DESIGN AND EVALUATION OF AN IMMNUNOEVASIVE CARBON NANOTUBE SHEET-COLLAGEN COMPOSITE NEURAL ELECTRODE

by

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Abstract

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Currently, long term neural interfacing is hindered by instability of the neural electrode-tissue interface. Many modes of electrode failure have been reported, though the tissue response to the foreign electrode body is commonly recognized as a major contributor. The encapsulation of electrode by fibrous tissue and a progressive inflammatory reaction leads to local neurodegeneration and isolation of the electrode from the electrically active tissue. To address this mechanism of failure, a new electrode material is presented and evaluated that uses low immunogenic carbon nanotube sheets that have been shown to be non-cytotoxic and promote neural growth as nanoscale electrode sites. It is proposed that because immune response on this scale typically occurs by molecular recognition, reducing the size of the site of charge exchange with the tissue to the nano-scale may potentially avoid the pathways that lead to the formation of an electrode isolating fibrous capsule. The design, characterization, and *in vivo* testing of such an electrode are presented.

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Chapter 1

Introduction

Neural Interfaces

A neural interface describes a combined system of hardware and software that records or stimulates electrically active nervous tissue via an implanted electrode to exchange data between the physiological state and the system. These implanted electrodes can sense changes in the electrical potential of the tissue corresponding to action potentials being propagated through the tissue. This translates into the ability to monitor and interpret the communications and information processing occurring within the brain or between the central nervous system and the rest of the body. Conversely, by rapidly altering the potential between two electrodes current can be injected to stimulate neural activity and elicit a physiological response or sensory perception.

The clinical implementation of neural interfaces may enable a number of bio-electronic based therapies, and provide for brain-machine/brain-computer interfaces to drive a new generation of neural prosthetics.[6] Neural controlled prosthesis provides a hitherto unavailable option in prosthetics for debilitating conditions including spinal cord injuries, paralysis, and robotic limbs for amputees. Bio-electronic medicine is a growing field of research interest, which functions under the principle of targeted neural stimulation to treat a broad range of conditions by using modulated peripheral nerve activity to regulate organs and functions distributed throughout the body.[34] Currently, neural electrode-tissue interfaces exhibit a characteristic loss of electrical contact with the nervous tissue that limits recording potential in long-term studies, and requires increasingly high stimulation amplitudes to elicit consistent physiological responses over the course of the study.[12,23,30,32,38,40,42,43] However, the future of bioelectronics based

therapies and brain-machine/brain-computer interfaces depends on overcoming this limitation to achieve reliable interfacing via neural electrodes.

Chronic Neural Interfacing

Many studies have explored the stability of chronic neural interfacing using currently available electrode technology.[7,31,42,43] Multi-site silicon probes and microwire arrays are the predominant platforms used for neural interfacing studies.[14,16,38,40,43] Both configurations perform well during short term studies (less than 1 month in vivo). However, all metal electrode-tissue interfaces have been reported to exhibit increasing failure rates of electrode channels over the course of longer duration studies and changes in electrode-tissue impedance. A study evaluating the UTAH microwire electrode array for chronic recordings stability reported after 6 months only 36.6% of the channels were recording activity and that none of the implanted units remained functional throughout the entire course of the 13 month study, with some devices losing and regaining functionality sporadically.[32] Silicon probes have been reported to have similar failure rates in long-term studies.[7] These high rates of failure limit the clinical translation of the current neural interface systems, since the application of the neural interface is compromised if none of the remaining functional electrode channels can provide the desired physiological function. This being the case, many systems are still able to obtain signals that can be used to drive neural prosthesis with limited degrees of freedom, even though the signal may not correlate with the intended action. For example, an electrode array implanted in the motor cortex may still be able to drive a robotic arm even though the patient reports thinking about moving a different part of their body to produce the action.

Many modes of electrode failure have been reported; however, there is a consensus in the literature that the progressive inflammatory response of the tissue

caused by the presence of the electrode material, also known as the foreign body response, contributes to the instability of the interface in long-term studies.[25,31,43] This process is characterized in both the peripheral and central nervous tissues by the formation of encapsulating fibrous tissue around the electrodes.[1,19,40] This capsule may cause increased impedance to charge exchange with the neural tissue by limiting the diffusion of charge carrying species to the electrode's surface and also increases the distance to the cellular processes of local neurons.[25] This response has been suggested to be a result of the combination of the trauma to the tissue by electrode insertion, mechanical mismatch between the nervous tissue and the hard electrode material, and a more generalized inflammatory response due to the recognition of a foreign body by the resident immune cells that accumulate at the site of insertion.[31]

The inflammatory foreign body response has been studied extensively in neural interface literature and spinal cord injury research.[37] Primarily, two cell types are implicated in the process of the central nervous system: microglia and astrocytes. Microglial cells are the resident phagocytic cells of the central nervous system and are attracted to sites of injury to degrade and remove foreign materials or pathogens. They are present during the early phase of the wound healing process following implantation and the number of microglia can be considered an indicator of the magnitude of the fibrous encapsulation that will occur during the later stages. Astrocytes are present during all stages of the wound healing.[19,30] Astrocytic cells form the encapsulating layer around the electrode characterized by dense fibrous proteins and will progressively isolate the electrode from the neuronal population. The astrocytic process is generally referred to as reactive gliosis or glial scarring. It is believed that this fibrous tissue is responsible for the high rates of electrode failure by limiting the access of ionic charge carriers to the electrodes surface and physically isolating the electrode from the electrically active

tissue.[23,30] Following the first few weeks of wound healing the distance to the neuronal cell bodies may increase by up to 60 μm.[7] At this distance isolating individual action potential signals from background noise and multi-unit potentials is extremely difficult.

