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Microscale Electrode Implantation during Nerve Repair: Effects on Nerve Morphology, Electromyography, and Recovery of Muscle Contractile Function

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Abstract

Background—Our goal is to develop a peripheral nerve electrode with long-term stability and fidelity for use in nerve-machine interfaces. Microelectromechanical systems (MEMS) use silicon probes that contain multi-channel actuators, sensors, and electronics. We tested the null hypothesis that implantation of MEMS probes do not have a detrimental effect on peripheral nerve function or regeneration.

Methods—A rat hindlimb, peroneal nerve model was utilized in all experimental groups: a) intact nerve (Control, n= 10); b) nerve division and repair (Repair, n= 9); and c) Nerve division, insertion of MEMS probe, and repair (Repair + Probe, n=9). Nerve morphology, nerve to muscle compound action potential (CMAP) studies, walking tracks, and extensor digitorum longus (EDL) muscle function tests were evaluated following an 80 day recovery.

Results—Repair and Repair + Probe showed no differences in axon count, axon size, percent non-neural area, CMAP amplitude, latency, muscle mass, muscle force, or walking track scores. Though there was some local fibrosis around each MEMS probe, this did not lead to measurable detrimental effects in any anatomic or functional outcome measurements.

Conclusions—The lack of a significant difference between Repair and Repair + Probe groups in histology, CMAP, walking tracks, and muscle force suggests that MEMS electrodes are compatible with regenerating axons and show promise for establishing chemical and electrical interfaces with peripheral nerves.

Keywords

peripheral nerve; electrode; MEMS; skeletal muscle; regeneration; nerve tissue

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Introduction

An unprecedented need has risen for prosthetic devices to return function to injured civilians and military personnel. Nearly 185,000 Americans undergo limb amputation each year, with one in 190 Americans affected by limb loss.(1, 2) In the military sector, advancements in body armor have reduced torso injuries and deaths, but have also resulted in a sharp increase in extremity injuries requiring amputation (3).

Therefore, much interest has been generated in robotic replacements for these amputees, particularly the upper extremity, complete with fine motor control and sensory feedback. Several groups have developed passive, body-powered, and myoelectric prostheses.(4) Recently, significant technologic advances have resulted in five-fingered robotic arms and hands with as many as twenty degrees of freedoms.(5) With the incorporation of shape memory alloy actuators, successive prototypes are becoming lighter and easier to use.(6, 7)

Despite these advances, the interface between biologic tissue and mechanical components continues to present challenges.(5, 8) This is particularly evident at the level of the peripheral nerve interface. In order to provide fine motor control and sensory feedback, we need a peripheral nerve interface with extended durability and high fidelity. Unfortunately, acute and chronic biologic responses lead to signal degradation over time, limiting the clinical utility of current peripheral nerve interfaces. In the acute phase of electrode implantation, edema and hemorrhage result from surgical placement of the device. In the chronic phase, the macrophage and fibroblast response causes a reactive gliosis within the nerve.(9-12) This chronic biofouling of the peripheral nerve interface is a notoriously difficult problem that ultimately results in signal loss over time.(13-15)

A number of electrode designs and implantation techniques have attempted to address these problems to bridge the nerve-machine interface. Sieve electrodes guide nerve regeneration along electrode arrays, creating microscale nerve fiber specificity. They offer ease of application and have successfully conveyed physiologic action potentials to artificial limbs in an acute human model.(16) Unfortunately, most sieve electrodes have resulted in diminished signal fidelity and nerve regenerative capacity with time.(17)

Cuff electrodes encircle nerves, with some designs impaling the nerve orthogonally to axon alignment. These electrodes often cause nerve compression as the nerve expands and shrinks with antegrade and retrograde axoplasmic transport of vesicles. Additionally, the cuff electrodes can lead to progressive scarring of the epineurium, create kinks in the nerve around the cuff, and cause axonal damage from the penetrating shanks. Constrictive designs result in tissue necrosis and axonal demyelination.(18-20)

Newer micro-electromechanical system (MEMS) electrode probes have the potential of interfacing between man and machine as well as minimizing biofouling. MEMS components are produced on a microscale level; this reduction in size typically confers greater biologic transparency.(14, 15) The silicon photolithographic fabrication technique allows for exquisite control over the texture of the probes, which has been demonstrated to influence biologic reactivity.(13, 21, 22) Spacing of the electrode sites on the probe is also far more precise with microfabrication techniques allowing more sites per unit surface area and therefore higher resolution signaling.(9, 23-25)

