Assessment of machine perfusion conditions for the donation after circulatory death heart preservation

Renee Cholyway1 | Oluwatoyin Akande2 | Adolfo Gabriele Mauro2,3 | Eleonora Mezzaroma2,4 | Rui Wang3 | Kristine Kenning1 | Stefano Toldo2,3 | Mohammed Quader1,2,5

1Division of Thoracic and Cardiovascular Surgery, Department of Surgery, Virginia Commonwealth University, Richmond, Virginia, USA
2Pauley Heart Center, Virginia Commonwealth University Health System, Richmond, Virginia, USA
3Division of Cardiology, Department of Internal Medicine, Virginia Commonwealth University, Richmond, Virginia, USA
4Department of Pharmacotherapy and Outcome Science, School of Pharmacy, Virginia Commonwealth University, Richmond, Virginia, USA
5McGuire Veterans Administration Medical Center, Richmond, Virginia, USA

Abstract

**Background:** Donation after circulatory death (DCD) hearts requires machine perfusion preservation, the conditions of which are not well defined.

**Methods:** To achieve this, rat hearts were procured following a DCD or control beating-heart donation (CBD) model, and perfused for 60 min with one of three machine perfusion solutions—St. Thomas (ST), University of Wisconsin (UW), or Polyethylene Glycol-20k (PEG)—at one of two temperatures, 4°C or 15°C. At 15-min intervals, perfusion pressure was measured as a marker of vascular resistance. Colored microspheres were added to capture the distribution of perfusate into the metabolically active sub-endocardium, and the eluate was collected for troponin assays. Analyses compared groups using Wilcoxon rank-sum and ANOVA.

**Results:** Perfusion pressure was significantly higher for DCD than CBD hearts at 15°C regardless of solutions. The lowest rise in perfusion pressure over time was observed with PEG at 15°C. Except for PEG at 15°C, ST and UW solutions at 4 or 15°C had decreased sub-endocardial perfusion in DCD hearts. Troponin release from DCD hearts with UW and PEG solutions was comparable to CBD hearts but was significantly higher with ST solution at 15°C.

**Conclusions:** Optimal preservation conditions for DCD hearts were observed with PEG machine perfusion solution at 15°C.
1 | BACKGROUND

Approximately 10% of patients with advanced heart failure will require heart transplantation in the United States.1–3 Currently, the donor hearts primarily come from patients with complete and irreversible brain damage (Donation after Brain Death, DBD), but a disproportionate increase in potential recipients outnumbers the supply due to improved medical and device management.2,4–6 Thus, there is an urgent need to expand the heart donor pool. A potential source of donor hearts is Donation after Circulatory Death (DCD) donors, these are patients with a terminal illness or with severe irreversible brain damage who has some residual brain stem activity that precludes designation “brain dead”.4,5,7 Utilizing DCD hearts could increase the number of heart transplants by up to 56%, significantly decrease the mortality of patients on the waitlist, and shorten the wait times for all patients.8–11

Unlike the DBD hearts, the DCD hearts sustain significant warm ischemic damage and do not maintain viability with cold storage for transportation.12 Reperfusion injury is also a well-established phenomenon, initiated at the moment of transplantation or ex situ organ perfusion.13,14 Current practice for DCD transplantation uses whole blood for organ preservation ex situ. However, using blood as perfusate has limitations such as securing adequate blood for perfusion and hemolysis from roller pumps that would plug the capillaries. In addition, the donor blood carries cytokines and high levels of catecholamines that can damage the DCD heart.15

Oxygenated non-blood-based organ preservation solutions can mitigate these shortcomings by blunting the deleterious effects of reperfusion and tissue edema while providing protection from ongoing ischemia and allowing for assessment of cardiac function before transplantation. The most commonly used organ preservation solutions are the University of Wisconsin machine perfusion solution (UW-MPS) and the Saint Thomas solution-2 (ST). While UW and ST solutions have an excellent record in preserving DBD hearts, they were not well studied in DCD heart preservation. A modified UW-MPS with added 20KDa polyethylene glycol (PEG), a better cell impermeant that limits tissue edema and improves intravascular volume, has been shown to be a superior organ preservative solution. Constituents and comparison of three solution used in our study are shown in Table 1.16

Unlike the DBD hearts, DCD hearts preserved on a machine perfusion system required a higher perfusion rate and may require a lower temperature to maintain the heart in an aerobic state with non-blood-based perfusate.17 Extreme hypothermic conditions (<4°C) are known to inflict endothelial damage and produce a lower ATP generation per oxygen consumed upon reperfusion as compared to midthermic temperature (21°C).6,18,19 Studies support mid-thermic temperature (roughly 12–21°C) for organ preservation to provide the best protection against reperfusion injury.18–21

The goal of this study is to identify an ideal non-blood-based machine perfusion condition including temperature and solution for a DCD heart that ensures homogeneous myocardial perfusion over time and superior myocardial preservation. We hypothesize that there is a difference between the extent of homogenous perfusion of metabolically active and ischemia-sensitive sub-endocardium among the three preservation solutions and that the heart is better preserved at a mid-thermic temperature (15°C) compared to hypothermic temperature (4°C).

