Decellular Biological Scaffold Polymerized with PEDOT for Improving Peripheral Nerve Interface Charge Transfer*

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Abstract— **Regenerative peripheral nerve interfaces (RPNIs) are for signal transfer between peripheral nerves inside the body to controllers for motorized prosthetics external to the body. Within the residual limb of an amputee, surgical construction of a RPNI connects a remaining peripheral nerve and spare muscle. Nerve signals become concentrated within the RPNI. Currently metal electrodes implanted on the RPNI muscle transfer signals but scarring around metal electrodes progressively diminishes charge transfer. Engineered materials may benefit RPNI signal transfer across the neural interface if they lower the power and charge density of the biologically meaningful signals. Poly3,4-ethylenedioxythiophene (PEDOT) is known to mediate ionic potentials allowing excitation across a critical nerve gap. We hypothesize that the capacity of an interface material to conduct electron mediated current is significantly increased by polymerized coating of PEDOT. SIS was either used plain or after PEDOT coating by electro-chemical polymerization. Muscle forces are a direct repre-sentation of stimulating current distribution within an RPNI. In situ muscle forces were measured for the same muscle by electrically stimulating: a) the muscle's innervating nerve, b) directly on the muscle, c) on plain SIS laid on the muscle, and d) on SIS polymerized with PEDOT laid on the muscle. Electro-chemically coating PEDOT on SIS resulted in a thin, flexible material. PEDOT coated SIS distributed electrical stimulation more efficiently than SIS alone. Conductive polymer containing biological material allowed ionic signal distribution within the RPNI like muscle at lower charge density.**

I. INTRODUCTION

One in 190 Americans has an amputated limb and over 185,000 new amputations are performed every year [1]. Neuroprosthetic arms available to patients rely on surface electromyographic (EMG) electrodes for motor control [2]. Electrodes are placed on the skin surface to measure EMG activity from underlying muscles in the residual limb. The surface area required for each EMG electrode and signal cross talk limits the number of channels available for control [3 - 5]. Most surface EMG driven prosthetics also require large amounts of muscle in the residual [6]. Recently, targeted muscle reinnervation (TMR) surgery has become available to patients without sufficient remaining muscle for traditional surface EMG systems [7, 8]. During TMR surgery, nerves remaining after amputation are routed to major muscles in the chest and/or residual arm. The muscle is subdivided into discrete regions; each is innervated by a rerouted nerve. Still TMR EMG activity is recorded through the skin surface. Although effective for patients with upper arm amputations, TMR suffers from problems with signal transfer as do traditional myoelectric prostheses [9 -11].

Our NeuroMuscular Lab has developed a method for neuroprosthetic control suited to wireless communication with motorized prosthetic limbs. By moving the recording electrodes to muscles inside the body, our Regenerative Peripheral Nerve Interface (RPNI) allows for controlling many degrees of prosthesis movement [12-14]. For each RPNI device, a small segment of the patient's muscle is freely transferred to the residual limb and neurotized with a remaining branch of the peripheral nerve. Each RPNI device contains an electrode which directly transduces EMG activity from the muscle to receiving electronics. Internalizing the electrode directly with the RPNI muscle, achieves better signaling due to a higher signal:noise ratio, reduced signal lag time, reduced electrode movement artifact, and consistency of electrode placement. Since RPNI devices are small (approximately 2 cm in length by 1 cm in diameter) and are not reliant on the limited available skin surface area, many RPNI can be implanted for providing more control channels [14]. RPNI devices have been implanted in a rat model for up to 18 months with no degradation in signal quality [15].

There are many considerations when selecting electrodes for implantation on the RPNI. These include balancing advantages of a large surface area which, contributes by decreasing charge density impedance, with the desire to minimize size to avoid any antigenic profile at the tissue interface. Stiffness in electrodes whether silicon, parylene, or metal based can mechanically damage the soft and compliant underlying tissues. Poly(3,4-ethylenedioxythiophene) or PEDOT has been deposited on metal electrodes with results indicating PEDOT consistently allowed recording high-quality neural activity [16] and PEDOT showed stable performance with almost no change in electrical properties, even at relatively high current densities which could cause electrode oxidation [17]. PEDOT when polymerized on decellular nerve tissue is known to mediate ionic potentials allowing the excitation of nerve conduction across a critical gap [18].

