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Biological and Electrophysiologic Effects of Poly(3,4-ethylenedioxythiophene) on Regenerating Peripheral Nerve Fibers

Ziya Baghmanli, M.D., Kristoffer B. Sugg, M.D., Benjamin Wei, M.D., Bong S. Shim, Ph.D., David C. Martin, M.S., Ph.D., Paul S. Cederna, M.D., and Melanie G. Urbanchek, Ph.D.

From the Division of General Surgery, Department of Surgery, Medical University of South Carolina; the Section of Plastic and Reconstructive Surgery, Department of Surgery, and the Department of Biomedical Engineering, University of Michigan; the Division of General Surgery, Department of Surgery, Henry Ford Hospital–Wayne State University; the Department of Chemical and Biological Engineering, Inha University; and the Department of Materials Science and Engineering, University of Delaware

Abstract

Background—Uninjured peripheral nerves in upper-limb amputees represent attractive sites for connectivity with neuroprostheses because their predictable internal topography allows for precise sorting of motor and sensory signals. The inclusion of poly(3,4-ethylenedioxythiophene) reduces impedance and improves charge transfer at the biotic-abiotic interface. This study evaluates the in vivo performance of poly(3,4-ethylenedioxythiophene)–coated interpositional decellularized nerve grafts across a critical nerve conduction gap, and examines the long-term effects of two different poly(3,4-ethylenedioxythiophene) formulations on regenerating peripheral nerve fibers.

Methods—In 48 rats, a 15-mm gap in the common peroneal nerve was repaired using a nerve graft of equivalent length, including (1) decellularized nerve chemically polymerized with poly(3,4-ethylenedioxythiophene) (dry); (2) decellularized nerve electrochemically polymerized with poly(3,4-ethylenedioxythiophene) (wet); (3) intact nerve; (4) autogenous nerve graft; (5) decellularized nerve alone; and (6) unrepaired nerve gap controls. All groups underwent electrophysiologic characterization at 3 months, and nerves were harvested for histomorphometric analysis.

Results—Conduction velocity was significantly faster in the dry poly(3,4-ethylenedioxythiophene) group compared with the sham, decellularized nerve, and wet poly(3,4-ethylenedioxythiophene) groups. Maximum specific force for the dry poly(3,4-ethylenedioxythiophene) group was more similar to sham than were decellularized nerve controls.

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Melanie G. Urbanchek, Ph.D., Section of Plastic and Reconstructive Surgery, Department of Surgery, University of Michigan, 2130 Taubman Center, 1500 East Medical Center Drive, Ann Arbor, Mich. 48109-0340, melurban@med.umich.edu

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Evident neural regeneration was demonstrated in both dry and wet poly(3,4-ethylenedioxythiophene) groups by the presence of normal regenerating axons on histologic cross-section.

Conclusions—Both poly(3,4-ethylenedioxythiophene) formulations were compatible with peripheral nerve regeneration at 3 months. This study supports poly(3,4-ethylenedioxythiophene) as a promising adjunct for peripheral nerve interfaces for prosthetic control and other biomedical applications because of its recognized ionic-to-electronic coupling potential.

Human-machine interfaces bear considerable potential for intuitive prosthesis control.¹ Restoration of purposeful movement and touch perception to an amputated limb relies on bidirectional information exchange mediated by the transformation of physiologic action potentials into electron-mediated transport and vice versa.^{2–4} This complex energy transfer scheme occurs between the biological substrate and electrode in a closed-loop design, with motor function being tightly coordinated by real-time graded somatosensory feedback. Although such interfaces have been studied extensively in animals⁵ and used in select human devices,^{1,6–8} robust and stable longterm recording and stimulation capabilities remain elusive.⁹ Ideally, human-machine interfaces require high biocompatibility and durability in vivo with low impedance and high charge-capacity profiles.^{10,11} Current generation human-machine interfaces, however, lack refinement of these characteristics and are subject to time-dependent signal degradation,^{10,12–16} an intrinsic process at the biotic-abiotic interface,¹⁷ or the result of foreign body response, encapsulation, and electrode breakage.^{18,19} With the burgeoning of more sophisticated robotics, several laboratories have investigated adding conductive polymers to human-machine interfaces, not only to improve their impedance profiles but also to alter the environmental conditions that lead to electrode biofouling.^{20–22}

Poly(3,4-ethylenedioxythiophene) is an anisotropic conductive polymer capable of both ionic and electronic signal transmission. Poly(3,4-ethylenedioxythiophene) has been selected in recent years for use in various biomedical applications, including neural cell signaling,^{23–25} neural interfaces,^{26–28} biosensors,^{29–31} and novel drug delivery systems.³² Its electrical conductivity is directly related to its highly ordered chemical structure consisting of an insulative polythiophene backbone with alternating single and double bonds between the carbon atoms (Fig. 1).³³ The net result is reduced impedance and improved charge transfer at the biotic-abiotic interface contributing to high-resolution signal transduction. In addition, because of its softer consistency compared with other biomaterials, poly(3,4-ethylenedioxythiophene) may also cause less tissue inflammation and foreign body response, resulting in less strain mismatch between the tissue and electrode.¹⁷

