

Responses to the Editor and Reviewers. Juuri and Balic, "Use of Trowell-type Organ Culture to Study Regulation of Dental Stem Cells".

Dear Editor,

We thank you and the Reviewers for the careful reading of our manuscript entitled "Use of Trowell-type Organ Culture to Study Regulation of Dental Stem Cells", which we have now corrected accordingly. We have addressed all the reviewers' comments (in the text below) and we hope that the revised version of the manuscript will now be acceptable for the publication.

Sincerely,

Emma Juuri, DDS PhD

Editorial

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

We have done thorough language revision for the manuscript.

2. Please revise the following lines to avoid previously published work: 42-45, 138-139, 140-141. Also please revise the summary to fit the 50-word limit.

We have revised the marked lines and the summary to fit the word limit.

3. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

We have removed all the personal pronouns.

4. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. 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.

For example: GlutaMAX, Eppendorf, etc.

We have removed the commercial language.

5. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

We have included the ethics statement.

6. Line 103-113: The Protocol should contain only action items that direct the reader to do something. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly.

We have changed the text in imperative tense and added some text as a "Note."

7. Line 109: Please include the specifications of the needles used for opening the mandible.

We have now specified the needles.

8. Line 125-129/131-136/138-141/143-147: Please ensure that the Protocol section has numbered steps. We cannot have non-numbered paragraphs/steps/headings/subheadings.

We have numbered the protocol steps.

9. Please include a one-line space between each protocol step and highlight up to 3 pages 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. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

We have added a one-line space between each protocol step and highlighted the essential steps of the protocol for the video.

10. For in-text formatting, corresponding references should appear as numbered superscripts after the appropriate statement(s).

References are now changed into numbered superscripts.

11. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage (YEAR).] For more than 6 authors, list only the first author then *et al.* Please use title case italicize journal titles and book titles. Do not use any abbreviations. Article titles should start with a capital letter and end with a period and should appear exactly as they were published in the original work, without any abbreviations or truncations.

Reference style is now according to JoVE style.

12. Figure 2: Please include scale bars in all the images of the panel. Please include the details of the magnification in the Figure Legends.

We have included scale bars in all the images and included the details of the magnifications.

13. Figure 3: Please include scale bars in all the images and define them in the Figure Legends.

We have included scale bars in all the images and included the details of the magnifications in figure legends.

14. Figure 4: Please include scale bars in all the images and define them in the Figure Legends. Please use a single space between the number and the time units in Figure 4E (revise “0h” to “0 h”).

We have included scale bars in all the images and included the details of the magnifications in figure legends. We have added single space between the number and the time units.

15. Please sort the Table of Materials in alphabetical order.

We have sorted the Table of Materials in alphabetical order.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The authors explained the protocol for the isolation and culture of the proximal end of the mouse incisor that houses stem cells and their niche using the modified Trowell-type organ culture protocol. The authors were able to clearly show the step wise protocol.

Minor concerns :

Hope the video and accompanying text will be able to completely depict the protocol explained.

We thank the reviewer for the positive review of our manuscript.

Reviewer #2:

Manuscript Summary:

The authors describe a protocol for the dissection and cultivation of the niche of epithelial and mesenchymal stem cells of mouse incisor.

The authors also describe the combination with transgenic reporter mouse to visualize characters of cells such as stemness and proliferation status.

The article will contribute to understanding the mechanisms of incisor development but also other ectodermal organs.

Major Concerns:

I have no issues.

We thank the reviewer for the positive review of our manuscript.

Minor Concerns:

Line 101, Is this step means that dissected tissues keep in a Petri dish containing PBS or culture media?

We have clarified this sentence in the text, and now it reads: in a Petri dish containing PBS.

Please unify some terms. e.g., 70% EtOH v.s. 70% ethanol

We have unified the terms.