nature portfolio

Peer Review File

A biodegradable and restorative peripheral neural interface for the interrogation of neuropathic injuries

Corresponding Author: Professor Lan Yin

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript 'A biodegradable and restorative peripheral neural interface for the interrogation of neuropathic injuries' This project represents advancement in nerve injury rehabilitation, combining biodegradable materials with cutting-edge machine learning to enable real-time monitoring and early detection of complications like traumatic neuroma. Its innovative design eliminates the need for retrieval surgeries, significantly reducing infection risks and improving patient outcomes. The ability to wirelessly track nerve recovery could transform clinical practices, offering a more dynamic, personalized approach to treatment. Reviewer has following concerns:
- The rate of degradation must align with the healing process, which varies between patients. Can authors comment on that?
- Piezoelectric materials are one of the kinds of sustainable materials? Reviewer has doubts on this statement.
- Introduction is redundant and should be shortened. Much info is already well known and well reported.
- A critical limiting factor is the resolution with which nerve inputs are mapped. How is this factored in to this work?
- False positives or negatives in detection might affect the quality of clinical decisions. How is that taken into account and tested?
- The device may provide valuable data during early recovery stages, but its effectiveness in chronic or later stages of nerve recovery might be reduced. How is the device and ML models suited for that?

Reviewer #2

(Remarks to the Author)
In this manuscript, the authors present a biodegradable nerve interface able to monitor and influence nerve regeneration following injury. Nerve interfaces that can be implanted during a surgical procedure and interact with nerves during this critical window, and then degrade without the need for a further surgical procedure, offer important clinical impact. Furthermore, there is little prior work on biodegradable nerve interfaces, making the technology the authors present both relevant and timely. The authors have clearly put a lot of work into this project and prepared the manuscript with care. I would recommend this work for publication in Nature Communications.
With this said, I do have some important criticism on the bio- side of things – specifically, many of the claims of the authors on the nerve interfacing capabilities of their technology are not sufficiently supported by their results. While I don't consider these critical flaws since the core innovation (a functional recording and stimulating biodegradable nerve device) is well supported by the data, I do think these should be addressed before publication.

Nerve recordings
1) The recorded nerve traces shown throughout the paper are not very convincing. Spontaneous freely-moving activity (e.g. Fig3h, 3j, 4f), which is notoriously difficult to do, in particular. Looking at the methods, the ephys data here was bandpass filtered between 10 and 300Hz. This is a relatively low frequency range, and I'm not convinced this removes movement artefacts like the authors claim. What do the higher frequencies look like? Are there spikes, or modulation of the amplitude of the baseline, when the animal moves? Furthermore, no processing has been carried out to remove EMG artefacts from the recorded trace as far as the methods describe, such as referencing recordings to the median or mean signal of all electrodes. These large, low frequency signals while the rat walks could originate from the leg muscles, rather than from the sciatic nerve. Nerve recordings from awake animals are very rare, so some further analysis to rule out alternative sources for the shown traces would be valuable. The same applies to recordings while the limb is manually flexed/extended (Fig2h), as

at such low frequencies movement artefacts could give rise to the recorded signals.
2) CAPs (FigS5-7, 5d-f) are also a bit confusing. According to the authors the stimulation pulse has a 1 Hz frequency (i.e. lasts half of a second if a single phase pulse is being delivered)? This would result in a stimulation artefact that would mask completely the CAP (particularly given the close proximity of the stimulation electrode). Typically a very short stimulation pulse would be used in CAP studies for this reason. The fact that CAP polarity reverses when the stimulation phase is inverted (S7) would also suggest that what is being recorded is the stimulation artefact, not the CAP.
3) The authors also claim (pg13) that their device can be used to track nerve recovery by localising the axon leading edge growing across the gap lesion. However, the only evidence to support this are two examples in Fig3h and 3j (with the latter not being particularly strong, since all electrodes being equally active is simply indicating that the edge is not within the electrode array region). This claim should be better supported. Even without the need for matching histology like in Figure3, can we see more examples from various animals of the leading edge localised via electrophysiology recording to the electrode array?

Nerve regeneration enhanced by the galvanic cell of the implant
4) The restorative capabilities of the device are enabled by the presence of a galvanic cell applying an electric field over the gap lesion. However, the analysis comparing device with (Bio-Restor) and without (Bio-Neuro) this cell is not terribly convincing. The authors rely on kinematics at weeks 2 and 3 post-implantation to evaluate regeneration (Fig4). It's a bit surprising to see significant motor recovery following a nerve lesion so soon, given the presence of such a large gap which must be first bridged (further supported by the location of the axon leading edge within the bridge at 3 weeks in Fig3g-l, and the fact that kinematics in Fig4c-d don't look like those of normal animals/limbs). Indeed the kinematics at these timepoints are not very convincing (no statistical difference between Bio-Restor3weeks and Bio-Neuro3weeks). Given that histology seems to be present, a quantification of something like axon density on the bridge or the distal stump would more convincingly show enhancement of regeneration by the Bio-Restor implant.
5) Referring to the CNN classification of gait using nerve recorded date, the authors claim that "The results indicate a significant reduction of swing time for the Bio-Restor group at week 3 compared to that of the Bio-Neuro group at week 2 ($p < 0.01$), suggesting a faster walking speed and therefore better recovery." While I find this classification strategy interesting as a way to monitor animal function without the need for a camera and gait analysis, this specific interpretation from the authors feels wrong. The authors have already evaluated recovery in Bio-Neuro vs Bio-Restor using ground truth (kinematic) gait analysis earlier in the paper. How does evaluating it again using an imperfect classifier based on nerve data provide any additional support to this claim?

Neuroma detection
6) In neuroma detection experiments (Fig5), uninjured nerves represent a poor control. Stimulation of a transected nerve will undoubtedly produce a smaller CAP due to axon retraction and death, regardless of whether regeneration across the gap is occurring correctly or a neuroma is developing. The same would apply for recorded activity in freely-moving animals in the bottom figure panels. A correct control to be able to claim that a neuroma is being detected using electrophysiology would be a transected and correctly aligned nerve with device such as those in Figure 3 and 4. Do the authors have CAP data from these they could use as control in Figure 5? If this data was not acquired, even just including recordings from awake animals with gap lesion but no neuroma in the confusion matrix of Fig5l would provide solid proof to back the ability of this technology to detect and track neuromas.

Other minor points
7) Please write out mean+/- SD values for impedances from Fig 2e (all, not just weeks 1 and 3)
8) Figure 2 legend "All data are presented as mean ± s.d. of n = 3 rats per condition" – does this refer to any data presented in this figure?
9) Fig2f is a bit confusing – degradation is much faster than that of electrodes (2e), I imagine due to the accelerated aging conditions. Can a real time equivalent to this accelerated aging time be estimated?
10) Fig4c and e – what statistical test is being employed here? The statistical comparison bars are also a bit confusing. For example, in 4e, does this mean all four groups are significantly different (e.g. one way ANOVA) or just Neuro2weeks vs Restor3weeks in a pairwise comparison? P values rather than asterisks* would also be preferable, particularly since the p values these represent are not indicated in the legend.
11) There is no description or citation for what the sciatic functional index score used in Fig4e is.
12) Referring to Fig5h the authors claim that "The results indicate a significant decrease in the relative energy within the high-frequency band (approximately 15-50 Hz) associated with the formation of the neuroma (neuroma 2 W and neuroma 3 W groups)." Since I can't see any statistical tests or quantification done on these, I would reword this claim.

The discussion is very short, and leaves several (in my opinion) relevant points un-discussed. Such as:
13) The interface is degradable, but the implanted wires are not. How do the authors foresee these connections be dealt with in a clinical implementation of this technology?
14) Applying an electric field over axons enhances regeneration, but the amplitude of this field will matter. Can an estimation of the field applied here be provided? How does this compare to other work such as ref32 which the authors cite? Would this scale adequately if this technology was applied in larger species such as humans?
15) Inducing and guiding regeneration using an electric field is a known concept, but many readers may not be aware. Discussing some prior literature on this (not necessarily from implantable device work) would be helpful.
16) Some discussion of existing biodegradable nerve interfaces to provide context to the novelty of the technology being presented would be useful. In addition to prior work from the authors, this paper from John Rogers comes to mind: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9534494/pdf/sciadv.abp9169.pdf

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Authors have made necessary and satisfactory corrections and manuscript can be accepted.

Reviewer #2

(Remarks to the Author)
The authors have done a good job addressing my suggestions. I believe the manuscript is close to a state acceptable for publication. I do have one point I really do think should be addressed. I also have some other minor points which, while less important, I think authors should also address to improve the manuscript.

