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Department of General Pediatrics, Neonatology, and Cardiology
Children's Hospital, Building 13.41, Level 3, Room 29

Vidhya Iyer, Ph.D.
Review Editor
JoVE

May 17, 2021

Dear Dr. Iyer,

On behalf of all authors, I thank you for considering our review manuscript and for sending it for revision. The input provided by the reviewers was indeed critical and it allowed us to significantly improve our manuscript.

We have responded to all the editorial concerns and points raised by the reviewers, and prepared a new version of the manuscript R1. We used the red font to indicate the revised portions in the manuscript.

Our point-by-point response to the reviewers' comments is provided below in blue font.

We hope that you will now be willing to consider publishing our work in *JoVE* and look forward to your reply.

Sincerely yours,
Alessandro Prigione

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Point-by-point response

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. Please define all abbreviations at first use.

We proofread the text.

2. Please provide an email address for each author.

We included the email addresses.

3. Please revise the following lines to avoid overlap with previously published work: 44-46, 288-290, 298-302, 317-323, 335-336, 339-346, 355-360, 363-364, 367-372

We revised the text.

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: Matrigel; Accutase; falcon tube; Glasgow-MEM; Knockout Serum Replacement (KSR); Glutamax; Benchkote®; Superfrost Plus slides; Trition-X; Nikon Eclipse 90i; Nikon, Duesseldorf, Germany; DS-Q1Mc camera; Nikon, Duesseldorf, Germany; NIS-Elements Advanced Research 3.2 software; Nikon, Duesseldorf, Germany; ImageJ software; NIH, Bethesda, MD (although this is open-source; mention only name “ImageJ”); Nikon Eclipse C1, Nikon, Duesseldorf, Germany; Nikon, Duesseldorf, Germany; Silver Version software; Nikon Instruments, Japan; Affinity Designer; Serif Europe Ltd, Nottinghamshire, UK; Worthington papain solution; Worthington DNase solution; Matrigel-coated XF96 cell culture microplate; XFe96 FluxPak; XF Calibrant Solution; Seahorse XFe96 Analyzer; Seahorse XF DMEM medium; XF Assay Medium; XFe96 sensor cartridge; CyQUANT®etc

We modified the text to use generic terms.

5. Please consider providing solution composition only in Tables in separate .xls or .xlsx files uploaded to your Editorial Manager account. These tables can then be referenced in the protocol text. Remove the composition from lines 112-115; 224; 310-312

We modified the text and included the information in separate excel tables.

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

We revised the text.

7. 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.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

We revised the text.

8. 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 ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. 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 revised the text to include as much details as possible.

9. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step.

We simplified the steps.

10. 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 and one-inch margins on all the side. Please include a one line space between each protocol step and then highlight up to 3 pages of protocol text for inclusion in the protocol section of the video. PLEASE LEAVE A ONE-LINE SPACE BETWEEN THE STEPS AND NOTES. AFTER ENSURING YOU HAVE ONLY 2-3 ACTIONS PER STEP AND NOTES ARE SEPARATED FROM STEPS, HIGHLIGHT ONLY STEPS (NOT NOTES) TO ENSURE YOU DO NOT EXCEED 3 PAGES OF HIGHLIGHTED TEXT.

We revised the text. We highlighted in yellow the 3 pages for inclusion in the video.

11. Please consider separating representative results from discussion so that it’s a little easier to read. The legends section will then go between the results and discussion sections.

We reorganized the sections as suggested.

12. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source (italics). Volume (bold) (Issue), FirstPage–LastPage (YEAR).] For 6 and more than 6 authors, list only the first author then et al. Please include volume and issue numbers for all references, and do not abbreviate the journal names.

We revised the reference format.

13. Please sort the Materials Table alphabetically by the name of the material.

We revised the Materials table and sorted the names alphabetically.

Reviewers' comments:

Reviewer #1:
Comments
The protocol will be a good addition to evolving field of brain organoids. I only have several questions to the authors.
- Reproducibility in independent cell lines. Would like to see how consistent this protocol would be in 2-3 different hPSC lines.

We included a sentence regarding the reproducibility in different human iPSC lines derived from controls and patients (lines 567-568).

- Some labs use feeder cells (MEF, SNL) to maintain hPSCs. Could you comment if the presence of feeders might render the process of organoid formation ineffective. If so, any recommendation to users?