While pure metal electrodes such as gold or platinum are easier to fabricate, recent evidence indicates that silicon offers greater flexibility in minimizing the foreign body response.(14, 26, 27) Utilizing a MEMS silicon electrode probe in the peripheral nervous system has the potential of preserving signal fidelity while causing less tissue response compared to conventional metal electrodes.(28)

In this study, silicon MEMS electrode probes are implanted in the distal stump of a peroneal nerve repair. Peroneal nerve sprouting and elongation is then studied in the presence of the silicon MEMS probe after 80 days. We hypothesize that implantation of a MEMS probe has minimal adverse impact on peripheral nerve morphology, electromyography, and end organ function following peroneal nerve regeneration and muscle reinnervation.

Materials and Methods

Animal Model

This study used 8 month-old retired breeder F344 male rats (Charles River Laboratories, Wilmington, MA). They were individually housed in a pathogen-free animal facility. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Michigan and were in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. For all surgical procedures, rats were given analgesic (buprenorphine, 0.05 mg/kg) prior to anesthesia and sodium pentobarbital (50 mg/kg) for anesthesia. All rats were euthanized with an IACUC approved procedure.

Silicon Probe

The MEMS based probe (Fig 2) is fabricated using photolithographic masks in a manner previously described.⁽²⁹⁾ Shape and thickness is defined by selective diffusion of boron into a silicon wafer. The probe measures 10mm × 0.2 mm, weighing approximately 1 mg. Insulating and conductive layers are deposited in a stepwise fashion. Recording sites are etched on the surface, followed by deposition of metal to form the electrode sites. The probes in this study had 16 electrode sites but the probes were not actively stimulating or recording..

Experimental Design and Procedure

A rat peroneal nerve repair model was used with three groups: Control, Repair, and Repair + Probe (Fig 1). Control animals (n=10) maintained an intact nerve throughout the study. Repair animals (n=9) underwent division of the left peroneal nerve 25 mm proximal to the fibular head, followed by an immediate end-to-end epineurial repair using (10-0) nylon suture. Repair + Probe animals (n=9) also underwent division of the left peroneal nerve at the same location. A 25 gauge needle was then inserted longitudinally into the distal segment of the nerve as an introducer, the introducer was removed, and a sterile MEMS probe (NeuroNexus Technologies, Ann Arbor, MI) was then inserted 1.0 cm into the peroneal nerve. This procedure was again followed by end-to-end epineurial repair using 10-0 nylon suture. In all animals, the wounds were closed in layers with simple interrupted 4-0 chromic sutures. Evaluation of walking tracks, CMAP, muscle force, and nerve histology took place after 80 days of recovery.

Histologic Evaluation

Peroneal nerve segments were taken 4 mm distal to the repair site on POD 80. The nerves were fixed in an immersion of cold 16% paraformaldehyde, 50% glutaraldehyde solution in 0.2M sodium phosphate buffer (pH 7.2). (Karnovsky's Fixative, Electron Microscopy Sciences, Hatfield, PA). The tissues were post-fixed with 1% osmium tetroxide, ethanol dehydrated, and embedded in Araldite 502 (Polysciences, Inc., Warrington, PA). One-micrometer thin sections were cut 4 mm distal to the repair site and stained with toluidine blue for examination under light microscopy.

Nerve sections were evaluated for total axon count, average axon size, and percent non-neural tissue. Six random fields per nerve were viewed through a Leitz Laborlux S fluorescent microscope (Leica, Wetzlar, Germany). Nerve sections were digitally captured in

24-bit RGB TIFF format images with an Optronics DEI-759, 3-chip color camera (Optronics Engineering; Goleta, CA) mounted to the microscope and evaluated with a digital imaging analysis system linked to morphometry software (BioQuant-R&M Biometrics, Inc, Nashville, TN). Only axons fully enclosed by the counting frame were quantified. The total number of axons is derived from the total area of the peroneal nerve and the number of axons counted per mm² of field area. Axon size is the average of the smallest diameters of visualized axons. Percent neural tissue includes axons, myelin and Schwann cells.

