DETERMINING THE FLOW CHARACTERISTICS OF A PERFUSION DEVICE FOR DONOR HEART TRANSPORT

BY

MICHAEL LOUIS COBERT

Presented to the Faculty of the Graduate School of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

MASTER OF SCIENCE IN BIOMEDICAL ENGINEERING

THE UNIVERSITY OF TEXAS AT ARLINGTON

MAY 2008

ACKNOWLEDGEMENTS

I would like to acknowledge the Biomedical Engineering Department at the University of Texas at Arlington and UT Southwestern Medical Center, and the faculty and staff of the Department of Cardiovascular and Thoracic Surgery at UT Southwestern Medical Center for the pursuit of improvement of treatments and quality of life. Without research grants and funding from Organ Transport Systems, Frisco, TX, this project would not be possible. Furthermore, the RealTime Group, Plano, TX, instrumental in the development and advancement of the device, is acknowledged.

I would personally like to thank Dr. Michael Jessen, MD for adding me to his stellar team of researchers and residents, LaShondra West, MS, Matthias Peltz, MD, and David Rosenbaum, MD. I would also like to acknowledge the research contributors of Organ Transport Systems, Tom Franklin, Howell Warner, Stacie Hyatt and the rest of able staff that are truly shifting the way we think about organ transplantation. I would also like to recognize the engineering team from the RealTime Group, Marshall Wenrich, Gerald Miles, Don Hurd, Mike Polcari, and Rob Davidson, in addition to the contributing companies that have developed this device. Finally, I would like to thank my family, Mom, Dad, Andrew, and Emily, you all are my inspiration and without your love and support I would not be the man I am today.

April 16, 2008

ABSTRACT

DETERMINING THE FLOW CHARACTERISTICS OF A PERFUSION DEVICE FOR DONOR HEART TRANSPORT

Michael Louis Cobert, MS

The University of Texas at Arlington, 2008

Supervising Professor: Dr. Michael Jessen

Current technology limits organ transport for transplantation to static storage, thereby reducing the reasonable distance of travel. Continuous machine perfusion has been proposed as a viable alternative to improving function and outcome of transported organs. A series of studies with such a device have been developed to elucidate the method by which optimal results can be achieved.

Adult mongrel dog hearts were established in a device that provided continuous perfusion of oxygenated fluid and enabled precise control of flow rate, oxygenation, and fluid temperature. The first series of experiments sought to examine the relationship between flow rate and tissue perfusion at six flow rates (between 5 and 30 mL/100 g/min). The second set of experiments looked at the effects of aortic attachment on tissue perfusion, employing initial high or low pressure, immersion, and aortic valve manipulations. Another set of experiments examined the effects of the inclination of the heart at different angles with and away from the non-coronary sinus on tissue flow characteristics. The final series of studies evaluated the influence of flow conditions on long term (10 hours) edema buildup and metabolic profile. Edema was evaluated by measuring weight change over the storage interval and by wet-dry analysis of stored tissue and intracellular metabolism was assessed by magnetic resonance spectroscopy.

Hearts extracted dissolved oxygen continuously throughout the perfusion period. The MVO_2 generally increased with increasing coronary flow but rose insignificantly at flows beyond 15 mL/100g/min. Higher pump flow rate correlated with increased tissue perfusate flow, as determined by microsphere analysis and perfusion at low flow rates was associated with an increased amount of non-nutrient flow. Different modes of attachment led to varying amounts of myocardial oxygen consumption, perfusate flow, and non-nutrient flow. Hearts deployed at low initial flow rate were found to have lower tissue perfusion rates and higher calculated non-nutrient flow than hearts attached at high initial flow rates. Heart inclination testing did not elucidate a definitive angle that would optimize tissue perfusion. Control angles ($\theta = 0°$) even displayed limited nutrient flow, despite the initial high flow protocol. For the 10 hour perfusion study, hearts that were found to have high tissue flow had low lactate:alanine ratios, but experienced significant weight gain (34±4%) ($p<.01$) and had higher myocardial water content. Those with low tissue flow were observed to have significant lactate accumulation with minimal myocardial weight gain over 10 hours (11±4%).

Based on prior studies it is believed that continuous machine perfusion of the heart can provide better outcomes, longer transport times, and expansion of the donor pool. However, this technology is predicated on the ability of the perfusion device to deliver nutrient (capillary) flow to the myocardium to allow ongoing aerobic metabolism and washout byproducts of metabolism. Two initializing factors are crucial to ensuring these goals and potentiating longer term viability of the organ. Starting high pressure flow, while attaching the aorta, improves valve leaflet apposition. Sequential adjustment to moderate flow rate provides adequate tissue perfusion and minimizes edema development. Heart inclination can affect tissue perfusion due to container and heart size; however, such an adverse outcome is not expected in the clinical device due to the improved design of the container.

This information may be important for the development of optimal protocols for perfusion preservation in the clinical arena.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Heart Transplant Overview

Over the past half century organ transplantation has gained much attention due to positive clinical results. However, the fact remains that the vital organs, the heart, lungs, liver, and kidneys, have a relatively short allowable period of ischemic time in which to complete the transplant operation. In general, the acceptable time for organ transport by simple immersion method is less than 6 hours. The United Network for Organ Sharing (UNOS) standard for heart transport is a 500 mile radius from the donor site.[1] Heart transplantation dates back to 1967, when Dr. Christaan Barnard of Cape Town, South Africa successfully transplanted a heart from a deceased 25 year old woman into a 55 year old man at the same institution. The recipient only lived for 18 days after the transplant, but the surgery quickly gained notoriety and inspired much developmental work. Due to limited knowledge about immunocompatability, patients were only able to survive a limited amount of time. Research at Columbia University Medical Center into immunosuppressive drugs, cyclosporine in particular, increased donor acceptance from days to years.[2] Furthermore, in 1984 the first successful pediatric heart transplant was performed at Columbia; the patient subsequently received a second transplant, in 1989, and with the aid of immunosuppression, is currently able to function normally. However, immunocompatability issues remain and require careful clinical attention.

The donor pool is markedly inadequate due to the ever increasing recipient demand. Current statistics from UNOS indicate that there are approximately 4000 registered recipients awaiting transplantation yet fewer than 2500 heart transplants are performed yearly due to the limited donor supply.[1] This disparity only increases, due to the time needed for graft assessment and tissue typing, and the limitations of transport time with existing procurement and storage techniques. For this and other reasons an improved method of long term heart preservation during transport from donor to recipient is required.

1.2 Limitations of Current Technology

The current approach for heart preparation for transport and transplant is similar to that of other organ preservation methods. The heart is arrested with a cardioplegic solution, and then immersed into a container filled with cold saline solution. The container is then placed into a cooler filled with ice, and allowed to cool down to approximately 0º C. As stated above this method of simple immersion greatly limits the time a usable organ can be transported. The primary concern with this treatment is the damage incurred by the myocardium, caused first by a lack of oxygen, which leads to the buildup of metabolic end products and ultimately, apoptotic death of myocytes. Once the tissue is set into an ischemic state, aerobic metabolism slows to a halt and anaerobic metabolism predominates. Compared to aerobic metabolism, which yields 36 ATP per glucose molecule, the net yield of ATP from of anaerobic metabolism in the form of ATP, is only two molecules.[3,4] Without a constant oxygen supply, tissue ATP production diminishes, and lactate levels increase dramatically due to catabolism of intracellular glycogen stores to provide more glucose for anaerobic metabolism. The tissue lactate buildup causes a condition known as lactic acidosis and the cells quickly fatigue, see Figures 1.1 and 1.2.

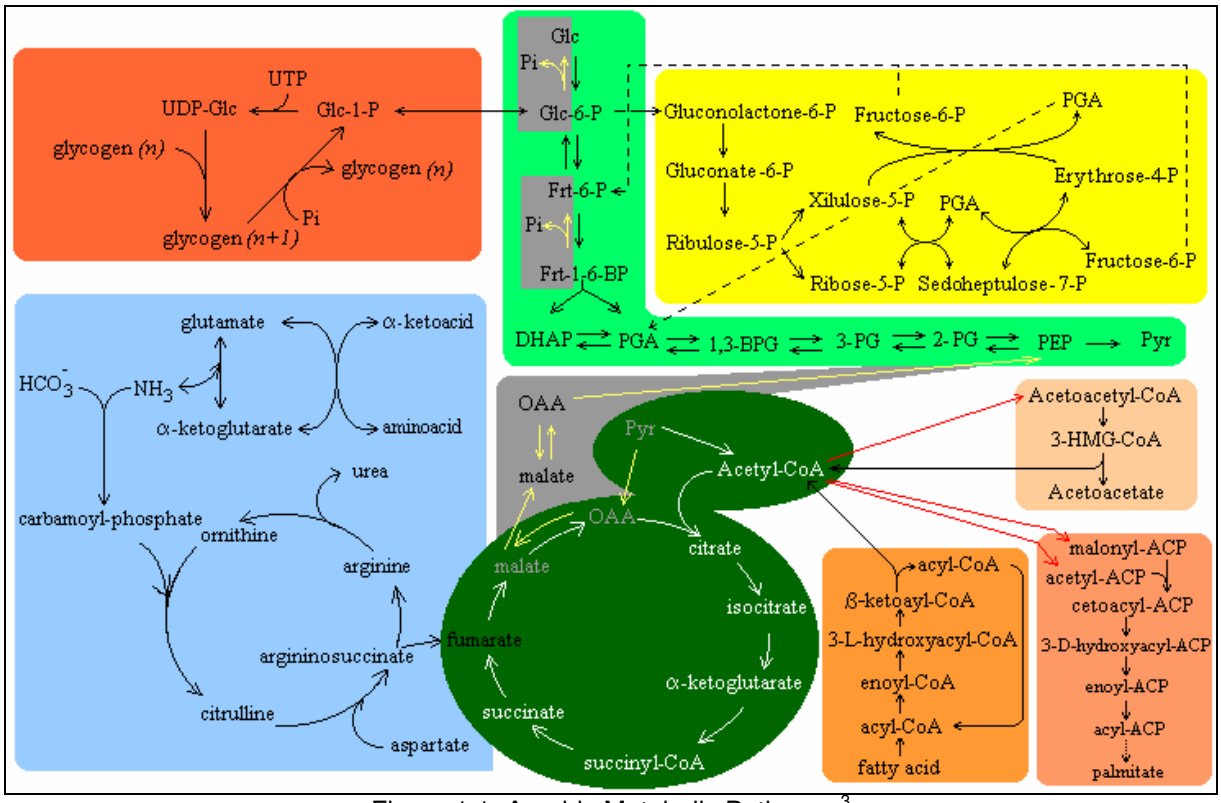


Figure 1.1: Aerobic Metabolic Pathways[3]

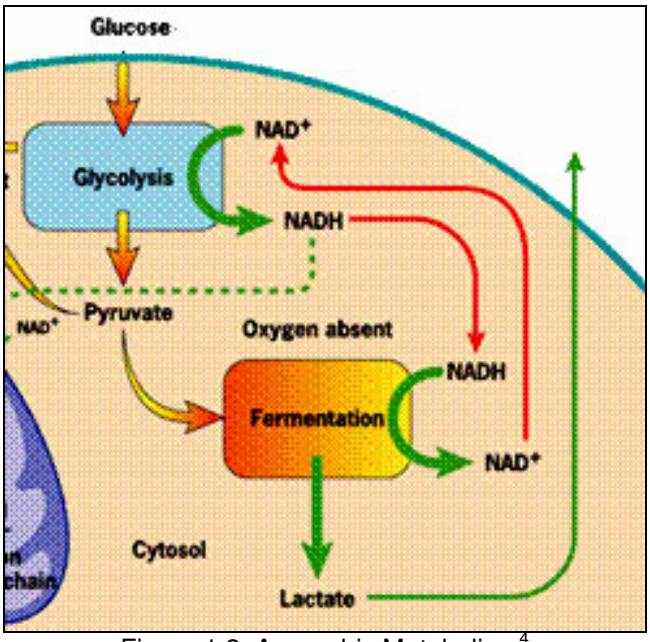


Figure 1.2: Anaerobic Metabolism[4]

At the time of heart arrival at the recipient site where the recipient has been placed on cardiopulmonary bypass and his/her diseased heart has been removed. The donor heart is then removed from the cooler and brought into the sterile operating field. The heart is then reattached (transplanted) by anastomoses of the left atrium, right atrium, pulmonary artery, and aorta, Figure 1.3. Removal of the cross clamp then restores normothermic blood to the transplanted heart to not only rewarm the heart, but to remove the metabolite burden.

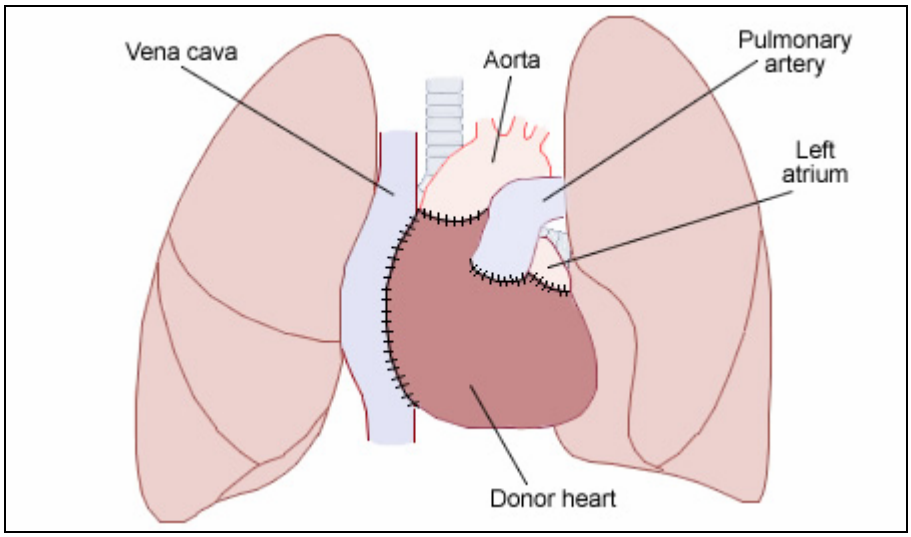


Figure 1.3: Heart Transplantation: Anastomoses performed in the following order – Left atrium, right atrium, pulmonary artery, and aorta.[5]

While this approach is logical, the cold anaerobic donor tissue must come into contact with the warm oxygenated recipient blood, with an increased risk for reperfusion injury. As it is now well known, the direct effects of reperfusion injury are inflammation and oxidative stress.[5] In prolonged ischemia, hypoxanthine is formed as breakdown product of ATP metabolism. The enzyme xanthine dehydrogenase acts in reverse, characteristically like xanthine oxidase, as a result of the higher availability of oxygen. This oxidation results in molecular oxygen being converted into highly reactive superoxide and hydroxyl radicals. Xanthine oxidase also produces uric acid, which may act as both a pro-oxidant and as a scavenger of reactive species such as peroxinitrite. Excessive nitric oxide produced during reperfusion reacts with superoxide to produce the potent reactive species peroxynitrite. Such radicals and reactive

oxygen species attack cell membrane lipids, proteins, and glycosaminoglycans, causing further damage. They may also initiate specific biological processes by redox signaling.[6] The net effect is a significant risk for damage to the donor heart, particularly if storage intervals are prolonged. If damage is too great the donor heart may fail. Primary graft failure remains a significant problem in transplant centers today.

1.3 Potential Benefits of Machine Perfusion

The theoretical benefits of a machine perfusion approach to heart transport for transplant include: increasing the donor-recipient transport time (thus the transport radius), and improved early graft function, potentially reducing ICU time, and enhancing long term outcome. Quantitatively, a reduced ischemic burden will reduce myocardial damage and the potential for reperfusion injury. In addition, the potential for increased aerobic metabolism and washout of metabolites during perfusion should aid in improved graft function.

By increasing the donor-recipient radius, more transport time is allowed which in turn allows a potentially higher need patient to receive the heart. Pumping the heart with an oxygenated solution mimics the body's natural perfusate, blood. If the tissue is able to maintain aerobic metabolism during machine perfused transport, the likelihood of myocardial damage is reduced.[36] Another potential benefit to this method would be to increase the donor pool through the inclusion of marginal and non-heart beating donors. The ability to provide nutrients to these hearts en route has been noted, with the potential to further expand the transport and storage interval.[7]

1.4 Previous Experimental Investigations

There have been several studies exploring the benefits of machine perfusion devices; however, there has not been a consensus on an ideal approach. There has been much debate over which preservation solution (intracellular or extracellular composition) works best, and which modifications or additives should be implemented. The optimal heart preservation solution remains elusive with a recent survey of transplant programs in the United States revealing the use of 167 different solutions in 147

transplant centers.[8] A review of literature of experimental studies of machine perfusion similarly reveals little consistency in the approach, as there appears to be a wide array of solutions used, a wide variation in devices and perfusion conditions, and even a lack of consensus on animal models. Different studies may or may not include transplantation or reperfusion of the stored heart, and have reported a wide range of outcome variables, including early reperfusion graft function and markers of myocardial metabolism. The most discussed issue in the reviewed studies was tissue water buildup (myocardial edema) with varying results based on perfusion conditions and preservation solution selection.

The nearly 50 studies available in the literature can be subdivided into three general categories:

1. Functional/Metabolic effects of perfusion preservation of animal hearts
2. Perfusion preservation and resuscitation of marginal hearts
3. Models development using various *ex vivo* studies, such as air persufflation

A review of these studies is provided in Appendix A.[10-72]

There has also been discussion as to which direction of perfusion is most effective.[9] Antegrade perfusion is delivery via the aortic root or via the innominate artery to the coronary arteries, and retrograde perfusion is the delivery to the coronary arteries through the coronary sinus, located on the posterior surface of the heart, Figure 1.4.

