8-2016

Biocompatible microchannel scaffold with microwires for recording regenerative peripheral nerve neural spikes

Manisankar Chennamsetti
The University of Texas Rio Grande Valley

BIOCOMPATIBLE MICROCHANNEL SCAFFOLD WITH MICROWIRES FOR RECORDING REGENERATIVE PERIPHERAL NERVE NEURAL SPIKES

A Thesis

by

MANISANKAR CHENNAMSETTI

Submitted to the Graduate College of
The University of Texas Rio Grande Valley
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2016

Major Subject: Electrical Engineering

BIOCOMPATIBLE MICROCHANNEL SCAFFOLD WITH MICROWIRES FOR RECORDING REGENERATIVE PERIPHERAL NERVE NEURAL SPIKES

A Thesis
by
MANISANKAR CHENNAMSETTI

COMMITTEE MEMBERS

Dr. Yoonsu Choi
Chair of Committee

Dr. Nazmul Islam
Committee Member

Dr. Jaimes S. Ramos - Salas
Committee Member

August 2016

ABSTRACT

Manisankar,Chennamsetti**,** Biocompatible Microchannel Scaffold with Microwires for Recording Regenerative Peripheral Nerve Neural Spikes. Master of Science (MS), August, 2016, 22 figures, 49 pp, references, 73 titles.

A new process for the fabrication of a microchannel scaffold with microwires for peripheral nerve applications is presented. This microchannel scaffold implemented between the ends of nerves guide the axons of which regenerate through microchannel in scaffold and fixed microelectrodes. This device is entirely handcrafted using commercially available materials such as microwires, PDMS film, liquid PDMS, dental cement, and epoxy glue. This device was implemented in a*Lewis rat* sciatic nerve to better analyze the electrical signals of regenerated axons. 64-electrode microchannel scaffolds were developed for both peripheral nerve interfacing and peripheral nerve regeneration. The microwires were used for recording electrode to capture neural signal from the regenerated peripheral nerves. To further differentiate the methodology, the new addition of a ribbon cable will facilitate the transmission of the electrical signals. A total of eight devices have been developed, the nerve regeneration were examined four weeks after device implantation.

DEDICATION

The completion of my master studies would not have been possible without the blessing and support of my family. I would like to thank and dedicate my work to my parents, Sai babu and Padmavathi for their love and support. I would also like to thank to my sisters', brother- in-laws. The completion of my masters would not possible without their support.

ACKNOWLEDGEMENT

I would like to formally thank:

Dr. Yoonsu Choi, my advisor and chair of committee, for his advice and, profound help. Ihave learned so much and without him,mythesis would have not been possible. I am truly grateful for hiscontinuingsupport and encouragement.I would also like to thank Dr. Nazmul Islam and Dr. Jaimes S. Ramos-Salas, for their willingness to serve as committee members.I would also like to express my appreciation to The University of Texas Rio Grande Valley Health Science Center-Regional Academic Health Center in Edinburg (ERAHC), for all the support they have given my work. Finally, I would also like to thank my friends in the research lab, for all the technical discussions.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

1.1 Introduction

Neural interface technologies are envisioned to facilitate direct connections between the nervous system and external technologies such as limb prosthetics or data acquisition systems for further processing. Today the number of people living with an amputation is estimated to be between 400,000 and 1,000,000 in the United States alone. Among those living with limb loss, the main causes are vascular disease including diabetes and peripheral arterial disease, trauma and cancer. But even though when you loss limbs, the peripheral nerves would still carry electrical command signals generated in the brain, but the signals would meet a dead end at the site of amputation and never reach the amputated muscles. So this issue encourages researchers to somehow by using the brain signals find a way to replacelosslimb with a prosthetic limb. Prosthetics are changing the way amputees live, work, and function in the world. Changing technologies are providing advances in prosthetic options for arms, legs, and hands that are now easier to build, more responsive, and sometimes even cheaper than earlier options. Prosthesis is a functional replacement for an amputated or congenitally malformed or missing limb. Throughout history, children who were born with congenital defects or malformed limbs were unfortunately viewed as ‘defective’ or ‘demonic’ and were not often allowed to live long enough to have need for prostheses[1]. In the 16th century, French surgeon his nameAmbroise Pare contributed major advances in prosthetics such as mechanical, hinged hands, and locking knee joints. These

implements were made of metal, but unlike those utilized by the Knights of the Middle Ages;these devices could be used in activities of daily life. A century later, a Dutch surgeon by the name of Pieter Verduyn created a lower-leg prosthesis that involved “specialized hinges and a leather cuff for improved attachment to the body. These drastic advances are still utilized today as some of the basic features of modern prosthetics. While these devices were highly useful, they were complicated and expensive, and few could afford them. The Civil War in America (1861–1865) served to make the need for prostheses commonplace among formerly able-bodied young men. Surgeons working on surviving soldiers who fought for the north conducted 300,000 amputations over the course of the war [2]. The high demand for prostheses due to the Civil War encouraged manufacturers to make advancements. World War I and II in 1945, the National Academy of Sciences, an agency funded by the United States government, established the Artificial Limb Program this program was designed to improve the design of prosthetic devices through the funding and coordination of research[3, 4]. The U.S. Veterans Association funded research that developed mechanical arms with a hook on the end that could open and close by shrugging the shoulder[2]. This gave the individual wearing it the ability to grasp things. The agencies combined funding to improve leg prostheses, ushering in improvements in both above and below-the-knee. Emphasis began to be placed on how lifelike the replacement limb appeared [3]. Doctors and engineers worked together to improve both the function and the appearance of prosthetic limbs. Creation of new materials such as plastic, the advent of computer aided design, and further improvement in surgical techniques all served to make prosthetic limbs more functional and lifelike [2,3].The cost of a typical prosthetic limb would fall in the range of several thousands to tens of thousands of dollars, depending on the type of limb desired by the patient. The costs depend on the type of leg and the level of amputation. For example, a basic

below-the-knee prosthetic that would allow a patient to walk on flat ground costs $5,000-$7,000, while one that would allow the patient to walk on stairs and bumpy ground could cost approximately $10,000. For a device that would allow a patient to walk and run as well as a non-amputee, the cost could go up to $15,000. Prosthetics with special hydraulic or mechanical systems that allow for movement control cancost more than $15,000, while a computer assisted prosthetic leg costs $20,000 or more. Computerized prosthetic leg, for above the knee amputees, can cost as much as $50,000or more, including the prosthetic foot. A prosthetic leg likely will need to be replaced several times during a patient's lifetime, and patients need ongoing adjustments. One of the advanced methods in artificial limbs is targeted muscle reinnervation(TMR) which a spare muscle of an amputated patient is denervated then reinnervated with residual nerves of the amputated limb. The resultant electromyographical signals of the targeted muscle now represent the motor commands to the missing limb, and are used to drive a motorized prosthetic device. TMR takes advantage of intact residual nerves that previously connected to muscles distal to the amputation. The intact residual peripheral nerves are transferred to surgically denervated areas of unused musculature in the residual limb or chest (Figure 1.1). During TMR surgery, severed arm nerves are transferred to spare target muscles in a person's residual limb or surrounding area. Over a period of months, these nerves grow into, or reinnnervate, the target muscles. Once this process is complete, the target muscles contract in response to attempted movements of the missing limb. EMG signals generated by these contractions can be used to control the prosthesis.TMR makes prosthesis control more intuitive because neural control signals intended for the missing limb are used to control analogous functions in prosthesis.For example when a person attempts to bend their missing elbow, the motor control information from the brain travels down the transferred nerve and makes the target

muscle contract. EMG signals from that target muscle are used to make the prosthetic elbow bend.TMR also creates additional control sites so the person can use different muscles to control different prosthetic functions [5]. TMR is a technique that is becoming available to patients in more places around the world.

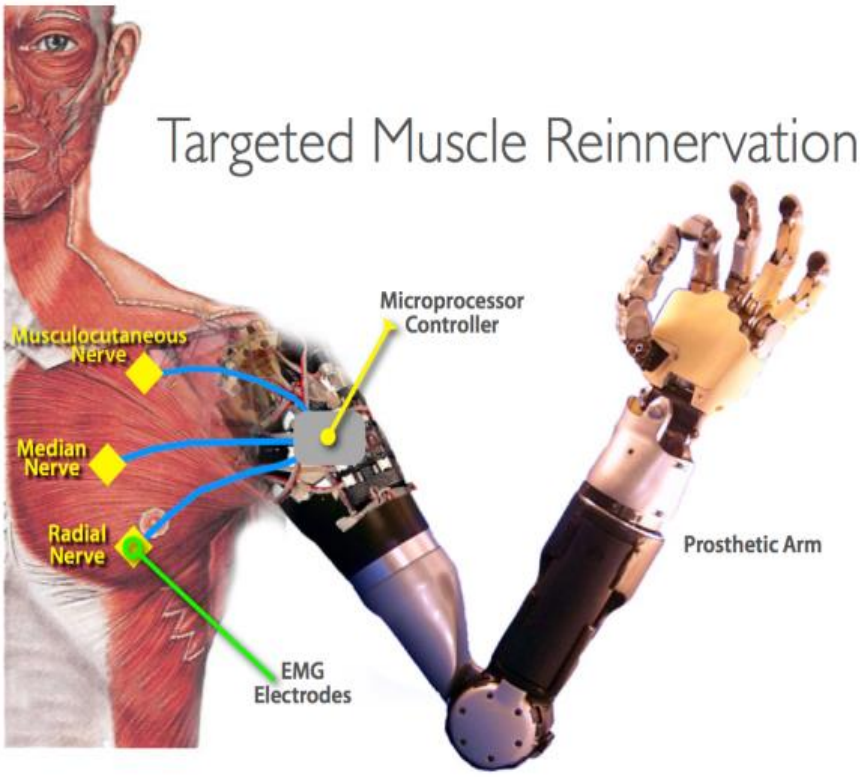

Figure 1.1: Targeted reinnervationsof the muscular nerve fibers [6].

