Conduction Properties Of Decellularized Nerve Biomaterials

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Abstract- **The purpose of this study is to optimize poly(3,4,-ethylenedioxythiophene) (PEDOT) polymerization into decellular nerve scaffolding for interfacing to peripheral nerves. Our ultimate aim is to permanently implant highly conductive peripheral nerve interfaces between amputee, stump, nerve fascicles and prosthetic electronics. Decellular nerve (DN) scaffolds are an FDA approved biomaterial (Axogen™) with the flexible tensile properties needed for successful permanent coaptation to peripheral nerves. Biocompatible, electroconductive, PEDOT facilitates electrical conduction through PEDOT coated acellular muscle. New electrochemical methods were used to polymerize various PEDOT concentrations into DN scaffolds without the need for a final dehydration step. DN scaffolds were then tested for electrical impedance and charge density. PEDOT coated DN scaffold materials were also implanted as 15-20mm peripheral nerve grafts. Measurement of *in-situ* nerve conduction immediately followed grafting. DN showed significant improvements in impedance for dehydrated and hydrated, DN, polymerized with moderate and low PEDOT concentrations when they were compared with DN alone ($a \leq 0.05$). These measurements were equivalent to those for DN with maximal PEDOT concentrations. In-situ, nerve conduction measurements demonstrated that DN alone is a poor electro-conductor while the addition of PEDOT allows DN scaffold grafts to compare favorably with the "gold standard", autograft (Table 1). Surgical handling characteristics for conductive hydrated PEDOT DN scaffolds were rated 3 (pliable) while the dehydrated models were rated 1 (very stiff) when compared with autograft ratings of 4 (normal). Low concentrations of PEDOT on DN scaffolds provided significant increases in electro active properties which were comparable to the densest PEDOT coatings. DN pliability was closely maintained by continued hydration during PEDOT electrochemical polymerization without compromising electroconductivity.**

Keywords— **poly(3,4,-ethylenedioxythiophene), peripheral nerve, decellular nerve, nerve conduction.**

I. Introduction

Health care professionals are challenged with enabling stable biological interfaces to currently available prosthetic arm devices which are microprocessor controlled and power out fitted [1]. Ultimately we see amputees using the peripheral nerves remaining in their stump to both control these motorized prosthetics and receive feedback from sensors located in the prosthetics [2]. Our aim is to permanently implant highly conductive peripheral nerve interface (PNI) connectors between amputee stump nerve fascicles and prosthetic electronics.

The purpose of this study is to increase the fidelity of signal transmission across the PNI. Poly(3,4,-ethylenedioxythiophene) (PEDOT) is intrinsically an electrical conductor. Acellular muscle when polymerized with the maximal density of PEDOT is shown to have electrical conduction properties similar to copper wire [3]. However materials maximally polymerized with PEDOT acquire a brittleness which is incompatible with coaptation to living peripheral nerve.

Decellular nerve (DN) scaffolds are an FDA approved biomaterial used clinically to repair peripheral nerve defects (Axogen™). They are extremely pliable, sized appropriately in diameter, and can be easily sewn to peripheral nerve for long term attachment without breaking off or causing injury to the native nerve. We plan to optimize the process by which PEDOT is polymerized into DN scaffolding. We seek both: a) increased electrical conductivity through DN by testing various concentrations of PEDOT deposition and b) maintenance of a pliable DN after PEDOT deposition.

II. Materials and Methods

A. Overview of Experimental Design

Our purpose is to optimize the electrical fidelity and gain seen when PEDOT is polymerized on DN scaffolding while minimizing the sharp rigidity which accompanies highly conductive but compact concentrations of PEDOT

Hypothesis: We hypothesize that electrochemical deposition of PEDOT allows DN scaffolds to remain pliable while it confers improved electro-conductive properties to the scaffold.

Bench test and In-situ experimental designs: PEDOT can be deposited onto DN scaffolds by methods that either include dehydration steps (chemical method) [4] or allow scaffolds to remain continuously hydrated (electro-chemical method) [5]. Using each method, various concentrations of PEDOT were polymerized onto DN scaffolds. We tested the DN scaffolds with bench tests which measured impedance

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(fidelity) and cyclic voltametry to determine charge transfer capacity (gain in amplitude). Then, based on the bench tests, we selected the "best" concentrations for dehydrated and hydrated DN scaffolds and conducted *in-situ* tests for measuring nerve conduction properties (biological signal conductivity) .