Major: With regards to the neuroma control group – I think this could still use some improvement. My issue here is that the authors are proving that they can detect the change from a smaller neuroma (Neuroma 2W) to a larger neuroma (Neuroma 3W). It would really help produce a complete story if the authors could include a CAP early on after transection, before the neuroma has formed. At the moment it is unclear whether the device could detect a neuroma <2W, or only in the 2-3W interval, which in my opinion matters quite a bit for an early intervention scenario as the authors suggest. It does not feel like this would be a difficult experiment to perform (measuring CAPs in a freshly transected nerve). Even if <2W detection is not possible, this device remains valuable for neuroma detection, but I believe it is important to complete the story.

Minor: The authors indicate that most of the “spontaneous” activity recorded by their electrodes was of very low frequency (1-90Hz) – a band were one may expect movement artefacts and EMG but not spontaneous nerve activity – and that no techniques were implemented to eliminate these non-nerve components. I am not convinced that the recorded signals are derived from nerve activity. Many of the recordings shown throughout the paper present very large amplitude, slow waves which look distinctly non-neural. To me, it looks like the tissue of the regenerated nerve segment (compared to the non-regenerated segment) has different properties leading to electrodes in contact with it being able to pick up more activity (whether from nerve, EMG, or movement). However, as the authors state, their novelty does not lie in the recording of nerve signals per se, so I don’t think this detracts from their work. While they acknowledge in the manuscript that EMG contamination could be playing a role in their recordings, this is currently somewhat hidden in the methods. I strongly encourage the authors to comment (and expand on this) in the discussion – readers knowledgeable in neural electrophysiology would likely be sceptical about the quality of the recording experiments otherwise.

Statistical analysis: the authors have included information on the test used for their comparison (one-way ANOVA). However, what they have done is not entirely clear. One-way ANOVA provides a p value for the probability that all group means being tested are equal. If this is what is being used, then I think this is a very strange choice of test (particularly in Fig 4c where two comparisons with overlapping groups are being used). What is more commonly used is a one-way ANOVA which, if statistically significant, is followed by a post-hoc pairwise comparison test (such as a Tukey test and or a Bonferroni-corrected t-test). If this is what is being used, it should also be indicated in the legends. It should also be indicated that pairwise comparisons not shown on graphs are $p > 0.05$ / not significant (I assume this is the case). The “statistical analysis” section in the methods is also too short and contradictory – it indicates that “for two-group comparisons with an unpaired t-test…” which makes me suspect that Fig4j, 6c and 6g are not one-way ANOVAs like the authors indicate in the figure caption. Please take the time to carefully revise the statistical tests being used and their description in figure captions and in the methods section, and update any p values in the figures if improper statistical tests were used.

Fig S18a: 90-900Hz magnification is too low. I’d suggest changing the amplitude of the traces to be able to visualise any activity or lack thereof.

Fig 2g-h: I appreciate the changes in the caption, but I don’t think it would be correct to indicate that these are $n = 3$ independent experiments. The authors are showing a trace from a single animal. Either remove this indication, or if the authors want to support the replicability of these recordings, include a trace in 2h for each of the three animals.

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
The authors have addressed all of my comments, I believe the manuscript should be published.

Reviewer #1

Summary Recommendation: *The manuscript 'A biodegradable and restorative peripheral neural interface for the interrogation of neuropathic injuries' This project represents advancement in nerve injury rehabilitation, combining biodegradable materials with cutting-edge machine learning to enable real-time monitoring and early detection of complications like traumatic neuroma. Its innovative design eliminates the need for retrieval surgeries, significantly reducing infection risks and improving patient outcomes. The ability to wirelessly track nerve recovery could transform clinical practices, offering a more dynamic, personalized approach to treatment. Reviewer has following concerns:*

Our response: We thank the reviewer for these favorable comments and valuable suggestions. We address these issues in the point-by-point response below.

- ***Comment #1:*** *"The rate of degradation must align with the healing process, which varies between patients. Can authors comment on that?"*

Our response: We thank the reviewer for this valuable suggestion. We agree with the reviewer that aligning the degradation rate with the healing process is crucial since individual patients' healing processes can vary due to factors such as age and overall health. Early repair is crucial for achieving optimal therapeutic outcomes following severe nerve injuries (Scandinavian Journal of Pain 20, 95-108 (2019)). Monitoring and intervention in the initial stages, typically within a couple of weeks (PloS one 8, e56484 (2013)), are therefore essential for enhancing functional restoration. The current device has an operational lifespan of approximately 3 weeks. Further optimization of electrode and encapsulation materials can customize degradation rates and extend the working timeframe. For example, substituting the bioactive components of the galvanic cells with Mg alloys or Zn can provide prolonged electrical cues to facilitate nerve regrowth. Utilizing electrodes with greater thickness and encapsulation materials with slower dissolution rates will extend the lifespan of the recording electrodes. Moreover, employing triggered degradable materials presents an alternative

materials strategy for precise control of degradation period, allowing devices to align with an individual's unique healing trajectory.

Our modification to the manuscript:

We added corresponding discussion in the discussion part on page 23:

"Further optimization of electrode and encapsulation materials can customize degradation rates and extend the working timeframe. For example, substituting the bioactive components of the galvanic cells with Mg alloys or Zn, and utilizing electrodes with greater thickness and encapsulation materials with slower dissolution rates will extend the life time of the neural interface. Moreover, employing triggered degradable materials presents an alternative method for precise control of degradation period. These material strategies have the potential to prolong the operational lifespan into the later stages of nerve recovery, allowing devices to align with an individual's unique healing trajectory."

- ***Comment #2:*** *"Piezoelectric materials are one of the kinds of sustainable materials? Reviewer has doubts on this statement."*

Our response: We thank the reviewer for the comment. We carefully checked the manuscript and ensure that we did not make the statement of piezoelectric materials being sustainable materials.

Our modification to the manuscript:

We ensure that the manuscript does not contain statements suggesting that piezoelectric materials are sustainable materials.

- ***Comment #3:*** *"Introduction is redundant and should be shortened. Much info is already well known and well reported."*

Our response: We thank the reviewer for the comment. We have removed the original first paragraph and the section on traditional diagnostic approaches from the second paragraph of the introduction to enhance conciseness.

Our modification to the manuscript:

We updated the introduction on page 4:

"Since early repair and diagnosis is crucial for optimal therapeutic results [5], monitoring the initial stages of healing after severe nerve injuries is essential, which can offer vital physiological and pathological insights for timely interventions and enhanced function restoration [10-13]. Multifunctional peripheral nerve interfaces present a promising approach for remote and continuous monitoring of nerve injuries, which however remains largely unexplored. By integrating both restorative and monitoring functions, the nerve interface has the potential to promote nerve regeneration and facilitate early detection of recovery challenges during the initial phases of nerve repair. This enables prompt interventions and enhances treatment optimization, ultimately maximizing rehabilitation outcomes and prosthetic integration."

- ***Comment #4:*** *"A critical limiting factor is the resolution with which nerve inputs are mapped. How is this factored in to this work?"*

Our response: We thank the reviewer for the comment. The resolution of nerve input mapping is directly correlated with the number of recording channels available. We currently have 7 channels distributed along the direction of nerve regrowth. Signals recorded at various positions facilitate the identification of the leading edge of axonal growth. Further increase of the number of channels can enhance the spatial resolution of input signals, allowing for the capture of more detailed and precise neural activity. This, in turn, offers deeper insights into the underlying neural processes at play.

Our modification to the manuscript:

We added corresponding discussion in the discussion part on page 23:

"The increase of the number of channels can enhance the spatial resolution of input signals, allowing for the capture of more detailed and precise neural activity. This, in turn, offers deeper insights into the underlying neural processes at play."

- ***Comment #5:*** *"False positives or negatives in detection might affect the quality of clinical decisions. How is that taken into account and tested?"*

  Our response: We thank the reviewer for the comment. The presence of false positives or negatives in detection can indeed impact the quality of clinical decisions. In the current study on neuroma detection, our algorithm has achieved a classification accuracy exceeding 90% at week 2, which represents an advancement compared to the traditional ultrasound method in identifying neuromas at an early stage. Nevertheless, to address the concern of false positives or negatives in detection, rigorous testing and validation processes are needed in the future. Strategies include optimizing algorithms through the collection of a large and diverse datasets, and employing cross-validation techniques such as splitting the dataset into training and testing subsets to assess the robustness of the algorithm. Additionally, in real diagnostic scenarios such as neuroma detection, a missed diagnosis may hold greater clinical significance than an incorrect positive result. Hence, we can strategically adjust parameter weights to minimize the missed detection rate, even if it leads to a slight increase in false positive results.

