until a donor heart is available. The use of stem cell therapy to treat heart failure has been gaining significant ground in recent years, specifically due to its regenerative properties, and both animal and human models have shown significant improvements in ventricular mass, ejection fraction, vascularization, wall thickness, and infarct size reduction. Using the patients’ HeartWare HVAD device diagnostics, we were

able to acquire our response variable; pulsatility. Pulsatility is a variable measure of the differential between minimum and maximum flow and is dependent on device motor speed, power, current, and fluid viscosity. This measurement is important as it relates to the contractility of the heart and could potentially be used as an end point in determining when a patient is healthy enough to have their HVAD explanted. We set out to develop a low cost and effective predictive model to determine amniotic mesenchymal stem cell's ability to repair compromised cardiac tissue of patients using the Total Artificial Heart (TAH) and Donovan Mock Circulation Tank (DMC).

Methods:

Predictive modelling was performed using the TAH and DMC. The system was set to a range from critical heart failure to a normal operating conditions through the variation of preload, afterload, and ventricular drive pressures with the intent of comparing the results to our patient population. Patients (n=7, 3 dilated, 4 ischemic) received intravenous and intra-myocardial injections of a heterogeneous amniotic mesenchymal stem cells mixture and liquid matrix (MSCs+LM) at HVAD implant. Groups were analyzed based on treatment; control (HVAD only, n=7) versus stem cells (HVAD + MSCs+LM) . HeartWare log files were acquired from patients' devices and analyzed in SAS and Matlab. Results from the patient study were compared to the predictive model to determine levels of stem cell response.

Results:

Pulsatility was found to increase with left drive pressure and afterload. Lower drive pressures resulted in a drop off in pulsatility at higher afterloads while higher drive pressures were able to

compensate for any afterload. Pulsatility also increased with preload but lower drive pressures were unable to fully eject at the highest preloads, resulting in a reduced pulsatility. We observed the effects of the stem cell injections on pulsatility and found that patients receiving therapy demonstrated statistically significant increases in pulsatility at 15-20 ($p=.0487$), 25-30 ($p=.0131$), 35-40 ($p=.0333$), and 75-80 ($p=0.0476$) days post implant. At minimum, when comparing the patient results to the in vitro model, the therapy resulted in a progression from end stage HF conditions to medium cardiac function conditions. At maximum, the therapy resulted in a progression from end stage HF to normal healthy operating cardiac function.

Conclusions:

Stem cells demonstrated a significantly increased rate of change in pulsatility within the first 40 days and at 80 days post implant when compared to control. They also demonstrated progression from end stage HF to normal healthy cardiac function at two time periods (Days 40, 90). These results justify expansion of the study to encompass a larger patient population to verify the results of the in vitro model to predict cardiac regeneration with multiple functional status indicators.

Chapter 1: Heart Failure and the Current Healthcare environment

Despite extensive research in the field heart disease and more specifically heart failure remain leaders in healthcare expenditures and mortality rates. In 2013, 1 in 9 of the deaths in the United States were related to heart failure with a total of over 58,000 being directly attributed to the disease [2]. Due to the high impact of heart failure many therapies are being actively investigated to reduce overall mortality. These therapies include angiotensin-

converting enzyme (ACE) inhibitors, β -blockers, coronary revascularization, implantable cardioverter-defibrillators, mechanical circulatory support, and cardiac resynchronization therapeutic strategies. While these therapy strategies have reduced mortality over the years, heart failure still remains a large risk factor for many patients.

Heart failure (HF) remains a complex diagnosis that relies on many factors. It is usually a progression of various cardiovascular diseases to a point of cardiac dysfunction. The working definition of HF states that it is a syndrome caused by cardiac dysfunction resulting from myocardial muscle dysfunction and is characterized by left ventricular dilation or hypertrophy[1]. Symptoms may vary but are most commonly fluid retention, shortness of breath, and fatigue. Patients with HF may have trouble during physical activities and exertion. Cardiac dysfunction will also become apparent physiologically through increased cardiac chamber pressures, thickened or thinned chamber walls, and compromised peripheral oxygen delivery[1]. A popular diagnostic tool for determining HF is left ventricular ejection fraction (LVEF). The most common cause of reduced LVEF is dilation of the LV though HF with a non-dilated LV is still common. For this reason, there are sub classifications of HF; HF with reduced LVEF due to chamber dilation, HF with preserved LVEF with non-dilated chambers most commonly the result of valvular disease, and myocardial remodeling due to increased myocardial stress[3].

Due to the difficulty of treating HF, prevention of the risk factors has become a primary concern when addressing potential HF patients. The major risk factors leading to HF are cigarette smoking, alcohol consumption, hypertension, old age, physical inactivity, obesity, genetics, high serum cholesterol, cholesterol fractions, low levels of high-density lipoprotein

(HDL) cholesterol, myocardial infarction, and diabetes mellitus[1]. The majority of these risk factors can be identified and modified to reduce the prevalence of HF development. Treatment of hypertension and preventative care of MI has been shown to significantly reduce the onset of HF [4].

Assessment of patients for HF is dependent on whether they are at risk of developing HF, suspected of having HF based on symptoms, or have symptomatic HF. Patients at risk of developing HF are recommended to have routine check ups including chest x-rays, electrocardiograms (ECG), and echocardiograms[1]. Indications of HF in these patients would be sustained arrhythmias, evidence of left bundle branch block, pathologic Q waves, and abnormalities in LV size and function. In addition to the tests performed for at risk patients, patients suspected of having HF should relay their symptoms and receive tests for BNP or NT-proBNP. Patients who have been identified of having HF should have the severity of their HF identified through evaluation of cardiac structure and function, status of coronary disease and myocardial ischemia, prevalence of life threatening arrhythmias, ability to perform physical tasks, and comorbidities that may influence therapy.[1]

The New York Heart Association has provided a framework of patient symptoms in addition to objective assessment to determine the stage of HF prevalence. They divide HF into 4 categories. Class I states the patient has no limitations to physical activity and ordinary activity does not cause fatigue. Class II is classified when slight limitations to physical activity are noted during ordinary physical activity but comfortable at rest. Class III suggests that less than ordinary activity causes fatigue, shortness of breath, or palpitations while still comfortable at

rest. The final and most severe classification, class IV, states that the patient is unable to perform any physical activity without discomfort including at rest.

Patients with HF must first consider nonpharmacologic management of their disease. Diet and physical activity play a large role in reducing mortality and improving quality of life. Proper diet includes but is not limited to reduced sodium intake, reduced fluid intake, appropriate caloric intake, and appropriate vitamin consumption through the use of multi-vitamins[1]. Physical activity should be monitored by the patient's physician and adjusted optimally.

Pharmacological Treatment of Heart Failure

A common treatment of HF has been the administration of ACE inhibitors. The mechanism is well understood in that ACE converts angiotensin I to angiotensin II in the renin-angiotensin-aldosterone system. Angiotensin II is responsible for vasoconstriction, therefore blocking the synthesis decreases blood pressure and oxygen demand from the heart. The Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) explored the effects of an ACE inhibitor in NYHA IV patients on mortality, NYHA classification, and requirement of other HF medications. They found a significant reduction in mortality of 40% within 6 months and 31% within 1 year of the trial. They also noted a significant improvement in NYHA classification in addition to a reduction in heart size and other HF medications [5]. Another study, Effect of Enalapril on Survival in Patients with Reduced Left Ventricular Ejection Fractions and Congestive Heart Failure, explored the effects of the ACE inhibitor in terms of mortality and hospitalization in patients classified as NYHA II or III. They found a significant reduction in mortality of 16% and

a 26% reduction in hospitalization in the treatment group during their 41-month study. [6] These trials justified the consistent prescription of ACE inhibitors for the majority of HF patients.

In addition to using ACE inhibitors to reduce angiotensin II production in the renin-angiotensin-aldosterone system, angiotensin receptor blockers (ARBs) also gained popularity once the mechanism was understood. Unlike ACE, ARBs do not block the production of angiotensin II but blocks the AT-1 receptor. This makes ARBs independent of angiotensin II production while still blocking the synthesis of aldosterone. To determine the beneficial effects of ARBs a meta analysis of over 147,000 patients was conducted. The investigating group found a significant reduction of 13 % in HF onset, 10% reduction in relative risk of stroke, and 15% reduction in new onset diabetes. However, they did not find a significant reduction in risk of MI occurrences. [7]

Another method of altering the renin-angiotensin-aldosterone system is to use mineralocorticoid receptor antagonists (MRAs) which block receptors that bind aldosterone and other corticosteroids. In the Randomized Aldactone Evaluation Study (RALES) 1,663 patients classified as NYHA III were randomized to receive MRAs. The treatment group demonstrated a RRR of 30% for mortality and 35% for HF hospitalization within 2 years of treatment. [8] Another trial, EMPHASIS-HF tested for similar endpoints in 2,737 patients classified as NYHA II. Treatment with the MRA resulted in a RRR in cardiovascular death of 24% and HF hospitalization of 42% within 21 months of beginning treatment. [9]

Another pharmacological approach in treating HF is beta-blocker treatment. The Metoprolol in Dilated Cardiomyopathy (MDC) trial investigated the effects of the beta-blocker on morbidity and survival. 94% of their trial subjects were classified as either NYHA II or III. 12 months after the initiation of the trial there was a 34% reduction in morbidity in the treatment group when compared to the control. They also evaluated EF% and found the metoprolol treated group demonstrated a significantly greater increase of 13% compared to the 6% increase in the placebo group. The treatment group also showed improvement in exercise at 12 months [10]. Three additional key trials; CIBIS II, COPERNICUS, and MERIT-HF investigated adding beta-blockers to other HF medication. 90% of the patients were on either on ACE inhibitors or ARBs. All three trials showed a relative risk reduction (RRR) in mortality of 34% and a 28-36% RRR in HF hospitalization. [11, 12]

Due to the success of each of the mentioned drugs individually, many physicians prescribe a combination of ACE inhibitors, beta-blockers, MRAs, and ARBs as the first line of treatment for HF patients. This is the case due to the varying mechanisms of action along with the understanding that ACE inhibitors may lead to LV remodeling and beta-blockers increase EF while ARBs and MRAs work complementary to improve HF survivability. In addition to these HF medications, diuretics are commonly prescribed to relieve dyspnea and edema in patients with symptoms of congestion.

A common arrhythmia found in HF patients is atrial fibrillation (AF). Reducing AF becomes critical in HF patients due to hemodynamic instability and the potential of a thromboembolic event. There is also a risk of conduction of AF contractions to the ventricles leading to rapid ventricular response (RVR). RVR can develop into a dangerous tachyarrhythmia

which could potentially lead to cardiac arrest and at minimum reduces EF in patients. For this reason, medical intervention is required to control the ventricular rate during AF. Beta-blockers are commonly administered for patients to induce rate control and it has been shown that the addition of digoxin is more effective than beta-blockers alone. [13] The only antiarrhythmic drug to show reduction of AF in HF patients is amiodarone. [14]

ICD Implantation for Treatment of Heart Failure

Almost half the deaths in HF patients are unpredictable and occur suddenly due to ventricular arrhythmias. [1] For this reason, implantable cardiac defibrillators (ICDs) are critical for reducing mortality in HF patients prone to ventricular arrhythmias. When patients without an ICD go into ventricular fibrillation they must wait for a paramedic or bystander to externally defibrillate them and the time to defibrillation may be prolonged. If the patient does not receive a shock within 5 minutes they will most likely die or receive permanent brain damage. For this reason, ICD therapy has provided a much needed solution to dangerous ventricular arrhythmias. Primary prevention and justification of ICD implant occurs when a patient has symptomatic HF in the form of NYHA II or III and an EF < 35% despite pharmacological therapy for at least 3 months. Patients are anticipated to survive > 1 year with good functional status and the goal of implantation is to reduce the risk of sudden death. Secondary prevention states that an ICD is recommended for those who are hemodynamically unstable due to ventricular arrhythmias. The SCD-HeFT trial enrolled 2,521 patients with HF classified as NYHA II or III, EF <35%, and no prior symptomatic ventricular arrhythmias. Patients were randomized to receive a placebo, Amiodarone, or an ICD in addition to standard pharmacological treatment with ACE inhibitors or beta-blockers. ICD treatment led to a RRR in mortality of 23% over solely

pharmacological approaches in a median of 45 months post implantation. [15] The MADIT-II trial verified the findings in the SCD-HeFT with a similar trial for patients with EF \leq 30%. Subjects received either conventional treatment or conventional treatment plus an ICD. ICD therapy demonstrated a 31% RRR in mortality when compared to their control. [16] There is limited clinical data to suggest ICDs provide a significant reduction in mortality for patients with non-ischemic dilated cardiomyopathy. A study published in the New England Journal of Medicine investigated ICD implant in 458 dilated cardiomyopathy patients and found a trend towards reduction in mortality ($p=0.08$). 2 years post implantation they found that their control group had a mortality rate of 14.1% while the ICD group was only 7.9%. Their final end point was sudden deaths from arrhythmias and they found a significant reduction with the ICD therapy ($p=0.006$). [17] This trial justified ICD implant in non-ischemic HF patients as evidence level B.

Reduction in EF is commonly caused by either right or left bundle branch block (RBBB, LBBB) which creates a dyssynchrony between the right and left ventricles. When the ventricles are not synergistically pumping blood they reduce the total cardiac output of the heart. To combat this issue, pacing investigators created cardiac resynchronization therapy (CRT). CRT functions by biventricular pacing and timing the contractions of the ventricles to occur simultaneously. Two major clinical trials, COMPANION and CARE-HF investigated the effects of CRT pacing on advanced HF patients in sinus rhythm but with widened QRS complexes > 120 ms and EF \leq 35%. Both trials compared CRT pacing with optimal medical therapy versus optimal medical therapy. The COMPANION trial demonstrated a total RRR of death to be 24 % with a CRT-pacemaker and 36% with a CRT-defibrillator. [18] The CARE-HF trial reported similar results with a RRR of death to be 36% with a CRT-pacemaker and 52% reduction in HF hospitalization.

[19] These trials demonstrated that CRT pacing decreased HF symptoms while improving quality of life and global ventricular function in patients classified as NYHA III or IV. Two additional trials, MADIT-CRT and RAFT, investigated CRT pacing in NYHA I or II patients. Both trials required patients to have an EF \leq 30% while the MADIT-CRT trials required a QRS duration \geq 130 ms and the RAFT trial required a QRS duration \geq 120 ms. The MADIT-CRT trial demonstrated a 34% RRR and the RAFT demonstrated a 25% RRR in HF events. In addition, the RAFT trial reported a 25% RRR reduction in mortality while the MADIT-CRT trial did not show a significant decrease. [20, 21] Similar to the COMPANION and CARE-HF trials, these trials demonstrated that CRT pacing improved cardiac function and reduced HF symptoms.

Coronary Revascularization for Heart Failure

Coronary artery disease (CAD) when left untreated results in ischemic heart failure due to limited blood supply to regions of the heart. For this reason, it is important to perform a coronary artery bypass graft (CABG) procedure to restore adequate perfusion to the myocardium. Patients with multiple vessel stenosis, especially in the left anterior descending (LAD) or the left main artery are the primary candidates for a CABG. In the Surgical Treatment for Ischemic Heart Failure (STICH) trial patients classified as NYHA I-III with CAD and an EF \leq 35% were enrolled to receive a CABG procedure in addition to medical treatment and were compared to solely medical intervention. The investigating group reported a 19% RRR in cardiovascular death and a 26% RRR in cardiovascular hospitalization when compared to their control.[22]

Heart Transplants

Cardiac transplantation is regarded as the optimal therapy for patients with end stage heart failure classified as NYHA IV. Cardiac transplants grew drastically in popularity during the 1980s but the increase was halted due to the lack of donor hearts. In 2010, less than 4,000 transplants were conducted worldwide and less than 2,500 in North America. 53.8% of recipients were diagnosed with non-ischemic cardiomyopathy, 37.1% had ischemic cardiomyopathy, and 2.6% had valvular cardiomyopathy between 2006 and 2011.[23]

The Primary causes of death after transplant are acute rejection, graft failure, cardiac allograft vasculopathy (CAV), infection, renal failure and malignancy. [23] Graft rejection and infection are the most common causes of acute morbidities while malignancy and CAV are the primary causes long term. Patients who survive the first year post-transplant have a 63% chance of remaining alive 10 years post transplant and a 27% chance of being alive 20 years post-transplant.

Due to the limited supply of donor hearts, mechanical circulatory support (MCS) in the form of a left or right ventricular assist device (LVAD, RVAD) or the Total Artificial Heart (TAH) are used as bridge to transplant options. These bridge to transplant devices maintain circulation in the absence of a functioning heart to increase survival until a donor heart can be found. MCS options have been growing in popularity in recent years with over 33% of transplant recipients having some form of MCS at the time of heart transplant [23].

Mechanical Circulatory Support

Due to the severely limited supply donor hearts MCS devices have gained popularity in sustaining life in patients with end stage heart failure. Patients deemed to be “end-stage” HF

generally have an EF $\leq 25\%$ and are classified as NYHA IV. Many of these patients may be candidates for cardiac transplantation. As many as 50,000 of these end stage HF patients would benefit from a new heart but as stated earlier, less than 2,500 transplants are performed in North America annually. This has triggered the investigation of MCS devices as long term solutions rather than solely bridge to transplants, which is termed as destination therapy.

There are multiple MCS devices on the market. When a patient has a compromised left ventricle they receive an LVAD which is connected near the apex of heart and funneled into the aorta to aid in pumping blood systemically. When the right ventricle is unable to sufficiently pump blood to the lungs an RVAD is implanted in the right ventricle and connected to the pulmonary artery. When failure occurs in both ventricles patients will receive a bi-ventricular VAD (BiVAD). BiVADs are also considered for patients who may be at risk of RV failure after implantation of an LVAD. Earlier VADs were pulsatile flow pumps while newer devices have migrated toward continuous flow. The final choice for an MCS device is the TAH and is only implanted when patients are critically ill. Implant of a VAD prior to multi-organ failure drastically improves surgical outcomes, so careful monitoring of HF patients is required to find the optimal time for MCS implants.

