Texas A&M University College of Dentistry
Department of Periodontics

05/07/2021

Vineeta Bajaj, Ph.D.
Review Editor, JoVE

Revision of our manuscript, JoVE62690 "Growing Cells and Tissues in Microgravity: Cervical Loop, Ameloblasts, Periodontal Progenitors, and Lung Alveoli"

Dear Dr. Bajaj,

Thank you very much for your thorough review of our manuscript. We appreciate your and the reviewer's comments and have now revised the manuscript according to your suggestions. Please find below our response to individual concerns.

Editorial comments:
Changes to be made by the Author(s):
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
We have now thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

2. Please revise the title to be more concise and avoid punctuations.
We have now revised the title to read "Propagation of dental and respiratory cells and organs in microgravity"

3. Please provide an email address for each author.
These have now been provided.

4. Please rephrase the Summary to clearly describe the protocol and its applications in complete sentences between 10-50 words: "This protocol presents…"
We have now rephrased the summary according to your guidance.

5. Please define all abbreviations during the first-time use.
We have now introduced abbreviations at the time of first-time use.

6. JoVE cannot publish manuscripts containing commercial language. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.
For example: Synthecon RCCS bioreactor (Synthecon, Houston, TX), Advanced Biomatrix, SpongeCol, Millipore, Fitton-Jackson, etc.
We have now removed all commercial language from the manuscript and placed these in the Table of Materials and Reagents.

7. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep and note in the protocol section. Please use Calibri 12 points
We have now used your recommended format.

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8. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note."
We have modified the protocol text to match the imperative tense style.

9. The Protocol should contain only action items in complete sentences that direct the reader to do something.
We have now modified the protocol accordingly.

10. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please ensure that individual steps of the protocol should only contain 2-3 actions sentences per step.
We have now made sure that the protocol only contains discrete steps.

11. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.
We have now added additional detail to address answers to "how" questions.

12. There is a 10-page limit for the Protocol, but there is a 3-page limit for filmable content. Please highlight 3 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.
We have now highlighted 3 pages for the filmable content.

13. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."
We have not reused any figures.

14. As we are a methods journal, please ensure that the Discussion explicitly cover the following in detail in 3-6 paragraphs with citations:
a) Critical steps within the protocol
The four critical steps of our protocol have now been described and discussed in the first four paragraphs.
b) Any modifications and troubleshooting of the technique
These are included in the discussion of the four critical steps.
c) Any limitations of the technique
d) The significance with respect to existing methods

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Limitations and significance over existing methods have now been mentioned in paragraph 5 of the discussion.

e) Any future applications of the technique

These are no listed in paragraph 6 of the discussion.

Reviewers' comments: Reviewer #1:

Manuscript Summary: In this manuscript, Pandya et al., describes the utilization of rotary cell culture bioreactor and showed specific applications for different cell types.

Major Concerns:

* In general, I expected some material to in video format because the journal is particularly suitable for such media. I recommend authors to use this availability, if possible, which would communicate their protocol better.

We will submit video format material as a second step following the manuscript.

* For the microgravity protocols used for tissue engineering, I advise authors to mention work from RPM bioreactors (numerous to select from, perhaps include 3D Clinostats as well) and also novel magnetic levitation studies (with scaffold: https://doi.org/10.1002/adhm.201500092; scaffold-free: https://doi.org/10.1002/bit.27631 AND https://doi.org/10.1038/s41598-018-25718-9)

We have now also mentioned work from RPM bioreactors and magnetic levitation studies.

* Information for the scaffolds used in the study comes at the results section, unexpectedly. It might be beneficial to dedicate a short paragraph in the introduction.

We have now added information about the scaffolds used in the introduction.

Minor Concerns:

* I failed to see the reason for highlighting the odd protocols.

Highlighting was performed to identify relevant section for the video script.

* In the results section bioreactor is introduced as "RCCS-4D" though it is not clear if this refers to a model or a specific working mode. The Table of Materials section also does not have the information. I suggest giving a brief description.

RCCS-4D is the name of the model. This is listed in the Table of Materials section.

* In results 1 section, authors argue that scaffolds allow "for cells to attach to scaffold surfaces and form cytoskeletal" networks. Alterations in the cellular cytoskeleton is not directly shown in the study, therefore I would either use citations to the claim, or revise.

We have now listed references in support of our claim.

* In results 5 section, authors mention "a significantly higher level of extracellular matrix", a statement that generally requires quantification and statistical tests.

We have now removed this statement as it is part of another study.

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Reviewer #2:

I have agreed to review this manuscript because I was intrigued by the title, but already reading the abstract, and even more the text, I found something different from what I expected.

We apologize if this reviewer found the title misleading. We have now revised the title of the manuscript.

It is not clear what the authors pursue: to study the effects of microgravity or to create 3D cultures for in vitro studies?

I think I understand that the authors propose the RCCS bioreactor as a tool for the establishment of 3D cultures and their characterization. The authors' study proposal could be interesting, even if not entirely new, but formulated in a confused way and hidden by many arguments that are not always pertinent and sometimes incomplete.

This manuscript is written to follow the format of a JoVE Methods paper as required by the Journal guidelines. The technology described in the manuscript serves as a means for readers to generate 3D cultures in microgravity for the study of unique cell populations in a gravity-free environment.

In my opinion, the manuscript should be re-written in order to focus and emphasize the real aim of the authors. The authors showed two models but their usefulness is not clearly described. The description of results is not always supported by the data shown in the figures. For example the description of results coming from fig. 5 (lines 254-259) are not supported by only the images, the author wrote: ..... The cells survived a two-week culture period in the 3D culture.....(are there vitality assay?)..... the galanin-coated scaffold group demonstrated a significantly higher proliferation rate....(are there proliferation curves and statistical analyses?)..... The cells in the experimental group also demonstrated a significantly higher level of extracellular matrix containing connective tissue fibers....(are there a specific staining?).

These are excellent suggestions. The author team is afraid that they go beyond the scope of JoVE, which by default focuses on establishing and sharing Methods with their readers. However, these are outstanding ideas for future studies.

Some suggestions.

If the aim is to establish 3D cultures, the description of the effects induced by microgravity should be avoided, also because the functioning principle of RCCS is to create a free-fall environment simulating microgravity and allowing cells to interact to each other or to scaffolds. They presented two models: dental-derived cell co-cultures in presence of scaffolds and lung organ cultures. The use of this second model is only roughly described. The quality of the images in the figures (for example fig. 1 and 4, but not only) should be improved. In particular the fields acquired appeared to be out of focus. The English language and style should be accurately revise also to avoid typing errors.

Micrographs are in focus and highlight an optical cross-section through a 3-dimensional environment. We have now thoroughly revised the use of language and style.

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We appreciate the thorough review of our manuscript and look forward toward your response.

Sincerely,

Thomas G.H. Diekwisch, DMD, Ph.D., Ph.D.

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