  Our modification to the manuscript:

  We added corresponding discussion on page 19:

  "Nevertheless, to address the concern of false positives or negatives in detection, rigorous testing and validation processes are needed in the future. Strategies include optimizing algorithms through the collection of a large and diverse datasets, and employing cross-validation techniques such as splitting the dataset into training and testing subsets to assess the robustness of the algorithm. Additionally, we can also strategically adjust parameter weights to minimize the missed detection rate."

- ***Comment #6:*** *"The device may provide valuable data during early recovery stages, but its effectiveness in chronic or later stages of nerve recovery might be reduced. How is the device and ML models suited for that?"*

  Our response: We thank the reviewer for the comment. Current device is designed to

monitor and facilitate nerve repair at the early stage of nerve injuries, as it is crucial for achieving optimal functional restoration (Scandinavian Journal of Pain 20, 95-108 (2019)). Nevertheless, further optimization of electrode and encapsulation materials can extend the working timeframe. For example, substituting the bioactive components of the galvanic cells with Mg alloys or Zn can provide prolonged electrical cues to facilitate nerve regrowth. Utilizing electrodes with greater thickness and encapsulation materials with slower dissolution rates will extend the lifespan of the recording electrodes. Moreover, employing triggered degradable materials presents an alternative materials strategy for precise control of degradation period. These material strategies have the potential to prolong the operational lifespan into the later stages of nerve recovery. Additionally, machine learning algorithms can be designed to analyze the characteristics of recorded signals at various stages and evaluate the device's stability. By leveraging this comprehensive data, the algorithm can be enhanced to predict outcomes effectively throughout different phases of nerve recovery.

Our modification to the manuscript:

We added corresponding discussion in the discussion part on page 23:

"Further optimization of electrode and encapsulation materials can customize degradation rates and extend the working timeframe. For example, substituting the bioactive components of the galvanic cells with Mg alloys or Zn, and utilizing electrodes with greater thickness and encapsulation materials with slower dissolution rates will extend the life time of the neural interface. Moreover, employing triggered degradable materials presents an alternative method for precise control of degradation period. These material strategies have the potential to prolong the operational lifespan into the later stages of nerve recovery, allowing devices to align with an individual's unique healing trajectory."

"Additionally, optimized machine learning algorithms can be designed to analyze the characteristics of recorded signals at various stages and evaluate the device's stability. By leveraging this comprehensive data, the algorithm can be enhanced to predict outcomes effectively throughout different phases of nerve recovery."

Reviewer #2

Summary Recommendation: *In this manuscript, the authors present a biodegradable nerve interface able to monitor and influence nerve regeneration following injury. Nerve interfaces that can be implanted during a surgical procedure and interact with nerves during this critical window, and then degrade without the need for a further surgical procedure, offer important clinical impact. Furthermore, there is little prior work on biodegradable nerve interfaces, making the technology the authors present both relevant and timely. The authors have clearly put a lot of work into this project and prepared the manuscript with care. I would recommend this work for publication in Nature Communications.*

With this said, I do have some important criticism on the bio-side of things – specifically, many of the claims of the authors on the nerve interfacing capabilities of their technology are not sufficiently supported by their results. While I don't consider these critical flaws since the core innovation (a functional recording and stimulating biodegradable nerve device) is well supported by the data, I do think these should be addressed before publication.

Our response: We thank the reviewer for these favorable comments and valuable suggestions. We address these issues in the point-by-point response below.

- ***Comment #1:*** *"The recorded nerve traces shown throughout the paper are not very convincing. Spontaneous freely-moving activity (e.g. Fig3h, 3j, 4f), which is notoriously difficult to do, in particular. Looking at the methods, the ephys data here was bandpass filtered between 10 and 300Hz. This is a relatively low frequency range, and I'm not convinced this removes movement artefacts like the authors claim. What do the higher frequencies look like? Are there spikes, or modulation of the amplitude of the baseline, when the animal moves? Furthermore, no processing has been carried out to remove EMG artefacts from the recorded trace as far as the methods describe, such as referencing recordings to the median or mean signal of all electrodes. These large, low frequency signals while the rat walks could originate from the leg muscles,*

rather than from the sciatic nerve. Nerve recordings from awake animals are very rare, so some further analysis to rule out alternative sources for the shown traces would be valuable. The same applies to recordings while the limb is manually flexed/extended (Fig2h), as at such low frequencies movement artefacts could give rise to the recorded signals."

Our response: We thank the reviewer for the comment. We apologize for the confusion regarding data collection and analysis aimed at reducing the influence of EMG and movement artifacts. The wireless circuit utilized a differential method to capture electrical signals, calculating the difference between the signals from the recording electrode and the reference electrode, which facilitated partially reducing electromyographic interference. Moreover, the data collected were band filtered between 1 to 90 Hz, instead of 10 to 300 Hz which was mistakenly described in the manuscript. We have updated these details in the methodology session.

Low frequency band filters were adopted because the measured electrical signals were distributed mainly in the range of 1 to 90 Hz, as the frequency-time diagram shown in Figure S17. Similar low frequency band filters were also adopted in some prior research (e.g., Biosensors and Bioelectronics 216, 114600 (2022)). The recorded electrical signals applied with low frequency and high frequency band filters were given in Figure S18. We did not observe significant spikes or modulation of the baseline in the high frequency range while animals were moving normally on the treadmill. There is some modulation of the baseline during manual flexion and extension movements; however, this (~0.2 Hz) can be effectively filtered out using a high-pass filter set at 1 Hz.

For the signals recorded during treadmill walking, we did not reference recordings to the median or mean signal of all electrodes. Our intention is to investigate electrical signals on the injured nerve, where the signals from different channels may vary significantly, which can be utilized to quantitatively identify the leading edge of axonal growth. Referencing signals to the mean signals of all electrodes would not enable the capture of variations among different electrode channels. In fact, the demonstration of

distinct signals recorded in different electrode channels across the nerve defects (Figure 3h) may indicate not significant EMG interference, as significant interference would typically lead to similar signals across all channels. For the signals recorded during flexion-extension movement of the hindlimb, we processed the data by referencing the recordings to the mean signal of all electrodes, as suggested by the reviewer. The updated data is presented in Figure 2h, showing a trend similar to the previous results.

Overall, we do agree with the reviewer on the inherent challenge of recording neural signals in freely-moving animals and the persistent EMG interference in the low frequency range, even with reference electrodes and data filtering. Future efforts to optimize electrode configurations and filtering algorithm may assist further reduction of such interferences. Nevertheless, for the present study, our focus remains on detecting signal variations during nerve regrowth processes and neuroma presence, with the expectation that moderate EMG interference will not significantly impact our analysis.

Our modification to the manuscript:

We added Figure S17, S18 and update Figure 2h, S8 and the description on page 10, 12, 30 and 31:

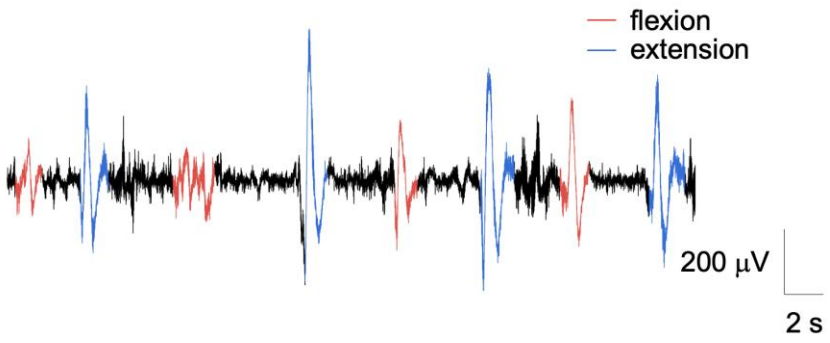


Figure 2 h, Recorded signal traces using the Mo/Si electrode array on sciatic nerves during the flexion-extension movement of the hindlimb.

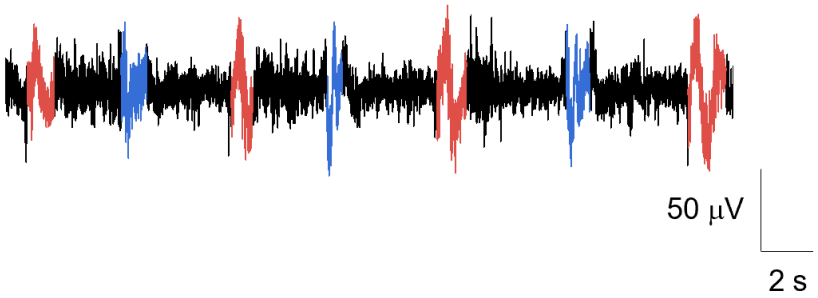


Figure S8 Recorded signal traces using cuff electrodes on sciatic nerves during the flexion-extension movement of the hindlimb.

