Peer Review File

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Reviewer A:

The review "Modeling congenital brain malformations with brain organoids: a narrative review" by Xiao-Shan et al. provides an overview of the development of the brain organoid field with a focus on modeling brain malformations. This is a very concise and clear review that is interesting to read and that provides a comprehensive overview of the modeling of brain malformations in the brain organoid system. I think that this review is suitable for publication in Translational Pediatrics.

Q1: However, before acceptance the English language of this manuscript needs to be substantially improved.

Reply to Q1: We really appreciate this comment. We did very careful proofreading throughout the paper, and the grammar and inappropriate descriptions have been revised and highlighted in red in the manuscript.

Q2: Moreover, I suggest to include the following minor changes:

Comment 1: The SFEBq approach mentioned in line 95 is normally not considered as a 3D model but as 2.5D model.

Reply 1: Thank you for pointing out our inappropriate description. We have changed the sentence "The first 3D neural tissue was established in 2008" to "The first ESC-derived cortical neuroepithelia, which was considered as a 2.5D brain model, was established in 2008, using a technique known as SFEBq (serum-free floating culture of EB-like aggregates)". (see Line 100)

Comment 2: For the statement in lines 164-165 the authors should provide references.

Reply 2: We are sorry about the lack of references. We have added the relevant references (Subramanian et al. 2017, Andrews et al. 2020) to support the statement

that the expansion of human cerebral cortex is the result of an increase in the number and diversity of progenitor cells (see Line 171).

Comment 3: I agree in principle with the author's statement in lines 207-209 that "real" cortical folding was not achieved so far in brain organoids. However, some publications claim that they have achieved folding of brain organoids (eg. Karzbrun et al. 2018, Li et al. 2017). I would suggest to mention and to discuss these publications so that the reader can form an own opinion on the topic of cortical folding in brain organoids.

Reply 3: We really appreciate this constructive comment. We have added some description and discussed relevant publications as follows: "There have been efforts to engineer neuroepithelial 'wrinkling' or 'pseudo-folding' during early differentiation, either by inducing enhanced proliferation of NPCs through genetic manipulation or mechanical internal constraint in a microfluidic device(29,46-48)." (see Line 216-220)

Comment 4: The authors are inconsistent with the nomenclature of progenitor cells. Sometimes for basal/outer radial glia they refer to bRG and sometimes to oRG. I think it would be good to be consistent here (either bRG or oRG or bRG/oRG) so that the reader does not think that these are different progenitor cells.

Reply 4: We are sorry about the inconsistent nomenclature in our context. We employed oRGs to refer to basal/outer radial glia cells in our manuscript (see Line 68, 152, 177 and 235).

Comment 5: In lines 244-245 the authors mention the use of spinning bioreactors for improving the quality of brain organoids. However, spinning bioreactors are used since the first cerebral organoid protocol. Moreover, the reference for this statement is actually not focusing on bioreactors but on the addition of patterning factors and using a scaffold to increase embryoid body size. Do the authors actually mean other methods to improve oxygen and nutrient supply as suggested for example by culturing

cerebral organoids at the air-liquid interphase? I think this would be an interesting option to discuss here. Another option would be to discuss Qian et al. 2016 as in this publication spinning mini-bioreactors were generated which can be produced by 3D-printing.

Reply 5: Thank you so much for pointing out the inappropriate description and quotation. We discussed the methodologies built to maintain oxygen and nutrient supply of brain organoid as follows: "Many methodologies, such as modifications of EB size, combination with bioengineering constructs and air-liquid interface culture, have been built to maintain oxygen and nutrient supply(48,56,57). Organoids with larger continuous cortical lobes were generated using a microscale internal scaffold to shape the organoids at the EB stage(58).". (see Line 254-258)

Comment 6: In lines 259-260 the authors claim that feeder-free culture conditions would improve the brain organoid technology. I wonder if this statement is true, as at least to my knowledge most of the well-known brain organoid groups use feeder-free conditions.

Reply 6: Thanks a lot for the comment. What we wanted to explain in our manuscript was that feeder-dependent hPSC cultures were more technique dependent, and properties of each hPSC line may sometimes be inconsistent. However, as you mentioned, we agree that most of the well-known brain organoid groups use feeder-free conditions, so we have deleted the statement and re-written the paragraph about batch-to-batch heterogeneity as follows: "It has been found that inconsistent neural induction efficiency could be a main source of variability, and attempts to increase the homogeneity included the use of micro-scaffolds to arrange cells in an organ-like configuration(58), the addition of exogenous patterning factors to generate region-specific organoids(65,66) and the use of mini spinning bioreactors with minimized volumes of variable ingredients and better-controlled conditions(59)." (see Line 271-276)

Comment 7: In line 261 do the authors actually mean “qualitatively” instead of “quantitatively”?

Reply 7: Thank you for pointing out our mistake. We have replaced the word “quantitatively” with “qualitatively” in our context. (see Line 277)

Comment 8: In line 268 what do they authors mean by state-of-the-art technology. Could they please provide some examples?

Reply 8: Thank you for the comment. We have provided three examples of state-of-the-art technology as follows: “integrating brain organoids with state-of-the-art technologies, such as lineage-coupled single-cell transcriptomics, long-term live imaging and automated read-outs for high-throughput analyses will help to exploit organoids to their full potential in clinical settings and translate well to patients’ bedside.” (see Line 280-281)

Comment 9: In figure 1, I would suggest to first show panel B followed by panel A, as panel B provides an overview for the two principal ways of generating brain organoids and panel A shows a specific protocol of one of these two ways.

Reply 9: Thanks for your valuable suggestion. We have adjusted the sequence of panel A and B in Figure 1 to make it more logical and reasonable.

Comment 10: In figure 1, what is labelled as a spinning bioreactor is actually an orbital shaker (which is very frequently used for organoid culture). The authors should relabel it or provide an image of a spinning bioreactor.

Reply 10: We are sorry for providing the inaccurate information. We have relabeled the orbital shaker in Figure 1.

Reviewer B

The paper is organized into two parts.

The first one is supposed to describe what is known about brain development. This is centerde on neocortex, there is no explanation of the other nervous centers like deep central nuclei, thalamus, hippocampus... Furthermore, the developement of the neocortex is far from being correctly presented. No explanation about preplate, subplate, origins of interneurons, migration, the different germinal layers... This part should be revised completely otehrwise the paper would be of no value for a didactic point of view.

The second part id more technical and is interesting but it has no sense without knowing the normal aspect of developement.

I suggest to reject this paper.

The first part is totally problematic and I guess that it has not been written by developemental biologists. Without correct informations about normal developement, the paper is of no value for the readers. Thank you for considering me as a reviewer.

Reply: Thank you for your comments and we are sorry for our incomplete description about brain development. We agree that providing correct information about normal development is the basis of discussing brain organoid and congenital brain malformations. We have added more detailed explanation of derivation of neural tissue, neocortex histogenesis and neuronal migration in our manuscript (see Line 52-59). However, as the focus and innovation of our review is how brain organoid mimicking congenital brain malformations, and neocortex is the major impaired region, we don't think it's necessary to explain the development of all nervous centers in our introduction. Thank you again for your comments.