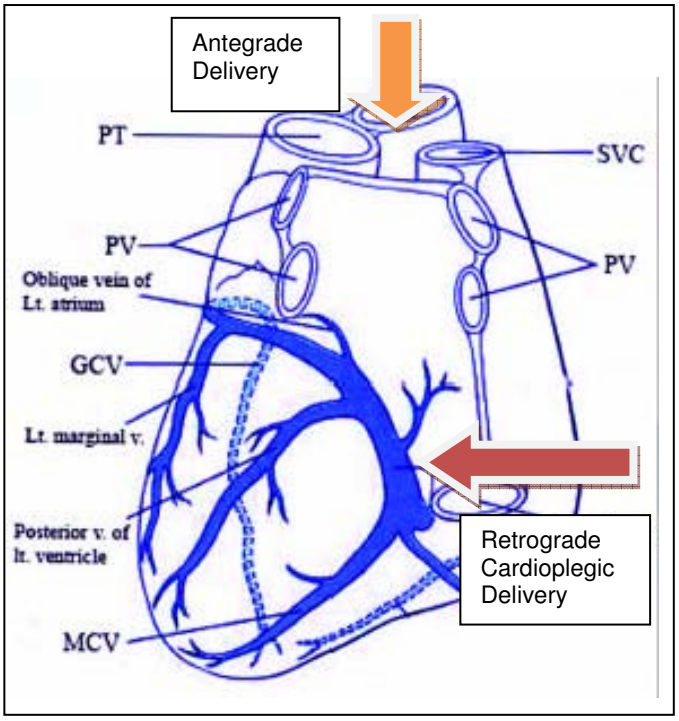


Figure 1.4: Diagram of Heart Perfusion. Antegrade aortic perfusion (Orange) and Retrograde cardioplegic delivery (Red).[9]

Studies by Aizaki et al suggest that a retrograde perfusion allows for better nutrient flow, whereas studies by Ferrera et al argue for antegrade flow under microperfusion conditions. Though varied in design, one theme remains consistent: all articles demonstrated beneficial effects on long term preservation with perfusion preservation.[10-14]

1.5 LifeCradle Development and Engineering

The LifeCradle™ is a new technology designed to overcome limitations of the current paradigm of organ donation and procurement. The parent company, Organ Transport Systems, Frisco, TX, acquired patent rights and developed the technological concept almost a decade ago. The company has since grown and matured, gaining the resources necessary to make the transition from concept to product.

The basic concept of the device is to provide a more physiological environment for organ transport between donor and recipient site. The first prototype consisted of a heat and mass exchanger,

fed by bulky oxygen tanks and a large fluid pump attached to the organ container [Appendix B, Figure B1]. The next prototype was designed around large igloo coolers which were packed with ice around the organ container, with a roller pump and membrane oxygenator to allow oxygenated fluid pumping through the organ [American Pneumatic Tools, Gardena, CA]. The third model incorporated externally mounted temperature and pressure sensors. While the methods embodied in these prototypes incorporated the idea of pumping fluid across the organ, they were still cumbersome and difficult to transport [Appendix B, Figures B2 and B3]. The next generation of design benefited from the inclusion of an experienced engineering prototype company to increase functionality and transportability [The RealTime Group, Plano, TX]. The prototype LifeCradle, as used in the swine and canine experiments described below, shifts from a large cooler containing the components to a small isolated cooler for the organ only with the other components in a surrounding shell [Appendix B, Figure B4]. The major change to this more portable unit is the incorporation of a dual Peltier cooling method, in which the temperature can be controlled by manually adjusting a dial, with a range from 5° to 12°C. In addition to manual temperature control the roller pump can also be dialed into the desired flow rate from 0 to ~75 ml/min [OEM Series, Watson-Marlow Pumps, Falmouth, England]. Third, a new miniature membrane oxygenator was included in the fluid path, with a pore size of 20 microns, replacing the bulkier oxygenator [Membrana GmbH, Obernburg, Germany]. A final incorporation to this new portable design is the ability to run off of AC power in addition to battery power [Inspired Energy, Inc., Newberry, FL]. Both power sources are approved for medical device usage, and the battery power life of the two batteries is approximately 3 hours when fully charged.

The transition from prototype into a clinical/commercial model has once again required an overhaul of the design. In order to facilitate user control, the unit has been redesigned to incorporate a touch screen computer [Appendix B, Figure B4]. As mentioned above, the current standard for heart transport is a simplistic sterile method involving a three layer sterile barrier. Keeping this in mind, the device evolved into two components, the durable unit and the disposable circuit [Appendix B, Figure B5]. The durable unit contains the computer, which runs a Visual C++ program to allow user interface, one OEM 102R low flow roller pump, four Inspired Energy 14.4V Li-Ion batteries that are supplemented with a

medical grade AC power connector, and locations for the single use oxygen tank and disposable circuit. The disposable circuit contains the chiller, Membrana Mini-Module membrane oxygenator, and organ container interface and bag [Appendix B, Figure B6]. The portability of the device has also been effectively addressed with an ergonomic design to the durable shell and the inclusion of light weight components; the final fully loaded unit weighs a mere 35 pounds. The clinical unit also addresses the issues of the sterile field inside the operating room by allowing the user to set up the disposable circuit and all of its components on a sterile back table. Once complete the same technician then activates and initiates the device on a separate non-sterile table. All priming and device activation takes approximately 10 minutes, almost the same time it would take to lay out and organize the components for a simple storage transplant.

1.6 Prior Canine and Swine Studies

A comparison study was performed with a group of eight pigs to determine the efficacy of machine perfusion versus static storage. Hearts were arrested and stored with glucose modified Celsior solution for durations of four hours. For the machine perfused hearts a normalized flow rate of 10mL/100g/min was used. Left ventricular (LV) function was evaluated using a load-independent analysis of ventricular performance from dimension data acquired by sonomicrometry crystals and pressure data acquired from an intraventricular micromanometer-tipped catheter. Metabolic activity was determined by magnetic resonance spectroscopy of excised left atrial appendage samples. After preservation the hearts were then transplanted into recipient pigs and reperfused for six additional hours, at which point left ventricular water content and serum creatine kinase-MB isoenzyme levels were measured. Results of the experiments revealed that LV function and water content were similar in both groups. Tissue lactate levels were lower in continuously perfused hearts and serum creatine kinase-MB levels were significantly higher in statically preserved hearts. This study suggests that continuous perfusion of donor hearts reduces the production of undesirable metabolites and has the potential to improve graft function.[15]

A similar study was performed using a canine model. A total of twelve dogs were studied using a four hour preservation interval with a six hour recipient reperfusion. In addition to measuring post-reperfusion LV water content and serum creatine kinase-MB isoenzyme levels, myocardial apoptosis was evaluated using a TUNEL test. Results of this study again showed that tissue lactate levels were reduced in the machine perfused hearts and LV water content remained similar. The CK-MB levels however, were similar in both groups. The apoptotic cell count although low, was markedly higher in the statically preserved hearts, further suggesting that continuous perfusion offers significant benefits for tissue viability.[16]

1.7 Specific Aims

From the preliminary studies with the prototype device, several more questions were raised and evaluated. The first major issue was to determine an optimal flow rate, by varying the flow parameters for short durations of time. We note that in the heart, unlike other organs the flow of perfusate is directed into the ascending aorta, and not directly into the coronary arteries supplying the capillary bed of the myocardium where oxygen exchange and washout of metabolites occurs. We refer to flow through the capillaries as “nutrient flow”. It is assumed that increasing nutrient flow will offer the greatest potential to maximize the benefits of machine perfusion for organ preservation. However, simply dialing up the flow rate may not achieve this effect, as some flow might bypass the capillaries through alternate (Thebesian) channels or might cross an insufficient aortic valve and circulate through the chamber without providing flow to the capillaries. If nutrient flow rates are too low, myocardial metabolism may be suboptimal, reducing the effectiveness of the perfusion preservation technique. Alternatively, if nutrient flow rates are high, myocardial edema development may accelerate. By evaluating the nutrient flow characteristics over a range of device conditions, an optimal flow rate and device protocol could be derived. .

Based on this rationale and the prior experience in the use of the prototype device, the following specific aims were set, and the corresponding assertions evaluated:

1. Device flow rate is a primary determinant of tissue perfusion.

2. Aortic attachment conditions affect tissue perfusion.
3. Heart inclination influences tissue perfusion.
4. Adequate tissue perfusion permits a better tissue metabolic profile.
5. Elevated flow rate causes myocardial edema development.

CHAPTER 2

MATERIALS AND METHODS

2.1 LifeCradle Prototype

The perfusion device used for these experiments was the fourth iteration of the LifeCradle device, Figure 2.1. As described above, both the temperature and the flow rate can be controlled manually. The heart attaches to the lid via aortic cannula which is held in the aorta and secured by surgical silk ties and umbilical tape, Figures 2.2 and 2.3.

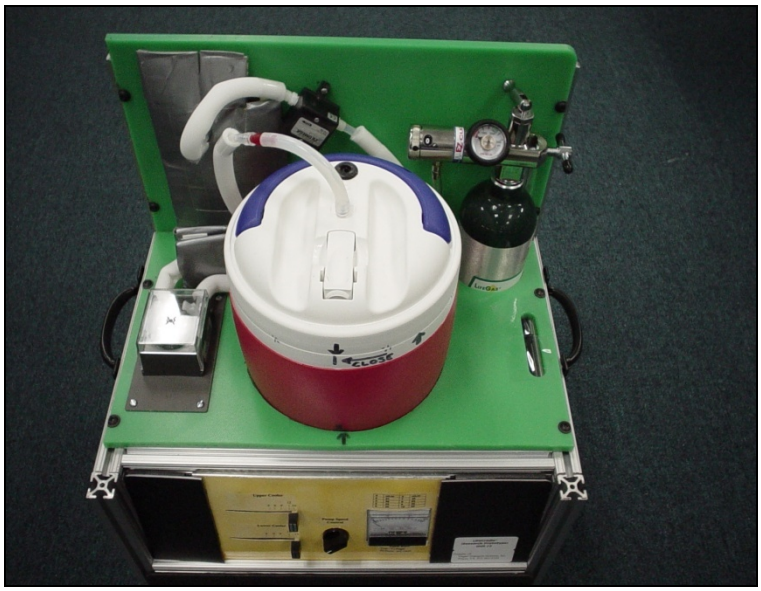


Figure 2.1: LifeCradle Model 4. Designed by Marshall Wenrich et al. at The RealTime Group, Plano, TX and OTS, Inc., Frisco, TX 2003-2005.

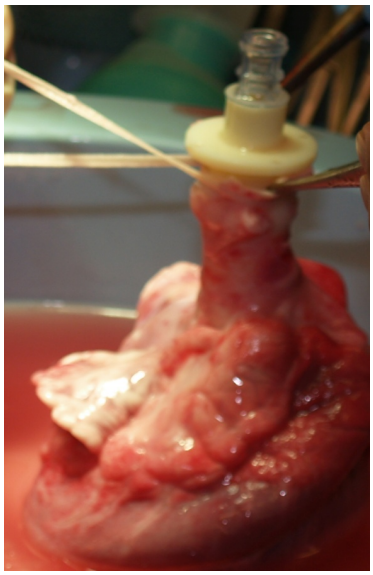


Figure 2.2: Aortic adaptor insertion into aorta.

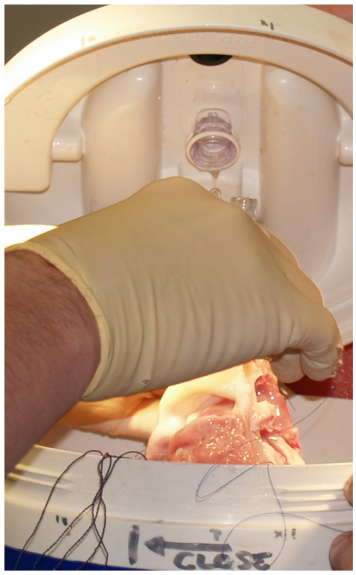


Figure 2.3: Heart attachment to lid.

The device oxygenates the perfusate fluid through a porous membrane filter which is connected to an oxygen tank and replaced at the end of each experiment.

2.2 Experimental Protocol

The protocol for this study was approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center. All animals were treated within guidelines set forth in the *Guide for the Care and use of Laboratory Animals* [National Institutes of Health Publication No. 86-23, revised 1996]. Adult mongrel dogs were used in these experiments. In each experiment, hearts were removed from anesthetized dogs and established in a perfusion device that provided continuous perfusion of oxygenated fluid through a calibrated pump system that enabled precise control of flow rate, oxygenation, and fluid temperature [LifeCradle™, Organ Transport Systems, Inc, Frisco, TX].

2.3 Donor Protocol

Each animal was premedicated with 0.07 mg/kg atropine IM and 4.4 mg/kg telazol IM, then intubated and ventilated with 100% oxygen at VT of 10 ml/kg, rate of 10/min, and PEEP of 5 cm H_2O. Anesthesia was maintained with 1-4% isofluorane. Ventilator settings were adjusted based on arterial

blood gas measurements to keep the pCO_2 at 35-45 mmHg, pH 7.35-7.45, and oxygen saturation > 95%. After sternotomy and exposure of the heart, 300 units/kg of heparin was administered intravenously and an ascending aortic cardioplegia catheter was inserted. The aorta was clamped and the heart was arrested with one liter of cold Celsior organ preservation solution [Genzyme Corporation, Cambridge, MA] which had been supplemented with 1g/L (5.5mmol/L) of glucose. The inferior vena cava and right superior pulmonary vein were incised to decompress the heart, and the donor cardiectomy was completed. Hearts were rapidly weighed and then attached to the perfusion device via a connector in the ascending aorta. The device then delivers retrograde ascending aortic flow, providing continuous antegrade coronary perfusion with an oxygenated, machine perfusate solution at a specified flow rate at 5 ± 2°C. A small polyethylene catheter was placed in the coronary sinus for serial measurements of oxygen tension during preservation. pH, oxygen tensions, and lactate levels in the preservation solution were measured with commercial analyzers ABL 5 and EML 105 [Radiometer Copenhagen, Bronshoj, Denmark].

2.4 Analytical Methods

Unlike perfusion of other organs where all delivered flow must enter the vascular bed, not all flow from the device entering the ascending aorta must enter the coronary capillary bed. Some may be lost through leakage at connector sites; some may enter the coronary arteries, but pass into the Thebesian veins, bypassing the capillaries; or a fraction of the delivered flow may cross an incompetent aortic valve and fail to reach the coronary microcirculation.[17] The last phenomenon might be of greater importance in a system that features non-pulsatile flow at low flow rates and low aortic root pressure. In these experiments, any flow that entered the ascending aorta but passed into the collection chamber (i.e. did not enter the coronary capillary system) was considered non-nutrient flow.

The fraction of flow (in %) that was nutrient flow was calculated as:

*[(inflow bead count - bead count in collection chamber)/ (inflow bead count)] * 100*

where

inflow bead count = (concentration of beads in bead container x flow rate x 2 minutes), and

bead count in collection chamber = (concentration of beads in sampling container x final volume of sampling container)

The fraction of flow (in %) that was non-nutrient flow was calculated as:

*[(bead count in collection chamber)/ (inflow bead count)] * 100*

where

inflow bead count = (concentration of beads in bead container x flow rate x 2 minutes), and

bead count in collection chamber = (concentration of beads in sampling container x final volume of sampling container)

Tissue flow rate (in mL/100 gram/min) was calculated from bead counts measured in the tissue samples and bead containers as follows:

myocardial blood flow = [(*bead count in tissue/ tissue weight*) * *concentration of beads in bead container*] *100/ *2 minutes*

2.5 Experimental Design

2.5.1 Effect of Device Flow Rate on Tissue Perfusion

For this group of six dog hearts, the selected machine perfusion solution was a modified Celsior solution. Our hypothesis was that the lower flow rates may lead to lower pressure in the ascending aorta, resulting in a less competent aortic valve and thus leading to a diminished nutrient flow via coronary circulation. To test this theory, the perfusion device was modified to include a side-branch from the recirculating tubing. This side-branch led to a Y-connector that enabled the device to infuse one of two alternate solutions for a limited time. One of these solutions consisted of modified Celsior solution cooled to 5℃ and supplemented with 15 micron colored microspheres [IMT Laboratories, Irvine, CA] at a final

concentration of 15,000 beads/mL and 0.01% TWEEN 80. The other solution consisted of cold modified Celsior solution without beads. A sampling container the same size as the perfusion device chamber containing one liter of cold modified Celsior solution was also prepared.

Each dog heart was initially established in the device at one of six flow rates (5, 10, 15, 20, 25 or 30 mL/100 g/min) in random order. This flow rate was continued with the recirculating preservation solution for 20 minutes (Figure 2.4); pressure in the ascending aorta and fluid temperature were continuously recorded.

