Author

From the start of her undergraduate education, Jessica Reddy has had an interest in cardiovascular physiology. *(continued on page 114).*

Comparison of Two Methods of Donor Heart Preservation in a Transplantation Model

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Biological Sciences

Abstract

Cold crystalloid ischemic storage is the method currently used for heart preservation. With this method, safe myocardial preservation time is limited to 4–6 hours, which restricts the geographic distance between donor and recipient to an approximate 500-mile radius. Due to these inherent restrictions, this preservation method prevents full access to the nationwide donor pool. Our laboratory has developed a method to extend cardiac preservation time using continuous perfusion with a 20 °C modified bovine polyethylene glycol hemoglobin (PEG-Hb) solution. Cardiac function has been restored *in vitro* to extirpated rabbit hearts that had been preserved for up to 24 hours. Our goal was to compare cardiac function *in vivo* of heterotopically transplanted hearts preserved by ischemic storage in cold crystalloid solution to that of hearts continuously perfused for 12 hours with a modified PEG-Hb solution. Measurements of heart rate, developed left ventricular pressure, maximum rate of contraction, and maximum rate of relaxation were obtained and assessed over a two hour time period following transplantation. Results demonstrate superior post-transplant cardiac function in hearts that underwent extended periods of perfusion preservation compared to those hearts subjected to short-term cold crystalloid storage.

Justin Zelones saw an online abstract on cardiothoracic surgery and decided to apply for the undergraduate research position it offered. *(continued on page 114).*

Key Terms

- Cardiac Perfusion
- Crystalloid Preservation
- Heterotopic Transplantation
- Polyethylene Glycol Hemoglobin (PEG-Hb)
- Reperfusion Injury

Faculty Mentor

Our laboratory has been working for some time on methods to extend the time a heart can be safely outside the donor's body before being transplanted to a recipient. Currently, the heart is limited to a few hours, which limits more specific tissue typing and the distance a heart can be transported. Additionally, some hearts are discarded because of uncertainty regarding their function. Prolonged preservation of the myocardium could ameliorate many of the time restrictions, allow for better donor-recipient matching, and perhaps even influence long-term outcomes. Participating in the Undergraduate Research Opportunities Program has been a very rewarding for us, as we have had the opportunity to work with some outstanding students, many of whom have gone on to medical school and other research activities.

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Introduction

Heart transplantation is clinically accepted as the treatment for end-stage heart disease. According to the United Network of Organ Sharing (UNOS), more than 3,000 people are on the waiting list for a heart transplant, and this number is increasing each year, due in part to the inability to make full use of the available nationwide donor pool.

Cold crystalloid ischemic storage is the clinical method most commonly used for heart preservation. There are 167 known variations of heart preservation solutions used by the 147 transplant centers in the United States; most, if not all, use hypothermic (cold) ischemic preservation (Demmy et al., 1997). A cold temperature is essential for ischemic organ preservation because it dramatically decreases cellular metabolism, suppresses the activity of hydrolytic enzymes, and stabilizes lysosomal membranes (Baumgartner et al., 2002). Temperatures implemented by clinical institutions range between 0 °C and 7 °C, with an optimal temperature for cardiac graft protection thought to be approximately 4 °C (Swanson, 1980).

Crystalloid preservation solutions are commonly classified by their sodium and potassium concentrations. They mimic either an extracellular (high sodium, low potassium) or intracellular (low sodium, high potassium) physiological composition. One common solution is Celsior. This is an extracellular solution with an additive, glutamate, for energy production and to act as an antioxidant. An alternate, intra-cellular-based solution—University of Wisconsin (UW)—has been shown to preserve the myocardium with excellent efficacy for up to 6 hours (Yeh et al., 1990).

Cold ischemic storage limits a safe preservation time to 4–6 hours. It has been shown that 30 day, 1 year, and 5 year mortalities of transplant recipients correlate with the duration of the donor heart's ischemic period while it is outside the body. This correlation is evident within the current clinically accepted preservation time range of 4–6 hours (Bourge et al., 1993). Also, transplant recipient mortality increases exponentially beyond 3 hours of storage under the clinically accepted heart preservation method (Taylor et al., 2003).