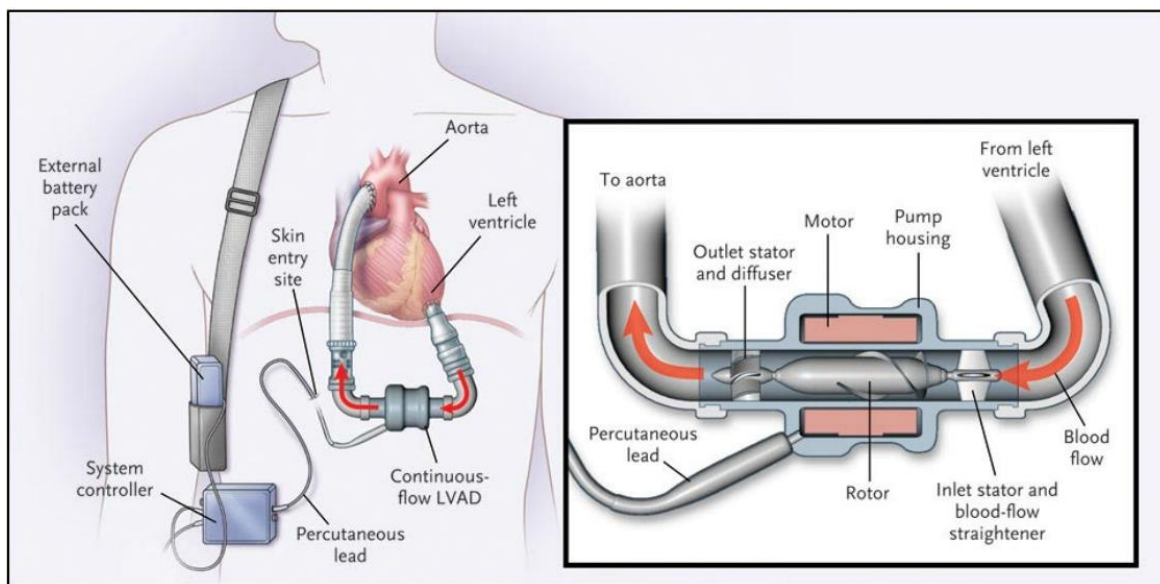
Two clinical trials investigated pulsatile LVAD support for patients ineligible for cardiac transplantation. The Investigation of Nontransplant-Eligible Patients Who Are Inotrope Dependent (INTrePID) trial compared LVAD therapy to medical therapy in end-stage HF patients. They reported improved survival rates among LVAD patients at 6 months (46% vs. 22%) and at 12 months (27% vs 11%) while also reporting that a large portion of the LVAD group did not have any HF symptoms. [24] Another study, the Randomized Evaluation of

Mechanical Assistance for the Treatment of Congestive Heart Failure (REMATCH), investigated a similar comparison in patients ineligible for cardiac transplantation. They reported an improvement in survival for the device group compared to the optimal medical therapy group at 1 year (52% vs. 25%) and at 2 years (23% vs. 8%). [25] While these trials demonstrated improvement with device intervention, the mortality rates were still less than optimal which decreased widespread application of pulsatile LVADs. Much of the mortality in these device patients were not necessarily attributed to cardiac failure but to infection, bleeding, and malfunction of the device. This brought criticism to the durability of these pulsatile LVADs and warranted further development to improve functionality.

The potential solution to the issues observed in pulsatile pumps was realized with continuous flow alternatives. Rather than having a large reservoir for volume displacement in pulsatile pumps, continuous flow pumps use either axial or centrifugal rotors to pump the blood through the device (Figure 1). This reduced the mechanical wear within the device in addition to greatly reducing its size. One of the first trials to investigate the efficacy of continuous pumps was performed in patients awaiting heart transplantation. The prospective trial investigated 133 patients and reported a 75% survival rate with the device at 6 months and a 68% survival rate at 12 months. [26] They also reported improvement in NYHA class and quality of life. This was the first major trial to demonstrate continuous flow LVADs could provide hemodynamic support in end stage HF patients awaiting cardiac transplantation. Another similar study supported these findings when they implanted continuous flow LVADs in 222 patients waiting for cardiac transplantation. At 6 months they noted significant improvements in functional status where 83% of the patients moved from NYHA IV to either I

or II. At 18 months post implantation survival was 72%.[27] These trials demonstrated that survival and quality of life improved significantly in comparison to optimal medical therapy and pulsatile flow LVADs in end stage HF patients awaiting transplants.

With the success of continuous flow LVADs in patients awaiting transplants it was necessary to investigate the potential of using these devices in patients ineligible for transplants. A trial set out to determine the long term effects of the continuous flow LVAD versus the pulsatile flow LVAD on survival, risk of stroke, frequency of adverse events, quality of life, and functional capacity. They enrolled end stage HF patients to receive one of the LVAD types and found a larger improvement in survival in the continuous flow group at 2 years (58% vs. 24%) as well as survival without stroke (46% vs. 11%). They also noted significantly less adverse events and device replacements with the continuous flow LVADs while both device types improved functional capacity.[28] This trial demonstrated that the use of continuous LVADs had better clinical outcome and greater durability while reducing size, weight, and noise when compared to pulsatile flow LVADs.



Left Ventricular Assist Device: “LVAD”

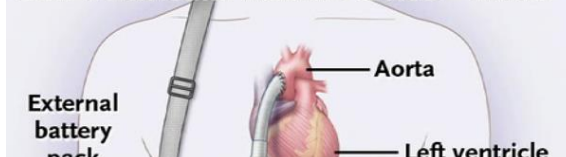


Figure 1: Schematic comparison between continuous flow LVAD and Pulsatile flow LVAD[26]

The final MCS device commonly used on the market is the Syncardia Total Artificial Heart (TAH). Unlike VADs, the TAH completely replaces both the ventricles and valves, in turn eliminating the adverse effects observed in many VAD applications. Until recently the TAH was only classified as a bridge to transplant device in the United States, therefore all patients who received the device were eligible for cardiac transplantation. A study was performed to determine the rates of survival with the TAH in comparison to medical management prior to and after cardiac transplantation. The investigating group reported the rate of survival to transplantation was 79% in the TAH group compared to 46% in the control. One-year survival in the therapy group was 70% compared the 31% among controls. One and five-year survival rates among the bridge to transplant group after transplantation were 86% and 64% respectively.[29] This study demonstrated the use of the TAH in patients with irreversible biventricular failure had higher survival rates to and after cardiac transplantation, making it a viable option for physicians and patients.

Stem Cell Treatment for Cardiac Regeneration

The use of stem cells to treat HF and potentially regenerate fibrotic or compromised cardiac tissue has been gaining much attention in recent years. Preclinical animal models have suggested cardiac remodeling is possible through the use of various stem cells and that has led

to the transition to human testing. Stem cell trials and background will be covered in chapter five of this paper.

Chapter 2: Using the Total Artificial Heart and Donovan Mock Circulation System as a Heart Failure Model

A Heart Failure model has been previously created and described in the dissertation, *Expanding the performance envelope of the total artificial heart: Physiological characterization, development of a heart failure model, and evaluation tool for mechanical circulatory support devices* by Crosby.[30] In the experiments outlined in paper the Donovan Mock Circulation (DMC) tank is used in conjunction with the Syncardia TAH to emulate the physiological conditions of the circulatory system. The combination of the TAH with the DMC has been named the Donovan Mock Circulation System (DMCS). With this system, both normal and HF conditions can be emulated to represent the hemodynamics under certain conditions.

The DMC is constructed of multiple chambers as seen in figure 2 filled with a mixture of glycerol and water to mimic the viscosity of blood. Right Atrial Pressure (RAP), pulmonary arterial pressure (PAP), left atrial pressure (LAP), and aortic pressure (AoP) are all created by the fluid levels in each of the chambers. Aop and PAP can be manipulated by adjusting the height of the systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) bellows. The SVR bellow alters the resistance between the Aop and RAP chambers while the PVR bellow alters the resistance between the PAP and LAP chambers. The TAH is controlled through a Syncardia Companion 2 driver which has the ability to alter the LDP, right ventricular

drive pressure (RDP), left ventricular vacuum, and right ventricular vacuum. The vacuums allow for an increased distention within the ventricles, in turn increasing fill volume. The system also has the ability to include a VAD through the use of T-junctions, CPC connectors, and the addition of an artificial valve to allow for evaluation of hemodynamics under different conditions with a MCS device. Experiments were conducted to demonstrate the startling like behavior of the system and similarities to the physiological circulatory system. [30]

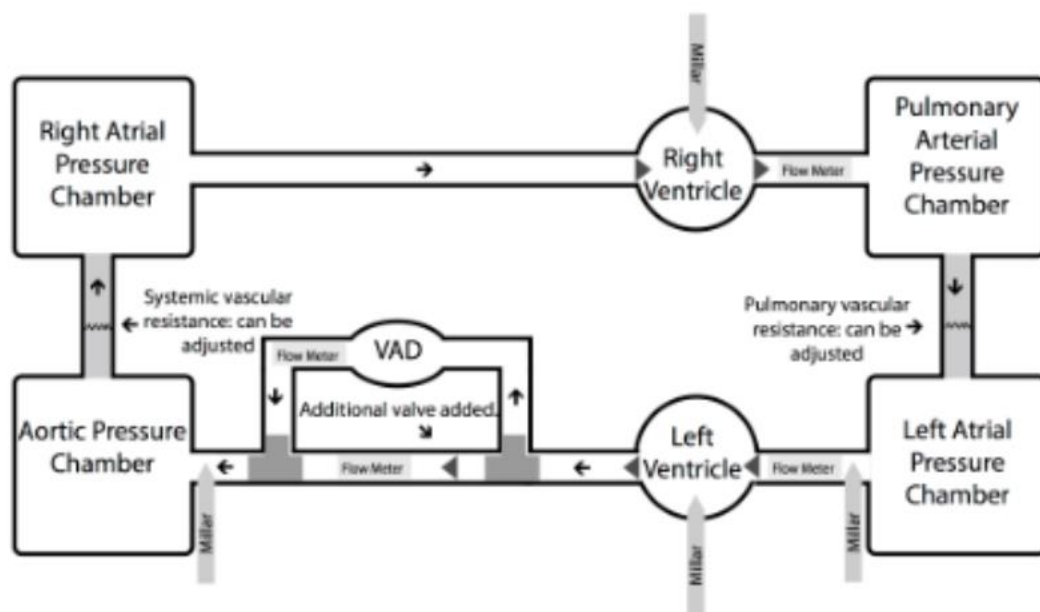


Figure 2: Schematic of DMC tank in conjunction with the TAH in the experiments performed by Crosby. Experiments were performed initially without the VAD but future studies explored the effects of a VAD on the hemodynamics of the circulatory system under different conditions [30]

To induce HF conditions the system was first set to normotensive conditions with a left ventricular drive pressure (LDP) of 180 mmHg, RAP of 7 mmHg, LAP of 9 mmHg, and mean Aop ranging from 85-95 mmHg. Once hemodynamics is established under normal conditions, HF conditions are induced by setting the lowest possible LDP in Companion 2 driver to 120 mmHg,

left ventricular vacuum to 0 mmHg, RDP to 60 mmHg, right ventricular vacuum to -10 mmHg, percent systole to 50% and heart rate to 100 beats per minute. Under these conditions the DMC tank pressure values were an AoP of 95 ± 5 mmHg, left atrial pressure of 30 ± 5 mmHg, right atrial pressure of 12 ± 5 mmHg and pulmonary arterial pressure of 27 ± 5 mmHg. Experiments included varying preload by adjusting the right vacuum and afterload by adjusting the SVR bellow. When preload was increased by varying the right vacuum between 0 and -20 mmHg there was a corresponding increase in LAP and left ventricular end diastolic volume (EDV). When afterload was increased there was a corresponding decrease in RAP and EDV while there was an increase in LAP left ventricular pressure (LVP) as seen in the pressure volume loops shown in figure 3.[30] These results correspond with the hemodynamics present in physiological HF conditions. In conclusion, this experiment demonstrated the the DMC tank in conjunction with the TAH can serve as a reproducible and accurate model for varying degrees of HF.

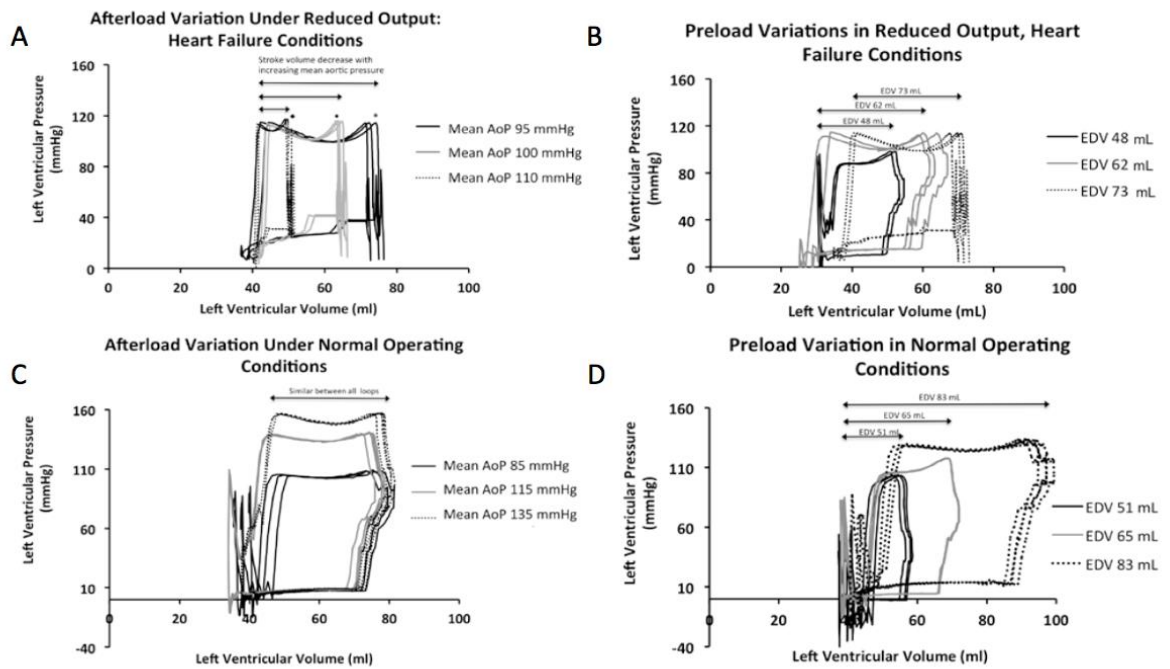


Figure 3: Pressure-Volume loops for A. varying afterload under HF conditions B. varying preload in HF conditions C. varying afterload under normal operating conditions and D. varying preload in normal operating conditions [30]

Chapter 3: Research Aims

Project Significance

MCS devices have provided a much needed alternative for patients either awaiting cardiac transplantation or ineligible for transplant. While these devices have prolonged life, increased functional status, and improved quality of life they fail to address the root cause of HF. Stem cell therapies have been gaining significant ground in recent year due to their potential of cardiac regeneration and LV remodeling. To this point, there has been no cost efficient, consistent or completely reproducible method of determining stem cell response in

patients undergoing therapy. There has also been no literature on the effects of incorporating micronized liquid matrix in conjunction with stem cells to determine if there is a provided benefit. In the studies outlined in this paper end stage HF patients were provided a combination of intra-myocardial and intravenous injections of allogeneic amniotic mesenchymal stem cells (MSCs) and micronized liquid matrix (LM) at the time of a Heartware ventricular assist device (HVAD) implantation. Using the HVAD diagnostics we were able to acquire our response variable, pulsatility, and compare patients with and without MSC intervention. In addition to implanting the HVAD in patients, the MCS was also incorporated into the DMC tank and TAH to characterize our response variable under different conditions. The aim of this thesis is to provide a cost effective and reproducible method of determining stem cell therapy response in patients with HVADs using device diagnostics. These goals were set out to be accomplished through the following aims.

- I. Create a LV Failure Model for Varying Degrees of Heart Failure using the TAH and DMC tank in Conjunction with the Heartware Ventricular Assist Device

Purpose and Approach

Characterization and creation of a HF model using the TAH and DMC tank has been previously performed [30] but there is a need to characterize the HVAD diagnostic parameters, more specifically pulsatility, under varying degrees of heart function. In this study, the previously described HF model was used with the addition of the HVAD to determine the pulsatility response under both HF and normal operating conditions. Preload and afterload were varied to compare the pulsatility response in the HVAD and ultimately compared to the results observed in patients undergoing MCS+LM therapy.

Comparison of True Flow Pulsatility to Calculated Pulsatility in the HVAD

The HVAD provides diagnostics data in the form of log files that record data every 15 minutes once the device is operational. When using the device diagnostics for clinical assessment of patients it is paramount the values are accurate. The sampling frequency of the HVAD provided limitations in testing the device with the TAH and DMC tank so it was necessary to use real time flow meters and compare the true flow pulsatility to the pulsatility recorded by the implantable device. To ensure the HVAD calculations were accurate the device was set to record log files for 45 minutes to acquire three separate data points and the results were compared to the data acquired by the Transonic Flow Meter sampling flow velocity at 1000 Hz.

II. Correlate Pulsatility Waveform Amplitude to Varying Degrees of Heart Failure

Purpose and Approach

Using the DMCS, it is possible to vary conditions from end-stage HF to normal heart function. With this functionality it is important to relate increases in pulsatility to clinical observations noted in patients. Correlating the pulsatility data in the DMCS to end stage HF, moderate HF, mild HF, and a healthy circulatory system will allow for seamless comparison in patients. This aim sets out to determine how percent increases in pulsatility relate to the progression of a recovering heart.

Comparing Patient Pulsatility to DMCS Pulsatility

Pulsatility increases were correlated to increases in heart function based on the results acquired from the HF model created with the DMCS. End stage HF conditions were compared to normal operating conditions and percent increases were correlated to similar increases observed in patients undergoing stem cell therapy.