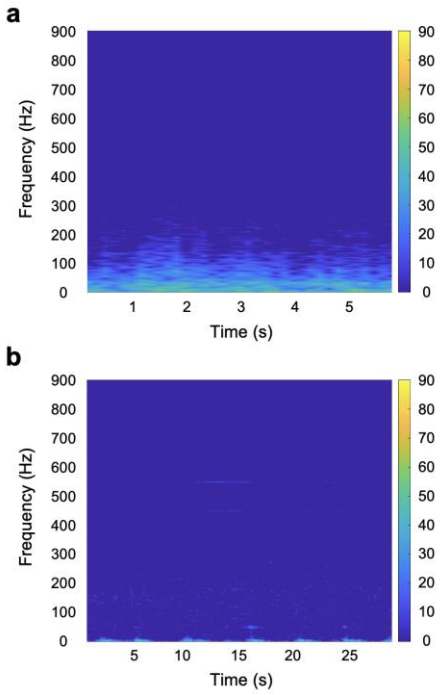


Figure S17 Representative time-frequency spectrograms of the Bio-Neuro group. **a,** walking on a treadmill at 3 weeks postimplantation and **b,** during the flexion-extension movement of the hindlimb.

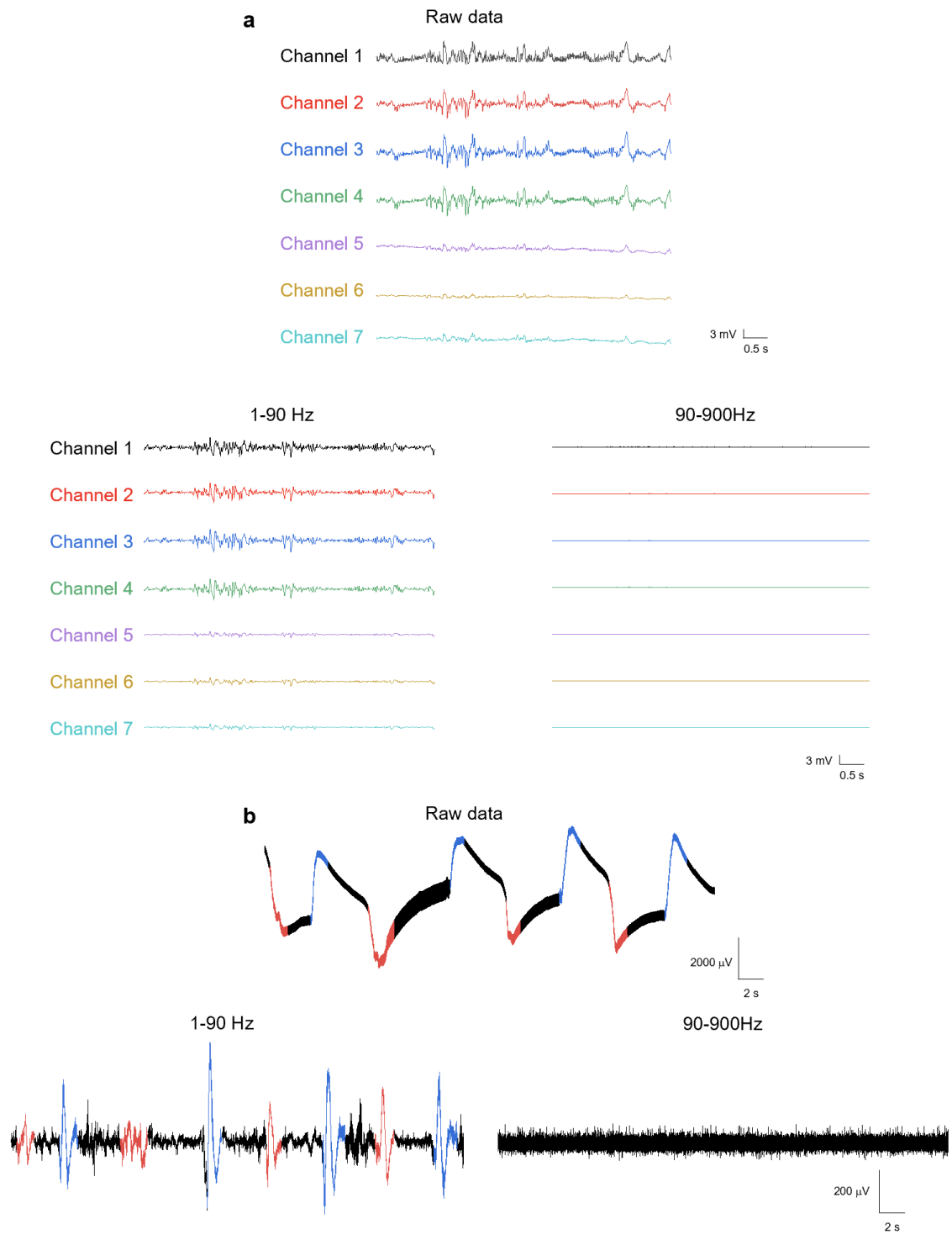


Figure S18 Recorded raw signal traces and corresponding signals after low-frequency and high-frequency band filtering of the Bio-Neuro group. **a,** walking on a treadmill at 3 weeks postimplantation and **b,** during the flexion-extension movement of the hindlimb.

"To eliminate the electromyography (EMG) artifact, we processed the data by referencing the recordings to the mean signal of all electrodes."

"For signal processing, we utilize low-frequency band filters, as the measured electrical signals were primarily distributed in the range of 1 to 90 Hz, as illustrated in the frequency-time diagram in Figure S17. The electrical signals recorded using both low-frequency and high-frequency band filters are presented in Figure S18. During normal movement, we do not observe significant spikes or baseline modulation in the high-frequency range. The demonstration of distinct signals recorded in different electrode channels across the nerve defects (Figure 3h) may indicate not significant EMG interference, as significant interference would typically lead to similar signals across all channels."

"A Butterworth high-pass filter at 1 Hz is used to remove low-frequency motion artifact noise, while a low-pass filter with a 90 Hz cut-off frequency removes high-frequency environmental noise [41]."

"It is noted that there is an inherent challenge in recording neural signals from freely-moving animals due to persistent EMG interference in the low-frequency range, even with reference electrodes and data filtering. Future efforts to optimize electrode configurations and filtering algorithm may assist further reduction of such interferences. Nevertheless, for the present study, our focus remains on detecting signal variations during nerve regrowth processes and neuroma presence, with the expectation that moderate EMG interference will not significantly impact our analysis."

- ***Comment #2*** *"CAPs (FigS5-7, 5d-f) are also a bit confusing. According to the authors the stimulation pulse has a 1 Hz frequency (i.e. lasts half of a second if a single phase pulse is being delivered)? This would result in a stimulation artefact that would mask completely the CAP (particularly given the close proximity of the stimulation electrode). Typically a very short stimulation pulse would be used in CAP studies for this reason. The fact that CAP polarity reverses when the stimulation phase is inverted (S7) would also suggest that what is being recorded is the stimulation artefact, not the CAP."*

Our response: We thank the reviewer for the comment. We apologize for the

confusion. The stimulation pulse we used is a 1 Hz square wave with a duty cycle of 1%, which is very short pulse duration of 10 ms. We have revised Figure 5d-f, S6, and S7 to include legends specifying the actual evoked signal. The initial peak marked with a purple square represents the artifact signal, whereas the subsequent peak denotes the evoked signal. The artifact signal reverses upon the inversion of stimulation polarity, whereas the evoked signals maintain the same polarity. The amplitude of the evoked signal decreases with a reduction in stimulation voltage.

Our modification to the manuscript:

We updated the captions of Figure 5 d-f, S6, S7 and the description on page 27 specifying the pulse duration:

Figure 5 d–f, Representative recorded evoked signals by Bio-Restor of the control (intact nerve), neuroma at 2 weeks, and neuroma at 3 weeks respectively, under the electrical stimulation at the proximal side with a voltage amplitude of 1 V, 5 V and 5 V respectively. Stimulation frequency: 1 Hz, pulse duration: 10 ms. Purple square represents artifact signals.

Figure S6 Evoked neural signals recorded by the Mo/Si electrode array and a commercial cuff electrode as a control. Stimulation frequency: 1 Hz, pulse duration: 10 ms. Purple square represents artifact signals.

Figure S7 Evoked neural signals recorded by the Mo/Si electrode array with **a,** ± 1 V and **b,** ± 0.25 V. Stimulation frequency: 1 Hz, pulse duration: 10 ms. Purple square represents artifact signals.

"Evoked neural signals were recorded upon the application of electrical stimulation (frequency:1 Hz, pulse duration: 10 ms, amplitude: ± 1 and 0.25 V) at the proximal side of the sciatic nerve."