The standard immersion technique precludes the removal of metabolic waste and the delivery of oxygen to the myocardium. This results in a period of static storage, ischemia, and anaerobic metabolism. With this method, preservation beyond 4–6 hours causes irreversible damage due to the accumulation of metabolic waste byproducts,

ischemia that leads to anaerobic metabolism, and reperfusion injury. When a donor heart is immersed in a cold crystalloid solution, oxygen is not delivered to the myocardial cells. In the absence of oxygen, the donor heart works twenty times harder to produce ATP than it does under aerobic metabolism (Opie, 2004). The myocardium must resort to glycogen and lipid stores to meet its decreased, but persistent metabolic demands. These finite energy stores are eventually depleted over a sustained ischemic period. Beyond 4–6 hours of ischemic cold storage, ATP levels are depleted, leading to rigor mortis (Germann et al., 2005). This forces the heart to resort to anaerobic metabolism, which leads to an accumulation of lactate. This increase in lactate promotes mitochondrial damage (Armiger et al., 1974), which causes a decrease in contractility (Opie, 2004) and inhibits glycolysis (Rovetto et al., 1975). Furthermore, when a donor heart is reperfused following a period of ischemic cold crystalloid storage, the reintroduction of oxygen to the heart leads to the generation of oxygen free radicals, which are harmful to the heart and cause the buildup of intracellular calcium. These effects lead to reperfusion injury following resumption of blood flow to the donor allograft (Bolli et al., 1999).

Development of a technique to extend cardiac preservation times beyond the current clinical standard would allow for better donor-recipient matching due to an extended shelf life, improve post-transplant cardiac performance, and lead to a more efficient use of all donor hearts. A nutrient rich perfusion preservation solution would continuously provide essential nutrients to the donor heart cells, while simultaneously removing metabolic waste byproducts from the myocardium. Our laboratory has previously demonstrated that longer safe preservation times can be achieved using a similar method; however, experts have yet to reach a consensus on one optimal perfusion preservation solution. Blood plasma based preservation solutions, while used frequently, are not suitable due to the viscosity changes of blood plasma at low temperatures. Another method is the use of blood substitutes, which have been shown to have oncotic and oxygen carrying properties that are similar, if not superior, to those of blood. For example, fluorochemical emulsions have higher oxygen carrying capabilities especially at lower temperatures (Kioka, 1986).

Our group has explored the efficacy of hemoglobin based blood substitutes as part of the optimal perfusion preservation solution. Hemoglobin based preservation solutions are capable of unloading significant amounts of oxygen at low temperatures. Perfusion with such solutions allows the cardiac allograft to maintain contractility similar to normal

beating hearts. Conjugating polyethylene glycol (PEG) to hemoglobin (Hb) changes the diffusive properties by altering molecular size, viscosity, and oxygen affinity. PEG modified Hb releases oxygen in a manner similar to that of native red blood cells. PEG stabilizes the α2β2-hemoglobin tetramer to prolong intravascular retention. Also, because of its increased molecular size, polyethylene glycolated hemoglobin (PEG-Hb) is unable to pass into the interstitial space from the vasculature. Because of this, PEG-Hb may aid in reducing fluid accumulation in cardiac tissue during long perfusion preservation periods (Smulowitz et al., 2000).

We have demonstrated that continuous perfusion of the extirpated rabbit myocardium for up to 24 hours, using an oxygen carrying 20 °C modified bovine PEG-Hb solution, allows for return of cardiac function *in vitro*. The purpose of this study is to compare post-transplant left ventricular function *in vivo* of hearts that underwent a 4-hour period of cold crystalloid storage to hearts continuously perfused for 12 hours using a normokalemic, hypocalcemic 20 °C bovine PEG-Hb solution. We hypothesize that the transplanted hearts perfused for 12 hours with a PEG-Hb solution will display superior performance—determined by increased blood pressure—and increased rates of contraction and relaxation, compared to hearts subjected to a 4-hour preservation under cold ischemic conditions.