Design Approaches for Chronic Neural Interfacing

Material science based approaches have been attempted to minimize the adverse tissue reaction and local neuronal cell death. These studies have suggested that mechanically compliant materials and nano-featured surfaces may mitigate the tissue response.[13,15,20,28] Furthermore, the response has been shown to be dependent on electrode geometry and size; reduced material diameter is directly correlated with a reduced tissue response.[35] Additionally, related studies have shown that incorporating anti-inflammatory compounds or growth factors to be released in a sustained manner at the electrode site can utilize molecular biology to manipulate the response, though these methods have not yet shown substantial improvements in chronic recordings.[17,47]

The mechanical stiffness of electrode materials has also been implicated as a contributor to the inflammatory response at the site of implantation.[2,15] Trauma during insertion and shifting of the material with respect to the tissue causes damage to the cells that initiate inflammatory processes and exacerbate scar tissue formation.[19] Nervous tissue of the cortex has been reported to have stiffness in the range of 0.5 to 1 kilopascals.[10,39] Previous work by Capadona et al. developed a mechanically responsive electrode substrate that softened upon implantation to reduce chronic mechanical mismatch.[2] The goal of the work was to use a substrate much thicker than the recording metal electrode that was stiff enough to penetrate the meninges protecting the brain's surface when dry, but that softened upon implantation to evaluate the role of the material's elastic modulus in the tissue response.[2]

Carbon nanotube based materials have gained substantial interest in neural interface and neural cell culture studies due to their unique capacity to enhance neural growth and electrical activity in culture.[3,9,36,44] Carbon nanotube immobilized materials have previously been demonstrated to be uniquely biocompatible and have low immunogenicity.[8,29] Nayagam et al. demonstrated that a biopolymer substrate with aligned and immobilized CNTs showed no substantial increase in foreign body response when compared to negative controls and minimal surface fouling.[29] Furthermore, it has been demonstrated that the functionalization of microelectrode tips with electrodeposited CNTs both improves the Signal-to-Noise Ratio of recordings and reduces the impedance, likely by increasing the available surface area for charge transfer; the biocompatibility of such surface treatments has also been reported to be superior to metal electrode.[18,22,24,27,33,41,46]

Zhang et al. developed a macroscale material based on aligned sheets of carbon nanotubes. These sheets have high tensile strength, anisotropic conductivity in the direction of fiber alignment, a very large aspect ratio derived from their nanoscale topography, and have dimensions of both the individual fibers and the thickness of the sheet in the nanoscale.[45] The high aspect ratio is ideal for charge transfer in an aqueous medium because the surface area available is much larger. Most importantly, the macro-material nature of drawn Carbon Nanotube Sheets (CNTS) allows for them to be easily incorporated in the fabrication of new devices and sensors since they can be handled in this form. Previous work with CNT-metal interfaces have provided methods for developing reliable, covalent, electrical bonding between certain transition metals and CNTs using thermal annealing or current welding approaches.[4,5,21,46]

Research Objective

Based upon an understanding of the literature and proposed mechanisms of neural electrode failure it is hypothesized that a low-immunogenic material with dimensions of the electrode in the nanometer scale and which minimally disrupts the surrounding tissue by being mechanically compliant may be able to avoid complete encapsulation by reactive gliosis and potentially avoid or reduce the proposed mechanisms of electrode failure.

The work presented is the design and evaluation of such an electrode that attempts to address both the phenomenon of fibrous encapsulation and of the mechanical mismatch between nervous tissue and conventional metal electrodes. The electrode has been designed to have a CNT sheet extending into the surrounding tissue with charge exchange occurring through the sheet. The CNT sheet is embedded in a hydration-responsive, softening collagen film that degrades during the tissue remodeling phase of the wound healing process. The CNT sheet electrode fabrication and characterization, histological evaluation of the material, and its demonstration in electro-physiological recording are presented in the following.

Chapter 2

Design of an electrode to avoid tissue response related failure

Design of a novel Carbon Nanotube Sheet-Collagen composite material

To address the modes of electrode failure that have been hypothesized, a new material was developed. Using aligned Carbon Nanotube Sheets (CNTS) embedded in Type 1 Collagen film a conductive material with a low modulus under wet conditions was created. The material can be seen in Figure 1. Embedding the CNTS into the collagen film substrate immobilizes the CNTS and prevents it from aggregating upon exposure to aqueous solution. The collagen acts as a delivery vehicle for the conductive CNTS fibers; the non-crosslinked collagen film will biodegrade quickly following implantation leaving only the extended nanoscale fibers within the tissue.[11] The extended geometry of the CNTS away from the known immunogenic metal electrode provides for a conductive material that may be beneath the size limit to be encapsulated individually. At this scale the immune system responds to foreign substances and pathogens using cellular recognition; however, pristine CNTS are comprised of a sp2 hybridized carbon structure not known to be antigenic.[48] Thus it is hypothesized that a material with these properties could both function as a neural electrode material and evaluate two proposed modes of electrode failure in chronic recordings.

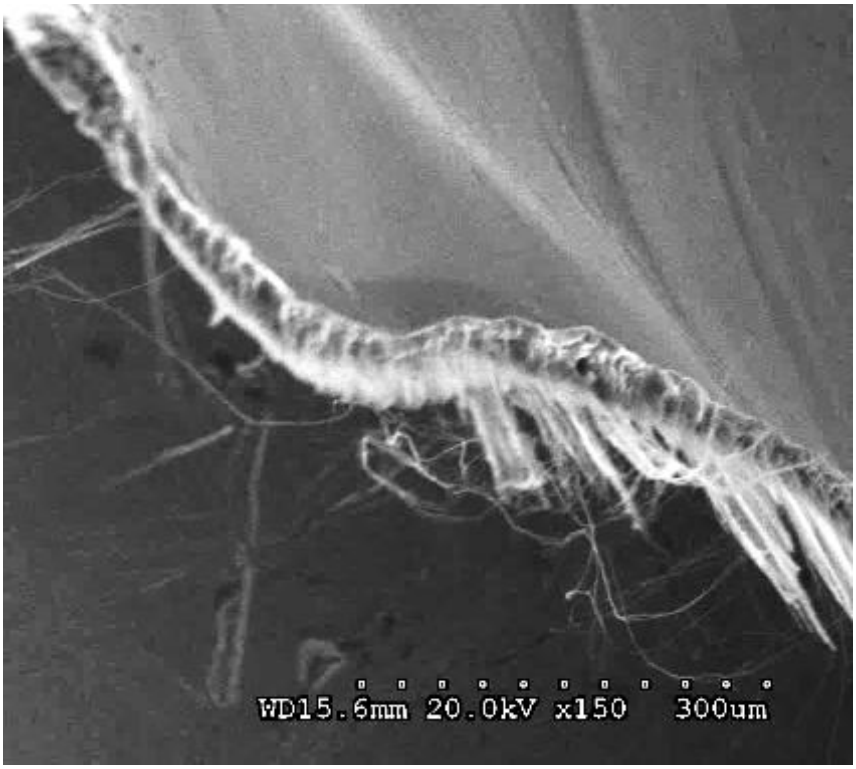

Figure 1 SEM picture of carbon nanotube sheet-collagen composite

Carbon Nanotube Sheets

The CNT sheets used were synthesized by a catalytic vapor deposition method developed by Zhang et al. and were provided by Dr. Ray Baughman of the NanoTech Institute at the University of Texas at Dallas.[45] These sheets have anisotropic conductivity higher in the direction parallel to the fibers. Additionally, the sheets can be considered "pristine" meaning that they have no trace metal catalyst contaminants. Sheets used in the formation of the CNTS-collagen composite were drawn from the as synthesized "forest" using a pair of adjustable platforms to draw the aligned CNT sheets horizontally from the wafer on which they were synthesized. This process can be seen in Figure 4