2 | METHODS

These experiments were conducted under the guidelines of the “Guide for the care and use of laboratory animals” published by the National Institutes of Health.22 The study protocol was approved by the Virginia Commonwealth Institutional Animal Care and Use Committee.

2.1 | Groups

Seventy-two Sprague–Dawley rats of 10–12 weeks old were assigned half to controlled beating-heart donor (CBD) and the other half to a DCD protocol as published before (Figure 1).23 The 36 hearts in each protocol were assigned for perfusion with one of the three machine perfusion solutions (ST, UW, or PEG) at two temperatures (4°C or 15°C) to create six groups; six hearts were perfused with Saint Thomas (ST) solution at 15°C, six with ST at 4°C, six with University of Wisconsin (UW) solution at 15°C, six with UW at 4°C, six with polyethylene glycol-20k (PEG) solution at 15°C, and the six with PEG at 4°C (Table 2).

Rats were anesthetized by administering ketamine/xylazine (100/10 mg/kg) intramuscularly, followed by
| Perfusate solutions composition     | ST. Thomas (ST)  | University of Wisconsin (UW) | Polyethylene glycol-20k (PEG) |
|------------------------------------|-----------------|-----------------------------|-------------------------------|
| Chemical name                      | Concentration   |                             |                               |
| HES (Starch)                       | 5% (w/v)        |                             |                               |
| PEG-20kDa                          | 2.5% (w/v)      |                             |                               |
| Glucose                            | 11 mM           | 10 mM                       | 10 mM                         |
| Na-Octanoate                       |                 |                             |                               |
| NaCl                               | 110 mM          |                             |                               |
| NaHCO₃                             | 10 mM           |                             |                               |
| KCl                                | 16 mM           |                             |                               |
| MgCl₂                              | 16 mM           |                             |                               |
| CaCl₂                              | 1.2 mM          | 0.5 mM                      | 0.5 mM                        |
| KH₂PO₄                             | 15 mM           | 15 mM                       |                               |
| K-Gluconate                        | 10 mM           |                             |                               |
| Mg-Gluconate                       | 5 mM            | 5 mM                        |                               |
| Ribose                             | 5 mM            |                             |                               |
| Adenosine                          | 5 mM            |                             |                               |
| Adenine                            | 5 mM            |                             |                               |
| HEPES                              | 10 mM           | 10 mM                       |                               |
| Allopurinol                        | 1 mM            | 1 mM                        |                               |
| Glutathione                        | 3 mM            | 3 mM                        |                               |
| **Amino acids**                    | **Concentration (g/L)** |                   |                               |
| L-Arginine (free base)            | 10.0            |                             |                               |
| L-Asparagine•H₂O                  | 2.84            |                             |                               |
| L-Aspartic acid                    | 1.0             |                             |                               |
| L-Cystine                          | 2.5             |                             |                               |
| L-Glutamic acid                    | 2.5             |                             |                               |
| L-Glutamine                        | 0.29            |                             |                               |
| Glycine                            | 0.5             |                             |                               |
| L-Histidine (free base)            | 0.75            |                             |                               |
| Hydroxy-L-proline                  | 1.0             |                             |                               |
| L-Isoleucine                       | 2.5             |                             |                               |
| L-Leucine                          | 2.5             |                             |                               |
| L-Lysine•HCl                       | 2.0             |                             |                               |
| L-Methionine                       | 0.75            |                             |                               |
| L-Phenylalanine                    | 0.75            |                             |                               |
| L-Proline                          | 1.0             |                             |                               |
| L-Serine                           | 1.5             |                             |                               |
| L-Threonine                        | 1.0             |                             |                               |
| L-Tryptophan                       | 0.25            |                             |                               |
| L-Tyrosine                         | 1.16            |                             |                               |
| L-Valine                           | 1.0             |                             |                               |
| pH                                 | 7.8             | 7.4                         | 7.4                           |
endotracheal intubation for respiratory support. The depth of anesthesia was monitored by checking for the absence of pedal reflexes. Heparin (400 Units) and vecuronium bromide (2 mg) were administered through the right internal jugular vein and allowed to circulate for 5 min. For the DCD model, the animals were then released from ventilator support to induce respiratory arrest leading to cardiac asystole. Thoracotomy was performed and hearts procured to be perfused via cannula attached to the ascending aorta on Langendorff setup. The total time from ventilation withdrawal to initiating reperfusion on the Langendorff apparatus was standardized to 25 min. The control CBD rats were ventilated until the moment of heart procurement; hearts were then mounted on the Langendorff system similar to the DCD hearts. Time from procurement to perfusion of the control heart was less than 5 min.