Electromyographic Studies

In-situ nerve stimulated muscle compound action potential (CMAP) studies were performed prior to measurement of muscle contractile properties.(30) Analgesia and anesthesia were administered. Standard 25 gauge, 5/8 inch stainless steel needle electrodes were used with a Synergy clinical Nerve Conduction System running customized Synergy software (TECASynergy Plinth T2X System, Viasys NeuroCare, Madison, WI). The recording electrode was inserted through the skin into the belly of the EDL muscle, the reference electrode was inserted through the distal EDL muscle tendon, and the ground electrode was inserted between the 4th and 5th metatarsals. Percutaneous stimulation was applied proximal to the nerve repair site at the mid-peroneal with a pediatric bipolar electrode. A single pulse lasting 0.1 ms was applied for each stimulation. The current was serially increased until an CMAP response with maximum amplitude was reached. The current was then serially increased to 20% greater than that used for the maximal response. This stimulation procedure was repeated at a site over the sciatic nerve close to the sciatic. The distances between the stimulating and recording electrodes was measured for nerve conduction velocity determination. The values recorded at each site were latency, amplitude, negative spike area, nerve conduction velocity and the current equal to 20% greater than that used to maximize amplitude.

Measurement of Muscle Contractile Properties

Contractile properties of the EDL muscle were measured *in situ* in a manner similar to that described previously.(31-33) Each rat was prepared for surgery as described above. Briefly, the EDL muscle was isolated and the foot and femoral condyle were fastened to a platform, A tendon loop was formed with the distal EDL tendon and connected to a strain gauge, The repaired peroneal nerve was isolated. Supramaximal square wave pulses (0.2 ms pulse duration, 2-6 volts) were delivered through a shielded bipolar silver wire electrode (Harvard Apparatus, South Natick, MA) to the proximal peroneal nerve. A BG 1000 strain gauge (Kulite Semiconductor, Leonia, NJ) with IBM-compatible custom designed software (LabVIEW 5.1, National Instruments, Austin, TX).measured change in voltage with isometric contraction and converted it to EDL muscle force during isometric contraction. The muscle length was measured, and the muscle was excised and weighed. The muscle physiological cross-sectional area (CSA) was determined by dividing the mass of the muscle by the product of L_f and the density of mammalian skeletal muscle (1.06 g/cm³). The maximum specific force (sF₀) was calculated as F₀ normalized by the total muscle fiber cross sectional area.

Walking Track Studies

Walking track data were recorded for each rat preoperatively, at early during recovery, and at the final evaluation as previously described.(34, 35) Intermediate toe spread distances were measured for the normal and the operated foot. Scores were computed according to equation 1.

$$100 - \frac{\text{normal foot intermediate toe spread} - \text{experimental toe spread}}{\text{normal toe spread}}$$

Equation 1

Data Analysis

Quantified axons were assumed to have circular circumferences (36, 37) and derived variables were calculated using equation 2:

$$axdiam = 2 * \left(\sqrt{\frac{axoarea}{\pi}} \right)$$

Equation 2

where: axoarea = axoplasm area (μm^2) and: axdiam = axon diameter (μm)

The mean and standard deviation for each dependent variable measured were computed for every surgical group. Contractile property measurements were compared with morphometric measurements for each group. To test for statistically significant differences, data were assessed via a single factor analysis of variance (ANOVA). *Post hoc* comparison of individual group means was performed if the F ratio for the overall ANOVA was significant; appropriate Bonferroni corrections were applied to *post hoc* comparisons if the criteria for equal variance was met. When variance was not homogeneous, then a Brown-Forsythe robust test for equality of means was used and the Tamhane multiple comparisons correction was applied if the model was significant. The only variable which did not show equality of variance was the average axon. Spearman's rho correlation coefficients were calculated for comparisons between maximal force and nerve fiber morphological variables. Statistical computations were performed using a microcomputer and statistical software (SPSS Science, Chicago, IL). The level of significance was set at $\alpha < 0.05$ (2-tailed). The power of the model to detect differences was calculated for our most valued variable, the maximal muscle force. With significance set at $\alpha < 0.10$, statistical power for finding a difference between Repair and Repair + Probe groups for muscle force was equal to .50.