Despite all the advantages, researchers believe that the sensors commonly used to control robotic prostheses are too unreliable. These sensors are placed on the skin, where normal physiological processes, like sweating, can interfere with the way they work. The question is why can't we just go to the source of the information and measure the electrical signals carried in the nerves, or even the brain. The new control system works with a direct link to nerve endings

and muscles in the patient. The system should provide more natural control of the arm because it allows the patient to feel what the hand is doing. Information goes both ways through the wires, both to and from the arm, and the patient gets feedback from various pressure points. A nerve provides a structured pathway that supports the electrochemical nerve impulses transmitted along each of the axons. In the central nervous system, the analogous structures are known as tracts. Neurons are sometimes referred to as nerve cells, although this term is misleading since many neurons do not occupy nerves, and nerves also include non-neuronal support cells that contribute to the health of enclosed neurons. Each nerve is a cable-like structure that contains many axons that are sometimes referred to as "fibers." Within a nerve, as shown in Figure 1.2, each axon is surrounded by a layer of connective tissue called the endoneurium. The axons are bundled together into groups called fascicles. Each fascicle is wrapped in a layer of connective tissue called the perineurium. Finally, the entire nerve is wrapped in a layer of connective tissue called the epineurium. The endoneurium consists of an inner sleeve of material called the glycocalyx and a mesh of collagen. Nerves are bundled along with blood vessels, which provide essential nutrients and energy to the enclosed, and metabolically demanding, neurons. Within the endoneurium, individual nerve fibers are surrounded by a liquid called the endoneurial fluid. The endoneurium has properties analogous to the blood-brain barrier. The blood supply to nerves is provided by coiled segmental arteries that enter the epineurium periodically along the length of the nerve and form the vasa nervorum. Arteries divide into epineurial arterioles that form an anastomotic network running primarily longitudinally within the epifascicular epineurium and the interfascicularepineurium.

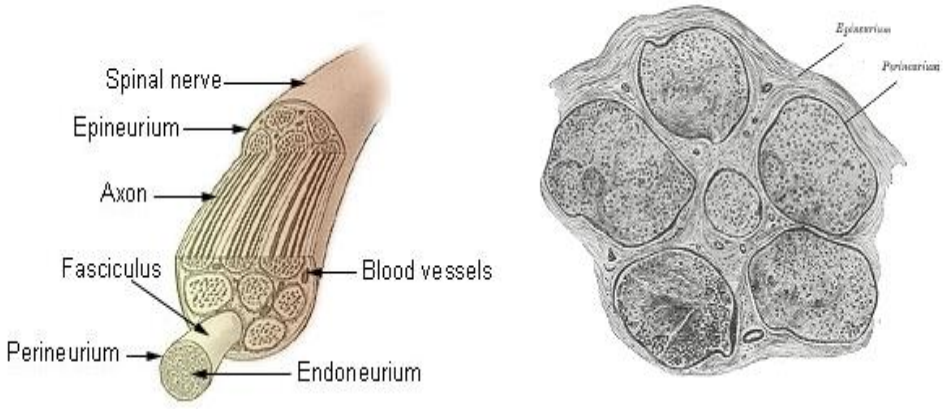

Figure 1.2: Cross sectional view of peripheral nerve [7].

In recent years, many scientific and technological efforts have been devoted to develop hybrid bionic systems that link, via neural interfaces, the human nervous system with electronic and/or robotic prostheses. Robotics appear promising for the recovery of some motor functions through the use of prosthetics, but neural control of these devices remains incomplete and unreliable. The limitations of nerve electrode interfaces vary depending on the type of electrode and the response of the implantation tissue, however, signal decay overtime has remained an insurmountable challenge despite the multiple electrode technologies available [8,9]. Current clinical alternatives through the use of electromyography signals from re-innervated pectoral muscles allow to control robotic prosthetics with some success, even though motor control is limited to gross voluntary movements and sensory feedback is lacking or incomplete [10–12]. Fine motor control and sensory function will require more extensive integration between the nervous system and implantable electronics. Recent advances in electrode designs have moved away from extraneural cuff and extrafascicular electrodes which provide a low number of small signals [13-14] andinstead have focused on penetrating electrodes. Indwelling arrays have been studied extensively in cortical implantations and have shown the ability to provide some level of

manual dexterity [15-16] and cortical stimulation of sensory areas has also yielded some spatiotemporal feedback in primates [17]. While these pursuits have produced promising, if not long lasting results, the invasiveness of cortical implantation is an obstacle to clinical implementation. As such, investigators have more recently evaluated the feasibility of interfacing with residual sensory and motor activity in the peripheral nervous system (PNS) of amputees [18, 19]. Penetrating electrodes in the PNS can provide both a high number and better quality of action potential recordings but still have many limitations, which lead to signal decay. These include poor tissue–electrode interface, tissue damage by probe micromotion within the soft nerve tissue, and electrode insulation due to tissue scar formation [20-24]. Recently reported that peripheral nerves, whether acutely injured or implanted after months of chronic amputation, could be interfaced early by enticing them to grow in close proximity to electrodes placed in a tridimensional open regenerative multi electrode interface (REMI) [25]. The REMI was able to detect signals as early as eight days post-implantation, however nerve regeneration during the first two weeks is a crucial time period thus, the REMI early recorded activity is expected to be highly valuable as well. While this activity might represent normal neural depolarization during axonal regrowth, it is also possible that the recorded action potentials are the result of altered nerve regeneration in the presence of REMI electrodes.A peripheral nerve interface is the bridge between the peripheral nervous system and a computer interface which serves as a bi-directional information transducer recording and sending signals between the human body and a machine processor. Interfaces to the nervous system usually take the form of electrodes for stimulation and recording, though chemical stimulation and sensing are possible[26-29]. Several designs, such as cuff electrodes, flat interface nerve electrodes (FINE) [30, 31], longitudinal intrafascicular electrode (LIFT) [32-34] and regenerative sieve and microchannel electrodes [35-

42]demonstrated selective recording and stimulation. Research in this area is focused on developing peripheral nerve interfaces for the restoration of function injury on nerves to minimize associated losses. Peripheral nerve interfaces also enable electrical stimulation and recording of the peripheral nervous system to study the form and function of the peripheral nervous system. Many researchers also focus in the area of neuroprosthesis, linking the human nervous system to bionics for natural sensor motor control.

Successful implantation of peripheral nerve interfaces depend on a number of factors which include appropriateindication perioperative testing, differentiated planning, and functionaltraining. Typically microelectrode devices are implanted adjacent to, around, or within the nerve trunk to establish contact with the peripheral nervous system. Different approaches may be used depending on the type of signal desiredand attainability. Many fabrication techniques are developed for recording neural signal from peripheral nerves such as cuff, sieve, shift and longitudinal intra fascicular electrodes.

Cable tie type cuff electrode is one of the designs for peripheral nerve interface. This cuff electrode consists of an insulating sheath encircling a nerve with a number of electrode sites arranged on the inside walls and lead wires. To minimize passively-induced neural damage, the cuff electrode itself should be made as small and flexible as possible. Among the factors related to safety, it is cuff nerve diameter ratio (CNR) that requires the most attention. In general, a small CNR prevents the flow of blood, nutrients and other critical fluids into nerves [43], while a large CNR leads to poor contact between electrode and nerve tissue [44]. The Association for the Advancement of Medical Instrumentation (AAMI) recommends that CNR should be larger than 1.5 for safe implantation. Thus, CNR is important for safe implantation and effective stimulation or high-quality recording. A typical cuff electrode is the split-cylinder that has a longitudinal slit

along the silicon rubber tube. The slit has to be closed by sutures, wax, or silicon rubber flaps. The split-cylinder cuff electrode that is commercially available from Micro Probes for Life Sciences (Gaithersburg, MD) has been used for many years by Loeb's group. Another widely used type is the spiral cuff, which consists of electrode sites embedded within a self-curling sheet [45]. The cuff is fabricated by bonding a pre-stretched polymer sheet to an un-stretched polymer sheet. The spiral cuff electrode is expandable so that it can be sized to fit snugly around a nerve or adjust for neural swelling. However, Loeb and Peck [46] reported that it is difficult to stretch and place the tightly wrapped spiral cuff around the nerve. There exist many variants of the split-cylinder type and the spiral type. In most cases, the method of fabrication is based on the assembly of discrete components, and thus the device has a bulky size and a limited number of electrode sites, which may limit its use in clinical practice. In contrast, themicroelectromechanical system (MEMS) techniques offer great capability in minimization and system integration. Recently, micro fabricated, polyimide-based spiral cuff electrodes have been developed and characterized [47]. To achieve a cylinder spiral shape, a thermal treatment is required, after the flat cuff electrode is released from substrate and rolled using a designed tool.

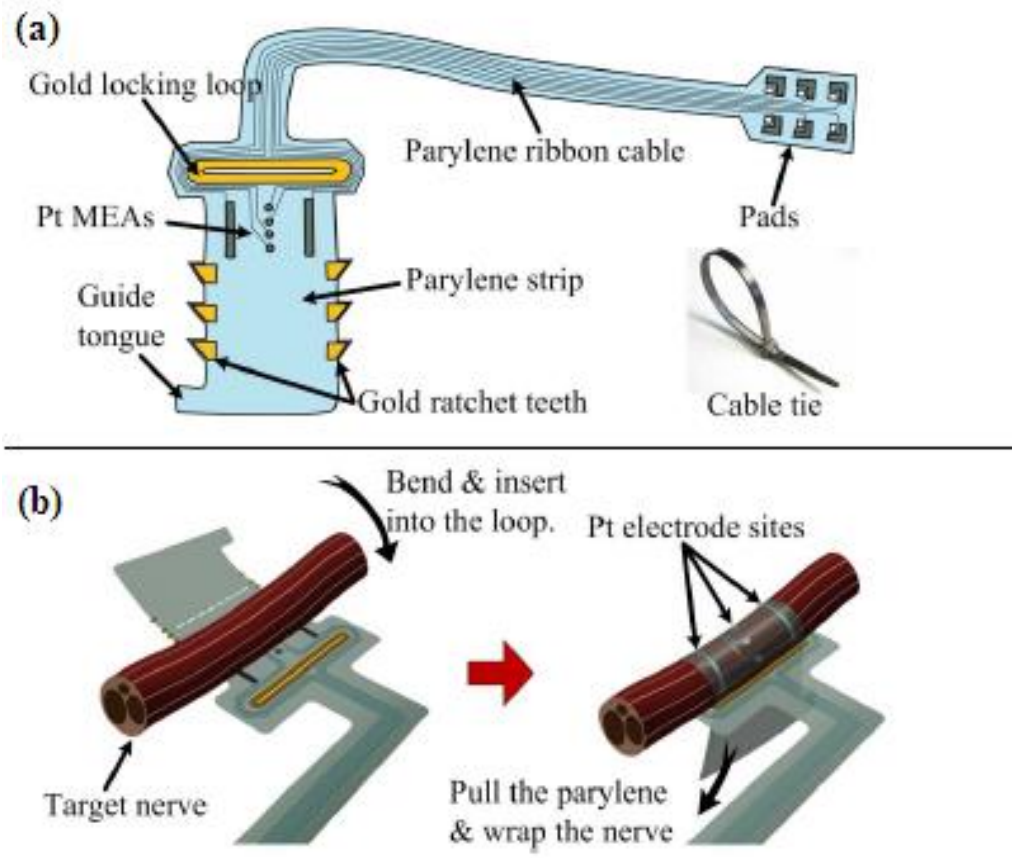

Figure1.3:(a) Schematic view of cable tie type cuff electrode. (b) Implementation on nerve [48]

