Author’s response to reviews

Title: A multi-chamber tissue culture device for load-dependent parallel evaluation of tendon explants

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APPENDIX

Point-by-Point response to the reviewer comments.

Reviewer#1:

Point 1
“This manuscript examines the use of an ex vivo culture system for tendon explants to understand homeostasis and healing. Developing an explant system could be beneficial to the field, but this reviewer has concerns regarding this current system. The novelty of this system is low, a similar system was used by Ed Grood in the 1990's to examine rat tail fascicles.”
We appreciate the comments of the reviewer that our model has limitations and undoubtedly may have design similarities with previously published devices. We gladly acknowledge the limitations of our device and prior art on the topic. Therefore, we have included a new paragraph that discusses the original papers by Grood and others using cells and allografts, as well as a more recent review by Galloway and colleagues (see references below). We note that the Wang paper focused on cell stretching, while the Nabeshima paper uses a device that can provide loads to explant tissues but has only one chamber. Other groups at Mayo Clinic have used static single load devices using one chamber. The key innovation of our device is the use of multiple vertical cylinders as parallel chambers for explant culture to test multiple concentrations of drugs, growth factors and morphogens under different load conditions. If such a device had been available to us, we would not have been motivated to design it. We have modified the Discussion of our paper to clarify the novel aspects of our device, what it achieves to do, and address the similarities and differences with the device described by Grood and colleagues.

The following changes have been incorporated.

Manuscript title has been changed to: “A multi-chamber tissue culture device for load-dependent parallel evaluation of tendon explants”
Method section, page 5, lines 14-17
Discussion section: page 11, line 24-page 12, line 16

Extra References


Point 2
“It has a static load applied to a tendon, which is not physiologic.”

We agree that the device as tested provides only static load, because that is the simplest version of the device that permits ease of use and can provide proof of concept. Static load is still better than applying no load in ex vivo explant culture and a single-chamber version of this device was tested by other groups at Mayo Clinic. We have revised the Discussion to clarify that static loading is not entirely physiological and that the prototype can be adapted in principle with an add-on device that can provide cyclical strain and desired levels of force.
Changes incorporated to the manuscript to address the comments of the reviewer in the Discussion section, page 11, line 13-16.

Point 3
“The loaded tendon show genetic differences compared to the snap frozen controls so this system does not maintain the tendon phenotype during culture, but this was not addressed in the discussion. Are these changes critical and how would that be answered?”

While the loaded tendons do not show genetic differences (i.e., changes in DNA), we observed changes in gene expression (i.e., differences in mRNA levels). We clarify that these differences are expected because explant tissues are subjected to different microenvironments (in vivo versus cell culture conditions). Tendon tissues may change their phenotype during culture as has been observed for cartilage explant cultures. We note that because we expected differences between frozen and cultured explants, it was critical to include this control and establish the nature and magnitude of this unavoidable fact under our culture conditions. However, key comparisons with this device will ultimately be made only between cultured explants maintained in parallel chambers, thus accounting for variability due to ex vivo culture in the studies that we envision. We have modified the text to provide a better explanation of our findings.

Changes has been incorporated to the manuscript to clarify these points in the Discussion section, page 11, line 7-17

Point 4
“The gene analysis has high levels of variability. What is the cause of this variability?”

Variability in gene expression within the same set of samples may in part be due to normal regional differences within the tendon or perhaps be due to limited necrosis. Both conditions may confound the expression values we measured. We now discuss these interpretations in our modified paper.

Changes were incorporated in the Discussion section, page 11, line 7-17

Point 5
“Figure 1 is difficult to comprehend.”

We have modified Figure 1 and its legend to improve the presentation.

Reviewer#2:
“The device described in this manuscript is unique and the authors showed many data of DNA expression. Since the viability of the cells inside tissue is very important for this study, authors should show histology samples of the tissue after culture. The cells at mid region of tissue may suffer low nutrient and oxygen and this may lead different results from native tissue.”
We thank the reviewer for the thoughtful comments. We appreciate that histology samples after culture would be desirable. However, our group at present has neither the manpower nor resources to conduct these studies prior to the resubmission deadline of February 2018. We have amended the Discussion and included the explicit acknowledgment of the limitation of the device that tissue necrosis may occur and that this limitation can potentially be remedied by modifying the device to include gas and nutrient exchange. We also included an additional reference that clarifies that our device may also have utility for tissue engineered tendon constructs under multiple experimental conditions.

Changes have been incorporated in the Discussion section, page 12, line 7-17

Extra Reference

Editor Comments:

1) Please include the email addresses of all authors on the title page. All email addresses have been added.

2) Please change the heading “Materials and Methods” to “Methods”. We have revised this to read “Methods”.

3) Please include a Conclusions section after the Discussion. A conclusions section has been added.

4) Please include the list of abbreviations after the Conclusions and the Declarations after the abbreviations. Do not upload these as separate files. A list of abbreviations has been included in the main manuscript.

5) In addition, please describe the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared. Also, please include a statement in the Authors' contributions section to the effect that all authors have read and approved the manuscript, and ensure that this is the case. The role of the funding body has been defined, and all authors have read and approved the manuscript, with a statement confirming this fact added to the text.

6) As this study is using tissue donated from other researchers, please include a statement in “Ethics approval and consent to participate” section of the Declarations that all necessary permissions were obtained to use this tissue and that the animals were maintained and euthanised in accordance with proper institutional and national ethical guidelines. All necessary permissions were acquired and have been described.
7) You have uploaded multi-panel figures as separate files. Please note that these figures should be submitted as a single composite file that contains all parts of the figure. If required, the figures can be separated into individual figures. Please note that the tables would need to be formatted according to our Submission Guidelines for tables (https://bmcmusculoskeletdisord.biomedcentral.com/submission-guidelines/preparing-your-manuscript#preparing+tables).

Figures have been separated and numbered individually. The smaller tables have been added directly to the text in the required format. The additional file 1 (Supplementary table of animal primers) has been included as a PDF.