April 10, 2023

Manuscript NO: 83876

Title: Current overview of induced pluripotent stem cell-based BBB-on-a-chip

Editors-in-Chief: Shengwen Calvin Li
The World Journal of Stem Cells

Dear Editor

We would like to thank the reviewers for the careful and thorough reading of this manuscript and for the thoughtful comments and constructive suggestions, which have helped to considerably improve the quality of this manuscript.

We are sending the revised version of the manuscript entitled***: Current overview of induced pluripotent stem cell-based BBB-on-a-chip***, Manuscript NO: 83876, with point-by-point corrections (see below) suggested by you and the reviewers.

We sincerely appreciate your time and consideration into this review. We hope the paper is now suitable for publication in *The World Journal of Stem Cells* and we are looking forward to hearing your decision.

Sincerely,
Lionel Gamarra

Reviewer #1 (code: 03979761)

Language Quality: Grade B (Minor language polishing)

Scientific Quality: Grade C (Good)

It provides a comprehensive analysis of the literature on microfluidic BBB models involving iPSCs, describing microfluidic devices, BBB in vitro constructs and applications. The review is well written and the results are presented in a clear and concise manner. However, there are some issues that need to be improved before publication.

1) *First, the introduction should provide more background knowledge about the blood-brain barrier. Describe how the current iPSC-based BBB-on-a-chip can overcome the limitations of traditional in vitro models and possible future directions.*

Answer: Thank you for your suggestion. We have divided the third paragraph of the Introduction into two paragraphs and we have added more background knowledge about the blood-brain barrier and the limitations of traditional in vitro models. The possible future directions have been addressed in the last two paragraphs of the Discussion Section. Please refer to the text highlighted in yellow.

2) *Second, the methods section should be more detailed. Please write in detail the literature search keywords, inclusion and exclusion criteria, search strategy and data extraction details.*

Answer: Thank you for your observation and suggestion. We followed the PRISMA guidelines, but we agree that more detail is necessary to improve methodological comprehension. We added more detailed information in the Materials and Methods section of the manuscript according to your request.

3) *Third, in the section "Microfluidic devices design and fabrication", in addition to an overview of the materials and specifications used in the literature, these design differences should be discussed, what their advantages and disadvantages are, and which aspects of the BBB model evaluation are more important.*

Answer: Thank you for your suggestion. We added one paragraph to the Discussion section of the manuscript about these differences in design and the

advantages and disadvantages of the presence of a membrane dividing the channels within the chip in the BBB model.

4) *Fourth, please add learning related to iPSC culture and differentiation induction. In general, mTeSR1 and Essential Medium 8 culture systems are both widely used iPSC culture systems, but these media are not involved in the differentiation process. This paper should focus on summarizing the media used in the differentiation of iPSC cells called BBB process. Also, why was doxycycline used in Middelkamp's study? This is a reagent that is not normally required in cell culture, please explain.*

Answer: Thank you for your observation. We corrected some parts of this text in the manuscript to clarify the information about the culture media and supplements applied in cell differentiation. In fact, mTeSR1 and Essential Medium 8 are media used in culture, but not in cell differentiation. Differentiation was usually reported using Unconditioned Medium (UM) and some variations. In addition, information on human endothelial serum-free medium has been added. Regarding the paper by Middelkamp, doxycycline induced the overexpression of Neurogenin 2 in GM25256 iPSCs, being one of the factors for the differentiation of GM25256 into iNeurons. This information was also added to the fourth paragraph of the topic "Characteristics of the iPSCs used in the BBB models and their cultivation and differentiation conditions" in the Results section.

5) *Fifth, in the section "Applications of BBB microfluidic 3D models using iPSCs", you mentioned "Neuronal functionality", why do neurons appear in the BBB microfluidic system? Why do neurons appear in BBB microfluidic systems? Please elaborate.*

Answer: Thank you for your interesting question. The NVU, which makes up the BBB, is mainly composed of strict interactions between BMECs, the extracellular matrix, basal lamina, pericytes, astrocytes and adjacent neurons [1, 2]. Most BBB models depicted in this review have built their BBB-on-a-chip from co-cultures of iBMECs and astrocytes [3-5] or even tricultures of iBMECs, astrocytes and pericytes [6-10]. This might be due to the fact that, even though neurons are indeed considered to be part of the BBB, they are physiologically positioned farther from the microvessels that make up the NVU (10 to 20 µm) than astrocytes or pericytes

[11]. However, some literature reports state that co-culture of iBMECs with neurons induces the upregulation of membrane transporters typical of the BBB, promoting a more robust BBB function [10, 12]. Even though the studies that report co-culture of endothelial cells with neurons mentioned in the current review do not clearly state the reasons for using neurons in their BBB models, the study by Wevers mentioned that co-culture with neurons induced tight barrier function and expression of relevant endothelial transporters [3].

6) *Finally, please add the prospect of iPSC-based BBB-on-a-chip development, i.e., what are the current challenges that need to be addressed and what are the foreseeable potential impacts of this model in drug screening or medical research. Overall, this study provides valuable insights into the use of iPSCs to construct BBB models. However, the manuscript could benefit from some revisions and more detailed information to improve its clarity and impact.*

Answer: Thank you for your suggestion. We added two paragraphs to the Discussion section of the manuscript about the prospects of iPSC-based BBB-on-a-chip development, including the current challenges that need to be addressed and the foreseeable potential impacts of this model in drug screening or medical research.