This cable-tie-type cuff electrode discussed in [48] is fabricated by electroplating and parylene deposition. In contrast to the conventional cuff electrodes, the cuff diameter of this typecan be intra operatively adjusted to fit the nerve properly by using the locking structure including paired ratchet teeth and a locking loop. The cuff is made out of thin, flexible and biocompatible parylene, which can minimize the likelihood of mechanically-induced neural damage after implantation. The integrated parylene ribbon cable facilitates connection with external circuits through wired or wireless interfaces. Further, the acute neural recoding and stimulation tests were performed on the rat sciatic nerve using the fabricated cable-tie-type

parylene cuff electrodes. In this work the cable-tie parylene cuff electrode is designed to fit the rat sciatic nerves (1.0-1.5 mm in diameter), illustrating the flexibility of the approach in its capability to produce customized electrodes to fit various sized nerves. The cuff diameter can be adjusted from 1.5 to 2.0 mm with an increment of 0.25 mm. The width and length of the opening in the locking loop are 70 μm and 8 mm, respectively. The width of the parylene strip is also 8 mm, while the length of the projection portion of the ratchet teeth is 100 μm. It should be noted that a long projection will increase the difficulty for the ratchet teeth in passing through the locking loop, while the locking loop will fail to catch the ratchet teeth if it is too short. A tri-polar configuration is used to reduce the noise caused by sources outside the cuff. The microelectrode array is composed of two outer reference electrode sites and six central working electrode sites. The longitudinal distance (center to center) between the reference electrode and working electrode is 3 mm. The pads and through holes in the parylene connection sheet are designed to match the position and size of the pins of the 1.27 mm pitch double row female header, which can be connected to some conventional male connectors such as the, Omnetics connectors. Surface micromachining processes have been used to fabricate the cable-tie-type parylene cuff electrode with an integrated flexible parylene ribbon cable. Parylene C was chosen as the substrate material for its biocompatibility, mechanical strength, electrical insulation properties, chemical resistance and low permeability to gas and moisture. Gold electroplating technique was used to create the locking structure for its simplicity and low cost. Furthermore, gold isbiocompatible, ductile and malleable. Commercial 40-pin 1.27 mm pitch double row femaleheaders were used as an adaptor allowing the flexible cuff electrodes to be connected to some male connectors such as, Omnetics connectors. The connection sheet with contact pads was carefully assembled along the pins of female header and subsequently the electrical

connection was created by using conductive glue. This contraction has the disadvantage that the epineurium covering the nerve is between the electrode and the fibers the perineurium works as a kind of insulator and there by reduces the recording signals and increases stimulation threshold.

Another way to create a neural interface is to implant a micro machined sieve electrode in a nerve trunk and regenerate nerve fibers through the via holes in the electrode and further into the distal nerve stump. Regenerative electrodes are designed to interface a high number of nerve fibers by using an array of via holes, with electrodes built around them, implanted between the severed stumps of a peripheral nerve [49]. One of the most logical and challenging applications of regenerative electrodes will be its implantation in severed nerves of an amputee limb for a bi-directional interface in a feedback-controlled neuroprosthesis. On the one hand, recording of neural efferent signals can be used for the motion control of a mechanical prosthesis [50], and on the other, sensory feedback from tactile and force sensors might be provided to the user through stimulation of afferent nerve fibers within the residual limb [51]. The applicability of regenerative electrodes is dependent upon the success of axonal regeneration through the perforations or via holes, the possible nerve damage from the mechanical load imposed by the electrode or from constrictive forces within the via holes, and the biocompatibility of the components, especially over long term implantation [52,53]. Different techniques and materials have been used during the last 30 years in the construction of regenerative electrodes. With the advent of microelectronic technologies, it became possible to construct silicon electrodes with dimensions and number of via holes compatible with the characteristics of peripheral nerves. Using multiple holes silicon arrays, axonal regeneration and even neural activity recording was demonstrated in peripheral nerves of rat[53–57]. Such silicon interfaces cause frequent signs of axonopathy [50, 53], and constitute a physical barrier that limits the elongation of regenerating

axons depending on the size of the via holes [52, 53, 58]. Because of their more adaptive physical characteristics, polyimide-based electrodes were introduced more recently [59, 60]. Polyimide allows to make a higher number of holes than silicon dice of the same total area and to be micro machined in a variety of designs suitable for implantation in different nerve models. Polyimide-based electrodes have been shown to be biocompatible [59, 61] and stable over several months of in vivo implantation and testing [60, 62]. The proportion of regenerates and the quality of the regenerated nerves were better than those found through silicon dice [53]. In a more recently reported paper mentions that nerve regeneration was sustained at long term, up to 12 months, and that the electrodes allowed for selective stimulation of different regenerated nerve fascicles [63]. The knowledge of the long-term functional and structural changes in the nerve due to implanted electrodes is highly relevant if they have to become a valuable tool for providing an interface between peripheral nerves and distal target organs or limb prosthetic devices in humans [52,64].

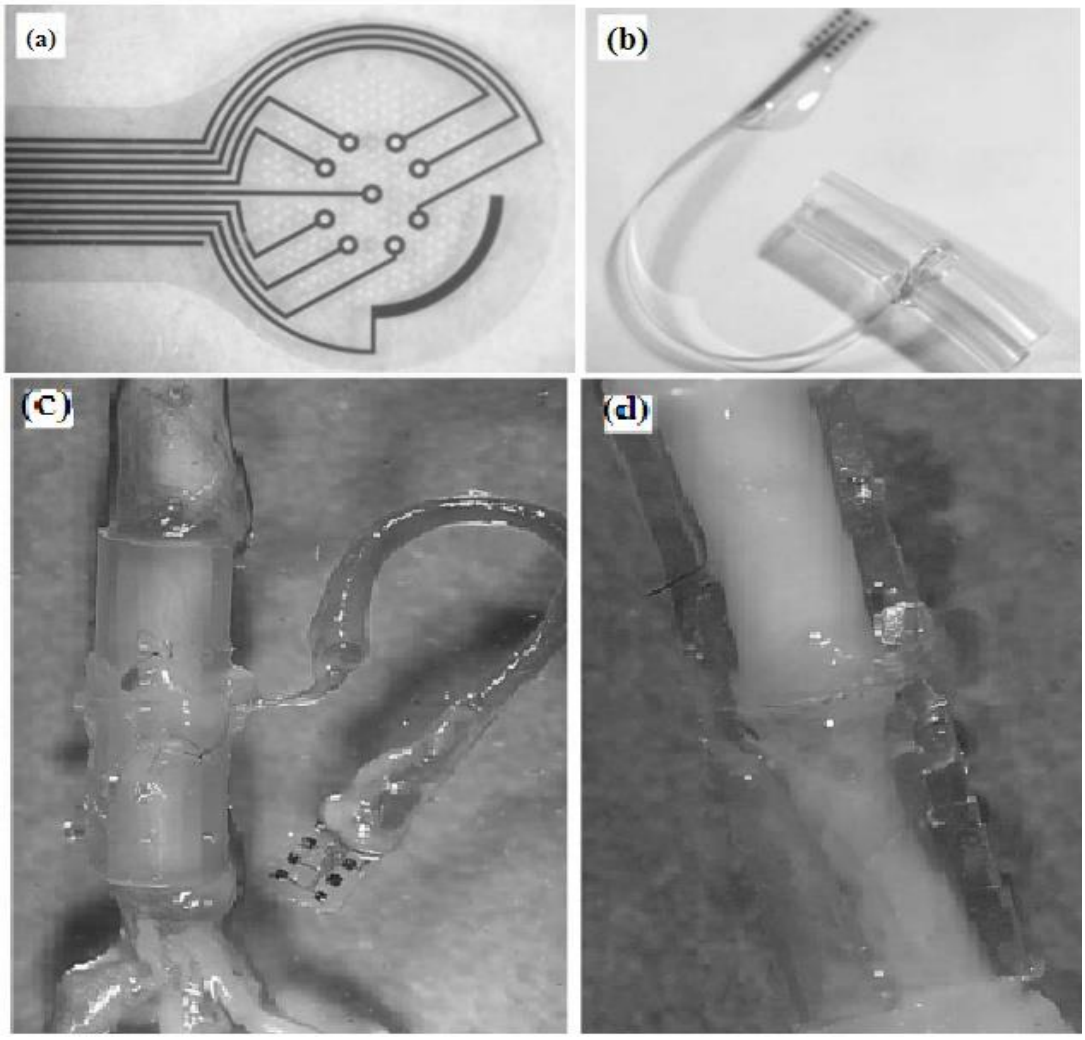

Figure 1.4 (a) Enlarged view of the sieve portion of the regenerative electrode, (b) the silicone guide with the sieve electrode encased. (c) A sciatic nerve regenerated through the polyimide sieve electrode. (d) View of the regenerated nerve after opening the silicone tube. [65]

The regenerative electrodes were micro machine fabricated using a polyimide resin (PI2611, DuPont) as substrate and insulation material with a single metallization layer. The used polyimide has low water absorption, it is biocompatible, and it has been shown to be mechanically stable after long*in vitro* and *in vivo* testing [60,62,63,66]. The regenerative electrode is a flexible structure, with a thickness of 10 mm and a weight of 4 mg. It consists of

three portions: a round sieve interface (2 mm diameter), a narrow ribbon cable (1 mm wide, 21 mm long) and an ending connector (2 mm 3 mm). The sieve part of the interface has 281 round via holes of 40 mm diameter, with nine integrated Pt electrodes arranged around via holes, occupying an area of 1473 mm^2. The Pt electrodes were connected through integrated leads in the ribbon to nine-squared connects placed at the ending pad. The sieve portion was placed between two silicone tubes (2 mm diameter. 4 mm length) and glued by plasma etching, and the interconnection covered with silicone glue. Silicone tubes served for implantation and as guidance for nerve regeneration.

Shaft electrodes and thin film longitudinal intra fascicular electrodes also another neural interface technology, Shaft electrodes have a needle shape with multiple electrode sides. The concept is to insert these electrodes into the neural tissue. This results in a closer contact between the electrode side and the nerve fibers. The principal difficulty is the implantation method because of the mechanical stiffness of the peripheral nervous system.

