



Administrative Offices

2130 Taubman Health Center
1500 E. Medical Center Drive
Ann Arbor, MI 48109-5340
Phone (734) 936-5895
Fax (734) 763-5354

Chief

Paul S. Cederna, MD

Section Administrator

Nadine Lewis, MHSA

Adult Appointments:

Phone (734) 998-6022
Fax (734) 998-6696

Pediatric and Craniofacial Appointments:

Phone (734) 763-8063
Fax (734) 232-6973

Plastic Surgeons

David L. Brown, MD
Steven R. Buchman, MD
Paul S. Cederna, MD
Kevin C. Chung, MD, MS
Robert H. Gilman, MD, DMD
Steven C. Haase, MD
Steven J. Kasten, MD, MHPE
Jeffrey H. Kozlow, MD, MS
Theodore Kung, MD
William M. Kuzon, MD, PhD
Benjamin Levi, MD
Adeyiza O. Momoh, MD
Erika D. Sears, MD, MS
Christian J. Vercler, MD, MA
Jennifer F. Waljee, MD, MPH, MS
Edwin G. Wilkins, MD, MS

Research Faculty

Dennis R. Claflin, PhD
Stephen W.P. Kemp, PhD
Shuli Li, PhD
Elham Mahmoudi, PhD

Emeritus Faculty

Cynthia I. Marcelo, PhD

November 12, 2021

Vidhya Iyer, PhD

Science Editor

JoVE

Dear Dr. Iyer

Thank you very much for your letter regarding the review of our submission to *JoVE* entitled, “**The Muscle Cuff Regenerative Peripheral Nerve Interface (MC-RPNI): A Surgical Method to Facilitate Amplification of Intact Peripheral Nerve Signals**” (Manuscript #: JoVE63222). We are also appreciative of the reviewer’s very constructive and insightful comments regarding our manuscript, and we believe that these changes have strengthened our paper. We believe that we have addressed all of the reviewer’s concerns, and we have subsequently incorporated these changes into a revised manuscript (changes are displayed in track changes). We include not only our response to each comment, but the specific portion of the manuscript at which any changes were made. We have set out a point-by-point list that highlights the changes that we have made with respect to each reviewer’s comments in our Response to Reviewers. We look forward to the review of the revised manuscript, and if you have any further questions or require any additional clarification, please don’t hesitate to contact me.

Sincerely yours,

Stephen W.P. Kemp, Ph.D
Director, Neuromuscular Lab
Assistant Research Professor
University of Michigan, Section of Plastic Surgery
Department of Biomedical Engineering
1150 W Medical Center Drive
MSRB II, A570 Ann Arbor, MI, 48109-5456
P: (734) 615-2598
swpkemp@med.umich.edu

Response to Reviewers

Editorial comments:

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. Please define all abbreviations at first use.
2. Please avoid abbreviations in the title and shorten the title to something along the lines of “The Muscle Cuff Regenerative Peripheral Nerve Interface for the amplification of intact peripheral nerve signals.”

-We have removed the abbreviation and have changed the title to the one suggested by the Editor.

3. Please provide an email address for every author.

4. Please keep the summary within 50 words.

-Summary was shortened to 48 words

5. Please revise the following lines to avoid previously published work: lines 150-153, 197-199, 200-201, 202-204, 254-255, 295-297.

-Lines were all changed to avoid previously published work; however, lines 197-204 all describe how we follow institutional protocol for euthanasia, rat strain selection, etc and as these steps are identical to previous work, it cannot be altered without causing misrepresentation of the experiment.

6. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s), but before punctuation.

7. JoVE policy states that the video narrative is objective. The goal of this policy is to focus on the science rather than to present a technique as an advertisement for a specific item or laboratory. To this end, we ask that you please reduce the number of instances of " Our laboratory has developed/we developed the RPNI " within your text.

-All mention of our laboratory developing these constructs was removed

8. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

-All instances were removed

9. Please ensure that all text in the protocol section is written in the imperative tense; any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly.