Materials and Methods

Cardiac Procurement
Fourteen New Zealand white rabbits received an intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) to induce anesthesia. Following the onset of anesthesia, each rabbit was intubated and placed onto a ventilator/anesthesia machine. Throughout surgery, general anesthesia was maintained with gaseous halothane, 0.8%–4%, adjusted periodically. This study was approved by the Institutional Animal Care and Use Committee (IACUC) of University of California, Irvine, under protocol number #2001-2247.

A median sternotomy was performed, followed by a pericardial incision, to expose the donor heart and major vessels. The brachiocephalic trunk, left common carotid artery, left subclavian artery, superior vena cava, and inferior vena cava were isolated after excision of the thymus (Figure 1). Heparin was administered to prevent blood clotting. Following occlusion of venous drainage into the heart and blood flow into the aortic arch, cold cardioplegia was administered through the ascending aorta to achieve cardiac
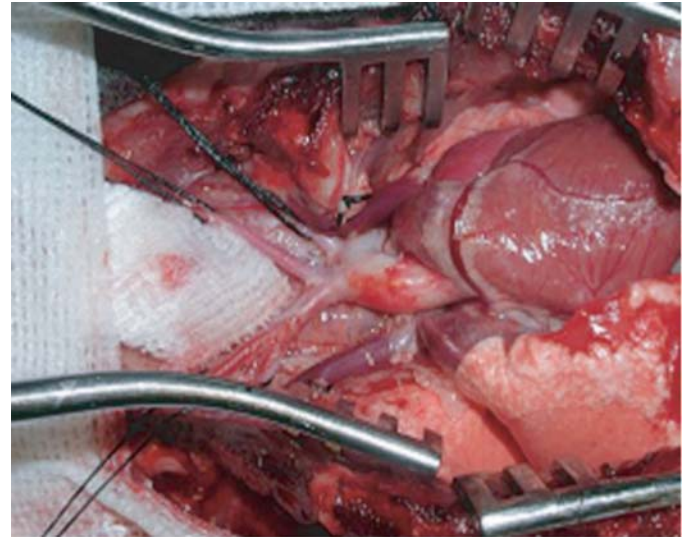

Figure 1
Donor heart and exposed isolated vessels

arrest. During this period the heart was topically irrigated with cold saline to slow metabolic activity. Following extirpation of the donor heart, the lung and other extraneous tissue were removed.

Preservation Solution
Group 1 (n=8) hearts were cannulated via the ascending aorta and placed onto a modified Langendorff perfusion circuit (Figure 2) filled with a 20 °C PEG-Hb based solution containing a mixture of additives (Table 1).

Table 1
PEG-Hb solution composition used for donor heart preservation

Agent	PEG-Hb Solution
3% Bovine PEG-Hb	324 mOsm/Kg
KCl	4.7 mEq/L
NaCl	148.7 mmol/L
NaH_2PO_4	2.5 mmol/L
NaHCO	2.5 mmol/L
$MgSO_4$	5.0 mEq/L
$CaCl_2$	1.0 mEq/L
Lidocaine HCl	12.5 mg/L
Heparin Sodium	1250 units/L
Dextrose	6.1 mOsm/L
Albumin	1.5 gm/L
Insulin	30.6 units/L
Tromethamine	7.3 cc/L