- ***Comment #3:*** *"The authors also claim (pg13) that their device can be used to track nerve recovery by localising the axon leading edge growing across the gap lesion.*

However, the only evidence to support this are two examples in Fig3h and 3j (with the latter not being particularly strong, since all electrodes being equally active is simply indicating that the edge is not within the electrode array region). This claim should be better supported. Even without the need for matching histology like in Figure3, can we see more examples from various animals of the leading edge localised via electrophysiology recording to the electrode array?"

Our response: We thank the reviewer for the valuable suggestions. We have added additional examples of immunofluorescent images of the longitudinal section of the nerve segment, along with the corresponding recorded signal traces from the implanted Bio-Neuro at 2 weeks postimplantation.

Our modification to the manuscript:

We added Figure S19 and updated the following description on page 13:

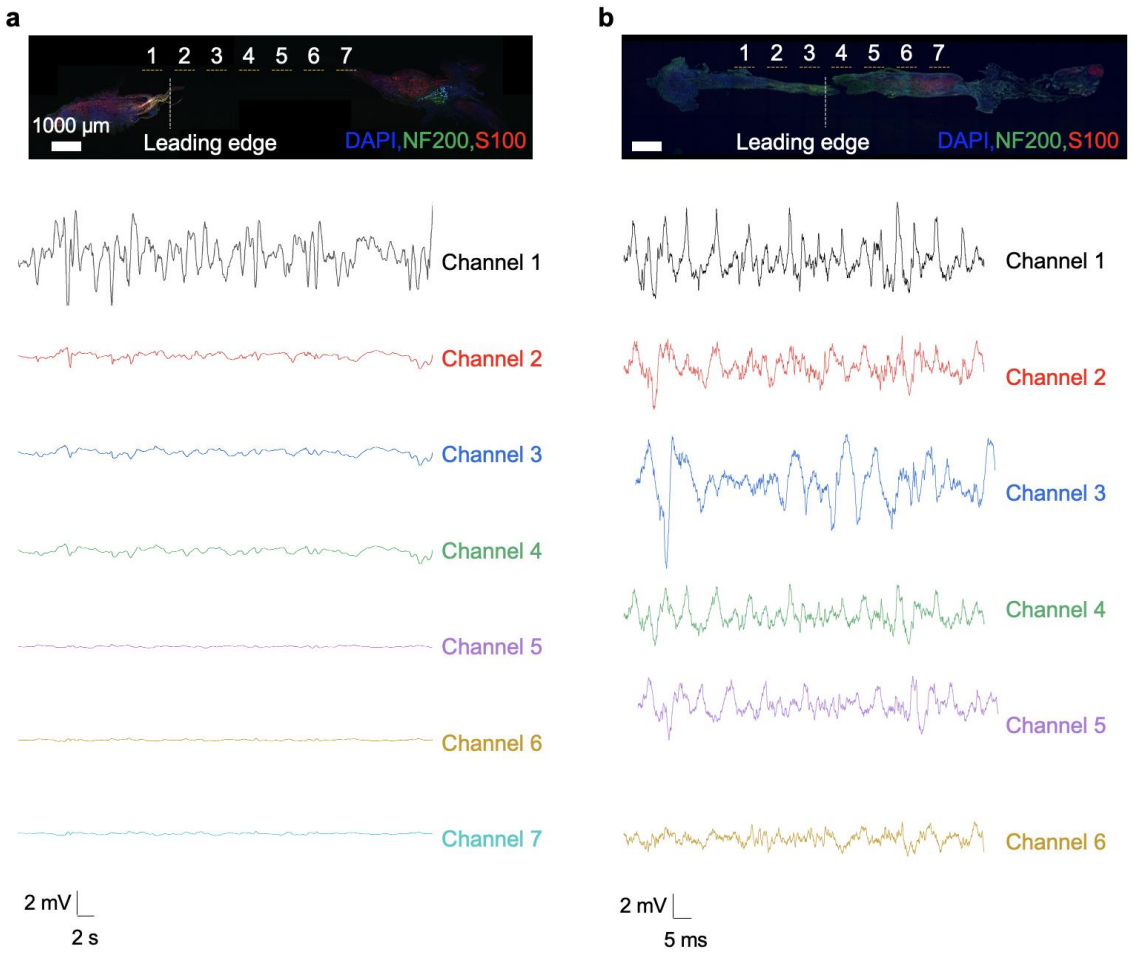


Figure S19 Immunofluorescent images of the longitudinal section of the nerve segment and the corresponding recorded signal traces with implanted Bio-Neuro at 2 weeks postimplantation. **a**, case 1; **b**, case 2. Yellow dashed lines indicate the position of neural electrodes. Note: channel 7 in **b** is broken and therefore no signal was recorded.

"Similar correlation between histological and signal results are also observed at week 2 in the Bio-Neuro group (Figure S19)**."**

- ***Comment #4:*** *"The restorative capabilities of the device are enabled by the presence of a galvanic cell applying an electric field over the gap lesion. However, the analysis comparing device with (Bio-Restor) and without (Bio-Neuro) this cell is not terribly convincing. The authors rely on kinematics at weeks 2 and 3 post-implantation to evaluate regeneration (Fig4). It's a bit surprising to see significant motor recovery*

following a nerve lesion so soon, given the presence of such a large gap which must be first bridged (further supported by the location of the axon leading edge within the bridge at 3 weeks in Fig3g-I, and the fact that kinematics in Fig4c-d don't look like those of normal animals/limbs). Indeed the kinematics at these timepoints are not very convincing (no statistical difference between Bio-Restor3weeks and Bio-Neuro3weeks). Given that histology seems to be present, a quantification of something like axon density on the bridge or the distal stump would more convincingly show enhancement of regeneration by the Bio-Restor implant."

Our response: We thank the reviewer for this valuable suggestion. We do agree that although the kinematics data at week 3 showed some improvement, it is not sufficient to fully support the restorative capabilities, because it is still at an early stage as the reviewer pointed out. Besides the histology results given in Figure 3g-i, we reperformed statistical analysis on axon density at week 3, and the results indicate a significant increase in axon density in the Bio-Restor group than that of the Bio-Neuro group (Figure S19). We also performed evoked stimulation at week 3 at the proximal site of the injured nerves, and the movement of hindlimb was observed in the Bio-Restor group, indicating the reinnervation of target muscles has been initiated. By contrast, such hindlimb movement was observed upon evoked stimulation in the Bio-Neuro group at week 3. We added this evidence of the Bio-Restor group at week 3 in the supplementary movie.

Our modification to the manuscript:

We added Figure S20 and updated the description on page 13:

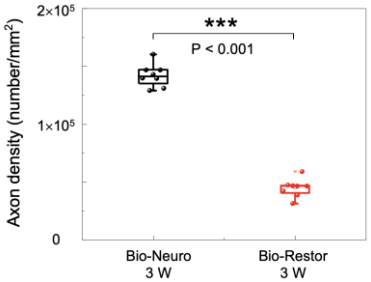


Figure S20 Statistical analysis of the axon density. One-way ANOVA was used for the statistical analysis (***P < 0.001).

"Additionally, we perform statistical analysis on axon density at week 3 following the method in the previous work [23], and the results indicate a significant increase in axon density in Bio-Restor group than that of the Bio-Neuro group (Figure S20)."
"Furthermore, we observe hindlimb movement in the Bio-Restor group at week 3 upon evoked stimulation on the proximal nerve segment, indicating muscle reinnervation has been achieved (Movie S4). By contrast, such hindlimb movement was observed upon evoked stimulation in the Bio-Neuro group at week 3."

- ***Comment #5:*** *"Referring to the CNN classification of gait using nerve recorded date, the authors claim that "The results indicate a significant reduction of swing time for the Bio-Restor group at week 3 compared to that of the Bio-Neuro group at week 2 ($p < 0.01$), suggesting a faster walking speed and therefore better recovery." While I find this classification strategy interesting as a way to monitor animal function without the need for a camera and gait analysis, this specific interpretation from the authors feels wrong. The authors have already evaluated recovery in Bio-Neuro vs Bio-Restor using ground truth (kinematic) gait analysis earlier in the paper. How does evaluating it again using an imperfect classifier based on nerve data provide any additional support to this claim?"*

  Our response: We thank the reviewer for the valuable comment. As the reviewer suggested, we intent to employ CNN classification to identify swing and stance time, to demonstrate the potential of using machine learning analysis as a novel approach to monitor kinematic function without the need for camera or gait analysis. The traditional gait analysis is used as the gold standard for comparation. It is not our focus to use the CNN classification results to support the restorative capability of the Bio-Restor group. We have updated the corresponding interpretation to avoid confusion.