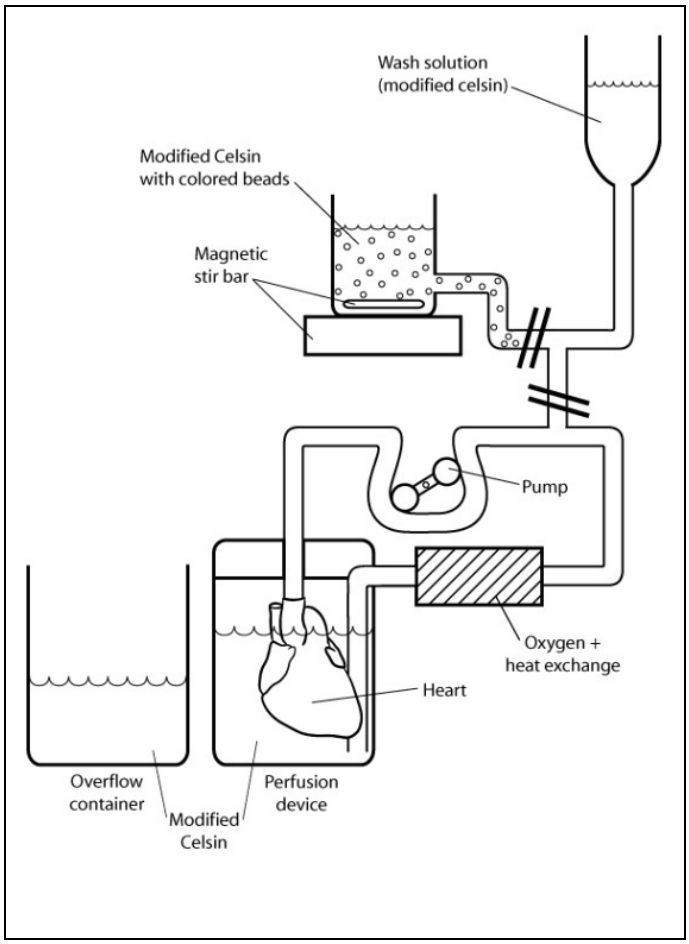


Figure 2.4: Standard perfusion setup of device

Myocardial oxygen consumption (MVO_2) was calculated, based on pO_2 levels measured in the aortic inflow line and from a small catheter placed in the coronary sinus. At this time the heart was gently repositioned from the perfusion chamber to the sampling container, the side-branch was opened and the

recirculating line was clamped to allow delivery of the bead-containing solution into the device through the inflow cannula for 2 minutes at the same flow rate without recirculation, Figure 2.5.

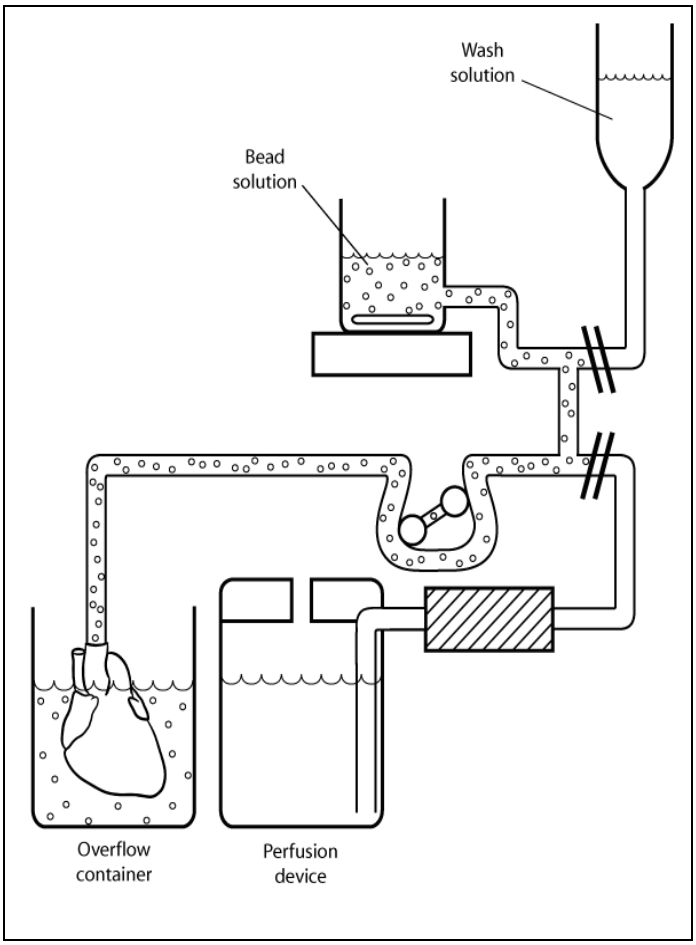


Figure 2.5: Bead solution delivery with organ in overflow container

This enabled measurement of tissue perfusion. A sample of the bead solution was collected for control measurement of bead concentration in perfusate, necessary for quantification of total inflow bead count, a component of the tissue perfusion calculation. After 2 minutes, the inflow was switched to the washout solution to clear any beads from the tubing by flowing at the same rate for a time calculated to deliver 120 mL of solution from the chamber, Figure 2.6.

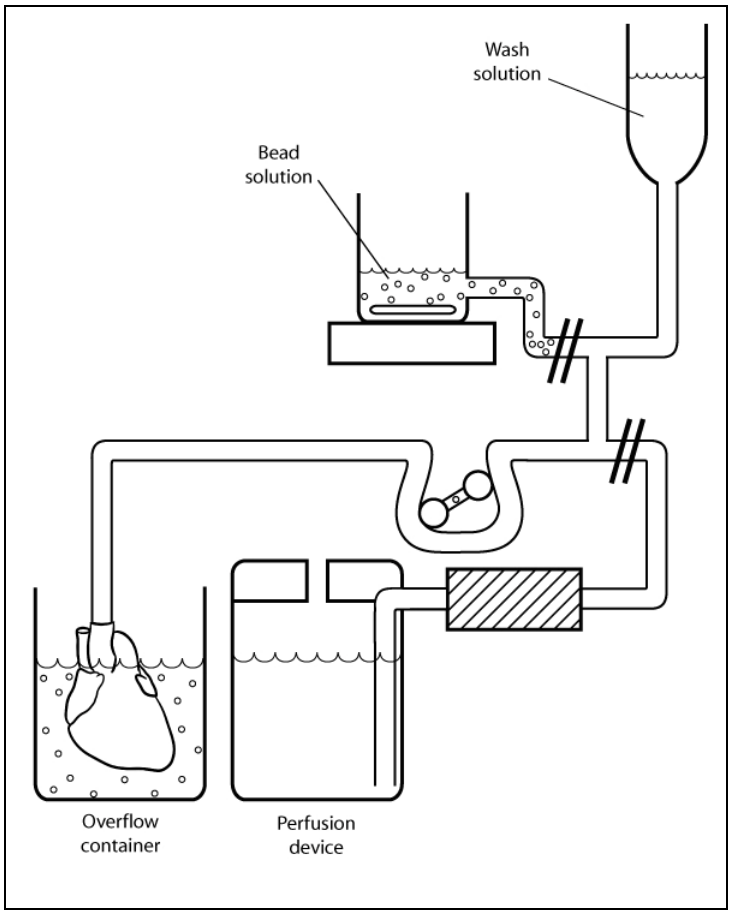


Figure 2.6: Bead washout solution flowing through the heart

At this point the heart was returned to the initial perfusion chamber and the second randomly selected flow rate was applied. The microsphere solution was then changed to one containing one of the other five bead color preparations. After 20 minutes of perfusion with this solution, the sequence was repeated until all six flow rates with separate bead labels had been completed.

After the final washout, the heart was removed from the sampling chamber. The heart was sliced and a 1 cm slice at the mid-ventricular level was selected for analysis. Tissue sections, approximately 2-3g each, comprising samples of endocardial and epicardial tissue taken from the anterior, lateral and posterior LV, the left and right ventricular septa, the RV free wall, and the left atrial appendage were harvested, labeled, bagged and stored in a -80℃ freezer until further use. Furthermore, a fluid sample from the overflow chamber of the perfusion circuit was collected and the final volume of the overflow

chamber was measured. Tissue and fluid samples were sent to IMT Laboratories in Irvine, CA, where all measurements of tissue and fluid bead counts were performed. Myocardial tissue perfusion rates and the fraction of non-nutrient flow were calculated from these data, as described in the analytical methods section.

2.5.2 Effect of Aortic Attachment Configuration on Tissue Perfusion

In this study, a group of four dog hearts were harvested as described above and the ascending aorta was attached to an adaptor allowing connection to the perfusion device, as shown above. Each heart was then attached to the device in one of six different conditions described in Table 2.1.

Table 2.1: Conditions under which the heart was attached to the perfusion device

Condition	Heart Position when Connector Attached	Initial Flow Rate when Attached	Final Flow Rate	Status of Aortic Valve
1	Immersed in solution	60 mL/min	10 mL/100g/min	No manipulation
2	Immersed in solution	5 mL/min	10 mL/100g/min	No manipulation
3	Suspended above solution, then lowered	60 mL/min	10 mL/100g/min	No manipulation
4	Suspended above solution, then lowered	5 mL/min	10 mL/100g/min	No manipulation
5	Immersed in solution	60 mL/min	10 mL/100g/min	Valve sewn closed
6	Immersed in solution	60 mL/min	10 mL/100g/min	Valve excised

The conditions varied in that the heart was either first immersed in the solution prior to attachment or attached to the device out of the solution and with a starting flow rate that was high (60 mL/min) or low (5 mL/min). For the final two conditions, the connector was removed and the aortic valve was first sutured closed for the perfusion period and then excised for the final duration, the connector was re-attached and reconnected to the device for both. For every set of conditions, after the connector was attached, the flow

rate was gradually altered, to arrive at a final rate of 10 mL/100g/min over the course of 2 minutes at a temperature of 5℃; perfusion was then maintained at this rate for 20 minutes. A measurement of myocardial perfusion and non-nutrient flow was then made by a similar technique to the one described above; in this instance the heart was not moved to a sampling container. After completion of the bead infusion and washout steps, the heart was detached from the device and re-attached to a second, identical device for the next loading condition. This was repeated for each heart until all 6 conditions were studied. At this point the heart was removed, tissue was harvested and fluid samples were collected for microsphere analysis as described previously.

2.5.3 Effect of Heart Inclination on Tissue Perfusion

Two hearts were harvested as described above and the ascending aorta of each was attached to an adaptor. The heart was then attached to the device in one of six different inclinations, Table 2.2.

Table 2.2: Conditions under which the heart was inclined in the perfusion device.

Angle	Heart Position	Heart Position when Connector Attached	Initial Flow Rate when Attached	Final Flow Rate
45°	Away from Non-coronary Sinus	Immersed in solution	60 mL/min	10 mL/100g/min
0°	Control	Immersed in solution	60 mL/min	10 mL/100g/min
75°	Away from Non-coronary Sinus	Immersed in solution	60 mL/min	10 mL/100g/min
15°	Away from Non-coronary Sinus	Immersed in solution	60 mL/min	10 mL/100g/min
30°	Away from Non-coronary Sinus	Immersed in solution	60 mL/min	10 mL/100g/min
60°	Away from Non-coronary Sinus	Immersed in solution	60 mL/min	10 mL/100g/min

Table 2.2 - *Continued*

Angle	Heart Position	Heart Position when Connector Attached	Initial Flow Rate when Attached	Final Flow Rate
60°	Away from Non-coronary Sinus	Immersed in solution	60 mL/min	10 mL/100g/min
30°	Away from Right Coronary Sinus	Immersed in solution	60 mL/min	10 mL/100g/min
60°	Away from Left Coronary Sinus	Immersed in solution	60 mL/min	10 mL/100g/min
30°	Away from Left Coronary Sinus	Immersed in solution	60 mL/min	10 mL/100g/min
0°	Control	Immersed in solution	60 mL/min	10 mL/100g/min
60°	Away from Right Coronary Sinus	Immersed in solution	60 mL/min	10 mL/100g/min

Two sets of inclinations, maintained a similar control angle of 0°, but differed in angles of tilt in relation to the coronary sinus, Figure 2.7.

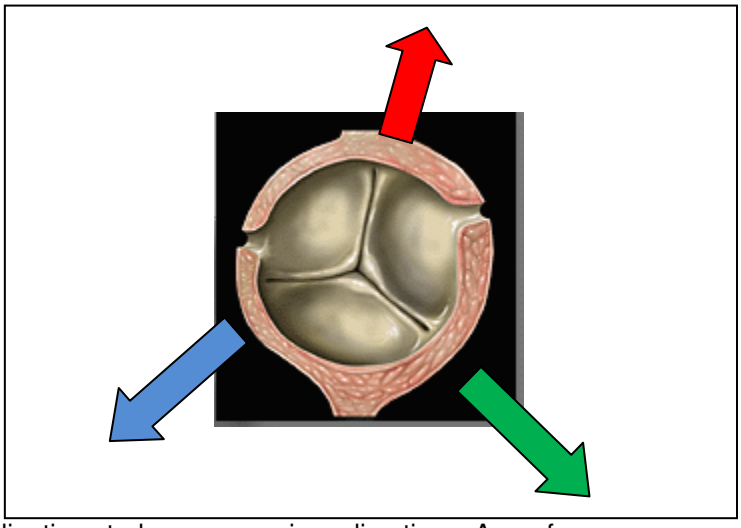


Figure 2.7: Heart inclination study coronary sinus directions. Away from non-coronary sinus (red). Away from left coronary sinus (green). Away from right coronary sinus (blue).

The heart was first immersed in the perfusate solution with a high starting flow rate, 60 mL/min, and over the course of 2 minutes, the flow rate was gradually brought to a tissue perfusion rate of 10 mL/100g/min, while maintaining a temperature of 5 ± 2°C; perfusion was then maintained at this rate for 20 minutes. A measurement of myocardial perfusion and non-nutrient flow was then made by a similar technique to the one described above; again, the heart was not moved to a sampling container. After completion of the bead infusion step and washout the heart was detached from the device and re-attached to a second identical device for testing of the next loading condition. This process was repeated until all 6 inclinations were studied. At this point the heart was removed, tissue was harvested and fluid samples were collected for microsphere analysis as previously described.

2.5.4 Effect of Flow Rate on Myocardial Metabolism and Edema Development

For this series, seven dog hearts were prepared and established in the perfusion device, using varied attachment techniques. Hearts were each perfused for 10 hours at one of 3 flow rates: 10, 15, or 30 mL/100g heart tissue/min, thereby creating conditions where a range of nutrient flow rates at the cellular level would be expected. Perfusion solution used in this series was modified Celsior Solution. At the end of the perfusion period, a measurement of flow distribution was made, using the technique described above. Hearts were removed from the device, drained of residual perfusion solution, and reweighed. Samples of left ventricular tissue were harvested, immediately freeze-clamped, and cooled in liquid nitrogen, then labeled and bagged. The tissue was stored in a -80° C freezer until use. For metabolic measurements, samples were subsequently extracted with perchloric acid. Purified extracts were reconstituted in D_2O and pH was adjusted to 7.0-7.4 for Magnetic Resonance Spectroscopy (MRS).[15] MR Spectra were then acquired with a 14.1 Tesla Varian spectrometer operating at 600 MHz over a spectral width of 8000 Hz. Lactate-to-alanine ratios were measured and compared with 1H proton spectra as measures of cellular metabolism during perfusion as previously described.[16] Separate samples (~2-3g) were collected for measurement of water content. The samples were placed in an oven and tissue weights were recorded until no further weight loss was observed. The percentage change

between the initial and final weights was calculated for each sample and data from hearts with high and low nutrient flow were compared.

2.6 Statistical Analysis

Results are reported as mean and standard error of the mean (SEM). Groups were compared by a one-way analysis of variance or t-test, as appropriate, using commercially available statistical software (SigmaStat®, Chicago, IL). A p-value less than 0.05 was considered significant.

CHAPTER 3

RESULTS

3.1 Effect of Device Flow Rate on Tissue Perfusion

Selected flow rates remained constant at the desired rate in all studies in addition to maintaining fluid temperature stability within the specified range. Flow rates from the device readout were compared with the change in weight of the bead solution beaker before and after a 2 minute infusion and found to correlate well. Hearts extracted dissolved oxygen continuously throughout the experiment and MVO_2 was measured at each flow rate. MVO_2 increased with increasing coronary flow but this difference was not significant at flows beyond 15 mL/100g/min, Table 3.1, Figure 3.1.

Table 3.1: Variables Measured at each Perfusate Flow Rate

Flow Rate (mL/100g/min)	MVO_2 (mL/100g/min)	Tissue Flow (mL/100g/min)	Epicardial/ Endocardial Ratio	Non-nutrient Flow (%)	Aortic Root Pressure (mm Hg)
5	0.10±.04	5.0±0.5	1.1±0.1	38±15	9±1
10	0.13±.03	5.1±1.0	1.7±0.5	35±13	13±3
15	0.30±.05	15.4±1.1*	1.0±0.1	15±12	10±3
20	0.26±.04	15.2±0.6*	0.9±0.1	16±10	17±3†
25	0.47±.10†	25.1±1.1*	0.9±0.1	13±9	13±3
30	0.33±.11†	40.0±2.5*	1.0±0.1	11±10	22±2*

Data are expressed as mean± SEM, n=6. “Tissue flow” represents a mean value of all LV and RV segments sampled.

