

OPEN PEER REVIEW REPORT 1

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Title: Toward three-dimensional in vitro models to study neurovascular unit functions in health and disease

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COMMENTS TO AUTHORS

Summary. In the manuscript entitled “Toward three-dimensional in vitro models to study neurovascular unit functions in health and disease,” authors discuss the recent advances, which have been made to model in vitro, the brain parenchyma, and the NVU. The review covers the applications with organoids, microfluidic devices, and tissue engineering techniques. Additionally, the authors compare and discuss the advantages and disadvantages of the existing models, which show promise for studying neurodegenerative diseases.

Overall, this is a good report presenting several sets of the novel and highly relevant results, which meaningfully improves the knowledge of the NVU function in health and disease, and summarizes the existing knowledge on in vitro BBB models.

However, in its current form, this study has several issues that should be carefully addressed.

The main issue of this review is the absence of figures. The article could benefit substantially from good visualizations, which should make the content more understandable and much clearer.

In my opinion, at least two figures should be introduced. The first one is showing the structure of the BBB in the healthy brain and the changes that are associated with neurodegenerative diseases. The second one may represent the existing models. Additionally, a table summarizing the existing in vitro models may significantly improve the paper.

An excellent example of a review with very well organized figures is a recent paper by Choi et al., which could be quoted as well [1]. In this article, the authors discuss a recent introduction of the blood-brain barrier in neurological disorder models by leveraging a recently developed microfluidic system and human organ-on-a-chip system. Moreover, they underline that such models provide an effective drug screening platform and facilitate personalized therapy of several neurological diseases. Therefore, I believe that it might be mentioned.

References and their format should be carefully checked. In some cases, the references that are provided by the authors are not related to the context. For example, page 4, line 50, reference #9 “Modeling neurological diseases using iPSC-derived neural cells,” does not fit the sentence. I suggest citing the article of Abbott et al.[2]. In other places, the text is not supported by references—for example, page 5, line 76. Here, the seminal work by Kim et al. could be mentioned [3].

Page 6, line 98, reference 28 should be replaced by a more appropriate. For instance, a paper by Payam Rezaie & David Male [4], or another one by Ginhoux et al. [5], which precisely describe the origin and differentiation of microglia in the brain.

Additionally, the review might benefit from mentioning a brilliant paper by Bergmann et al., which has been published in Nature protocols (2018) [6]. The article describes a new method of creating the BBB organoids via use of coculture of endothelial cells, pericytes and astrocytes under low-adhesion conditions. Of note, these organoids reproduce many features of the BBB, including the expression of tight junctions, molecular transporters, and drug efflux pumps. They hence can be used to model drug transport across the BBB.

Minor issues. The review needs English editing. Several grammar and punctuation mistakes should be carefully corrected. Example: page 4, line 54, failure is singular, so it suggests but not suggest. The writing style should be unified and polished. Additionally, several typos should be corrected. Example:

page 2, line 54, **couplingbut**.

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- [2] N.J. Abbott, L. Rönnbäck, E. Hansson, Astrocyte-endothelial interactions at the blood-brain barrier, *Nat. Rev. Neurosci.* (2006). doi:10.1038/nrn1824.
- [3] Y.H. Kim, S.H. Choi, C. D'Avanzo, M. Hebisch, C. Sliwinski, E. Bylykbashi, K.J. Washicosky, J.B. Klee, O. Brüstle, R.E. Tanzi, D.Y. Kim, A 3D human neural cell culture system for modeling Alzheimer's disease, *Nat. Protoc.* (2015). doi:10.1038/nprot.2015.065.
- [4] P. Rezaie, D. Male, Mesoglia and microglia - A historical review of the concept of mononuclear phagocytes within the central nervous system, *J. Hist. Neurosci.* (2002). doi:10.1076/jhin.11.4.325.8531.
- [5] F. Ginhoux, S. Lim, G. Hoeffel, D. Low, T. Huber, Origin and differentiation of microglia, *Front. Cell. Neurosci.* (2013). doi:10.3389/fncel.2013.00045.
- [6] S. Bergmann, S.E. Lawler, Y. Qu, C.M. Fadzen, J.M. Wolfe, M.S. Regan, B.L. Pentelute, N.Y.R. Agar, C.F. Cho, Blood-brain-barrier organoids for investigating the permeability of CNS therapeutics, *Nat. Protoc.* (2018). doi:10.1038/s41596-018-0066-x.