  Our modification to the manuscript:
  We updated the following description on page 15–16:
  "The results indicate a significant reduction of swing time for the Bio-Restor group at week 3 compared to that of the Bio-Neuro group at week 2 ($p < 0.01$). This trend is

consistent with the gait analysis obtained by conventional methods (Figure 4b–e). These results altogether suggest that the real-time electrical signals recorded by the bioresorbable interface can be utilized to remotely interrogate the leading edge of axonal growth and gait parameters, therefore predicting the recovery status of injured nerves without the need for camera-based gait analysis."

- ***Comment #6:*** *"In neuroma detection experiments (Fig5), uninjured nerves represent a poor control. Stimulation of a transected nerve will undoubtedly produce a smaller CAP due to axon retraction and death, regardless of whether regeneration across the gap is occurring correctly or a neuroma is developing. The same would apply for recorded activity in freely-moving animals in the bottom figure panels. A correct control to be able to claim that a neuroma is being detected using electrophysiology would be a transected and correctly aligned nerve with device such as those in Figure 3 and 4. Do the authors have CAP data from these they could use as control in Figure 5? If this data was not acquired, even just including recordings from awake animals with gap lesion but no neuroma in the confusion matrix of Fig5l would provide solid proof to back the ability of this technology to detect and track neuromas."*

  Our response: We thank the reviewer for this valuable suggestion. In Figure 5d, we first utilized the uninjured nerve as the control group to compare signal amplitudes between conditions with and without neuroma formation. Our findings indicate significantly greater evoked signal amplitude in the uninjured nerve compared to the presence of a neuroma. Additionally, in Figure 5h-l, we did use transected and regenerated nerves (Bio-Restor group at week 3) as the control as the reviewer suggested. The recorded signals exhibit distinct patterns between the control and neuroma groups. We have updated the discussion to clarify the different control groups employed in Figure 5.

  Our modification to the manuscript:

  We added a description to clarify the motivation using the uninjured nerve as the control group on page 17 and 18:

"The initial use of the uninjured nerve as the control group is intended to facilitate a comparison of signal amplitudes between conditions with and without neuroma formation."

"Here, the regenerated nerves without neuroma formation at week 3 (Bio-Restor group at week 3) is adopted as the control group, to rule out the uncertainty associated with impaired signals resulting from nerve injuries."

- ***Comment #7:*** *"Please write out mean+/- SD values for impedances from Fig 2e (all, not just weeks 1 and 3)"*

Our response: We thank the reviewer for this valuable suggestion. We have listed all the mean ± s.d. values for impedance from Figure 2e.

Our modification to the manuscript:

We updated the following description on page 8:

"Moreover, the impedance of Si/Mo electrodes demonstrates reasonable stability after soaking in PBS at 37 °C up to week 7, with an increase in impedance from 35.6 ± 9.8 kΩ (week 1) to 61.1 ± 3.9 kΩ week 3, 89.6 ± 7.7 kΩ week 5 and 451.9 ± 131.5 kΩ week 7 (Figure 2e)."

- ***Comment #8:*** *"Figure 2 legend "All data are presented as mean ± s.d. of n = 3 rats per condition" – does this refer to any data presented in this figure?"*

Our response: We thank the reviewer for this valuable suggestion. We have updated the caption of Figure 2 to improve clarity.

Our modification to the manuscript:

We updated the figure caption of Figure 2:

Figure 2 Biodegradable, restorative and self-morphing neural interface. In **e**, data are presented as mean ± s.d.. In **g** and **h**, n = 3 independent experiments.

- ***Comment #9:*** *"Fig2f is a bit confusing – degradation is much faster than that of electrodes (2e), I imagine due to the accelerated aging conditions. Can a real time equivalent to this accelerated aging time be estimated?"*

  Our response: We thank the reviewer for this valuable suggestion. The degradation process observed in Figure 2f occurs at a much faster rate compared to that depicted in Figure 2e. This is attributed to the accelerated kinetics at an elevated temperature of 60 °C. The dissolution rates of the recording electrode are expected to be regulated by Si membranes. By leveraging the Arrhenius equation, as detailed in prior literature (Advance Materials 27, 1857-1864 (2015)), we can project the degradation timeframe at 37°C based on the results at 60°C. Specifically, the degradation rate of Si is estimated to be approximately 4-7 times slower at 37°C than at 60°C. Consequently, extrapolating from the observed lifetime of the Si membrane at 60°C (around 9 days), the lifespan of the recording electrodes at 37°C is estimated to be around 5.5-8.5 weeks, aligning well with the results demonstrated in Figure 2e.

  Our modification to the manuscript:

  We updated the following description on page 9:

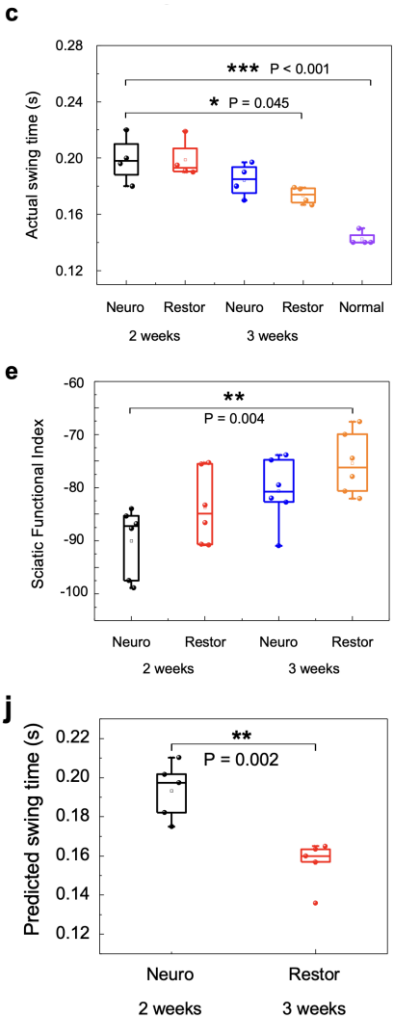
  "The degradation rate of Si is estimated to be approximately 4-7 times slower at 37 °C than at 60°C [27]. Therefore, the lifespan of the recording electrodes at 37 °C is estimated to be around 5.5-8.5 weeks, aligning well with the results demonstrated in Figure 2e."

- ***Comment #10:*** *"Fig4c and e – what statistical test is being employed here? The statistical comparison bars are also a bit confusing. For example, in 4e, does this mean all four groups are significantly different (e.g. one way ANOVA) or just Neuro2weeks vs Restor3weeks in a pairwise comparison? P values rather than asterisks* would also be preferable, particularly since the p values these represent are not indicated in the legend."*

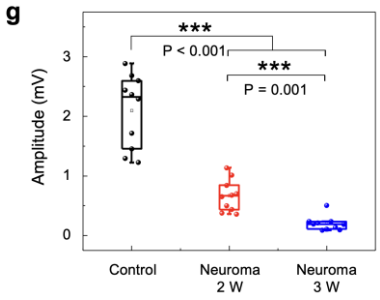
  Our response: We thank the reviewer for this valuable suggestion. One-way ANOVA was used for the statistical analysis. We have specified the p values and updated the

statistical comparison bars for better clarity. Additionally, we included an explanation of the relationship between asterisks (*) and the p value in the figure captions.

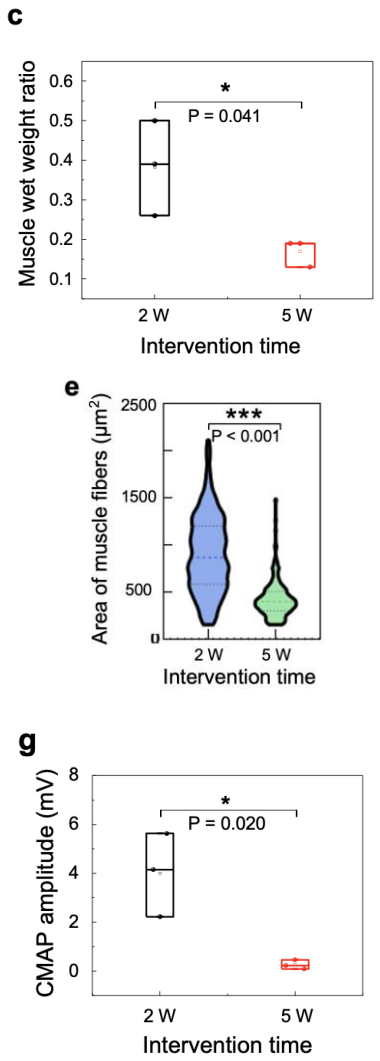
Our modification to the manuscript: We updated Figure 4c, 4e, 4j, 5g, 6c, 6e 6g, and S24, added description in corresponding figure captions and updated corresponding description on page 20:



"Figure 4 One-way ANOVA was used for the statistical analysis (*P < 0.05, **P < 0.01, ***P < 0.001)."