2.2 | Perfusion

The Langendorff perfusion system was primed with 200 ml of one of the three organ preservation solutions (ST, UW, and PEG), and set to run in a closed circuit at a fixed flow of 140–170 ml/100 g/min for 60 min at one of the two temperatures, 4°C or 15°C. The three solutions used are cardioplegic and therefore prevent the heart beat and lowers the ATP consumption during organ preservation. The perfusion flow at the selected temperatures was chosen based on prior studies performed to determine the critical flow needed to maintain a DCD heart in aerobic condition during preservation. Heart weight was measured to adjust for perfusion flow. Mean weight of heart and standard deviation were 1.34 ± 0.16 and 1.32 ± 0.21 g in the CBD and DCD groups, respectively. A schematic representation of the perfusion system used, is reported in Figure 2A. Colored DYE-TRAK® microspheres were used to track the regional tissue flow. These microspheres have a diameter of 12 microns and are designed to distribute with the flow of blood (or other perfusates), and get dispersed and trapped in the terminal microvasculature, which has an average diameter of 5 microns, too small for the microspheres to pass through. Approximately 50,000 colored microspheres (12-micron diameter) were added to 3 ml of respective perfusate, agitated, and delivered via three-way stopcock into the perfusion system, directly into the column above the aortic cannula providing perfusion. (Figure 2A). To determine whether the perfusion pattern changes over the 60 min of perfusion, we delivered different colored microspheres at 15 min interval. From the onset of perfusion to 15 min red color microspheres (Persimmon DYE-TRAK® VII+,
Cat# 145-0545, Triton Technology Inc., San Diego, CA, USA) were injected, at 30 min, blue color microspheres (Blue DYE-TRAK® microspheres, Cat# 145-0672, Triton Technology Inc.) were injected, and at 45 min the yellow color microspheres (Yellow DYE-TRAK® VII+ microspheres, Cat# 145-0448, Triton Technology Inc.) were injected and allowed to distribute for 15 min. Figure 2B shows a diagram of the timing and colored microspheres administration, which, thanks to the use of different colors, reflects the flow distribution at the precise moment the microspheres were trapped while moving through the vasculature. At 30 min, when all the red microspheres are trapped in the tissue, the blue microspheres will be injected, followed, after 45 min, by the yellow microspheres. After a total of 60 min, the hearts are collected to measure the microsphere distribution in left ventricular slides.

2.3 Statistics

Median (IQR 25%, 75%) or mean (SD) is compared based on the distribution of data. Non-parametric Wilcoxon rank-sum test is used for comparisons, then Steel-Dwass each pair for comparisons including more than two groups, reporting the p-value. For data with normal distribution, equal variance ANOVA followed by Tukey HSD is performed for comparisons between more than two groups with p-value reported. Data outliers due to technical errors (8 CBD hearts from different groups, and 5 DCD hearts from different groups) were excluded from the microsphere count analysis to prevent artificially skewed
data. Significance was set to a p-value of <0.05. JMP (SAS Institute Inc. Version 15) statistical software was used for calculations. All raw data generated from this study are available upon request.

3 | RESULTS

3.1 | Coronary vascular resistance

Perfusion pressure at the fixed target flow rate was used as a surrogate for coronary vascular resistance. The average perfusion pressure was calculated for each group from measurements obtained at 15, 30, 45, and 60 min of perfusion. The overall median values were compared between CBD and DCD hearts perfused with the same solution (ST, UW, or PEG) at 4 and 15°C. In addition, within the DCD group, the coronary vascular resistance was studied with the three solutions at 4 and 15°C, and with the same solution comparing perfusion pressure at 4 and 15°C (Figure 3). For temperature and perfusate conditions, all DCD hearts had elevated perfusion pressures compared to CBD hearts; however, the difference was statistically significant only at 15°C (ST p = 0.007, UW p = 0.01, and PEG p = 0.01). Within the DCD groups, there was no significant difference in perfusion pressure with ST or PEG between 4 and 15°C. UW solution had higher perfusion pressure at 15°C compared to 4°C (p = 0.016). These results suggest that the ischemic damage to the DCD hearts likely causes microvascular injury leading to tissue edema and high vascular resistance, especially at 15°C. Within the DCD groups, there was no significant difference in perfusion pressure with any of the three solutions.