Rat sciatic nerves were harvested at the University of Michigan and decellularized by Axogen™. The DN scaffolds were polymerized with PEDOT. A chemical polymerization method used an EDOT monomer (Clevios™ M, H.C. Starck, Coldwater, MI) and iron chloride as a dopant [4]. The DN scaffolds must be dehydrated for the PEDOT to adhere. EDOT solutions were made in low (Low), moderate (Mod), and high (High) concentrations which corresponded to the amount of PEDOT deposited. The electrochemical method for PEDOT deposition used a PEDOT polymer and polystyrenesulfonic acid (Clevios™ P, H.C. Starck, Coldwater, MI); DN scaffold dehydration was not needed [5]. Low and Mod concentrations of PEDOT were deposited using the method which allowed constant hydration of the DN scaffolds

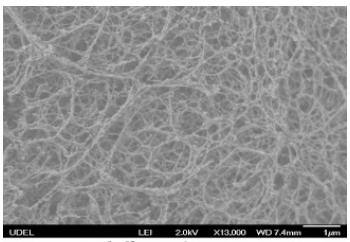

Figure 1. Fluffy PEDOT connections polymerized on decellular nerve (DN) by using an electro-chemical method.

B. Measurement of Test Material Impedance and Specific Charge Density

Electrical impedance spectroscopy (EIS) testing was applied to determine electrode impedance (Frequency Response Analyzer; Version 4.9.007; Eco Chemie B.V.; Utrecht, The Netherlands) and cyclic voltametry (CV) to determine charge transfer capacity (n ~ 4 per 9 groups) [General Purpose Electrochemical System, Version 4.9.007, Eco Chemie B.V., Utrecht, The Netherlands) [5]. Graphs were viewed using MatLab (Version 7.8.0.347 R2009a; MatchWorks, Inc). Materials tested were between 15 and 20 mm in length. Impedance values were sampled for frequencies of 10, 100, and 1000Hz. For CV, a scan rate of 10 mV/s was used and the potential on the working electrode was swept between -1.0 to 1.0 V. Specific charge density was calculated by dividing the charge transfer capacity by each sample's surface area (surface area of a cylinder).

C. Measurement of Nerve Conduction

For *in-situ* measurements, dehydrated (DPEDOT) and hydrated (HPEDOT) DN scaffolds were polymerized with moderate concentrations of PEDOT. Selection of the moderate concentration for further testing was based on favorable results from the bench tests. Five experimental groups were tested; these groups were: Intact nerve, Autograft, DN (hydrated as shipped frozen), DPEDOT, and HPEDOT. Using 10-0 nylon suture, DN scaffold materials were sewn to the ends of divided, rat, peroneal nerve as 15-20mm peripheral nerve grafts ($n \geq 5$ per 5 groups). Measurement of *in-situ* nerve conduction immediately followed grafting (Synergy T2X System,Viasys NeuroCare, Madison, WI). Stimulation was applied with a bipolar electrode placed on the nerve proximal to the nerve graft and as close to the sciatic notch as possible. Muscle electromyographic (EMG) responses were recorded with a needle electrode in the extensor digitorum longus muscle located distal to the nerve graft. Reference and ground needle electrodes were placed distal to the recording electrode [6]. Values recorded were EMG response latency, maximal amplitude, and spike area; as well as nerve conduction velocity, rheobase, and the stimulation amperage equal to 20% greater than that used to maximize EMG.

D. Graft Stiffness Rating Scale

Graft stiffness was rated using a scale from 4 to 0. A score of 4 meant the DN scaffold handled as native nerve; 3 = pliable, slight resistance to bending; 2 = rigid, resistant to needle insertion; 1= brittle, very stiff, cut the suture; and 0 meant a needle could not be placed through the material.

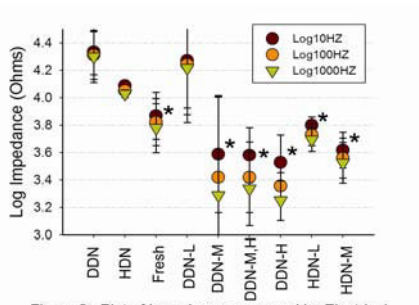

Figure 2. Plot of Impedance measured by Electrical Impedance Spectroscopy. * Indicates significantly different from dehydrated decellular nerve, a<=0.05. Abbreviation are: DN= decellular nerve, HDN = hydrated decellular nerve, DDN = dehydrated decellular nerve, L = low condentration of PEDOT, m = moderate concentration of PEDOT, h = high concentration of PEDOT, Fresh = fresh dissected nerve.