Results

Demographics

There were no significant differences among the surgical groups for rat age or body mass, at entry into the study. Also the groups did not vary in post operative days before final evaluation or body mass at evaluation (Table 1). None of the animals died during the operative procedure. One rat in the Repair group died 2 days following surgery from unknown causes. We were not able to perform all outcome measurements on all rats due to technical failure (muscle force collection) or because the equipment was unavailable (CMAP)..

Histological Findings

Examples of nerve cross sections cut 4 mm distal to the nerve repair are presented in Fig 3. Nerve photomicrographs showing the repair area including the electrode scar are presented in Fig 4. There were no differences between the Repair and Repair + Probe groups in average axon diameter or percent non-neural area (Table 2). Both operative groups had smaller axon diameters and more non-neural tissue than the Control group. There were no differences between any groups for axon count.

Electrophysiological Studies

A number of animals were evaluated with evoked electrophysiological studies. Stimulation was applied proximal to the nerve repair site. The results for area under the negative amplitude curve (Area), nerve conduction velocity, and latency were measured once amplitude was maximized (Table 3). For these measurements, no differences were elicited between the Repair and Repair + Probe groups. Both groups had lower amplitudes, Areas, and conduction velocities than the controls.

Muscle Force Generation

Force testing of the EDL muscle revealed significant decreases in maximal isometric force for both the Repair and the Repair + Probe groups when compared with the Control group; but no significant differences when compared with each other (Table 4). When force measurements were normalized to optimal muscle length, the resulting specific force measurements were not different between any of the groups indicating normality of nerve fiber reinnervation of skeletal muscle. Maximal muscle force was significantly correlated with average axon size ($r = 0.7$); average neural percent area ($r = 0.7$); average non neural percent area ($r = -0.7$), and wet muscle mass ($r = 0.9$) with $\alpha = .01$ (2tailed).

Muscle Mass

No significant differences in muscle mass were noted between the Repair and Repair + Probe groups, and both were significantly less than the controls.

Walking Track Score

No significant differences in Walking Track Scores were noted among the groups either prior to surgery or at the final evaluation date.

Discussion

When mechanical components are interfaced with peripheral nerves, conventional experience has shown eventual loss of signal fidelity. This study presents several different outcome measures highlighting robust axonal sprouting and elongation, muscle reinnervation, and preservation of nerve function in the presence of an intrafascicular MEMS probe.

Histologically, we expected to see a reduction in axon size and an increase in non-neural area by dividing and repairing the nerve. While this was reflected in the data, the surprising outcome was that the additional traumatic insult of an intrafascicular MEMS probe did not yield a significant difference in either of these measurements when compared to the Repair group alone. These data suggest that the MEMS probe had minimal influence on inflammation, wound healing, and nerve regeneration at 80 days post-insertion.

The electrophysiological data corroborates these findings. Electrical stimuli proximal to the repair site were successfully transmitted to a distal recording site in the Repair + Probe group. Furthermore, the amplitude, velocity, and latency of these signals were no different than the Repair group alone. The similarity of amplitudes between these groups reflect preservation of a coordinated signal from nerve stimulus to muscle response. The velocity data shows consistent myelination between the groups. The similarity in latency indicates the integrity of the neuromuscular junction.

End-organ function provides the closest approximation in this study to clinical significance. The muscle masses between the Repair and Repair + Probe groups had no differences. Taken together with similar findings from the maximal force and specific force

measurements, these data indicate the reinnervation of muscle and a return of function that is as robust with the MEMS probe as it is without. The Walking Track evaluations provide reassuring evidence that the animals continue to have end-organ function even in the presence of an intrafascicular probe.

We chose a nerve repair model in this study although its application is primarily for amputees. The repair model allows evaluation of end organ function after nerve division and repair. Specifically, we were able to evaluate the impact of the probe on axonal sprouting, elongation, and muscle reinnervation. Significant adverse effects from the probe on nerve regeneration would have been easily identified with this model in both an acute and chronic setting, but none were seen. These results suggest that the silicon MEMS probe is relatively transparent to the peroneal nerve with minimal impact on end organ reinnervation.

These data do not directly imply that MEMS probes should be used in normal nerves. However, for amputees that no longer have normal nerves, these probes may comprise a reasonable peripheral nerve interface for closed-loop neural control of a prosthesis.