Thin-film longitudinal intrafascicular electrode combines a loop of a thin wire electrode with a filament loop including a thin needle.The new version of LIFEs has been recently designed and fabricated [67, 68]. The new electrode, named as thin film -LIFE, is composed of thin-film polyimide on which eight active sites have been placed. The flexibility of the innovative electrode combined together with a larger number of active sites reduces drifts and enhances signal to noise ratios, thus making tf-LIFE a good solution for long-term implantations and for controlling hybrid bionic system [69]. Another drawback of conventional LIFEs is that chronic intrafascicular electrodes cannot be moved after their implant. This means that electrodes may not be positioned near selected cells and there is no flexibility in targeting specific cell types

or receptive field positions. So it would be desirable to control the electrodes positions once they are implanted in order to adjust it in the tissue and to improve longevity of cells recordings.

Tf-LIFEs are a new kind of intrafascicular electrodes which have been recently fabricated [67]. They have a 5µm thin-film polyimide substrate. Flexible polyimide acts as substrate and as insulation on which platinum tracks are sputtered. All tracks are 10 µm in width and 300 nm in thickness. Total length of the device is 60 mm comprising left and right parts. Tf-LIFEs structure is shown in Figure 1.5 (a), while a detail of one end of the device is illustrated in Figure 1.5 (b). There are eight electric contacts that are used for recording and stimulation.The great number of active sites enhance significantly the signal to noise ratio. Four sites are placed on the left part of the device (L1-L4) and the other four are on the right part (R1-R4). Relative position between two consecutive active sites in the same side of the electrode is 1.5 mm. L0 and R0 are the recording reference electrodes. Furthermore, two large ground electrodes are placed at the end of the electrode area.

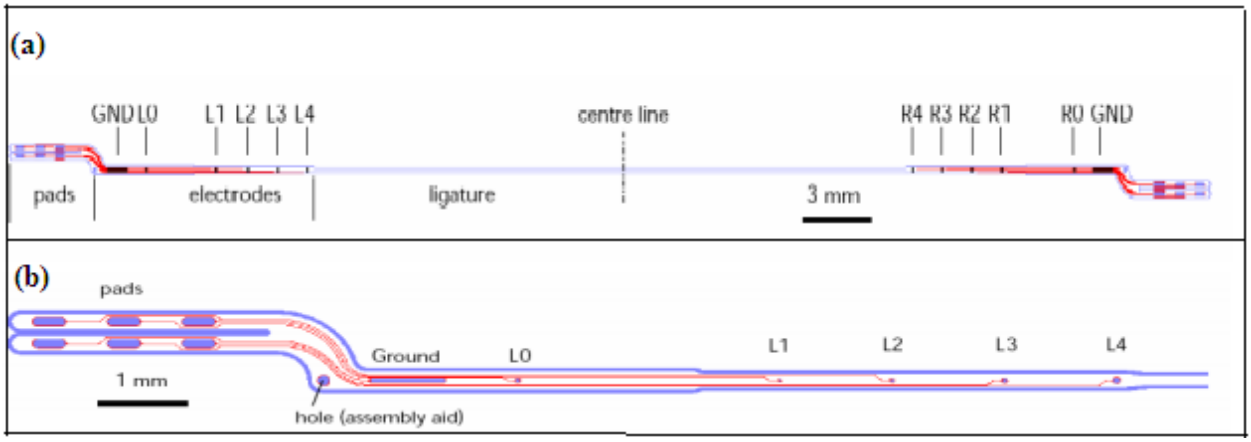

Figure 1.5: (a) Scheme of a tf-LIFE electrode. (b)Detail of one end of the tf-LIFE showing the pad area and the electrode contacts. [70]

However, all this devices have limited electrode sites and recordings can only be obtained from the limited number of nerve fascicles. Moreover, they require advanced micro fabrication techniques that not all labs have access to them and it makes the devices very expensive. A regenerative peripheral nerve interface, developed here, can be utilized to address these goals.

A scalable microwire peripheral nerve interface was developed, which interacted with regenerated peripheral nerves in microchannel scaffolds. Neural interface technologies are envisioned to facilitate direct connections between the nervous system and external technologies such as limb prosthetics or data acquisition systems for further processing. Presented here is an animal study using a handcrafted microwire regenerative peripheral nerve interface, a novel neural interface device for communicating with peripheral nerves.The neural interface studies using animal models are crucial in the evaluation of efficacy and safety of implantable medical devices before their use in clinical studies 4-electrodes, 8-electrodes and 16-electrode microwire microchannel scaffolds were developed for both peripheral nerve regeneration and peripheral nerve interfacing. Here we report an advanced generation of the 64-electrodes peripheral nerve interface (Figure1.6). The microchannels were used for nerve regeneration pathways as a scaffolding material and the embedded microwires were used as a recording electrode to capture neural signals from the regenerated peripheral nerves.

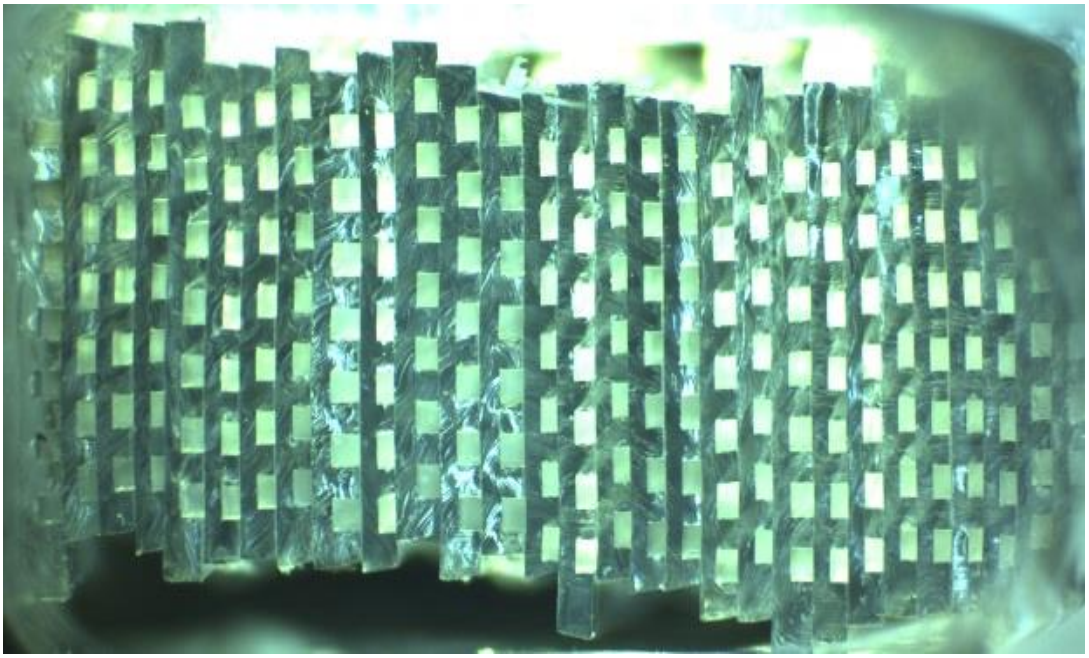

Figure 1.6: 20 layers of multi-channel stacks

The whole implantable micro device nerve grafting involves directly suturing both ends of the affected nerve. Although nerve grafting has proven to be an effective method in numerous cases, a critical concern becomes inevitable when having an injury affecting multiple nerve paths which can be problematic and has greatly limit its medical and technological applications. Consists of a μPNI for recording was placed on the transection site of the sciatic nerve and three pairs of electromyography electrode pairs were implanted on muscles on the right hind leg to record the muscle signals during the animal's locomotion tests. It gives us the capability of both electrophysiological recording and stimulation to develop a communication pathway from the brain to the endings of peripheral nerves. Peripheral nerve stimulation from one end of the μPNI initiates a neural signal pathway. Animal locomotion was observed in the animal facility at UTRGV and the μPNI has enabled us to analyze any sophisticated behavioral patterns. Independent microchannel neural interfaces will be creatively achieved by microwires embedded

inside the microchannel scaffolds which can be occupied by regenerated nerves and develop an isolated neural signal communication. The microchannel and microwires are long enough to cover and record neural signals from the isolated nerve by structural selectivity during nerve regeneration. The 64 channel recordings system covered 58- electrode microwires in the peripheral nerve interface, three pairs of electromyography electrodes.Two commercially available Molex 33-channel ribbon cables were be inserted into the TDT input connectors andbe a bridge to the stablohm wires. Each of these ribbon cables have 33 conducting wires running parallel to each other on the same flat plane while the 17^{th} conducting wire was marked and used as ground.This device was successfully implemented in *Lewis* rat and signals were demonstrated in the result section.

1.2 Problem statement

In recent years, many scientific and technological efforts have been devoted to develop hybrid bionic systems that link, via neural interfaces, the human nervous system with electronic and/or robotic prostheses, with the main aim of restoring motor and sensory functions in patients with spinal cord injuries, brain injuries, or degenerative diseases. Several designs, such as cuff electrodes, shaft electrodes, longitudinal intra fascicular electrodes, regenerative sieve, and microchannel electrodes demonstrated selective recording and stimulation. However, the devices have limited electrode sites and recordings can only be obtained from the limited number of nerve fascicles. Moreover, they require advanced micro fabrication techniques that not all labs have access to and it makes the devices very expensive. A regenerative peripheral nerve interface, developed here, can be address following goals.

- More number of electrodes inside the microchannel
- Reduce the cost and size of the device.

CHAPTER II

FABRICATION PROCEDURE

The multilayer microchannel scaffold is made up of 20 layers of PDMS structures with each layer having nine channels to cover a larger cross-sectional area of the target nerve and, therefore, obtain signals from a larger number of regenerated axons for examination. The total number of microwires being implemented into this design is divided in half, where 29 microwires were placed at each end of the scaffold and reserved 6 EMG wiresfor a total of 64 wires. 58 wireswereinserted into individual channels to keep at least an open end on each channel. The other end of the microwires was soldered to a ribbon cable which facilitated the connection toa TDT interface board. The fabrication process will describe the two ends of the device: the development of the multilayer microchannel scaffold and the connection for the connector. Prior to the fabrication process described below, a PDMS wafer with the desired design structure of the layers had been manufactured and reported in previous workreference to previous work [61]

As mentioned, the multilayer microchannel scaffold for this device is made of a stack of 20 (3 mm x 2 mm) PDMS layers that were cut from a structured PDMS wafer with a sharp blade. The 20 layers were cleaned with alcohol, and an ultrasonic cleaner was used to remove any residue. These layers are laid out in Figure 1(a).

Before continuing with the fabrication, it is essential tocheck that each layer was properly cut to avoid axons or wires from going into another channel which would comprise a portion of this study.