E. Animal Care and Compliance

Rats used were male Fischer-344 rats which were retired as breeders (Charles River Laboratory, Kingston, NY). All procedures were approved by the Institutional Animal Care and Use Committee of the University of Michigan and were in strict accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals [7]. For all surgical procedures, rats were given an analgesic (buprenorphene, 0.05 mg/kg) prior to anesthesia with sodium pentobarbital (65 mg/kg). All rats were euthanized with an UCUCA approved procedure.

F. Statistical Analysis

A one-way analysis of variance (ANOVA) was performed, followed by Tukey's *post hoc* test to determine significant differences between experimental groups in the bench test and in the *in-situ* studies. A p value with $a \leq 0.05$ was considered to be significant.

III. Results

Data indicate that deposition of PEDOT on DN scaffolds significantly lowers (improves) impedance across all the hydrated as well as all dehydrated DN materials when compared to dehydrated DN with the exception of the Low dehydrated DN scaffold (Fig 2). Specific charge density was increased (improved) only for the Mod dehydrated DN when compared with dehydrated DN (Fig 3.). Some PEDOT broke off the dehydrated DN scaffolds during EIS and CV testing. No charge density could be measured for three of seven scaffolds in the High dehydrated DN group. These zero scores, most likely due to PEDOT cracking and falling off, were included in the statistics and explain the drop in specific charge density seen for this group when compared with the Mod dehydrated DN group. The EIS and CV data taken together may indicate there was a ceiling effect on how much PEDOT was enough.

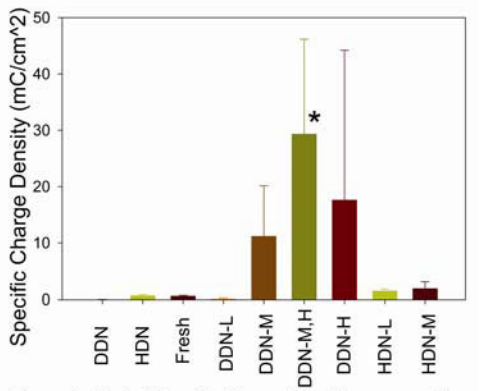

Figure 3. Plot of Specific Charge Density measured by Cyclic Voltametry. * Indicates significantly different from dehydrated decellular nerve, a<=0.05. Abbreviations are: DN= decellular nerve, HDN = hydrated decellular nerve, DDN = dehydrated decellular nerve, L = low condentration of PEDOT, m = moderate concentration of PEDOT, h = high concentration of PEDOT, Fresh = fresh dissected nerve.

In-situ, nerve conduction measurements demonstrated that hydrated DN scaffolds were poor electro conductors. While intact nerve was best, addition of PEDOT allowed grafted DN scaffolds to compare favorably with the "graft gold standard", autograft nerve (Table 1). Autograft, DPEDOT, and HPEDOT grafts did not vary from each other.for all EMG data (latency, maximal amplitude, spike area) and nerve conduction measurements (velocity, rheobase, and the stimulation voltage). Statistical power for these findings exceeded β=0.80. Though not significant, greater stimulation amperage is needed to initiate a twitch response (rheobase) and maximal response amplitude for conduction to pass through the DN scaffold graft with PEDOT when compared with the Autograft.

Surgical handling characteristics for the hydrated DN scaffold and the Autograft nerve were rated 4 (as native nerve). The highly conductive HPEDOT DN scaffolds were rated 3 (pliable). DPEDOT DN scaffolds were rated 2 (rigid). The dehydrated DN with High PEDOT group from the bench studies was rated 1 (very stiff). Polymerization of PEDOT by the electrochemical method allowed DN scaffolds to remain hydrated and therefore to behave almost like native nerve during surgery.

Our hypothesis said that electrochemical deposition of PEDOT would allow DN scaffolds to remain pliable while PEDOT conferred improved electro-conductive properties to the scaffold. The hypothesis was supported by most of the data. Bench test findings for impedance indicated that PEDOT does confer improvements in conduction fidelity and signal to noise ratio. I*n-situ* tests showed that PEDOT deposition on DN facilitated biological signal conduction across a nerve gap. However, our bench cyclic voltametry results did not show convincing improvements in charge transfer capacity (signal gain) for the PEDOT coated DN scaffolds. The electrochemical polymerization process did allow the DN scaffolds to remain pliable following polymerization with PEDOT.

VI. Discussion

Bench tests measured improvements in impedance for DN scaffolds polymerized with PEDOT. Lower impedance indicated the electrical signal has better fidelity or signal to noise ratio. Devices with low impedance generally have lower overall power requirements leading to extended battery life. Decreased impedance is a favorable quality for a PNI scaffold.

