

## Peer Review Overview

### Manuscript Title: “Neuroimmune Interactions and Immunoengineering Strategies in Peripheral Nerve Repair”

Received	29-Apr-2021
1 <sup>st</sup> Decision	17-Jun-2021
Revision Submitted	11-Aug-2021
Accepted	02-Sep-2021

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### Decision Letter

Dear Dr. Wofford,

Thank you for submitting your manuscript to Progress in Neurobiology.

We have completed our evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following major revision. We invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Aug 16, 2021.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

To submit your revised manuscript, please log in as an author at <https://www.editorialmanager.com/proneu/>, and navigate to the "Submissions Needing Revision" folder.

Progress in Neurobiology values your contribution and we look forward to receiving your revised manuscript.

Kind regards,

Jeannie Chin  
Associate Editor  
Progress in Neurobiology

Sabine Kastner  
Editor-in-Chief  
Progress in Neurobiology

Editor and Reviewer comments:

Reviewer #1: The manuscript is well written and focused on a topic very interesting for the field of peripheral nerve regeneration. The manuscript is clear with regard to the aims of the review and well structured. The value of the work could, however, be further increased by revision.

- 1) It could be graphically illustrated more the different aspects of macrophages - other cell - cross talk during nerve regeneration. While figure 1 presents a quite good summary it is not sufficiently referred to it in the text and it also lacks supportive information on the abbreviations used.
- 2) In addition, the abbreviation list is incomplete it should either be completely omitted or completed.
- 3) When looking at the title, this reviewer would expect that the authors could elaborate more on the

"(immuno-)engineering" part of graft material for nerve repair. This relates also to the sentence written on page 32: "Indeed, it is feasible to imagine utilizing immunomodulatory biomaterials to supplement existing nerve conduits and grafts."; from the point of view of this reviewer, this aspect is worth to be looked at a bit more in detail within the review. For example, other authors have already speculated that nerve guide materials on their own could have immunomodulatory effects, e.g. doi: 10.1186/s12868-017-0374-z and there is likely similar evidence from the literature to be included into an additional paragraph or section.

4) Could the authors be more specific, also, on how they consider therapeutic use of macrophages becoming controllable in location and time in the future, without the risk of negative systemic side effects?

Reviewer #2: This review of the role of macrophages in peripheral nerve regeneration with development of arguments to pursue their role and to utilize their potential for augmenting regeneration, is quite novel. The review is well written and comprehensive. There are several small errors, but my primary concern is the limited referencing of literature concerning peripheral nerve regeneration. The authors tend to focus on single studies which are well described. Nonetheless, there are many references that have been overlooked and I encourage the authors to quote several of them.