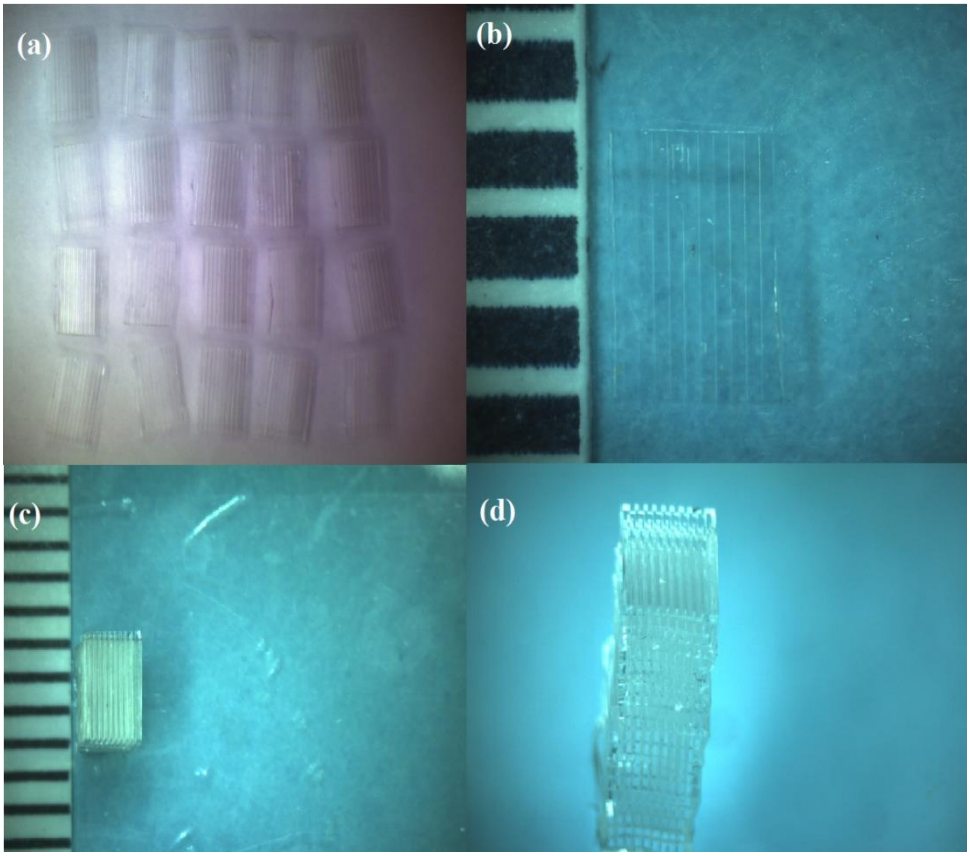

Figure 2.1: (a) 20 layers of PDMS micro-channel scaffolds, eachwith dimensions of 3 mm x 2 mm. (b) Asingle layerplacedon a thin PDMS film. Ruler scale 1 mm. (c) 10 layers placed on thin PDMS film. (d) Three-dimensional array structure of PDMS multilayer microchannel scaffold on thin film.

After checking the quality of each layer, PDMS thinfilm was placed on a glass plate and cut into a 25 mm x 12 mm rectangle. Then the bottom side of a single layer was bonded with 10:1 PDMS tocutting agent mixture (Sylgard® 186, Dow Corning®, MI) onto thethin film at the center edge of a 12 mm side, as seen in Figure1(b). After the PDMS had cured,

20 microchannellayers were stacked together as shown in Figure 1(d).The thin film was then wrapped around the microchannel scaffold structure twice and 10:1 PDMS to cutting agent mixture (Sylgard® 186, Dow Corning®, MI) was once againapplied to the thin film to secure the microchannel layers together into a cohesive structure, Figure 2(a). Once the PDMS cured, dental cement was applied around the PDMS thin film at each end of the scaffold as another measure of security for the microchannel scaffold

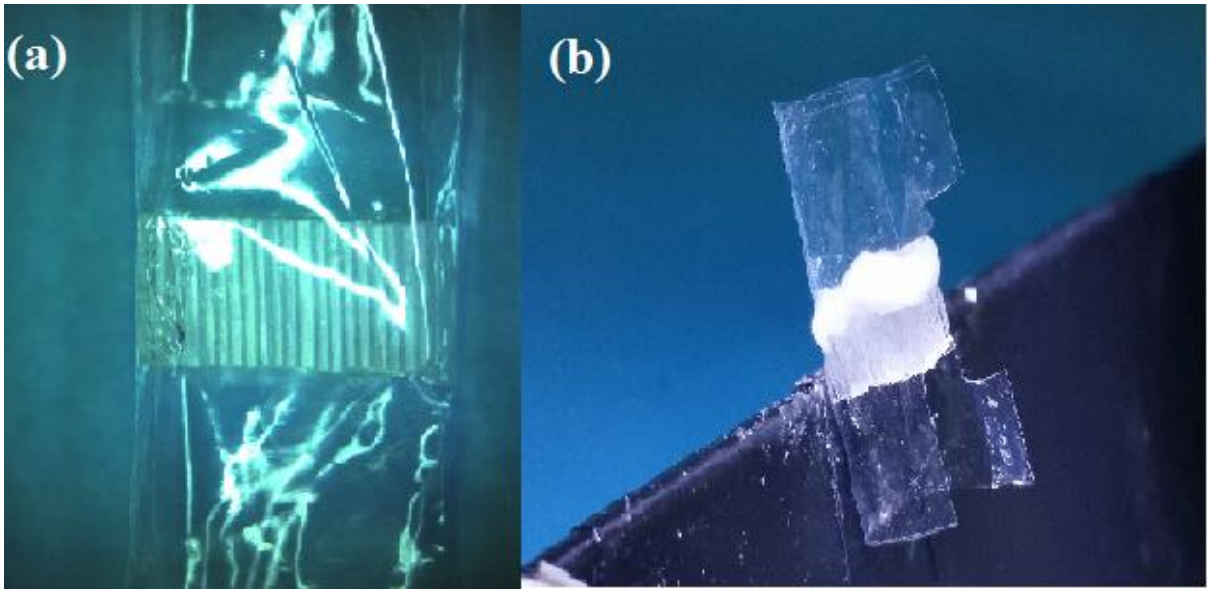

Figure 2.2: (a) Thin PDMS film wrapped around the multilayer microchannel scaffold. (b) An example demonstration of dental cement applied to one end of the scaffold and the opening made before inserting the wires

The dental cement on the device was then subjected to ultraviolet (UV) light for 8 seconds for it to harden and tighten the thin film to the structure while keeping it in place as it may be subjected to move throughout the fabrication process as seen in Figure2.2(b).

In order to place the microwires into the scaffold with a sense of ease, an X-Acto knife was used to cut along a length of the thin film and the edge of the dental cement at the bottom, on both ends of the device. But must remain attached to the rest of the thin film for it will be closed once the microwires have been inserted. On the portion of the thin film that was cut, a small areaof 1.5 $mm^2$was removed from the side closer to the dental cement. Removing this small area will create an opening where the microwires will pass through. An example result of these actions can clearly be seen in Figure 2.2(b).Once the PDMS scaffold has been constructed and prepared,commercially available microwires of 75 μm diameter (Stablohm 800A, California fine wire, Grover Beach, CA) were embedded within the microchannels. The wires were arranged in order to have 58 microwiresacting as probes and 6 EMG wires,whichwere all cut into 9-inch segments.With the use of a sharp blade and ruler, 0.5 mm ofinsulation wastrimmed off from the tip of the wires shown in Figure 2.3(b). Furthermore, 2 mm of each wire was alsobent to a 90° anglewith the help of precise tweezers under the microscope as shown in Figure 2.3(b).

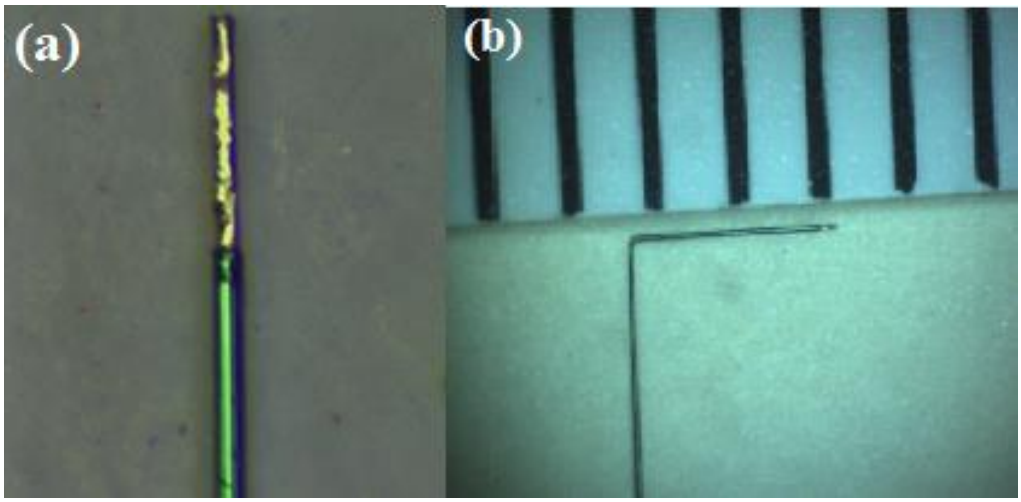

Figure 2.3: (a) 0.5 mm un-insulated green wire. (b) Single microwire bent to 90 degrees

The 58 uninsulated microwires were then placed one-by-one into the desiredmicrochannels through the opening made on the PDMS thin film and glued to the dental cement, previously applied,with more dental cement using the same technique in order to restrict any movement of the wires, as seen in Figure 2.4 (a). It is crucial that the microwires on both ends of the scaffold are kept within the 1.5 mm^2 cut-off areato keep the space occupied by the wire to a minimum, shown in Figure 2.4(b), which will increase the chances of nerves to regenerate into the microchannel scaffold.Dental cement was then used to seal the opening that was made, being careful not to allow any dental cement into the regenerating path of axons.