To study the change in perfusion pressure with time, we collected data on the perfusion pressures measured at the end of 15 min and compared them to the pressure measured at 60 min of perfusion. We collected this data with the three solutions at 4 and 15°C. The lowest median rise in perfusion pressure was seen for PEG at 15°C and highest median rise in pressures was seen with ST solution. However, these pressure increases were not statistically significant (Figure 4). An increase in perfusion pressure over time likely suggests ongoing tissue ischemia–reperfusion injury, swelling, and increased vascular resistance. A solution with the least rise in perfusion pressure over time might be a better preservative for the heart. Our data showed a better cardioprotection trend with PEG solution at 15°C, but the difference did not reach statistical significance.

3.2 | Microsphere distribution

The ratio of colored microspheres perfused deeply into the sub-endocardial space to the myocardial space (E/M ratio) was used as a visible surrogate for even distribution of perfusate. The E/M ratio is displayed as a percent of microspheres perfused into the sub-endocardium from the total amount of microspheres counted (Figure 5A). The overall median E/M ratios were compared between CBD and DCD hearts using the same solution at 4 and 15°C, between DCD hearts using the three solutions at 4 and 15°C, and with the same solution comparing 4 and 15°C (Figure 5B,C). Compared to CBD hearts, all DCD hearts perfused a significantly lower proportion of microspheres into the sub-endocardial tissue except for those perfused with PEG solution at 15°C (p = 0.2). In comparing the three solutions for DCD hearts at 4°C, DCD hearts perfused with UW had a significantly lower proportion of

Figure 3
Coronary vascular resistance, demonstrated by perfusion pressure, (A) displayed at 4°C and (B) at 15°C. CBD, controlled beating-heart donor; DCD, donation after circulatory death; PEG, polyethylene glycol 20k solution; ST, ST. Thomas solution; UW, University of Wisconsin solution. Data are expressed as median perfusion pressure. Number of samples per group: CBD ST 4°C n = 6, UW 4°C n = 6, PEG 4°C n = 6, ST 15°C n = 6, UW 15°C n = 6, PEG 15°C n = 6. The statistical method used was non-parametric Wilcoxon rank-sum test followed by Steel-Dwass each pair when three groups were compared, and p < 0.05 considered significant denoted with an (*). CBD vs. DCD 4°C ST (p = 0.297), UW (p = 0.055), PEG (p = 0.337). CBD vs. DCD at 15°C using ST (p = 0.007), UW (p = 0.01), PEG (p = 0.01). DCD ST vs. UW vs. PEG at 4°C (p = 0.849) and 15°C (p = 0.119). DCD 4°C vs. 15°C ST (p = 0.749), UW (p = 0.016), and PEG (p = 0.575)
microspheres into the sub-endocardial space compared with PEG and ST \( (p = 0.009) \) because the median E/M ratio from hearts with UW was 0 (IQR 0, 0.002). There was no difference in the E/M ratio between the three solutions when examined at 15°C \( (p = 0.83) \). The E/M ratio compared at 4°C versus 15°C for the three solutions was comparable except when using UW solution \( (p = 0.005) \), likely due to a particularly low median and IQR for UW hearts at 4°C.

Along with an increased coronary vascular resistance, a reduction in perfusion rate was anticipated. Expressed as a percent change in the mean total number of microspheres over time, we compared the microsphere count with three solutions from after 15 min of perfusion to after 45 min of perfusion (Figure 6). At 4°C using ST solution, more microspheres actually perfused after 45 min with a mean of +136.5%, which significantly differed from the hearts perfused with UW solution by 86% \( (p = 0.037) \). DCD hearts perfused with UW solution at 4°C experienced a reduction in the number of microspheres perfused by a mean of 50%. At 15°C, there was no difference in the percent reduction of microspheres perfused at 15 min versus 45 min among the three solutions \( (p = 0.273) \).