During the 12-hour antegrade perfusion preservation period, Group 1 hearts' pH, pO_2, and heart rate were moni-
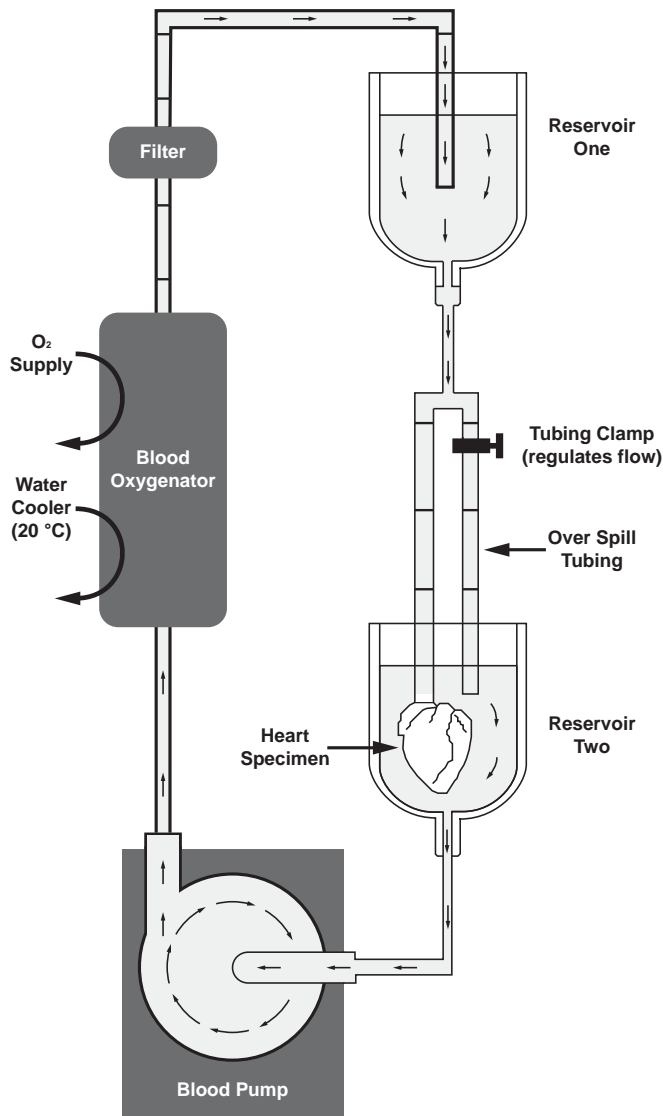
Figure 2
Isolated donor heart perfusion preservation circuit

tored: (1) pH was maintained between 7.15 and 7.35, (2) pO_2 between 400 to 600 mmHg, and (3) heart rates varied between 15 and 40 bpm. The pH and pO_2 were measured by in-line arterial gas electrodes located within the modified Langendorf perfusion circuit. Heart rate was monitored by counting each cardiac contraction during one-minute intervals. Group 2 (n=6) hearts were immersed into a 4–7 °C crystalloid solution composed of 0.9% NaCl for a 4-hour ischemic period. Group 1 hearts received cardioplegia to achieve cardiac arrest of the beating heart at the end of the 12-hour preservation period. This was not necessary for Group 2 as these hearts were not contracting throughout their 4-hour preservation period (static storage in cold crystalloid solution).

Heterotopic Transplantation

At the conclusion of the preservation periods, each donor heart was transplanted into a recipient New Zealand white rabbit in an infrarenal position. Following stabilization of general anesthesia, all recipients underwent a laparotomy incision overlying the lower abdomen and surgical preparation of the intra-abdominal heterotopic transplant site. Heparin was administered to prevent thrombosis, and blood flow was occluded at the planned anastomosis sites at the abdominal aorta and inferior vena cava. An anastomosis was performed between the donor aorta and recipient abdominal aorta that allowed for arterial blood flow into the donor coronary arteries. A second anastomosis was performed between the donor pulmonary artery and recipient inferior vena cava to allow for venous drainage from the transplanted donor heart.

Assessment of Cardiac Function

Following transplantation and the resumption of blood flow into the grafted donor heart, a deflated latex balloon connected to a pressure transducer was inserted through an incision into the left atrium and passed into the left ventricle where it was secured. The balloon was connected in-line to a force transducer (Biopac Systems, Inc., Santa Barbara, CA), analog to digital converter (Biopac Systems, Inc., Santa Barbara, CA), and generic desktop computer. Developed left ventricular (LV) pressure (systolic minus diastolic), peak rates of left ventricular contraction (+dP/dt) and relaxation (-dP/dt), and heart rate were measured at 30 minute intervals consecutively, over a period of 2 hours. Each testing subjected the donor allograft to preload volumes increasing with 0.1 mL increments. Statistical analysis was performed at a preload volume of 0.5 mL across both groups and all time points. Significance between groups was tested; a p value of <0.05 was considered significant, according to the Students' t-test.