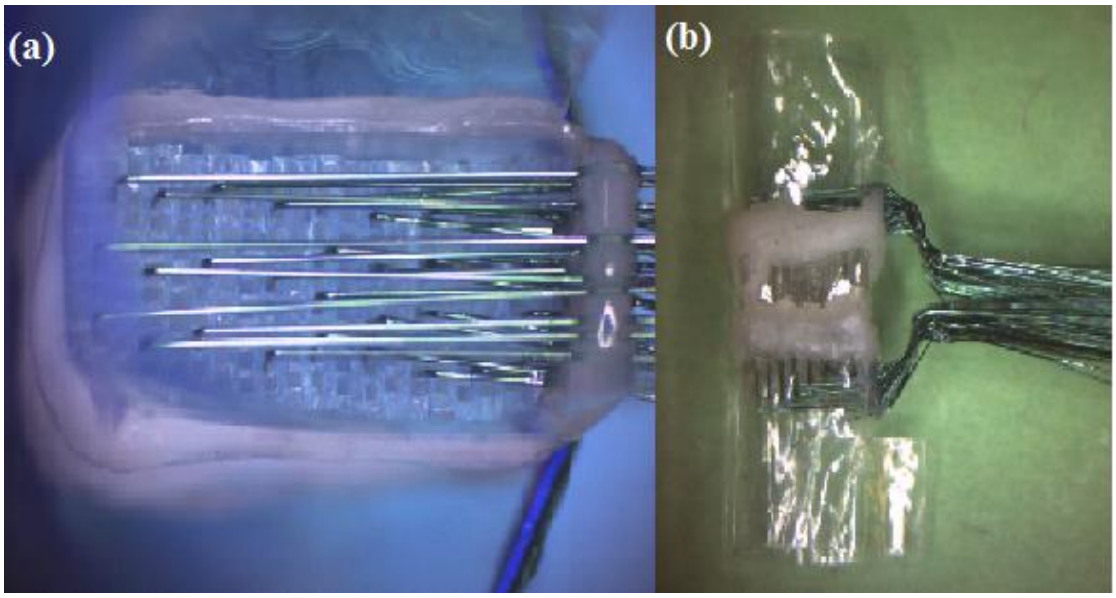

Figure 2.4: (a) A cross-sectional view of 32 wires placed into the microchannels.
(b) A side view showing all 58 wires placed within the openings.

All 64 wires were braided together to make them as compact as possible. Next, the nerve entry points of the device were reduced from 6 mm to 2 mm(Figure 2.5(a)), as an attempt to make them approximately the same diameter as the target nerve. To reduce the entry points

each end of the thin film was cut at an angle to the desired measurement and was then closed using tweezers and secured with dental cement.Additionally, any space between the wires and microchannel scaffold were filled with epoxy glue to provide structural support while also acting as an additional layer of insulation. The end product of these steps is shown in Figure 2.5(b).

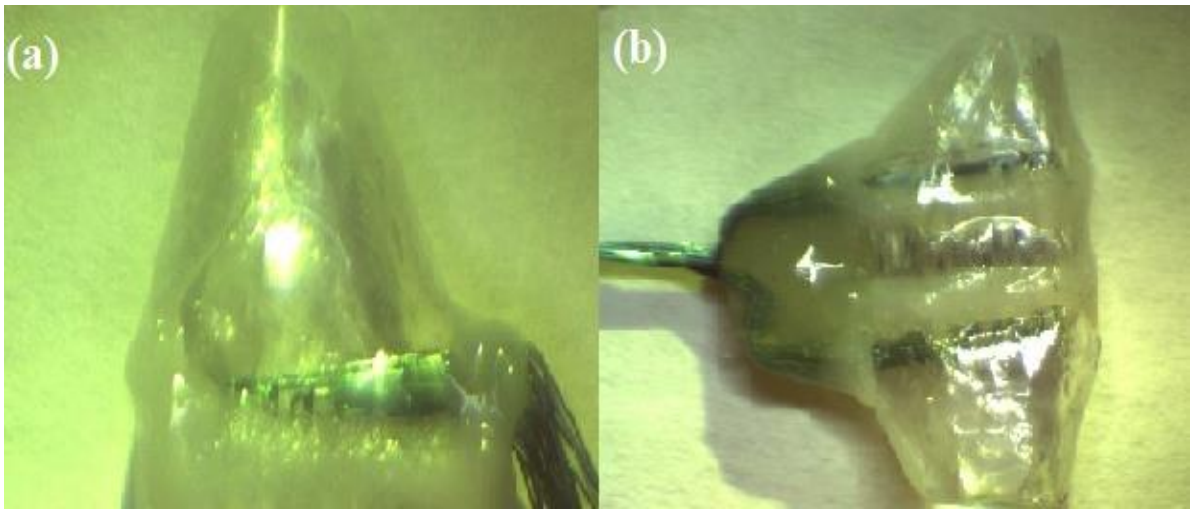

Figure 2.5: (a) Reduced the diameter with 2 mm. (b) Shows the openings sealed, and open spaces filled with epoxy glue.

Once the multilayer microchannel scaffold has been completed, the next part of the process is to organize the wires, So they could properly be linked to the TDT 128-channel interface board connector that will act as the interface between the computer and our device. In this implementation, we used twocommercially available Molex 33-channel ribbon cablesthat will be inserted into the TDT input connectors and be a bridge to the stablohm wires. Each of these ribbon cables have 33 conducting wires running parallel to each other on the same flat plane, shown in Figure 2.6(a), whilethe 17^{th} conducting wire was marked and used as ground.

Each ribbon cable was cut to a length of 15 mm with a sharp blade and removed 0.2 mm of insulation at the end of the ribbon cable that would connect to the other end of stablohm wires. Up to 5 mm of each wire of the ribbon cable was separated from the rest of the cable, as shown in Figure 2.6(b), to prevent the wires from making contact after they have been soldered with the stablohm wires. Next, the 6 EMG wires were evenly organized among the two ribbon cables and all 64stablohm wires were trimmed 0.2 mm from other end before soldering to the ribbon cables with the help of a sharp blade to isolate a single wire from the rest of the ribbon cable and a precise soldering iron. The result of 32 microwires (29 probe wires and 3 EMG wires) soldered onto a ribbon cable is shown in Figure 2.7(a). Furthermore, two 1.5 inch uninsulated wires (AS631, Cooner Wire, Chatsworth, CA)were connected to the 17th unused wire on both ribbon cables ribboncables to act as the ground.

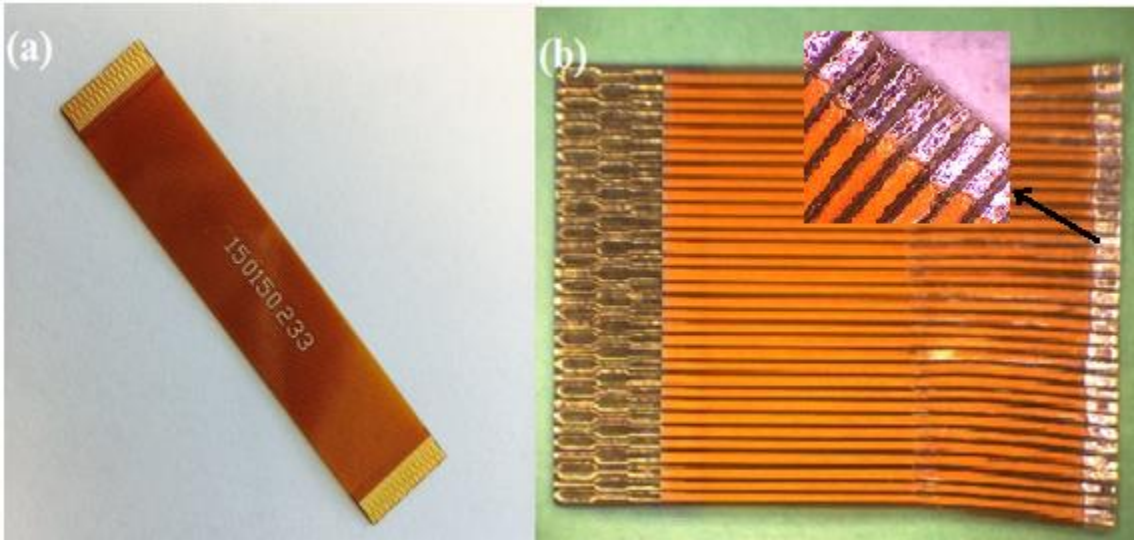

Figure 2.6: (a) Single ribbon cable. (b) Uninsulated 15 mm ribbon cablesepara

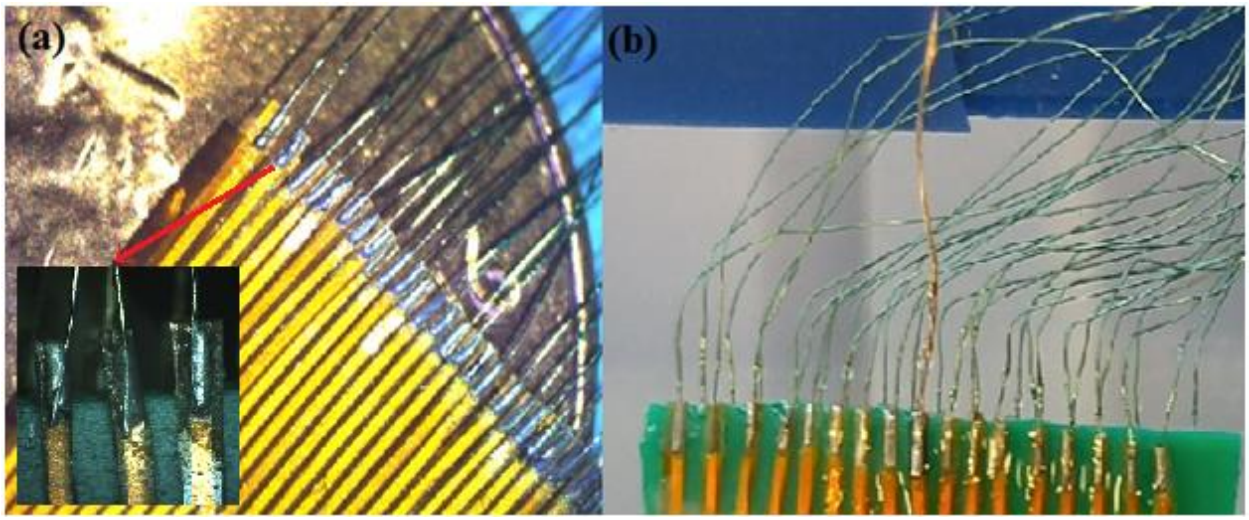

Figure 2.7: (a) Stablohm wires soldered to a ribbon cable. (b)Ribbon cables after soldering and separation of the wires with tape.

