

Development of a new decellularization protocol for the whole porcine heart

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Development of a new decellularization protocol for the swine heart: a standard preservation of left ventricular compliance

Journal of Clinical and Translational Research

Dear Dr Miglino,

Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, reviewers' comments are appended below.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you submit the revised manuscript. Also, please ensure that the track changes function is switched on when implementing the revisions. This enables the reviewers to rapidly verify all changes made.

Your revision is due by Jun 20, 2021.

To submit a revision, go to <https://www.editorialmanager.com/jctres/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Reviewer #1: In this paper, authors describe three methods of decell of the swine heart aimed at the obtention of bio-scaffolds able to maintain the properties of extracellular matrix of the myocardium and therefore to support recell new transplantable myocardium. The paper is strongly limited by a confuse wording a too long extension and lack of novelty and usefulness for the field.

Reviewer #2: While I can commend the authors on the extensive work undertaken to develop a protocol to support a promising field of tissue engineering, these are some of the more substantial limitations to the submitted work:

- 1) It is unclear what problem the authors seek to solve for. In the introduction, they propose the field is constrained by the ability to 'remove all cell debris' in 'minimal time'. However, others (several years ago now) have been quite successful in achieving this. Best described across species in Guyette et al Nat Protocols (2014), human heart decellularization can be successfully achieved in approximately 7 days. The field is rather limited by the ability to functionally recellularize and re-endothelialize the organ graft. This is not attempted in this work.
- 2) The experimental protocols differ in at least 4 ways (mentioned above), which make it impossible to identify which parameters are ideal
- 3) Significant focus is placed on the structural dynamics of the organ (volume, compliance) as the authors point out (rightfully) that this is an important attribute of a transplantable graft. However, these evaluations are done on the decellularized organ, which should not be expected to perform in any way comparably to an organ with cells. In particular the heart, where cardiomyocytes are responsible for cardiac function. In addition, organ shrinkage is said to be a negative, which is unclear to this reviewer. In personal experience, successful decellularization results in a volume increase as a result loss of structural integrity that goes with the removal of cells.
- 4) The experimental design or protocol optimization is unclear. Twenty-one porcine hearts are described in the methods, but it cannot be derived from the data how many organs were used for what appears to be sequential phase of the research. I recommend clarifying this so that the data can be better evaluated by readers.

Reviewer #3: Please provide some more data concerning techniques for heart decellularization and methods used in the paper. It should be briefly justified and confirmed in the introduction section. Additionally Authors used 3 different methods of decellularization but did not provide any scientific support for them. According to Materials and Methods. Please provide some

more data concerning rinsing of the decellurized hearts from detergents used. Please provide more justification for the type of cells you have used. The conclusion that ECM is suitable for cell adhesion is well known fact. There are studies that depicts revitalized hearts able to heart contraction. Please verify the correctness of the quotations used - in some works the year of publication does not match.

Authors' response

Manuscript title: Development of a new decellularization protocol for the swine heart: a standard preservation of left ventricular compliance

Rebuttal Letter

Dear Reviewers,

We would like to thank you for your comments on our manuscript. All the comments were extremely valuable for the improvement of our article, and we are sure that you made excellent work. Regarding this, we hereby inform you of the changes made to this manuscript, following the order of the questions raised.

Answer to Reviewer 1:

In view of the comments of Reviewer 1, the writing of the article underwent changes and was redirected, emphasizing the content in line with our objective, which was the development of a new protocol for porcine hearts decellularization. All topics, from the introduction until the conclusion of our study underwent important changes. And we are sure these changes contributed for an intense improvement of the content covered in the manuscript. Because of this, we decided to change the manuscript title title to “**Development of a new decellularization protocol for the whole porcine heart**”. The literature about the biomaterials encourages the bioscaffolds production for field application, reinforcing the importance of developing and adapting decellularization protocols to acquire different forms to obtain these scaffolds.

Answer to Reviewer 2:

Considering the observations made by the Reviewer 2, we placed the objective of the work focusing on obtaining a new protocol for the decellularization of the porcine heart in the last paragraphs of the abstract and introduction of the manuscript. In addition, the reviewer perfectly mentioned the existence of effective protocols in the heart decellularization; however, based on literature, we reinforce that only some of these works are specifically had focus in the porcine hearts - and most of them address the decellularization of porcine heart valves, few of them address the decellularization of the whole heart. Sometimes they also deal with expensive

protocols to be reproduced. In this way, we emphasize the experimental protocol established by our work is cheaper, although we recognize that it needs improvement in future studies.