III. Correlate Modelled Pulsatility and Patient Data to Determine Stages of Patient Recovery with Stem Cell Intervention

Purpose and Approach

Due to the severely limited supply of donor hearts and transplants worldwide the use of MCS devices has increased substantially. Although, as noted previously, they fail to address the root cause of the disease and cardiac remodeling rarely occurs with MCS devices alone. The primary goal is not only to support the circulatory system of patients, but to ultimately cure them of their ailment through the regeneration of their cardiac tissue. In this case, once patients have recovered sufficiently HVAD explant may be warranted. Many groups must use invasive or expensive imaging techniques to verify stem cell efficacy using ejection fraction, infarct size, or contractile function as the primary endpoint. Not only are these diagnostic methods time consuming, they also require a cardiologist to read and interpret the data. This leads to inconsistent interpretations that are difficult to compare among different physicians or institutions. With the opportunity of using device diagnostics as a method of determining stem cell response, consistent and reproducible results are guaranteed. To fully understand the meaning of a variation in pulsatility, the data gathered

from the model described in aim 1 and 2 was compared to the data acquired from patient devices.

Comparing Pulsatility in the Stem Cell Therapy Group versus Control

Study groups were divided into the therapy group, which consisted of end stage HF patients receiving a combination of MSCs+LM in addition to HVAD implantation and the control group, which consisted of patients solely receiving an HVAD. Patient data was acquired from the Heartware log files for the first 100 days following implantation and grouped into 5 day windows. Pulsatility data was normalized to each patients' baseline values and normalized pulsatility was compared between groups. Significant increases in normalized pulsatility were noted and percent increases were correlated to the results of the DMCS experiments.

Chapter 4: Characterization of Pulsatility Index using the TAH and DMCS

Introduction

Stem cell therapies are being actively researched due to their potential of ventricular remodeling and restoration of contractile function in patients with reduced cardiac function or HF. Generally, groups investigating the efficacy of stem cells in patients must use expensive and time consuming methods such as echocardiograms or MRIs to determine the therapy's response. These methods are not only cost and time inefficient but also require a physician to interpret the data. This leads to inconsistent reporting across different physicians or

institutions. With this in mind, there is a need to develop a cost effective, consistent, and reproducible method of determining cardiac recovery in patients receiving stem cell therapy.

An advantage in our study is that our patient cohort all received implantation of an HVAD at the time of stem cell administration. This provides an opportunity to apply objective device diagnostics as a mean of determining cardiac recovery with stem cells. The HVAD is a continuous axial flow device and uses a centrifugal rotor to pump blood. The ADVANCE trial investigated the success of the device through survival, survival to transplantation, or explant of the device for ventricular recovery. They found that the device was successful in 90.1% of patients, making it a viable bridge to transplant option[31]. The HVAD records multiple parameters including flow rate, minimum flow (trough), device power, and pulsatility. The parameter of interest in our study was pulsatility. Pulsatility is the measure of peak systolic flow velocity minus the minimum diastolic flow velocity over a single cardiac cycle. Pulsatility provides insight of individual flow waveforms which can be critical for diagnostic purposes. This is an important measure as it is the derivative of the flow calculation of stroke volume, therefore provides insight on the contractility of the heart. The device records the pulsatility value every 15 minutes from the time of implant. This provides 96 data points a day which allows for close monitoring of patient status.

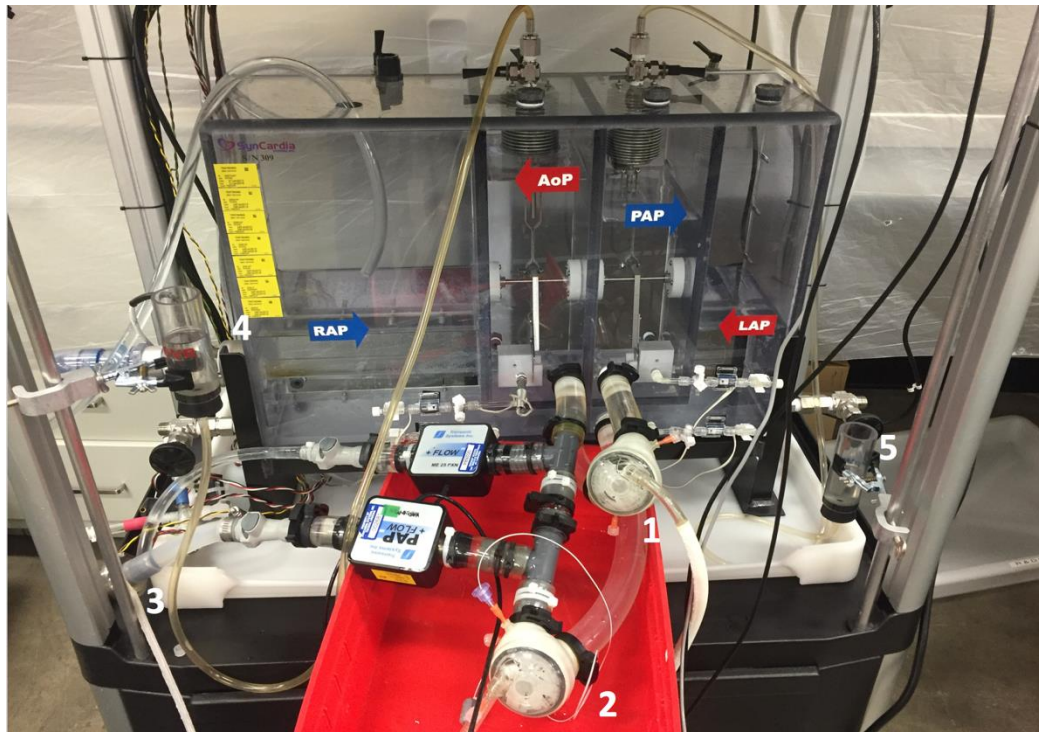
To understand how changes in pulsatility relate to the cardiac status it was important to characterize pulsatility under various conditions. A well established and characterized HF model has been created previously using the TAH and DMC tank [30] and was used as a framework for modelling pulsatility through the HVAD. The goal of this study was to model changes in

pulsatility under varying degrees of HF to ultimately compare percent increases to the patients receiving MSCs+LM therapy.

Methods

The system was constructed similarly to the previously created model [30] with the TAH and DMC tank. Syncardia's TAH was connected with 1 inch tubing and controlled through the Syncardia Companion 2 (C2) driver. The Heartware HVAD was connected between the LV and the AoP chamber using T-junctions and CPC connectors (Figure 4). The tank was filled with a 35% glycerol to water ratio to mimic the viscosity of blood. Transonic flow meters were connected pre and post VAD to record flow rate at 1 KHz and to ensure suction was not occurring within the HVAD. The Transonic Flow meters were connected to a NiDAQ board. A Millar Catheter was inserted in the LV to record left ventricular pressure (LVP) sampled at 1 KHz. RAP, PAP, LAP, and AoP were recorded using tank pressure sensors. All calculations and post processing were performed through Syncardia's Labview based program.

Experimental design included varying preload and afterload. Preload was varied through altering the fill volume of the RV by adjusting the right vacuum on the C2 between 0 and -20 mmHg. Afterload was varied between 65 and 115 mmHg by adjusting the height of the SVR bellow (Figure 4 #4) to increase the resistance between the AoP and RAP chambers. Data was recorded at multiple points of preload and afterload. Pulsatility was calculated post data acquisition using the data from the Transonic Flow meter.



1. RV
2. LV
3. HVAD
4. SVR
5. PVR

Figure 4: TAH and DMC tank with the Heartware HVAD connected (3) to the LV using CPC connectors

Results

Afterload Variation:

LDP	Pulsatility	HVAD Pulsatility	Delta puls	puls sem	hvad sem
120	2.912	2.928	-0.016	0.022	0.021
140	3.564	3.524	0.040	0.032	0
160	3.906	3.907	-0.001	0.03	0.036
180	3.945	3.966	-0.021	0.047	0.005
200	3.987	3.937	0.050	0.029	0.006

Table 1: Comparison of pulsatility recorded by the Transonic Flow meters and the pulsatility calculated through the HVAD device

As seen in Table 1, the true value of pulsatility recorded in real time through the Transonic Flow Meter was nearly identical to the HVAD calculated pulsatility.

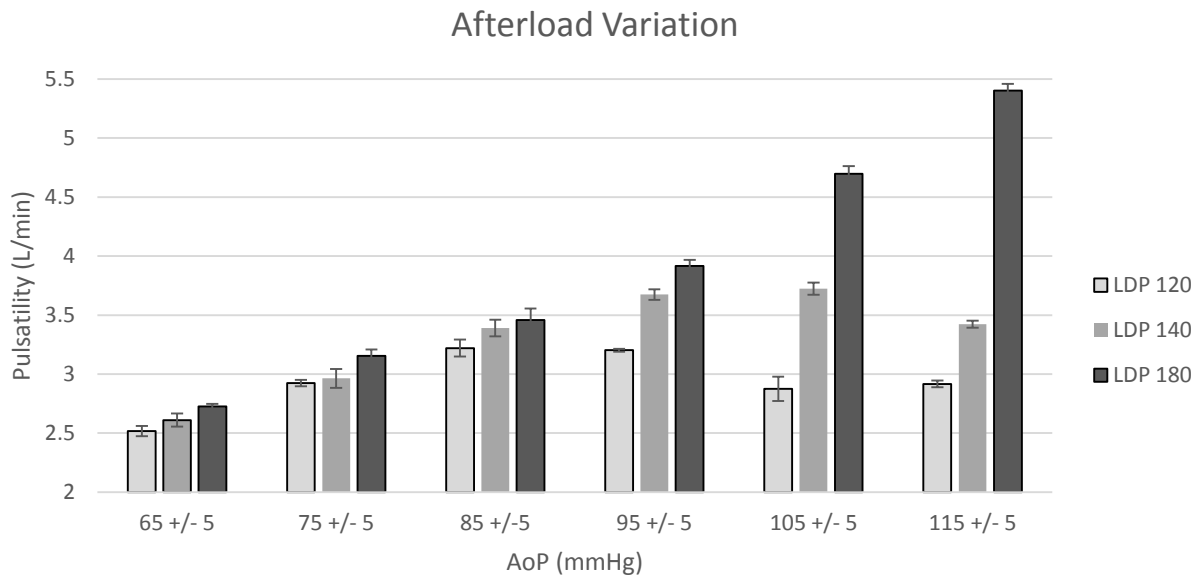


Figure 5: Varying afterload (AoP) while recording pulsatility under a low ventricular drive pressure (120 mmHg), medium drive pressure (140 mmHg), and normal drive pressure (180 mmHg)

Running afterload variation under various degrees of left ventricular drive pressure correlates to varying degrees of heart function. LVP increased with an increased AoP. At a low drive pressure (120 mmHg) and low afterload (65 mmHg) LVP was 32.467 mmHg, at a high afterload (95 mmHg) LVP was 49.94 mmHg, and at the maximum afterload (115 mmHg) LVP was 61.515 mmHg. At a medium drive pressure (140 mmHg) and low afterload LVP was 32.32

mmHg, at a high afterload LVP was 51.55 mmHg, and at the maximum afterload LVP was 69.69 mmHg. At a normal drive pressure (180 mmHg) and low afterload LVP was 29.79 mmHg, at a high afterload LVP was 48.453 mmHg, and at the maximum afterload LVP was 71.667 mmHg.

At the low drive pressure, pulsatility increased with AoP until 85 mmHg and dropped at higher afterloads. Similarly, at a medium drive pressure pulsatility increased with AoP until 105 mmHg and dropped at the higher afterload. In normal operating conditions pulsatility increased with AoP to the maximum afterload with no drop in pulsatility noted. This translated to a 3.7% increase in pulsatility from a low to medium drive pressure at a low afterload, a 14.7% increase in pulsatility from a low to medium drive pressure at a high afterload, and a 17.36% increase in pulsatility from a low to medium drive pressure at the maximum afterload. Calculating the increases from a low to high drive pressure yielded an 8.3% increase at a low afterload, 22.32% increase at a high afterload, and an 85.25% increase at the maximum afterload. Calculating the increases from a medium to high drive pressure yielded an 4.4% increase at a low afterload, 6.63% increase at a high afterload, and an 57.85% increase at the maximum afterload.

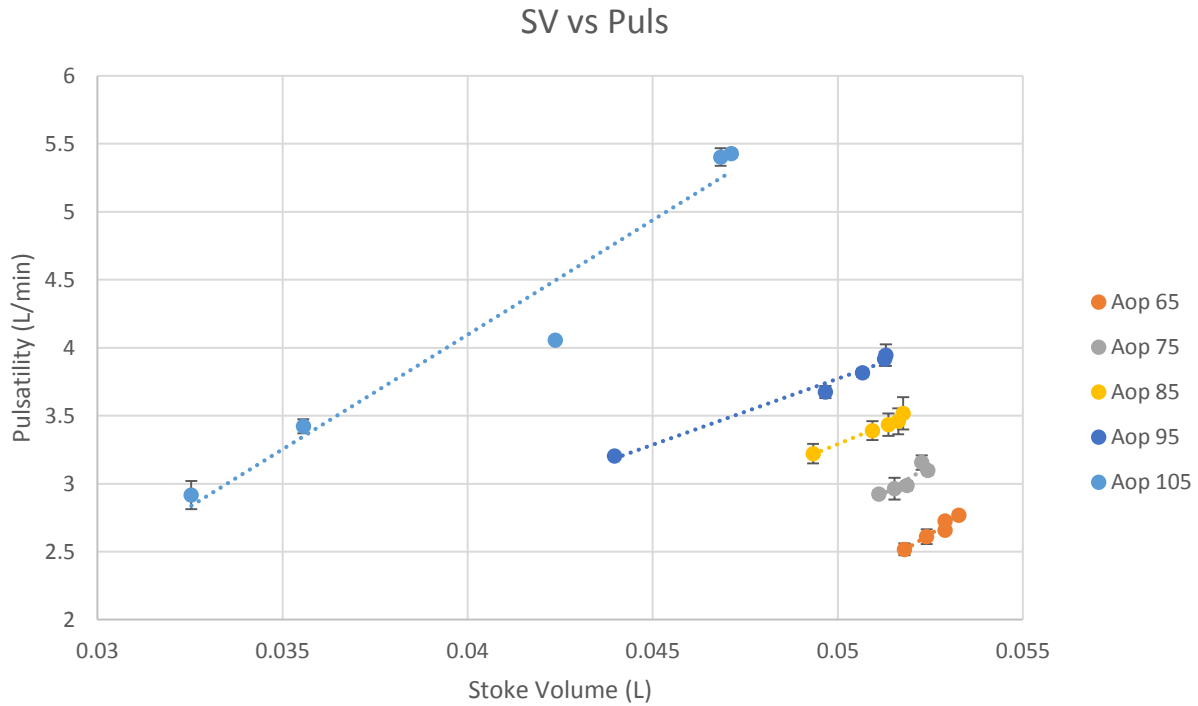


Figure 6: Comparing stroke volume to pulsatility under varying afterload (different color lines) and left ventricular drive pressures (points along the lines)

All afterloads demonstrated a linear relationship between stroke volume and pulsatility. The range of stroke volume and pulsatility increased with an increase in afterload. As an example, at a low afterload the minimum values for pulsatility and stroke volume were 2.517 L/min and .0518 L respectively while their maximum values were 2.767 L/min and .0532 L. At a very high afterload (105 mmHg) the minimum values for pulsatility and stroke volume were 2.875 L/min and .0393 L respectively while their maximum values were 4.761 L/min and .0499 L.

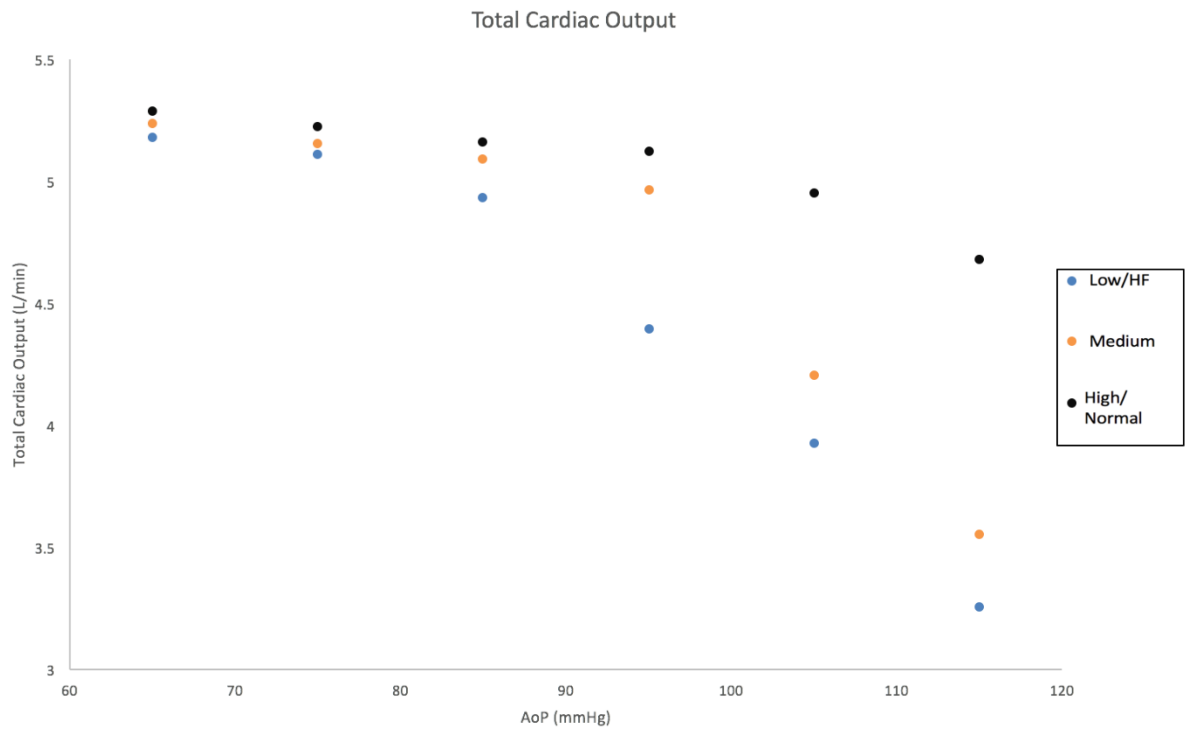


Figure 7: Total Cardiac Output at low, medium, and normal operating conditions under various afterloads

At a low afterload total cardiac output (TCO) is nearly identical in all cardiac status cases, similar to pulsatility. As afterload increased, low and medium cardiac conditions reduced drastically while normal operating conditions maintained a higher level of TCO throughout the range.