Building on previous work demonstrating in vivo electrical conductivity of a biosynthetic acellular muscle–poly(3,4-ethylenedioxythiophene) interface in an acute rat hind-limb amputation model,²⁶ we studied the long-term effects of poly(3,4-ethylenedioxythiophene) on regenerating peripheral nerve fibers. Because of its beneficial reduction in tissue impedance, poly(3,4-ethylenedioxythiophene) was chemically polymerized onto nonconductive, processed decellularized nerve grafts, rendering them highly conductive, but stiff.^{34,35} Moreover, we developed a novel polymerization process known as wet

polymerization, in which the end product is softer and more pliable secondary to constant hydration. Products of both polymerization processes were tested for their impact on peripheral nerve regeneration across a critical nerve conduction gap as determined by evaluations of nerve electrical conductivity, end-organ functional recovery, and histomorphometry at 3 months after nerve surgery. We hypothesized that (1) peripheral nerve regeneration across a critical nerve conduction gap occurs in the presence of both dry and wet poly(3,4-ethylenedioxythiophene) formulations and (2) poly(3,4-ethylenedioxythiophene) increases electrical conductivity in peripheral nerves at 3 months.

Materials and Methods

Experimental Design

Forty-eight adult, male, specific pathogen– free Fisher 344 rats (Charles River Laboratories, Inc., Wilmington, Mass.) were assigned randomly to one of six groups with eight rats per group. A unilateral 15-mm gap in the common peroneal nerve was repaired using an equivalent length biological or biosynthetic electrically active nerve graft. Experimental groups included processed decellularized nerve graft either chemically (dry) or electrochemically (wet) polymerized with poly(3,4-ethylenedioxythiophene), compared with intact nerve (sham), fresh reversed autograft (autograft), processed decellularized nerve graft alone, and unrepaired nerve gap (gap) controls.

Preparation of Dry and Wet Poly(3,4-ethylenedioxythiophene) Formulations

Rat sciatic nerves were harvested at the University of Michigan and shipped to Axogen, Inc. (Alachua, Fla.) for decellularization. The dry poly(3,4-ethylenedioxythiophene) variant was then produced by oxidative chemical vapor deposition of poly(3,4-ethylenedioxythiophene) onto these processed decellularized nerve grafts using a method described previously,³⁵ whereas the wet poly(3,4-ethylenedioxythiophene) variant was prepared by a novel polymerization process known as wet polymerization, in which poly(3,4-ethylenedioxythiophene) was electrochemically deposited onto processed decellularized nerve grafts through an intermediary gel-forming stage. The graft was first dipped in an aqueous dispersion of poly(3,4-ethylenedioxythiophene) poly(styrene sulfonate) (PEDOT:PSS, Clevios P; H. C. Starck, Inc., Coldwater, Mich.) and then transferred to a mixture of 1% poly(diallyldimethylammonium chloride) (Sigma-Aldrich Corp., St. Louis, Mo.). The sample was air dried in a fume hood until a thin gel coat formed on its surface. It was then immersed in a solution containing 1% 3,4-ethylenedioxythiophene monomer, 0.5% poly(vinyl alcohol) (molecular weight, 195,000; Sigma-Aldrich), 48.5% ethanol, and 50% deionized water, supporting adherence of poly(3,4-ethylenedioxythiophene) to the underlying graft when direct current (10^{-4} A) was applied for 1 hour. The end product was subsequently stored in $1\times$ phosphate-buffered saline at pH 7.4 until surgical implantation. The chemical properties of poly(3,4-ethylenedioxythiophene) have been previously well-studied, demonstrating superb biocompatibility, little to no cytotoxicity in vitro, and shielding from biodegradation because of steric hindrances.³⁵

Operative Technique

Using microsurgical techniques, the left sciatic nerve was exposed through a biceps femoris muscle-splitting approach and the common peroneal nerve was identified. For the sham group, no additional dissection was performed, whereas for the remaining groups, a 15-mm gap was created in the common peroneal nerve 10 mm proximal to the lateral compartment of the calf of the lower extremity (Fig. 2). Next, the nerve gap was either repaired or not repaired depending on the rat's experimental group. Sequential coaptation of all nerve grafts was performed from proximal to distal, and decellularized small intestinal submucosa (Surgisis; Cook Medical, Inc., Bloomington, Ind.) was lightly wrapped around either the neurorrhaphy site or its corresponding region in the sham and gap groups. The surgical site was uniformly closed by approximating muscle, fascia, and skin.

Nerve Conduction Studies

Nerve conduction studies were performed at 3 months using methods described previously.^{26,34-41} The nerve was stimulated with a commercial electromyographic device (TECA Synergy N; VIASYS Healthcare, Inc., Madison, Wis.) at three locations: (1) fibular head (distal to the graft); (2) midgraft; and (3) sciatic notch (proximal to the graft). Recordings were made at the extensor digitorum longus muscle. Rheobase (minimal current of infinite duration required to reach the depolarization threshold) and chronaxie (minimal time of an electrical current twice the rheobase to reach the depolarization threshold) were measured followed by standard compound muscle action potential testing using supramaximal stimuli with a duration of 0.1 msec.