#### Specific comments

1. The manuscript should have had page numbers and where I refer to these page numbers, I have numbered the pages from the title page being page 1.
2. P6. Lines 3-4. Nervous systems do not regenerate but nerves do or neurons regenerate their axons
3. Don't use split infinitives. Page 3, paragraph 2 line 10 Correct to diagnose certain types of nerve injuries appropriately.
4. P9 Include reference to work of Tajdaran in discussion of FK506.
5. Page 12:
  - a. Very strange references used here.
  - b. P 12, para 2: Following injury but before the process of fragmentation, macrophages increase in numbers along the .....
  - c. P12, para 2 Please include the actual timing of events in this paragraph and refer to figure 1
6. P13. Please quote earlier work including that of Kliot.
7. P14. Para 2. Indeed, since the 1980s (Perry et al., 1987), myelin fragment removal ..... (refs)
  - a. In the same para 2, not even Schwab is quoted. Another example of ignoring references to key works.
8. Page 15 para 1 Transfer the sentence beginning to 'While macrophage....' To before the second sentence Schwann cells can complete.....
  - a. Page 15 para 1: in their lysosomes by an mTOR-independent pathway
  - b. Page 15 para 2. Start first sentence with Removal and not with 'Clearly'
  - c. Page 15, para 2, line 9: cells - which cells?
  - d. Page 15 para 3. ECM in the title should be written in full
9. Page 16 para 2 line 3 Delete However and proceed with Following tranma,
  - a. Page 16 para 2 Be careful in this paragraph to state the cells expressing and releasing the MMPs and TIMPS. In the final sentence starting with In this way, macrophages contribute to the preservation of the basement membrane via TIMPs ...
  - b. Page 16, para 3. ....adhesion molecules that guide regenerating nerves
  - c. Page 16, paragraph 3: line 4 delete completely
  - d. Page 16 para 3 line 6; surgical intervention, axons that grow out from the proximal stump ca grow toward distal end targets if they can locate .....
  - e. Page.16 para 3, last line quote earlier references here and at the end of the last sentence that goes onto page 17..
10. Page 17, para 2. Lines 5-8 please be more specific about the secreted factors being polarized to differing phenotypes
  - a. Final sentence: will identify more mechanisms by which macrophages...
  - b. Para 3 Line 5-6. Delete an paper from the Lloyd group discovered that.... And Start with Macrophages
  - c. Para 3 line 7 they respond and not they are responsive.
  - d. Para 3 VEGF Transcription in what cells?
11. Para 2 line 4: delete first names from Long et al
  - a. Para 3, lines 2-3: After a crush injury to peripheral nerve, the number of S-100 positive Schwann cells within the injured nerve distal to the crush, drop to half .....
  - b. Para 3 last 3 lines: Macrophages upregulate .....to induce Schwann cell migration and thereby, increase the numbers (don't repeat the reference twice).  
HOW does the SC migration increase their numbers?



12. Para 1 line 1 Please delete can
  - a. Line 3 TAM define
  - b. Line 4: receptor, TYRO3, cueing.....
  - c. Line 6: secreted from macrophages increases ...mature SOX 10+ cells and their subsequent myelination of regenerating axons
  - d. Lines 8 and 9: Thereby, Gas6 increases .....action potentials in the regenerating nerves indirectly
  - e. Line 10: Genetic deletion of ....
  - f. Para 2. line 1 ... across any gap between proximal and distal nerve stumps, and thereafter, through
  - g. Lines 4 and 5 locations as described first by Ramon y Cajal (reference 1928). Axonal misdirection after PNS is difficult to correct.....
  - h. Line 8 determinants of
  - i. Para 3 line 2 to guide the distribution of Schwann cells in an injured nerve. Perhaps be more specific in where these SCs are.
13. Page 21, para 2; LIF is increased in neurons after nerve injury.
14. Page 23. Here and elsewhere do NOT abbreviate laboratory.  
Para 2. Sentence 1: Refer to the reference.
15. Page 27, para 1, line 1. Clearly, as macrophages play a critical role in tissue regeneration, several... (delete thus)
16. Page 32. Para 3, last line. I would chose a word other than 'drastic'

## Author Response Letter

We are grateful for the insightful suggestions made by the reviewers for the purpose of improving the clarity and sources for this manuscript.

**Reviewer #1:** It could be graphically illustrated more the different aspects of macrophages - other cell - cross talk during nerve regeneration. While figure 1 presents a quite good summary it is not sufficiently referred to it in the text and it also lacks supportive information on the abbreviations used.

***Authors' Response:** We thank the reviewer for their suggestion. We have increased the references to the figure and edited the figure to increase the interpretability by the readers.*

In addition, the abbreviation list is incomplete it should either be completely omitted or completed.