3.3 | Myocardial protection

The amount of cardiac troponin I (cTnI) released from the hearts was compared to identify a protective benefit of the perfusates from reperfusion injury. The overall median troponin concentrations was compared between CBD and DCD hearts using the same solution at 4 and 15°C, between DCD hearts using the three solutions at 4 and 15°C, and with the same solution comparing 4 and 15°C (Figure 7). A significantly higher level of troponin was released from the DCD hearts compared to CBD hearts at 4°C, especially from hearts perfused with ST solution. At 15°C the troponin release from DCD hearts perfused with UW and PEG solutions was less and comparable to CBD hearts. The highest amounts of troponin release were noted with ST solution both at 4°C and 15°C \( (p = 0.004) \). When comparing DCD hearts perfused with the three solutions, ST solution at 15°C was associated with highest release of troponin compared to PEG or UW solution \( (p = 0.005) \). DCD hearts perfused with ST solution had a significantly higher troponin level at 15°C than at 4°C \( (p = 0.025) \) while PEG had a significantly lower troponin level at 15°C than at 4°C \( (p = 0.025) \), and UW was comparable at either temperature \( (p = 0.631) \). These results suggest that DCD hearts sustain significant ischemic damage and when perfused release cTnI. Among the three perfusates we tested in DCD hearts, PEG solution afforded the least troponin release, particularly at 15°C. ST solution appears to afford the least protection for DCD hearts from reperfusion injury.

When the difference in troponin levels from first 15 min of perfusion to the last 15 min of perfusion were compared between DCD hearts with the three solutions (Figure 8), ST solution had the highest release in troponin in the last 15 min, particularly at 15°C compared to UW or PEG solutions \( (p = 0.005) \). These data suggest that the ST solution is not a suitable preservative solution for DCD hearts and that the reperfusion injury continues to get worse with time.

4 | DISCUSSION

DCD hearts sustain ischemic damage prior to procurement and cannot be stored in ice for preservation and transportation. Optimal preservation conditions for a DCD heart are not well defined. Our study examined the preservation strategy for the DCD hearts using three non-blood-based perfusates at hypothermic and mid-thermic
conditions. We measured perfusion pressure as a surrogate marker of coronary vascular resistance, examined the distribution of perfusate into the myocardial and sub-endocardial layers with colored microspheres, and measured troponin release in eluate as a marker of ischemia/reperfusion injury. We observed, (1) perfusion pressure was higher for DCD hearts compared to CBD hearts, the difference became statistically significant at 15°C with all three perfusates, (2) among the DCD hearts the lowest rise in perfusion pressure over the duration of perfusion was observed with PEG solution at 15°C, (3) except for PEG solution at 15°C, all other solutions had decreased sub-endocardial perfusion in DCD hearts, (4) troponin I release from DCD hearts was significantly high with ST solution compared to UW or PEG solution at 15°C, and (5) troponin release in DCD hearts perfused with UW and PEG solutions was comparable to CBD hearts. Overall, the PEG solution at 15°C provided the optimal perfusion and preservation to a DCD heart.

At present, limited number of DCD heart transplantations are done using a machine perfusion system that uses donor whole blood as perfusate. There are limitations to using whole blood for perfusion; due to multiple organs besides heart are procured, removing a large volume of donor blood takes time and adds to the critical warm ischemia time, potentially jeopardizing the use of other organs. Machine perfusion systems utilize roller pumps, which cause hemolysis and are known to plug capillaries, which may further compromise tissue perfusion. In addition the blood cells when interacting with the pump tubing promote the release of cytokines that can further damage the donor heart. Medications administered to
the donor, and the high levels of catecholamines which are well known to be present in the donor’s blood can have detrimental effects on DCD heart and can lead to primary graft dysfunction. For these reasons, we propose the use of non-blood-based preservation solutions for a DCD heart. We selected the two most commonly used heart preservation solutions, the UW and ST solution, and a novel modified UW solution with PEG-20K as the impermeant to test DCD heart preservation. Polyethylene glycol (PEG) is a colloid as well as a cell impermeant that affects the oncotic and osmotic gradients at the capillary and cell membrane level, augmenting the expansion of intravascular volume while reducing cellular edema.

In hemorrhagic shock models of low volume resuscitation PEG-20K based solution has been shown to maintain higher mean arterial pressures, less tachycardia, and better capillary perfusion to the left ventricle, resulting in significantly improved mortality compared to crystalloid based resuscitation. Based on these qualities we modified UW solution with added PEG-20K and several substrates for enhanced myocyte metabolism (Table 2) culminating into a novel preservative solution, which we tested, in DCD hearts.

