Author’s response to reviews

Title: Investigating the physiology of normothermic ex vivo heart perfusion in an isolated slaughterhouse porcine model used for device testing and training

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Author’s response to reviews:

Dear Dr. Byrne, Dr. Freed and Dr. Ghodsizad,

we would like to thank you for the time you took to review this manuscript. The reviewers have made valuable suggestions, which we thoughtfully considered. We have added a table with changes for each point raised by the reviewers on the following pages. Due to the suggestions the text underwent a major overhaul.

In summary, we toned-down the title and several aspects of the text. We have highlighted that the presented data are representative for our platform only but could be of potential interest for other platforms as well.
We believe our study will be a contribution to the field and matches the scope of the journal. If there remain any questions left, don’t hesitate to let us know.

Kind regards,

Benjamin Kappler

Editor

1) In the section Ethics approval and consent to participate, please include information on the ethics approval for this study.

We have specified the ethical approval.

“All protocols followed by the slaughterhouse and laboratory were consistent with EC regulations 1069/2009 regarding the use of slaughterhouse animal material for diagnosis and research, supervised by the Dutch Government (Dutch Ministry of Agriculture, Nature and Food Quality) and were approved by the associated legal authorities of animal welfare (Food and Consumer Product Safety Authority).”

Reviewer 1

The authors present a limited experience using the PhysioHeart platform to 'revive and resuscitate' hearts procured from a slaughterhouse. This is presented as an analogous situation to the DCD context. While investigations on ex situ heart perfusion are topical given the current context of donor heart shortage, this report suffers from the fundamental flaw of an appropriate control group. Without comparing these results to those obtained from carefully procured hearts, one cannot conclude anything meaningful on the suitability of hearts procured from the slaughterhouse in this manner.
We thank the reviewer, Dr. Freed, for his precious time and valuable comments as an expert in the field of ex vivo cardiac perfusion.

We have carefully evaluated this general comment of the reviewer and understand the point raised. As a consequence, the manuscript underwent a complete overhaul. We have toned down the entire manuscript and tailored our introduction, discussion as well as conclusion to the use of our platform only.

The new aim, which is to report a comprehensive inventory of time-dependent changes in our setup only, does not require a control group in our point of view anymore.

However, we have added a subtitle “possible clinical relevance” as part of the discussion, in which we dared to speculate on the eventual clinical relevance of our study.

1) Why were hearts paced at the RVOT rather than atrial pacing

The choice between atrial and ventricular pacing followed experimental con-straints. It happens from time to time that some hearts have no atrial activity. For this reason and to remain consistent in the experiment protocol, we only paced at the RVOT. Conversely, we have chosen the RVOT pacing because it provides good conduction and hemodynamic properties.

2) Page 6 line 20 - do the authors mean 'atrial' pressure rather than 'arterial'?

We have corrected the word which now spells: “atrial”.

3) The authors have conducted an elegant analysis of surface ECG; how does this compare to a 'normal' heart?

In our reply we assume that by “normal” the reviewer refers to a human heart.
Even though the purpose of this paper is not to draw similarities between porcine and human hearts, we can speculate on the translational proper-ties of this experiment. Given the similarities between the porcine and hu-man cardiac physiology [2-4], we would expect the discussion in L301-323 to be largely equivalent on human hearts. The main difference between porcine and human hearts would be in the magnitudes of the quantities measured. For instance, in human hearts, we would expect the sinus rhythm to be closer to 75 bpm and the wave propagation velocity to be between 60-70 cm/s at that heart rate. However, we believe that the main contribution of the electrophysiological analysis is to show that, under this setup, the abnormalities in the ion con-centrations in blood have a quantifiable effect on the electrical properties of the tissue. Hence, monitoring the electrical activity of a heart in normo-thermic perfusion platforms using an array of electrodes could be a valua-ble asset when determining the physiological condition of the heart. We argue that monitoring the electrical activity, in concert with the hematolo-gy, gives the means to understand the causes for reduced heart perfor-mance and the means to mitigate these causes. This argument was ex-pressed in our manuscript in L319-323.


4) The authors describe 'unphysiologically' high electrolyte concen-trations. It appears from the system photographs that the apparatus is not enclosed. Was this high concentration due to evaporation of free water? Was any attempt made to restore homeostatic values? If not why not?

We thank the reviewer for the comment. In case, evaporation would be the reason for the rise of the ions, all measured biomarkers would rise. How-ever, this is not the case. We have added an additional explanation in the text.
“Finally, the static concentrations of albumin, triglycerides, urea, creatinine, calcitriol but also potassium exclude the possibility that the rise of electrolytes could arise from evaporation of free water in our system.” (page 15, line 352)

For the second part of the question we would like to highlight that this manuscript should be a first step to understand the occurring biochemical changes over time, to closely evaluate the issues and to discuss future necessary effort. To take action to overcome these observed issues was out of scope for this manuscript.