Preload Variation

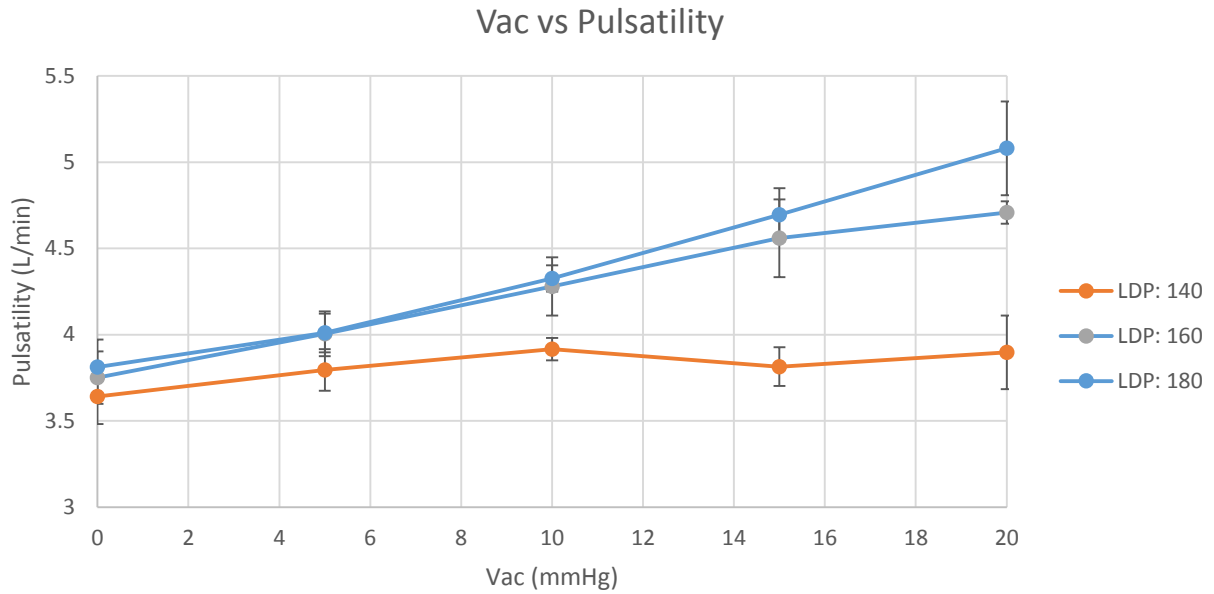


Figure 8: Varying left ventricular vacuum and therefore fill volume of the LV in relation to pulsatility under varying LDPs

At the lower drive pressure pulsatility increases with vacuum filling until -10 mmHg and decreases at higher fill volumes. At 160 and 180 mmHg LDPs both demonstrate increases in pulsatility with fill volume, but 180 mmHg demonstrates a larger trend.

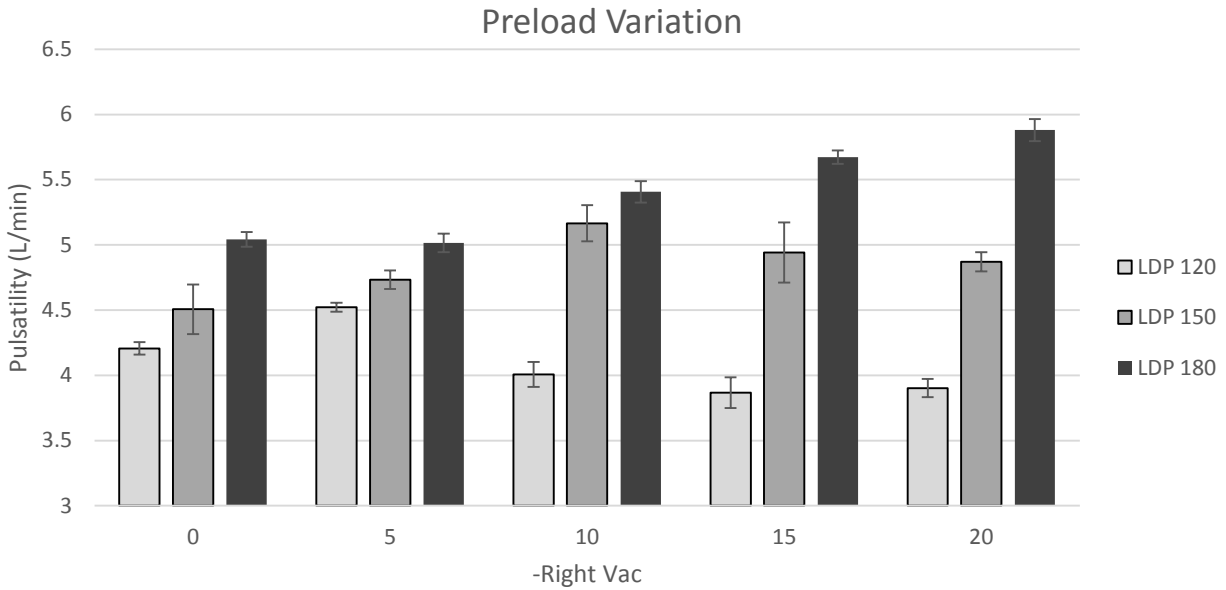


Figure 9: Increasing RV output by varying RV vac to induce varying degrees of preload pressures

Varying preload on the LV in a HF emulated setting resulted in a slight increase in pulsatility at -5 mmHg right vacuum but decreased at higher preloads. In the medium operating conditions pulsatility increased to -10 mmHg right vacuum but dropped at higher preloads. In the normal operating conditions pulsatility increased up to maximum preload pressures. When progressing from low to medium cardiac conditions there was a 7.1% increase in pulsatility at a low preload, a 28.89% increase at a medium preload, and a 24.83% increase at a high preload. When progressing from low to high cardiac conditions there was a 19.9% increase in pulsatility at a low preload, a 34.9% increase at a medium preload, and a 50.69% increase at a high preload.

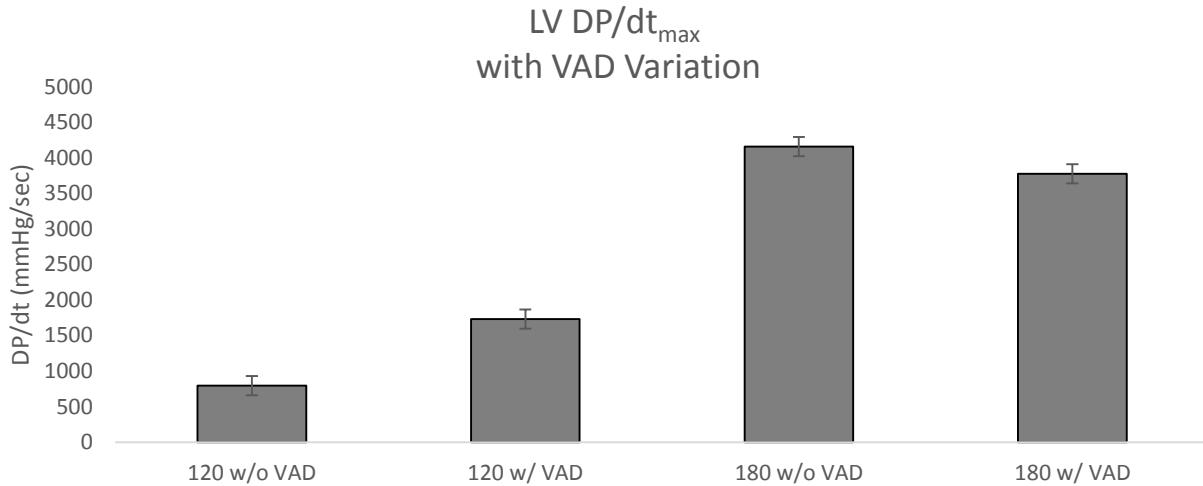


Figure 10: DP/dt_{max} measured in the left ventricle at HF and normal operating conditions with VAD variation

There was no significant difference in left ventricular DP/dt_{max} at normal operating conditions with and without the HVAD (p=.255). At HF conditions there was a significant increase (p<.001) in DP/dt_{max} from approximately 800 mmHg/sec without the HVAD to 1700 mmHg/sec with the HVAD.

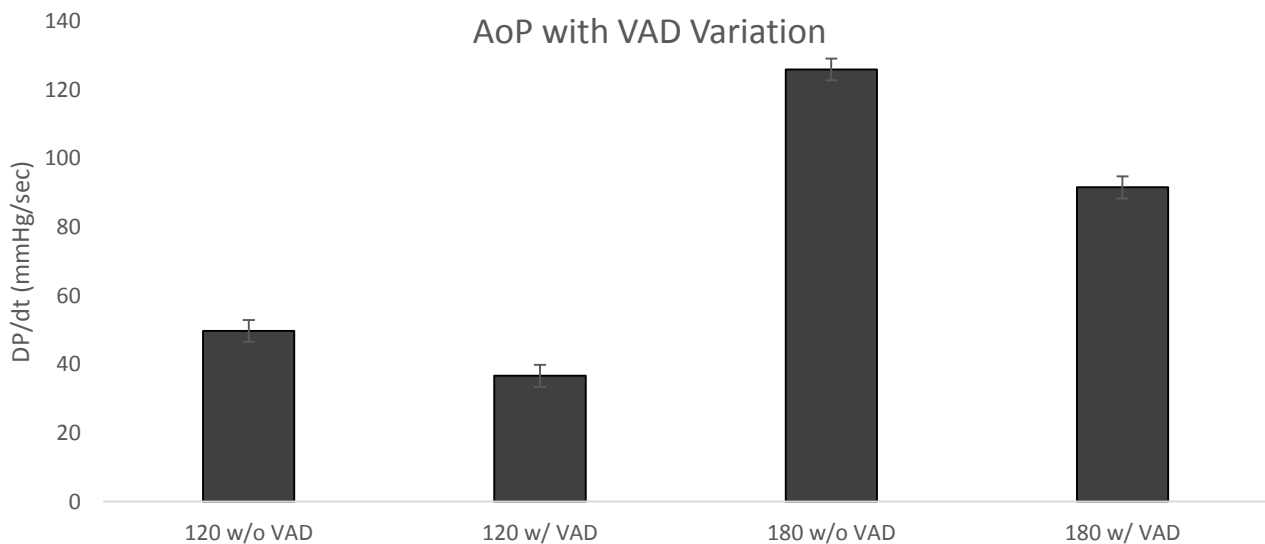


Figure 11: DP/dt measured in the aorta at HF and normal operating conditions with VAD variation

The AoP DP/dt decreased with addition of the HVAD in both HF and normal operating conditions. There was a large increase in AoP DP/dt when comparing HF and normal operating conditions.

Discussion

Prior to using the Transonic Flow Meters to calculate pulsatility it was necessary to demonstrate that the true flow recorded from the flow meters were equivalent to the pulsatility reported through the HVAD algorithm. This was critical due to the low sampling rate of the HVAD, which only records data points every 15 minutes. The results in Table 1 prove the accuracy of the HVAD algorithm and allowed for data collection through the flow meters. This allowed for a more efficient experimental design. Contractility and therefore pulsatility are dependent on afterload. An increase in afterload should result in an increased contractility (Figure 12). Afterload determines the amount of work necessary from the heart to successfully eject blood. The results of the afterload variation experiment support these concepts (Figure 5). As afterload was increased, pulsatility also increased. In the low LDP and high afterload cases, pulsatility decreased due to the fact the the drive pressures and therefore the strength of the contractions were not sufficient to compensate for the amount of work necessary to eject blood at high afterloads. These cases indicate compromised cardiac function and the 120

mmHg LDP illustrates the pulsatility response in an end stage HF patient. In the normal LDP case, pulsatility increased up to the maximum afterload, suggesting healthy cardiac function.

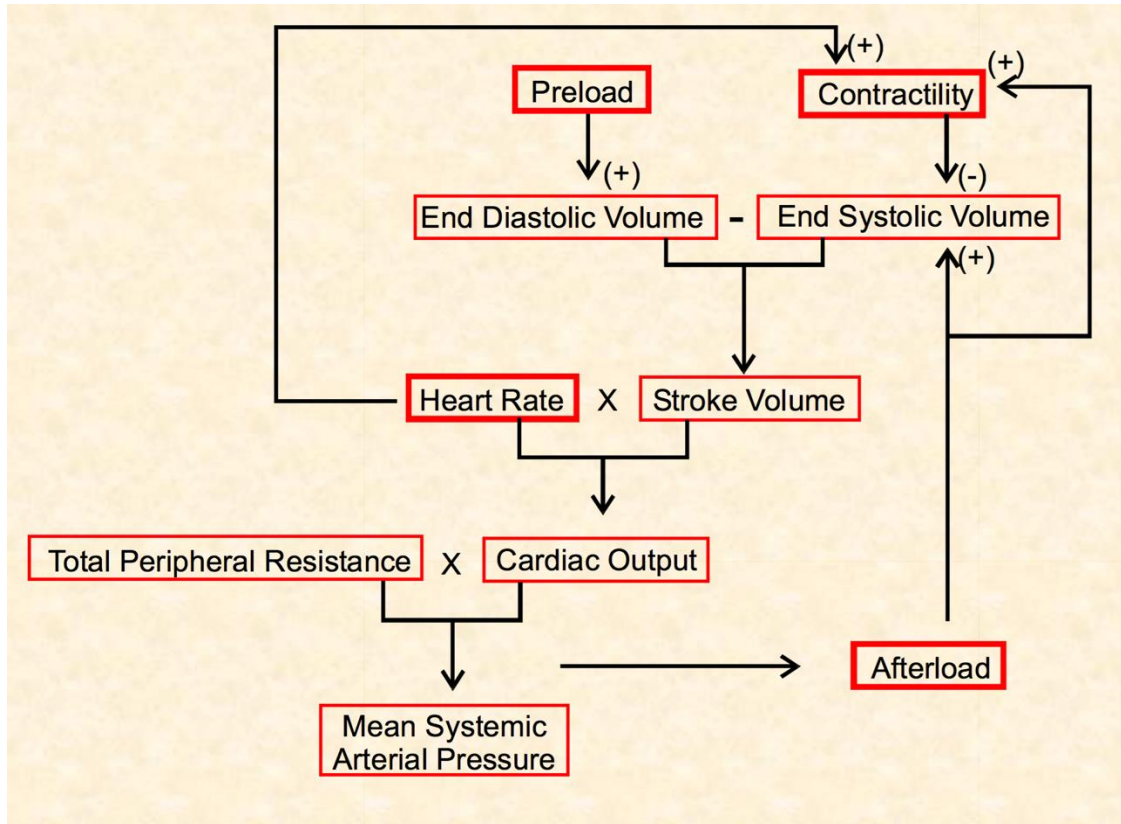


Figure 12: Dependence and effects of the cardiac variables on one another (Konhilas BME 511 slides, Contractile function of the Heart)

An increase in contractility reduces end systolic volume, which ultimately increases stroke volume (Figure 12). Pulsatility is the derivative of stroke volume which makes it a valuable variable for determining heart function. Figure 6 demonstrated the direct relationship between the recorded stroke volumes and pulsatility. It also supported the concept that a low drive pressure resulted in a reduced stroke volume and pulsatility. The range of stroke volume and pulsatility was narrow in the low afterload cases and increased as afterload increased. This

was apparent due to the ability for a low LDP to compensate for the low afterloads while they were unable to compensate at high afterloads. This suggests that patients with HF and high afterloads will demonstrate lower pulsatility responses that will increase as cardiac function is restored. Figure 7 demonstrates that at a low afterload, there is not a substantial difference in cardiac output between low and high LDPs. As afterload increases the low LDP conditions results in a significant reduction in total cardiac output while normal operating conditions are able to compensate and maintain or only slightly drop in cardiac output.

Varying preloads was accomplished with two methods. The first being varying LV fill volume through the variation of the LV vacuum and the second being the more established method, where preload is varied by increasing RV output though the variation of the RV vacuum. An increased preload will result in a higher end diastolic volume and stroke volume when contractility is not compromised (Figure 10). Figure 8 and 9 demonstrates that in HF conditions a higher preload actually resulted in a reduced pulsatility. This suggests a higher end systolic volume resulted from a reduced contractility. At normal operating conditions, pulsatility increased to the maximum preload, suggesting that a healthy heart has sufficient contractility to eject blood at high fill volumes.

Measuring Dp/dt has been shown to be an indicator of cardiac contractility in patients [32]. At normal operating conditions there was not a significant difference in DP/dt_{max} between the HVAD and no HVAD tests (Figure 10). This indicated that in a healthy heart the HVAD does not improve cardiac function but at HF conditions there was a significant increase, suggesting the HVAD improved contractility when additional support was needed. Figure 10 also demonstrated a 117% increase in DP/dt_{max} when comparing HF and normal operating

conditions with the HVAD. This was associated with a 22.3% increase in pulsatility. This may suggest a correlation between DP/dt_{max} and pulsatility.

The results from both the afterload and preload experiments demonstrate that pulsatility is a dynamic and valuable variable that can be used for diagnostic purposes. It may provide insight on increased cardiac function and longitudinal analysis through the HVAD is both accurate and beneficial for long term assessment of patient conditions. These results justify our use of pulsatility to investigate the effects of stem cell therapy in patients with HVADs.