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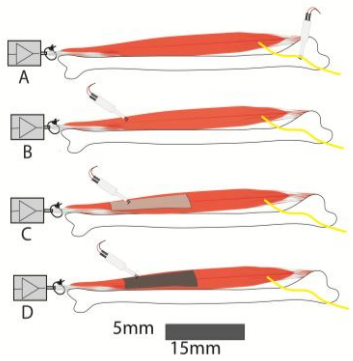

Figure 1. Muscle activation methods. A) Nerve Stim; B) Direct Stim; C) Direct SIS; D) Direct PEDOT.

In this study, we combined the conductive properties of PEDOT with a biotic material to determine if the combination would increase the signal capture of an electrode and provide a soft padding between the electrode and the underlying tissue. Porcine small intestinal submucosa (SIS) is a commercially available decellular scaffold that has desirable characteristics including softness and pliability similar to muscle tissue, high strength, and a low antigenic profile. SIS can be made electro-conductive through polymerization with a coating of electrically conductive poly-3,4-ethylenedioxythiophene (PEDOT) [19]. We hypothesize that the capacity of a soft interface material to conduct electron mediated charge transfer is significantly increased by a polymerized coating of PEDOT.

II. Methods

A. Animal Model

All procedures were approved by the Committee on Use and Care of Animals and were in strict accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals [20]. Rats were dosed with the analgesic buprenorphine HCl (0.05mg/kg) followed by anesthesia with sodium pentobarbital (50mg/kg). The study utilized male, retired breeder rats weighing between 332-352g (Charles River, Wilmington, MA).

B. Decellular Scaffold Polymerization with PEDOT

Coating of PEDOT (Clevios, HC StarckM, Coldwater, Michigan) on a 1-layer porcine decellular intestinal submucosa (SIS) scaffold (Cook Biotech, West Lafayette, IN) was a two-step process. SIS is a collagenous mixture of extracellular matrix proteins and has a low immunogenicity. First a soft gel was formed by dipping a dried sheet of SIS into a solution of PEDOT and poly(styrene-sulfonate) (PEDOT:PSS). The sample was then dipped in a 2% chitosan solution (Sigma Aldrich, St. Louis MO) and then rinsed in water to remove excess chitosan. This process was repeated until a stable, soft conducting gel of PEDOT:PSS was formed. Then the SIS was electrochemically plated with PEDOT:PSS at a charge density of $6*10^{-7}$ A/mm^2. The electroplating solution consisted of EDOT monomer (Baytron, H.C. Stark, Coldwater, MI) and poly(sodium-4-styrenesulfonate) (PSS). Samples were then rinsed in ethanol. Electroconductivities of the bare SIS and SIS+PEDOT interface materials were measured using impedance spectroscopy.(Gamry Instruments, Warminster, PA). The real and imaginary components of the impedance were measured as a function of frequency from 1 to 10,000Hz.

C. Evaluation of Conduction of Charge Transfer using Measurement of Muscle Force

The performances of SIS and SIS+PEDOT as soft interface materials for conducting electron mediated charge transfer were assessed using muscle force testing. Four tests were performed with each rat of five rats. Two control conditions were muscle stimulation through the peroneal nerve (NerveStim) and direct stimulation of the muscle (DirectStim). Experimental conditions were direct muscle stimulation through the SIS (DirectSIS) and the SIS+PEDOT (DirectPEDOT) [21].Test order for DirectStim, DirectSIS, and DirectPEDOT was randomly assigned; the muscle was rested 5 minutes in between each test.

For the NerveStim test, the peroneal nerve was dissected free in the hip compartment. The extensor digitorum longus (EDL) muscle was then exposed through a lower leg skin incision. The distal tendon of the extensor digitorum longus was dissected free below the extensor retinaculum and connected to the force transducer (Kulite BG 1000, Cambridge, MA).