After finishing all soldering parts, all ribbon cable wires were kept separated by intertwining a small piece of tape in between the wires shown in Figure 2.7(b).Dental cement glue was applied, using the same UV light procedure to harden it, on each and every wire to secure them in place and keep them from accidently making contact during implementation, shown in Figure 2.8

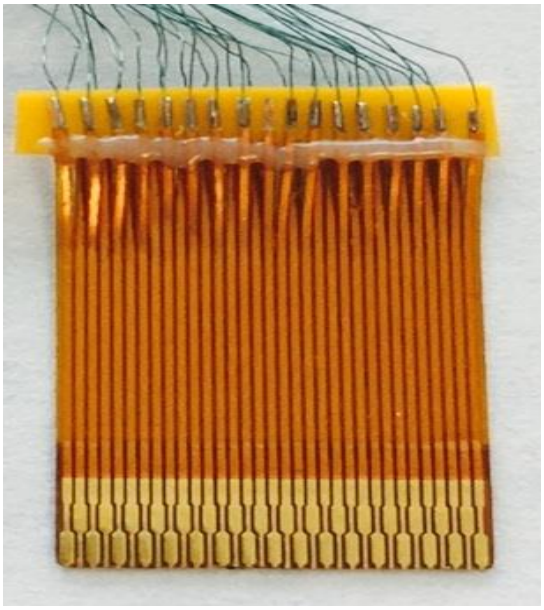

Figure 2.8: Dental cement glue was applied on separated wires.

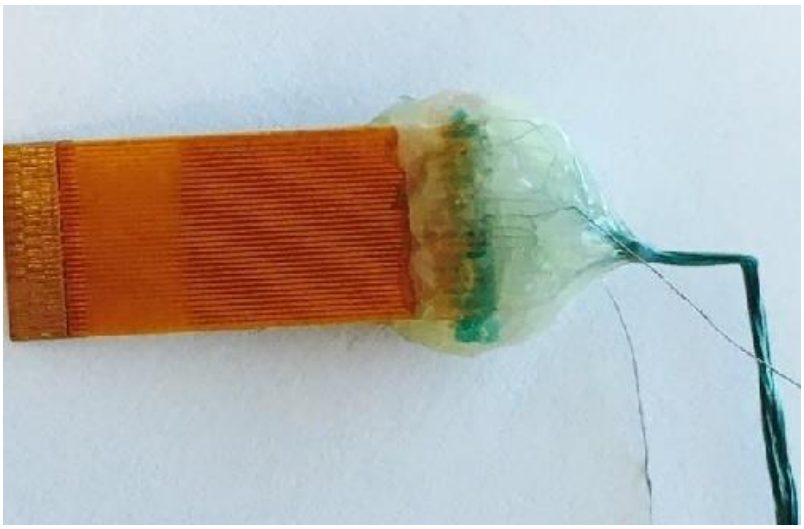

Figure 2.9: Two ribbon cables after covering all spaces with epoxy glue.

Once all connections had been checked for proper contact, epoxy glue was applied betweenall wiresto ensure that all connections will be kept separated and prevented from becoming unattached shown in Figure 2.9. This epoxy glue will act as insulation for each wire and provide physical support for the two ribbon cables and wires. The connection of the ribbon cables to the TDT interface board is shown in Figure 2.10. After twisting and ensuring the braided stablohm wires will not become loose, the device was completed.

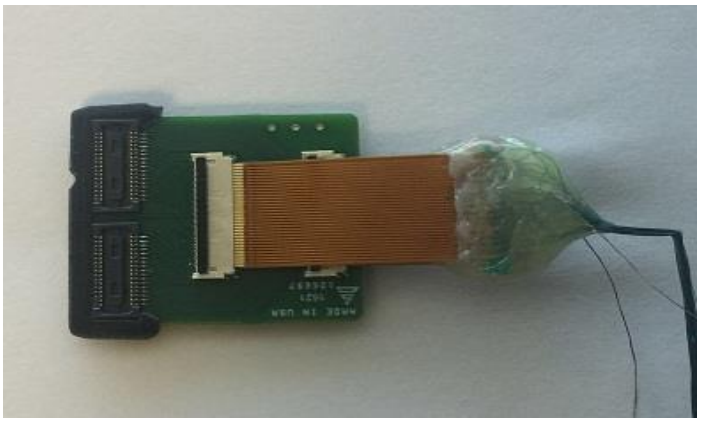

Figure 2.10: Ribbon cables connected to the TDT 128-channel interface board connector

CHAPTER III

SURGICAL PROCEDURE

All surgical procedures were performed under aseptic condition at the UTRGV Animal Facility as seen in (figure 4.1). Prior to implantation, the*Lewis* rat was placed into an induction chamber and subjected to gas anesthesia (Isoflurance) until unconscious.

Figure 3.1: Surgery setup and implementation.

All surgical procedures were done under stringent ethical standards at the UTRGV animal facility. The surgery location (right thigh and top of the head) were shaved and cleaned using a betadine scrub and isopropyl alcohol. Its maxillary central incisors were hooked into a gas mask through which it continued to receive small doses of anesthesia. It was secured to surgery table and its body temperature was regulated with the placement of a heat pad. Incisions were made along the right thigh to expose the rat sciatic nerve.

The designed μPNI was implanted by suturing both the distal and proximal ends of the nerves to the guides of the device. EMG signals were obtained by implanting pairs of microwires (Stablohm 800A, California Fine Wires, CA) (75 μm diameter) into the TA and SOL. All electrodes were guided subcutaneously to an incision made at the top of the head and henceforth attached to a ribbon cable connector, which was secure to the skull using dental cement and stainless steel screws (Figure 3.2). All procedures conformed to the Guide for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (National Academy Press, Washington, DC, 1996) and were reviewed and approved by the Institutional Animal Care and Use Committee UTRGV.

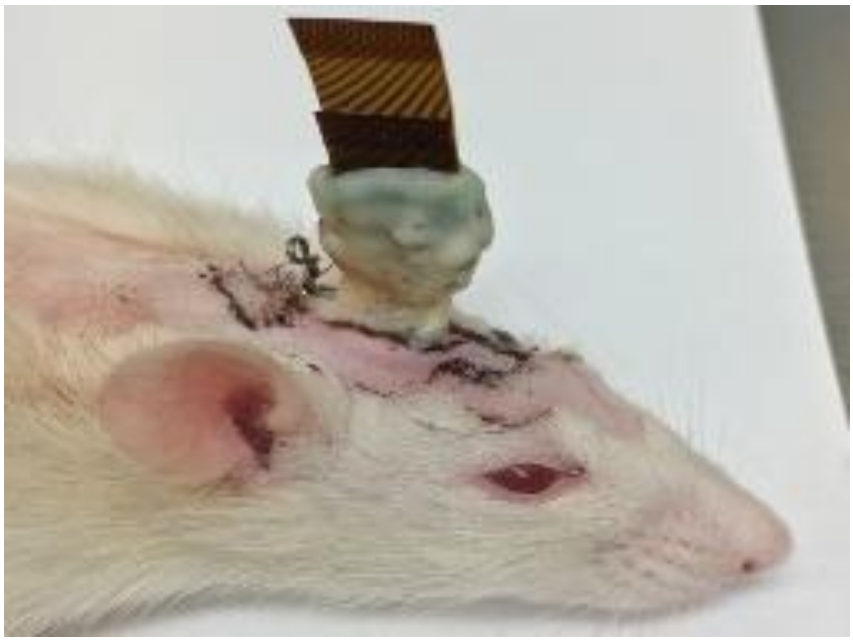

Figure 3.2μPNI device implemented rat sciatic nerve and dental cement was applied to skull for securing the cables.

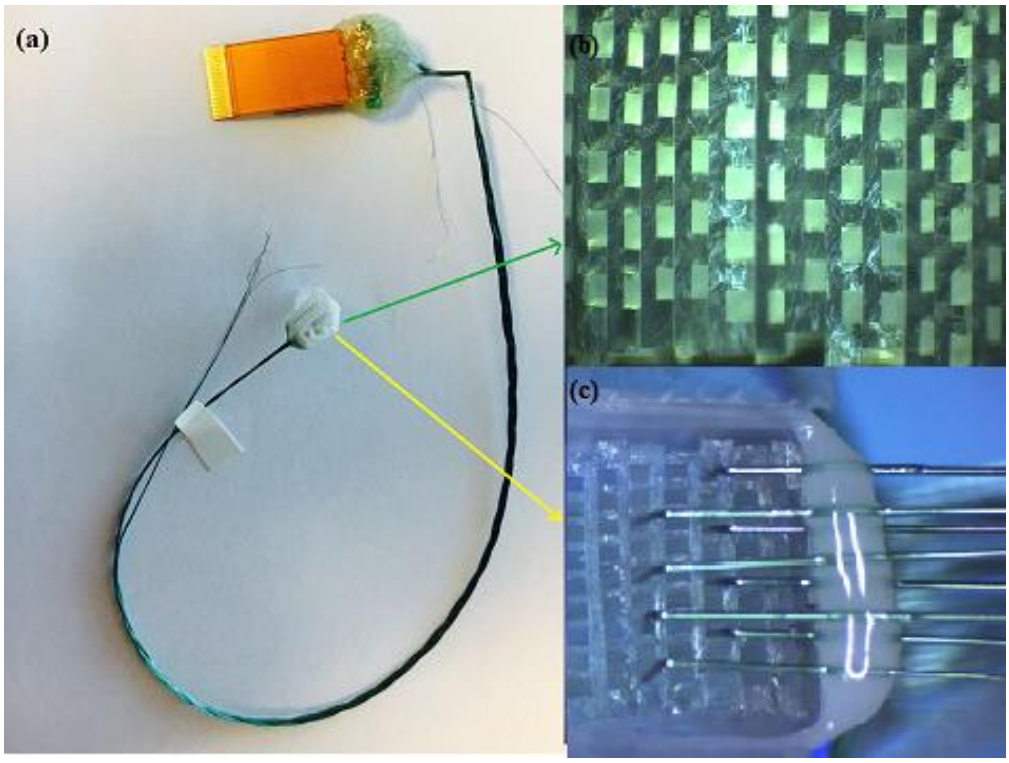

Figure 3.3: Implantable micro devices (a) whole configuration.(b) 120μm microchannel scaffold before inserting microwires.(c) μPNI after microwires were inserted inside microchannels.