Muscle Force Testing

Following completion of nerve conduction studies, muscle force testing was performed using methods described previously.^{26,34-41} The distal tendons of the extensor digitorum longus muscle were transected, folded into a loop, and attached to a force transducer (Kulite Semiconductor Products, Inc., Leonia, N.J.). Supramaximal stimuli were delivered proximal to the repair site, resulting in isometric contractions of the extensor digitorum longus muscle. Strain gauge voltage was output to a microcomputer and converted to force values. The optimum length and maximum isometric tetanic force were recorded, and then the maximum specific force was calculated as maximum isometric tetanic force normalized to the total muscle fiber cross-sectional area.^{42,43}

Nerve Histomorphometry

The nerve grafts containing the regenerating axons of the common peroneal nerve were stained with 1% toluidine blue (Electron Microscopy Sciences, Inc., Hatfield, Pa.)⁴⁴ and examined at 40× magnification using a modified peripheral nerve grading system adapted from Murji et al. (Table 1).⁴⁵ Images were acquired at 100× magnification for further analysis using commercial digital processing software (MetaMorph Offline; Molecular Devices LLC, Sunnyvale, Calif.). For each specimen, the total cross-sectional area (in microns squared) was first measured and then three regions were randomly selected representing approximately 50 to 60 percent of that value. Further calculations were then

performed to determine axon density (1/micrometers squared), estimated total axon count, and estimated total neural area (microns squared).

Statistical Analysis

Data were analyzed using commercially available statistics software packages including IBM SPSS Version 19 (IBM Corp., Armonk, N.Y.). Oneway analysis of variance was performed for normally distributed interval data values, and ordinal data were analyzed with a Kruskal-Wallis analysis of variance by ranks. A post hoc comparison using the Tukey-Kramer method of correction for multiple comparisons was applied to determine which groups, if any, were different from another. For each comparison, a value of $p < 0.05$ was considered statistically significant.

Results

All animals survived experimental surgery and none suffered from surgical-site infection or foot autophagy. Average body mass was 372.7 ± 40.4 g at the time of grafting and 401.2 ± 35.2 g at 3 months postoperatively ($p = 0.0001$). No difference in body mass was found between groups.

Nerve Conduction Studies

For all three stimulation sites, only recordable data were included in further analyses. There were no electrophysiologic recordings obtained in the gap group. No difference in chronaxie was found among any of the groups. Nerve conduction studies with stimulation distal to the graft evaluates the quality of neural tissue that regenerated through the distal stump. Axons regenerated across five of the dry poly(3,4-ethylenedioxythiophene) grafts but only two of the wet poly(3,4-ethylenedioxythiophene) grafts (Table 2). Multiple comparisons demonstrated higher rheobase in the dry poly(3,4-ethylenedioxythiophene) group compared with the sham, autograft, and decellularized nerve groups, but shorter latency and faster velocity compared with the decellularized nerve group alone. These values indicate faster nerve conduction at the expense of more stimulating energy with other conduction parameters similar to sham for the dry poly(3,4-ethylenedioxythiophene) group. No difference in rheobase was found between the wet poly(3,4-ethylenedioxythiophene) group compared with the sham, autograft, and decellularized nerve groups, but longer latency was found compared with all groups. The velocity of the wet poly(3,4-ethylenedioxythiophene) group was slower than that of the dry poly(3,4-ethylenedioxythiophene) group.

Nerve conduction studies with stimulation at the midgraft indicate conduction facilitation afforded by the addition of poly(3,4-ethylenedioxythiophene) (Table 3). Multiple comparisons demonstrated higher rheobase and shorter latency in the dry poly(3,4-ethylenedioxythiophene) group compared with the sham, autograft, and decellular nerve groups, and faster velocity compared with the decellularized nerve group alone. Again, these values indicate faster nerve conduction in the dry poly(3,4-ethylenedioxythiophene) grafts, but at the expense of more stimulating energy. No difference in rheobase was found between the wet poly(3,4-ethylenedioxythiophene) group compared with the sham, autograft, and decellularized nerve groups. The wet poly(3,4-ethylenedioxythiophene) group also

demonstrated longer latency compared with the sham, autograft, decellularized nerve, and dry poly(3,4-ethylenedioxythiophene) groups, and slower velocity compared with the sham, autograft, and dry poly(3,4-ethylenedioxythiophene) groups.

Nerve conduction studies with stimulation proximal to the graft require action potentials to cross two repair sites and the graft. Both the dry and wet poly(3,4-ethylenedioxythiophene) groups demonstrated higher rheobase compared with the sham, autograft, and decellularized nerve groups (Table 4). The velocity of the dry poly(3,4-ethylenedioxythiophene) group was faster compared with the sham and decellularized nerve groups, whereas no difference in velocity was found in the wet poly(3,4-ethylenedioxythiophene) group. No difference in latency was found among any of the groups.

