# OPEN PEER REVIEW REPORT 1

**Name of journal:** Neural Regeneration Research
**Manuscript NO:** NRR-D-21-00483
**Title:** Neurovascular unit-on-a-chip: culture and differentiation of human neural stem cells in a three-dimensional microfluidic environment
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## COMMENTS TO AUTHORS

The authors have developed a culturing system for the parallel study of the vasculature with NSCs. NSCs are a hot topic and will remain so for several years, and more so when suitable technologies to study them become available. I think the device the authors propose has beneficial usage to study NSCs. With further improvements, the authors in followup studies may have an in vitro system which mimic in the in vivo environment, which will be very useful for basic research purposes and high-throughput screening.
As the manuscript stands, there are improvements mostly on the text and interpretation of the results to make the manuscript sound and accurate to enable it receives a wider reception to investigators in the field.

Major -
1 - The authors should provide an in-depth supplementary instructions and exact product codes for assembling the device correctly other than shown in Figure 3. Otherwise, only the authors would be able to use such a system.
2 - Higher magnification crops are required for the immunofluorescence images. For example, I cant see clearly any MBP+ oligodendrocyte morphology. The same goes for the other images.
3 - The discussion isnt accurate. Neurogenesis in mammalian brains has been thought to exist in adults long before the availability of NSC cultures. This is one example out of others. Another example is where NSCs are not considered multipotent anymore. See for example Ortega et al 2013 Nature Cell Biology amongst others. Work from the lab of Laura Lopes Masqueraque shows that even during in vivo postnatal development, the same clones that make astrocytes dont seem to make oligodendrocytes. See Dulken et al 2017 Cell Reports. They show that cultured adult SVZ NSCs compared to in vivo are not the same at passage 3. In fact, it is becoming taboo to use NSCs using typical protocols. The discussion could be changed to go over methods which improve the use of NSCs in vitro with additional cells in the vicinity, such as endothelia. The authors have a nice system and I wonder if they should be talking about how improvements can be made using adaptations as they have done.
There are other several aspects which also needs attention but I have given some to start with.
4 - How is this in vitro system a chip? There doesnt look like some recording device that allows some sort of automated quantification? There does not seem to be any computerized component. It seems more of a scaffold-type device. The "organ-on-chip" is not justified.
5 - I dont see the rationale for the use of such high FBS. Is it because the NSCs die? This much FBS can permanently transform NSCs. I understand the need for FBS, but I wonder if keeping the cells more naive and natural appearing to allow them to differentiate is the best way forward. This could be why oligodendrocytes are too few in this system.

Minor -
1 - There are only 5 primers and might as well be included in the methods rather than a whole table.
2 - This section "Stimulation of inflammatory factors" should be called something like "Stimulation of inflammation via TNFa" - since only one inhibitory ligand was used.
3 - Figure 5 and 6 can be easily merged as one figure. The same goes for other figures.

4 - There are no stats in Figure 5. Some of of ANOVA+Bonferroni's test is needed to show significance between the data.
5 - In conclusions, it is an overstatement to mention that NVU is a whole system. There are other cells such as glial cells that interact with the BBB to adapt their signalling secretome accordingly and then pass the information over differently to other cells which eventually reach NSCs. There are other cells/tissues as well as the BBB that influence NSCs. I find that the conclusions in general has other overstatements in general. To simply tone them down.
6 - Can the system be modified for high-throughput experiments? If so, how is this possible?
7 - What other systems are out there where the current system has its advantages?