There were several limitations of this study. We made our measurements 80 days after neurotomy. Application to humans would require a study of greater duration for long-term use.⁽³⁸⁾ The probe used did not include any other electrical system components such as circuitry or wiring, which could contribute to fibrosis and inflammation with time. In addition, we did not include any measurements of sensation or fine motor control. However, enhanced signal resolution and fidelity are a known advantage of MEMS-based probes, so these measurements of sensation or fine motor control would theoretically be enhanced with this type of neural probe. These will be very interesting avenues of future research.

Conclusion

The presence of a MEMS probe in the regenerating peripheral nerve shows little to no disturbance in nerve morphology, electromyography, or muscle force. Our results suggest that these probes have long-term *in vivo* compatibility and show promise for establishing chemical and electrical interfaces with peripheral nerves. The sophistication of these components coupled with their microscopic size makes them a vital piece of the nerve interface puzzle.

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References

1. Ziegler-Graham K, MacKenzie EJ, Ephraim PL, et al. Estimating the prevalence of limb loss in the United States: 2005 to 2050. *Arch Phys Med Rehabil*. 2008; 89:422–429. [PubMed: 18295618]
2. Dillingham TR, Pezzin LE, MacKenzie EJ. Limb amputation and limb deficiency: epidemiology and recent trends in the United States. *South Med J*. 2002; 95:875–883. [PubMed: 12190225]
3. Gawande A. Casualties of war--military care for the wounded from Iraq and Afghanistan. *New England Journal of Medicine*. 2004; 351:2471–2475.
4. Biddiss E, Chau T. Upper limb prosthesis use and abandonment: a survey of the last 25 years. *Prosthetics and orthotics international*. 2007; 31:236–257. [PubMed: 17979010]

5. Hijjawi JB, Kuiken TA, Lipschutz RD, et al. Improved myoelectric prosthesis control accomplished using multiple nerve transfers. *Plast Reconstr Surg*. 2006; 118:1573–1578. [PubMed: 17102730]
6. De Laurentis KJ, Mavroidis C. Mechanical design of a shape memory alloy actuated prosthetic hand. *Technol Health Care*. 2002; 10:91–106. [PubMed: 12082214]
7. Loh, C. Siong; Yokoi, H.; Arai, T. New shape memory alloy actuator: design and application in the prosthetic hand. *Conf Proc IEEE Eng Med Biol Soc*. 2005; 7:6900–6903. [PubMed: 17281861]
8. Lamb DW. State of the art in upper-limb prosthetics. *J Hand Ther*. 1993; 6:1–8. [PubMed: 8343869]
9. Kipke DR, Shain W, Buzsaki G, et al. Advanced Neurotechnologies for Chronic Neural Interfaces: New Horizons and Clinical Opportunities. *Journal of Neuroscience*. 2008; 28:11830–11838. [PubMed: 19005048]
10. Polikov VS, Tresco PA, Reichert WM. Response of brain tissue to chronically implanted neural electrodes. *Journal of Neuroscience Methods*. 2005; 148:1–18. [PubMed: 16198003]
11. Schmidt S, Horch K, Normann R. Biocompatibility of silicon-based electrode arrays implanted in feline cortical tissue. *Journal of biomedical materials research*. 1993; 27:1393–1399. [PubMed: 8263001]
12. Turner JN, Shain W, Szarowski DH, et al. Cerebral astrocyte response to micromachined silicon implants. *Experimental Neurology*. 1999; 156:33–49. [PubMed: 10192775]
13. Edell DJ, Toi VV, McNeil VM, et al. Factors influencing the biocompatibility of insertable silicon microshafts in cerebral cortex. *IEEE transactions on bio-medical engineering*. 1992; 39:635–643. [PubMed: 1601445]
14. Polikov V, Tresco P, Reichert W. Response of brain tissue to chronically implanted neural electrodes. *Journal of Neuroscience Methods*. 2005; 148:1–18. [PubMed: 16198003]
15. Szarowski DH, Andersen MD, Retterer S, et al. Brain responses to micro-machined silicon devices. *Brain Research*. 2003; 983:23–35. [PubMed: 12914963]
16. Jia X, Koenig MA, Zhang X, et al. Residual motor signal in long-term human severed peripheral nerves and feasibility of neural signal-controlled artificial limb. *J Hand Surg Am*. 2007; 32:657–666. [PubMed: 17482005]
17. Castro J, Negredo P, Avendaño C. Fiber composition of the rat sciatic nerve and its modification during regeneration through a sieve electrode. *Brain Research*. 2008; 1190:65–77. [PubMed: 18086465]
18. Larsen JO, Thomsen M, Haugland M, et al. Degeneration and regeneration in rabbit peripheral nerve with long-term nerve cuff electrode implant: a stereological study of myelinated and unmyelinated axons. *Acta Neuropathologica*. 1998; 96:365–378. [PubMed: 9797001]
19. Loeb GE, Peck RA. Cuff electrodes for chronic stimulation and recording of peripheral nerve activity. *Journal of Neuroscience Methods*. 1996; 64:95–103. [PubMed: 8869489]
20. Thil MA, Duy DT, Colin IM, et al. Time course of tissue remodelling and electrophysiology in the rat sciatic nerve after spiral cuff electrode implantation. *J Neuroimmunol*. 2007; 185:103–114. [PubMed: 17343923]
21. Abidian MR, Martin DC. Multifunctional Nanobiomaterials for Neural Interfaces. *Advanced Functional Materials*. 2009; 19:573–585.
22. Pancrazio JJ. Neural interfaces at the nanoscale. *Nanomed*. 2008; 3:823–830.
23. Kewley DT, Hills MD, Borkholder DA, et al. Plasma-etched neural probes. *Sensors and Actuators A: Physical*. 1997; 58:27–35.
24. Nicolelis MAL, Dimitrov D, Carmena JM, et al. Chronic, multisite, multielectrode recordings in macaque monkeys. *Proceedings of the National Academy of Sciences of the United States of America*. 2003; 100:11041–11046. [PubMed: 12960378]
25. Seymour JP, Kipke DR. Neural probe design for reduced tissue encapsulation in CNS. *Biomaterials*. 2007; 28:3594–3607. [PubMed: 17517431]
26. Johansson F, Kanje M, Linsmeier CE, et al. The influence of porous silicon on axonal outgrowth in vitro. *IEEE Trans Biomed Eng*. 2008; 55:1447–1449. [PubMed: 18390337]
27. Rohatgi P, Langhals NB, Kipke DR, et al. In vivo performance of a microelectrode neural probe with integrated drug delivery. *Neurosurg Focus*. 2009; 27:E8. [PubMed: 19569896]