Nevertheless, we would like to emphasize that we recently have made valuable attempt with dialysis to restore the homeostatic values which resulted in an additional manuscript which is under review elsewhere.

5) The authors describe a steady increase in plasma free hemoglobin. What was the source of hemolysis? Clearly the system needs optimization.

We have added the following explanation in the manuscript:

“The rise of plasma free hemoglobin in our study was not significant. However, in only one experiment free hemoglobin passed 0.08 mmol/L, which occurred already from the beginning of the experiment. That could have resulted from pre-experimental blood handling. We identified the centrifugal pump as the source with the highest risk to induce hemolysis.”(page 15, line 350)

6) Was the 10L blood acquired from alternate animals filtered and leukocyte reduced? If not, how did immunocompatibility affect preservation of function, inflammation etc?

We thank the reviewer for this valuable question.

We did not use leukocyte-filtered blood in our study. We have elaborated on this issue in the manuscript:

“Generally, pooling blood leads to transfusion reaction in humans, but the particular characteristics of the porcine hematopoietic system make porcine blood pooling less harmful as it causes transfusion reaction in very rare cases.” (page 13)
“Throughout the experiment, we observe a rise in cardiac injury markers caused by reperfusion injury and inflammatory responses of leukocytes and platelets.” (page 14)

“In view of improved and prolonged preservation of the PhysioHeart™ model is the mitigation of the immune response of the pig blood. This can be achieved by separating lymphocytes and platelets, to obtain platelet and lymphocyte-poor blood in combination with administering inflammatory and autoimmune depressing drugs (i.e. dexamethasone, prednisone). The use of antibiotics and fungistatic medication would further serve to avoid infections.” (page 16)

7) Although the authors have presented a method for research on ex situ heart perfusion, there appear to be a number of opportunities for optimization of the procurement protocol, the perfusion system/hardware, and perfusion protocol that need to be done to prove whether the changes presented are an artifact of the overall protocol vs a real phenomenon that occurs during ESHP generally.

We thank the reviewer for the very important remark. As a consequence, we have entirely revised the manuscript with the focus on our own device and the need for it.

Reviewer 2

The conclusion of the abstract does not reflect all relevant facts in summary.

We thank Dr. Ghodsizad as an expert for his comments and his time to re-view the manuscript. The manuscript underwent major changes. In this context, we have also revised the abstract.

1. Please mention the only CE and about to be FDA approved clinical model for human ex vivo device Transmedics. It is important to mention because that device never found its way to be used in a working mode.

We strongly advise to add following citation:

We have cited the study in page 3 line 40

“The optimal perfusion with warm oxygenated blood enables realistic device validation, while these setups can be also medical devices themselves (e.g. donor heart transportation). [2,3]“

2. Please mention why you decided to go with St Thomas solution

We thank the reviewer for the valuable comment and added the following sentences in the text:

“A more complex composed cardioplegic solutions like Custodiol, Somah, Cel-sior or UWS, could be of favor during hypothermic storage of slaughterhouse hearts. However, the use of a more complex solution also requires a careful consideration of price and advantages, which are currently under evaluation.” (page 13, line 317)

3. Metabolic panel data should be presented in a diagram, Figures 6 to 8 should be summarized

We thank the reviewer for this suggestion. However, we are not sure what the reviewer means exactly with “diagram”, as the data are already presented in a diagram. Moreover, the aim of the study was to present a comprehensive data set of the biochemical changes in our setup to understand the source of cardiac degradation over time in detail. Therefore, we think it is only possible to entirely understand the ongoing biological processes, when the data are presented as they are.

4. Please refer to the pig anatomy and differences to human anatomy

We have added an explanation in the introduction ( page 3, line 50).

“However, potentially significant differences (i.e. shape, opening of superi-or and inferior caval veins into the atrium, prominent left azygous vein drainage, number of pulmonary veins, etc.) are known between porcine and human hearts.[9]”

5. How did you protect the coronary sinus as venous branches drain into it in pigs
We have applied the cardioplegic solution after heart removal from the thorax. Therefore, we were able to directly cannulate the dissected aorta and apply the 2 L of cardioplegia with no hesitation, where it might have caused issues in an in vivo situation.

6. Please comment the fact that animals were killed by electric shock, do you expect an effect on the physiology of cardiomyocytes?

We thank the reviewer for this comment. We have specified that the pig got only head-stunned in the method section. We therefore do not expect any damages to the cardiomyocytes, as the electrical current should occur between the electrodes.

7. Did you give the animals heparin before the cardiac arrest?

As we had to follow the guidelines for food production. No heparin was administered to the animals prior harvest. This has been specified in the methods section:

“The procedure for harvesting the hearts was equivalent in all the animals and is summarized in this section. Before heart harvesting, the pig was electrically stunned, hung and exsanguinated, but not heparinized.”