Our previous work showed that the DCD hearts require higher oxygen supply on ex vivo perfusion setup compared to CBD hearts. One of the main limitations of non-blood-based perfusate is its inability to deliver enough oxygen above 15°C. As DCD hearts do not function when preserved in cold storage (ice) and that a non-blood-based perfusates cannot deliver adequate oxygen above 15°C, we designed our study at 15°C that also has an advantage of mid-thermic preservation. Cardiomyocyte microtubules subjected to 4°C have increased dysfunction, with elevated basal lactate dehydrogenase release and superoxide production. Canine DCD hearts stored at 4°C had significantly higher lactate levels and lower left
ventricular developed pressure as compared to hearts perfused with warm oxygenated blood. Cold storage is also associated with cellular swelling from reduced enzymatic activity of ATPases leading to passive intracellular sodium leak. Porcine hearts stored at midthermic (13°C) and sub-normothermia (21°C) temperatures, showed lower levels of creatinine kinase, cTnI release, and preserved ejection fractions compared to hearts storage at 4°C. Due to the reasons mentioned above and the inability of non-blood-based solutions to support aerobic conditions in DCD hearts above 15°C, we elected to test the three perfusion solutions at 15°C.

Results of this study suggest that perfusion pressures are generally higher for DCD hearts than CBD hearts, regardless of temperature condition or perfusate choice, and that perfusion pressure increases with the duration of perfusion. This is likely attributed to perivascular edema from ischemic injury and vasoconstriction at reduced temperatures. The lowest rise in perfusion pressures over 60 min of perfusion was observed with PEG solution at 15°C, while ST solution had the greatest increase in perfusion pressure over 60 min of perfusion. Although these observations do not reach statistical significance, we favor PEG or UW solution over ST due to the steady rising coronary resistance with the duration of perfusion.

The sub-endocardial component of myocardium is metabolically more active compared to myocardium. Sub-endocardial area has terminal capillary flow without watershed boundaries, hence it is more susceptible to ischemia. The capillary density in sub-endocardium is 10% higher compared to other layers and is maximally dilated at rest; in addition, compressive effects of increased intramyocardial/intraventricular pressure are greater in the sub-endocardial layer than elsewhere in the myocardium. Adequate perfusion into this layer is, therefore, a strong indicator of optimal perfusion and preservation against reperfusion injury. In our study the DCD hearts perfused with PEG at 15°C had better perfusion into sub-endocardial layer which was comparable to CBD hearts. UW and ST solutions did not perfuse the sub-endocardium well. The possible explanations for the better preservation of the DCD hearts perfused with PEG solution are likely the result of superior osmotic and oncotic properties that would allow for efficient capillary perfusion, decreased cellular edema, and less reperfusion injury. In addition, the added amino acids in the PEG solution are easily taken by cardiomyocytes and used in the Krebs cycle to produce electron donors for the electron transport chain in mitochondria. For example, L-arginine, the amino acid in highest concentration in PEG, specifically has been found to activate nitric oxide synthesis during resuscitation of hemorrhagic shock, which improves arterial flow, decreases lactate production, decreases histologic evidence of reperfusion injury, and improves overall survival in rat studies.

The UW solution uses hydroxyethyl starch as colloid, which imparts higher viscosity at lower temperatures and had poor perfusion through all myocardial layers. ST solution relies on negatively charged chloride ion alone to electrochemically moderate the flow of water but otherwise lacks gluconate or other additives serving as impermeant to prevent cellular swelling and vascular compression. This was evident in our study where the initial low perfusion pressures progressed rapidly to high perfusion pressures and evidence of myocyte breakdown in the form of very high troponin release. Troponin (cTnI) release is an indicator of ischemic myocardial damage. DCD hearts released higher amounts of troponin compared to CBD hearts, especially at 4°C compared to 15°C. DCD hearts perfused with ST solution particularly had the highest concentration of troponin release, both at 4 and at 15°C. Interestingly, DCD hearts perfused with UW and in particular, PEG solution had the least amount of troponin
release and was comparable to troponin release from CBD hearts. Our troponin data support DCD heart preservation with PEG solution at 15°C over UW or ST solutions.

A limitation of the study is the adoption of a modified donation after brain death model, in which a fatal traumatic brain injury has not been incurred in our rats. Although our controlled beating-heart donor (CBD) group mimicked the continued oxygenation and function of cardiac tissue that serves as a premise for transplant suitability, inflicting brain injury can cause a sympathetic storm and systemic inflammatory response that would potentially affect cardiac structure and function. The small sample size in six groups may have limited the statistical power of some reported results. The blood-based perfusate is physiologic and we did not compare our preservative solution against blood as perfusate. The results from our study can develop future studies investigating cardiac function after midthermic preservation on a Langendorff setup with reanimated hearts or in heterotopic heart transplant murine models.

5 CONCLUSION

Our study findings suggest optimal preservation conditions of DCD hearts are with PEG solution at 15°C.