The same measurements were made for each of the four tests except the location for stimulation was at the nerve during NerveStim tests and at the muscle during direct stimulation tests [22]. The muscle was first activated with a single one 0.2 ms square wave pulse eliciting a single muscle twitch. Stimulation voltage was increased for each twitch until the maximum muscle twitch force was reached. The muscle was then stimulated with 30, 50, 100 and 150 Hz pulses delivered for 300 msec at the voltage determined from maximal twitch testing. The maximal tetanic force of the muscle was recorded. The length of the muscle was then measured and the cross sectional area calculated [23].

Stimulation of the peroneal nerve was uniformly applied two cm distal to the sciatic notch using a bipolar stimulating electrode (Harvard Apparatus, Holliston, MA). For direct stimulation of the EDL muscle the bipolar stimulating electrode was placed at the distal 1/3 of the muscle. For stimulation using SIS and SIS+PEDOT interface materials, a 5 x15mm segment of SIS was placed along the distal length of the muscle. A 3 x 5 mm rubber gasket was placed underneath the SIS at the distal 1/3 of the muscle. Stimulation was again with a bipolar stimulating wire electrode with the tips placed on top of the interface material over the rubber gasket. Data

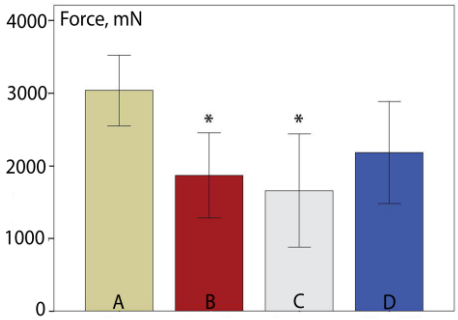

Figure 2. Maximal tetanic force, mN for A) NerveStim; B) DirectStim; C) DirectSIS; D) Direct PEDOT tests. Bars are mean +/- SD. * indicates different from A, p<0.05.

acquisition and recording were performed using LabView (National Instruments Corp., Austin, TX). Fresh, unused sections of SIS and SIS+PEDOT were tested with each individual rat.

D. Statistics

Data were analyzed using SPSS V17 (IBM, Baltimore, MD). Statistical significance for data of muscle force study was determined using a multivariate analysis of variance test. Significance was set a priori at $\alpha \leq 0.05$ and a Bonferroni correction factor for multiple comparisons was applied.

III. Results

PEDOT polymerization deposited a dark and evenly distributed coating of PEDOT which covered the entire active portion of the SIS. PEDOT polymerization reduced the porosity of the SIS when examined with transmission electron micrographs. On a macroscopic level however, the SIS+PEDOT remained flexible with a high wettability similar to uncoated SIS. The PEDOT coating remained uniform throughout ex-vivo bench testing and in-situ muscle force testing. Fresh, unused sections of SIS and SIS+PEDOT material were used for each individual rat.

Bare SIS was found to possess properties of a bio-electrical insulator with high impedance across the biologically relevant range of 1 to 10,000 Hz. Electrochemical polymerization of SIS with PEDOT reduced the impedance by an average of 3-5 orders of magnitude across the 1 to 10,000 Hz spectrum. SIS polymerized with a single layer of PEDOT had lower impedance than PBS soaked SIS alone. Thus electrical polymerization of PEDOT on SIS greatly improved electrical impedance while maintaining the mechanical properties.

The maximum specific muscle force production with NerveStim was significantly higher than for DirectStim ($p=0.04$) and DirectSIS groups ($p<0.02$). Maximum specific force was not significantly different between NerveStim and DirectPEDOT groups ($p=0.67$, power = 0.82). SIS+PEDOT materials more closely approximated the gold standard of stimulating through the nerve than SIS alone or direct stimulation of the muscle. Higher force production with nerve stimulation was expected since the peroneal nerve provides innervation to all of the motor fibers in the muscle tested. Electrical stimulation of the other three groups relied on ionic conduction throughout the extracellular fluid and t-tubule system of the muscle. Increased stimulation frequency corresponded to an increased mean muscle force production. NerveStim required significantly less voltage than DirectStim ($p=0.002$), DirectSIS ($p<0.001$), or DirectPEDOT ($p<0.001$) testing. The reduced stimulation required to reach maximal muscle force for NerveStim compared to the other three groups was expected as well. Threshold potential for stimulation of a nerve action potential is far lower than muscle stimulation due to salutatory conduction along peripheral nerve.