Chapter 5: Stem Cell Therapy for Cardiac Applications

Background of Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) were first discovered in 1970 when Friedensten et al. found stromal cells residing in the bone marrow. [33] These cells readily expanded in culture and were later renamed MSCs. MSCs are identified by their surface markers CD73, CD90, and CD 105 and their ability for pluripotent differentiation. MSCs are distributed and can be extracted from various sources of the body. These include bone marrow, adipose tissue, lungs, liver, amniotic fluid, and the heart. [34, 35] Our specific source of interest are MSCs derived from the amniotic fluid. Amniotic MSCs have been demonstrated to have pluripotent characteristics similar to that of embryonic stem cells by exhibiting the embryonic stem cell markers Oct-4 and SSEA-4. [36] While amniotic MSCs are our main interest, it is still important to compare the results of other sources. Isolation of MSCs is relatively simple through the use

of density gradient centrifugation which allows for separation of the nucleated MSCs. Once isolated the MSCs can be grown on plastic culture.

MSCs are viable candidates for allogeneic transplantation due to their immunomodulatory and immunosuppressive characteristics. When cultured with T cells, MSCs reduce T cell activation and proliferation through PD-L1 and PD-L2 ligands expression which results in decreased proinflammatory cytokines interferon- γ , tumor necrosis factor- α , and interleukin-2. [38] Additionally, MSCs downregulate natural killer cells activation and inhibit B-cell maturation, which results in reduced expression of antigens and T cell activation. [39, 40] These in vitro studies justified the use of allogeneic MSCs for applications in humans. The first major trial to test the immunologic reaction in patients with implantation of allogeneic MSCs showed no reaction 12 months post implant. [41]

MSCs in vivo mechanisms have shown reduction in fibrosis, stimulation of angiogenesis, and improvement in contractile function by differentiation and stimulation of endogenous cardiac stem cells to proliferate. [41-44] Endogenous stem cell differentiation occurs through the transfer of genetic information from the MSCs in the form of miRNA and mRNA through exosomes [45]. Exosomes play a role in apoptosis, angiogenesis, inflammation and coagulation. [46] Additionally, exosomes facilitate cell to cell communication between separated locations in the body to allow for protein, lipid, and nucleic acid exchange. [47] These transfers trigger downstream signaling events which enable endogenous repair.

Respiratory rescue may also be performed by MSCs through mitochondrial transfer. It has been shown that cells depleted of mitochondrial DNA co-cultured with MSCs demonstrated

reparatory rescue through the transfer of mitochondria from the MSCs to the injured cells. [48] The complete cellular mechanism is not understood but it is believed that mitochondrial transfer occurs through actin based nanotubes and gap junctions mediated by Rho-GTPase. [49] The stem cell niche within the heart also plays a crucial role in regulating the mechanisms of cardiac repair. MSCs induce endogenous repair by activating chemokine and cytokine cascades within the niche.[50] CXCR4 has been shown to induce MSC homing to damaged myocardium to stimulate endogenous repair. [51] CXCR4 expression has also been found on the surface of MSCs[52], further suggesting they play a pivotal role in regulating cardiac repair.

Preclinical Studies of MSCs

MSCs have demonstrated the ability to differentiate into cardiomyocytes in vivo[53] while also demonstrating immunoprivileged characteristics through long term engraftment using allogeneic cell without triggering an immune response [54]. This is beneficial due to allogeneic sources being easier to procure for off the shelf applications. Many animal models have demonstrated that mesenchymal precursor cells(MPCs) and MSCs have antifibrotic effects and reduce scar size while improving EF in an acute MI setting[54-57]. Two of these trials explored the effects of altering the dose of administered cells to determine whether there was a threshold for therapy response. The first administered different doses of MSCs in an acute MI setting and found no dose dependent changes in scar size or EF[56]. The second administered various doses of MPCs and found that the lower doses had a greater reduction in scar size and EDV while all doses increased EF[57]. This data suggested that there may be an upper threshold that determines the efficacy of MPCs and an optimal dose is likely.

While administering MSCs in an acute MI setting had proven to be beneficial, exploring MSCs effects in a chronic setting was also important. Once an infarct region has stabilized, a network of inefficient vasculature is created which progresses the diseased area. A group explored injecting MSCs intramyocardially in canines 30 days post MI. They reported a significant increase in EF with a trend toward increased vascular density and reduced fibrosis in their treated group[58]. Another group performed a similar study in swine 12 weeks post MI. After transendocardial injections they found their treatment group had an increase in vessel formation, a reduction in infarct size, improved contractility, and an increase in EF[59]. These trials demonstrated that MSCs can reverse the negative remodeling that occurs in the chronic MI setting and restore perfusion while improving heart function.

With the success of MSCs, many groups have explored genetically modifying the cells or adding components to improve their efficacy, retention, and survival. Combining MSCs with simvastatin in swine increased EF, systolic wall thickening, stem cell retention, and survival [60]. Another group treated MSCs with IGF-1 which increased expression of CXCR4 in mice. Overexpression of CXCR4 led to an increase in survival proteins which increased global LV function[61]. Combining MSCs with VEGF reduced cellular stress, increased expression of phosphorylated Akt and Bcl-Cl, and improved cell survival [62]. One of the first groups to genetically modify MSCs explored modifying the cells with Bcl-2 and found a reduction in MSC apoptosis, increased VEGF secretion, improved cell survival, increased capillary density, and reduced infarct size[63]. These trials demonstrated that MSC viability, retention, and survival could be increased by pretreating MSCs with additional components.

Clinical Trials of MSCs

With the success of MSCs in animal models, many clinical trials have followed. A phase I double blind trial determining the safety of intravenously administering MSCs reported similar adverse events among the MSC and placebo groups. They also noted a significant reduction in tachyarrhythmia events and an increase in EF [64]. The success of this trial led to the randomized phase II trial, POSEIDON. They set out to compare the safety and efficacy of autologous versus allogeneic MSCs in different doses for ICM patients. They reported no ventricular arrhythmias, no allo-immune reactions, a reduced EDV, a reduced MI size, and an increased EF among the allogeneic group. This study found that both MSC types improved functional capacity, quality of life, and ventricular remodeling [41]. One of the first trials to administer adipose derived MSCs found a significant reduction in the % of LV infarcted mass and an improvement in EF 6 months post injection [65]. The PRECISE trial set out to compare similar adipose derived MSCs in a chronic HF setting and did not find a significant reduction in LV infarcted mass but did report a significant reduction in total LV mass [66]. The C-CURE trial evaluated the effects of transendocardial injections of cardiopoietic stem cells derived from MSCs. They enhanced the cardiopoietic commitment through the ex-vivo treatment of the MSCs with a cocktail of cytokines. They reported a significant increase in EF and reduction in ESV 6 month after therapy administration [67]. These clinical trials demonstrated the MSCs are not only safe in humans but also improved perfusion, infarct mass, contractile function, EF %, and quality of life in ICM patients.

Human Amniotic Allograft Liquid Matrix Can Provide a Bridge to Regeneration in Patients with Cardiac Mechanical Circulatory Support

Abstract:

Background- Mechanical circulatory support (MCS) systems have emerged as vital life-saving therapy, either as bridge to transplant or as destination devices, for patients suffering from end-stage heart failure (HF). Rarely, cardiac offloading from MCS leads to remodeling and functional “recovery”. Recently, use of mesenchymal cells to modulate HF in this setting has shown promise, possibly due to intrinsic regenerative properties. We hypothesized that administration of amniotic allograft liquid matrix (LM) containing mesenchymal stem cells could increase the efficacy of MCS as a bridge to regeneration.

Methods and Results- KardiaFlow™ (KF) amnion-derived mesenchymal stem cells (aMSCs) and liquid (micronized) extracellular matrix (LM) were characterized using flow cytometry, mass spectroscopy and ELISA. Their effects were investigated in cardiomyopathy patients with MCS (n=14). KF was found to demonstrate CD+ percentages consistent with aMSCs and contain vital growth factors, cytokines, and proteins necessary for cell retention, proliferation, and survivability. Leveraging HeartWare® left ventricular assist device (HVAD®) diagnostics, pulsatility, an available response variable, was utilized as a marker of cardiac contractility and followed for response to aMSC treatment. The group receiving KF (n=7) demonstrated a significant increase in normalized pulsatility when compared to the control group (n=7) at serial time points: 15-20 (p=0.0487), 25-30 (p=0.0131), 35-40 (p=0.0333), and 75-80 (p=0.0476) days post-implant.

Conclusions- These findings suggest that administration of KF can increase cardiac contractility in MCS patients with advanced heart disease and potentially improve the efficacy of MCS devices as a bridge to regeneration. Pulsatility downloaded from implanted HeartWare left ventricular assist devices may be useful for assessment of recovery in experimental protocols.

Introduction:

Despite therapeutic advances in recent years, heart failure (HF) remains a growing epidemic and a leading healthcare cost. Recent estimates indicate that end-stage HF (AHA Stage D, NYHA Class IV) is associated with two-year mortality rates of 70-80%, affects over 100,000 patients in the US each year, and has costs exceeding \$32 billion [2]. While medical management is continued in these patients, cardiac transplantation is regarded as the optimal therapy. Presently less than 2,500 cardiac transplants are performed annually in the United States due to the severe limitation of donor organs. This has led to increased reliance on mechanical circulatory support (MCS), to bridge patients with end-stage heart failure until a donor heart is available (bridge to transplant). While MCS provides patients with lifesaving alternatives, the therapy fails to address the root cause of the HF. In rare circumstances, off-loading provided by MCS allows the patient's native heart to heal sufficiently to allow explant of the device (bridge to regeneration). There is significant interest in the potential of stem cell therapy to increase the likelihood of "bridge to regeneration", eliminating the need for cardiac transplantation.

Mesenchymal stem cells (MSCs), specifically amniotic (aMSCs), have been successful in *in vivo* applications due to their pluripotency, anti-inflammatory and immunosuppressive

capabilities, making them ideal candidates for allogeneic transplantation. Wolbank, et al. demonstrated amniotic fluid derived stem cells have the ability to reduce peripheral blood mononuclear cell (PBMC) immune response, reporting a mean reduction in PBMC proliferation of 66% and a maximum reduction of 97% in culture compared to baseline [68]. Perin, et al. verified that c-kit⁺ amniotic fluid derived aMSCs express surface markers similar to those found in embryonic stem cells, including OCT-4 and SSEA-4, suggesting they retain pluripotent characteristics. They also reported successful myogenic differentiation both in vitro and in vivo with expression of muscle gene markers MyoD, Myf-5, sarcomeric tropomyosin, and desmin [36]. These results substantiate MSCs ability to stimulate endogenous cardiac stem cells to proliferate and differentiate.

The regenerative properties of MSCs were demonstrated in both animal and human models. Molina, *et al.* performed LV protein analysis of extracellular matrix metalloproteinases in rats and found the MSC treated groups down-regulated fibrotic tissue markers when compared to control [42]. The PROMETHEUS and TAC-HFT clinical trials demonstrated that MSC-treated cardiac tissue improved in contractile function, perfusion, and reduced infarct size [69, 70]. One trial investigated the effects of bone marrow derived MSCs in dilated cardiomyopathy patients and found a 37% reduction in cardiac events when compared to placebo [71].

A major criticism of stem cell therapies in humans is low retention in the myocardium with groups reporting rates as low as 0.44% [53]. This provides incentive to improve cell retention and regeneration in the heart. A potential solution is incorporation of extracellular matrix proteins in conjunction with stem cell administration. Extracellular liquid matrix (LM) contains numerous adhesion and signaling proteins important in cell survival and localization. Groups have

explored encapsulation of stem cells with extracellular proteins including fibrinogen and fibronectin, which were found to increase pro-angiogenic cytokines and long-term retention of cells under hypoxic conditions in humans [72]. A separate study explored the feasibility of combining left ventricular assist device (LVAD) support and particulate extracellular matrix (P-ECM) through intramyocardial injections in a bovine model. The authors concluded that P-ECM+LVAD, LVAD, and P-ECM therapies all provided increases in ejection fraction (EF) while the P-ECM+LVAD treatment provided the most significant increase in EF. P-ECM+LVAD also reduced cardiac fibrosis 60 days post treatment [73]. A group investigating LVAD wean success 90 days post implant in humans after delivery of mesenchymal precursor cells found that more patients in the treatment group tolerated LVAD weaning than in the control group [74]. These trials identify MSCs as viable candidates for cardiac regeneration and suggest that administration of KardiaFlow™ (KF), which contains an allogeneic mixture of micronized LM and aMSCs, could enhance recovery when coupled with an HVAD®.

The goal of this study was to utilize on-board LVAD diagnostics, specifically Heartware HVAD® pulsatility, to identify functional differences between patients administered KF with aMSCs+LM at the time of implant and control (MCS only) patients with either ischemic cardiomyopathy (ICM) or dilated cardiomyopathy (DCM). The response variable of interest, pulsatility, is an indirect measurement of contractility. It is calculated by subtracting the end diastolic flow from the peak systolic flow during a single cardiac cycle. As contractility increases, end systolic volume decreases, subsequently increasing stroke volume. Therefore, pulsatility is an important measurement as it relates to cardiac contractility and could potentially be used as an endpoint in determining when a patient has recovered sufficiently to warrant HVAD® explant

(Figure 13). This study presents a retrospective analysis of 7 patients who underwent HVAD implantation and received concurrent myocardial aMSCs+LM and 7 patients who underwent HVAD implantation alone.

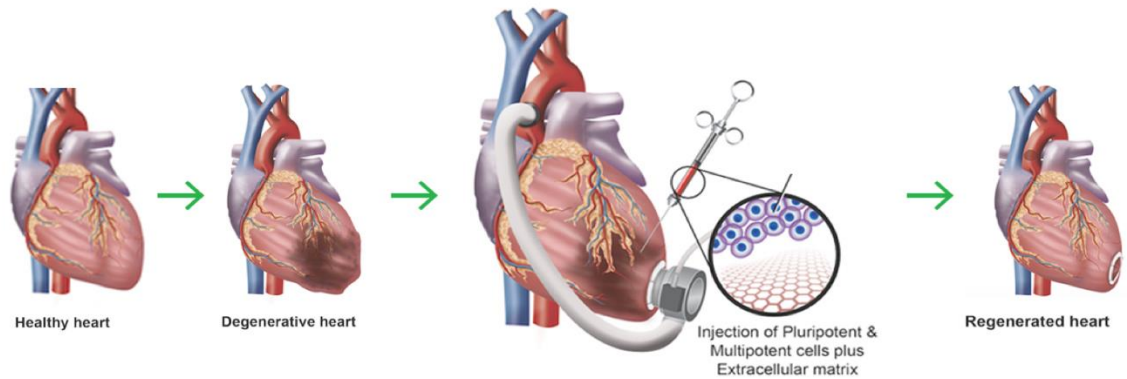


Figure 13: Schematic of the potential sequence of an advanced heart failure patient receiving mesenchymal stem cells and liquid matrix therapy at HVAD[®] implant and the subsequent regeneration and removal of HVAD[®]

Results:

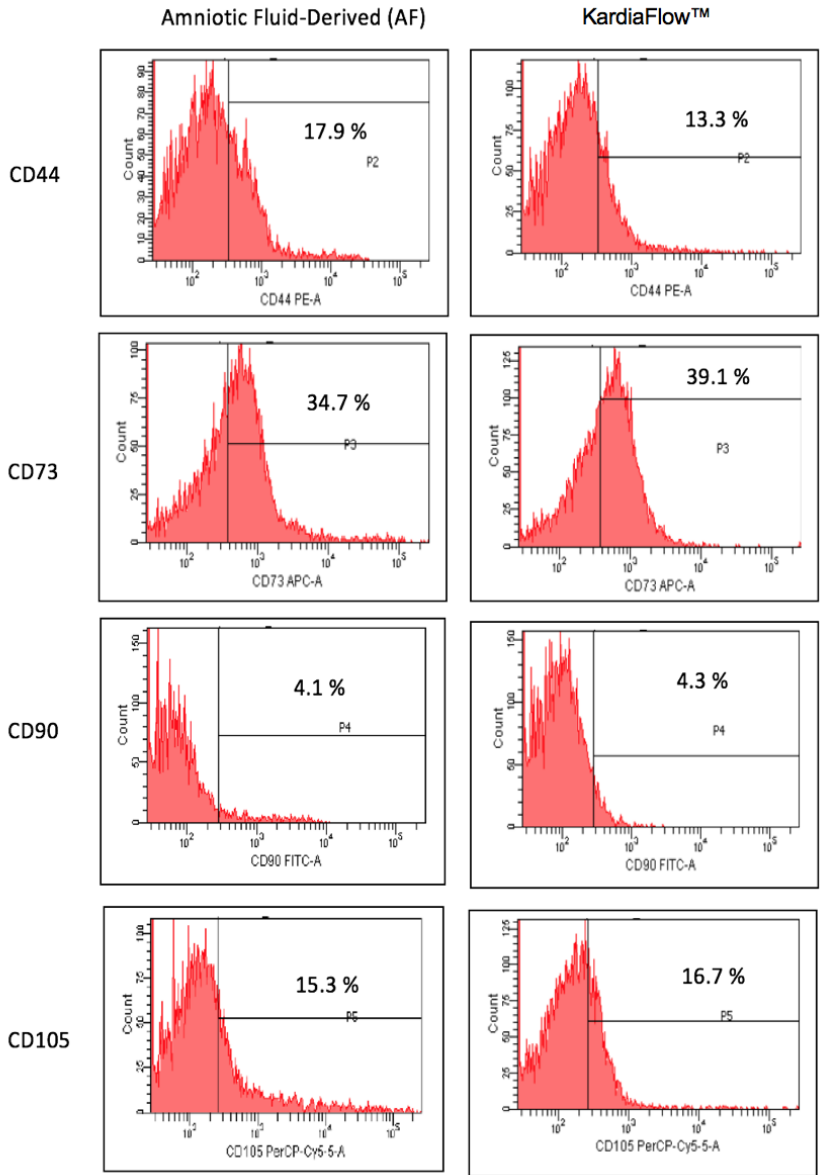


Figure 14: Histogram of flow cytometric analysis against CD44, CD73, CD90, and CD105 using amniotic fluid-derived cells and KardiaFlow™ cells.

