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● **INVITED REVIEW**

Interfacing peripheral nerve with macro-sieve electrodes following spinal cord injury

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Abstract

Macro-sieve electrodes were implanted in the sciatic nerve of five adult male Lewis rats following spinal cord injury to assess the ability of the macro-sieve electrode to interface regenerated peripheral nerve fibers post-spinal cord injury. Each spinal cord injury was performed *via* right lateral hemisection of the cord at the T_{9-10} site. Five months post-implantation, the ability of the macro-sieve electrode to interface the regenerated nerve was assessed by stimulating through the macro-sieve electrode and recording both electromyography signals and evoked muscle force from distal musculature. Electromyography measurements were recorded from the tibialis anterior and gastrocnemius muscles, while evoked muscle force measurements were recorded from the tibialis anterior, extensor digitorum longus, and gastrocnemius muscles. The macro-sieve electrode and regenerated sciatic nerve were then explanted for histological evaluation. Successful sciatic nerve regeneration across the macro-sieve electrode interface following spinal cord injury was seen in all five animals. Recorded electromyography signals and muscle force recordings obtained through macro-sieve electrode stimulation confirm the ability of the macro-sieve electrode to successfully recruit distal musculature in this injury model. Taken together, these results demonstrate the macro-sieve electrode as a viable interface for peripheral nerve stimulation in the context of spinal cord injury.

Introduction

Spinal cord injury (SCI) is a debilitating condition that is detrimental to the wellbeing and productivity of affected individuals. In the United States alone, 282,000 individuals are estimated to be living with a SCI, with ~17,000 new cases occurring each year, primarily in young adults (National Spinal Cord Injury Statistical Center, 2016). Injury to the mammalian spinal cord causes neuron death at lesion site with local loss of anterior horn cells. This ultimately results in injury-dependent losses to motor function distal to the site of injury. While axons in the peripheral nervous system (PNS) are capable of robust regeneration post-injury, axons in the central nervous system (CNS) are not and thus loss of function due to SCI is typically permanent (Huebner and Strittmatter, 2009). One promising approach to restoring motor function to SCI patients is the use of peripheral nerve interfaces (PNIs). A PNI is a micro-electrode array used to stimulate or record from a peripheral nerve, in this case one distal to the spinal cord lesion. Many types of PNIs are being developed as potential modalities for neuromuscular control and delivery of functional electrical stimulation. In this paper, we discuss the applicability of the macro-sieve electrode (MSE) as a potential target for restoring motor function following SCI.

Interfacing Peripheral Nerve in the Context of SCI

PNIs are broadly classified into three types—extraneural electrodes, penetrating intraneural electrodes, and regenerative electrodes. Extraneural electrodes, such as the cuff electrode (Veraart et al., 1993) or the flat interface nerve electrode (Tyler and Durand, 2002), are minimally invasive but achieve only limited selective muscular recruitment. Penetrating intraneural electrodes, such as the Utah slant electrode array (Branner et al., 2004) and the transverse intrafascicular multichannel electrode (Boretius et al., 2010), are inserted directly into the target nerve to gain selective intrafascicular control, but suffer from complications such as the breakage of the tines and fibrous encapsulation (Gasson et al., 2004; Christensen et al., 2014). Regenerative electrodes, such as the MSE, interface peripheral nerve that has regenerated across the electrode interface from two surgically opposed nerve stumps (Thompson et al., 2016). By interfacing directly with this regenerated nerve, the MSE is able to provide selective control while avoiding issues such as breakage and scarring (MacEwan et al., 2016).