-Text was altered as needed

10. Please format the manuscript as: paragraph Indentation: 0 for both left and right and special: none, Line spacings: single. Please include a single line space between each step, substep, and note in the protocol section. Please use Calibri 12 points and one-inch margins on all the side. Please include a ONE LINE SPACE between each protocol step and then HIGHLIGHT up to 3 pages of protocol text for inclusion in the protocol section of the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader. The video must present the main message of your paper clearly, as indicated by your title.
11. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please ensure the inclusion of specific details (e.g., button clicks for software actions,

numerical values for settings, etc) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

i) Line 208: please specify how to determine that anesthetization is adequate.

ii) Line 210: please specify the injection-needle gauge

iii) Line 221: Please specify the type of temperature probe.

iv) Lines 316, 334, 340, 345: “[**MCRPNI CITE HERE]” - please cite the appropriate references.

-Requested items were specified/changed.

12. Please submit individual figures (PDF, TIF, or JPEG) and tables (.xls) without associated legends in your editorial manager account.

13. Figure 3: Please increase the font size of scale for legibility.

-Increased font size was added

14. Figure 4: Please include a scale bar for all images taken with a microscope to provide context to the magnification used. Define the scale in the appropriate figure or its legend.

-Scale bar added

15. Please define the abbreviation Lo in the last column in Table 2.

-Defined in legend

16. As we are a methods journal, please ensure the Discussion explicitly covers any limitations of the technique and indicates when this technique would be useful (current and future applications).

-Have made some elaborations when addressing other reviewers comments in the Discussion

17. Please ensure that the references appear as the following: [Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source (ITALICS). Volume (BOLD) (Issue), FirstPage–LastPage (YEAR).] For 6 and more than 6 authors, list only the first author then et al. Please include volume and issue numbers for all references, and do not abbreviate the journal names. Make sure all references have page numbers or if early online publication, include doi.

18. Please add all items (plastic and glassware, solvents, equipment, software etc) in the Table of Materials so that it serves as a handy reference for users to get everything ready for the protocol. Please sort the Materials Table alphabetically by the name of the material.

-Added additional materials including some requested by Reviewer #2

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

this work challenges to the problem of having a clear signal of motor intent for communication between neuromuscular system of the patient and an exoskeleton. This approach works through a muscle graft that envelops the nerve and its activation amplifies the nerve signal.

Major Concerns:

This work tackles a really important problem of peripheral nerve interfaces. Therefore I consider it very interesting and useful.

Despite this I have some concerns:

1) I would like to better understand which are for the Authors the clinical settings in which an exoskeleton with this technology could be used. As it needs the graft muscle activation, I imagine it cannot be used in neuromuscular disorders in which the muscles cannot contract. Therefore, I would like the Author to state for which clinical problems it should be used (spinal cord injuries? Strokes? Traumas? Muscular dystrophies? Neuropathies?...).

-An additional paragraph was added towards the end of the discussion to provide additional clinical context for the function of this construct. A significant limitation of the MC-RPNI is when there is complete absence of lower motor neurons, as in severe stroke and complete spinal cord injury. A complete lack of functional lower motor neurons would prevent MC-RPNI reinnervation and thus signal amplification. In the majority of other neuronal diseases, some degree of lower motor neurons are present which would enable MC-RPNI function, albeit at lower degrees of signal amplification (studies are currently underway to better define the required minimum). Similarly in muscular dystrophy, motor units are functional although reduced in number and weakened/damaged, similarly producing lower signal amplification. As for neuropathies, although we have not explicitly studied the MC-RPNI in that scenario, it is assumed that given more proximal placement on the nerve and reliance on motor neuron reinnervation, neuropathies would be less likely to affect MC-RPNI function except in the most extreme of cases.

2) In which way the Authors imagines the recording of the signal in an in-vivo setting and the following communication with the exoskeleton (Wireless I imagine; in this case maybe wirelessly rechargeable sensors should be used)?