Type I Collagen Film

Collagen film was formed on the surface of Polydimethylsiloxane (PDMS) by a solvent casting method using lyophilized collagen dissolved in dilute acetic acid. The collagen was extracted from rat tail tendons of Sprague-Dawley rats. The tendons were manually pulled using tissue forceps from surgically removed rat tails and transferred to an ice cold 1xPBS solution for a thorough rinsing to remove blood and other biological contaminants. Next a 5 min wash in ice cold acetone was used to make the tendons more fragile and further wash away impurities. The treated tendon fibers were mixed in a 0.02% acetic acid solution at 4 °C for 24 hours to allow collagen to dissolve into the solution. Finally, the collagen solution was frozen and lyophilized to obtain Type I collagen sponge.

Lyophilized Type 1 collagen protein as extracted from the tail of Sprague-Dawley rats was mixed into a solution of 0.02% acetic acid to a concentration of 6 mg/mL of collagen and centrifuged to remove impurities and large aggregates. 200 µL of the solution was dispersed onto an area of 3 cm x 3 cm on the surface of a piece of cured PDMS. The film was allowed to dry to a thickness of approximately 3-5 µm. The length of PDMS was chosen to be of a size that it could accommodate the length of the wire or electrode, with the de-insulated tip to be functionalized with the CNT sheet laying on the surface of the film.

Polydimethylsiloxane Preparation

Polydimethylsiloxane was used as a substrate for adhering the collagen-CNT film composites to. This material worked well for this purpose because it is optically transparent which made imaging of the work possible, the hydrophobic surface allowed the film to adhere and remain flat during fabrication but allowed for easy removal when the electrode fabrication was complete, and finally the polymer is easily to handle and

prepare in small batches. PDMS is a commonly used polymer in many biomedical labs due to its lack of toxicity and is useful for fabricating implantable devices and cell culture devices.

PDMS substrates used in this research were prepared using Sylgard 184 (Dow Corning) by the standard methods as recommended by the manufacturer. The prepolymer solution was mixed with the curing agent in a 10:1 ratio. The mixture was mixed vigorously, degassed in a vacuum chamber of approximately 20 in mmHg. Next the mixture was transferred to a glass dish and cured on a hot plate at 150 C° for 10min. Finally, the cured polymer was cut to the desired size for the experiments described herein.

Cell culture

To test the biocompatibility of the new material, cell culture using the CNT-collagen composite sheet as a substrate was performed. This test demonstrates the relative toxicity of the material on neuronal cells. For the material to be useful in a neural electrode design, it must allow for the healthy growth of cells.

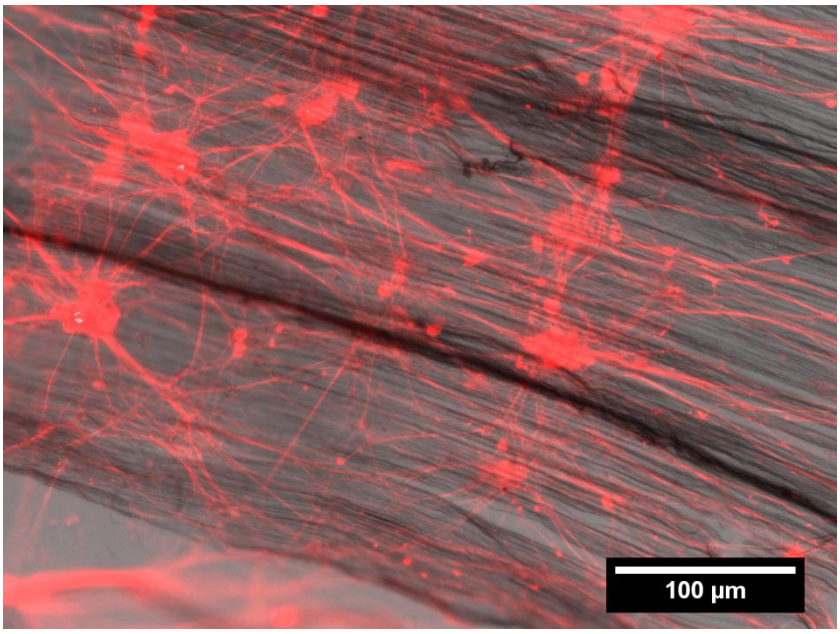

Figure 2 Superimposed image showing robust neuronal cell growth on CNTS-collagen substrate.

E18 rat cortical neurons were cultured using the material as a substrate for neuronal growth. The sheets were adhered to glass cover slips by wetting the surface of the glass with water before placing the CNT-collagen sheet and allowing it to dry completely to adhere the collagen to the glass. This resulted in a semi-permanent bonding of the collagen to the glass. Control cultures were performed with collagen only and PDL coated glass coverslips. The coverslips were seeded with the cells disassociated by Trypsin EDTA for a total density of approximately 100,000 cells per coverslip. Culture was performed at 37 °C, 5% CO_2, in Neural Basal Media supplemented with B-27.

Figure 2 shows robust growth of the neurons cells using the CNT-collagen sheet as a substrate. The growth and interconnection of cellular processes is typical of healthy

cell growth in the absence of a toxic material. From this study, it is concluded that the material may be viable to use for implantation in close contact with host neuronal tissue.

Electrical Bonding

To provide reliable electrical bonding between the conductive CNTS and the electrode, voltage controlled resistive welding was performed by passing current through the CNTS-metal interface. Initial experiments showed that without a bonding method the CNTS loses contact with the metal following periods of hydration exceeding one hour. This was indicated by the return of electrochemical impedance to untreated levels.

Previous literature has shown that permanent covalent bonding between carbon nanotubes and transition metal such as titanium and tungsten can be achieved by bringing the carbon nanotubes in contact with the metal to a high temperature. The work in this area has shown that at sufficiently high temperature, tungsten carbide heterostructures are formed at the interface between the carbon nanotubes and the transition metal. To achieve high temperatures at the interface, current can be passed through the CNTS and metal interface.[5] The resistance of the electrical circuit is highest at this point and thus the most heat will be generated at the material interface.

