ADVANCED HEALTHCARE MATERIALS

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Experimental

Animal Model: The hydrogel tubes were rinsed in DI water for 48 hrs to remove any THF and non-reactive EDOT monomers that remained inside the hydrogel tube. Experiments were performed using 8 to 10 month-old adult male, specific-pathogen-free Fischer-344 rats (Charles River Laboratory, Kingston, NY). All animal care, housing, and operative procedures were conducted in accordance with the U.S. Public Health Service Guide for the Care of Laboratory Animals (NIH Publication Number 85–23, 1985); the University Committee on the Use and Care of Animals approved and monitored the experimental protocol. Rats were individually housed in a restricted-access, pathogen-free facility in the Unit for Laboratory Medicine at the University of Michigan, given food and water ad libitum, and were exposed to a 12-hr light-dark cycle. For all surgical procedures, rats were anesthetized with an initial intraperitoneal injection of sodium pentobarbital (60 mg kg^{-1});

supplementary doses were administered to maintain a deep plane of general anesthesia. Surgical procedures were conducted under aseptic conditions.

After induction of anesthesia, surgical sites were shaved and prepared with Betadine and 70% surgical alcohol. After gluteal and posterior thigh incisions were made, the sciatic nerve was exposed deep to the biceps femoris muscle, a 10 mm segment of the peroneal nerve (a branch of the sciatic nerve) was excised, and then the appropriate 12 mm-long tube was sutured into the resulting gap. To create a gap of 10 mm, the proximal and distal nerve stumps were inserted into the inner lumen of the tube at a distance of 1 mm from each end. To secure the nerve conduit in place, interrupted sutures of 10-0 nylon were placed from the epineurium of the nerve stump and through the conduit under the operating microscope. The identical procedure was performed both proximally and distally to complete the nerve gap repair. In the nerve autograft group, the 10 mm segment of peroneal nerve was sharply excised, the segment of nerve was reversed in direction, and an end-to-end neurorrhaphy was performed with interrupted epineurial sutures of 10-0 nylon. All surgical procedures were performed atraumatically with the use of the operating microscope. The wounds were then closed in layers with absorbable sutures.

Measurement of Contractile Properties: Measurement of left extensor digitorum longus (EDL) muscle contractile function was performed 12 weeks following the initial nerve graft surgery [1]. Each rat was anesthetized and the left EDL was isolated, while taking care to avoid damage to the neurovascular pedicle. The distal tendons of the EDL were identified on the dorsum of the foot, divided, and folded to create a tendon loop. The loop was secured at the musculotendinous junction with 4-0 silk suture, and later used to affix the distal tendons to the force transducer. The tibial and sural nerves, as well as the distal tendon of the tibialis anterior muscle, were then divided to avoid motion artifact.

The rat was placed on a platform maintained at 37° C by a warming pad and a heating lamp, and the EDL tendon loop was secured to the force transducer. To stabilize the left leg during force measurements, the distal femur and foot were secured to the platform. Throughout the evaluation, the EDL muscle and peroneal nerve were regularly bathed in warm mineral oil (36° C); muscle temperature was monitored and maintained between 35° C and 37° C. The EDL muscles were activated indirectly by delivering supramaximal electrical stimuli (square pulses, 0.2-msec pulse duration, 6 to 10 V) to the peroneal nerve proximal to the nerve graft. The EDL tendon loop was secured to a BG 1000 strain gauge (Kulite Semiconductor, Leonia, NJ).