*-p<.05 vs. 5, 10mL/100g/min groups

†-p<.05 vs. 5 mL/100g/min groups

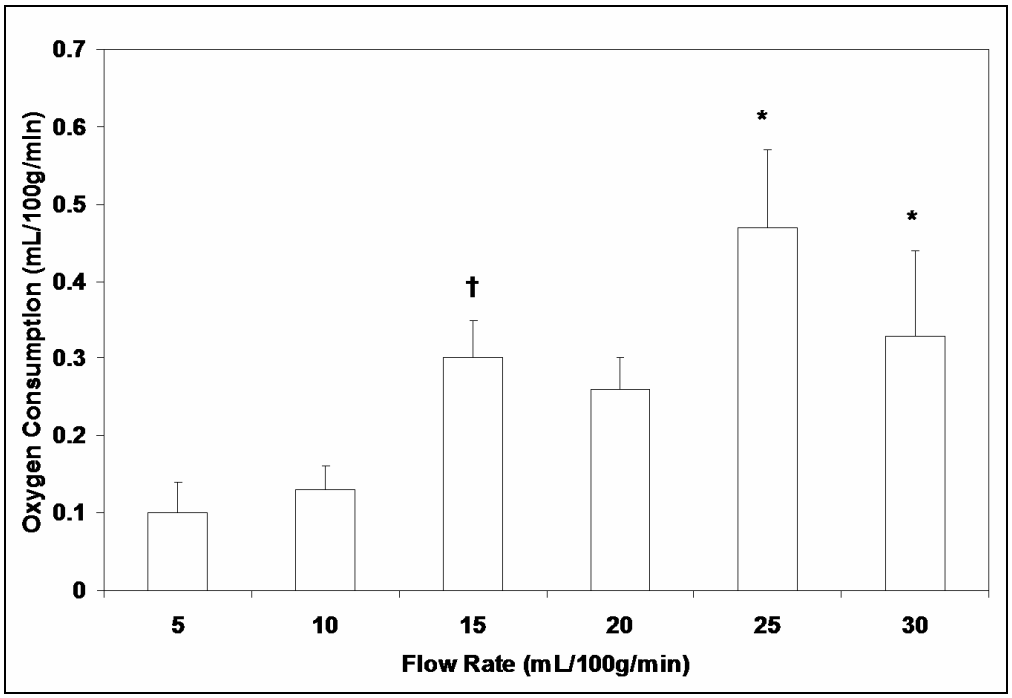
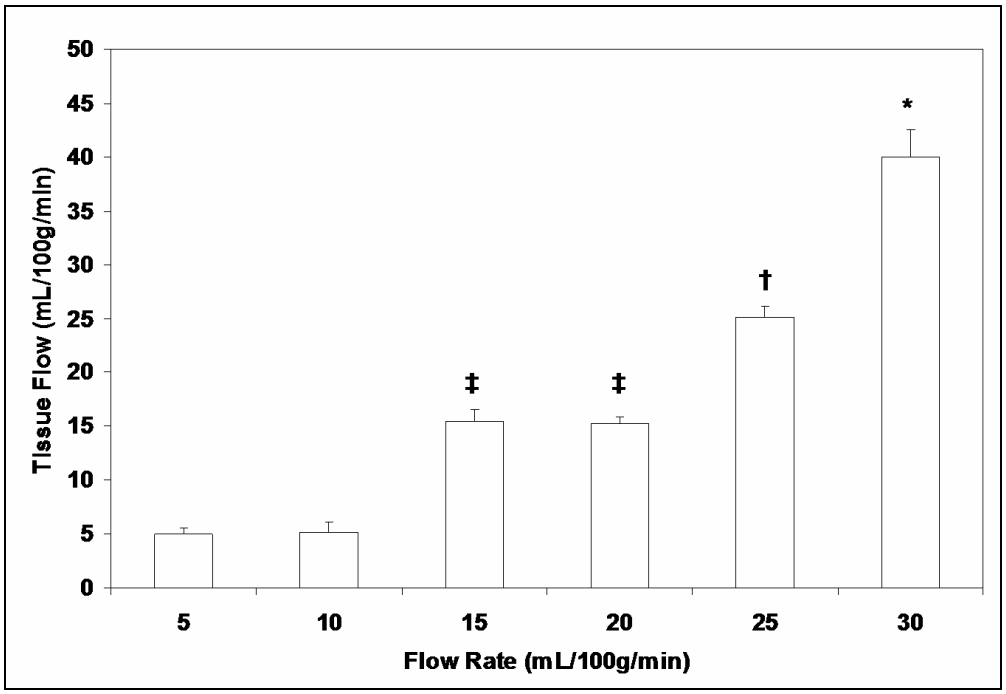
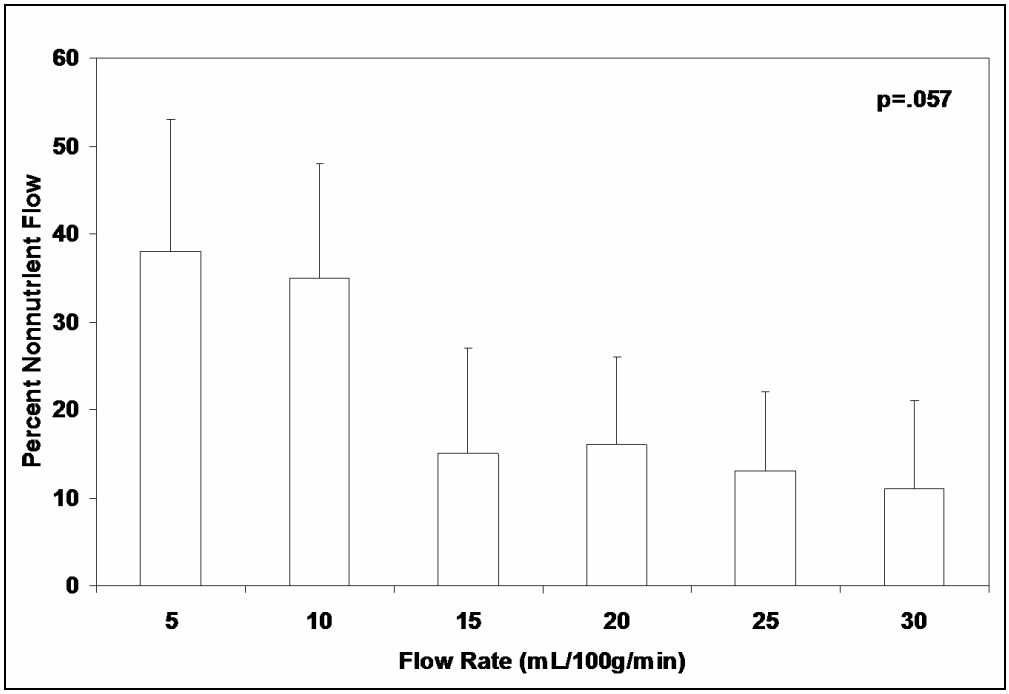


Figure 3.1: Effects of altering device flow on myocardial oxygen consumption.

Higher flow rates, taken from the visual readout on the device, also correlated with increased tissue perfusate flow, as determined by microsphere analysis of the tissue, Figure 3.2. Non-nutrient flow was observed to increase at the lowest flow rates, although this change did not reach a statistically significant correlation ($p = 0.57$), Figure 3.3.



Figures 3.2: Effects of altering device flow on calculated tissue flow.



Figures 3.3: Effects of altering flow on non-nutrient flow.

For most flow rates the epicardial:endocardial perfusion ratio remained close to 1:1. A trend towards increasing ascending aorta pressure was observed as flow rates were increased, Table 3.1.

3.2 Effect of Aortic Attachment Configuration on Tissue Perfusion

It was observed that different modes of attachment of the donor heart to the apparatus influenced myocardial oxygen consumption, myocardial tissue perfusate flow and, to different degrees, the non-nutrient flow, Table 3.2.

Table 3.2: Variables Measured at each Attachment Condition

Condition	MVO_2 (mL/100g/min)	Tissue Flow (mL/100g/min)	Epicardial/ Endocardial Ratio	Non-nutrient Flow (%)	Aortic Root Pressure (mm Hg)
1	0.15±0.02	10.0±1.2	1.1±0.1	5.2±1.5	12±2
2	0.11±0.04	3.2±2.5	2.2±.8	41.1±21.8	8±2
3	0.19±0.03	12.7±1.6	2.5±1.0	9.5±0.7	13±4
4	0.07±0.04	4.2±2.4	1.5±0.4	59.0±24.5*	15±3
5	0.15±0.03	10.2±2.6	1.2±0.1	9.7±3.2	15±2
6	0.01±0.01‡	0.1±0.01‡	1.0±0.2	96.2±5.6†	10±3

Data are expressed as mean± SEM, n=4. “Tissue flow” represents a mean value of all LV and RV segments sampled.

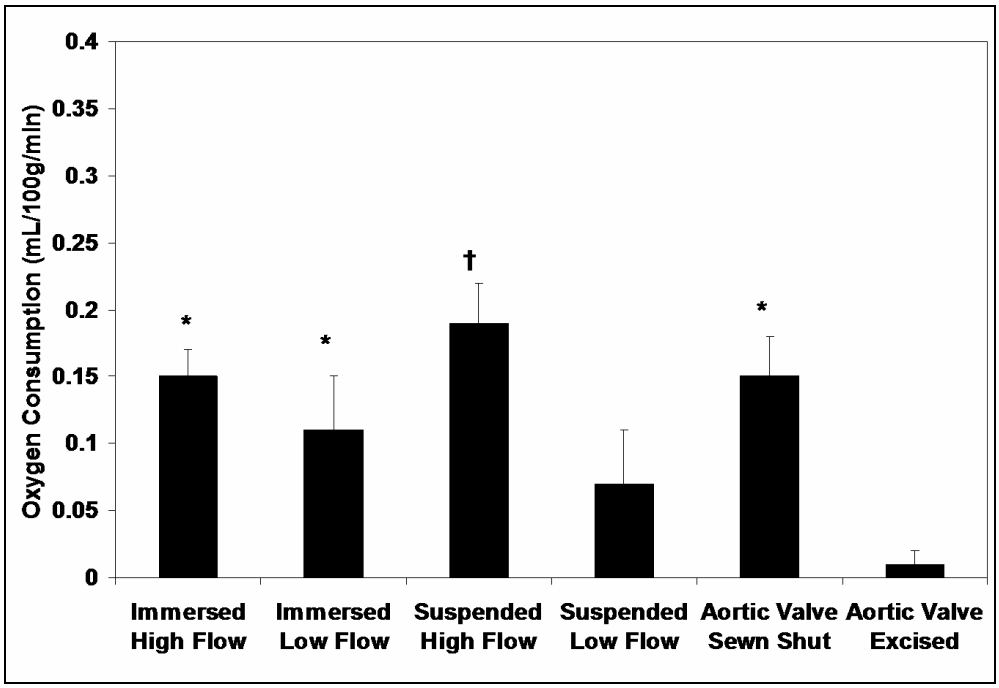
*-p<.05 vs. Condition 1

†-p<.05 vs. Conditions 1,2,3 and 5

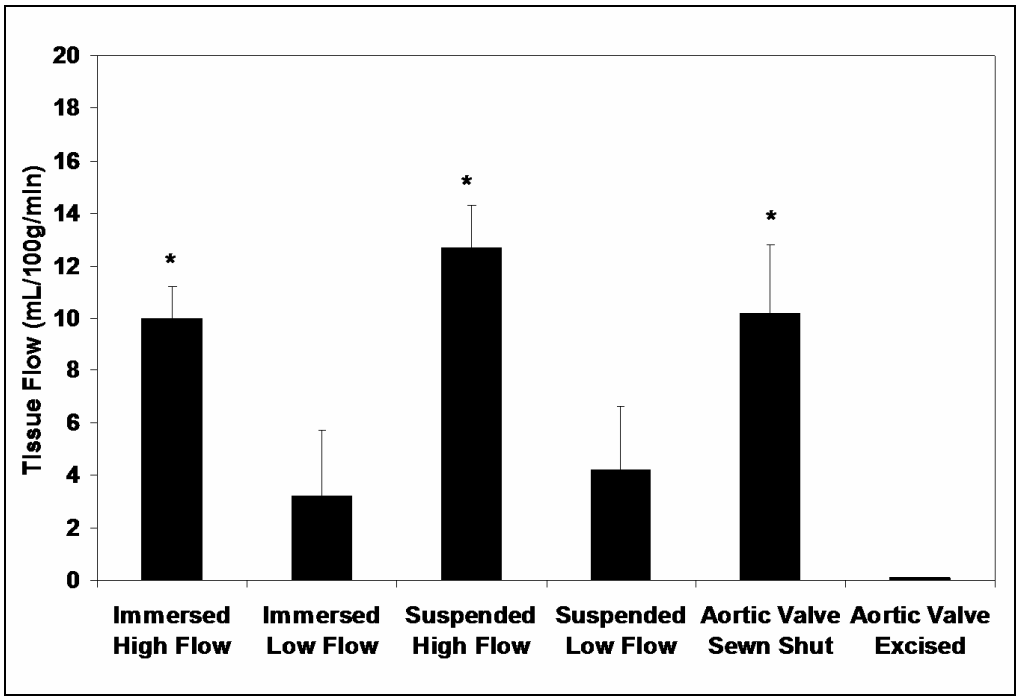
‡-p<.05 vs. Conditions 1,3,and 5

The starting flow rate was also found to influence myocardial nutrient flow. The high (60mL/min) and low (5mL/min) initial flow rates were selected to approximate practical minimum and maximum absolute flow rates for the altered flow rate experiments. The final flow rate resulting in a tissue perfusion rate of 10mL/100g/min was selected, as this rate had been shown effective in previous studies with this device.[15,16] Hearts attached at the low initial flow rate, conditions 2 and 4, generally were found to have lower tissue perfusion rates and higher calculated non-nutrient flow rates than hearts attached at the higher initial flow rate, conditions 1 and 3. The study of heart immersion prior to connection to the inflow line, compared to connection *ex vivo*, was designed to evaluate whether the mass effect of the preservation solution in the perfusion chamber might affect aortic valve competence. The initial

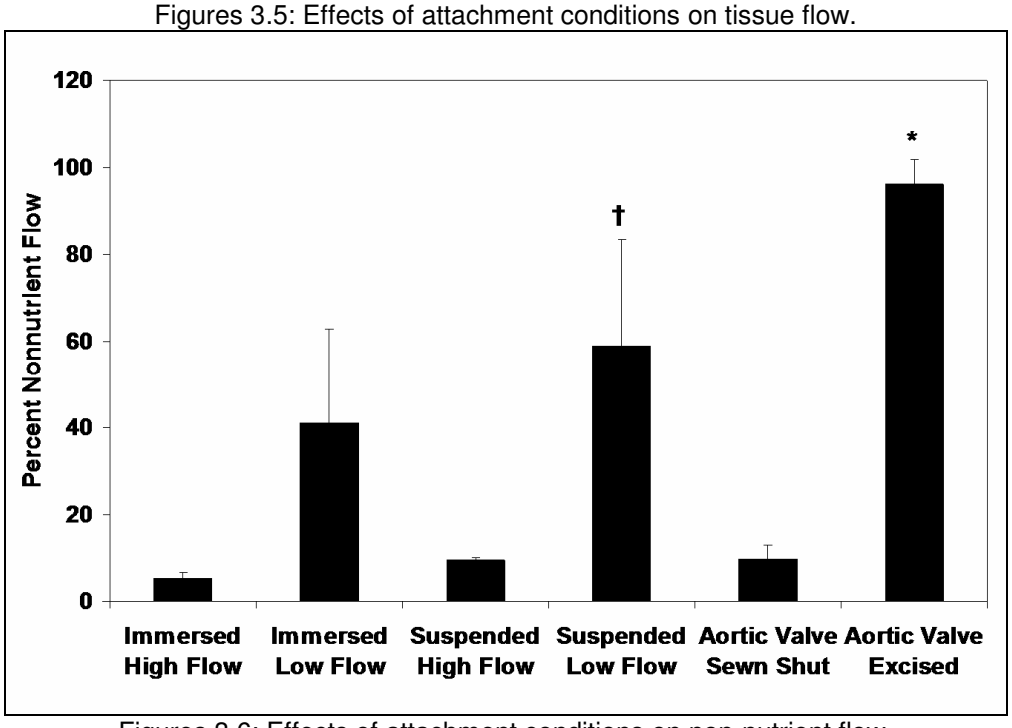
attachment position, immersed or suspended, did not seem to have much influence on perfusate flow distribution. In some animals more than 70% of the labeled microspheres bypassed the coronary capillary network and passed into the container. Conditions 5 and 6 were studied to determine nutrient flow, when maximal efforts were made to ensure aortic valve competence and incompetence, respectively. The measurements made under condition 5, with the aortic valve sutured closed, revealed consistently high myocardial oxygen consumption, tissue flow rates and low non-nutrient flow fraction, while measurements made under condition 6, with the aortic valve excised, showed minimal myocardial oxygen consumption, tissue perfusion and extremely high non-nutrient flow, see Figures 3.4-3.6.



Figures 3.4: Effects of attachment conditions on myocardial oxygen consumption



Figures 3.5: Effects of attachment conditions on tissue flow.



Figures 3.6: Effects of attachment conditions on non-nutrient flow.

The epi/endocardial perfusion ratio was more variable for these experiments but these differences were not significant. No changes in aortic root pressures were noted, Table 3.2.

3.3 Effects of Heart Inclination on Tissue Perfusion

The hearts tested in these experiments revealed varying results with the angles tested, Table 3.3. The first heart was studied at an angle of instance bearing between 0° and 75°. Surprisingly the fraction of flow that was non nutrient was extremely high under almost all conditions in this animal, including the control condition.

Table 3.3: Variables measured at each Heart Inclination

	Heart inclination	Flow Rate ml/100g/min	Non-Nutrient Flow (%)	Tissue Flow (mL/100g/min)
Dog 23				
1	45°	10	47.5	12.0
2	0°	10	96.7	0.1
3	75°	10	15.2	11.8
4	15°	10	71.1	4.3
5	30°	10	100.6	1.0
6	60°	10	90.6	0.8
Dog 24				
1	60°	10	77.8	1.4
2	30°	10	7.1	13.3
3	60°	10	82.3	0.0
4	30°	10	81.8	0.0
5	0°	10	71.1	0.0
6	60°	10	74.8	0.1

Tissue flow rates, as calculated by bead entrapment were correspondingly low. Similarly when hearts were angled at 30° or 60° away from any of the aortic sinuses the fraction lost remained very high and tissue flow was low. The control position (0°) also displayed very high levels of non nutrient flow and low tissue perfusion.