ACKNOWLEDGMENTS

This work was supported by Merit Review Grant awarded to Dr. Mohammed Quader (1I01 BX003859) and funds from the Pauley Heart Center to Mohammed Quader and Dr. Stefano Toldo. Services and products in support of the research project were generated by the Virginia Commonwealth University Cancer Mouse Models Core Laboratory, supported, in part, with funding to the Massey Cancer Center from NIH-NCI Cancer Center Support Grant P30 CA016059.

CONFLICT OF INTEREST

None of the authors has conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Stefano Toldo and Mohammed Quader designed the study. Stefano Toldo and Mohammed Quader secured funding for the study. Renee Cholyway, Stefano Toldo, Oluwatoyin Akande, Kristine Kenning, performed the experiments. Adolfo Gabriele Mauro and Eleonora Mezzaroma developed the imaging and analysis protocol. Renee Cholyway, Stefano Toldo, and Mohammed Quader collected, analyzed, and interpreted the data. Renee Cholyway and Stefano Toldo performed the statistical analysis. Renee Cholyway drafted the article. Stefano Toldo and Mohammed Quader performed a critical revision of the article. All the authors gave final approval of the article.

ORCID

Stefano Toldo https://orcid.org/0000-0002-1588-4019

REFERENCES

1. Mozaffarian D, Benjamin EL, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics—2015 update: a report from the American Heart Association. Circulation. 2015;131:e29-322.
2. Colvin M, Smith JM, Hadley N, Skeans MA, Carrico R, Uccellini K, et al. OPTN/SRTR 2016 annual data report: heart. Am J Transplant. 2018;18(Suppl 1):291–362.
3. Mancini D, Lietz K. Selection of cardiac transplantation candidates in 2010. Circulation. 2010;122:173–83.
4. Steinbrook R. Organ donation after cardiac death. N Engl J Med. 2007;357:209–13.
5. Quader M, Toldo S, Chen Q, Hundley G, Kasirajan V. Heart transplantation from donation after circulatory death donors: present and future. J Card Surg. 2020;35:875–85. https://doi.org/10.1111/jocs.14468
6. Ribeiro RVP, Alvarez JS, Yu F, Paradiso E, Adamson MB, Maria Ruggeri G, et al. Hearts donated after circulatory death and reconditioned using normothermic regional perfusion can be successfully transplanted following an extended period of static storage. Circ Heart Fail. 2019;12:e005364.
7. Potter KF, Cocchiola B, Quader MA. Donation after circulatory death: opportunities on the horizon. Curr Opin Anaesthesiol. 2021;34:168–72.
8. Osaki S, Anderson JE, Johnson MR, Edwards NM, Kohimoto T. The potential of cardiac allografts from donors after cardiac death at the University of Wisconsin Organ Procurement Organization. Eur J Cardio-thorac Surg. 2010;37:74–9.
9. Noterdaeme T, Detry O, Hans MF, Nellessen E, Ledoux D, Joris J, et al. What is the potential increase in the heart graft pool by cardiac donation after circulatory death? Transplant Int. 2013;26:61–6.
10. Quader MA, Wolfe LG, Kasirajan V. Heart transplantation outcomes in patients with continuous-flow left ventricular assist device-related complications. J Heart Lung Transplant. 2015;34:75–81.
11. Messer S, Page A, Rushton S, Berman M, Tsui S, Catarino P, et al. The potential of heart transplantation from donation after circulatory death donors within the United Kingdom. J Heart Lung Transplant. 2019;38:872–4.
12. Repse S, Pepe S, Anderson J, McLean C, Rosenfeldt FL. Cardiac reanimation for donor heart transplantation after cardiocirculatory death. J Heart Lung Transplant. 2010;29:747–55.
13. Rosenfeldt F, Ou R, Salamonson R, Marasco S, Zimmet A, Byrne J, et al. A novel combination technique of cold crystalloid perfusion but not cold storage facilitates transplantation of canine hearts donated after circulatory death. J Heart Lung Transplant. 2016;35:1358–64.
14. Toldo S, Quader M, Salloum FN, Mezzaroma E, Abbate A. Targeting the innate immune response to improve cardiac graft recovery after heart transplantation: implications for the donation after cardiac death. Int J Mol Sci. 2016;17:958. https://doi.org/10.3390/ijms17060958
15. Sutherland FJ, Hearse DJ. The isolated blood and perfusion fluid perfused heart. Pharmacol Res. 2000;41:613–27.