Specific charge density measurements did not increase significantly for all PEDOT coated DN scaffolds except for the moderately high coated dehydrated PEDOT DN group. Charge density is thought to determine charge transfer capacity or gain. A PNI needs to contribute some as yet unknown quantity of charge transfer. PEDOT is known to accumulate greater charge density because the fluffy PEDOT structure increases surface area (Fig 1). However, too much specific charge density could damage the native peripheral nerve. The meaning and benefits of charge density need further study.

DN scaffolding alone, although not a good electrical conductor, may be a fine base material for a PNI. Peripheral nerves grow through it and, though it is a xenograft mate-

rial, inflammation and immune response to it are minimal [8].

This is the first study to show that addition of PEDOT to DN allowed action potential type signals to pass across a 15 to 20 mm nerve graft. A 15 mm distance is the desired length for a PNI. Higher stimulation was needed to initiate a twitch response and a maximal response across the graft because signals are obstructed by scarring at two nerve to graft coaptation sites. Whether the "biologic like" signals across the graft were purely electrical, ionic, or a mixture of the two was undefined.

Table 1. Summary for In-Situ, Nerve Conduction Electro-diagnositic Properties Acutely Measured Across Coaptations of Decellular Nerve Interface Scaffolding (15-20 mm) in a Rat Peroneal Nerve Grafting Model.

Variables	Main Effect				
	Intact Control (n=27)	Autograft (n=4)	DN (n=5)	DPEDOT (n=8)	HPEDOT (n=8)
Latency (ms)	1.3 ± 0.2 a	1.6 ± 0.4 a	40.9 ± 53.9 b	1.2 ± 0.1 a	1.8 ± 1.0 a
Amplitude (mV)	16.7 ± 6.1 a	6.4 ± 3.7 b	3.6 ± 7.5 b	13.1 ± 6.7	7.2 ± 6.3 b
Velocity (M/s)	25. ± 4.2	22 ± 5.0	12 ± 18 b	36 ± 12 a	29 ± 18
Area 1-3 (mV*ms)	18 ± 7 a	7 ± 4	5 ± 12 b	13 ± 5	8 ± 7 b
Rheobase (mA)	0.22 ± 0.47 a	1.72 ± 2.47 a	55.5 ± 25.9 b	4.5 ± 4.18 a	2.52 ± 2.37 a (n=6)
Stimulation (mA)	1.15 ± 3.86 a	3.36 ± 5.09 6	21.20 ±11.78 b, d	11.42 ± 9.61 b	7.52 ± 4.52 (n=6)

Main Effect indicates type of interface coapted to the divided peroneal nerve. All grafts were 15-20 mm in length. 10-0 nylon suture was used for proximal and distal stump approximations to interface grafts. **Intact Control** = no peroneal nerve lesions or graft; **Autograft** = peroneal nerve section removed transposed 180^0 and grafted; **DN** = Decellular Nerve alone as interface; **DPEDOT** = dehydrated, PEDOT coated, DN, interface graft; **HPEDOT** = hydrated, PEDOT coated, DN, interface graft. Stimulation was applied at the sciatic notch at least 5 mm proximal to the proximal interface coaptation site.

Significance: a = 0.05. a – b, and c - d pairs indicate significant difference with Bonferonni adjustment for multiple comparisons. For all electroconductive variables, **Autograft, DPEDOT**, and **HPEDOT** groups did not vary from each other which are supported by ANOVA statistical power, ß > 0.80.

Abbreviations are: milliseconds (ms), millivolts (mV), meters/second (M/s), and milliamps (mA).

The bench studies and the *in-situ* nerve conduction studies indicated that moderate concentrations of PEDOT on DN were enough to reduce impedance and facilitate conduction through the DN scaffold. Reduced PEDOT concentrations along with the electrochemical deposition allowed the DN scaffold to remain pliable. Still slightly higher concentration of PEDOT in the hydrated samples should be possible. Implanting this DN scaffold as part of a PNI is a realistic goal. Pliability allows DNs to move with the peripheral nerve endings rather than breaking off or injuring surrounding tissues.

This study was an acute *in situ* study and, therefore, carries certain limitations as the DN scaffolds were not maintained in-vivo as true PNIs would be. One cannot predict whether biological defenses would lead to degradation or encapsulation of the DN PNI materials without running a long term implant study.

V. Conclusions

Low concentrations of PEDOT on DN scaffolds can provide significant increases in electro active properties which are comparable to maximal High PEDOT coatings. DN pliability is closely maintained by continued hydration during PEDOT electrochemical polymerization without compromising electro conductivity.

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Conflict of interest statement: No potential conflicts of interest are disclosed.

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