3.4 Effect of Flow Rate on Myocardial Metabolism and Edema Development

Each heart in the 10 hour perfusion studies was perfused at a different set of conditions to attempt to create a range of flow characteristics in the “transported” organs. The microsphere analysis,

performed at the end of the perfusion period, found that these hearts had significant variations in myocardial perfusion, see Table 3.4, column 3.

Table 3.4: Variables Measured at End of 10 Hours of Perfusion

Flow Rate (mL/100g/min)	Aortic Root Pressure (mm Hg)	Tissue Flow (mL/100g/min)	Non-nutrient Flow (%)	Lactate: Alanine Ratio	Weight Gain (%)	Tissue Water Content (%)
15 (Dog 7)	15.6±5.0	16.9±3.2	3.7	0.7	37	81.5
30 (Dog 8)	21.1±1.0	26.6± 6.1	2.2	0.2	44	82.8
15 (Dog 12)	13.9±3.4	10.8±3.2	6.9	-	27	79.9
10 (Dog 13)	14.7±3.6	12.2±3.8	4.2	-	29	78.6
10 (Dog 9)	13.6±0.8	0.1±0.1	89.6	4.4	11	74.5
15 (Dog 14)	18.2±6.6	0.1±0.1	98.9	3.6	18	78.5
10 (Dog 10)	20.8±0.4	0.8±0.9	87.8	4.3	6	74.2

Data are presented for each individual heart. “Tissue flow” represents a mean value (±SEM) of all LV and RV segments sampled. Measurements of aortic root pressure were sampled hourly over the perfusion interval and data are shown as mean (±SEM).

These hearts were then assigned to one of two groups based on whether they had high (>10 mL/100g/min) or low (<1 mL/100 g/min) tissue perfusion. The metabolic state was assessed by measuring myocardial lactate:alanine ratios in the LV samples, Figure 3.7.

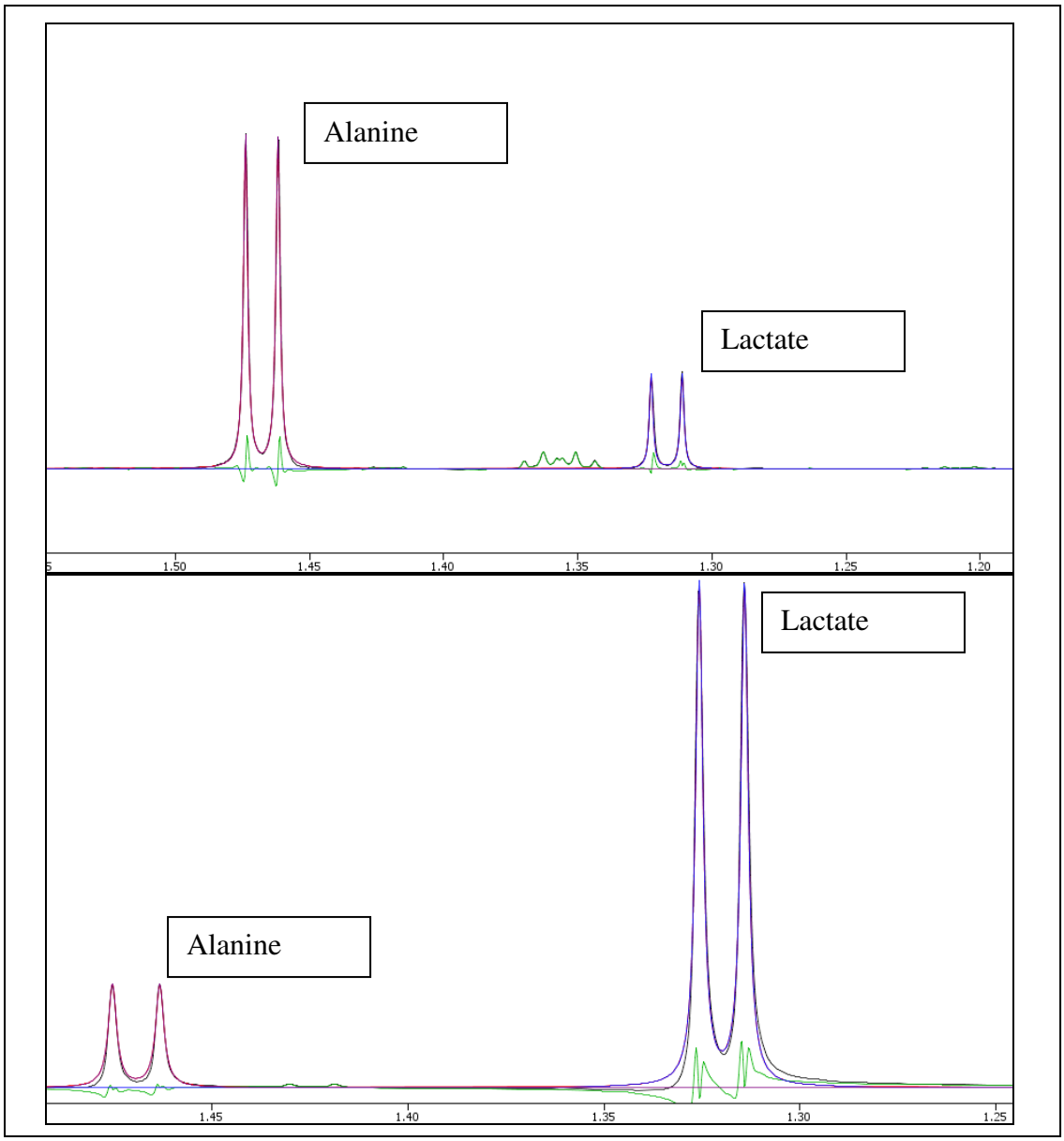


Figure 3.7: Representative magnetic resonance spectra of heart tissue samples from 2 experiments. Perfusion Preservation(Top), Static Preservation (Bottom)

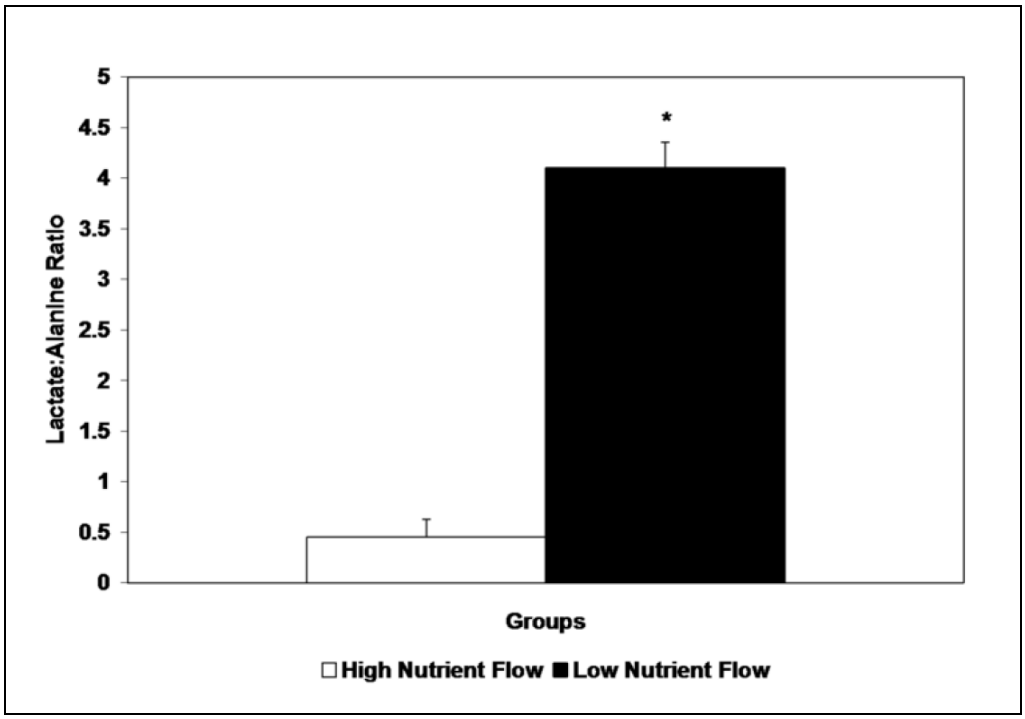


Figure 3.8: Lactate:Alanine ratios of perfused hearts at high and low nutrient flow

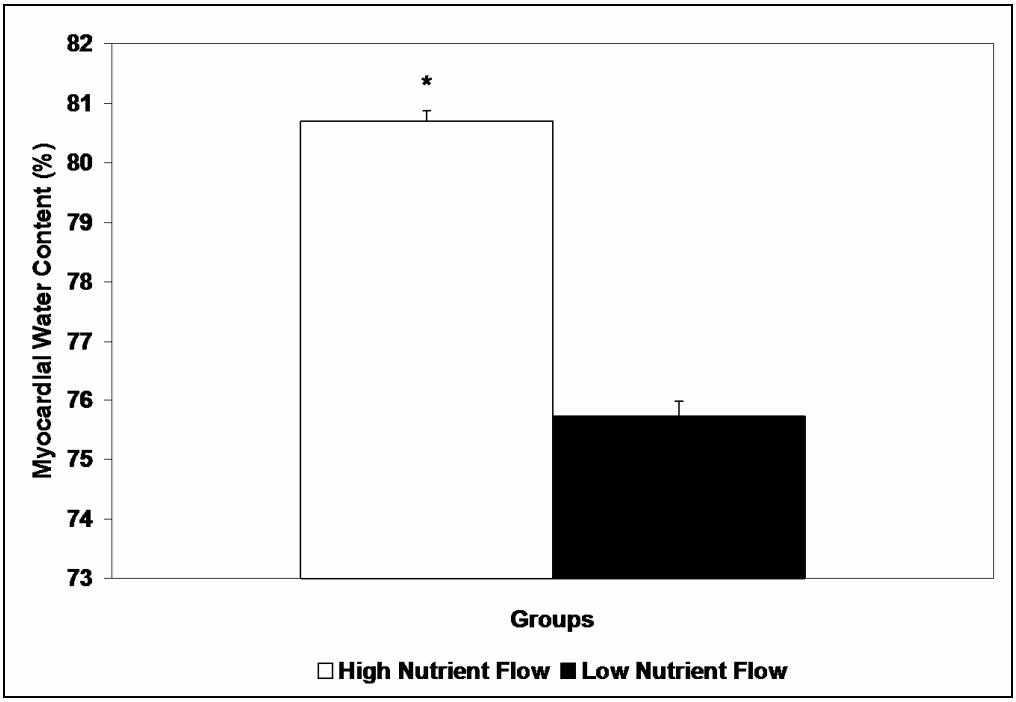


Figure 3.9: Myocardial water content of perfused hearts at high and low nutrient flow

Hearts that were found to have high perfusate flow had low lactate:alanine ratios, consistent with ongoing aerobic metabolism and/or tissue washout of metabolites. Those with low tissue perfusion were observed to have significant lactate accumulation in the myocardium (Figure 3.7). In contrast, hearts with low tissue perfusion had minimal myocardial weight gain over 10 hours (11±4%), while hearts with high tissue flow experienced significant weight gain (34±4%) ($p<.01$), and higher myocardial water content (Figure 3.8), suggesting greater edema formation.

CHAPTER 4

DISCUSSION

The current experiments suggest that myocardial perfusion in the organ transport device is influenced by the delivered flow rate and conditions under which the ascending aorta is interfaced with the device. In our model, device flow rates corresponding to 10ml/100g/min or less tissue perfusion resulted in higher percentages of non-nutrient flow. Non-nutrient flow was also increased when the aorta was attached at low flow rate conditions. These same perfusion changes were observed to a more extreme degree when the aortic valve was excised. The results suggest that initial pressurization of the aortic root results in stable coronary flow and tissue perfusion even if the device flow rate is subsequently decreased. The reason for this is not entirely clear. However, one clue may be that initial higher pressurization of the aortic root, by high perfusate flow delivery, may be required to achieve valve leaflet seating. Once satisfactory closure is obtained, lower flows may then be sufficient to maintain valve competence. At low flows and correspondingly lower aortic root pressures, satisfactory valve leaflet apposition may not be achieved, rendering the valve incompetent with resultant low nutrient flow.

Some clues as to the origins of the observed non-nutrient flow are provided by this study. A system leak would result in reduced preservation solution volume and would be easily detected; however, this was not observed. Intracardiac shunting through coronary arteriovenous connections is another potential source of non-nutrient flow. Under normothermic blood-perfused conditions, the physiologic shunt across the myocardium appears minimal. Human data from Ravin et al suggests only 0.26% of the total cardiac output bypasses the myocardial capillary network.[17] This would represent about 5% of coronary blood flow. The intracardiac shunt fraction during hypothermia has not been previously quantified. Our data from hearts where the aortic valve was sewn closed suggests that the intracardiac shunt was at most 9.7% of delivered flow, assuming that perfect competence was obtained by this manipulation. Measurements made when aortic valve incompetence was guaranteed through excision of the valve nearly eliminated myocardial capillary perfusion. It is likely that aortic valve incompetence of

lesser degrees also influences tissue perfusion, perhaps proportionally. Lower flows delivered by the device, either during aortic attachment or during the course of machine perfusion, result in lower distending pressure in the ascending aorta, a condition more likely to result in incomplete aortic valve closure. As a result, we feel that these data suggest that the majority of non-nutrient flow is due to aortic insufficiency.

It was hypothesized that heart inclination during the procurement and transport process has the potential to create improper valve seating resulting in poor tissue perfusion. However, based on the results of the two hearts sampled in the heart inclination studies, a firm conclusion cannot be drawn at this time. Further testing will be required to elucidate why such results were observed. A possible explanation for the observed data is that based on the randomized sequence of conditions, the hearts were not initially established in the control ($\theta = 0°$) position, and the aortic valve leaflets were never allowed to successfully appose. This lack of leaflet apposition may have persisted even under subsequent conditions where valve competence would be expected thereby preventing effective nutrient flow.

Aortic valve incompetence in a hypothermic perfusion device may have minimal clinical consequence for standard ischemic intervals since even in the worst case scenario, i.e. absent coronary (nutrient) flow, standard static storage conditions would result in reasonable organ preservation in a controlled temperature environment. However, as the storage interval is extended, or the organ harvested starts with suboptimal cardiac function, this scenario becomes more problematic, and inadequate myocardial perfusate delivery might result in an injured heart. Data from the fourth phase of experiments in this study supports this hypothesis. In this phase hearts were perfused for prolonged intervals under conditions that resulted in high or low fractions of non-nutrient flow. In every case, high levels of non-nutrient flow correlated with the expected low levels of tissue perfusion, measured by beads entrapped in the myocardium.

MRS analysis of tissue samples from these perfused hearts found other interesting associations. Myocardial lactate accumulation, expressed as lactate to alanine ratio, was higher when tissue perfusion was low and much lower when tissue perfusion matched the delivery rate of the device. It has been

previously shown that the lactate to alanine ratio is significantly reduced with perfusion preservation, and is associated with improved cardiac function during early reperfusion in a swine model.[15] Thus, designing the perfusion protocol to optimize tissue perfusion would seem to be a desired goal.

However, ongoing perfusion preservation for prolonged intervals has the potential to create myocardial edema, as identified by other investigators.[20,37,65-67] In our study, after 10 hours of simulated transport, hearts with higher degrees of myocardial perfusion and low non-nutrient flow fractions had greater weight gain and higher water content compared to hearts with the reciprocal perfusion pattern. The clinical relevance of these seemingly contradictory results is unclear. Small animal heart perfusion preservation studies and clinical data from kidney transplantation suggest that increased weight gain may be of little importance. However, unlike renal allografts, transplanted hearts must function immediately and myocardial edema, if not reversed prior to weaning from cardiopulmonary bypass, might result in impaired graft function.[32-35] It is possible that edema observed in some of the perfused hearts could be a function of the preservation solution used in this study. Celsior does not contain a large molecule that would exert an oncotic pressure effect within the coronary vasculature, and may therefore be prone to developing myocardial edema. Other solutions now undergoing examination such as Belzer-Machine Perfusion Solution (MPS) have superior oncotic properties and preliminary studies with this solution have shown either minimal weight change or an overall weight loss for perfused hearts. This, or other solutions with similar oncotic properties, could prove to be a more viable alternative.

CHAPTER 5

CONCLUSIONS

Based on the analysis of these studies, the following factors are deemed important to ensuring the optimal delivery of perfusate to the myocardium by the device:

1. Device flow rate is a primary determinant of tissue perfusion.
2. Aortic attachment conditions affect tissue perfusion.
3. Heart inclination may influence tissue perfusion.
4. Adequate tissue perfusion permits a better tissue metabolic profile.
5. Elevated flow rate contributes to myocardial edema development.

The collection of results suggests that initial high pressure, high flow aortic attachment is recommended to ensure proper valve leaflet apposition. Subsequent downward adjustment of device flow is also recommended, to provide adequate tissue perfusion while minimizing the potential for edema development. In addition to reducing edema, our results suggest adequate tissue perfusion will result in a better tissue metabolic profile. By adopting these protocols for device operation better nutrient perfusion of the myocardium in the transported donor heart may be achieved. This in turn may lead to better early graft performance and improved long term patient outcomes.