16. Plant V, Parrish DW, Limkemann A, Ferrada P, Aboutanos M, Mangino MJ. Low-volume resuscitation for hemorrhagic
shock: understanding the mechanism of PEG-20k. J Pharmacol Exp Ther. 2017;361:334–40.
17. Quader M, Toldo S, Torrado J, Mishra S, Mauro AG, Mezzaroma E, et al. Determination of optimal coronary flow for the preservation of "donation after circulatory death" in murine heart model. ASAIO J. 2018;64:225–31.
18. Lowalekar SK, Treanor PR, Thatte HS. Cardioplegia at subnormothermia facilitates rapid functional resuscitation of hearts preserved in SOMAH for transplants. J Cardiothorac Surg. 2014;9:155.
19. Lowalekar SK, Cao H, Lu XG, Treanor PR, Thatte HS. Subnormothermic preservation in somah: a novel approach for enhanced functional resuscitation of donor hearts for transplant. Am J Transplant. 2014;14:2253–62.
20. Feemster JA, Lillehei RC. Hypothermic-hyperbaric pulsatile perfusion for preservation of the canine heart. Transplant Proc. 1969;1:138–46.
21. Rivard AL, Gallegos R, Ogden IM, Bianco RW. Perfusion preservation of the donor heart: basic science to pre-clinical. J Extra Corpor Technol. 2009;41:140–8.
22. National Research Council Institute for Laboratory Animal Research. Guide for the care and use of laboratory animals. Washington, DC: National Academies Press; 1996.
23. Quader M, Cholyway R, Wickramaratne N, Akande O, Mangino M, Mezzaroma E, et al. Refining murine heterotopic heart transplantation: a model to study ischemia and reperfusion injury in donation after circulatory death hearts. Animal Model Exp Med. 2021;4:283–96.
24. Quader M, Torrado JF, Mangino MJ, Toldo S. Temperature and flow rate limit the optimal ex-vivo perfusion of the heart—an experimental study. J Cardiothorac Surg. 2020;15:180.
25. Dhital KK, Iyer A, Connellan M, Chew HC, Gao L, Doyle A, et al. Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series. Lancet. 2015;385:2585–91.
26. Whitlock RP, Chan S, Devereaux PJ, Sun J, Rubens FD, Thorlund K, et al. Clinical benefit of steroid use in patients undergoing cardiopulmonary bypass: a meta-analysis of randomized trials. Eur Heart J. 2008;29:2592–600.
27. Plant V, Limkemann A, Liebrecht L, Blocher C, Ferrada P, Aboutanos M, et al. Low-volume resuscitation using polyethylene glycol-20k in a preclinical porcine model of hemorrhagic shock. J Trauma Acute Care Surg. 2016;81:1056–62.
28. Parrish D, Lindell SL, Reichstetter H, Aboutanos M, Mangino MJ. Cell impermeant-based low-volume resuscitation in hemorrhagic shock: a biological basis for injury involving cell swelling. Ann Surg. 2016;263:565–72.
29. Parrish D, Plant V, Lindell SL, Limkemann A, Reichstetter H, Aboutanos M, et al. New low-volume resuscitation solutions containing PEG-20k. J Trauma Acute Care Surg. 2015;79:22–9.
30. Devillard L, Vandroux D, Tissier C, Dumont L, Borget J, Rochette L, et al. Involvement of microtubules in the tolerance of cardiomyocytes to cold ischemia-reperfusion. Mol Cell Biochem. 2008;307:149–57.
31. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. Transplantation. 1988;45:673–6.
32. Rubio R, Berne RM. Regulation of coronary blood flow. Prog Cardiovasc Dis. 1975;18:105–22.
33. Duncker DJ, Bache RJ. Regulation of coronary blood flow during exercise. Physiol Rev. 2008;88:1009–86.
34. Arora TK, Malhotra AK, Ivatury R, Mangino MJ. L-arginine infusion during resuscitation for hemorrhagic shock: impact and mechanism. J Trauma Acute Care Surg. 2012;72:397–402.
35. Bratton SL, Chestnut RM, Ghajar J, McConnell Hammond FF, Harris OA, Hartl R, et al. Guidelines for the management of severe traumatic brain injury. I. Blood pressure and oxygenation. J Neurotrauma. 2007;24(Suppl 1):S7–13.
36. Lu J, Goh SJ, Tng PY, Deng YY, Ling EA, Moochhala S. Systemic inflammatory response following acute traumatic brain injury. Front Biosci. 2009;14:3795–813.

SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

How to cite this article: Cholyway RP, Akande O, Mauro AG, Mezzaroma E, Wang R, Kenning K, Toldo S, Quader M. (2022). Assessment of machine perfusion conditions for the donation after circulatory death heart preservation. Artif Organs. 2022;46:1346–1357. https://doi.org/10.1111/aor.14208