Results

Developed LV pressure

At a preload volume of 0.5 mL, Group 1 hearts demonstrated significantly superior LV pressure and ventricular performance throughout testing. Group 2 hearts exhibited an upward trend in LV pressure towards Group 1 hearts (Figure 3).

Maximum Rate of LV Contraction

At a preload volume of 0.5 mL, Group 1 hearts maintained +dP/dt values that were statistically superior to Group 2 hearts at all time points except at 120 min (Figure 4).
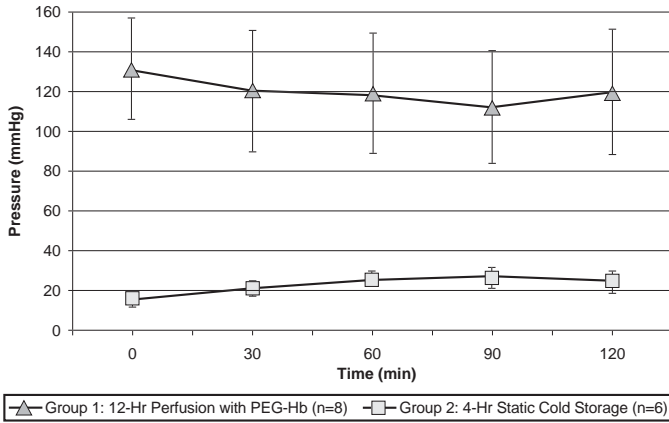
Figure 3

Developed LV Pressure at 0, 30, 60, 90, and 120 min following heterotopic transplantation. Developed LV Pressure in Group 1 hearts were superior to Group 2 hearts at all time points (p<0.05).
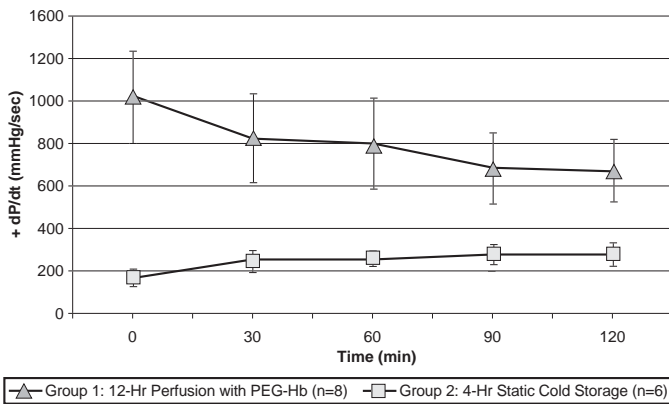

Figure 4

Maximum rate of LV Contraction at 0, 30, 60, 90, and 120 min following heterotopic transplantation. The +dP/dt values in Group 1 hearts were superior to Group 2 hearts at all testing time points with the exception of 120 min (p<0.05).
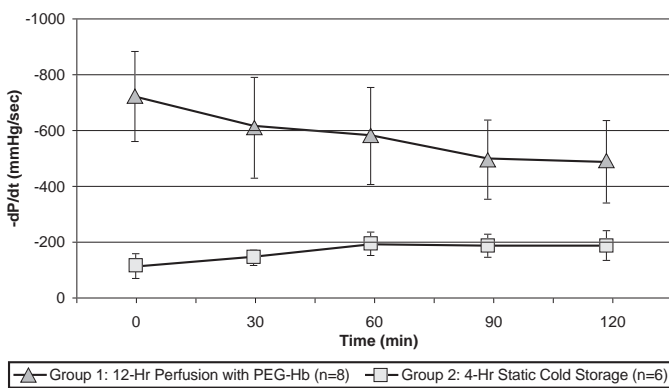

Figure 5

Maximum rate of LV Relaxation at 0, 30, 60, 90, and 120 min following heterotopic transplantation. The -dP/dt values in Group 1 hearts were superior to Group 2 hearts at all testing time points, with the exception of 90 min (p<0.05).

