OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-21-00641
Title: Chitin-polydopamine conduits with sustained release of bioactive peptides enhance peripheral nerve regeneration
Reviewer's Name: Aldo Calliari
Reviewer's country: Uruguay

COMMENTS TO AUTHORS

The manuscript is a technical work that proposes the use of a Chitin-polydopamine (Chi/PDA) film-coated conduits designed to bridge and reconnect the proximal and distal stumps of severed peripheral nerves. The performance of the conduits on nerve regeneration was improved by the addition of short peptides that mimics the action of tropic factors. As a whole, I found the manuscript interesting; however it contains missing information, basically scarce information in regards to experimental details. Additionally I found some methodological aspects that still need to be addressed, mostly in reference to control experiments. Finally I recommend to re-write the Discussion, aiming to make it more straightforward and concise.

Specific concerns:

1- In order to visually display the binding performance of the two different peptides to the conduits, they were tagged using fluorescent compounds. Could you provide information in regards to the nature of the label and how was it made, please?

Additionally, was the green and red tagging done only in this experiment or were a constituent part of the peptide used in all the experiments?

2- Which was the methodology used to detect and measure the kinetics of peptide release? Did you use ELISA? Could you provide additional methodological information in this regard?

3- It surprised me the use of collagenase to eliminate fibroblasts from cell culture. Could you please further explain this approach?

4- Cell staining was used to measure live/dead cells present over conduits surfaces. Which label did you used, how was the labeling done? Please, clarify.

5- Cell viability was evaluated using "CCK-8 solution". What is this?. Specifically, what are you measuring in this way? Please, explain.

6- How did you labeled S-100 antigen in Schwann cells?

7- Could you provide information of the exact region were the nerve injury was done? Additionally, how were the conduits fixed to the nerve? Were they sutured?

8- What does "NIT" means in "Behavioural analysis" section? Please, clarify.

9- Under "Histological analysis of regenerated nerves", what is TEM? It stand for Transmission electron microscopy? The cited reference, correspond to the microscope used? Please, clarify.

Additionally, it is important to clearly state what part of the nerve was used for the histological analysis: the 2 mm space between nerve stumps; a region distal to the conduit? Please, provide additional information.

10- Concerning "Confirmation and release efficiency of mimic-peptides on the Chi/PDA conduits" (Results section). Did you confirmed the absence autofluorescence by the Chi/PDA ?

Here the authors speculate that release differences between both peptides may be due their different molecular weights. At this point I wonder which kind of chemical interaction is the responsible for the peptide-substrate binding? Could do provide additional information in this regard?

11- Figure 3B. As figure 1 depicts, the chitin (Chi) surface is rather hydrophobic. Thus it is surprising such a high cell density growing on it. Moreover, the density seems to be similar to the other more hydrophilic layers. How can be explained this result?

A scale bar reference must be included in all the figures of micro photographies

12- Figure 3C. I suggest to express the dead cell number as percentage of total cells (live cells and dead) or as a live/dead ratio. The use of the absolute number of cells may lead to confusion. Thus, the statistical contrast should be made between dead cells (or the ratio live/dead) in each condition, using ANOVA.
13- Figure 4B. The comparison here depicted is difficult to interpret because the absolute number of cells depend upon the number of cells originally seeded en each layer. If authors want to demonstrate proliferation rate, they need to compare the number of cell at the end of the experiment (expressed as a %) against the number of cells at beginning. Alternatively, they can show proliferation rate in an indirect way, ie counting the number of mitosis or using markers of cell proliferation.
14- Figure 5D is referred to an experiment that was not declared at material & methods section. It must be included there.
15- Figure 6A is confuse. What is "stress area"? What do the 3-D plots means? Please, clarify.
16- The results showed in figure 6B are difficult to evaluate without a reference consisting in a control condition representing a normal animal. Should a healthy uninjured rat yield a SFI score of 0? Should a just injured animal yield a score near 100%? I a am right?. Could you please further explain this in the text or provide any kind of control (an uninjured animal or alternatively a recently injured one) to the experiment?
17- I have also a similar concern about the result shown in 6C. In order to realize the importance of the atrophy degree observed in each experimental condition, an addition control condition (a muscle that remained denervated for 12 weeks) is necessary. Otherwise it is difficult to evaluate the real effect of each treatment.
18- Figure 7A needs a scale of mV against ms, as is usual used o illustrate these type of results.
19- Figure 8B is highly unsatisfactory. Axonal profiles cannot be distinguished at all, so one cannot evaluate what the authors quantified. Instead, an enlarged image or a myelin specific stain (ie osmium, luxol, etc) should be used.
20- Figures 7C appears to be inconsistent with quantification shown in 7E and 7F. I cannot see such differences in axonal diameter or myelin thick. Please, revise. Additionally, to better evaluate such differences an additional image depicting a healthy nerve is needed.
21- Discussion. Considering the results displayed so far, I would not claim that "nerve conduits have achieved excellent curative effects in PNI". Authors need to be more caution on this point or provide additional evidence demonstrating a clear improvement when compared to control healthy animals.