Reviewer #2 (code: 05191118)

Language Quality: Grade B (Minor language polishing)
Scientific Quality: Grade D (Fair)

Reviewer's Comment In this review, the authors have presented an overview of induced pluripotent stem cell (iPSC)-based BBB-on-a-chip. The authors have analysed the literature for BBB models on-a-chip involving iPSCs, described the microdevices, the BBB in vitro construction, and applications. The development of models based on BBB-on-a-chip using iPSCs is promising and is a potential alternative to the use of animals in research. The manuscript is well written and the work is well conducted. The results of different studies are well presented and discussed. The topic is interesting and may be helpful for future studies. I feel this study deserves to be published after addressing the minor points:

1) *The authors should thoroughly discuss the challenges of iPSC-based BBB-on-a-chip models, which is missing in the current draft.*

Answer: Thank you for your observation and dedication to this review. Other reviewers have pointed out this issue to us and it has been addressed in the fourth paragraph of the Introduction section and in new paragraphs that have been added to the Discussion section. Please refer to the text highlighted in yellow to analyze the changes made.

2) *"Different from primary cells, iPSCs are easily attainable, able to mature into almost any desired cell type. In general, they may be obtained from biopsied tissues or from more accessible sources, such as peripheral blood, renal epithelial cells or dental pulp [3]." The above sentence is not clear and correct. iPSCs cannot be obtained from biopsied tissues or more accessible sources. Please edit it. The revised sentence can be "Different from primary cells, iPSCs are easily attainable, able to mature into almost any desired cell type. In general, these can be formed by reprogramming cells obtained tissue biopsy or more accessible sources, such as peripheral blood, renal epithelial cells or dental pulp [3]." The more appropriate reference to cite to these sentences are listed below:*

https://link.springer.com/article/10.1007/s12015-021-10200-3

https://link.springer.com/chapter/10.1007/5584_2021_660.

Answer: We appreciate your comments. We changed the paragraph as indicated to make it clearer according to the notes made. The cited references were also added to fit more appropriately in the sentence.

3) *Correct the spelling "disfunction" throughout the manuscript.*

Answer: Thank you for pointing out this mistake. We corrected the word to "dysfunction" and also reviewed the spelling throughout the manuscript for other mistakes.

4) *"paralyze the cells". "paralyze" word is not appropriate.*

Answer: Thank you for your comment. We changed the word "paralyze" in the sentence for a more appropriate term.

Reviewer #3 (code: 03948836)

Language Quality: Grade B (Minor Language Polishing)

Scientific Quality: Grade C (Good)

In this systematic review, the authors searched published articles that used iPSCs to mimic the BBB and its microenvironment in microfluidic devices. According to the inclusion and exclusion criteria, 14 articles were selected and analyzed in this study. Data extracted from this articles were organized into four topics: (1) Microfluidic devices design and fabrication; (2) Characteristics of the iPSCs used in the BBB model and their differentiation conditions; (3) BBB-on-a-chip reconstruction process; and (4) Applications of BBB microfluidic 3D models using iPSCs. And the result suggested that: (1) Conventional polydimethylsiloxane was the most used material to fabricate in-house chips; (2) IMR90-C4 from human fetal lung fibroblast was the mainly used iPSC cell line; (3) The construction process of the BBB-on-a-chip involved previous coating mostly with fibronectin/collagen IV, followed by cell seeding in single cultures or co-cultures. The manuscript is consistent with the scope of the World Journal of Stem Cells. And this study will be interesting to the readers. However, there are still some questions that need to be addressed.

1) *This review aimed to analyze recent literature about BBB models on-a-chip involving iPSCs, and 86% of selected studies have differentiated their iPSCs into BMECs. However, like most iPSC-derived cells, BMECs do not fully recapitulate all aspects of their in vivo counterparts. BMECs express some epithelial markers that may not have a purely endothelial cell identity. Thus, whether the articles selected in this review described this question and pointed out the solution or future directions? We hope the authors can discuss this if possible.*

Answer: Thank you for this observation, which has helped us improve our work. The reviewer is correct. iBMECs are not able to fully recover their in vivo function upon differentiation from iPSCs. However, it is still a more reliable and easily attainable alternative to primary or immortalized cells. We have added a discussion on this topic to the tenth paragraph of the Discussion section (please refer to the text highlighted in yellow). We hope we have clarified the reviewer's questions with this addition.

2) *The permeability of iPSC-derived BBB models is an important factor. We suggest the author list the data of 14 selected articles, if applicable, such as TEER or other evaluation indicators.*

Answer: Thank you for your suggestion. Some further information on assays and markers used in the analyzed studies has been added to the third paragraph of the topic "Applications of BBB microfluidic 3D models using iPSCs" in the Results section. Also, more information on TEER measurements has been added to the sixteenth paragraph of the Discussion section and to Table 3. We hope to have properly addressed the reviewer's suggestion with these additions.

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