These studies have significant implications for the clinical perfusion device, LifeCradle HR. It cannot be assumed that all flow delivery by the device will provide nutrient flow to the myocardium. Protocols for use of the LifeCradle HR should consider high initial flow rates to establish aortic valve competence. Predicting aortic valve competence from aortic pressures may be difficult or inaccurate as our studies have shown little variation in aortic root pressure despite widely varying perfusion conditions. We suspect that hearts positioned at high inclinations should be avoided. However, while heart inclination was observed to affect tissue perfusion in the prototype device, this is not suspected to be a problem for the clinical model since extreme tilt angles will not be encountered simply due to the improved design engineering of the clinical device. Finally, other solutions that avoid edema

accumulation may be preferable in organs perfused at higher flow rates particularly for longer storage intervals.

CHAPTER 6

FUTURE STUDIES

These studies have advanced our understanding of the effects of device conditions on myocardial nutrient perfusate delivery, a concept that is critical to the success of any machine perfusion strategy. Armed with this information, we now plan to move forward with testing of the device using protocols that have been adapted to optimize perfusion conditions.

The first of these trials will test the effects of the perfusion device on heart preservation over a longer (8 hour) storage interval. This interval is beyond the current limits of conventional static storage techniques and, if successful, could offer an expanded storage time that could safely increase transport distance. This in turn might allow time for better donor-recipient matching and allow transplantation on a less urgent basis. Studies will be performed in a canine model and will evaluate early reperfusion function and myocardial metabolism using the techniques that we have applied in prior studies in our laboratory. An extension of these trials is under consideration that would test the effects of machine perfusion preservation on donor hearts that have already sustained some degree of damage. These experiments will model the scenario of "donation after cardiac death (DCD)" whereby the donor has undergone an interval of cessation of cardiac activity prior to organ harvest. This results in a donor heart that has sustained a brief interval of ischemic damage. As the perfusion device can deliver oxygenated solution to the heart and washout by-products of metabolism, it may offer an opportunity to render some of these hearts useable for transplantation. If successful, this could substantially increase the donor pool, making more organs available to more patients.

Another area for future studies will involve testing of the clinical device with human hearts procured from donors whose hearts have been deemed unacceptable for transplantation due to advanced age, existing cardiac disease, or viral diseases. These hearts will be harvested, attached to the device at the clinical sites, and stored for 12 hours. Metabolic assessments and measurements of myocardial edema will be done. These studies will provide information about the effects of machine

perfusion on myocardial metabolism in humans and will also enable us to refine donor procurement protocols for the device in a real-world setting. To date, five discarded human hearts have been recovered from donor sites. Four of the five trials have been perfusion studies and one trial was a static control. It is hoped that a series of twelve hearts will be evaluated to thoroughly gain experience and understanding with the clinical device. Preliminary results from the Discarded Human Heart (DHH) trials have revealed similar metabolic profiles to those observed in the canine studies described above. Of interest, we have also observed a decrease in heart weight over the 12 hour storage interval in most human hearts that have been perfused using Belzer-MPS.

Finally, participation of our research group in the clinical trial, currently scheduled for next year, will also provide valuable knowledge of the potential for machine perfusion preservation in clinical heart transplantation. It is our hope that this process will lead to improvements in the results of clinical heart transplantation, offering improved outcomes to the large population of patients suffering from heart failure.

APPENDIX A

PREVIOUS MACHINE PERFUSION INVESTIGATIONS

Authors	Journal Citation		Species	Duration of preservation	Preservation Solution	Perfusion Temperature (℃)	Benefits of Perfusion
Aizaki M et al.	Transplant Proc	Nov;32(7):2409-10	Canine	12 hours	Celsior and UW	4	Myocardial phosphocreatine, inorganic phosphate, Beta-ATP, and pH levels were higher in Celsior vs. UW
Calhoon JH et al.	Ann Thorac Surg	Jul;62(1):91-3	Canine	12 hours	UW - Viaspan	4	Cardiac output and rate of change of LV pressure were comparable to control. Minimal inotrope doses required for recipient resuscitation
Copeland JG et al.	Ann Surg	Dec;178(6):687-92	Canine	24-28 hours	Krebs	0-3	Perfusate Potassium and CPK levels rose while perfusate glucose fell. No consistent trend in lactate conc. was seen
Ferrera R, Hadour G.	Transplant Proc	Dec;30(8):4340-3	Pig	24 hours	St. Thomas	4	Ultrastructural tissue samples were comparable to normal tissues with slight damage observed in mitochondria
Ferrera R et al.	J Heart Lung Transplant	Aug;19(8):792-800	Pig	24 hours	St. Thomas, "New Perfusion Solution" (NPS)	4	Ultrastructural damage with microperfused NPS samples scored similarly to control whereas the St. Thomas microperfused samples revealed slight damage to cellular components
Fitton TP et al.	Clin Transplant	18 Suppl 12:22-7	Canine	24 hours CHP, 4 hours SP	Not stated	5	CHP stored hearts demonstrated adequate hemodynamic function to support systemic circulation
Fitton TP et al.	Ann Thorac Surg	Nov;80(5):1812-20	Canine	24 hours CHP, 4 hours SP	Modified Belzer	5	Reduced oxidative stress and preserved DNA repair processes (OGG1, MYH, MSH2)
Koike N et al.	J Heart Lung Transplant	Jul;22(7):810-7	Canine	1 hr perfusion with 3 hrs SP	Celsior	4	Cariac output recovery was 80% 1hr after weaning from CPB. End-systolic maximal elastance fell only 2 mmHg/ml compared to baseline of 11.9 mmHg/mL
Hassanein WH et al.	J Thorac Cardiovasc Surg	Nov;116(5):821-30	Pig	12 hours	Normothermic Blood	37	Blood gas measurements over perfusion interval remained within normal limits. After reperfusion hearts returned to normal sinus rhythm without pacing or direct current
Jones BU et al.	ASAIO J	May-Jun;47(3):197-201	Rabbit	8 hours	PEG-Hemoglobin	20	PEG-Hb hearts maintained greater baseline function in developed LV pressure, maximum rates of LV contraction and relaxation, and coronary flow
Wicomb WN et al.	J Surg Res	Mar;40(3):276-84.	Pig/Baboon	48 hours	Unnamed Cardioplegic Solution	4-10	Baboon heart autotransplant saw no loss of function and functional recovery based on tissue histology
Ogiwara H et al.	J Cardiovasc Surg (Torino).	Jun;39(3):313-20.	Canine	12 hrs SP; 1 hr CHP	UW and Modified Kawakami	4	Phosphocreatine, β-ATP, and myocardial pH levels all rose after coronary perfusion recovery. CPK and lactic acid values both decreased dramatically after perfusion.
Hasegawa Y et al.	J Cardiovasc Surg (Torino).	Jun;41(3):363-70.	Canine	12 hrs SP; 1 hr CHP	UW and Diluted Blood (Both Oxygenated)	4 and 15	Phosphocreatine, β-ATP, and myocardial pH levels all rose after coronary perfusion recovery. CPK and lactic acid values both decreased dramatically after perfusion.
Ferrera R et al.	Ann Thorac Surg	May;57(5):1233-9.	Pig	24 hours	St. Thomas	4	Glucose production was increased, noting that St. Thomas solution does not include glucose or sugars.
Hudon MP et al.	J Heart Lung Transplant	Sep-Oct;10(5 Pt 1):704-9	Pig	5 hours	Unnamed Cardioplegic Solution	8-10	Fatty acid turnover was maintained 2 hours post-op.
Kitamura M et al.	ASAIO J	Jul-Sep;38(3):M163-6	Canine	3-5 hours	UW	Not defined	Ability to be weaned from bypass without catecholamine support
Ohtaki A et al.	J Heart Lung Transplant	Mar;15(3):269-74.	Canine	12 hrs SP; 1 hr CHP	Oxygenated UW	4	P-NMR of PCr, α-,β-,γ-ATP revealed significant increases after 1-hour coronary perfusion as compared to static preservation. CPK and Lactic acid values decreased significantly after perfusion
Suehiro K et al.	Ann Thorac Surg	Jan;71(1):278-83	Canine	Up to 60 minutes	St. Thomas	4	All subjects who were successful in ejecting 80 mmHg of afterload for t> 15 min after coronary perfusion were able to be weaned from CPB and Dopamine. These hearts showed significantly higher systolic pressure associated with lower LA pressure
Wicomb W et al.	J Thorac Cardiovasc Surg	Jan;83(1):133-40	Human	Variable from 6 to 15 hrs	Unnamed Oxygenated Cardioplegic Solution	4-10	Heterotopic transplant method used; 3 of the 4 patients were able to accept the heart within the first 20 hours post-transplant
Toledo-Pereyra LH et al.	Ann Thorac Surg	Jan;27(1):24-31	Canine	24 hours	Sacks' solution II	7	Significant damage and lactic acid buildup were observed at the highest pressure, 80 mmHg, whereas minimal to moderate damage was observed in the lower groups, 25 and 50 mmHg respectively. Hearts transplanted from the 25 mmHg group had the greatest post-transplant recovery

Authors	Journal Citation		Edema in Perfusion Group	Other findings
Aizaki M et al.	Transplant Proc	Nov;32(7):2409-10	No significant water difference for either group	Blood washout from coronaries performed earlier with Celsior vs. UW
Calhoon JH et al.	Ann Thorac Surg	Jul;62(1):91-3	No significant water difference for perfused group, subjective edema in nonperfused	Nonperfused hearts were unable to support recipient bypass and led to failure
Copeland JG et al.	Ann Surg	Dec;178(6):687-92	An average of 38% increase in heart weight was observed	Light and electron microscopic analysis were performed on biopsies revealing a few intracellular vacuoles and a large number of capillary pinocytotic vacuoles
Ferrera R, Hadour G.	Transplant Proc	Dec;30(8):4340-3	No significant water difference for either group	ATP, Total amount of Adenine Nucleotides, and Energetic Index decreased between the preserved groups, however, the perfused group remained significantly higher. LV Developed Pressure was higher for perfused group than cold storage over 24hr interval
Ferrera R et al.	J Heart Lung Transplant	Aug;19(8):792-800	Microperfusion storage with St. Thomas solution yielded an average of 40% increase in heart weight whereas microperfusion and simple storage with the unnamed solution led to a net water loss	Post-ischemic coronary flow of microperfused NPS hearts was more than two times greater than microperfused St. Thomas hearts, and equal to that of control
Fitton TP et al.	Clin Transplant	18 Suppl 12:22-7	An average of 18% increase in heart weight was observed	Oxidative stress evaluated by expression of 8-oxoG was significantly increased in the SP group vs. the CHP group
Fitton TP et al.	Ann Thorac Surg	Nov;80(5):1812-20	An average of 27% increase in heart weight was observed	Decreased expression of DNA repair enzymes in SP gorup may be indicative of low temperature environment
Koike N et al.	J Heart Lung Transplant	Jul;22(7):810-7	Water content was 76.3 ± 1.3% after 2hrs of weaning from CPB, significantly different than the SP group 81.1 ± 1.7%	H&E staining revealed myocardial necrosis, dissolution of myocardial cells, and changes in the structural framework. These findings were more severe in the SP subjects than the CPs. Periodic acid Schiff staining showed that glycogen was better preserved in the CP group
Hassanein WH et al.	J Thorac Cardiovasc Surg	Nov;116(5):821-30	No siginficant water difference between blood perfused group and control.	Severe acidosis and edema were achieved in UW stored subjects. Coronary endothelial function evaluated by vasodilatory response of preconstricted vasular rings revealed diminished response in UW stored hearts compared to the blood-perfused group
Jones BU et al.	ASAIO J	May-Jun;47(3):197-201	Qualitatively less edema was observed in PEG-Hb based on greater oncotic properties	Performance testing was doubled from 4 hours to 8 hours with test group and no significant changes were observed
Wicomb WN et al.	J Surg Res	Mar;40(3):276-84.	All groups demonstrated at least a 47% weight gain, the greatest in the pig hearts at 70%	LV pressure buildup in perfused pigs was less than freshly excised hearts. Maintaining temperature above 1℃ is critical for functional preservation.
Ogiwara H et al.	J Cardiovasc Surg (Torino).	Jun;39(3):313-20.	No significant water difference for any groups	The inclusion of calcium may play an important role in the use of an extracellular perfusion solution
Hasegawa Y et al.	J Cardiovasc Surg (Torino).	Jun;41(3):363-70.	Mean Water Content in diluted blood group (79 ± 0.4%) was significantly higher than UW group (74 ± 1.1%) after perfusion interval	Myocardial Interstitial Tissue Space (ITS) rate of the subepicardium rose significantly after 12hrs and then dropped back down after coronary perfusion, with the diluted blood group being slightly higher. For the subendocardium, levels continued to rise even after perfusion with the UW group increasing dramatically
Ferrera R et al.	Ann Thorac Surg	May;57(5):1233-9.	Hearts preserved in the low pressure group and low flow group contained a severe amount of edema compared to control and simple storage	Microperfusion indicated slight damage to mitochondria, compared to none observed with low pressure perfusion. Overall the total adenine nucleotide levels were not statistically different.
Hudon MP et al.	J Heart Lung Transplant	Sep-Oct;10(5 Pt 1):704-9	Not discussed	Radioionated free fatty acid imaging of I-IPPA may be a useful tool and comparative to tissue lactate levels
Kitamura M et al.	ASAIO J	Jul-Sep;38(3):M163-6	Not discussed	Waterproof packed device that provided sterile in-storage hypothermic perfusion
Ohtaki A et al.	J Heart Lung Transplant	Mar;15(3):269-74.	No significant water difference was obtained after the 1-hour coronary perfusion	Ventricular fibrillation occurred rapidly after removal of recipient cross-clamp, but restarted using DC shock. LVP and LV dp/dt values were significantly better in the perfused group versus statically stored hearts
Suehiro K et al.	Ann Thorac Surg	Jan;71(1):278-83	Not discussed	No thrombi were found in the major coronary arteries and veins after perfusion and transplantation, yet thrombi were found in both ventricles and inferior vena cava.
Wicomb W et al.	J Thorac Cardiovasc Surg	Jan;83(1):133-40	Not discussed	Slight reduction (< 25%) of coronary flow during perfusion for all hearts
Toledo-Pereyra LH et al.	Ann Thorac Surg	Jan;27(1):24-31	Low pressures generated edemic buildup comparable to statically preserved hearts, approximately 10%. Hearts preserved at higher pressures of 50 mmHg and 80 mmHg generated edemic buildup of 20% and 40% respectively	Systolic perfusion pressure increased with higher pressures, and decreased at lower pressures. Perfusion flow of the different pressures converged to approximately 1 mL/min/g during the preservation interval.

Authors	Journal Citation		Species	Duration of preservation	Preservation Solution	Perfusion Temperature (°C)	Benefits of Perfusion
Wicomb WN, Collins GM.	Transplantation	Jul;48(1):6-9.	Rabbit	24 hours	UW	0	Hearts microperfused with fresh UW and UW with PEG20 displayed the best cardiac output and ablility to generate systolic pressures of 100 cm H2O
Wicomb WN et al.	Transplantation	Jan;43(1):23-9	Baboon	48 hours	2 Unnamed Oxygenated Cardioplegic Solutions	4-10	Coronary flow in Group A did not change during the storage interval. Coronary sinus lactate dehydrogenase increased significantly in Group B. Coronary sinus lactate for both groups decreased over time, however the two values differed from each other significantly.
Wicomb WN et al.	Transplantation	Nov;34(5):246-50.	Pig	20-24 hours	Unnamed Oxygenated Cardioplegic Solution	4-10	Mean myocardial oxygen uptake and coronary flow increased significantly over preservation period
Watson DC Jr.	Transplant Proc	Mar;9(1):297-9	Canine	24 hours	Unnamed Oxygenated Cardioplegic Solution	3	All perfused hearts survived transplantation and observatory 24 hour post-op. Mild left ventricular damage was seen.
Peltz M et al.	Surgery	Oct;138(4):795-805	Rat	200 minutes	Celsior and UW	4	MVO2 during storage was much higher in the perfusion preservation groups. Significant utilization of exogenous substrate by oxidative metabolism was identified in the Celsior perfusion group
Tsutsumi H et al.	J Surg Res	Apr;96(2):260-7	Canine	24 hours	Celsior	4	Significantly higher (SH) ß-ATP levels, pH in perfused group.
Nameki T et al.	J Surg Res	Sep;135(1):107-12. Epub 2006 Feb 28	Canine	12 hours	Celsior	4	No significant difference in hemodynamic.
Masters T et al.	J Heart Lung Transplant	May;21(5):590-9	Canine	18 hours	UW	4-4.5	Regained 50-60% original function. Substrate uptake normal >>glycolytic and lipolytic processes functional.
Smulowitz P et al.	ASAIO J	Jul-Aug;46(4):389-96					
Stowe D et al.	Am J Physiol Heart Circ Physiol	Apr 13; [Epub ahead of print]	Guinea Pig			37.2 ± 0.1	
Minten J et al.	J Heart Lung Transplant	Jan-Feb;10(1 Pt 1):71-8	Rat, Dog	24 hours	Bretschneider-HTK	2 to 4	Higher levels of ATP and Creatine Phosphate
Ferrera R et al.	J Heart Lung Transplant	May-Jun;12(3):463-9.	Pig	6,12,24 hours	St. Thomas' solution	4	Higher levels of HEPs. Higher left ventricular developed pressure (LVDP) than cold storage alone.
Tsutsumi H et al.	Transplant Proc	Nov;32(7):2415-6.	Canine	24 hours	Celsior		Myocardial phosphocreatine, inorganic phosphate, Beta-ATP, and pH levels were higher in perfused group vs static preservation (simple immersion)
Tsutsumi H et al.	Int J Angiol	Jan;10(1):15-19	Canine	12 hours	Celsior		Myocardial phosphocreatine, inorganic phosphate, Beta-ATP, and pH levels were higher in perfused group vs static preservation (simple immersion)
Rosenstrauch D et al.	Tex Heart Inst J	30(2):121-7	Pig	not described, probably <30 min	Houston cardioplegia		Hearts could achieve approx 60% of pre-excision stroke work in this device
Osaki S et al.	Ann Thorac Surg	Jun;81(6):2167-71	Pig	30 min warm arrest, then 5, 20 or 60 min of blood CP perfusion	4:1 leukocyte-depleted blood with Modified St Thomas		Hearts reperfused for 20 minutes before transplanation had better functional recovery than those reperfused for 5 or 60 minutes
Rao V et al.	J Thorac Cardiovasc Surg	Sep;122(3):501-7	Pig	5 hours	Arrested with crystalloid CP, perfused with harvested shed donor blood		Both groups perfused during storage. Group perfused at 25oC had improved recovery of developed pressure, both groups had worsened diastolic compliance after transplantation.
Kadipasaoglu KA et al.	Tex Heart Inst J	1993;20(1):33-9	Human	< 90 min	Unspecified CP, modified donor blood for reperfusion		11/12 hearts resumed beating in this modified Langendorff model. They could be supported for 96+/-49 min. Low pressures were generated. O2 consumption was observed. Reperfusion flow rates were 32-150 cc/min/100 g heart tissue.
Kuhn-Regnier F et al.	Eur J Cardiothorac Surg	Jan;17(1):71-6	Pig	14 hours for 3 groups, 3 hours for control group	HTK, mHTK (HTK with hyaluronidase), or mHTK with coronary oxygen perfusion		The perfusion applied inthis study is only gaseous oxygen (no crystalloid). Hearts protected with mHTK and coronary oxygen persufflation (COP) had better functional recovery (CO and +dP/dt), ATP and total nucleotides better preserved with mHTK and COP, lactate levels higher in mHTK and COP (?)
Kay L et al.	Cardiovasc Res	34(3):547-56	Rat	6, 15, or 24 hours	St. Thomas		Flow rate used in the perfused group was 0.3 ml/min (~30 ml/min/100 g). Perfusion with St Thomas slowed the decline in ATP/Pi ratio and led to better LVDP (although recoverfy was substantially impaired after 6 hours). After 15 hours of storage, perfused hearts did not show evidence of damage to the outer mitochonndrial membrane as was seen with static preservation. The degree of activation by creatine of the respiration in skinned cardiac fibers was minimally impaired at 6 hours but significantly depressed at 15 hours in static groups. This change was avoided by perfusion preservation at 15 hours.