"Figure 5 One-way ANOVA was used for the statistical analysis (***P ≤ 0.001)."



"Figure 6 One-way ANOVA was used for the statistical analysis (*P < 0.05, ***P < 0.001)."

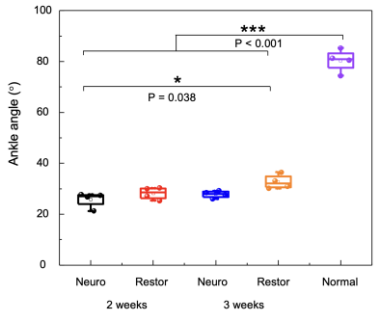


Figure S24 Statistics of ankle angle for Bio-Neuro, Bio-Restor and normal groups on terminal stance at 2 and 3 weeks after implantation (*P < 0.05, ***P < 0.001).

"Statistical analysis of the cross-sectional area of muscle fibers demonstrates that the muscle fiber area in the early intervention group (2 W) are significantly greater than that of the later intervention group (5 W) ($p < 0.001$) (Figure 6e)."

- ***Comment #11:*** *"There is no description or citation for what the sciatic functional index score used in Fig4e is."*

Our response: We thank the reviewer for this valuable suggestion. We have added the description and the corresponding citation for sciatic functional index score in the methodology session.

Our modification to the manuscript:

We updated the following description on page 14 and 29:

"The associated sciatic functional index (SFI) [23] , calculated from these footprints, shows a significantly greater value ($p < 0.01$) in the Bio-Restor group at week 3 compared to the Bio-Neuro group at week 2, signifying improved motor functional recovery."

"SFI was calculated using the formula outlined previously [23]:

$$\mathrm{SFI}=\frac{109.5(\mathrm{ETS}-\mathrm{NTS})}{\mathrm{NTS}}-\frac{38.3(\mathrm{EPL}-\mathrm{NPL})}{\mathrm{NPL}}+\frac{13.3(\mathrm{EIT}-\mathrm{NIT})}{\mathrm{NIT}}-8.8$$

where ETS is experimental toe spread (the distance between the first and fifth toes), while NTS denotes the normal toe spread. EIT is the experimental intertoe spread (the distance between the second and fourth toes), and NIT is the normal intertoe spread. EPL denotes the experimental print length, and NPL denotes the normal print length."

- ***Comment #12:*** *"Referring to Fig5h the authors claim that "The results indicate a significant decrease in the relative energy within the high-frequency band (approximately 15-50 Hz) associated with the formation of the neuroma (neuroma 2 W and neuroma 3 W groups)." Since I can't see any statistical tests or quantification done on these, I would reword this claim."*

  Our response: We thank the reviewer for this valuable suggestion. We have revised the corresponding statement.

  Our modification to the manuscript:
  We updated the description on page 18:
  "The results indicate a decrease in the relative energy within the high-frequency band (approximately 15-50 Hz) associated with the formation of the neuroma (neuroma 2 W and neuroma 3 W groups)."

- ***Comment #13:*** *"The interface is degradable, but the implanted wires are not. How do the authors foresee these connections be dealt with in a clinical implementation of this technology?"*

  Our response: We thank the reviewer for this valuable suggestion. Although the implanted wires are not degradable yet, they can also be designed to be biodegradable in the future as implied in reported works (Nature 530, 71-76 (2016)), eliminating the need for a second surgery to retrieve them.

  Our modification to the manuscript:
  We added the following discussion on page 23:

"Additionally, the implantable wires can also be designed to be biodegradable in the future [39], negating the need for surgical removal and reducing the risk of complications."

- ***Comment #14:*** *"Applying an electric field over axons enhances regeneration, but the amplitude of this field will matter. Can an estimation of the field applied here be provided? How does this compare to other work such as ref32 which the authors cite? Would this scale adequately if this technology was applied in larger species such as humans?"*

Our response: We thank the reviewer for this valuable suggestion. The electric field adopted in the current studies is estimated to range from 20 to 250 mV/mm, similar to the previous reported works, including reference 32 (Science Advances 6, eabc6686 (2020)). Specifically, electric field in the range of 10 to 600 mV/mm has been prove to promote nerve regeneration, both in rodents and human (Experimental Neurology 223, 192–202 (2010); Interdisciplinary Neurosurgery 24, 101117 (2021)). Further increase in electric field could also be achieved by placing multiple galvanic cells in consecutive segments to adapt to larger nerve defects.

Our modification to the manuscript:

We added the following description on page 22:

"The electric field strength along the conduit is expected to range from 20 to 250 mV/mm [23], consistent with the electric field needed to promote nerve regeneration reported in previous research [35,36]."

- ***Comment #15:*** *"Inducing and guiding regeneration using an electric field is a known concept, but many readers may not be aware. Discussing some prior literature on this (not necessarily from implantable device work) would be helpful."*

Our response: We thank the reviewer for this valuable suggestion. We have included discussions of the effects of an electric field on guiding regeneration.

Our modification to the manuscript:

We added the following discussions on page 22:

"The enhanced regeneration effect is attributed to the electrical cues generated by the galvanic cell. Electric field has been proved to promote nerve repair. The beneficial effects could results from accelerated Wallerian degeneration [32], and prompt and continuous upregulation of regeneration associated genes [33,34], therefore facilitating more rapid axonal regrowth."

- ***Comment #16:*** *"Some discussion of existing biodegradable nerve interfaces to provide context to the novelty of the technology being presented would be useful. In addition to prior work from the authors, this paper from John Rogers comes to mind: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9534494/pdf/sciadv.abp9169.pdf"*

Our response: We thank the reviewer for this valuable suggestion. We have added discussions of the currently available biodegradable nerve interfaces.

Our modification to the manuscript:

We added the following description on page 22:

"While the therapeutic effects for tissue regeneration and pain management based on optoelectronic or electrical stimulation have been demonstrated previously [23,37,38], there is limited prior work on biodegradable nerve interfaces that are capable of signal recording. We now showcase the capability of remote monitoring nerve recovery status by tracking the leading edge of axonal growth and the prediction of gait parameters."

Reviewer #2

Summary Recommendation: *The authors have done a good job addressing my suggestions. I believe the manuscript is close to a state acceptable for publication. I do have one point I really do think should be addressed. I also have some other minor points which, while less important, I think authors should also address to improve the manuscript.*

Our response: We thank the reviewer for these favorable comments and valuable suggestions. We address these issues in the point-by-point response below.

- ***Comment #1:*** *"Major: With regards to the neuroma control group – I think this could still use some improvement. My issue here is that the authors are proving that they can detect the change from a smaller neuroma (Neuroma 2W) to a larger neuroma (Neuroma 3W). It would really help produce a complete story if the authors could include a CAP early on after transection, before the neuroma has formed. At the moment it is unclear whether the device could detect a neuroma <2W, or only in the 2-3W interval, which in my opinion matters quite a bit for an early intervention scenario as the authors suggest. It does not feel like this would be a difficult experiment to perform (measuring CAPs in a freshly transected nerve). Even if <2W detection is not possible, this device remains valuable for neuroma detection, but I believe it is important to complete the story."*

  Our response: We thank the reviewer for this valuable suggestion. We measured the evoked signal under electrical stimulation immediately after nerve transection, at the proximal end near the transection site, which has now been included as a new control group. The amplitude of the evoked signals is smaller at the freshly transected nerves compared to that of the intact nerves. We also used the new data in the confusion matrix. The corresponding updates have been made to Figure 5d, 5g, and 5k.

  Our modification to the manuscript:

  We updated Figure 5d, 5g, 5k, corresponding figure caption and description in

manuscript on page 17-19:

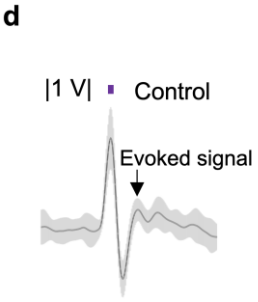


Figure 5 d, Representative recorded evoked signals by Bio-Restor of the control (freshly transected nerve, measured at the proximal end close to the transected site), under the electrical stimulation at the proximal side with a voltage amplitude of 1 V. Stimulation frequency: 1 Hz, pulse duration: 10 ms. Purple square represents stimulation artifacts.

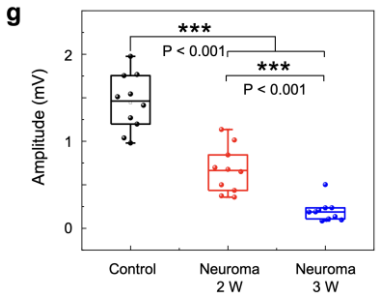


Figure 5 g, Statistical analysis of the amplitude of evoked neural signals.