Muscle Force Testing

Axons successfully regenerated across the grafts in all groups (except the gap) and reinnervated their respective end-organs (Table 5). Multiple comparisons demonstrated that the maximum isometric tetanic force was lower in both the dry and wet poly(3,4-ethylenedioxythiophene) groups compared with the sham and autograft groups. The wet muscle mass in the dry poly(3,4-ethylenedioxythiophene), wet poly(3,4-ethylenedioxythiophene), and gap groups was also lower compared with the sham, autograft, and decellularized nerve groups. However, no differences in maximum specific force and cross-sectional area were noted among any of the groups.

Nerve Histomorphometry

In general, a lower total score on the modified peripheral nerve grading system reflects more favorable neural regeneration. Multiple comparisons demonstrated a higher total score for the dry poly(3,4-ethylenedioxythiophene) group compared with the sham, autograft, and decellularized nerve groups. Although the total score for the wet poly(3,4-ethylenedioxythiophene) group was also higher compared with the sham, autograft, and decellularized nerve groups, it was significantly lower compared with the dry poly(3,4-ethylenedioxythiophene) group. This is attributed to the presence of more fibrous tissue in the dry poly(3,4-ethylenedioxythiophene) group. The total score for the gap group was higher compared with the sham, autograft, decellularized nerve, and wet poly(3,4-ethylenedioxythiophene) groups, but no difference was noted when compared with the dry poly(3,4-ethylenedioxythiophene) group. Representative nerve conduit histology is shown in Figure 3.

Nerve Quantification

Multiple comparisons demonstrated a lower axon density and percentage neural area per region for the dry poly(3,4-ethylenedioxythiophene) group compared with the sham and autograft groups, and a lower regional axon count, neural area per region, estimated total axon count, and estimated total neural area compared with the autograft group alone (Table 7). No differences in neural area per region, axon density, and estimated total neural area were noted between the wet poly(3,4-ethylenedioxythiophene) group compared with the sham, autograft, and decellularized nerve groups, but the wet poly(3,4-ethylenedioxythiophene) group demonstrated lower regional axon count, percentage neural

area per region, and estimated total axon count compared with the autograft group alone. No difference in regional area and total cross-sectional area was noted among any of the groups. Nerve histology (Fig. 3) and the histomorphometry data in Tables 6 and 7 indicate that neural regeneration can take place through decellularized nerve polymerized with either dry or wet poly(3,4-ethylenedioxythiophene). These also indicate that neural regeneration through decellularized nerve is not ideal and is even less ideal when poly(3,4-ethylenedioxythiophene) forms a physical barrier to axonal growth.

Discussion

Poly(3,4-ethylenedioxythiophene) is a conductive polymer that has gained prodigious recognition in recent years for its use in various biomedical applications.^{26–28} Its ionic-to-electronic coupling potential permits high-resolution signal transduction by reducing tissue impedance and improving charge transfer at the biotic-abiotic interface. This salient feature is perhaps its greatest utility, because poly(3,4-ethylenedioxythiophene) functions directly at the site of information exchange, preserving the fidelity of the transmitted signal. More importantly, by altering the impedance profile, less energy is required to reach stimulation threshold, which in turn protects the surrounding soft tissues from unnecessary insult and mitigates the risk of electrode biofouling.^{21,22} Despite these promising findings, the long-term influence of poly(3,4-ethylenedioxythiophene) on regenerating peripheral nerve fibers has yet to be determined. In this report, we provide both biological and electrophysiologic confirmation of peripheral nerve regeneration across a critical nerve conduction gap at 3 months in the presence of two different poly(3,4-ethylenedioxythiophene) formulations.

Our laboratory previously demonstrated that acellular muscle polymerized with poly(3,4-ethylenedioxythiophene) improved nerve conduction immediately following nerve division and repair in a rat hind-limb amputation model.²⁶ Although these findings spotlight the potential use of conductive polymers in neural interfaces, further investigation is required to confirm the stability of the electrophysiologic properties of poly(3,4-ethylenedioxythiophene) over time, because most microfabricated bioelectronics succumb to poor long-term performance.^{12–16} The results of the present study confirm the in vivo electrical responsiveness of poly(3,4-ethylenedioxythiophene) at 3 months in the presence of a regenerative tissue. Improved nerve conduction in the dry poly(3,4-ethylenedioxythiophene) grafts was demonstrated by faster velocity compared with decellularized nerve alone, with other conduction parameters more similar to sham than were decellularized nerve results; however, this occurs at the expense of more stimulating energy, indicating lower overall nerve excitability. Interestingly, slower nerve conduction was noted in the wet poly(3,4-ethylenedioxythiophene) grafts, with no difference in velocity compared with decellularized nerve alone. In contrast to oxidative chemical vapor deposition, the process of wet polymerization includes an intermediary gel on the surface of the scaffold. Because poly(3,4-ethylenedioxythiophene) is electrochemically deposited directly onto the gel, a relative deficiency of poly(3,4-ethylenedioxythiophene) is created within the interior of the construct, forming a less electrically dense graft, which accounts for the observed differences in nerve conduction between the dry and wet poly(3,4-ethylenedioxythiophene) groups.