Direct nerve integration with any regenerative electrode, however, requires transection of the nerve of interest and
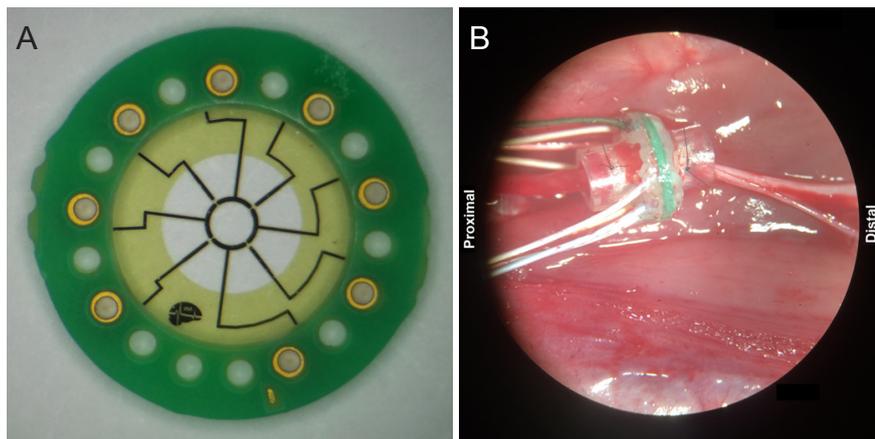
Figure 1 Macrosieve electrode.
(A) Optical micrograph of MSE (without connector assembly). (B) MSE with connector assembly implanted in transected sciatic nerve 5 mm proximal to the point of trifurcation. MSE: Macro-sieve electrode.
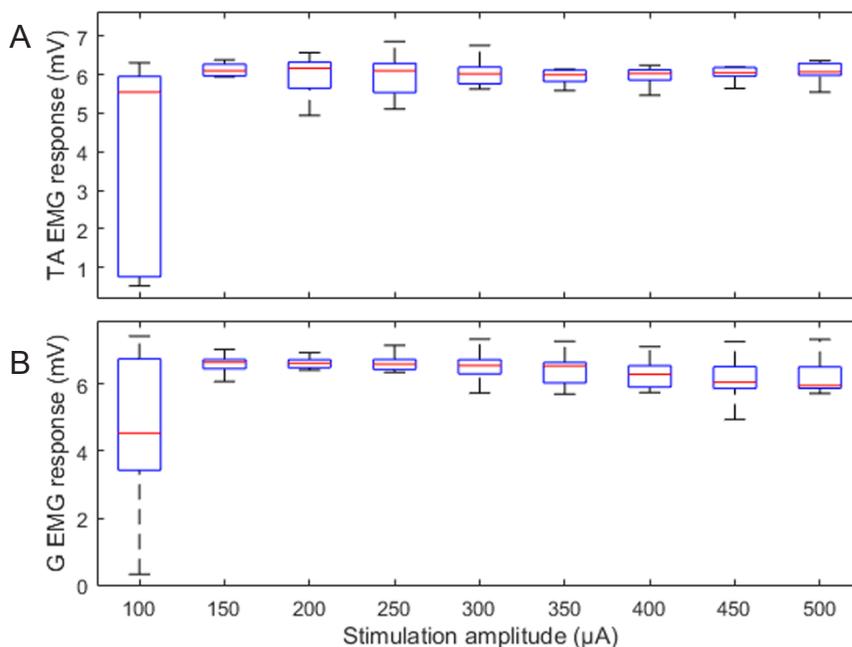

Figure 2 Population tibialis anterior (TA) and gastrocnemius (G) EMG data for stimulation through each macro-sieve electrode (MSE) site in each animal.
Red lines represent the mean signal response across all MSE sites in each animal, while the blue upper and lower bounds of the boxes represent the 75th and 25th percentile respectively. Maxima and minima are marked using black whiskers.

depends entirely on the regenerative capacity of the nerve – two complicating factors which determine the applicability of regenerative electrodes (Lago et al., 2007). Furthermore, implantation of an MSE subjects the PNS to a second injury following the initial CNS lesion, which intuitively may affect the peripheral nerve's regenerative capacity, as peripheral nerve axons distal to the SCI lesion have altered structural morphology (Redondo-Castro and Navarro, 2013). Our clinical experience and a growing body of literature demonstrating clinical recovery using peripheral nerve transfers distal to the site of injury suggests that regenerative electrodes such as the MSE may provide an interface that promotes a more widespread use of caudal spinal segments in SCI patients (Ray et al., 2016). We sought to investigate whether a MSE implanted post-SCI could still be used to selectively recruit distal musculature.