Authors	Journal Citation		Edema in Perfusion Group	Other findings
Wicomb WN, Collins GM.	Transplantation	Jul;48(1):6-9.	Not discussed	Stored solutions, 3-6 months, performed poorly, hypothesized to be related to oxidation of glutathione
Wicomb WN et al.	Transplantation	Jan;43(1):23-9	Significant increase was observed in both groups with perfusate Group A having the higher value. Perfusate Group A contained lower contaminant concentrations of Fe++, Pb, and arsentae than Group B	Group B hearts displayed decreased metabolic activity with none being able to be successfully transplanted. This was associated with cellular destruction seen before reperfusion.
Wicomb WN et al.	Transplantation	Nov;34(5):246-50.	Significant edema was observed, 79% weight increase, with loss of 7.5% during reperfusion.	Hystologically the myofibers and nuclei were well preserved with minimal edema and damage. Left ventricular pressure decreased compared to the fresh control hearts.
Watson DC Jr.	Transplant Proc	Mar;9(1):297-9	An average of 34% increase in heart weight was observed	Long term endogenous stores need to be documented
Peltz M et al.	Surgery	Oct;138(4):795-805	The Celsior perfusion group had the lowest myocardial water content of all groups	Exogenous glucose was oxidized significantly in the Celsior perfusion group only. Perfusion preservation groups recovered more quickly after reperfusion, compared to static.
Tsutsumi H et al.	J Surg Res	Apr;96(2):260-7	Histo showed slight vs. severe interstitial edema in perfused vs. static group	CO and LVP recovery rates not statistically significant but better hemodynaics observed in perfused group. Orthotopic Transplantation
Nameki T et al.	J Surg Res	Sep;135(1):107-12. Epub 2006 Feb 28	Lower water content & less swelling in the SI + CP group vs. the CP group.	Found their method to be suitable but optimal ratio of coronary perfusion to simple immersion has not been found.
Masters T et al.	J Heart Lung Transplant	May;21(5):590-9	Not mentioned	ATP and creatine phosphate leves decreased during preservation but returned to control during reperfusion. Apoptosis but no necrosis. Myocardial function decreased with increasing number of apoptotic cells over time.
Smulowitz P et al.	ASAIO J	Jul-Aug;46(4):389-96		REVIEW: Info for introduction and/or source for additional references
Stowe D et al.	Am J Physiol Heart Circ Physiol	Apr 13; [Epub ahead of print]		
Minten J et al.	J Heart Lung Transplant	Jan-Feb;10(1 Pt 1):71-8	Slight increase in tissue water content in both species (dog 76.3% ± 0.4% to 79.1% ± 1.2%). Suggests that functional recovery is directly proportional to ATP concentration though not tested in this study via reperfusion.	Perfused hearts were not immersed in solution. Hearts had significantly higher levels of HEPs than those in cold storage in saline solution only.
Ferrera R et al.	J Heart Lung Transplant	May-Jun;12(3):463-9.	Significantly higher edema in microperfused group resulting in higher hydrostatic pressure in this group.	
Tsutsumi H et al.	Transplant Proc	Nov;32(7):2415-6.	Not described	Note: low flow rates: 0.5 - 0.7 mL/min in perfusion group
Tsutsumi H et al.	Int J Angiol	Jan;10(1):15-19	Not described	Note: low flow rates: 0.5 - 0.7 mL/min in perfusion group. This study is probably a subset of the one described in #9 above.
Rosenstrauch D et al.	Tex Heart Inst J	30(2):121-7	Not quantified, but seen histologically in the one heart that was "re-resuscitated"	This is not truly a study of perfusion preservation - it is a description of a model
Osaki S et al.	Ann Thorac Surg	Jun;81(6):2167-71	Hearts reperfused for 20 minutes before transplanation had better less edema (10% weight gain) than those reperfused for 5 or 60 minutes (20% gain)	Flow rates not described - prefusion pressure of 40 mm Hg applied at 20oC. Lactate extraction after initial reperfusion returned to zero by 20 min. This is fundamentally a study of resuscitation conditions with perfusion preservation.
Rao V et al.	J Thorac Cardiovasc Surg	Sep;122(3):501-7	Not described	No difference between groups in lactate extraction or pH gardient across myocardium. Tepid group (25oC) had higher oxyegn extraction at all timepoints
Kadipasaoglu KA et al.	Tex Heart Inst J	1993;20(1):33-9	Not quantified, but seen histologically	This is not truly a study of perfusion preservation - it is a description of a model
Kuhn-Regnier F et al.	Eur J Cardiothorac Surg	Jan;17(1):71-6	Water content significantly increased after storage and reperfusion in all groups except mHTK and COP (unchanged from pregrafting values)	Air embolism after oxygen persufflation was prevented by perfusion of the coronary arteries with a low viscosity electrolyte solution prior to blood reperfusion. Not truly machine perfusion as only gaseous oxygen perfsued the coronaries in this study.
Kay L et al.	Cardiovasc Res	34(3):547-56	Not evaluated	This study shows that continuous perfusion of the heart even at low flow rates with a crystalloid solution reduces many of the changes seen under ischemic conditions. In particular, damage to the outer mitochondrail membrane (effect of cytochrome c), damage to the inner mitochondrial membrane (increase in initial respiratory rate with only substrates), decrease in delta VMAX, and loss of the stimulating effect of creatine are all reduced. Alterations to the process of channelling of energy from mitochondia occur before any other damage to mitochondrial respiratory function can be seen.

APPENDIX B

LIFECRADLE EVOLUTION

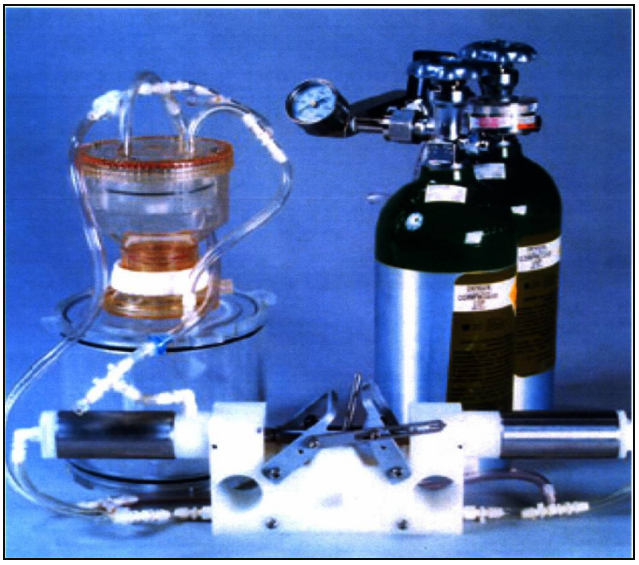


Figure B1: Model 1. Designed & fabricated in 1999 by Les Matthews, Ph.D.

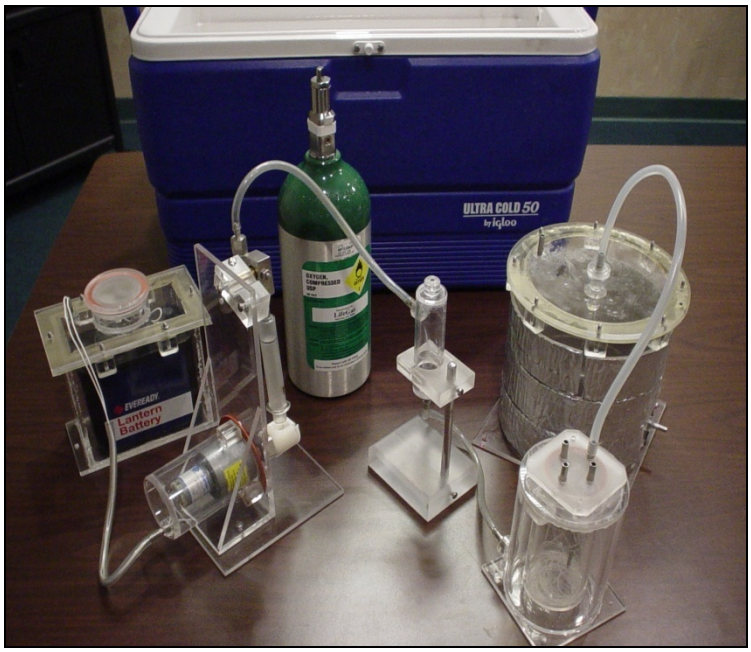


Figure B2: Model 2. Designed in 2000 at Penn State by George Panal-Design Engineer for Artificial Heart

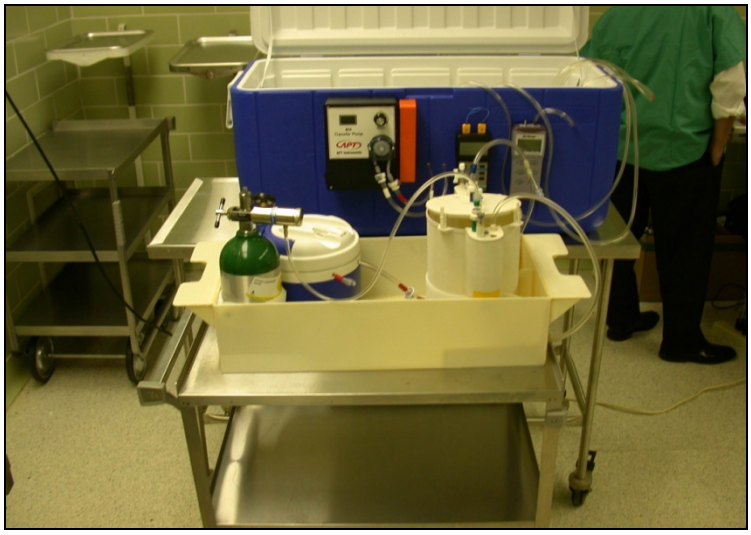


Figure B3: Model 3. Designed in 2001 by Tommy Davis

Figure B4: Model 5. LifeCradle™ HR. Designed by OTS, Inc.; Probasco; & The RealTime Group 2006-2008

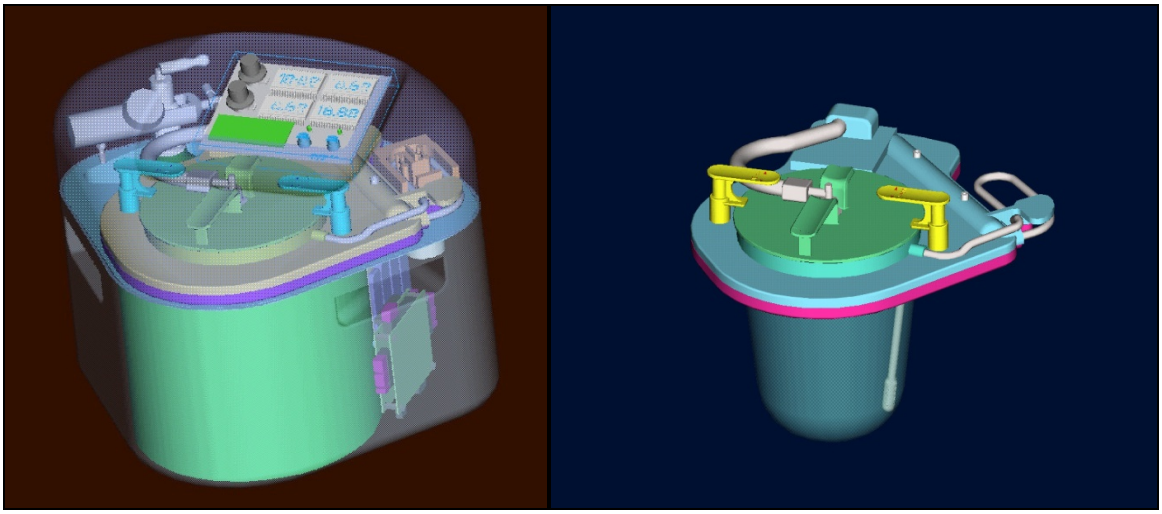


Figure B5: LifeCradle™ HR Components: Durable Unit (left) and Disposable Circuit (right)

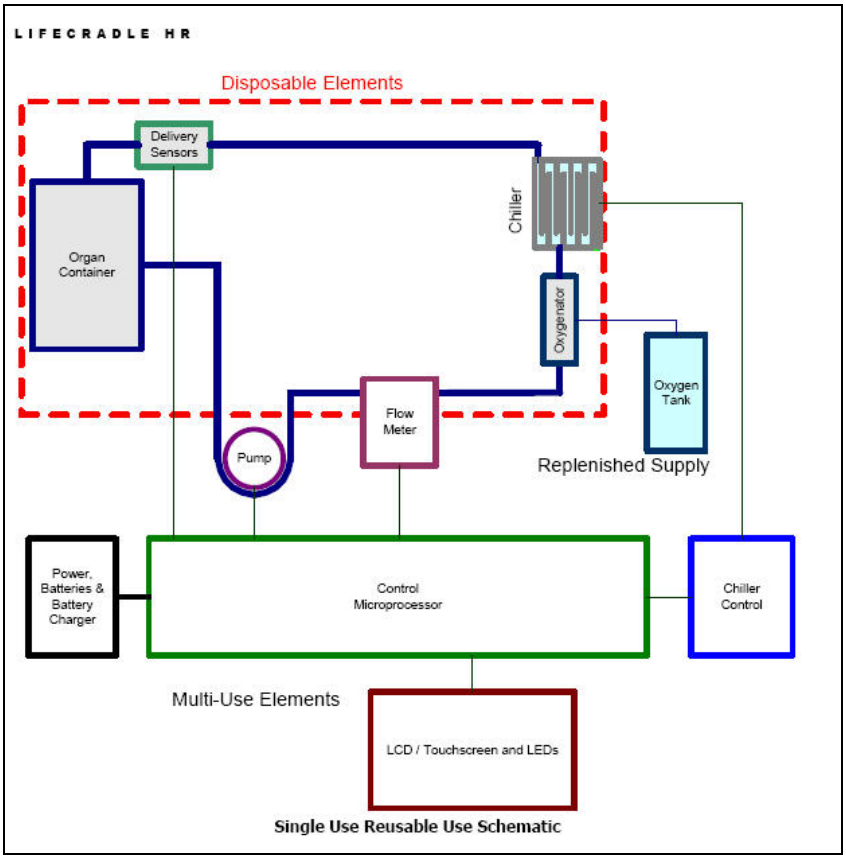


Figure B6: Schematic Drawing of LifeCradle™ HR. (Franklin)

REFERENCES

1. United Network for Organ Sharing. (2008, April 2). *United Network for Organ Sharing: Organ Donation and Transplantation*. Retrieved 2008 2, April, from UNOS website: http://www.unos.org/news/newsDetail.asp?id=147

2. Columbia University Department of Surgery. (2008). *Heart Transplant, History of Heart Transplantation*. Retrieved April 2, 2008, from Columbia University Department of Surgery: http://www.cumc.columbia.edu/dept/cs/pat/hearttx/history.html

3. Silva, D. (2002). *A General Overview of Metabolic Pathways*. Retrieved April 2, 2008, from Universidade Fernando Pessoa: http://www2.ufp.pt/~pedros/bq/integration.htm

4. Mallory, C. (2006, April 17). *Anaerobic Cell Metabolism & Regulation*. Retrieved April 2, 2008, from University of Miami Biology: http://fig.cox.miami.edu/~cmallery/bil257.htm

5. Dineen, D. (2008, 2008). *Heart Transplantation*. Retrieved April 2, 2008, from Wikipedia: http://en.wikipedia.org/wiki/Heart_transplant

6. Jurmann MJ, S. H., Dammenhayn L, Haverich A. (1988). "Oxygen-derived free radical scavengers for amelioration of reperfusion damage in heart transplantation." J Thorac Cardiovasc Surg 95(3): 368-377.