As an indicator of end-organ functional recovery, muscle force testing was performed to determine the extent of neural regeneration. The maximal forces produced by the extensor digitorum longus muscles in both poly(3,4-ethylenedioxythiophene) groups were significantly lower than in the sham and autograft groups. These findings are consistent with histologic and nerve conduction measurements, and further indicate that fewer nerve fibers crossed the graft in both poly(3,4-ethylenedioxythiophene) groups, with the wet poly(3,4-ethylenedioxythiophene) graft being especially obstructive. Maximum specific force roughly correlates with the proportion of muscle fibers that are reinnervated, indicating that the remaining muscle fibers of the dry poly(3,4-ethylenedioxythiophene) group conducted normally, whereas those in the wet poly(3,4-ethylenedioxythiophene) group exhibited compromised contraction. The unexpected finding of a lower wet muscle mass in both the dry and wet poly(3,4-ethylenedioxythiophene) groups suggests that some component of denervation atrophy persisted. Poly(3,4-ethylenedioxythiophene) may act as an incomplete physical barrier to axonal growth within the graft (Fig. 4).

Nerve histology provides further support for the presence of regenerating axons within processed decellularized nerve graft polymerized with either dry or wet poly(3,4-ethylenedioxythiophene) (Fig. 3). Using a modified peripheral nerve grading system adapted from Murji et al. (Table 1),⁴⁵ we found a higher total score in both the dry and wet poly(3,4-ethylenedioxythiophene) groups compared with the decellularized nerve group alone, but no difference was noted in axon density, estimated total axon count, and estimated total neural area between these groups. Moreover, we found a significantly lower total score in the wet poly(3,4-ethylenedioxythiophene) group compared with the dry poly(3,4-ethylenedioxythiophene) group, which again indicates that poly(3,4-ethylenedioxythiophene) may partially obstruct axonal growth within the graft. Less obstruction is observed in the wet poly(3,4-ethylenedioxythiophene) group because of the differential deposition of conductive polymer on the surface of the scaffold.

An alternative explanation for these differences between the dry and wet poly(3,4-ethylenedioxythiophene) groups is related to the stiffness of poly(3,4-ethylenedioxythiophene) and its influence on foreign body response and in vivo functionality.¹⁶ Peripheral nerve is soft and pliable, whereas most electrodes are hard and rigid, creating a measurable strain mismatch in which any physiologic stressor can cause micromotion at the nerve-electrode interface, resulting in fibrosis and signal interference.^{46–48} By altering the biomechanical properties of the electrode or its neurointegrative coatings, strain around the implanted electrode can be reduced.⁴⁹ In the present study, strain mismatch between the peripheral nerve and poly(3,4-ethylenedioxythiophene) explains why more fibrous tissue is observed in histologic samples from the dry poly(3,4-ethylenedioxythiophene) group. Oxidative chemical vapor deposition renders a stiff graft, whereas wet polymerization yields a malleable graft that more easily conforms to the recipient bed in which it is placed, creating a more favorable environment for regenerating axons to grow within the wet poly(3,4-ethylenedioxythiophene) graft compared with the dry poly(3,4-ethylenedioxythiophene) graft.

Conclusions

This report provides biological and electrophysiologic confirmation of peripheral nerve regeneration across a critical nerve conduction gap at 3 months in the presence of two different poly(3,4-ethylenedioxythiophene) formulations. Evident neural regeneration was demonstrated by end-organ functional recovery and the identification of normal regenerating axons on histologic cross-section. Dry poly(3,4-ethylenedioxythiophene) is stiffer, with improved nerve conduction, whereas wet poly(3,4-ethylenedioxythiophene) is softer, with less strain mismatch. This study supports poly(3,4-ethylenedioxythiophene) as a promising adjunct for peripheral nerve interfaces for prosthetic control and other biomedical applications because of its recognized ionic-to-electronic coupling potential.

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Evidence-Based Medicine: A New Emphasis

Plastic and Reconstructive Surgery has made evidence-based medicine a major initiative to improve the overall quality of published articles. Rather than the traditional uncontrolled case series and retrospective cohort studies, PRS strongly encourages submissions to employ the full spectrum of research methodology, including:

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Fig. 1.

(*Above*) Chemical structure of poly(3,4-ethylenedioxythiophene) showing four repeat units along the polymer backbone. The polymer is chemically synthesized by oxidizing the 3,4-ethylenedioxythiophene monomer with ferric chloride. (*Center*) Space-filling model for poly(3,4-ethylenedioxythiophene) where carbon molecules are *gray*, oxygen molecules are *red*, sulfur molecules are *yellow*, and hydrogen molecules are *white*. (*Below*) The molecular backbone of poly(3,4-ethylenedioxythiophene) is conjugated, meaning that it consists of

alternating single and double bonds. This allows the electrons to adopt extended orbitals, which in turn is related to its relatively high conductivity.