Measurement of EDL muscle force during isometric contraction was obtained using an IBM-compatible personal computer with custom designed software (LabVIEW 5.1, National Instruments, Austin, TX). Software allowed the investigator to control the trigger for data collection, to set electrical pulse properties, and to record voltage output from the force transducer. Supramaximal square wave pulses (0.2 ms pulse duration, 2-10 V) were delivered through a shielded bipolar silver wire electrode (Harvard Apparatus, South Natick, MA) to the proximal peroneal nerve by setting the software's virtual stimulator. The strain gauge was powered with a signal-conditioning amplifier (Vishay 2310, Measurements Group, Instruments Division, Raleigh, NC). Bridge excitation and bridge balance were set with the amplifier and strain gauge electrical voltage was filtered and passed from the amplifier to the computer program during force production. The measured change in voltage with isometric contraction was stored and converted to force by the software program. Procedures to determine maximum tetanic isometric force measurements began with finding the optimal twitch force and then the optimal muscle length (L_0) for force development. Twitch force was measured during single twitch contractions with the muscle at a slightly relaxed length. Applied voltage was set to 0 V during the initial stimulation and then increased between each

stimulation until muscle contractile force no longer increased. Next, with stimulation voltage held at the optimized value while the muscle was lengthened 0.5 mm between each twitch stimulation until a maximum twitch force was identified by decreased twitch force with increased muscle length. This muscle length at which optimal twitch force occurred was the L_0. Identification of maximum isometric force (F_0) now proceeded by serially increasing the frequency at which the optimal twitch voltage was applied with the muscle at L_0. Stimulation frequencies of 30, 50, 80, 100, 120, 150, 175, 200, and 220 Hz were applied for 300 ms each until an increase in frequency resulted in a lower isometric force. The muscle was allowed to rest for 2 minutes before stimulation at a higher frequency. The highest isometric contractile force was the F_0. Using calipers, L_0 was measured as the total muscle length not including tendons of origin or insertion. Muscle fiber length (L_f) was calculated as the product of L_0 and the ratio of average fiber length to muscle belly length (L_f/L_m), 0.44 for the rat EDL (unpublished laboratory for F344 rat). The muscle was dissected from the rat, tendons were trimmed, and the muscle was weighed. The muscle physiological CSA was determined by dividing the mass of the muscle by the product of L_f and 1.06 g cm^{-3}, the density of mammalian skeletal muscle. The maximum specific force (sF_0) was calculated as F_0 normalized by the total muscle fiber cross sectional area (CSA). All animals were sacrificed with a lethal injection of pentobarbital sodium followed by a double pneumothorax incision.

Nerve Histology: Prior to euthanasia, nerve grafts were harvested, pinned to length, and fixed in a formaldehyde/glutaraldehyde solution off cold 16% paraformaldehyde, 50% glutaraldehyde solution in 0.2 M sodium phosphate buffer (pH 7.2) (Karnovsky's Fixative, Electron Microscopy Sciences, Hatfield, PA). The tissues were post-fixed with 1 percent osmium tetroxide, ethanol dehydrated, and embedded in Araldite 502 (Polysciences, Inc., Warrington, PA). One-micrometer thin sections were taken from the middle of the nerve

grafts and stained with toluidine blue for examination under light microscope [2]. Morphological measurements were performed using Meta Imaging Software 7.5.0 (Molecular Devices Corporation, Dowingtonwn, PA). Images of nerve cross sections were electronically captured at 100 X magnification. Morphological measurements indicate the maturity and normality of nerve fibers.

Statistical Analysis: For each group, means and standard deviations were calculated. An one-way ANOVA, Tukey post hoc test, was used to calculate the significance of differences between mean values. A “p” value less than 0.05 was considered statistically significant. Appropriate Bonferonni corrections were applied to all post hoc comparisons. Statistical calculations were performed using commercially available statistical software SPSS V19 (SPSS Science, Chicago, IL).