The total body mass of the rats tested varied considerably (range 310-440 grams). The mass of each EDL muscle tested varied by a proportional amount, (range 118-176 mg). Since each rat was exposed to each experimental group, the average EDL and body mass remained constant throughout experimental testing groups. Similarly, the average length of each muscle varied very little between both individual rats and experimental groups. Test order was randomized a priori to direct stimulation tests and was not a significant predictor of muscle force production ($p=0.28$).

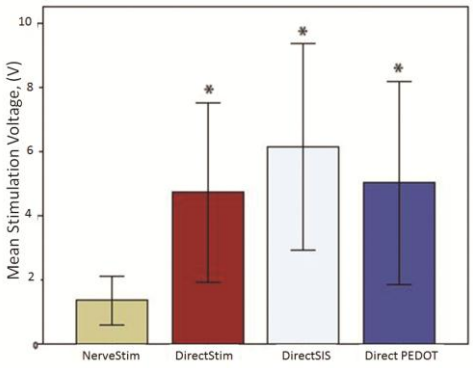

Figure 3. Voltage to ev0ke maximal tetanic force. Bars are mean +/- SD. * indicates different from A, $p<0.05$.

IV. Discussion

This study used muscle contractile force to indicate charge transfer from a stimulating electrode to an underlying excitable skeletal muscle. We included two control test situations. Activation of muscle contraction by stimulating the innervating nerve demonstrates the maximal measureable muscle force. Stimulation directly on the muscle indicates contraction of only the muscle fibers that are activated by spreading of the stimulating charge [22]. We used direct stimulation on SIS and SIS+PEDOT interface materials in contact with muscle tissue. We found that SIS+PEDOT distributed charge density to the muscle better than SIS alone as represented by differences in specific muscle force. Specific muscle force is muscle force normalized to muscle physiological cross sectional area. Thus the forces produced by large and small muscles can be compared.

Specific muscle force recorded when stimulation was directed through SIS+PEDOT materials closely resembled stimulation of the extensor digitorum longus muscle indirectly through the peroneal nerve than either SIS alone or direct muscle stimulation. This has important implications for future electrode design within the regenerative peripheral nerve interface [17]. The biocompatibility of SIS is very well established [24] the flexibility and electrical properties of SIS+PEDOT make it a promising choice for use in an RPNI. The SIS used in this study covered less than 50% of the total muscle area. Other geometric arrangements of the SIS as either a spiral or webbing might further help to distribute charge while maintaining adequate exchange of extracellular fluid and nutrients. Additionally the SIS+PEDOT material remained soft and pliable when placed on muscle tissue.

PEDOT has been explored by several groups for coating electrodes which are implanted in the central nervous system for long periods of time. They showed good short term improvement in recording fidelity for up to 6 weeks following implantation [24, 25]. Oxygen and sulfur

substitutions along the carbon backbone of the PEDOT molecule produce an insulated resonance pathway for ionic conduction along the molecular backbone. This ionic conduction helps bridge the differences between electrical conduction of the electrode and the ionic conduction of nerve impulses. It is also responsible for the high charge capacity of PEDOT. The electrochemical polymerization method used in this study has both excellent electrical characteristics and high biocompatibility but also relies on electrostatic adhesion to the electrode surface. This electrostatic adhesion is vulnerable to mechanical delamination. Although PEDOT delamination was not encountered in this acute study, delamination would present a potential problem for chronic applications. Other chemical polymerization procedures result in covalent bonding to the electrode substrate and show high PEDOT adhesion and retention out to 16 months. PEDOT's structure is similar to biological compounds such as melanin and the biocompatibility of PEDOT has been well established for acute implantation.

V. Conclusion

Electrochemical polymerization of SIS with PEDOT significantly reduced its impedance by three orders of magnitude. The SIS+PEDOT interface material distributed electrical stimulation more efficiently than SIS interface material alone. A conductive polymer interface material may allow ionic signal distribution within a RPNI at lower charge density.

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