Fig. 2.

Experimental surgery. (*Above*) Schematic representation of the nerve gap. Neurectomy of the common peroneal nerve is performed proximal to the lateral compartment of the calf, producing a 15-mm nerve gap. (*Below*) Intraoperative photograph from the gap group demonstrating no interpositional nerve graft. Decellularized small intestinal submucosa (*asterisk*) bridges the nerve gap and is secured to the proximal and distal stumps of the divided nerve. *EDL*, extensor digitorum longus; *PD*, peroneal division; *SN*, sciatic nerve; *TD*, tibial division.

Fig. 3. Representative nerve conduit histology in sham (*above, left*), autograft (*above, right*), decellularized nerve (*center, left*), dry poly(3,4-ethylenedioxythiophene) (*center, right*), wet poly(3,4-ethylenedioxythiophene) (*below, left*), and gap (*below, right*) groups. Note abundant fibrous tissue and absence of neural tissue in the gap group. *PEDOT*, poly(3,4-ethylenedioxythiophene); *A*, axon; *M*, myelin; *F*, fibrous tissue.

Fig. 4. Scanning electron microscopic image of processed decellularized nerve graft coated with poly(3,4-ethylenedioxythiophene) by means of oxidative chemical vapor deposition [dry poly(3,4-ethylenedioxythiophene)]. The conductive polymer partially obstructs the internal lining of the pores within the graft, acting as an incomplete physical barrier to axonal growth. *P*, poly(3,4-ethylenedioxythiophene); *B*, basal lamina.

Table 1
Modified Peripheral Nerve Grading System^{*,†}

Epineurium		Endoneurium		Perineurium	
Microfascicles	Free Space	Organization	Definition	Thickening	Microfascicles
None (1)	Normal (1)	Good (1)	Good (1)	None (1)	None (1)
Rare (2)	Mild (2)	Poor (2)	Poor (2)	Mild (2)	Rare (2)
Moderate (3)	Moderate (3)	None (3)	None (3)	Moderate (3)	Severe (3)
Severe (4)	Severe (4)			Severe (4)	
Extensive (5)					

^{*} Adapted from Murji A, Redett RJ, Hawkins CE, Clarke HM. The role of intraoperative frozen section histology in obstetrical brachial plexus reconstruction. *J Reconstr Microsurg.* 2008;24:203–209.

[†] None (1) indicates a score of 1, in which no microfascicles were identified outside the epineurium. Extensive (5) indicates a score of 5, which reflects barely recognizable epineurium/no epineurium; thus, microfascicles were observed throughout the entire tissue block. Rare (2), Moderate (3), and Severe (3) are graded by density of microfascicles interspersed within the epineurium. All tissue blocks were evaluated by two independent, blinded observers.

Table 2
Summary of Electrophysiologic Measurements for the Fibular Head Stimulation Site*

	Experimental Groups					
	Sham	Autograft	DN	Dry PEDOT	Wet PEDOT	Gap
No. of subjects with recordable data	8	8	8	5	2	0
Rheobase, mA	0.2 ± 0.2	0.5 ± 0.2	0.4 ± 0.1	11.9 ± 5.6 ^{†‡§§}	0.5 ± 0.4 ^{//}	NR
Chronaxie, msec	0.6 ± 0.2	0.05 ± 0	0.06 ± 0.02	0.04 ± 0.01	0.08 ± 0.04	NR
Latency, msec	1.0 ± 0.1	1.2 ± 0.2	1.7 ± 0.4 [†]	0.8 ± 0 [§]	2.3 ± 0.4 ^{†‡§//}	NR
Amplitude, mV	18.5 ± 4.6	9.2 ± 2.8 [†]	7.1 ± 3.7 [†]	12.1 ± 5.3	1.3 ± 0.7 ^{†‡§}	NR
Velocity, m/sec	11.2 ± 1.7	12.6 ± 5.0	8.4 ± 2.4	15.1 ± 3.3 [§]	5.0 ± 1.5 ^{†//}	NR
CMAP peak area, mV × msec	23.5 ± 7.0	11.4 ± 2.6 [†]	8.8 ± 5.1 [†]	12.1 ± 5.5 [†]	2.3 ± 1.2 [†]	NR
CMAP peak duration, msec	2.2 ± 0.3	2.3 ± 0.3	2.2 ± 0.5	2.0 ± 0.1	3.0 ± 0.1 ^{†‡§//}	NR

DN, decellularized nerve; PEDOT, poly(3,4-ethylenedioxythiophene), NR, not recorded; CMAP, compound muscle action potential.

* Parametric analysis of variance, $p < 0.05$. Values are listed as mean ± SD.

[†] Statistically significant difference compared with sham.

[‡] Statistically significant difference compared with autograft.

[§] Statistically significant difference compared with DN.

^{//} Statistically significant difference compared with dry PEDOT.