28. Sahin M. Information capacity of the corticospinal tract recordings as a neural interface. *Ann Biomed Eng.* 2004; 32:823–830. [PubMed: 15255213]
29. Vetter RJ, Williams JC, Hetke JF, et al. Chronic neural recording using silicon-substrate microelectrode arrays implanted in cerebral cortex. *IEEE Trans Biomed Eng.* 2004; 51:896–904. [PubMed: 15188856]
30. Chapin JK, Moxon KA, Markowitz RS, et al. Real-time control of a robot arm using simultaneously recorded neurons in the motor cortex. *Nat Neurosci.* 1999; 2:664–670. [PubMed: 10404201]
31. Cederna PS, Youssef MK, Asato H, et al. Skeletal muscle reinnervation by reduced axonal numbers results in whole muscle force deficits. *Plastic and reconstructive surgery.* 2000; 105:2003–2009. [PubMed: 10839398]
32. Urbanchek M, Ganz D, Aydin M, et al. Muscle-nerve-muscle neurotization for the reinnervation of denervated somatic muscle. *Neurological Research.* 2004; 26:388–394. [PubMed: 15198864]
33. Yoshimura K, Asato H, Cederna PS, et al. The effect of reinnervation on force production and power output in skeletal muscle. *The Journal of surgical research.* 1999; 81:201–208. [PubMed: 9927541]
34. Reynolds JL, Urbanchek MS, Asato H, et al. Deletion of individual muscles alters rat walking-track parameters. *J Reconstr Microsurg.* 1996; 12:461–466. [PubMed: 8905546]
35. de Medinaceli L, Freed WJ, Wyatt RJ. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. *Exp Neurol.* 1982; 77:634–643. [PubMed: 7117467]
36. Geuna S, Tos P, Guglielmone R, et al. Methodological issues in size estimation of myelinated nerve fibers in peripheral nerves. *Anat Embryol (Berl).* 2001; 204:1–10. [PubMed: 11506429]
37. Mackinnon SE, Dellon AL, O'Brien JP. Changes in nerve fiber numbers distal to a nerve repair in the rat sciatic nerve model. *Muscle Nerve.* 1991; 14:1116–1122. [PubMed: 1745287]
38. Institute ANS. Biological evaluation of medical devices - Part 11: Tests for systemic toxicity. ANSI/AAMI/ISO. 2006:10993.