7. Suehiro K, M. M., Yamaguchi H, Takagaki M, Hisamochi K, Morimoto T, Sano S. (2001). "Posttransplant function of a nonbeating heart is predictable by an ex vivo perfusion method." Ann Thorac Surg 71(1): 278-283.

8. Demmy, Todd L. et al., "Organ Preservation Solutions in Heart Transplantation--Patterns of Usage and Related Survival," Transplantation, Jan. 27, 1997, 63:262-9.

9. Iyer, B. (2005). *Indmedica*. Retrieved April 2, 2008, from Indmedica: http://www.indmedica.com/journals.php?journalid=8&issueid=87&articleid=1180&action=article

10. Aizaki M, T. I., Oshima K, Mohara J, Tsutsumi H, Koyano T, Tokumine M, Ishikawa S, Morishita Y. (2000). "Effects of Celsior solution on long-term preservation of canine hearts with a new portable hypothermic perfusion apparatus: a preliminary study." Transplant Proc **32**(7): 2409-10.

11. Ferrera R, H. G. (1998). "A reliable method for long-term (24-hour) hypothermic transport of cardiac graft." Transplant Proc 30(8): 4340-4343.

12. Ferrera R, L. A., Marcsek P, Guidollet J, Verdys M, Dittmar A, Dureau G. (1994). "Comparison of different techniques of hypothermic pig heart preservation." Ann Thorac Surg 57(5): 1233-1239.

13. Ferrera R, M. P., Larese A, Girard C, Guidollet J, Dittmar A, Dureau G. (1993). "Comparison of continuous microperfusion and cold storage for pig heart preservation." J Heart Lung Transplant 12(3): 463-469.

14. Ferrera R, M. P., Hadour G, Chiari P, Chambers D, Rodriguez C. (2000). "Microperfusion techniques for long-term hypothermic preservation." J Heart Lung Transplant 19(8): 792-800.

15. Rosenbaum DH, Peltz M, DiMaio JM, Meyer DM, Wait MA, Merritt ME, Brown R, Chao RY, Ring WS, Jessen ME. Perfusion preservation vs. static preservation for cardiac transplantation: effects on myocardial function and metabolism. J Heart Lung Transplant 2008; 27(1): 93-9.

16. Rosenbaum DH, , Peltz M, Merritt ME, Thatcher JE, Sasaki H, Jessen ME. Benefits of Perfusion Preservation in Canine Hearts Stored for Short Intervals. Journal of Surgical Research 2007;140:243-249.

17. Ravin MB, Epstein RM, Malm JR. Contribution of Thebesian veins to the physiologic shunt in anesthetized man J Appl Physiol 20: 1148-1152, 1965

18. Calhoon JH, B. L., Gelineau JF, Felger MC, Naples JJ, Miller OL, Sako EY. (1996). "Twelve-hour canine heart preservation with a simple, portable hypothermic organ perfusion device." Ann Thorac Surg 62(1): 91-93.

19. Chambers DJ, T. A., Hearse DJ. (1992). "Long-term preservation of the heart: the effect of infusion pressure during continuous hypothermic cardioplegia." J Heart Lung Transplant 11(4 pt. 1): 665-675.

20. Copeland JG, J. M., Spragg R, Stinson EB. (1972). "Successful orthotopic transplantation of canine hearts after in vitro preservation for 24 to 28 hours." Surg Forum 23: 202-204.

21. Copeland JG, J. M., Spragg R, Stinson EB. (1973). "In vitro preservation of canine hearts for 24 to 28 hours followed by successful orthotopic transplantation." Ann Surg 178(6): 687-692.

22. Copeland JG, S. E. (1974). "Evaluation of hypothermic perfusion for 24-hour preservation of canine hearts." Transplant Proc 6(3): 311-313.

23. Feemster JA, I. Y., Dietzman RH, Lillehei RC, Manax WG. (1965). "The use of hypothermia, hyperbaria, and metabolic inhibition in organ preservation." JAMA 22(191): 1006-1008.

24. Fitton TP, B. C., Bonde PN, Wei C, Gage F, Rodriguez R, Conte JV. (2005). "Attenuation of DNA damage in canine hearts preserved by continuous hypothermic perfusion." Ann Thorac Surg 80(5): 1812-1820.

25. Fitton TP, W. C., Lin R, Bethea BT, Barreiro CJ, Amado L, Gage F, Hare J, Baumgartner WA, Conte JV. (2004). "Impact of 24 h continuous hypothermic perfusion on heart preservation by assessment of oxidative stress." Clin Transplant 18(12): 22-27.

26. Gohra H, F. Y., Hamano K, Noda H, Katoh T, Zempo N, Esato K. (1997). "As originally published in 1989: The effect of fluorocarbon emulsion on 24-hour canine heart preservation. Updated in 1997." Ann Thorac Surg 63(2): 594-595.

27. Hasegawa Y, S. M., Ohtaki A, Takahashi T, Sato Y, Ishikawa S, Morishita Y. (2000). "The effect of short-term coronary perfusion using oxygenated diluted blood following cold storage for long-term heart preservation." J Cardiovasc Surg (Torino). 41(3): 363-370.

28. Hassanein WH, Z. L., Tyrrell TA, Healey NA, Crittenden MD, Birjiniuk V, Khuri SF. (1998). "Continuous perfusion of donor hearts in the beating state extends preservation time and improves recovery of function." J Thorac Cardiovasc Surg 116(5): 821-830.

29. Hill DJ, W. W., Avery GJ, Portnoy VF, Collins GM. (1997). "Evaluation of a portable hypothermic microperfusion system for storage of the donor heart: clinical experience." Transplant Proc 29(8): 3532-3531.

30. Hudon MP, J. W., Qayumi AK, Dougan H, Sartori C, Lyster DM. (1991). "Assessment of myocardial metabolic state after hypothermic heart preservation for transplantation utilizing radioiodinated free fatty acid imaging." J Heart Lung Transplant 10(5): 704-709.

31. Jahania MS, S. J., Narayan P, Lasley RD, Mentzer RM Jr. (1999). "Heart preservation for transplantation: principles and strategies." Ann Thorac Surg 68(5): 1983-1987.

32. Jones BU, S. D., Beckham G, West J, Smulowitz P, Farber A, Kahwaji C, Connolly P, Steward E, Purdy RE, Milliken JC. (2001). "Recovery of cardiac function after standard hypothermic storage versus preservation with Peg-hemoglobin." ASAIO J 47(3): 197-201.

33. Jr., W. D. (1977). "Heart and liver transplantation. Consistent survival after prolonged donor heart preservation." Transplant Proc 9(1): 297-299.

34. Kadipasaoglu KA, B. G., Conger JL, Birovljev S, Sartori M, Clubb FJ Jr, Noda H, Ferguson JJ, Frazier OH. (1993). "An ex vivo model for the reperfusion of explanted human hearts." Tex Heart Inst J 20(1): 33-39.

35. Kay L, D. Z., Saks VA, Rossi A. (1997). "Alteration in the control of mitochondrial respiration by outer mitochondrial membrane and creatine during heart preservation." Cardiovasc Res 34(3): 547-56.

36. Kitamura M, T. O., Akimoto T, Yamazaki K, Niinami H, Nojiri C, Hachida M, Endo M, Hashimoto A, Koyanagi H. (1992). "In-storage hypothermic perfusion for heart and lung transplantation." ASAIO J 38(3): 163-166.

37. Koike N, T. I., Ohki S, Tsutsumi H, Matsumoto K, Morishita Y. (2003). "The effect of short-term coronary perfusion using a perfusion apparatus on canine heart transplantation from non-heart-beating donors." J Heart Lung Transplant 22(7): 810-817.

38. Kuhn-Regnier F, F. J., Jeschkeit S, Switkowski R, Bardakcioglu O, Sobottke R, de Vivie ER. (2000). "Coronary oxygen persufflation combined with HTK cardioplegia prolongs the preservation time in heart transplantation." Eur J Cardiothorac Surg 17(1): 71-76.

39. Larson DF, G. L., Bowers M, Sethi G, Copeland JG. (1998). "Assessment of left ventricular compliance during heart preservation." Perfusion 13(1): 67-75.

40. Masters TN, F. A., Schaper J, Pool L, Gong G, Robicsek F. (2002). "Changes in the preserved heart that limit the length of preservation." J Heart Lung Transplant 21(5): 590-599.

41. Minten J, S. L., Van Belle H, Wynants J, Flameng W. (1991). "Differences in high-energy phosphate catabolism between the rat and the dog in a heart preservation model." J Heart Lung Transplant 10(1): 71-78.

42. Nameki T, T. I., Oshima K, Kobayashi K, Sato H, Matsumoto K, Morishita Y. (2006). "A comparative study of long-term heart preservation using 12-h continuous coronary perfusion versus 1-h coronary perfusion following 11-h simple immersion." J Surg Res 135(1): 107-112.

43. Nickless DK, R. M., Richards SM, Conyers RA, Rosenfeldt FL. (1998). "Continuous perfusion improves preservation of donor rat hearts: importance of the implantation phase." Ann Thorac Surg 65(5): 1265-1272.

44. Ogiwara H, I. H., Takahashi T, Ohtaki A, Ishikawa S, Morishita Y. (1998). "A comparison of the University of Wisconsin solution and the modified Kawakami solution for initial flush and coronary perfusion in long-term canine heart preservation." J Cardiovasc Surg (Torino). 39(3): 313-320.

45. Ohtaki A, O. H., Sakata K, Takahashi T, Morishita Y. (1996). "Long-term heart preservation by the combined method of simple immersion and coronary perfusion." J Heart Lung Transplant 15(3): 269-274.

46. Osaki S, I. K., Kotani Y, Honjo O, Suezawa T, Kanki K, Sano S. (2006). "Resuscitation of non-beating donor hearts using continuous myocardial perfusion: the importance of controlled initial reperfusion." Ann Thorac Surg 81(6): 2167-2171.

47. Oshima K, M. Y., Yamagishi T, Mohara J, Takahashi T, Hasegawa Y, Ishikawa S, Matsumoto K. (1999). "Long-term heart preservation using a new portable hypothermic perfusion apparatus." J Heart Lung Transplant 18(9): 852-861.

48. Oshima K, M. Y., Yamagishi T, Mohara J, Takahashi T, Ishikawa S, Hasegawa Y. (1999). "A new portable hypothermic perfusion apparatus for long-term heart preservation in canine models." Transplant Proc 31(1): 1072-1073.

49. Peltz M, H. T., Adams GA 4th, Koshy S, Burgess SC, Chao RY, Meyer DM, Jessen ME. (2005). "Perfusion preservation maintains myocardial ATP levels and reduces apoptosis in an ex vivo rat heart transplantation model." Surgery 138(4): 795-805.

50. Rao V, F. C., Weisel RD, Boylen P, Cohen G. (1997). "Donor blood perfusion improves myocardial recovery after heart transplantation." J Heart Lung Transplant 16(6): 667-673.

51. Rao V, F. C., Cohen G, Borger MA, Ross HJ, Weisel RD. (2001). "Effects of metabolic stimulation on cardiac allograft recovery." Ann Thorac Surg 7(1): 219-225.

52. Rao V, F. C., Cohen G, Borger MA, Boylen P, Ross HJ. (2001). "Is profound hypothermia required for storage of cardiac allografts?" J Thorac Cardiovasc Surg 122(3): 501-507.

53. Rosenstrauch D, A. H., Bolukoglu H, Behrens L, Bryant L, Herrera P, Eya K, Tuzun E, Clubb FJ Jr, Radovancevic B, Frazier OH, Kadipasaoglu KA. (2003). "Ex vivo resuscitation of adult pig hearts." Tex Heart Inst J 30(2): 121-127.

54. Scheule AM, H. J., Zurakowski D, Strotmann C, Michel F, Vogel U, Miller S, Wendel HP, Ziemer G. (2002). "A non-heart-beating donor model to evaluate functional and morphologic outcomes in resuscitated pig hearts." J Invest Surg 15(3): 125-135.

55. Segel LD, F. D., Iguidbashian JP, Contino JP, Castellanos LM, Berkoff HA, Kaufman RJ, Schweighardt FK. (1994). "Posttransplantation function of hearts preserved with fluorochemical emulsion." J Heart Lung Transplant 13(4): 669-680.

56. Smulowitz PB, S. D., Beckham GE, Milliken JC. (2000). "Ex vivo cardiac allograft preservation by continuous perfusion techniques." ASAIO J 46(4): 389-396.

57. Spray TL, W. D., Roberts WC. (1977). "Morphology of canine hearts after 24 hours' preservation and orthotopic transplantation." J Thorac Cardiovasc Surg 73(6): 880-886.

58. Stowe DF, C. A., Heisner JS, Aldakkak M, Harder DR. (2007). "10 h preservation of guinea pig isolated hearts perfused at low flow with air-saturated Lifor(R) solution at 26{degrees}C: comparison to ViaSpan(R)." Am J Physiol Heart Circ Physiol.

59. Takahashi A, H. D., Braimbridge MV, Chambers DJ. (1990). "Harvesting hearts for long-term preservation. Detrimental effects of initial hypothermic infusion of cardioplegic solutions." J Thorac Cardiovasc Surg 100(3): 371-378.

60. Toledo-Pereyra LH, C. M., Lillehei RC. (1979). "Effects of pulsatile perfusion pressure and storage on hearts preserved for 24 hours under hypothermia, for transplantation." Ann Thorac Surg 27(1): 24-31.

61. Tsutsumi H, O. K., Mohara J, Takeyoshi I, Aizaki M, Tokumine M, Matsumoto K, Morishita Y. (2001). "Cardiac transplantation following a 24-h preservation using a perfusion apparatus." J Surg Res 96(2): 260-267.

62. Tsutsumi H, T. I., Ohshima K, Mohara J, Aizaki M, Morishita Y. (2000). "Effect of coronary perfusion with an oxygenated Celsior solution on 24-hour preservation in canine hearts." Transplant Proc 32(7): 2415-2416.

63. Tsutsumi H, T. I. I., Oshima K, Mohara J, Aizaki M, Morishita Y. (2001). "The Effect of Coronary Perfusion with an Oxygenated Celsior Solution on 12-hour Cardiac Preservation." Int J Angiol 10(1): 15-19.

64. Ueno T, Y. T., Itoh T, Natsuaki M. (1992). "The optimal temperature for 24-hour heart preservation using continuous coronary perfusion method." Thorac Cardiovasc Surg 40(4): 204-208.

65. Wicomb W, B. S., Cooper DK, Rose AG, Barnard CN. (1981). "Ex vivo functional evaluation of pig hearts subjected to 24 hours' preservation by hypothermic perfusion." S Afr Med J. 60(6): 245-248.

66. Wicomb W, C. D., Hassoulas J, Rose AG, Barnard CN. (1982). "Orthotopic transplantation of the baboon heart after 20 to 24 hours' preservation by continuous hypothermic perfusion with an oxygenated hyperosmolar solution." J Thorac Cardiovasc Surg 83(1): 133-140.

67. Wicomb WN, C. D., Barnard CN. (1982). "Twenty-four-hour preservation of the pig heart by a portable hypothermic perfusion system." Transplantation 34(5): 246-250.

68. Wicomb WN, C. D., Novitzky D, Barnard CN. (1984). "Cardiac transplantation following storage of the donor heart by a portable hypothermic perfusion system." Ann Thorac Surg 37(3): 243-248.

69. Wicomb WN, C. D., Lanza RP, Novitzky D, Isaacs S. (1986). "The effects of brain death and 24 hours' storage by hypothermic perfusion on donor heart function in the pig." J Thorac Cardiovasc Surg 91(6): 896-909.

70. Wicomb WN, C. D., Novitzky D. (1987). "Loss of myocardial viability following hypothermic perfusion storage from contaminating trace elements in the perfusate." Transplantation 43(1): 23-29.

71. Wicomb WN, C. G. (1989). "24-hour rabbit heart storage with UW solution. Effects of low-flow perfusion, colloid, and shelf storage." Transplantation 48(1): 6-9.

72. Wicomb WN, N. D., Cooper DK, Rose AG. (1986). "Forty-eight hours hypothermic perfusion storage of pig and baboon hearts." J Surg Res 40(3): 276-284.

BIOGRAPHICAL INFORMATION

Michael Cobert has been drawn to the fields of medicine and science since he was a young boy. He earned a Bachelor's degree in Biomedical Engineering from the University of Texas at Austin in May 2006. He spent time there working in Dr. Christine Schmidt's lab with Fransisco (Kiko) Serna and Jon Nichols studying and developing applications for the use of the electroactive polymer Polypyrrole. During his graduate career in the Joint Biomedical Engineering Program with UT Arlington and UT Southwestern Medical Center he was mentored by Dr. Michael Jessen and studied the machine perfusion characteristics of a device for organ transport, on which this Master's Thesis is based. Michael will continue in Dr. Jessen's lab, and pursue his Doctorate of Philosophy in Biomedical Engineering as a graduate student of UT Southwestern.