Capabilities of the MSE

Details of the MSE design and fabrication have been de-scribed previously (MacEwan et al., 2016). Briefly, the MSE is a high-transparency regenerative sieve electrode featuring nine large transit zones, each with an area of approximately 0.285 mm^2. These transit zones are bordered by eight radial spokes and a central ring which are metallized with Pt-Ir to yield four central and four peripheral active electrode sites (**Figure 1A**). Each MSE assembly also features silicone nerve guidance conduits which project 3 mm from each face and enable the MSE to be secured to the epineurium during implantation.

Several characteristics of the MSE make it especially attractive in the context of SCI compared to other PNIs. The MSE can recruit highly selective groups of regenerated nerve fibers in uninjured animals (MacEwan et al., 2016), meaning that selective motor control can potentially be provided to multiple muscle groups affected by SCI through a single MSE. Specifically, in uninjured animals, the MSE has been able to recruit up to 50% of maximal

evoked muscle twitch force using only monopolar stimulation paradigms. Additionally, as the MSE is not implanted *via* penetration, it does not experience the mechanical or foreign-body response complications associated with intraneural electrodes (Christensen et al., 2014), furthering its applicability as a long-term PNI for use in SCI. However, as with any regenerative sieve electrode, the ability of the MSE to selectively interface the nerve relies on robust regeneration across its interface, and thus potential hurdles to this regeneration must be carefully evaluated. It is for this reason that we have sought to investigate whether there are any negative downstream effects of SCI on regeneration across the MSE interface.

Evaluation of the MSE in SCI

MSE were implanted in the sciatic nerve of five adult male Lewis rats following SCI to assess the ability of the MSE to interface regenerated peripheral nerve fibers post-SCI. Each SCI was performed *via* right lateral hemisection of the cord at the T_{9-10} site. Two weeks post-SCI, an MSE was implanted in the right sciatic nerve. For this procedure, the sciatic nerve was transected 5 mm proximal to the point of trifurcation. An MSE was placed in the transected nerve gap and the proximal and distal nerve stumps were sutured into either end of the silicone nerve guidance conduit by the epineurium (**Figure 1B**).

Five months post-implantation, the ability of the MSE to interface the regenerated nerve was assessed by stimulating through the MSE and recording both electromyography (EMG) signals and evoked muscle force measurements from distal musculature. An identical set of stimuli was used to stimulate the regenerated nerve for both EMG and muscle force recordings. Individual stimuli consisted of a biphasic, square, symmetrical pulse of current between 100 µA and 500 µA delivered over 1 ms (*i.e.*, 100 µA for 0.5 ms then −100 µA for 0.5 ms). Stimuli were delivered cathodically through the implanted MSE using a MS16 stimulus isolator (Tucker-Davis Technologies, Inc., Alachua, FL, USA) connected to a desktop PC *via* optical cable.

For the EMG measurements, recording needle electrodes were placed in the tibialis anterior (TA) and gastrocnemius (G) muscles. Additional counter and reference needle electrodes were placed subcutaneously in the lower back of the animal. Recorded EMG signals were routed through a RA-16LI-D 16-channel differential recording head stage and amplified using a RA16PA 16-channel medusa preamp (Tucker-Davis Technologies, Inc., Alachua, FL, USA) before being sent to a desktop PC *via* optical cable using custom OpenEx data acquisition software (Tucker-Davis Technologies, Inc., Alachua, FL, USA).

For muscle force recordings, the anterolateral aspect of the right hind limb was exposed to facilitate access to the tendons of the TA, extensor digitorum longus (EDL), and G muscles. Distal tendons of the target muscles were cut and

secured to separate stainless-steel S-hooks using 5-0 nylon suture. The right leg was then immobilized at the femoral condyles by use of a C-clamp. The stainless-steel S-hook was then connected to a 5 N thin-film load cell force sensor (Strain Measurement Devices, Inc., Meriden, CT, USA). Evoked muscle forces for each the TA, EDL, and G muscles were transduced individually *via* the force sensor and recorded on a desktop PC using the previously described hardware and software.