-At present in rats we utilize bipolar electrodes fabricated in our laboratory that are hooked up to either an implanted headcap or routed and buried at the level of the scapula. The wires that are buried are left in until time of eval when they are surgically explanted and secured externally to the rat. In human studies with the MC-RPNIs predecessor, the RPNI, we use implanted bipolar electrodes routed through the skin. We have recently received approval for implanted wireless electrodes that will be utilized in future human experiments. Specifically for the MC-RPNI, we anticipate utilizing wireless electrode systems in eventual human experiments. At present in rats, the wireless devices available are too expensive and not small enough in scale, unfortunately.

3) Do the Authors think that this technology could be used even for inside body artificial muscles in the future or just for exoskeletons? I imagine this technology useful for detecting the nerve activation and then communicating with a sensor implanted in the natural muscle for triggering activation or entirely communicating with an artificial actuator.

-This is an incredibly interesting point, and we don't see why this couldn't be an eventual application given that the neuromuscular interaction is the same in both cases. We have utilized the RPNI with muscle grown in vitro with success, and it is reasonable to assume this would work with the MC-RPNI as well. We have also discussed a variety of other applications, including its potential use for chronic pain, CRPS, and SCI-related muscle spasm. It will be interesting to see how its use will continue to develop over time.

Reviewer #2:

The article describes a protocol to create a muscle cuff regenerative peripheral nerve interface. The methods are clearly described, and a good fit for a JoVE article. I have only minor suggestions for improvements.

_ In terms of flow, I would have liked to see a description of standard methods to validate the interface included in the protocol, rather than scattered in the representative results section. Likewise, the length of time required for the construct to be mature and functional should be in the protocol rather than the results.

-The length of time for maturation was added to the protocol. The primary focus of this manuscript is to detail the fabrication of the construct with data from our prior experiments to support its viability. Detailing the validation of this construct is an additional protocol that lies outside of the scope of this manuscript, but it is an excellent point about more detail being needed. We would be happy to write an additional paper detailing all of our validation methods in an additional JoVE manuscript.

_ In the Table of Materials, little detail is provided about the surgical instruments. The protocol involves removing a window of epineurium, which sounds like a very delicate procedure. Readers may appreciate more details about the appropriate tools for this task.

-Details regarding the tools utilized to handle the nerve and perform the window were provided in the table of materials. The remainder of tools utilized in this protocol are general surgical tools that don't require special consideration/purchasing.

_ In figure 6, multiplying the amplitude of nerve recording by 10 is potentially confusing. It would be preferable to have separate axes if the range of amplitudes is too large to be visualized on a single axis.

-We have altered this figure to hopefully lessen confusion

_ Some discussion might be helpful of whether the technique would apply in any cases where there is some impairment of the lower motor neuron. The authors could be a little more specific about what would and would not be appropriate indications for this approach.

-The particular injury/ratios of affected lower motor neurons that are required for MC-RPNI function is currently being investigated. It is our assumption that if some functional lower motor neurons are present, they will to some degree reinnervate the construct and thus provide amplification. This would be beneficial over utilization of electrodes in distal muscle tissue and the functioning/reinnervated motor units would have a higher density within the smaller surface area of the MC-RPNI. We have added an additional paragraph towards the end of the discussion to provide some clinical scenarios for its use as well as its limitations.

Minor:

_ There are some missing references through the text ("**MCRPNI CITE HERE").

-Apologize, the incorrect final version of the document was submitted that omitted the finalized citation (we had been waiting on the final publication date for that particular citation). This is corrected in the current version.

_ l. 331: I suggest clarifying here that the EDL being harvested is contralateral, otherwise it is confusing since the ipsilateral EDL is the muscle being used for testing.

-Added the above clarification

_ Table 2: undefined abbreviation Lo.

-Lo is a standardized term utilized in muscle physiology assessments, its definition was added to the table legend to provide necessary definition/clarification.

_ The authors may wish to note that the video provided includes audio of someone saying "we'll trim this for the final video"...

-Apologize, the powerpoint version of the figures that was sent with peer review did not correctly strip the audio. The final .mov for the figures has the audio correctly stripped

_In the abstract, the term "notoriety" does not seem to match the intended meaning, given its negative connotation.

-Appreciate this mention as it does cause ambiguity/confusion, changed wording to better fit intended meaning.