We included a note regarding feeder cultures (lines 121-122).

- How uniform these organoids are? Could you quantify patterns of morphology (size, layer) and molecular markers in each organoids (-30?) and demonstrate uniformity?

We did not systematically quantify the uniformity of the organoids. We included a section in the discussion to address this point (lines 598-602).

- For future study, could you comment if it could be possible to generate organoids from a single cell of hPSC per well? It would be useful for genetic screening with CRISPR. I guess that current protocol still needs further optimization though. It would be encouraging for users to be shown the direction of the evolution of brain organoid technology.

We included a section in the discussion to address this point (lines 602-604).

Reviewer #2:
Manuscript Summary:
In this manuscript, titled "Generation of human brain organoids for mitochondrial disease modeling," the authors describe their technique in generating iPSC-derived brain organoids designed to study mitochondrial disorders and their neurodevelopmental consequences. The authors have adequately described in detail their technique, as well as disclosed the materials needed for this protocol. The major advantage to this proposed protocol is that the organoids are not required to be manually embedded in a scaffolding matrix. Another advantage of these organoids, aside from morphological study, is that they can be used to assess mitochondrial metabolism in terms of oxygen consumption rate and extracellular acidification rate, which the authors demonstrated well. Taking the above-mentioned into account, we recommend the production of video and publication of this manuscript, with a minor revision.

Major Concerns:
None.

Minor Concerns:
1. Please add number of organoids (n=?) used to plot Figures 2D and E to the figures and to the figure legends.

We defined the number of organoids used for Figures 2D-E (lines 551-553).

2. Please discuss the limitations of your protocol. One limitation for example is the absence of vasculature and microglia in the produced brain organoids. Discuss how the limitations affect the interpretation of results.

We included a discussion on how these limitations may affect the interpretation of results (lines 594-598).

3. Minor grammatical errors throughout the manuscript would need to be corrected such as consider revising the sentence on page lines 78-81 (too lengthy).

We corrected the typos and grammatical errors and we revised the mentioned sentence (lines 87-92).

Reviewer #3:
Manuscript Summary:
Manuscript of Li et al. entitled "Generation of human brain organoids for mitochondrial disease modeling" provides detailed protocol for the robust and reproducible generation of human iPSC-derived brain organoids and for their use in mitochondrial bioenergetics profiling and in-depth imaging analyses. Derivation of brain organoids is already well known and common procedure in many laboratories, however efficacy of the presented protocol is unquestioned, as for the first time it is adjusted to screen bioenergetics in brain organoids. The authors, are well experienced, working with modeling of mitochondrial disorders with induced pluripotent stem cells and patient-derived brain organoids - thus provided sufficient introduction and unbiased discussion for the presented protocol, especially regarding the use of this protocol to investigate metabolic and developmental dysfunctions in human 3D culture model. Couple of minor concerns are below.

Major Concerns:
No major concerns

Minor Concerns:
1) Page 2 lines 82-84. The sentence regards reconstruction - to long difficult to follow.

We revised the sentence (lines 93-96).

2) Page 9, lines 402-403. "Interconnection" between neuronal and glial cells cannot be seen on the level of magnification presented in the manuscript. I would rather say "mutual occurrence"

We modified the text as suggested (line 488).

3) In discussion please refer to other tools than bioengineering profiling and in-depth imaging used for analyzing the role of mitochondria in health, development and disease as presented in recent review Liput at al., Dev Neurobiol. 2021 Mar 16. doi: 10.1002/dneu.22818.

We included the mentioned reference (reference 15). Thanks for bringing this to our attention.

3) Page 10. In the Fig1 legend: "dissolved Matrigel" should be mentioned in description

We included a sentence in the legend of Figure 1 to clarify this point (lines 522-523) and included a more clear description of when the matrix component (i.e. Matrigel) is added to the cultures (lines 203-210).

Reviewer #4:
Manuscript Summary:
This manuscript describes in detail the generation of brain organoids for the modelling of mitochondrial disorders. bestie teh generation of the organoids they describe in detail how to assess the bioenergetic level of these brain organoids,

Major Concerns:
There are no main concerns. The protocol is clearly written and contains all necessary details in order to reproduce the protocol.