Figure 1. Experimental groups. Control group consisted of sham or nonoperative animals. Repair and Repair + Probe groups underwent division with immediate primary repair.

Figure 2.
Silicon 16-channel MEMS probe used for nerve implantation under a) electron microscopy,
and b) light microscopy.

Figure 3. Toluidine blue stained nerve cross sections from peroneal nerve on post operative day 80: a) sham control, b) divided nerve followed by primary repair, and c) divided nerve followed by implantation of MEMS probe in distal nerve, then primary repair.

Figure 4.
Examples of two epon embedded nerve in the distal nerve area where the electrode was implanted. a) Nerve was harvested 4 days after electrode implantation. b) Nerve was harvested 80 days after electrode implantation.

Table 1

Summary of rat demographics at final evaluation.

Test	Measurement	Peroneal Nerve Groups		
		Control (n=10)	Rep air (n=9)	Rep air+Probe (n=9)
Demographics	Body mass (grams)	404 ± 39.6	430 ± 20.0	428 ± 14
	Post operative day (days)		75 ± 12	79 ± 9
	Age (months)	10.2 ± 1	10.2 ± 0.4	10.7 ± 0.7

Values are means ± SD. No statistically significant differences were measured.

Table 2

Data summary for nerve morphology of cross sections cut 4mm distal to the nerve repair site.

Test	Measurement	Peroneal Nerve Groups		
		Control (n=10)	Rep air (n=9)	Rep air+Probe (n=7)
Peroneal Nerve Morphology	Estimated Axon Count	1863 ± 861	2359 ± 1008	1731 ± 634
	Average Axon Size (μm^2) [†]	46 ± 17	9.6 ± 5.4 [*]	10.2 ± 4.8 [*]
	Average Non- Neural Area (%)	59 ± 11	87 ± 5.2 [*]	84 ± 15 [*]

Values are means ± SD.

^{*} Indicates different from the Control Nerve group,

[†] Variance for this group is not homogeneous; therefore, Brown-Forsythe test applied for significance of the model. $\alpha < 0.05$ (2-tailed).

Table 3

Data summary for nerve electrodiagnostic measurements following 80 days of recovery form nerve lesion and repair or microscale electrode implantation and nerve repair.

Test	Measurement [†]	Peroneal Nerve Groups		
		Control (n=9)	Rep air (n=2)	Rep air+Probe (n=5)
Peroneal Nerve Conduction	Amplitude (mV)	31.1 ± 15.1	17.3 ± 6.2 [*]	10.9 ± 5.4 [*]
	Nerve Conduction Velocity (m/s)	11.5 ± 2.8	13.4 ± 8.0 [*]	7.4 ± 4.9 [*]
	Area of Curve ^{††} (mV*ms)	44.3 ± 19.7	17.8 ± 6.2 [*]	12.2 ± 5.4 [*]
	Latency (ms)	1.5 ± 0.2	1.2 ± 0.2	2.1 ± 0.6

Values are means ± SD.

^{*} Indicates different from the Control group, $\alpha < 0.05$ (2-tailed).

[†] Stimulation across repair site. Stimulus was applied percutaneously over the peroneal nerve.

^{††} Area under the negative amplitude curve. Recording electrode was located in the extensor digitorum muscle.

Table 4

Data summary for final muscle maximal isometric force and walking track function after microscale electrode implantation and nerve repair.

Test	Measurement	Peroneal Nerve Groups		
		Control (n=10)	Rep air (n=7)	Rep air+Probe (n=9)
EDL Muscle Force	Force (mN)	3613 ± 909	1925 ± 621 *	2328 ± 836 *
	Muscle Mass (mg)	151 ± 27	101 ± 14 *	112 ± 23 *
	Specific Force (kN/m ²)	313 ± 43	256 ± 59	277 ± 61
	Walking Track Score	99.9 ± 0.07	100.1 ± 0.13	100.1 ± 0.11

Values are means ± SD.

* Indicates different from the Control Nerve group, $\alpha < 0.05$ (2-tailed).