All channels in each animal reached an EMG response plateau atstimulation values of 150–200 µA (**Figure 2**), consistent with data from uninjured animals (MacEwan et al., 2016). A similar plateau was obtained for evoked muscle force recordings, in which recorded muscle force normalized by the maximum muscle force for each muscle also reached a maximum at stimulation values of 150–200 µA. When recording evoked muscle response due to electrical stimulation, a sigmoidal curve typically appears as smaller currents do not cause the motor fibers to reach threshold, while higher currents recruit increasingly more motor fibers until all fibers are recruited and a plateau is reached. However, due to the absence of lower stimulation values and the use of comparatively large current step sizes in this experiment, it is difficult to visualize the expected sigmoidal recruitment curve.

Successful sciatic nerve regeneration across the MSE interface following spinal cord injury was visually observed in all five rats. EMG and muscle force recordings obtained following stimulation through the MSE confirm the ability of the MSE to successfully recruit distal musculature in this injury model. Taken together, these results demonstrate that the MSE is a viable interface for providing functional neuromuscular stimulation following SCI.

Conclusion

The PNS offers an attractive biological target for neuroprosthetic devices aimed at restoring motor function following SCI. Unfortunately, microelectrode devices developed to date have not been able to achieve a stable, chronic, high-specificity interface with peripheral nerve tissue required for high resolution muscle activation and motor control. MSEs represent a novel approach to achieving a chronic, stable, high-specificity interface with peripheral nerve tissue for the purpose of muscle activation and motor control.

The present study represents the first instance of regenerative sieve electrodes being applied as a means of interfacing peripheral nerve tissue and providing motor activation in the context of SCI. Further work is needed to determine the clinical potential of MSEs in the context of SCI.

sition, and data analysis. MRM is responsible for study concept and study design. WZR was responsible for study concept, study design, manuscript review, and manuscript editing.

Conflicts of interest: *WZR declared provision of consulting services to Globus and Depuy and an ownership interest in Acera Surgical, Inc. All other contributing authors declared no other potential conflicts of interest.*

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Peer review: *A double-blind and stringent peer review process has been performed to ensure the integrity, quality and significance of this paper.*

Open peer review reports:

Reviewer 1: Athanasios Chatzisotiriou, Aristotle University of Thessaloniki, Greece.

Comments to the author: This is an interesting study in the field of neurorepair. The authors have performed spinal cord hemisection of the cord at the T9-T10 site in adult Lewis rats and two weeks after the lesion macrosieve electrodes were implanted in the right sciatic nerve. Five months post-implantation, electromyography was performed from distal musculature and the signal recorded from the tibialis anterior (TA) and gastrocnemius (G) muscles was similar to the one obtained from uninjured controls (MacEwan et al., 2016). The authors conclude that successful sciatic nerve regeneration occurred across the MSE interface and that MSE may be a viable option for providing functional neuromuscular stimulation following SCI. See details in the additional file.

Reviewer 2: Christof A. Leicht, Loughborough University, UK.

Comments to the author: This is an invited paper describing the use of Macro-Sieve Electrodes in peripheral nerve stimulation following spinal cord injury. The manuscript gives a nice little snapshot on MSE, including some original data, and makes an interesting read. There is a small experimental section at the end, and I appreciate, given the nature of this manuscript, that not all procedures are given in full detail. However, I wonder whether the authors are planning to publish these or whether the reader has opportunity to gain more insight in these methods and data. See details in the additional file.

Additional file: *Open peer review report 1, 2.*

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Additional file:

Open peer review report 1 on "Interfacing peripheral nerve with macro-sieve electrodes following spinal cord injury".

Reviewer: Athanasios Chatzisotiriou, Aristotle University of Thessaloniki, Greece

Comments to the author:

This is an interesting study in the field of neurorepair. The authors have performed spinal cord hemisection of the cord at the T9-T10 site in adult Lewis rats and two weeks after the lesion macrosieve electrodes were implanted in the right sciatic nerve. Five months post-implantation, electromyography was performed from distal musculature and the signal recorded from the tibialis anterior (TA) and gastrocnemius (G) muscles was similar to the one obtained from uninjured controls (MacEwan et al., 2016). The authors conclude that successful sciatic nerve regeneration occurred across the MSE interface and that MSE may be a viable option for providing functional neuromuscular stimulation following SCI.