Maximum Rate of LV Relaxation

At a preload volume of 0.5 mL, Group 1 hearts maintained -dP/dt values that were significantly superior throughout testing except at 90 min (Figure 5). Group 2 hearts showed myocardial recovery during the early and mid-stages of testing.

Discussion

Overall, the performance of hearts perfused with PEG-Hb was superior to that of hearts preserved using the clinical standard. Cold ischemic storage is limited in part by the fact that cellular metabolism, although diminished during hypothermia, still persists. Others have shown that during the cold crystalloid preservation period, sustained deep hypothermia causes tissue edema, which inactivates membrane-bound enzymes and disrupts lipid bilayer permeability. This occurs because inhibition of the Na^+/K^+ ATPase pump leads to a loss of membrane potential as sodium is allowed to enter the cell. Water passively follows and accumulates inside the cell as a result of the hyperosmotic intracellular environment and further diffuses into its organelles (Baumgartner et al., 2002). Furthermore, ischemia and reperfusion causes the buildup of reactive oxygen species (ROS), which cause peroxidation of membrane lipids. These contribute to a reperfusion injury by allowing for an increase in membrane permeability to calcium (Kloner et al., 2001). Another effect of ROS is that it reverses Na^+-Ca^{2+} channels, thereby allowing more Ca^{2+} into the cell (Opie, 2004). Because of the inherent limitations of cold ischemic storage, perfusion preservation methods that use aerobic conditions have been of interest. An oxygen carrying substance within the preservation solution would prevent ischemia and reperfusion injury caused by oxygen free radicals.

Furthermore, cold ischemic storage also causes cellular acidosis and endothelial injury. Acidosis results from the accumulation of carbon dioxide and protons during anaerobic metabolism. This can decrease the pH of ischemic tissue from about 7.0 to 6.5 (Cross et al., 1995). As a result of the low pH, lysosomes can become activated and release enzymes that irreversibly damage the cell.

In previous studies, our laboratory has demonstrated that extended preservation of the rabbit myocardium can be achieved for up 24 hours followed by return of cardiac function *in vitro*. Results of this study show superior post-transplant cardiac function in donor hearts perfused for 12 hours in a PEG-Hb based solution when compared to donor hearts preserved using the current clinical standard

of 4-hour cold static storage in crystalloid solution. Our results suggest a framework for future studies that would include biochemical, expanded functional, and morphological analyses of the graft myocardium. Our long-term goal is to develop a perfusion system that would standardize myocardial preservation and heart transplantation. This would include building a practical, cost-efficient transportable perfusion-preservation device. Lengthening cardiac preservation times has many promising applications and benefits, and our results demonstrate the potential of perfusion preservation with a PEG-Hb based solution in the effort to extend heart preservation times.

Jessica Reddy Biography

(continued from page 109) She has immersed herself in her research experience, and has been excited by the opportunity to perform surgery, a rare opportunity for undergraduates. Jessica hopes to move on to graduate school, pursuing an M.D./Ph.D. path in cardiovascular biomedical engineering, with a goal of becoming a physician in that field. In addition to her classes and research, Jessica has volunteered at the Orange Coast Memorial Hospital, and has been a suture instructor for the UCI College of Medicine Camp Med program.

Justin Zelones Biography

(continued from page 109) Working on ways to extend the length of donor heart preservation, Justin has enjoyed the hands-on aspects of his research—learning surgical techniques and applying them in the lab. He plans to become a physician and to continue doing research, in addition to having his own practice. Justin volunteers at Orange Coast Memorial Medical Center, and is a suture instructor for the UCI College of Medicine Camp Med program.

Acknowledgements

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