Specifically, a 3 second 5 volt monophasic pulse was used to form the bond between the composite sheet and the electrode. For the tungsten microwires with laminated CNTS on the metal it was observed that the resistance of the sheet-electrode circuit fluctuated randomly or was measured as an open circuit value; however, following the bonding process the resistances remained at 39.10 kohms $\pm$ 9.99 kohms (n=3). The importance was further validated by testing the electrochemical properties of the electrode in wet conditions. The electrodes were stable over multiple charge injection cycles when bonding was performed, but showed progressive loss of performance without bonding.

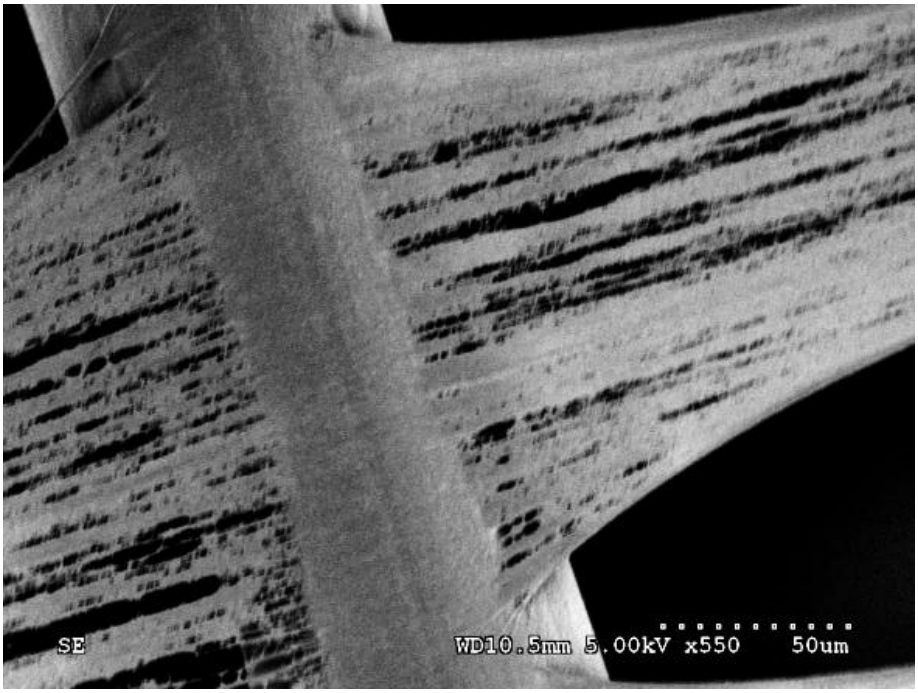

Figure 3 SEM image of CNTS resistively welded to tungsten microwire

Figure 3 shows a Scanning Electron Microscopy (SEM) picture taken of a tungsten electrode with CNTS bonded to the tip without collagen film. A 5nm thick layer of silver was sputter deposited on the sample to enhance the SEM contrast. The individual fibers of the sheet have been obscured by the silver layer; however a distinct interfacial layer between the CNTS and the tungsten can be visualized. This intimate contact is crucial for voltage transduction back to the metal electrode and out of the body. Perhaps most importantly, a mechanically stable bond enhances the overall robustness of the electrode design.

Fabrication of a CNTS-collagen composite electrode

Two types of electrodes were used to evaluate the technique: 25 µm diameter tungsten wires insulated with 1 µm of vacuum deposited parylene-c coating made in the laboratory setting with a de-insulated length of approximately 20µm generated using laser

ablation and commercially purchased tungsten microelectrodes (Microprobes for Life Sciences) with sharpened conical tips tapering to 1 µm at the point. The wires used were mechanically more stable to handle during fabrication and surgeries; however, the commercial electrodes were also used to demonstrate the versatility of the technique.

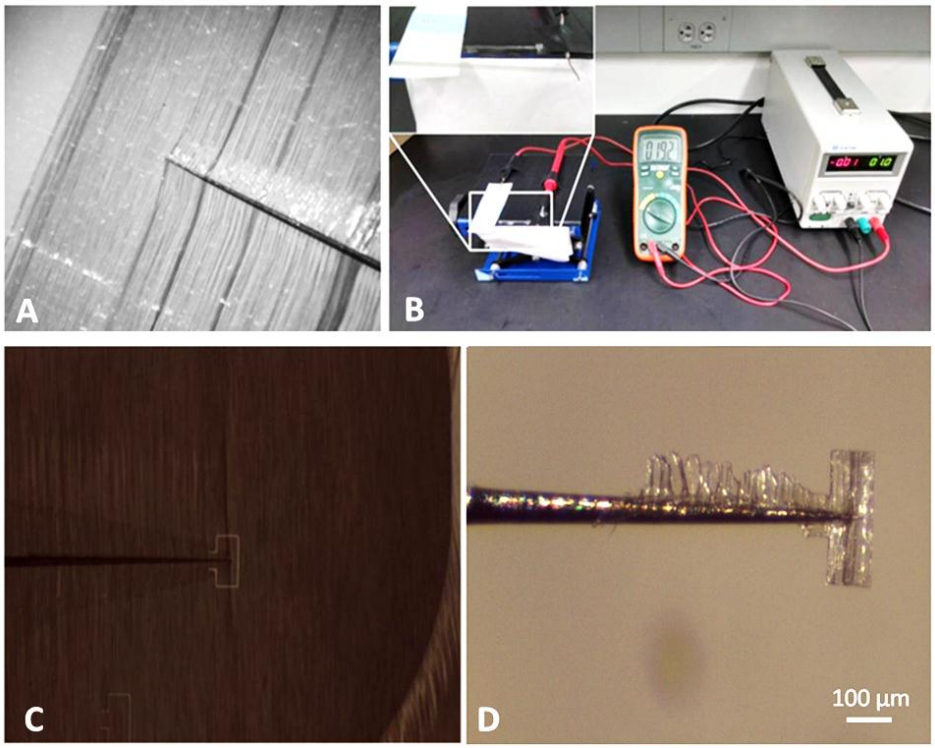

Figure 4 Schematic of electrode fabrication process. A)CNTS laminated on electrode tip and collagen substrate B) Resistive welding setup C) Laser micromachining D) Free standing electrode

To functionalize the wire with the CNT-collagen sheet, a four step process was performed to 1) form the collagen film on PDMS substrate 2) apply and densify the CNTS onto the electrode and collagen film 3) perform the resistive welding process and 4) laser micromachine the desired electrode geometry from the composite sheet. An electrode was placed with the de-insulated tip on prefabricated collagen film adhered on a PDMS

substrate. The sheets were drawn over the tip of the electrode, lowered, and laminated on to the surface of the collagen film. This was repeated 3 consecutive times to form a layer of CNT-sheet with three times the density of a single drawn sheet (Figure 4A). The CNT sheet, collagen, and the substrate were flooded with 200 proof isopropyl or ethyl alcohol to bring the sheet into maximum contact with the metal, to embed the CNTs into the collagen layer, and to reduce the sheet thickness into the nano-scale.[45] After exposure to isopropyl alcohol, the sheets have been collapsed or “densified” to a thickness of approximately 150 nm on the surface of the collagen. To perform the resistive welding and to confirm charge transfer through the CNT sheet to the metal, a 3 second 5 volt monophasic pulse was passed through the CNT sheet-metal interface to bond the CNT sheet to the wire (Figure 4B). Finally, a flag-like structure was laser micromachined (Spectra-Physics) at 355 nm 725 µW per cm^2 from the resulting composite material formed at the electrode tip (Figure 4C).