***Authors' Response:** We have updated the abbreviations list to comprehensively define all abbreviations in the manuscript.*

When looking at the title, this reviewer would expect that the authors could elaborate more on the "(immuno-)engineering" part of graft material for nerve repair. This relates also to the sentence written on page 32: "Indeed, it is feasible to imagine utilizing immunomodulatory biomaterials to supplement existing nerve conduits and grafts."; from the point of view of this reviewer, this aspect is worth to be looked at a bit more in detail within the review. For example, other authors have already speculated that nerve guide materials on their own could have immunomodulatory effects, e.g. doi: 10.1186/s12868-017-0374-z and there is likely similar evidence from the literature to be included into an additional paragraph or section.

***Authors' Response:** We thank the reviewer for their suggestion. We agree that a more in-depth review of immunoengineered graft materials would strengthen the manuscript. As such, we have expanded the "Current Immunoengineering Strategies" section. We have also incorporated the suggested reference.*

Could the authors be more specific, also, on how they consider therapeutic use of macrophages becoming controllable in location and time in the future, without the risk of negative systemic side effects?

*Authors' Response: We agree that reviewing potential methods for controlling macrophages could strengthen the manuscript. We have expanded the "Future Directions" section of the manuscript to add a discussion of potential ways to control macrophages in the future.*

Reviewer #2: The manuscript should have had page numbers and where I refer to these page numbers, I have numbered the pages from the title page being page 1. Page 6, Lines 3-4: Nervous systems do not regenerate but nerves do, or neurons regenerate their axons.

*Authors' Response: We thank the reviewer for their thoughtful and thorough feedback. We have corrected the referenced sentence.*

Don't use split infinitives. Page 3, paragraph 2 line 10: Correct to diagnose certain types of nerve injuries appropriately.

*Authors' Response: We thank the reviewer for their grammatical corrections. We have corrected the split infinitive in the referenced sentence.*

Page 9: Include reference to work of Tajdaran in discussion of FK506.

*Authors' Response: We thank the reviewer for their feedback and agree that the manuscript could be strengthened with more references to previous literature. We have added a paragraph discussing Tajdaran's FK506 work and several relevant references.*

Page 12: Very strange references used here. Page 12, paragraph 2: Following injury but before the process of fragmentation, macrophages increase in numbers along the ..... Also, Page 12, paragraph 2: Please include the actual timing of events in this paragraph and refer to figure 1.

*Authors' Response: We have corrected the sentence structure to reflect the reviewer's suggestion. We included more precise timing of the described events. We have also incorporated more references to the figure throughout the paper.*

Page 13: Please quote earlier work including that of Kliot.

*Authors' Response: We thank the reviewer for their suggestion. We have included a couple references to Kliot's work within this section.*

Page 14, paragraph 2: Indeed, since the 1980s (Perry et al., 1987), myelin fragment removal ..... (refs). In the same paragraph 2, not even Schwab is quoted. Another example of ignoring references to key works.

*Authors' Response: We have corrected the sentence structure to reflect the reviewer's suggestion. We thank the reviewer for their feedback on key literature to include in this manuscript. We have included several references to Schwab's work, which we believe has strengthened this section of the manuscript.*

Page 15, paragraph 1: Transfer the sentence beginning to 'While macrophage....' To before the second sentence Schwann cells can complete..... Page 15, paragraph 1: in their lysosomes by an mTOR-independent pathway. Page 15, paragraph 2: Start first sentence with Removal and not with 'Clearly'. Page 15, paragraph 2, line 9: cells - which cells? Page 15, paragraph 3: ECM in the title should be written in full.



*Authors' Response: We have corrected all the sentences and their organization to reflect the reviewer's suggestions. We have added more information to clarify which cells can become loaded with myelin. We have written "extracellular matrix" in full, as directed.*

Page 16, paragraph 2, line 3: Delete However and proceed with Following trauma. Page 16, paragraph 2: Be careful in this paragraph to state the cells expressing and releasing the MMPs and TIMPS. In the final sentence starting with In this way, macrophages contribute to the preservation of the basement membrane via TIMPs ... Page 16, paragraph 3: ...adhesion molecules that guide regenerating nerves. Page 16, paragraph 3, line 4: delete completely. Page 16, paragraph 3, line 6: surgical intervention, axons that grow out from the proximal stump ca grow toward distal end targets if they can locate ... Page 16, paragraph 3: last line quote earlier references here and at the end of the last sentence that goes onto page 17.