KardiaFlow™ (KF) is a preparation of micronized amniotic membrane and amniotic stem cells. To evaluate the preparation and ensure MSC lineage we compared the cells and proteins in the material to amniotic mesenchymal stem cells (aMSCs) isolated and cultured from an amniotic fluid sample and amniotic membrane material donated at cesarian section. CD surface

markers were analyzed using flow cytometry against CD44+ (13.3%), CD73+(39.1%), CD90+(4.3%) and CD105+(16.7%) for both amniotic derived MSCs and the mixture of aMSCs+LM in KF. CD positive percentages found in the two preparations were similar. (Figure 14)

Proteins	Properties
Collagen VI	Extracellular Matrix Structural
Collagen VII	Extracellular Matrix Structural
Collagen XII	Extracellular Matrix Structural
Fibronectin	Extracellular Matrix Structural
Vimentin	Extracellular Matrix Structural
Laminin subunit alpha-3	Extracellular Matrix Structural
Laminin subunit alpha-5	Extracellular Matrix Structural
Transforming Growth Factor β -induced protein ig-h3	Anti-Inflammatory, cell differentiation
S100A3	Anti-Microbial
S100A9	Anti-Microbial
<u>Pentraxin-related protein PTX3</u>	Anti-Microbial

Supplement Table 2: The type and concentration of cytokines and growth factors in KardiaFlow™

CD surface markers were analyzed using flow cytometry against CD 44, 73, 90 and 105 for both amniotic derived MSCs and the mixture of MSCs+LM in KF. The corresponding levels of CD markers are similar between amniotic fluid derived cells and KF cells.

- Angiostatin
- Galectin-7
- TIMP-2
- IL-1 F10
- IGFBP-2
- FLRG
- IGFBP-6
- Pentraxin 3
- Resistin
- CA9
- OSM
- TRAIL
- Thrombospondin-5
- WISP-1
- S100A8
- RGM-B
- Marapsin
- PGRP-S
- Thrombospondin-2
- aFGF
- FABP2
- OPG
- Trappin-2
- Dkk-4
- Lipocalin-2
- Cystatin C
- FGF-9
- IL-1ra
- Leptin
- MCSF
- Cripto-1
- GASP-1
- TFPI
- GRO
- GDNF
- PIGF
- Eotaxin-2
- IGFBP-3
- Thyroglobulin
- OPN
- Furin
- DKK-1
- GROa
- PF4
- BMP-5
- Granulysin
- Galectin-1
- DAN
- IL-21
- Clusterin
- MIF
- GDF-15
- CEA
- MIP-1a
- CXCL16
- CNTF
- TPO
- Procalcitonin
- sFRP-3
- FGF-19
- PDGF-AA
- MCP-1
- Kallikrein 5
- PARC
- FGF-21
- VEGF
- IP-10
- NT-3
- IL-18
- IL-8
- IL-1 F8
- VEGF-D
- I-309
- Eotaxin
- ACE-2
- NSE
- PAI-1
- IL-1 F5
- IL-1 F7
- Gas 1
- CRP
- HGF
- 6Ckine
- EG-VEGF
- Cystatin B
- CHI3L1
- IL-17C
- SP-D
- uPA
- ANG-4
- Shh-N
- TSH
- Renin
- NT-4
- GASP-2
- ANGPTL3
- FGF-6
- NAP-2
- BDNF
- ST2
- IL-34
- BAFF
- EGF
- GH
- IGF-I
- BTC
- TGFb3
- MIP-1d
- Ck beta 8-1
- IL-12p40
- Adiponectin
- TSP-1
- Angiotensinogen
- Serpin A4
- Midkine
- TGFb1
- IL-1 F6
- Dkk-3
- IL-1 F9
- Osteoactivin
- DcR3
- Fractalkine
- LAP(TGFb1)
- IGF-2
- DLL1
- PDGF-BB
- Angiogenin
- Cystatin A
- BMP-7
- MBL
- Cystatin E M
- NOV
- Eotaxin-3
- PDGF-AB
- IL-33
- SDF-1b
- IL-6
- BMP-9
- LIGHT
- TNFb
- IL-1a
- NRG1-b1
- FGF-7
- IL-32 alpha
- IL-7
- HB-EGF
- Pref-1
- Follistatin-like 1
- gp130
- RBP4
- hCgb
- Legumain
- Prolactin
- biG-H3
- RANTES
- WIF-1
- Galectin-3
- Follistatin
- APRIL
- Insulin
- IL-24
- CF XIV
- ULBP-1
- Chemerin
- CSa
- MIG
- IL-23
- IL-17B
- VEGF-C
- IL-6sR
- MIP-1b
- ENA-78
- IL-20
- TGFb2
- Lymphotactin
- AgRP
- TNFa
- I-TAC
- FIt-3L
- IL-1b
- G-CSF
- IL-2
- Fetuin A
- ANGPTL4
- IGFBP-5
- Adipsin
- TIMP-1
- LRIG3
- IGFBP-1
- BMP-2
- HAI-2
- CXCL14
- IGFBP-4
- FSH
- TRANCE
- TWEAK
- Galectin-9
- ADAMTS13
- ANG-2
- MCP-2
- IL-27
- HCC-1
- Kallikrein 14
- bFGF
- ANG-1
- IL-16
- IL-11
- BLC
- IL-17E
- TIMP-4
- IL-3
- Galectin-2
- SCF
- GCP-2
- GM-CSF
- Activin A
- IL-15
- IL-4

Supplement Figure 1: Components found in amnion

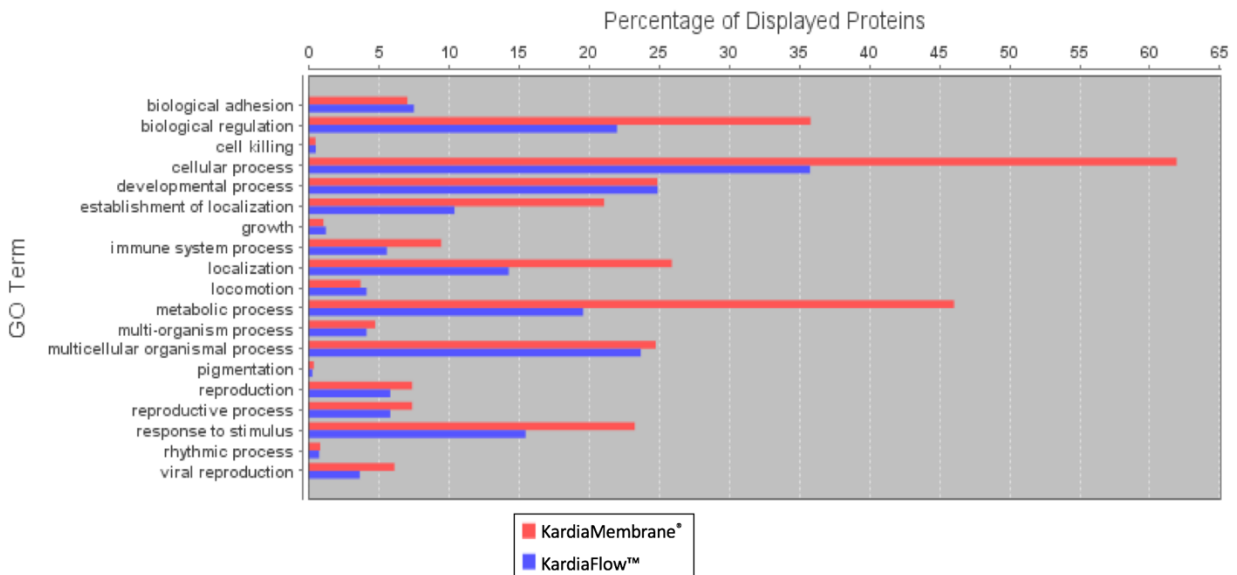
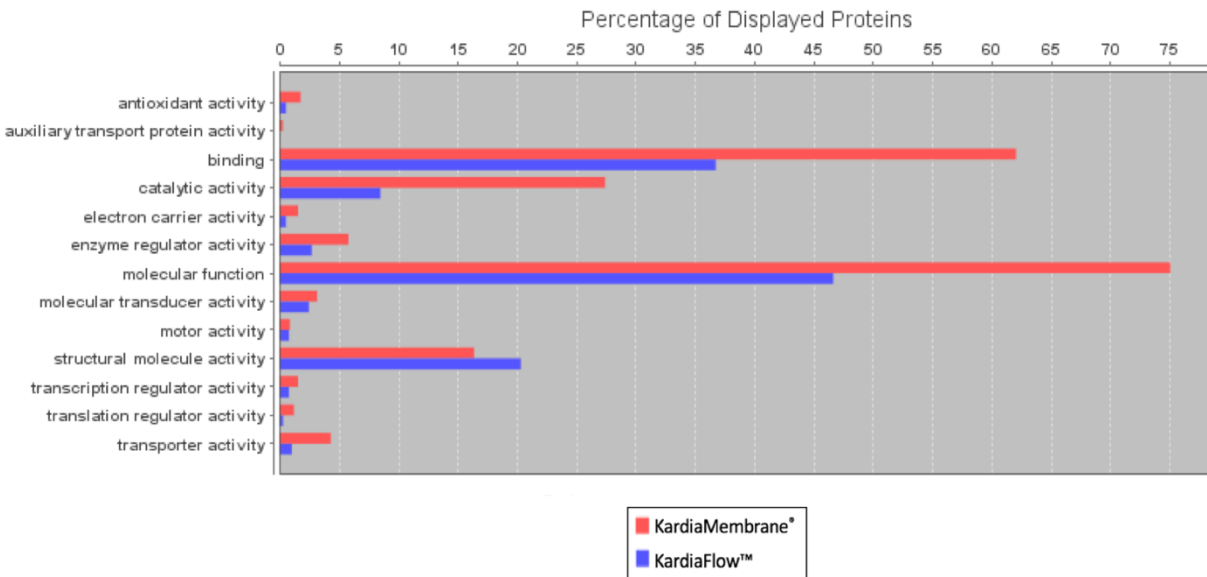


Figure 15: Percentage of displayed proteins by biological function. Red bars represent KardiaFlow™ Flow sample and blue bars represent the KardiaMembrane™ sample.



Supplement Figure 2: Molecular function of proteins found in KardiaFlow™ and KardiaMembrane®. Red bars represent KardiaFlow™ sample and blue bars represent the KardiaMembrane®.

Mass Spectrometry:

Mass spectrometry was utilized to analyze proteins contained in KardiaFlow™ to ensure protein content was preserved in the transition from the membrane source to the liquid allograft (Fig 15). It was also of interest to identify and correlate protein biological functions, to provide insight on possible operative cellular mechanisms of retention and efficacy of transplanted aMSCs, which are not fully understood. The primary biological functions of proteins found in the KF mixture pertained to biological adhesion, regulation, developmental mechanisms, cellular

localization, metabolic mechanisms, and cellular reproduction. These functions may underlie aMSCs mechanisms of action for myocardial regeneration and improved aMSC retention. These data show that the aMSCs and the LM proteins are representative of the source materials utilized in the preparation of KardiaFlow™.

	Average (pg/mL)	Standard Deviation	n
FGF2	430.9	234.3	24
EGF	260.3	100.5	24
PDGF-AA	8.9	3.4	12
PDGF-BB	10.8	8.4	12
VEGF	68.3	20.9	8
TGF-β1	1249.2	557.0	10
TIMP-1	1350.3	0	1
TIMP-2	3789.9	0	1
PTX3	114.4	0	1

Supplement Table 1: The type and concentration of cytokines and growth factors in KardiaFlow™

Cytokines and growth factors regulate many cellular processes critical for cell growth, retention, proliferation, and survivability in addition to having anti-apoptotic functions and contributing to the integrity of the extracellular matrix. To identify the concentration of critical proteins, cytokines and growth factors present within samples we used ELISA assays. Cytokines and growth factors identified in KF included: fibroblast growth factor 2 (FGF2), epidermal growth factor (EGF), platelet derived growth factor (PDGF-AA,-BB), vascular endothelial growth factor (VEGF), transforming growth factor beta 1 (TGF-β1), TIMP metalloproteinase inhibitors 1 and 2

(TIMP-1, TIMP-2), and pentraxin related protein (PTX3). The presence of these components may provide further insight on the mechanisms of aMSCs ability to facilitate regeneration.

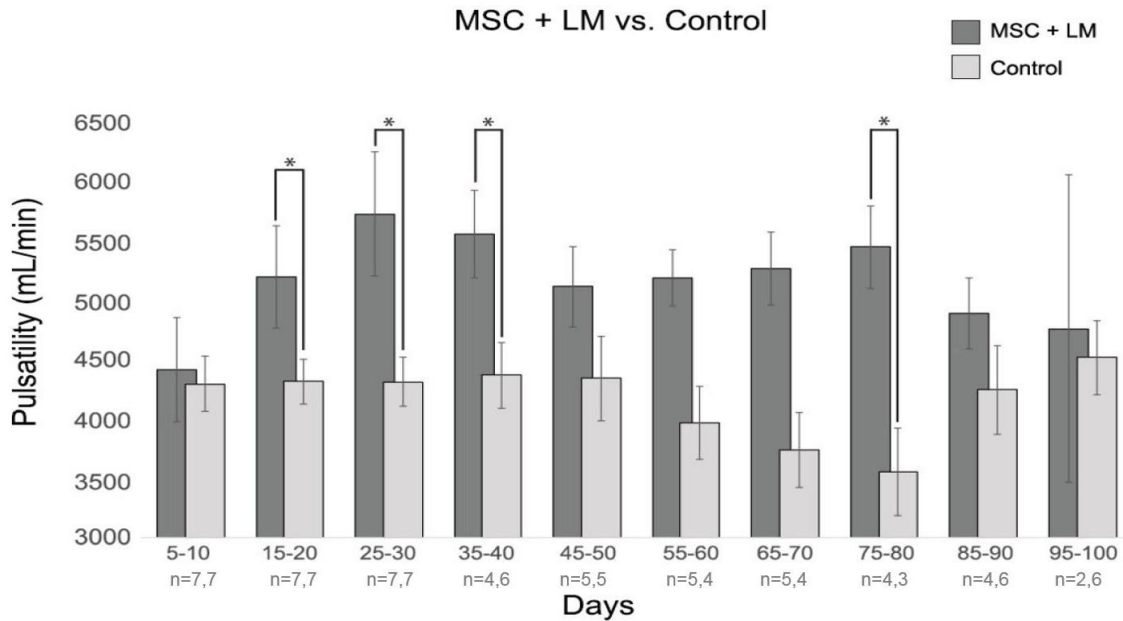


Figure 16: Pulsatility recorded in ml/min of groups prior to normalization from days 5 to 100 post implantation of HVADs® (control) and KF (MSCs+LM)

Pulsatility of KF and control groups were nearly identical at baseline with values of approximately 4,400 mL/min(Fig 4). The KF group demonstrated significant increases in pulsatility while the control group did not . When normalizing pulsatility to baseline, the KF group demonstrated significant increases in pulsatility of 20±9% compared to control at 20 days, 32±11% at 30 days, 39±18% at 40 days, and 32±27% at 80 days post implant (Figure 16/Table 2). The KF group also demonstrated significant increases in pulsatility compared to baseline at 30 days (p=0.008), 40 days (p=0.003), and 50 days (p=0.036), while the control group did not. It was

found that at 30 days post implant the DCM group (n=3) demonstrated 35.6±11.1% increase in pulsatility compared to baseline while the ICM group (n=3) showed an 18.0±17.3% increase. Conversely, at 50 days post implant the DCM group (n=3) had 18.4±12.2% increase compared to 22.2±26.7% for the ICM group (n=2). These differences were not significant.

Days	KardiaFlow™			MCS only			P-value
	N	NP	SD	N	NP	SD	
5-10*	7	1.000	0	7	1.000	0	N.S.
15-20	7	1.216	0.248	7	1.014	0.100	0.0487
25-30	7	1.339	0.294	7	1.014	0.127	0.0131
35-40	4	1.421	0.366	6	1.031	0.166	0.0333
45-50	5	1.199	0.241	5	1.016	0.105	0.2143
55-60	5	1.198	0.329	4	0.958	0.076	0.1111
65-70	5	1.142	0.496	4	0.939	0.172	0.4206
75-80	4	1.227	0.542	3	0.911	0.166	0.0476
85-90	4	1.402	0.346	6	1.004	0.198	0.0857
95-100	2	1.148	0.037	6	1.064	1.506	0.2143

Table 2: Representation of normalized pulsatility to baseline through 100 days post implant and significance between control and therapy treated groups

Days	KardiaFlow™					MCS only				
	N	EF%	StD.	N EF	N StD.	N	EF %	StD.	N EF	N StD.
Baseline	7	15.25	3.536	1.000	0	7	17.14	0.944	1	0
5-25	5	15.8	7.328	1.025	0.2123	4	17.5	2.041	1.047	0.2020
25-50	4	19.25	1.500	1.390	0.4095	2	17.5	3.535	1.071	0.1010
50-75	2	22.5	3.536	1.402	0.0970	3	21.66	9.464	1.238	0.5408
75-100	3	23.67	12.05	1.289	0.5734	2	22.5	7.071	1.416	0.5892
125-150	2	34	1.414	2.137	0.2772	1	17.5	N/A	1	N/A
160-185	2	22.5	3.536	1.735	0.3743	1	17.5	N/A	1	N/A
>200	1	33	N/A	1.941	N/A					

Table 3: Table displaying average EF and normalized EF to baseline of the mesenchymal stem cell liquid matrix group (KardiaFlow™) and MCS only group (control) within time frames from baseline to 200 days

At baseline, 7 KF and 7 MCS patients had echocardiograms, but fewer patients had echos at later time points. There was a trend toward an increased EF in the KF patients which was not seen in the control patients (Table 3). Echocardiograms were also used to evaluate aortic insufficiency (AI) and the ability for the aortic valve to open during systole. Six of the eight KF evaluated patients demonstrated AI and the inability for the aortic valve to open during systole at some point within 100 days post operation. Four of the six MCS only patients demonstrated AI and the inability for the aortic valve to open during systole.