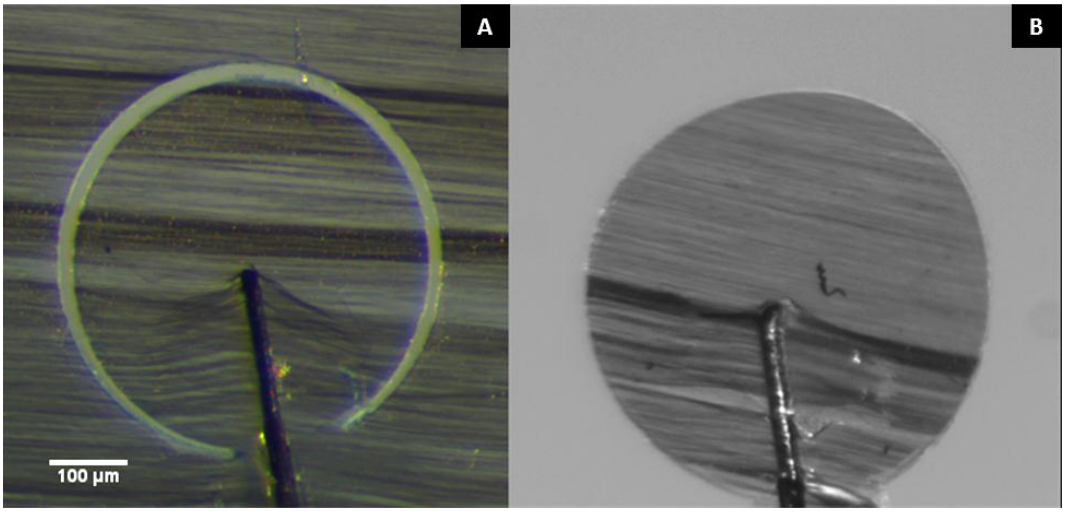

Figure 5 Representative images of laser micromachined CNTS-collagen composite on microwires

Electrochemical Characterization

To evaluate an electrode's electrical performance in a wet environment, a series of electrochemical measurements must be performed. Charge transfer through an electrolyte solution utilizes capacitive mechanisms as well as ionic charge carriers to pass current through the solution. In neural stimulation scenarios, the majority of current exchange happens through faradaic mechanisms (by ionic charge carriers in solution). This involves the reaction of particles in solution with excess electrons at the metal-fluid interface. Therefore, the current is limited by the kinetics of the reaction, mass transport of the charge carrying species, and voltage applied to the electrodes. These phenomena can be represented as a series of resistances and capacitances that result in the electrode material specific charge transfer characteristics and the phase delay seen in the current conduction.

In the neural interface literature, two characteristic tests are typically run to evaluate a potential neural electrode. Electrochemical Impedance Spectroscopy (EIS) determines the relationship between signal frequency and the electrode-electrolyte impedance. This defines how sensitive the electrode is to detect signals of varying frequencies. Relevant neuronal frequencies can range from 1 Hz to 1KHz, therefore the electrode must be able to detect signals across this frequency spectrum of very small amplitudes.[26] Cyclic Voltammetry (CV) measurements give an indication of how an electrode behaves over a charge exchange cycle with the solution. To prevent irreversible faradaic reactions at the surface of the neural electrode, the charge injected by current controlled pulses must have balanced positive and negative polarities. This prevents the accumulation of potentially toxic redox byproducts. Ideally, an electrode material should be able to inject the maximum amount of charge by reversible processes (capacitive and reversible faradaic). Plots of CV measurements show how much charge

can be injected within a window of safe voltages to prevent hydrolysis of water and tissue damage, and they also show how reproducible the charge injection is. The integrated area of the curve within the potential range can be considered to be the electrode's effective charge storage capacity.[26]

Electrical Impedance Spectroscopy and Cyclic Voltammetry readings were taken using a CH Instruments potentiostat in order to evaluate the fabricated electrode's viability as a neural electrode. Triplicate measures were taken for each both non-functionalized wire and microelectrodes and wires/electrodes with 300 µm CNT-collagen flags. Measurements were taken in 3M NaCl. Graphs of the electrochemical study results were generated in Microsoft Excel.