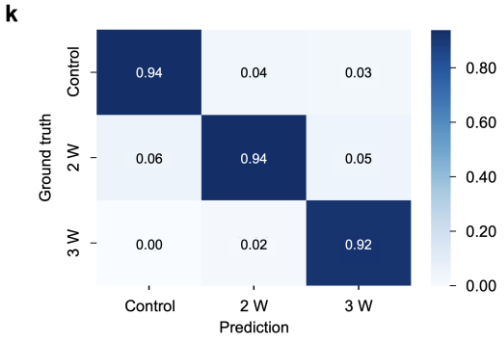


Figure 5 k, Confusion matrix of the prediction and ground truth of the control, neuroma 2 W and neuroma 3 W groups based on evoked neural signals.

“We study signal traces recorded by the Bio-Restor device in the control (freshly transected nerve, measured at the proximal end close to the transected site), neuroma 2

W and neuroma 3 W groups, upon electrical stimulation on the proximal site."
"The identification accuracy of neuroma formation at week 2 is 94% and 93%, based on signals recorded during evoked stimulation and treadmill walking respectively."
"Moreover, the identification accuracy for Neuroma 3 W is 92% and 96%, respectively, based on signals recorded during evoked stimulation and treadmill walking."

- ***Comment #2:*** *"Minor: The authors indicate that most of the "spontaneous" activity recorded by their electrodes was of very low frequency (1-90Hz) – a band were one may expect movement artefacts and EMG but not spontaneous nerve activity – and that no techniques were implemented to eliminate these non-nerve components. I am not convinced that the recorded signals are derived from nerve activity. Many of the recordings shown throughout the paper present very large amplitude, slow waves which look distinctly non-neural. To me, it looks like the tissue of the regenerated nerve segment (compared to the non-regenerated segment) has different properties leading to electrodes in contact with it being able to pick up more activity (whether from nerve, EMG, or movement). However, as the authors state, their novelty does not lie in the recording of nerve signals per se, so I don't think this detracts from their work. While they acknowledge in the manuscript that EMG contamination could be playing a role in their recordings, this is currently somewhat hidden in the methods. I strongly encourage the authors to comment (and expand on this) in the discussion – readers knowledgeable in neural electrophysiology would likely be sceptical about the quality of the recording experiments otherwise."*

  Our response: We thank the reviewer for this valuable suggestion. We have incorporated additional discussions regarding EMG and movement artifacts of the measured electrical signals.

  Our modification to the manuscript:

  We added the following description on page 13-14:

  "It is noted that for the signals recorded during treadmill walking, we did not reference recordings to the median or mean signal of all electrodes. Our intention is to investigate electrical signals on the injured nerve, where the signals from different channels may

vary significantly, which can be utilized to quantitatively identify the leading edge of axonal growth. Referencing signals to the mean signals of all electrodes would not enable the capture of variations among different electrode channels. There is an inherent challenge in recording neural signals from freely-moving animals due to persistent EMG interference in the low-frequency range, even with reference electrodes and data filtering. The currently measured electrical data exhibits relatively large amplitude and low frequency, which may be attributed to the characteristics of the regenerated nerve segment. Compared to the non-regenerated segment, the regenerated segment may cause the electrodes in contact with it to detect increased activity, whether originating from the nerve, EMG signals, or movement. Future efforts to optimize electrode configurations and filtering algorithm may assist further reduction of EMG and movement interferences. Nevertheless, for the present study, our focus remains on detecting signal variations during nerve regrowth processes and neuroma presence, with the expectation that moderate EMG interference will not significantly impact our analysis."

- ***Comment #3:*** *"Statistical analysis: the authors have included information on the test used for their comparison (one-way ANOVA). However, what they have done is not entirely clear. One-way ANOVA provides a p value for the probability that all group means being tested are equal. If this is what is being used, then I think this is a very strange choice of test (particularly in Fig 4c where two comparisons with overlapping groups are being used). What is more commonly used is a one-way ANOVA which, if statistically significant, is followed by a post-hoc pairwise comparison test (such as a Tukey test and or a Bonferroni-corrected t-test). If this is what is being used, it should also be indicated in the legends. It should also be indicated that pairwise comparisons not shown on graphs are p > 0.05 / not significant (I assume this is the case). The "statistical analysis" section in the methods is also too short and contradictory – it indicates that "for two-group comparisons with an unpaired t-test…" which makes me suspect that Fig4j, 6c and 6g are not one-way ANOVAs like the authors indicate in the figure caption. Please take the time to carefully revise the statistical tests being used and their description in figure captions and in the methods section, and update any p*

values in the figures if improper statistical tests were used."

Our response: We thank the reviewer for the comment. We apologize for the confusion. The reviewer is right. One-way ANOVA followed by Turkey test was used for the statistical analysis. We updated the corresponding figure captions and methods.

Our modification to the manuscript:

We updated the caption of Figure 4, 6, S24 and statistical analysis methods in manuscript on page 35:

"**Figure 4** In 4c and 4e, One-way ANOVA followed by Turkey test was used for the statistical analysis (*P < 0.05, **P < 0.01, ***P < 0.001, pairwise comparisons not shown on graphs are p > 0.05 (not significant)). In 4j, unpaired t-test was used for the statistical analysis."

"**Figure 5** One-way ANOVA followed by Turkey test was used for the statistical analysis (***P < 0.001)."

"**Figure 6** Unpaired t-test was used for the statistical analysis (*P < 0.05, ***P < 0.001)."

"**Figure S20** Statistical analysis of the axon density. Unpaired t-test was used for the statistical analysis (***P < 0.001)."

"**Figure S24** Representative images and statistics of ankle angle for Bio-Neuro, Bio-Restor and normal groups on terminal stance at 2 and 3 weeks after implantation. One-way ANOVA followed by Turkey test was used for the statistical analysis (*P < 0.05, ***P < 0.001, pairwise comparisons not shown on graphs are p > 0.05 (not significant))."

"Statistical analysis was performed using GraphPad Prism (version 6.0) for two-group comparisons with an unpaired t-test, and SPSS software (version 23.0) for multi-group comparisons using one-way ANOVA followed by Tukey test. Significance levels were set at *P < 0.05, **P < 0.01, ***P < 0.001, pairwise comparisons not shown on graphs were p > 0.05 (not significant). Data are presented as mean ± standard deviation."

- ***Comment #4:*** *"Fig S18a: 90-900Hz magnification is too low. I'd suggest changing the amplitude of the traces to be able to visualise any activity or lack thereof."*

Our response: We thank the reviewer for the comment. We amplified the signal traces and corresponding scale bar to enhance the visibility any activity for Figure S18a.

Our modification to the manuscript:

We updated Figure S18a:

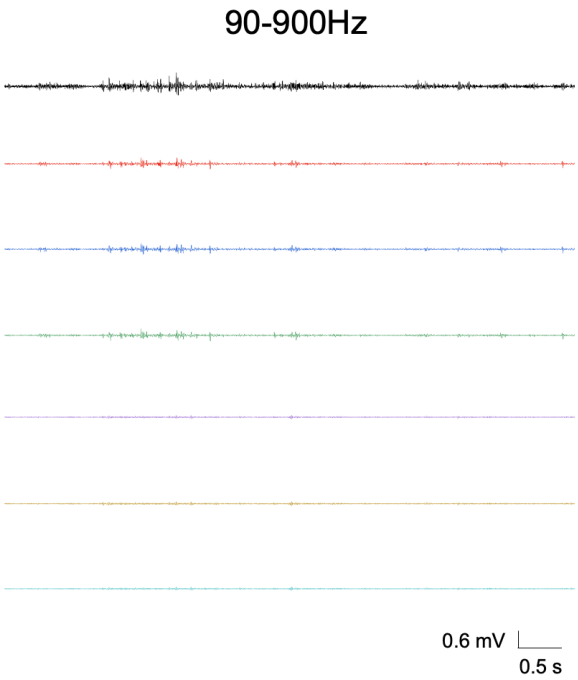


Figure S18 Recorded raw signal traces and corresponding signals after low-frequency and high-frequency band filtering of the Bio-Neuro group. **a,** walking on a treadmill at 3 weeks postimplantation.

- ***Comment #5:*** *"Fig 2g-h: I appreciate the changes in the caption, but I don't think it would be correct to indicate that these are n = 3 independent experiments. The authors are showing a trace from a single animal. Either remove this indication, or if the authors want to support the replicability of these recordings, include a trace in 2h for each of the three animals."*

Our response: We thank the reviewer for the comment. We removed the n = 3 independent experiments indication from the figure caption.