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Layer-by-layer heparinization of decellularized liver matrices to reduce thrombogenicity of tissue engineered grafts

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1st editorial decision:

Date: 9 July, 2015

Ref.: Ms. No. JCTRes-D-15-00002
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Dear Mr. Bruinsma,

Reviewers have submitted their critical appraisal of your paper. The reviewers' comments are appended below. Based on their comments and evaluation by the editorial board, your work was FOUND SUITABLE FOR PUBLICATION AFTER MINOR REVISION.

If you decide to revise the work, please itemize the reviewers' comments and provide a point-by-point response to every comment. An exemplary rebuttal letter can be found on at http://www.jctres.com/en/author-guidelines/ under "Manuscript preparation." Also, please use the track changes function in the original document so that the reviewers can easily verify your

responses.

Your revision is due by Jul 15, 2015.

To submit a revision, go to http://jctres.edmgr.com/ 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

Rowan van Golen
Associate Editor
Journal of Clinical and Translational Research

*****Editorial comments*****

Dear Dr. Bruinsma,

Two experts in the field have reviewed your manuscript, based on which the editorial board has decided to accept your paper with minor revisions despite the negative decision by Reviewer 1.

Reviewer 1 posited that the manuscript should be rejected because of the negative end result, further stating that negative results should be published for clinical studies but not in case of experimental studies.

This is in contradiction to the philosophy of JCTR, where we specifically advocate the publication of negative results for several reasons. First, publication of negative results, especially when obtained in a technically sound study (i.e., your study), provides cues as to why a certain procedure or process did not work, thereby guiding alternative strategies and approaches. In that sense, something not working can be considered ‘part’ of the mechanism. Second, negative results prevent colleagues in the field from conducting redundant work. An expedient trajectory to the clinical setting, during which redundancy is minimized, is ultimately more beneficial for patients.

So, in your revision, please address the points of Reviewer 2 only.

Lastly, I kindly ask you to implement the following:

1) Restructure the abstract according to journal guidelines: Background; Aim; Methods; Results; Conclusions; and Relevance for patients. It suffices to copy/paste the first sentence of the Discussion in the ‘Relevance for patients’ section.

2) When proofreading, please ensure that the manuscript is written in past tense where applicable (e.g., Discussion, first paragraph, “Recently, the group reports…” should read “reported.” In the same sentence, “we incorporate…” should read “we incorporated.” Moreover, in the Abstract, last sentence of the Background section, “DLM to thrombogenicity…” should read “DLM on thrombogenicity…”
3) Since this is study reports negative findings, you should consider including a more elaborate directionality of future studies beyond only endothelialization of the matrix (last sentence Discussion) to further increase the value of your work. This may be brief as long as it is guiding future research efforts. For example, are the intravascular plugs that you observe the result of coagulation only or also of platelet aggregation? Perhaps a combinatorial inhibition modality employing heparinization with anti-CD41 or anti-CD62P antibodies constitutes a potential avenue? I am sure you have good ideas of your own.

Congratulations on this study and thank you for contributing your work to JCTR.

Kindest regards,

Michal Heger, Editor-in-Chief.

*****Reviewer comments*****

Reviewers' comments:

Reviewer #1:

Basically the manuscript reports negative results. Negative results are worth being published when they refer to clinical data. Instead, when they refer to experimental data, publication is of not interest to the scientific community at all.

Reviewer #2:

The authors report on Layer-by-layer immobilization of heparin inside decellularized rat liver matrices to reduce thrombogenicity of the tissue engineered grafts. Decellularized rat livers were heparinized with four different methods and then recellularized with primary hepatocytes. Non heparinized decellularized liver grafts served as controls. Tissue engineered livers were cultured in vitro, perfused with blood ex vivo or implanted heterotopically in vivo to evaluate the effects of heparin coating on graft function and thrombogenicity.
Graft function did not significantly differ in any of the groups. Heparinization could significantly reduce thrombogenicity during ex vivo blood perfusion. However, transplantation remained unsuccessful with this method.
The manuscript is well structured and nicely written. Methods are described clearly and results appear logical. The topic is of interest for the field. I would suggest the manuscript for publication in JCTR with minor revisions:

1. Although the presented methodology shows clear advantages in vitro the in vivo results are frustrating. Authors should discuss in more detail why heparinization is capable to prevent clotting in vitro but not in vivo.

2. Ko et al. (2014) showed that it is possible to implant a reendothelized porcine liver graft for 24 hours in vivo with low thrombogenicity. I think adding a group of heparinized decellularized liver matrices recellularized with hepatocytes AND endothelial cells for implantation experiments would be beyond the scope of this manuscript. However, this possibility should at least be mentioned and discussed.

3. Authors state that 4BL scaffolds appeared to produce albumin and urea at lower levels. This could be discussed in more detail.

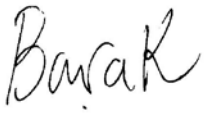
4. From my point of view it would be better for the comprehensibility and perception, if the images with scale bars also have a numeric display.

5. Authors could show some histological images in a smaller magnification to see how the cells are distributed in the matrix, to see if there are vessels occluded with hepatocytes. Cell occlusion might also have an effect on blood clotting.

Authors' rebuttal:

Dear Dr. Heger,

Thank you for giving us an opportunity to resubmit a revised version of our manuscript entitled "Layer-by-layer heparinization of decellularized liver matrices to reduce thrombogenicity of tissue engineered grafts." We appreciate the editor's and reviewers' feedback and have addressed all comments with changes tracked. Changes made to the manuscript have also been outlined below. We believe the manuscript has been significantly improved as a result.
On behalf of the authors, kindest regards,



Basak Uygun, PhD

1. Although the presented methodology shows clear advantages in vitro the in vivo results are frustrating. Authors should discuss in more detail why heparinization is capable to prevent clotting in vitro but not in vivo.

Main differences between the in vivo and in vitro setting are dilution of the blood and a flow-driven, rather than pressure-driven, perfusion of the graft. Both are likely to contribute to better preserved patency of the vasculature in vitro. This has been added to the discussion.

2. Ko et al. (2014) showed that it is possible to implant a reendothelized porcine liver graft for 24 hours in vivo with low thrombogenicity. I think adding a group of heparinized decellularized liver matrices recellularized with hepatocytes AND endothelial cells for implantation experiments would be beyond the scope of this manuscript. However, this possibility should at least be mentioned and discussed.

We had addressed the potential benefit of additional endothelialization in paragraph 3 of the discussion. As this is an essential component of the engineered graft it warrants particular emphasis and we have expanded on this discussion as suggested.

3. Authors state that 4BL scaffolds appeared to produce albumin and urea at lower levels. This could be discussed in more detail.

Since this result is not statistically significant we wouldn't want to overemphasize this. To avoid this we've restated these results.

4. From my point of view it would be better for the comprehensibility and perception, if the images with scale bars also have a numeric display.

This has been added as suggested.

5. Authors could show some histological images in a smaller magnification to see how the cells are distributed in the matrix, to see if there are vessels occluded with hepatocytes. Cell occlusion might also have an effect on blood clotting.

We have added a lower magnification, which in addition to the other images shows that vessels are not obstructed. The text has also been modified to include mention of the absence of vascular obstruction.

2nd editorial decision:

Date: 13 July, 2015

Ref.: Ms. No. JCTRes-D-15-00002R1
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Dear Mr. Bruinsma,

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

Please do not hesitate to contact us with any questions you may have about the production process.

Thank you for submitting your work to JCTR.

Kindest regards,

Rowan van Golen
Associate Editor
Journal of Clinical and Translational Research