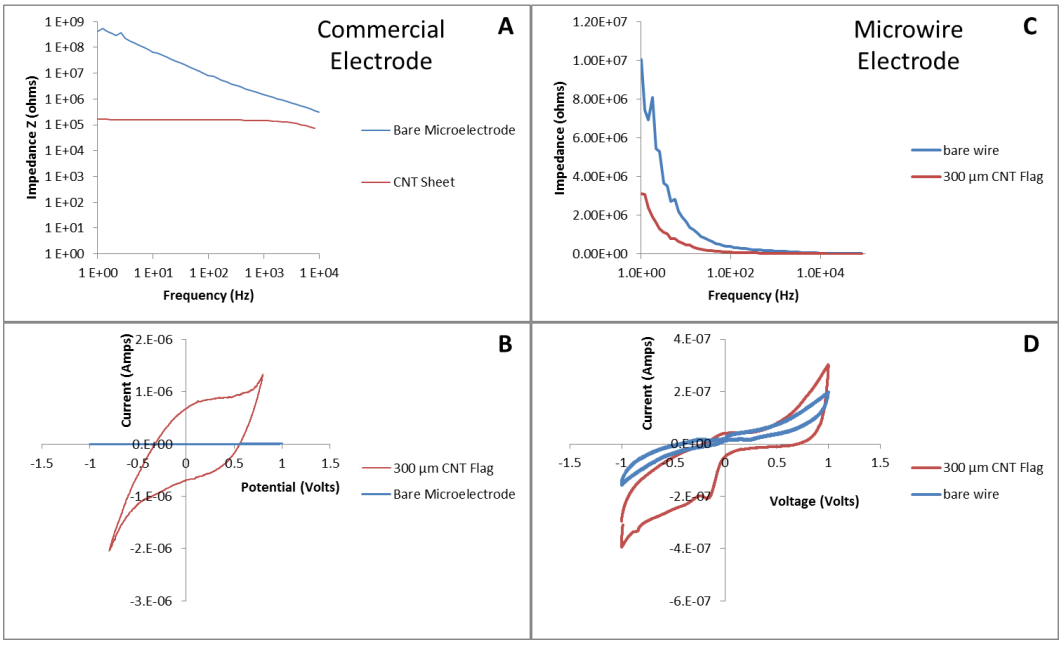

Figure 6 Electrochemical Characterization A) & B) Electrochemical Impedance Spectroscopy of commercial electrodes and microwires untreated and functionalized with CNTS-collagen composite C) & D) Cyclic Voltammetry of commercial electrodes and microwires untreated and functionalized with CNTS-collagen composite

Figures 6A and 6C show EIS results for both commercial microelectrodes and microwires functionalized with the CNTS-collagen composite compared to untreated electrodes. In both cases, the treated electrodes showed significantly reduced impedance at 1kHz ($n=3$ $p<0.05$). In the case of the commercial electrode the sheet showed a remarkable lack of frequency dependence, indicating most of the impedance was derived from resistive contribution as opposed to capacitive. In the case of the microwires, a greater surface area of metal was exposed in the untreated case. This resulted in the untreated impedance being two orders of magnitude less than the commercial electrode. Correspondingly, the reduction was less dramatic in this case. Additionally, it was noted that there was substantial frequency dependence in the case of the microwire. The results can be interpreted to mean that the reduced impedance increases the sensitivity of the electrodes and that the lack of frequency dependence may be advantageous in recording various different frequencies of brain waves. It should be noted that while the impedance is reduced, the size of the recording site is also increased which reduces the selectivity of the electrode to record from small populations of neurons.

Figures 6B and 6D show CV results for both electrode models. The plots include only one representative cycle for clarity; however, it was observed that the charge cycles were consistent past 10 sweeps. The integrated area of the contour represents the charge storage capacity and is a graphical representation of how much charge can be injected over a reversible cycle. As is apparent from the figure, the increased surface area of the sheet increased the charge storage capacity substantially. From these results it can be concluded that the CNTS-collagen composite significantly improves the stimulation capabilities of the electrode.

Atomic Force Microscopy

Atomic Force Microscopy is a method that can be used to measure the bulk elastic modulus of a material. Atomic Force Microscopy utilizes a cantilever bar with a sharp pyramidal or conical tip on the end of the bar that is brought into contact with the substrate being examined. The spring constant of the tip is known and matched to the expected modulus of the substrate so that the deflection of the bar is proportional to the force applied (by the constitutive equation for a spring, Hook's Law). The position of the cantilever bar can be precisely monitored using a laser and set of photodiodes to track the angle of laser reflection. This method can provide information on the elastic modulus or stiffness of a sample, various small forces such as surface tension or magnetic forces, and can also be used to "scan" the surface of a substrate to generate a topological profile.

These measurements indicated the mechanical compliance of the sheet to allow for comparison with native brain tissue. To address the role of mechanical mismatch between the host tissue and the electrode, the material should have a small difference in mechanical stiffness from the surrounding host tissue. If the material modulus is close to the tissue modulus it can be assumed that mechanically induced inflammation, excluding the initial trauma of implantation, can be neglected as a cause for electrode-tissue interface phenomena.

Atomic Force Microscopy (nanonics) measurements of the bulk modulus for the CNT-collagen sheet composite were taken for both dry and wet samples. The wet measurements were taken using samples that had been hydrated in 1x phosphate buffer solution for 24 hours. The nominal spring constant of the tips used for the wet samples was 0.07N/m (Bruker MLCT) and the spring constant for dry was 1.6N/m (Appnano).

Table 1 shows the values for the CNT-collagen composite film under hydrated and dry conditions. The values show that not only is the film responsive to hydration by softening, but that the modulus under wet conditions approaches that of the native tissue. The wet modulus differs by only about 1 order of magnitude when compared with estimates of cortical tissue modulus in the literature of 1 kPa.[39] This indicates that the material does well to match the material properties of the tissue. Thus, it is expected that the mechanical properties of the material will not be a significant factor in the tissue response elicited during implantation.

Table 1 Atomic Force Microscopy measurement of elastic modulus

Cortical Brain Tissue	0.5 to 1 kPa
Dry CNT-Collagen Film	716 $\pm$ 217.7 kPa
Hydrated CNT-Collagen Film	111 $\pm$ 22.6 kPa

Chapter 3

In vivo Characterization

Recordings

Recording capabilities of the CNTS-collagen flag electrode were confirmed by recordings taken from the surface of the M1 motor cortex of SD rats. N=3 rats were used to take recordings from 300 μm^2 CNT flags compared to non-functionalized tungsten wires. All surgeries were performed according to an approved Institution Review Board protocol. Data analysis of the recording data was performed in Neuroexplorer 4 and the graphs for power spectral densities were generated in MATLAB version 7.0.4.

The quality of recordings taken, the sensitivity of the electrode in detecting neural activity, can be evaluated by considering the amplitude of signal voltages compared to noise. If the electrode being tested is able to respond to a wide range of physiologically relevant frequencies (up to 1 kHz), then the electrode can be feasibly implemented. If the signals can be acquired at a higher power, then the electrode can be considered to be more sensitive. Power Spectral Densities (PSD) of the recordings are shown in Figure 7. The PSD is a graphical representation of the relative signal power of the frequency components detected during a given period of recording. Higher power indicates that signals at the given frequency are detected with greater amplitude.