*Authors' Response: We have corrected all the sentences to reflect the reviewer's suggestions. We have added more information to clarify which cells release MMPs and have added the reference to the end of the paragraph.*

Page 17, paragraph 2, Lines 5-8: please be more specific about the secreted factors being polarized to differing phenotypes. Page 17, paragraph 2, Final sentence: will identify more mechanisms by which macrophages... Page 17, paragraph 3, Line 5-6. Delete an paper from the Lloyd group discovered that.... And Start with Macrophages. Page 17, paragraph 3, line 7: they respond and not they are responsive. . Page 17, paragraph 3: VEGF Transcription in what cells?

*Authors' Response: It remains unknow which macrophage-released factors drive fibroblast behavior. Gene analyses of macrophages suggest that it could be some combination of ECM- promoting and angiogenesis-promoting cues. We have added language to this section to clarify this point for the readers. We have corrected all the sentences to reflect the reviewer's suggestions. We have added information about which cells transcribe VEGF to this section.*

Page 18, Para 2 line 4: delete first names from Long et al. Paragraph 3, lines 2-3: After a crush injury to peripheral nerve, the number of S-100 positive Schwann cells within the injured nerve distal to the crush, drop to half .... Paragraph 3, last 3 lines: Macrophages upregulate ... to induce Schwann cell migration and thereby, increase the numbers (don't repeat the reference twice). HOW does the SC migration increase their numbers?

*Authors' Response: We have corrected the requested references. We have corrected all the sentences to reflect the reviewer's suggestions. We have added language to clarify that CCL3 increases the number of immature Schwann cells.*

Page 19, paragraph 1, line 1: Please delete can. Line 3: TAM define. Line 4: receptor, TYRO3, cueing... Line 6: secreted from macrophages increases ...mature SOX 10+ cells and their subsequent myelination of regenerating axons. Lines 8 and 9: Thereby, Gas6 increases ... action potentials in the regenerating nerves indirectly. Line 10: Genetic deletion of .... Paragraph 2, line 1: ... across any gap between proximal and distal nerve stumps, and thereafter, through. Lines 4 and 5: locations as described first by Ramon y Cajal (reference 1928). Axonal misdirection after PNS is difficult to correct... Line 8: determinants of. Paragraph 3, line 2: to guide the distribution of Schwann cells in an injured nerve. Perhaps be more specific in where these SCs are.

*Authors' Response: We have corrected all the sentences to reflect the reviewer's suggestions. We have restructured the sentence to eliminate the TAM abbreviation. We have also added more description of where Schwann cells are located.*

Page 21, para 2; LIF is increased in neurons after nerve injury.

*Authors' Response: We have corrected the sentence to reflect the reviewer's suggestion.*

Page 23. Here and elsewhere do NOT abbreviate laboratory.

*Authors' Response: We have changed 'lab' to 'laboratory' throughout the manuscript.*

Para 2. Sentence 1: Refer to the reference.

*Authors' Response: We have added the relevant references to the end of the appropriate sentence.*

Page 27, para 1, line 1. Clearly, as macrophages play a critical role in tissue regeneration, several.... (delete thus)

*Authors' Response: We have corrected the sentence to reflect the reviewer's suggestion.*

Page 32. Para 3, last line. I would choose a word other than 'drastic'

*Authors' Response: We have changed the word 'drastic' to 'profound' in the relevant sentence.*

We sincerely appreciate the constructive feedback provided by the reviewers and editors. We believe the criticism has strengthened this manuscript's interpretability by making them clear to the reader and better founded in literature.

Respectfully Submitted,



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Corporal Michael J. Crescenz VA Medical Center in Philadelphia