Clinical Demographics

	Control (n=7)	KardiaFlow™(n=7)
Age (years)	58.8±6.35	61±6.69
Sex	4M, 3F	6M, 1F
Renal Failure	1	3
Diabetes	3	3
Hypertension	3	5
Previous Cardiac Surgeries	4	4
Mini-thoracotomies / Full sternotomies	5/2	3/4
Hospitalization (days)	41.07±31.03	31±18.74 days
Bypass Time (mins)	106.4±54.25	122.75±29.51
Concomitant Procedures	None	2 Tricuspid valves, 1 Aortic valve, 2 LAAL, 1 LV cryoablation

Table 4: Clinical demographics of groups. LAAL denotes left atrial appendage ligations. The left ventricular cryoablation was performed to mitigate ventricular tachycardia.

Adverse Events

No adverse events felt by investigators to be related to device implantation or stem cell administration , including acute device failure, thrombosis, immune reactions, stroke, or anaphylaxis, were reported in either group. There were no device replacements and no device-related deaths.

Discussion:

In this retrospective analysis, it was found that pulsatility, a derivative of stroke volume, increased in patients with advanced heart failure receiving KF therapy simultaneous with implantation of MCS. We found pulsatility a useful indirect measure of contractile function in patients with an HVAD®. Proteins, cytokines, and growth factors identified within the KF samples

are capable of aiding cellular retention, growth proliferation, and ECM integrity. These functions may contribute to mechanisms of aMSC regeneration. This may also provide an explanation on why patients receiving KF therapy demonstrated significant increases in pulsatility over control and trends towards improved EF within the study period. Improvement of pulsatility in the KF group may be attributed to the immunomodulatory and paracrine factors of the administered allograft containing aMSCs+LM. Studies investigating paracrine factors involved in cardiac regeneration suggest that IGF-1, VEGF, TGF- β , interleukin-1, HGF, IL-6, and various exosomes contribute to increased cell survival, proliferation, migration, angiogenesis, cardiac differentiation and cardiac function while demonstrating immunosuppressive characteristics. These components are all found in the amnion from which the cells are extracted (Supplement Figure 1). The proteins found in KardiaFlow™ (Supplement Table 2, Table 1), including collagen VI and fibronectin, have been demonstrated to increase stem cell endogenous self-renewal, muscle regeneration, and efficacy of satellite cells [75, 76]. The introduction of a decellularized amniotic ECM may also play a role in efficacy of the mesenchymal cell differentiation by improving aMSCs retention, in turn improving stimulation of resident stem cells. It has been shown that amniotic ECM components maintain higher expression of tenascin-C (TnC) and fibronectin (Fn1) when compared to adult samples[77]. TnC has been shown to mediate many processes including cell adhesion, inflammation, and cellular communication while Fn1 has been shown to augment Wnt signaling [75, 78]. The same combination of aMSCs+LM used in this study was shown to remodel external scar tissue in humans, which may be indicative of paracrine factors for in vivo remodeling [16].

Use of aMSCs allows delivery not only of stem cells themselves to the diseased myocardium, but potentially allows delivery of an accompanying extracellular milieu to promote cell survival. Characterization of aMSCs through flow cytometry demonstrated surface antigens CD73+, CD90+, and CD105+ consistent with aMSC lineage (Figure 14). Mass spectroscopy identified proteins involved in cell adhesion, regulation, cellular processing, and metabolism, potentially important for stem cell viability and efficacy (Figure 15). A substantial portion of the proteins found in KardiaFlow™ contribute to structural integrity of the extracellular matrix, which may improve mesenchymal cell retention and viability. The largest molecular function of the proteins present within KardiaFlow™ is cell-cell interactions which we postulate may have increased mesenchymal cell retention (Supplement Figure 2). Additionally, proteins as such as ig-h3 are present which synergistically exhibit anti-microbial effects (Supplement Table 2). This may also provide advantages, particularly in VAD patients.

Standard practice uses invasive or expensive imaging techniques to verify mesenchymal cell efficacy including echocardiography and invasive hemodynamics. These diagnostic methods may be inaccurate or difficult in patients on MCS and may lead to inconsistent interpretations that are difficult to compare between clinicians or institutions. Pulsatility offers the advantage of being a reproducible and consistent method through the use of device diagnostics available after HVAD® implantation.

KF patients expressed significant increases in pulsatility from baseline values when compared to control at all time points in the first 40 days and at 80 days post operatively. The most common cause of the reduction in sample size at post-operative time points was patient failure to attend clinic visits preventing device interrogation. Importantly, mean normalized

pulsatility for the KF group never fell below 1.00, suggesting contractile function only improved in these patients during the 100-day time frame post operatively. The control group did not improve pulsatility, and many patients demonstrated reductions in pulsatility over time after implantation. The observed reduction in pulsatility contradicts current literature that states unloading the left ventricle increases contractility in end stage heart failure patients [79]. During the study 4 of the control patients demonstrated NP values less than 1.0. This may indicate that some hearts are unable to restore contractile function or remodel even in unloaded conditions. The KF group demonstrated a larger positive trend in EF when compared to control, while EF in the control group returned to baseline within 200 days. These data support the hypothesis that use of KF improves heart function in comparison to MCS alone.

There is limited literature on the differing response of mesenchymal cell therapies in ICM versus DCM. At 30 and 50 days there were varying trends in pulsatility between the DCM and ICM subgroups. These data suggest that cardiomyopathy type may have a role in KF response in patients. Further longitudinal studies with MCS and KF will be needed to determine whether there is a true difference based on etiology of cardiomyopathy. Future animal studies to observe the effect of aMSCs alone vs LM on potential mechanisms for improved myocardial contractility with MCS therapy also warrant further investigation.

Limitations:

Allograft administration was nonrandomized. Patients who received KF were the most recent HVAD implant candidates, while the control group were those prior. Further, patients were implanted within a single center and by a single surgeon. Sample size for efficacy measures

decreased over time due to inconsistent patient follow-up, limiting data availability and power at later time points. This created limitations on HVAD[®] data retention, as files are only stored on the device for 31 days. Therefore, patients unable and/or unwilling to make scheduled visits for subsequent evaluation generated gaps in collected data on pulsatility. A solution for acquiring patient files remotely in the future would be optimal. The patient population consisted of both DCM and ICM subjects but statistical comparisons were unable to be performed due to limited sample size. The mechanism of benefit of the treatment is speculative, as pathologic or cellular specimens have not been obtained for analysis from included patients as only one of the bridge to transplant patients have been transplanted thus far. Hearts that have been explanted at the time of LVAD explant will have their left ventricle harvested and analysed to determine cellular mechanisms. Furthermore, a residual sample of KF aMSCs used in each patient at the time of implant have been banked in our IRB approved cardiopulmonary biobank and stem cell directory (CAPTURED, IRB#1300000194). Following the explant of the patients in the KF group, FISH analysis will be performed coupled with the phenotypic and DNA information of the aMSCs. This will help characterize the anterior, inferior and lateral left ventricular muscle of the explanted hearts to evaluate the contribution, if any, of the aMSCs involved at the injection sites. These future studies may allow us to explain, in part, the significant pulsatility findings in this study.

Conclusion:

The findings demonstrate that administration of KF appeared to increase contractility in an off-loaded environment in MCS patients with advanced heart disease. HVAD diagnostics

provide pulsatility, which appears to be a meaningful measure of cardiac contractility and could potentially be used as an endpoint in determining when a patient has recovered sufficiently to warrant HVAD[®] explant. The results from this initial study suggest a need for future studies with larger patient populations to determine if this strategy provides sufficient regeneration of compromised cardiac tissue to permit HVAD[®] explantation. In addition, study of aMSCs administration with LM in ICM versus DCM patients should be done, to better characterize how efficacious the intervention may be for different types of cardiomyopathies.

Comparison of Patient Data to In Vitro Model

Patients who received MSCs+LM therapy in addition to a HVAD device were initially considered end stage HF. The group demonstrated a range from 14.2% to 42.1% increases in pulsatility within the 100 day study (Table 2). The minimum increase is associated with a progression from end stage HF to medium operating conditions at a high afterload (14.74%) (Figure 5). The minimum increase is also associated with an increase from end stage HF to medium functioning status at a low preload (12.46%) (Figure 9). The maximum increase demonstrated in the patient population was associated with either a moderately high cardiac function at a moderately high afterload (40.0%) or a normal healthy operating function at a moderately high afterload (46.73%) (Figure 5). The maximum pulsatility increase may also be associated with a progression of end stage HF to healthy normal operating condition at a moderately high preload (41.58%) (Figure 9). This data suggests that although the patient population did not maintain >40% increases in pulsatility from baseline, they demonstrated an association to normal cardiac functional status at 2 time points (days 40, 90) (Table 2). It also suggests functional status was improved from end stage HF to medium cardiac function at minimum. Of the 7 therapy patients, one maintained >40% NP for 8

time periods, one maintained for 7 time periods, one maintained for 4 time periods, and 1 maintained for 3 time periods. If patients maintain >40% increases in pulsatility from baseline for extended periods, it may warrant HVAD explant. Based on the previously created TAH/DMCS HF model that demonstrated the startling like behavior and relationship to clinical observations [30] we can conclude that the pulsatility model presented here is both accurate and clinically translatable. Additionally, a larger study associated with other cardiac functional status markers would be beneficial to further verify this model.

Methods:

Patient Population:

Patients received implantation of a HeartWare HVAD[®] as a bridge to cardiac transplant at Banner University Medical Center - Tucson (Tucson, AZ) between April 2013 and June 2015. The HVAD[®] is a continuous flow pump with a frictionless impeller . All patients received the HVAD[®] as a bridge to cardiac transplantation. Patients consented to receiving aMSCs+LM with the option of opting out of the administration.

This retrospective analysis of human subjects was approved through the University of Arizona Institutional Review Board (#1507990305).

Procedures:

All patients included in the analysis, following formal diagnosis of either ischemic or dilated cardiomyopathy, underwent left ventricular assist device implantation of a HeartWare HVAD[®] as a bridge to cardiac transplantation at Banner University Medical Center - Tucson. For

all patients, implant and postoperative care were performed per standard of care. During the operation and following the VAD implant, seven consecutive patients received administration of heterogeneous KF aMSCs and micronized LM prior to chest closure. The KF mixture was delivered via a 22G needle into the left ventricular myocardium in three distinct locations: left ventricular anterior wall (1.2 million aMSCs+LM, 1ml); inferior wall (1.2 million aMSCs+LM, 1ml); and lateral wall (1.2 million aMSCs+LM, 1ml). In addition to intramyocardial injections, the KF mixture was injected, off-pump (2.4 million aMSCs+LM, 2ml mixed with 5cc normal saline) intra-atrially into the right atrial appendage with a 22G needle, steadily over 5 minutes by the surgeon. The seven control patients underwent HVAD[®] implant and did not receive aMSCs+KF.

Allograft Procurement:

KardiaFlow[™] and KardiaMembrane[®] are proprietary, minimally manipulated amniotic allografts (Amnio Technology, LLC, Phoenix, AZ), derived from donated human birth tissue and intended for human homologous use. The KardiaFlow[™] allograft is provided in an aseptic state. The KardiaMembrane[®] allograft is provided in a sterile state. Both allografts are intended for one-time use. The products are manufactured and regulated for clinical use under 21 CFR Part 1271 and Section 361 of the Public Health Service Act. Allografts are processed and packaged at an FDA registered and American Association of Tissue Banks (AATB) accredited facility in accordance with Current Good Manufacturing Practice (CGMP) standards.

Flow Cytometry Analysis:

For identification of the phenotype of cells present in KF, cytometric analysis was performed using a LSRII flow cytometer (BD Biosciences, San Jose, CA) equipped with lasers tuned

to 488nm, 532nm and 640nm. FITC-CD90 was detected using a 525/50nm bandpass filter, PE-CD44 was detected using a 575/26nm filter, PerCP-Cy5.5-CD105 was detected using a 710/50nm filter and APC-CD73 was detected using a 670/14nm filter. Data files were acquired and analyzed using BD FACSDiva software (BD Biosciences, San Jose, CA). Appropriate electronic compensation was achieved by acquiring cell populations stained with each dye/fluorophore individually, as well as an unstained control. Briefly, cells were collected by centrifugation at 300 x g for 5 min, washed once in phosphate buffered saline (PBS) with 0.2% fetal calf serum (FCS), and centrifuged again. A total of 5×10^5 amniotic cells were resuspended in 100 μ L of PBS with 1% BSA and incubated with the above conjugated mouse anti-human antibodies using the BD Human MSCs analysis kit (562245) and appropriate isotype controls. Samples were incubated in darkness at 4°C for 30 min, centrifuged and fixed with PFA/PBS for 5 minutes. The final stage of preparation was centrifugation and resuspension with 1 mL PBS for future analysis.

Characterization of membrane and flow proteins:

Two 4cm x 8cm KardiaMembrane® (KM) were cut into approximately 5mm pieces. The pieces were transferred to microcentrifuge tubes and 200uL of RIPA buffer or TFA:CAN (0.1-60%) was added. The membranes were extracted for 10 minutes at room temperature (RT). Samples were then centrifuged for 5 min at 16K x g at RT and the supernatants were removed. The membranes were washed with 100uL extraction solution and the washes were combined with the supernatants. Proteins were precipitated with acetone incubated at -20°C for 1 hr. After centrifuging at 16K x g for 10 min at RT, the supernatants were removed and discarded and the tubes were washed with ice-cold 80% acetone. After air drying for approximately 30 minutes, 30uL of Laemmli sample buffer was added and the samples were heated at 95°C for 5 min. 4.6ug

of proteins were then loaded onto a 12% acrylamide gel. After SDS-PAGE, the gel was stained with Coomassie Blue. The four sections in all 3 lanes (1, 2, and 3) were excised, areas were digested, and sections were then combined to yield 4 samples. Samples were analyzed by a Thermo Scientific Velos Orbitrap mass spectrometer after in-line fractionation by a C18 column using Proxeon liquid chromatography. The data were searched against a human database using SEQUEST. The SEQUEST output was then analyzed using Scaffold.

Protein concentration from KardiaFlow™ human amniotic tissue allograft was measured using the Pierce 660nm reagent. 4.2 µg of proteins was then loaded onto a 4-15% acrylamide gel. After SDS-PAGE, the gel was stained with Coomassie Blue. The eight sections in all 3 lanes (1, 2, and 3) were excised, areas were digested, and sections were then combined to yield 8 samples. Samples were analyzed by a Thermo Scientific Velos Orbitrap mass spectrometer after in-line fractionation by a C18 column using Proxeon liquid chromatography. The data were searched against a human database using SEQUEST. The SEQUEST output was then analyzed using Scaffold.

Evaluating concentration of cytokines and growth factors in KardiFlow™:

The content of specific growth factors in samples of processed and cryopreserved KF was measured with Quantibody multiplex enzyme-linked immunosorbent assay (ELISA) (RayBiotech, Inc., Norcross, GA). Cryopreserved KF samples were thawed, centrifuged at room temperature (RT) to remove tissue residues from suspension. The supernatant was retained and measured in undiluted aliquots with a Quantibody for multiplex ELISA analysis to determine the content of target growth factors, cytokines and proteins. A calibration curve was developed for each reference growth factor with an acceptance criterion of $R^2 \geq 0.95$. Limits of detection (LOD) were

established for each reference growth factor and values above the LOD were used for the analysis.

Acquisition of HeartWare® log files:

Logs are maintained in the device controller memory. The clinician can transfer alarm and trend data from the controller to a USB flash drive. Parameters in the data file are labeled as follows: Time, Speed (RPM), average flow values recorded (Flow, mLPM), minimum flow values recorded (Trough, mLPM), and Pulsatility (mLPM). Pulsatility is calculated through a device algorithm by recording the differential between the peak systolic flow velocity and minimum diastolic flow velocity over a 3 second windows every 15 minutes, which has been shown to be accurate [80] (Figure 17). Maximum flow values can be calculated by adding the Pulsatility and Trough values. Data are recorded on each HVAD® device every 15 minutes and stored for a 30 day window. Log files exported from the HeartWare® monitor were analyzed in STATA and Matlab.

$$\text{Log File Pulsatility (L/min)} = \text{HVAD Estimated Flow}_{max} - \text{HVAD Estimated Flow}_{min}$$

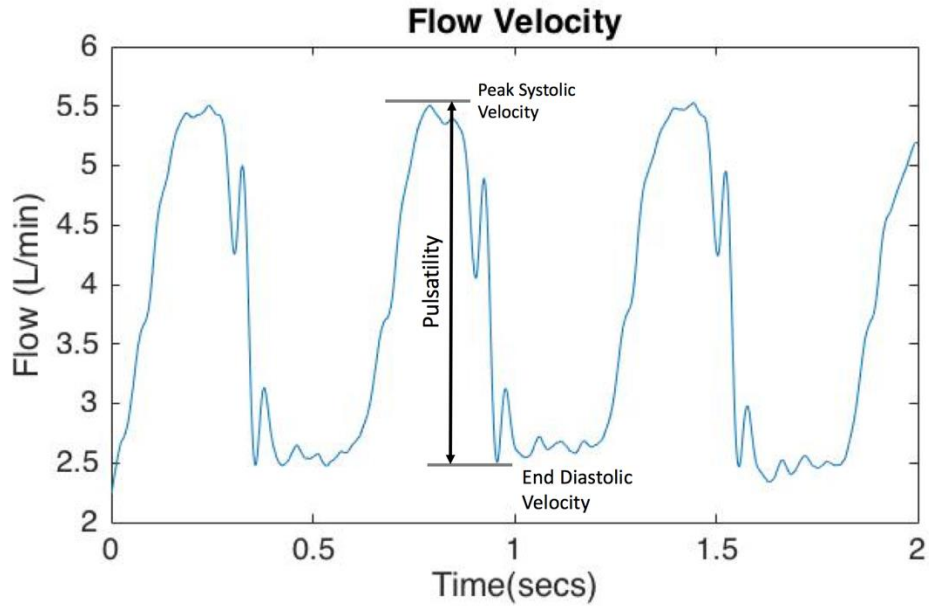


Figure 17: Flow waveform and visual representation of pulsatility

Echocardiography

Echocardiograms were obtained as part of clinical care and interpreted per standard of care protocols at the investigating institution or as needed determined by the investigating cardiologist [81]. Ejection fraction as recorded by the reading cardiologist was used in this analysis. Echocardiograms were not available for all patients at every time point. EF values were normalized to baseline EF .