There are major concerns regarding this article. First of all, it is not clear why the authors have chosen a double crush model (spinal cord injury + peripheral nerve injury) in order to perform their study. If their principal aim was to study the regeneration of peripheral axons after nerve transection, then they should have performed an experimental procedure with control or sham operated animals, those with nerve crush or nerve transection and those with the MSE interface implanted. They ought to have supplemented these results with histological study of reinnervation (muscle fiber atrophy, muscle fiber enzyme conversion, end-plate immunohistochemistry etc), as regeneration cannot be proved only by electrophysiology. By this way, they could have proved that stimulation may provide the electrical activity which is necessary for nerve regeneration. One cannot evaluate this kind of electrode design, if they are not initially evaluated in a pure nerve injury.

In spinal cord injury, however, apart from the neurons lying in the traumatic cavity, the others are not traumatized, and usually no degeneration is noted. The citation of (Redondo-Castro and Navarro 2013), supports the lack of motoneuron death (and subsequent wallerian degeneration) and discusses the structural abnormalities of neurons. The purpose of these devices in spinal cord injury is not to restore normal function or induce regeneration (after all there is no degeneration) but to provide an external means of stimulating movements in an artificial way and this is how it is used in rehabilitation centres.

Due to the above comments and in combination with the fact that there is no statistical analysis, I think that the paper is not eligible for publication in the present form, but it could be published as a novel technique and be incorporated in another study with a more precise design.

Additional file:

Open peer review report 1 on "Interfacing peripheral nerve with macro-sieve electrodes following spinal cord injury".

Reviewer: Christof A. Leicht, Loughborough University, UK

Comments to the author:

Strengths:

- interesting field

- potential future applications in SCI

- inclusion of some experimental data

Weaknesses

- experimental data is very superficial

General feedback

This is an invited paper describing the use of Macro-Sieve Electrodes in peripheral nerve stimulation following spinal cord injury. I assume this paper falls under the category "Perspectives/Research Highlights"? It would be good if this could be made clearer (note - as reviewer I cannot see page 1 of the submission, should it had been indicated there).

The manuscript gives a nice little snapshot on MSE, including some original data, and makes an interesting read.

There is a small experimental section at the end, and I appreciate, given the nature of this manuscript, that not all procedures are given in full detail. However, I wonder whether the authors are planning to publish these or whether the reader has opportunity to gain more insight in these methods and data.
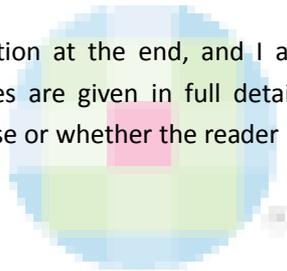
Specific comments

Page 1

- There does not appear to be an abstract. Please include one.

Line 33: "integrated directly" - could a very brief description be given how this is done practically? Surgical procedure?

Line 42: "distal": use consistent terminology (?) - two lines down "caudal" is used

Line 45/46: more detail needed: why should MSE provide a more widespread use of caudal segments (also, is "of" the correct word?)

Page 2

Line 33-36: in general - more detail needed? E.g., stimulation details, how was muscle force assessed, equipment details (EMG, forces...)?

Line 38: channel should be plural; reach should be reached

Line 38: Plateau: given the small sample size and no statistics, this has to be formulated with caution - I assume it is determined simply visually?

Line 41: more detail on muscle force recordings needed? There are no data at all on muscle forces. Would it be better to leave this aspect out, giving room for some more detail for other aspects?

Line 44-45: nerve generation is not directly observed (only measureable EMG and force signals).

Consider re-wording.

Could a conclusions section be added to present the experimental findings in the greater context?

Figure legends (Page 4)
Line 10: TA, G - define abbreviations.
Are the whiskers really outliers or do they just represent maxima/minima?