Table 3
Summary of Electrophysiologic Measurements for the Midgraft Stimulation Site*

	Experimental Groups					
	Sham	Autograft	DN	Dry PEDOT	Wet PEDOT	Gap
No. of subjects with recordable data	8	8	8	5	2	0
Rheobase, mA	0.3 ± 0.1	0.5 ± 0.2	2.0 ± 3.7	24.2 ± 16.5 ^{†‡§}	5.7 ± 3.1 ^{//}	NR
Chronaxie, msec	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 0	0.06 ± 0.03	0.08 ± 0.04	NR
Latency, msec	1.3 ± 0.2	1.5 ± 0.2	2.1 ± 0.3 ^{†‡}	0.9 ± 0.03 ^{†‡§}	2.6 ± 0.3 ^{†‡§//}	NR
Amplitude, mV	18.2 ± 5.0	8.1 ± 2.9 [†]	5.5 ± 3.2 [†]	12.1 ± 6.3	1.1 ± 0.6 ^{†‡§}	NR
Velocity, m/sec	13.4 ± 2.7	13.9 ± 3.8	9.3 ± 1.5 [†]	19.8 ± 2.8 [§]	5.8 ± 1.3 ^{†‡//}	NR
CMAP peak area, mV × msec	22.4 ± 6.0	10.0 ± 3.6 [†]	7.1 ± 4.4 [†]	11.4 ± 6.6	1.8 ± 0.8 ^{†‡}	NR
CMAP peak duration, msec	2.1 ± 0.2	2.3 ± 0.3	2.4 ± 0.6	1.7 ± 0.1 [†]	2.8 ± 0.04 ^{†‡//}	NR

DN, decellularized nerve; PEDOT, poly(3,4-ethylenedioxythiophene), NR, not recorded; CMAP, compound muscle action potential.

* Parametric analysis of variance, $p < 0.05$. Values are listed as mean ± SD.

[†] Statistically significant difference compared with sham.

[‡] Statistically significant difference compared with autograft.

[§] Statistically significant difference compared with DN.

^{//} Statistically significant difference compared with dry PEDOT.

Table 4
Summary of Electrophysiologic Measurements for the Sciatic Notch Stimulation Site*

	Experimental Groups					
	Sham	Autograft	DN	Dry PEDOT	Wet PEDOT	Gap
No. of subjects with recordable data	8	8	8	5	2	0
Rheobase, mA	0.2 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	5.9 ± 4.6 ^{†‡§}	8.9 ± 11.0 ^{†‡§}	NR
Chronaxie, msec	0.09 ± 0.02	0.07 ± 0.03	0.05 ± 0.02	0.05 ± 0	0.04 ± 0.02	NR
Latency, msec	5.0 ± 9.8	1.8 ± 0.2	2.5 ± 0.4	1.0 ± 0.1	2.8 ± 0.4	NR
Amplitude, mV	16.7 ± 8.0	8.4 ± 3.1	4.7 ± 3.4 [†]	19.2 ± 8.2	1.1 ± 0.4 ^{†‡}	NR
Velocity, m/sec	15.3 ± 3.0	16.1 ± 4.9	10.6 ± 1.0 [†]	22.9 ± 2.7 ^{†§}	9.1 ± 2.5	NR
CMAP peak area, mV × msec	21.3 ± 9.4	10.8 ± 3.8	6.5 ± 4.2 [†]	19.5 ± 8.0	1.7 ± 1.0 ^{†‡}	NR
CMAP peak duration, msec	6.5 ± 12.3	2.4 ± 0.4	5.7 ± 9.7	1.9 ± 0.2	2.6 ± 0.5	NR

DN, decellularized nerve; PEDOT, poly(3,4-ethylenedioxythiophene), NR, not recorded; CMAP, compound muscle action potential.

* Parametric analysis of variance, $p < 0.05$. Values are listed as mean ± SD.

[†] Statistically significant difference compared with sham.

[‡] Statistically significant difference compared with autograft.

[§] Statistically significant difference compared with DN.

Table 5

Summary Values for Muscle Force Testing*

	Experimental Groups					
	Sham	Autograft	DN	Dry PEDOT	Wet PEDOT	Gap
No. of subjects with recordable data	7	8	8	7	6	8
Maximum isometric tetanic force, mN	2471.7 ± 1278.3	1591.7 ± 520.2	1204.0 ± 1686.0	66.3 ± 142.3 ^{†‡}	27.4 ± 50.2 ^{†‡}	0 ± 0
Maximum specific force, mN/mm ²	259.1 ± 124.0	222.2 ± 58.1	198.0 ± 308.8	211.6 ± 46.7	85.9 ± 15.0	0 ± 0
Wet muscle mass, mg	140.2 ± 18.0	104.9 ± 15.5 [†]	89.4 ± 16.8 [†]	42.6 ± 7.7 ^{†‡§}	46.4 ± 5.4 ^{†‡§}	49.7 ± 7.0 ^{†‡§}
Cross-sectional area, mm ²	9.4 ± 0.9	7.0 ± 1.5	6.3 ± 1.2	3.1 ± 0.5	3.1 ± 0.3	3.5 ± 0.6

DN, decellularized nerve; PEDOT, poly(3,4-ethylenedioxythiophene).