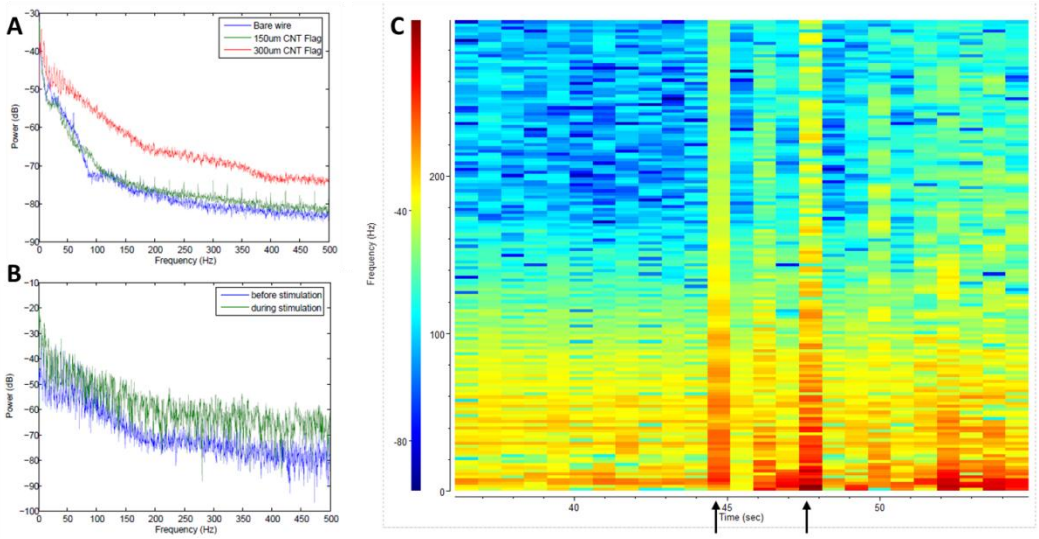

Figure 7 Cortical recording data analysis A) Power Spectral Density comparison of electrodes B) Power Spectral Density before and during stimulation C) Spectrogram of recording with stimulation (black arrows indicate contralateral hindlimb stroking)

Figure 7A is a PSD calculated using a 2048 point Fast Fourier Transform algorithm in the software package Neuroexplorer. The plot shows increased signal sensitivity across the entire frequency spectrum. This demonstrates the effect of reduced impedance by two different flag sizes on tungsten microwire. The 300 μm^2 was shown to have the highest power across the entire spectrum.

Figures 7B and 7C both show the effect of contralateral hindlimb stroking on the PSD. The PSD shown in Figure 7B shows a PSD calculated for 5 seconds before stimulation and 5 sec during stimulation. The increased power shows the signal being recorded is derived from neural activity. Figure 7C shows a spectrogram depiction of the recording. For this analysis a 256 point FFT was performed with each bin corresponding to 0.75 seconds. The black arrows indicate contralateral hindlimb stroking, note the frequency response of neural activity across the entire spectrum is localized to those time points.

Analysis of cortical recording showed substantial differences between functionalized and non-functionalized electrodes (Figure 7A). This indicates that signal was transmitted through the CNTS in the functionalized case. Results from recordings before and during stimulation further corroborate that signal characteristics are derived from neuronal activity (Figures 7B and 7C).

Cortical Implants

To evaluate the immunogenicity of the CNT sheet collagen composite material 1mm x 1mm pieces of the film were implanted into the cortex of 3 Sprague-Dawley rats. Implants were placed by drilling four equilaterally spaced holes approximately1 cm lateral to the midline and 1 cm to either side of transverse plane that along with the midline divided the surface of the cranium into four quadrants. Control implants of collagen were randomly assigned to two of the locations per animal. The dura mater was removed from the exposed area prior to implantation and the composite films were introduced by inserting approximately 2-3mm deep. After 14 days, the animals were sacrificed, the tissue was fixed with paraformaldehyde, and the entire brain was embedded in paraffin. Transverse sections were taken 7 μm thick and stained with DAPI, anti-Beta 3 tubulin, and ED1 using standard immunostaining methods. All surgeries were performed according to an approved Institutional Review Board protocol.

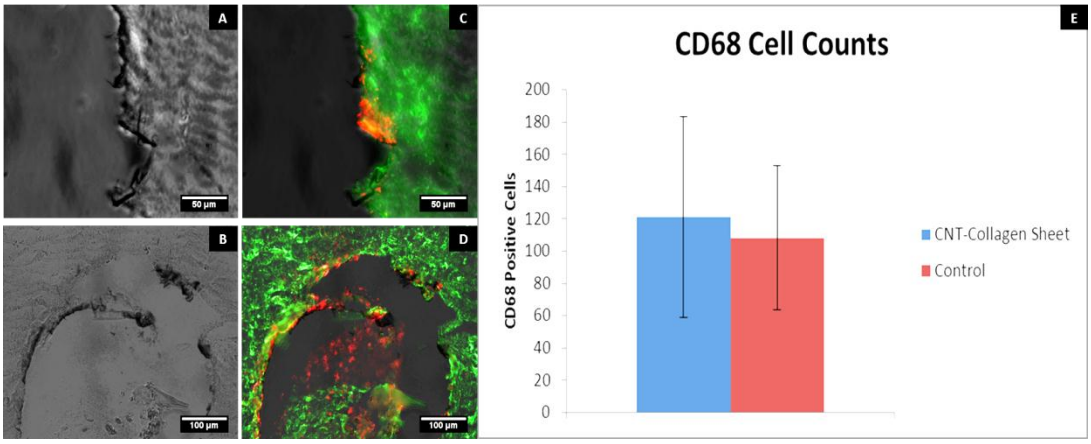

Figure 8 Histological analysis of cortical implant tissue sections A) & B) Bright field images of implant sites showing CNTS debris C) & D) Double immunostained images superimposed over bright field (red=CD68, green= Beta-III Tubulin) E) Comparative cell counts between control implants and CNT-collagen composite

Figure 8 shows sections stained with Beta-III tubulin and ED-1. These are two markers for neurons and microglia cells respectively. Statistical analysis (student t-test for difference of means n=10 p=0.575) revealed no significant difference in immune response of microglial cells accumulated at the injury site at the experiment end point between CNT containing implants and collagen implants (Figure 8E). The figure shows representative images of the tissue sections containing CNT debris with limited microglia recruitment compared to the surrounding injury.

Figures 8A and 8B show CNTS debris around the periphery of the injury site. CNT-sheet debris remained fibrous without aggregation. The dispersion of the CNTS debris may have been by mechanical disruption during sectioning of the tissue. Conversely, CNT sheets may have failed to maintain a continuous macrostructure during the 14 day implantation period. Further study is needed to determine whether the sheets

can maintain the extended fiber geometry in vivo necessary for long term signal acquisition or stimulation before implementing this type of electrode in chronic studies.

