Challenges in extended ex vivo heart perfusion in a porcine model of donation after circulatory death

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1. Abstract

Heart transplantation is the gold standard treatment for patients with advanced heart failure, but organ shortage remains a major limitation. Donation after circulatory death (DCD) combined with ex vivo heart perfusion increases the number of hearts available for transplantation; however, it is limited to approximately 6 hours in clinical practice. Prolonged perfusion would further increase organ availability. This is currently under development at the Department of Cardiac Surgery (Inselspital), but it is limited by severe hemolysis, which occurs after an average of 12 hours. This study investigates whether the experimental perfusion system itself contributes to red blood cell (RBC) damage. A blood-only perfusion model was established using the same circuit and conditions as heart perfusions. After 20 hours, little to no hemolysis was observed. Changes in free heme and oxidative stress were comparable to those observed during heart perfusions. These findings suggest that the perfusion system causes little RBC damage and is unlikely to be the main cause of sudden hemolysis. Further investigation is therefore needed to determine the role of the heart and its associated processes.

Keywords: Donation after circulatory death (DCD), ex vivo heart perfusion, blood-only perfusion, hemolysis

2. Introduction

Heart failure (HF) affects an estimated 64.3 million people worldwide and remains a major cause of morbidity and mortality [3]. Heart transplantation (HT) is the gold standard therapy for advanced HF [2], but the shortage of donor hearts remains a major challenge [3]. Donation after circulatory death (DCD) expands the donor pool [1], but exposes hearts to warm ischemia, increasing the risk of ischemia reperfusion injury [4]. Normothermic ex vivo heart perfusion (EVHP) enables continuous perfusion and functional assessment while reducing cold ischemia. Extending EVHP duration could further expand the donor pool, facilitate long-distance transport and improve donor-recipient matching.
While perfusion times of an average of 12 hours have been achived experimetally, sudden hemolysis remains a major limitation. This study investigates the contribution of the perfusion system itself to hemolysis using a blood-only perfusion model without a heart.

3. Aims and leading questions

Aim: Clarifying the mechanism leading to hemolysis and oxidative stress during prolonged ex vivo heart perfusion, ultimately providing insights to optimize preservation strategies for DCD hearts.

RBC lifespan: What is the lifespan of slaughterhouse pig RBCs during prolonged ex vivo perfusion in a porcine DCD model without a heart?

Effect of the heart: How do free heme and oxidative stress levels compare between DCD systems with vs. without a heart?

4. Methods and material

3 blood-only perfusion experiments were performed for 20 hours using porcine RBC in a sterile, closed EVHP circuit under conditions mimicking DCD heart perfusion. Perfusate and plasmapheresis waste samples were collected every 2 hours and analyzed for hemolysis related parameters, free heme and oxidative stress markers. The blood-only results were compared with samples from previous porcine DCD heart perfusions performed using the same protocol.

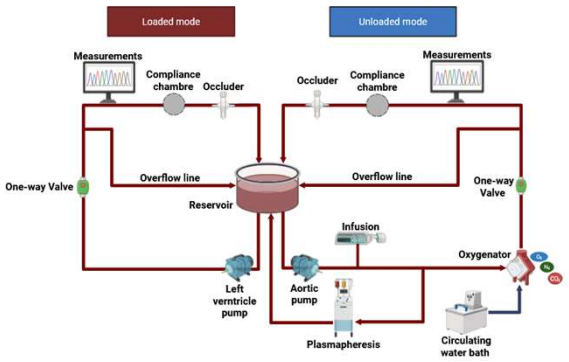

Fig 1 Depiction of the blood only system, with the loaded line (left) and the unloaded line (right), (Fürst C. 2026, created with Biorender)

Statistical analyses were performed using the Mann-Whitney test with $p < 0.05$ considered statistically significant.

5. Results

Blood-only perfusions maintained stable RBC-related parameters over 20 hours, with potassium, hemoglobin and hematocrit remaining relatively constant. In contrast, pig heart perfusions terminated earlier (9.0-17.54 hours) and showed signs of hemolysis including increased potassium and decreased hemoglobin and hematocrit.

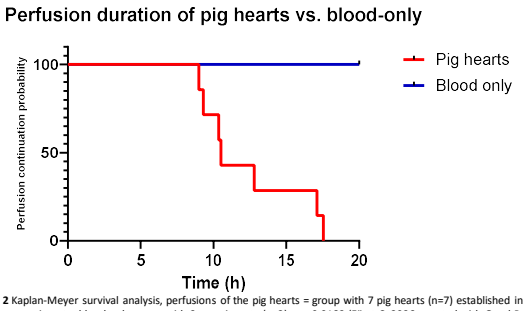

Fig 2 Kaplan-Meyer survival analysis, perfusions of the pig hearts = group with 7 pig hearts (n=7) established in prior experiments, blood-only group with 3 experiments (n=3), p = 0.0182 (Fürst C. 2026, created with GraphPad)

Free heme accumulation tended to be higher in pig heart perfusions (n=6) than in blood-only perfusions (n=3), with a significant increase in total release at the end of perfusion (p=0.0476). Similarly, 8OHdG accumulation and release tended to be higher in pig heart perfusions, reaching statistical significance for total release at the end of perfusion ($p<0.05$).

6. Discussion , conclusion and outlook

The RBC from slaughterhouse pigs survived 20 hours of EVHP without signs of hemolysis and the experiments could likely have continued longer. Free heme and oxidative stress, which is associated with RBC damage and hemolysis, gradually increased over time, but there was no sudden RBC breakdown in the blood-only system. Compared with heart perfusions, levels of oxidative stress were very similar during the first 8 hours, whereas heart perfusions showed a major increase in free heme and oxidative stress towards their endpoint.
These data suggest that the perfusion system itself is unlikely to be responsible for the sudden destruction of RBC and that the presence of the heart plays an important role. However, as only 3 blood-only experiments were performed, these conclusions should be interpreted cautiously.
The heart and its processes therefore need to be investigated more closely to determine the mechanisms leading to sudden hemolysis.

List of references

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Figures

Fig 1 Fürst C. 2026g. Depiction of the blood-only experiment system, medi
Fig 2 Fürst C. 2026h. Kaplan-Meyer survival analysis of perfusion times, medi