* Parametric analysis of variance, $p < 0.05$. Values are listed as mean ± SD.

[†] Statistically significant difference compared with sham.

[‡] Statistically significant difference compared with autograft.

[§] Statistically significant difference compared with DN.

Table 6
Summary of the Modified Peripheral Nerve Grading System*

	Experimental Groups					
	Sham	Autograft	DN	Dry PEDOT	Wet PEDOT	Gap
No. of grafts quantified	5	6	8	8	8	7
Epineurium						
Microfascicles	1.0 ± 0	2.3 ± 0.8	2.5 ± 0.9	4.3 ± 0.5	3.3 ± 1.3	5.0 ± 0
Endoneurium						
Free space	1.0 ± 0	1.8 ± 0.8	2.6 ± 0.9	4.0 ± 0	3.0 ± 1.0	4.0 ± 0
Organization	1.0 ± 0	1.0 ± 0	1.5 ± 0.8	2.9 ± 0.4	2.3 ± 0.8	3.0 ± 0
Perineurium						
Definition	1.0 ± 0	1.0 ± 0	1.4 ± 0.5	2.8 ± 0.5	2.1 ± 0.5	3.0 ± 0
Thickening	1.0 ± 0	1.7 ± 0.8	2.5 ± 0.8	3.9 ± 0.4	3.0 ± 0.7	4.0 ± 0
Microfascicles	1.0 ± 0	1.3 ± 0.5	2.3 ± 0.5	2.8 ± 0.5	2.6 ± 0.5	3.0 ± 0
Total score	6.0 ± 0	9.1 ± 1.9	12.8 ± 2.6 ^{††}	20.7 ± 1.4 ^{††§§}	16.3 ± 3.2 ^{††§§}	22.0 ± 0 ^{††§§¶¶}

DN, decellularized nerve; PEDOT, poly(3,4-ethylenedioxythiophene).

* Kruskal-Wallis analysis of variance, $p < 0.05$. Values are listed as mean ± SD.

[†] Statistically significant difference compared with sham.

^{††} Statistically significant difference compared with autograft.

[§] Statistically significant difference compared with DN.

^{§§} Statistically significant difference compared with dry PEDOT.

^{¶¶} Statistically significant difference compared with wet PEDOT.

Table 7

Summary Values for Nerve Quantification*

	Experimental Groups					
	Sham	Autograft	DN	Dry PEDOT	Wet PEDOT	Gap
No. of grafts quantified	4	7	6	8	6	7
Regional area, μm^2	44,910 $\pm$ 14,750	46,960 $\pm$ 24,220	57,790 $\pm$ 19,510	57,890 $\pm$ 17,180	44,740 $\pm$ 1339	42,260 $\pm$ 30,630
Regional axon count	877.3 $\pm$ 460.7	862.7 $\pm$ 296.8	551.7 $\pm$ 256.2	169.1 $\pm$ 239.6 [‡]	190.2 $\pm$ 99.7 [‡]	0 $\pm$ 0
Neural area per region, μm^2	15,170 $\pm$ 5639	9409 $\pm$ 5191	4442 $\pm$ 2810	998 $\pm$ 1380 [‡]	1289 $\pm$ 815	0 $\pm$ 0
Axon density, 1/ μm^2	0.019 $\pm$ 0.005	0.022 $\pm$ 0.012	0.011 $\pm$ 0.008	0.003 $\pm$ 0.004 ^{‡‡}	0.004 $\pm$ 0.002	0 $\pm$ 0
Neural area per region, %	35 $\pm$ 10.0	21 $\pm$ 7.6	9.0 $\pm$ 6.3 [‡]	1.7 $\pm$ 2.4 ^{‡‡}	2.7 $\pm$ 1.8 ^{‡‡}	0 $\pm$ 0
Total cross-sectional area, μm^2	117,200 $\pm$ 23,300	138,700 $\pm$ 59,100	118,500 $\pm$ 27,150	168,400 $\pm$ 14,800	72,400 $\pm$ 25,200	0 $\pm$ 0
Estimated total axon count	2197 $\pm$ 650	2731 $\pm$ 983.3	1418 $\pm$ 1001	636.1 $\pm$ 894.1 [‡]	300.4 $\pm$ 166.1 [‡]	0 $\pm$ 0
Estimated total neural area, μm^2	41,800 $\pm$ 19,870	28,660 $\pm$ 11,960	11,040 $\pm$ 8036	4045 $\pm$ 6210 [‡]	1983 $\pm$ 940.6	0 $\pm$ 0

DN, decellularized nerve; PEDOT, poly(3,4-ethylenedioxythiophene).

* Parametric analysis of variance, $p < 0.05$. Values are listed as mean $\pm$ SD.[‡] Statistically significant difference compared with sham.^{‡‡} Statistically significant difference compared with autograft.