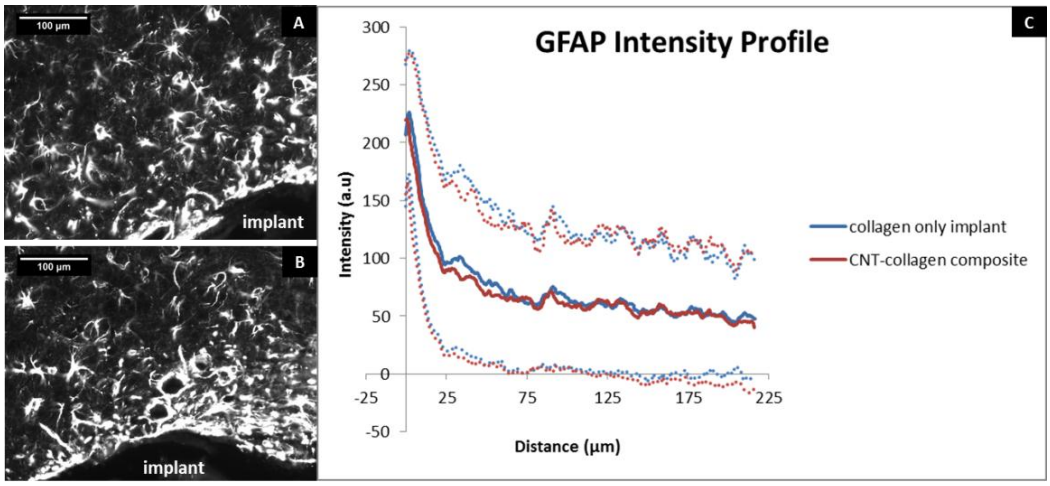

Figure 9 Glial Fibrillary Acidic Protein Intensity Profiles A) Representative image of CNTS-collagen composite implant B) Representative image of control collagen implant C) Intensity profiles of forming scar as a function of distance

Figure 9C shows intensity profiles of Glial Fibrillary Acidic Protein (GFAP), a marker for reactive astrocytes, staining as a metric for the developing scar, or pseudocapsule. GFAP intensity as a function of distance was plotted and no substantial difference between collagen only implants and CNT-collagen composite was observed. A student t-test for difference of means (n=20 sections with 10 profiles per image) revealed no significant difference between the collagen control implant and the CNTS-collagen composite at 10, 25,100, and 200 µm from the injury site ($p>0.10$ for all cases).

Chapter 4

Closing Remarks

Conclusions

The presented design strategy based off a novel CNTS-collagen composite shows several desirable qualities for use as neural interface electrode. The compliance of the material addresses one proposed mechanism of electrode failure while the nano-scale may help to mitigate increased tissue impedance and increasing capsule thickness that separates the electrode from the electrically active tissue. The electrical properties of the sheet demonstrate charge transfer characteristics desirable in a bio-electronic electrode intended for stimulation applications. Finally, the biocompatibility of the selected materials may mitigate deleterious host tissue responses.

CNT sheets were advantageous to this design for two primary reasons. Firstly, the sheets were an achievable method for forming nanoscale conductive fibers in the desired configuration. Secondly, there is a possibility that the chemical composition of CNT sheet may limit the immune response. Some work with immobilized carbon nanotubes has shown they elicited no substantial increase in foreign body response when compared to negative controls.[29] Furthermore, it is know that at the sub-cellular scale immune response is driven primarily driven by molecular recognition. Pristine carbon nanotubes have reduced antigenic site due to their lack of chemical moieties and may therefore elicit a reduced immune response via these pathways. There are other pathways that can lead to the foreign body response, such as the complement pathway; however, avoiding direct cellular recognition would mitigate the response enhance the scar tissue escape of the fibers.

Collagen was used as a substrate to stabilize the CNT sheet and prevent its aggregation in aqueous media. Specifically, collagen was chosen due to its robust

mechanical properties as a dry film, and its acceptance as a resorbable biomaterial. For a cortical implant, it may be more intuitive to use a film of proteoglycan or glycosaminoglycan composition, because they compose the majority of the extracellular matrix in brain tissue. For the presented work, collagen was chosen for ease of application and so the film could be handled and laser-cut in its dry form.

Other materials for neural electrodes are available and currently in use; however, most of these materials are limited to functional coatings. The fabrication processes involved in nanoscale fiber formation make them difficult to incorporate into the proposed design, though many of these materials have shown excellent electrochemical properties that would be especially useful as the electrode dimensions are reduced further. The conductive fibers need to extended from the electrode shaft by at least 50 microns to effectively escape the scar tissue region, depending on the method of surgical fixation. In this aspect the utilized carbon nanotube sheets are ideal, because the can be handled and used as a macro-scale material. The feature coupled with the presented resistive welding technique provided the means for the forming such an electrode.

In its current form the material may not be stable enough to remain in the body as a complete electrode structure with electrical continuity. This may have been a result of the tissue sectioning, a mechanical disruption of the CNT sheets. However, the similar location of CNT sheets across multiple depths of sectioning suggests that the structure was disrupted before sectioning. Regardless, the individual qualities of the material demonstrated suggest the merit of using similar design choices in future electrode designs and demonstrate the potential of addressing multiple suggested modes of electrode failure with a single design.

The conformable nature and adhesion to tissue demonstrated by collagen embody qualities sought in many other designs already published in the literature. This

suggests the potential applications of this material in surface recording based interfaces in either the peripheral or central nervous system. Highly conformable materials like the CNT collagen composite minimize mechanical mismatch between the tissue and electrode, limiting harmful tissue disruption that contributes to chronic inflammation and neuronal cell death.

In the field of neural interfaces it is truly the interface between electrode and tissue that is the challenge. The robotic prosthetics, signal acquisition, and processing capabilities are all realities. The remaining challenges involve designing methods to extended stability of theses interfaces into a clinically relevant lifespan and in clinical optimization. Should neural interfaces become a reality, many patients suffering from debilitating conditions will benefit from neural prosthesis and bioelectronic therapies and a multi-million dollar medical industry will be created.

Future Work

Future work with this biomaterial approach to electrode failure modes should continue to explore the impact of charge transfer through mechanically compliant materials with dimensions in the nanoscale. Substantial evidence suggests that reduced host tissue response is highly correlated with these two qualities. Potentially, electrically conductive polymer fibers can be fabricated to have qualities similar to the collagen-CNT composite developed and to maintain stability in the body over a chronic scale.

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Biographical Information

Ryan Landrith was born in Oklahoma City, Oklahoma April 29th, 1992. He graduated from Duncanville High School in 2010. During his undergraduate education at the University of Texas at Arlington (UTA) Ryan was involved in a variety of research projects including, epidemiological modeling of the neglected tropical disease Leishmaniasis, social network modeling using Markov chain Monte Carlo techniques, and air dispersion modeling of radiological substances. Ryan is currently a Graduate Student Intern at the University of Texas at Arlington Research Institute (UTARI) working with the biomedical division on the development of neural interfaces using the micro-electronics manufacturing facilities at UTARI.

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