As for the methodology, we decided that it would be better to focus on protocol 3; protocols 1 and 2 previously present in topic 2.1 have been removed from this article and its results have been removed from the rest of the text. We reiterate that several protocols were tested and adapted in our research until we attain the mentioned. Protocol 3 showed the best results, both in macroscopic and microscopic analysis; and the results obtained from this analysis were the parameters adopted for this conclusion; it should be noted that, for comparative purposes, a native heart was used in this work as well as cited in the text.

Thus, we explored the data obtained through this protocol, which was chosen by the article as a "new decellularization protocol for porcine heart", making the article's wording clearer and more cohesive throughout, as well as better adjusting the methodology used and the chosen results. Therefore, the results were better propagated in the discussion topic.

Topics that previously addressed the calculation of left ventricular volume (2.2), compliance of decellularized heart organs (2.2) and stereology (2.3) were taken from our manuscript. Consequently, Tables 1 and 2 and Figure 5 were removed from the text. Given the criticism raised by Reviewer 2, we chose to focus on the production of a bioscaffold that can be recellularized - since one of the main characteristics of a functional bioscaffold is the capacity of the matrix to receive and provide an adequate environment for the proliferation of new cells; cell adhesion and proliferation capacity. We preferred to keep the content of this article within tissue engineering, without explain the regenerative medicine area such as organ transplantation. Due this, we also reformulated the manuscript title.

All information regarding volume, sterology and organ compliance removed from the main text was reorganized and separated into a Supplementary data, which is attached to the review documents.

However, we emphasize in this letter that Montaham et al. (2015) performed a test of mechanical properties of the heart; decellularized heart samples compared to native hearts had their volume calculated, and then the compression was measured by stress as a function of the deformation of these samples, to assess their mechanical stiffness. They concluded that decellularized samples tend to have a smaller elasticity and resistance to compression stress than native tissue samples.

Additionally, Ferng et al. (2017) in their study which provide a protocol for whole porcine heart decellularization, also related a decrease of weight after decellularization process, indicating a volume retraction, as described in our findings.

Answer to Reviewer 3:

In response to the questions raised by the Reviewer 3; the focus porcine heart decellularization protocol is now discussed in more detail in its methodology included in the second paragraph of topic 2.1 and 2.2; therefore, it was decided to remove protocols 1 and 2 from the text, giving greater transparency and consistency to the manuscript, its methodology, results, and discussion.

We insert some of the main references by which we base ourselves for the formulation of the new protocol in topic 2.1. Additionally, we explain a little more about the detergents used in this technique in the discussion (topic 4).

As for the recellularization of the scaffold obtained, we chose to use equine fibroblast mainly for reasons of convenience, as it is a cell available in our Cell Bank and with a high proliferative capacity; described in topic 2.6. We reiterate that this part of the methodology was applied for proof-of-concept purposes only, to demonstrate that the extracellular matrix of the scaffold produced was suitable for cell adhesion. The results obtained were positive and relevant to our hypothesis and are described in topic 3.5 and 3.6 and discussed in topic 4.

Finally, many references were changed in the text; some present in the old version were removed, others were checked properly and added to the new version.

To all the Reviewers, we emphasize that, as well as the changes made throughout the text, changes in the sequence of images and their captions were also made, leaving the sequence of methodology and results shown in our manuscript more organized.

We are grateful for the valuable considerations and available for any necessary clarifications.

Sincerely,

The authors.

2nd Editorial decision
12-Jul-2021

Ref.: Ms. No. JCTRes-D-21-00021R1
Development of a new decellularization protocol for the whole porcine heart

Journal of Clinical and Translational Research

Dear authors,

I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers:

Reviewer #2: My thanks to the author for the careful consideration of the reviewers comments. The changes add clarity to the objective of the study and the added focus to a specific protocol and key outcomes remove concern about suggesting an optimization study and questions about the relevance of the endpoints evaluated.