Statistical Methods

Baseline was defined as 5-10 days post-implant, when the early variability in device readout had decreased to steady state. Normalized pulsatility (NP) for each patient was calculated by a ratio of pulsatility to baseline pulsatility. Time points were acquired from the

device log files and the entirety of the pulsatility data within the 5-day windows was used. Differences between investigational and control groups with respect to NP were analyzed using a Mann-Whitney nonparametric test with KF administration as the independent variable. A nonparametric test was chosen only after each distribution was tested for normality and found to be skewed. The limitation of the sample size also warranted a nonparametric test. Nonparametric tests were also used to compare the age of patients and bypass times during surgery due to the limited sample size.

Chapter 6: Future Studies with MSCs+LM

As stated earlier, due to the sample size constraints on our patient study we were unable to reach the end point of determining sufficient cardiac regeneration to warrant HVAD explant. Based on our preliminary results we believe expansion to a multicenter study with a larger patient population is warranted. To ensure sufficient cardiac regeneration occurs with MSCs+LM, pulsatility will need to be correlated with additional data, including imaging studies that investigate reduction in infarcted mass, perfusion and functional capacity experiments. It may also be beneficial to investigate the reduction in fibrotic tissue markers.

There is no literature stating the effects of administering recurrent intravenous injections of MSCs in patients. The data from our study demonstrated the largest trend within the first 40 days post therapy, suggesting that the benefits of the MSCs+LM therapy may drop off with time. With this in mind, it may be of interest to administer MSCs+LM through different time frames (ex. Every 2 weeks, 1 month, 2 months) to determine if recurrent injections

improve cell survivability, retention, and efficacy. There have been studies exploring dose dependence of MSCs but none that include MSCs+LM. So in addition to recurrent MSCs+LM injections, it would be important to determine the optimal dosage of cells through different times periods.

Continuing the theme of using device diagnostics to determine stem cell response in patients, gathering data from patients ICD devices may provide insight on the arrhythmia suppressive capabilities of MSCs+LM. Using remote monitoring, it would be of interest to evaluate patient's intra-thoracic impedance, heart rate variability, ventricular arrhythmias or defibrillations, AF burden, and patient activity. The combination of tracking these parameters has shown to have a specificity of 96.9% in predicting HF events [82]. Therefore, tracking improvements in these parameters through remote monitoring will be beneficial in determining the efficacy of therapy and improvement of patients receiving MSCs+LM.

References

1. McMurray, J.J., et al., *ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC.* Eur J Heart Fail, 2012. **14**(8): p. 803-69.
2. Mozaffarian, D., et al., *Executive Summary: Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association.* Circulation, 2016. **133**(4): p. 447-54.
3. *Executive summary: HFSA 2006 Comprehensive Heart Failure Practice Guideline.* J Card Fail, 2006. **12**(1): p. 10-38.
4. Verma, S., et al., *Plasma renin activity predicts cardiovascular mortality in the Heart Outcomes Prevention Evaluation (HOPE) study.* Eur Heart J, 2011. **32**(17): p. 2135-42.
5. Swedberg, K. and J. Kjekshus, *Effects of enalapril on mortality in severe congestive heart failure: results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS).* Am J Cardiol, 1988. **62**(2): p. 60a-66a.
6. *Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. The SOLVD Investigators.* N Engl J Med, 1991. **325**(5): p. 293-302.
7. Bangalore, S., et al., *Angiotensin receptor blockers and risk of myocardial infarction: meta-analyses and trial sequential analyses of 147 020 patients from randomised trials.* Bmj, 2011. **342**: p. d2234.
8. Pitt, B., et al., *The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators.* N Engl J Med, 1999. **341**(10): p. 709-17.
9. Girerd, N., et al., *Clinical benefits of eplerenone in patients with systolic heart failure and mild symptoms when initiated shortly after hospital discharge: analysis from the EMPHASIS-HF trial.* Eur Heart J, 2015. **36**(34): p. 2310-7.
10. Waagstein, F., et al., *Beneficial effects of metoprolol in idiopathic dilated cardiomyopathy. Metoprolol in Dilated Cardiomyopathy (MDC) Trial Study Group.* Lancet, 1993. **342**(8885): p. 1441-6.
11. *The Cardiac Insufficiency Bisoprolol Study II (CIBIS-II): a randomised trial.* Lancet, 1999. **353**(9146): p. 9-13.
12. *Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF).* Lancet, 1999. **353**(9169): p. 2001-7.
13. Khand, A.U., et al., *Carvedilol alone or in combination with digoxin for the management of atrial fibrillation in patients with heart failure?* J Am Coll Cardiol, 2003. **42**(11): p. 1944-51.
14. Piepoli, M., et al., *Overview and meta-analysis of randomised trials of amiodarone in chronic heart failure.* Int J Cardiol, 1998. **66**(1): p. 1-10.
15. Bardy, G.H., et al., *Amiodarone or an implantable cardioverter-defibrillator for congestive heart failure.* N Engl J Med, 2005. **352**(3): p. 225-37.
16. Moss, A.J., et al., *Improved survival with an implanted defibrillator in patients with coronary disease at high risk for ventricular arrhythmia. Multicenter Automatic Defibrillator Implantation Trial Investigators.* N Engl J Med, 1996. **335**(26): p. 1933-40.
17. Kadish, A., et al., *Prophylactic defibrillator implantation in patients with nonischemic dilated cardiomyopathy.* N Engl J Med, 2004. **350**(21): p. 2151-8.
18. Bristow, M.R., et al., *Cardiac-resynchronization therapy with or without an implantable defibrillator in advanced chronic heart failure.* N Engl J Med, 2004. **350**(21): p. 2140-50.
19. Cleland, J.G., et al., *The effect of cardiac resynchronization on morbidity and mortality in heart failure.* N Engl J Med, 2005. **352**(15): p. 1539-49.

20. Moss, A.J., et al., *Cardiac-resynchronization therapy for the prevention of heart-failure events*. N Engl J Med, 2009. **361**(14): p. 1329-38.
21. Tang, A.S., et al., *Cardiac-resynchronization therapy for mild-to-moderate heart failure*. N Engl J Med, 2010. **363**(25): p. 2385-95.
22. Carson, P., et al., *The STICH trial (Surgical Treatment for Ischemic Heart Failure): mode-of-death results*. JACC Heart Fail, 2013. **1**(5): p. 400-8.
23. Stehlik, J., et al., *The Registry of the International Society for Heart and Lung Transplantation: 29th official adult heart transplant report--2012*. J Heart Lung Transplant, 2012. **31**(10): p. 1052-64.
24. Rogers, J.G., et al., *Chronic mechanical circulatory support for inotrope-dependent heart failure patients who are not transplant candidates: results of the INTrEPID Trial*. J Am Coll Cardiol, 2007. **50**(8): p. 741-7.
25. Rose, E.A., et al., *Long-term use of a left ventricular assist device for end-stage heart failure*. N Engl J Med, 2001. **345**(20): p. 1435-43.
26. Miller, L.W., et al., *Use of a continuous-flow device in patients awaiting heart transplantation*. N Engl J Med, 2007. **357**(9): p. 885-96.
27. Pagani, F.D., et al., *Extended mechanical circulatory support with a continuous-flow rotary left ventricular assist device*. J Am Coll Cardiol, 2009. **54**(4): p. 312-21.
28. Slaughter, M.S., et al., *Advanced heart failure treated with continuous-flow left ventricular assist device*. N Engl J Med, 2009. **361**(23): p. 2241-51.
29. Copeland, J.G., et al., *Cardiac replacement with a total artificial heart as a bridge to transplantation*. N Engl J Med, 2004. **351**(9): p. 859-67.
30. Crosby, J.R., *Expanding the Performance Envelope of the Total Artificial Heart: Physiological Characterization, Development of a Heart Failure Model, And Evaluation Tool for Mechanical Circulatory Support Devices*. The University of Arizona.
31. Aaronson, K.D., et al., *Use of an intrapericardial, continuous-flow, centrifugal pump in patients awaiting heart transplantation*. Circulation, 2012. **125**(25): p. 3191-200.
32. Mason, D.T., *Usefulness and limitations of the rate of rise of intraventricular pressure (dp-dt) in the evaluation of myocardial contractility in man*. Am J Cardiol, 1969. **23**(4): p. 516-27.
33. Friedenstein, A.J., R.K. Chailakhjan, and K.S. Lalykina, *The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells*. Cell Tissue Kinet, 1970. **3**(4): p. 393-403.
34. Riekstina, U., et al., *Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis*. Stem Cell Rev, 2009. **5**(4): p. 378-86.
35. Tsai, M.S., et al., *Functional network analysis of the transcriptomes of mesenchymal stem cells derived from amniotic fluid, amniotic membrane, cord blood, and bone marrow*. Stem Cells, 2007. **25**(10): p. 2511-23.
36. Perin, L., et al., *Characterization of human amniotic fluid stem cells and their pluripotential capability*. Methods Cell Biol, 2008. **86**: p. 85-99.
37. Karantalis, V. and J.M. Hare, *Use of mesenchymal stem cells for therapy of cardiac disease*. Circ Res, 2015. **116**(8): p. 1413-30.
38. Di Nicola, M., et al., *Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli*. Blood, 2002. **99**(10): p. 3838-43.
39. Zhang, W., et al., *Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells*. Stem Cells Dev, 2004. **13**(3): p. 263-71.

40. Spaggiari, G.M., et al., *Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2*. Blood, 2008. **111**(3): p. 1327-33.
41. Hare, J.M., et al., *Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial*. Jama, 2012. **308**(22): p. 2369-79.
42. Molina, E.J., et al., *Reverse remodeling is associated with changes in extracellular matrix proteases and tissue inhibitors after mesenchymal stem cell (MSC) treatment of pressure overload hypertrophy*. J Tissue Eng Regen Med, 2009. **3**(2): p. 85-91.
43. Psaltis, P.J., et al., *Concise review: mesenchymal stromal cells: potential for cardiovascular repair*. Stem Cells, 2008. **26**(9): p. 2201-10.
44. Hatzistergos, K.E., et al., *Bone marrow mesenchymal stem cells stimulate cardiac stem cell proliferation and differentiation*. Circ Res, 2010. **107**(7): p. 913-22.
45. Mittelbrunn, M., et al., *Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells*. Nat Commun, 2011. **2**: p. 282.
46. Janowska-Wieczorek, A., et al., *Microvesicles derived from activated platelets induce metastasis and angiogenesis in lung cancer*. Int J Cancer, 2005. **113**(5): p. 752-60.
47. Verweij, F.J., et al., *Analysis of viral microRNA exchange via exosomes in vitro and in vivo*. Methods Mol Biol, 2013. **1024**: p. 53-68.
48. Spees, J.L., et al., *Mitochondrial transfer between cells can rescue aerobic respiration*. Proc Natl Acad Sci U S A, 2006. **103**(5): p. 1283-8.
49. Ahmad, T., et al., *Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy*. Embo j, 2014. **33**(9): p. 994-1010.
50. Mazhari, R. and J.M. Hare, *Mechanisms of action of mesenchymal stem cells in cardiac repair: potential influences on the cardiac stem cell niche*. Nat Clin Pract Cardiovasc Med, 2007. **4 Suppl 1**: p. S21-6.
51. Zhang, S.J., et al., *Effect of TGF-beta1/SDF-1/CXCR4 signal on BM-MSCs homing in rat heart of ischemia/perfusion injury*. Eur Rev Med Pharmacol Sci, 2016. **20**(5): p. 899-905.
52. Shi, M., et al., *Regulation of CXCR4 expression in human mesenchymal stem cells by cytokine treatment: role in homing efficiency in NOD/SCID mice*. Haematologica, 2007. **92**(7): p. 897-904.
53. Toma, C., et al., *Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart*. Circulation, 2002. **105**(1): p. 93-8.
54. Amado, L.C., et al., *Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction*. Proc Natl Acad Sci U S A, 2005. **102**(32): p. 11474-9.
55. Qi, C.M., et al., *Transplantation of magnetically labeled mesenchymal stem cells improves cardiac function in a swine myocardial infarction model*. Chin Med J (Engl), 2008. **121**(6): p. 544-50.
56. Hashemi, S.M., et al., *A placebo controlled, dose-ranging, safety study of allogeneic mesenchymal stem cells injected by endomyocardial delivery after an acute myocardial infarction*. Eur Heart J, 2008. **29**(2): p. 251-9.
57. Hamamoto, H., et al., *Allogeneic mesenchymal precursor cell therapy to limit remodeling after myocardial infarction: the effect of cell dosage*. Ann Thorac Surg, 2009. **87**(3): p. 794-801.
58. Silva, G.V., et al., *Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model*. Circulation, 2005. **111**(2): p. 150-6.
59. Quevedo, H.C., et al., *Allogeneic mesenchymal stem cells restore cardiac function in chronic ischemic cardiomyopathy via trilineage differentiating capacity*. Proc Natl Acad Sci U S A, 2009. **106**(33): p. 14022-7.

60. Yang, Y.J., et al., *Combined therapy with simvastatin and bone marrow-derived mesenchymal stem cells increases benefits in infarcted swine hearts*. *Arterioscler Thromb Vasc Biol*, 2009. **29**(12): p. 2076-82.
61. Guo, J., et al., *Insulin-like growth factor 1 improves the efficacy of mesenchymal stem cells transplantation in a rat model of myocardial infarction*. *J Biomed Sci*, 2008. **15**(1): p. 89-97.
62. Pons, J., et al., *VEGF improves survival of mesenchymal stem cells in infarcted hearts*. *Biochem Biophys Res Commun*, 2008. **376**(2): p. 419-22.
63. Li, W., et al., *Bcl-2 engineered MSCs inhibited apoptosis and improved heart function*. *Stem Cells*, 2007. **25**(8): p. 2118-27.
64. Hare, J.M., et al., *A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction*. *J Am Coll Cardiol*, 2009. **54**(24): p. 2277-86.
65. Houtgraaf, J.H., et al., *First experience in humans using adipose tissue-derived regenerative cells in the treatment of patients with ST-segment elevation myocardial infarction*. *J Am Coll Cardiol*, 2012. **59**(5): p. 539-40.
66. Perin, E.C., et al., *Adipose-derived regenerative cells in patients with ischemic cardiomyopathy: The PRECISE Trial*. *Am Heart J*, 2014. **168**(1): p. 88-95.e2.
67. Bartunek, J., et al., *Cardioprotective stem cell therapy in heart failure: the C-CURE (Cardioprotective stem Cell therapy in heart failURE) multicenter randomized trial with lineage-specified biologics*. *J Am Coll Cardiol*, 2013. **61**(23): p. 2329-38.
68. Wolbank, S., et al., *Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: a comparison with human mesenchymal stem cells from adipose tissue*. *Tissue Eng*, 2007. **13**(6): p. 1173-83.
69. Heldman, A.W., et al., *Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: the TAC-HFT randomized trial*. *Jama*, 2014. **311**(1): p. 62-73.
70. Karantalis, V., et al., *Autologous mesenchymal stem cells produce concordant improvements in regional function, tissue perfusion, and fibrotic burden when administered to patients undergoing coronary artery bypass grafting: The Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS) trial*. *Circ Res*, 2014. **114**(8): p. 1302-10.
71. Patel, A.N., et al., *Ixmyelocel-T for patients with ischaemic heart failure: a prospective randomised double-blind trial*. *Lancet*, 2016.
72. Mayfield, A.E., et al., *The effect of encapsulation of cardiac stem cells within matrix-enriched hydrogel capsules on cell survival, post-ischemic cell retention and cardiac function*. *Biomaterials*, 2014. **35**(1): p. 133-42.
73. Soucy, K.G., et al., *Feasibility study of particulate extracellular matrix (P-ECM) and left ventricular assist device (HVAD) therapy in chronic ischemic heart failure bovine model*. *Asaio j*, 2015. **61**(2): p. 161-9.
74. Ascheim, D.D., et al., *Mesenchymal precursor cells as adjunctive therapy in recipients of contemporary left ventricular assist devices*. *Circulation*, 2014. **129**(22): p. 2287-96.
75. Bentzinger, C.F., et al., *Fibronectin regulates Wnt7a signaling and satellite cell expansion*. *Cell Stem Cell*, 2013. **12**(1): p. 75-87.
76. Urciuolo, A., et al., *Collagen VI regulates satellite cell self-renewal and muscle regeneration*. *Nat Commun*, 2013. **4**: p. 1964.
77. Tierney, M.T., et al., *Autonomous Extracellular Matrix Remodeling Controls a Progressive Adaptation in Muscle Stem Cell Regenerative Capacity during Development*. *Cell Rep*, 2016. **14**(8): p. 1940-52.
78. Chiquet-Ehrismann, R., et al., *Tenascins in stem cell niches*. *Matrix Biol*, 2014. **37**: p. 112-23.

79. Heerdt, P.M., et al., *Chronic unloading by left ventricular assist device reverses contractile dysfunction and alters gene expression in end-stage heart failure*. *Circulation*, 2000. **102**(22): p. 2713-9.
80. Reyes, C., et al., *Accuracy of the HVAD Pump Flow Estimation Algorithm*. *Asaio j*, 2016. **62**(1): p. 15-9.
81. Pombo, J.F., B.L. Troy, and R.O. Russell, Jr., *Left ventricular volumes and ejection fraction by echocardiography*. *Circulation*, 1971. **43**(4): p. 480-90.
82. Calo, L., et al., *Comparison of partners-heart failure algorithm vs care alert in remote heart failure management*. *World J Cardiol*, 2015. **7**(12): p. 922-30.