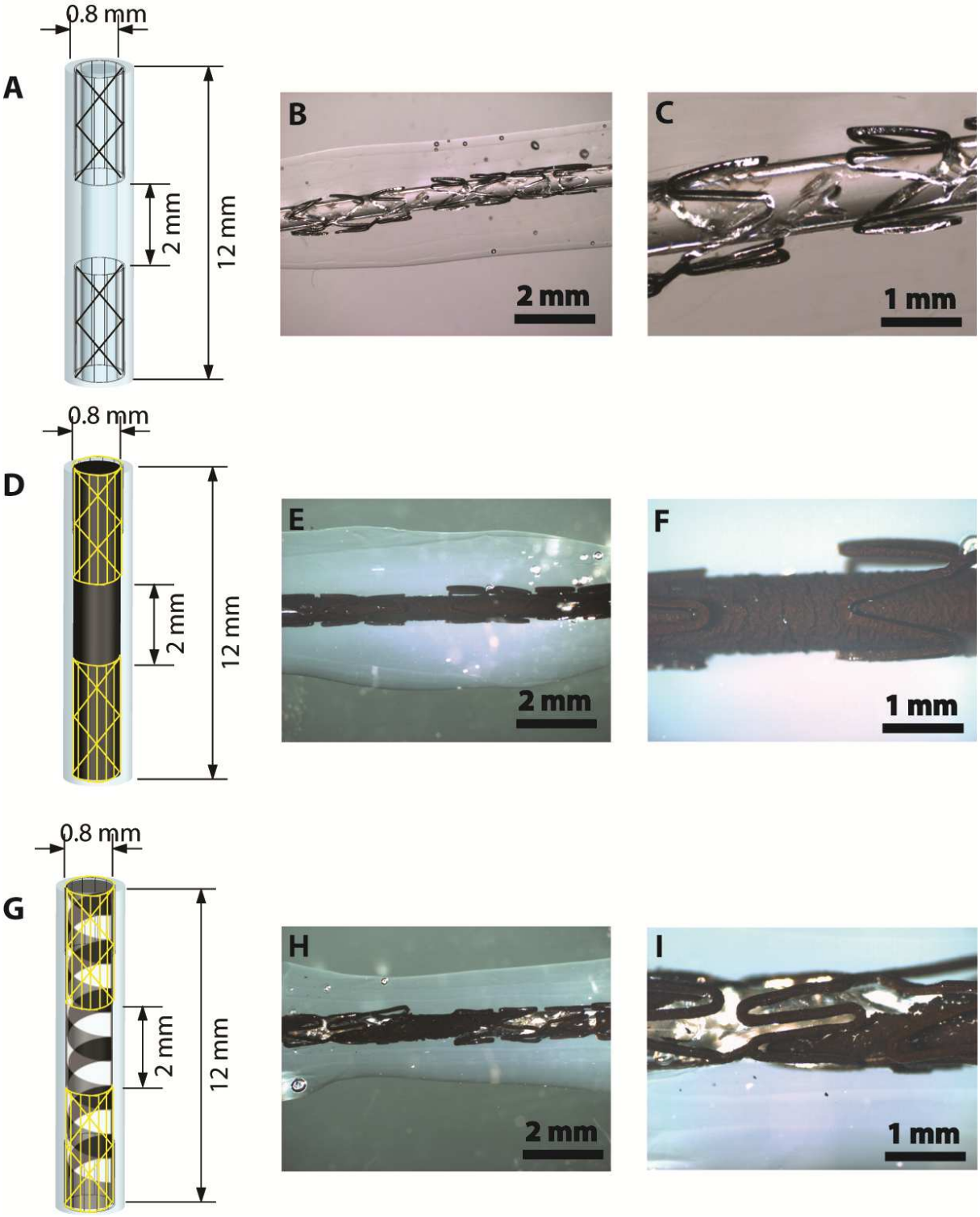

Figure S1. Schematic illustrations and optical micrographs of 12 mm long and 0.8 mm inner diameter nerve conduits with 5 mm stents at the ends. A-C) PA conduit. D-F) FPEDOTA conduit. G-I) PPEDOTA conduit (before electrochemical deposition of PEDOT within the inner lumen). As shown, the length of stents was 5 mm, therefore, there was a 2 mm gap between the stents which allowed for cross sectioning during histolomorphometric analysis.

[1] J. A. Faulkner, J. H. Niemeyer, L. C. Maxwell, T. P. White, *Am. J. Physiol.* 1980, 238, C120.

[2] S. C. Haase, J. M. Rovak, R. G. Dennis, W. M. Kuzon, P. S. Cederna, *J. Reconstr. Microsurg.* 2003, 19, 241.