CHAPTER IV

RESULTS

A fabricated implantable microdevice ready for the surgery. Both Molex 33-channel ribbon cables for TDT (Tucker-Davis Technologies) neuroware and TDT board for TDT stemware were placed at one end of the microwire bundle. The other end of the microwires bundle was connected with a μPNI and three pairs of electromyography electrodes. Peripheral nerve axons were targeted in the μPNI using microchannels that isolate different groups of axons. Since the developed fabrication technique is simple and adjustable, the scaffold parameters can be modified to fit different applications. The μPNI was implanted between the transected sciatic nerve stumps. All embedded electrodes were routed subcutaneously and connected to a head-mounted plug. The nerve stumps were sutured on each side of the μPNI while an animal was awake and free behavingfor behavioral pattern analysis, TDT-64 system recorded the electrophysiological signals from all implanted micro devices. TDT stimulator was used to generate the evoked signal to analyze the neural pathways and electrophysiological properties of the sciatic nerve branches. Neural recording and stimulation signals were forced to flow longitudinally within the microchannel scaffolds which make each microchannel independent from all other microchannels, making it possible to retrieve specific signals. The implantable devices of the μPNI, and the EMG electrodes were implanted in the animal and neural signal recordings were obtained, while the animal was actively moving around a closed perimeter(Figure4.1).

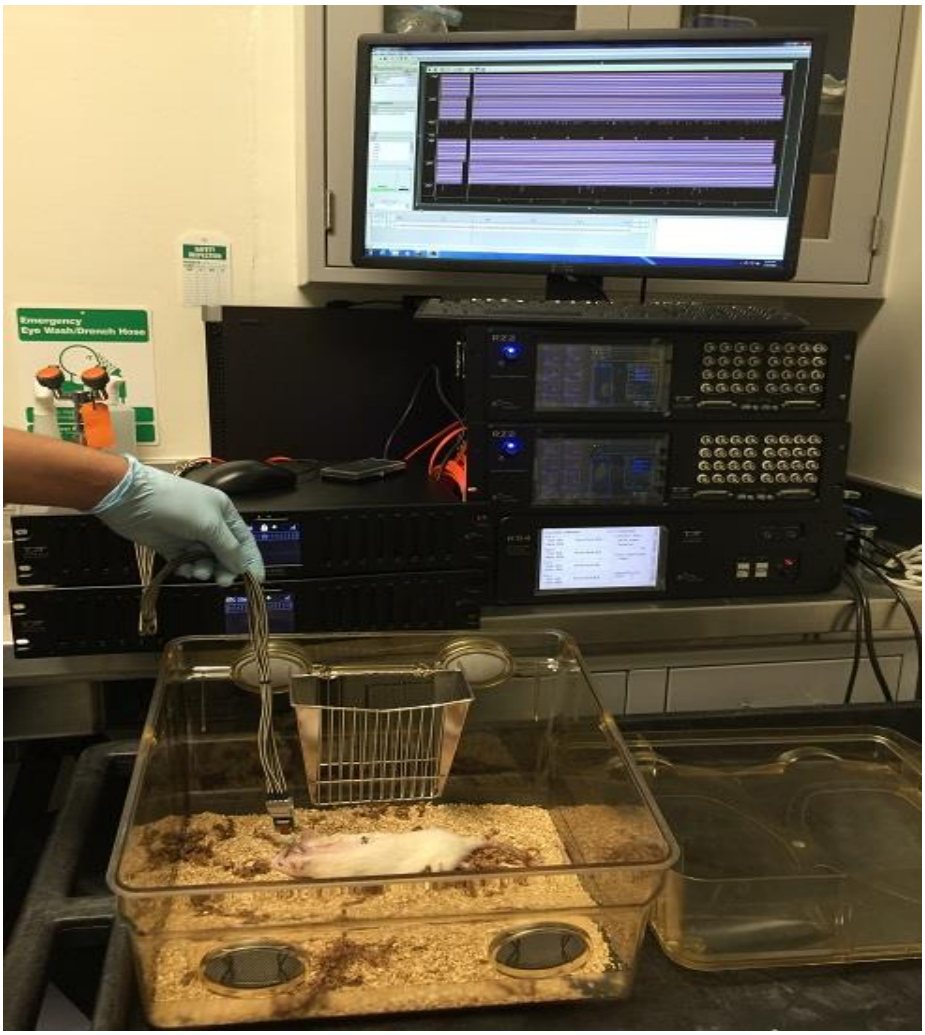

Figure4.1: The acquisition system setting. One end of a TDT connector isconnected to top of the rat's head and the other end is connected to PZ5

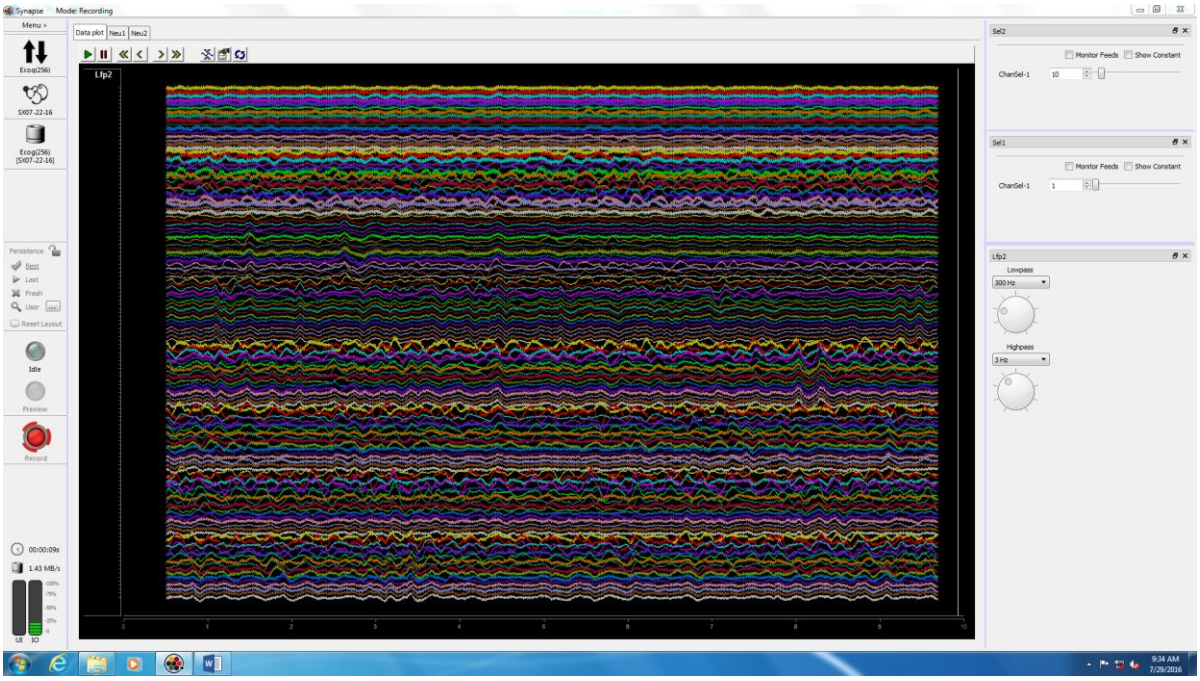

Figure 4.2: The 64 electrophysiological signals captured by the 64 electrodesμPNI using TDT acquisition system

The TDT acquisition system gave the maximum flexibility for the locomotion studies. Due to the robust nerve regeneration of the sciatic nerve, all channels were occupied with regenerated nerve.(Figure 4.2) shows the electrophysiological signals captured by the 64-electrode microwireμPNI using TDT recording system three weeks after implantation. The neural signals through the regenerated nerves in the μPNI were recorded and analyzed to retrieve data corresponding to animal behavior patterns. Electrophysiological signals were recorded from all 64 electrodes. The extracellular recording of neural signals consists of action potentials from

several neurons near the electrode site, and background noise. Since information of the nervous system is encoded in the form of firing frequency or firing time, the first procedure in the interpretation of neuronal signals is the detection of the action potential firing, i.e., the neural spike. In spite of the fundamental importance of this, only a few studies on neural spike detection have appeared in the literature. In most cases, major efforts have been made to optimize experiments so that the recorded waveforms are of sufficient quality to enable reliable detection by simple traditional methods. However, situations are often encountered where the signal-to-noise ratio (SNR) of the recording is so poor as to prohibit neural spike detection using simple thresholding, and in some cases, such as the recording from a long-term implanted electrode, precise experimental control cannot be achieved. Moreover, the statistical characteristics of background noise can be very similar to those of the target signal (action potential). In particular, longitudinal intrafascicular electrodes (LIFEs), waveletdenoising technique was used to improve the signal-to-noise ratio (SNR) in the neural signals recorded. Wavelet denoising is a set of techniques used to remove noise from signals. The main idea is to transformthe noisy data into an orthogonal time-frequency domain. In that domain, thresholding is applied to the coefficients for noise removal, and the coefficients are finally transformed back into the original domain obtaining the denoised signal. The denoised signals were then used to identify the dispatched motor commands by implementing the following procedure. For each trial, the different recording periods (’epochs’) related to the movement classes (e.g. grip types and rest) were labeled. The three desired movements and the rest were considered as separated classes. The performance metrics considered was the ratios between classes correctly identified out of those presented and the leave one out validation standard method has been used. Each epoch was a

example that was used to train the classifier or to test its generalization skills. The feature vector was made of the ratios between the number of spikes matching each spike template and the total number of spikes in the epoch. Therefore, the absolute spike rates were not used, but rather the relative spike rates of each waveform with respect to the others. This should prevent classification of the motor commands based on the "quantity of activity" and favor the use of the "quality of activity" intended in terms of different waveforms for different stimuli.The combined use of wavelet denoising and spike sorting algorithms could increase the amount of information that is decoded from intraneural recordings in the PNS. This could allow the development of more effective neuroprosthesis systems.

Here, in order to remove noise from the signals, we first used notch filter for removing 60 Hz and similar interferences from the waveforms and re-referenced them to one of the 64 channels. Next, we band pass filtered the signal from 300 – 5000 Hz.

The unique neural signal patterns of the μPNI, depending on the animal behavior patterns, will not only confirm the brain-controlled neural signals at the μPNI, but also pioneer the delicate neuronal networks in the brain linked to the sensory and motor feedback of peripheral nerves. Action potentials with similar waveforms were identified in the locomotion microelectrode recordings and extracted using a timeamplitude window discriminator routine. The average amplitude of the action potentials extracted from microchannels was about 100 μV withamplitudesranging from 40~200 μV.

A neural signal combination of all microwires of the μPNI, or part of them, will express a behavioral pattern at a specific temporal moment. A repeatable behavioral pattern may express a series of temporal neural signal patterns. Thin scar tissue formation covering outside PDMS scaffolds was observed from the harvested μPNI. However, no obstructing inflammation responses was observed inside microchannels with a two-month regeneration period. PDMS is an FDA approved biomaterial for several clinical applications. As a biocompatible material, PDMS has been used in a wide range of applications, such as a structure itself as part of the device and an insulator. PDMS cuff electrodes have been used on the extradural sacral root to sense the bladder response to stimulation in patients.

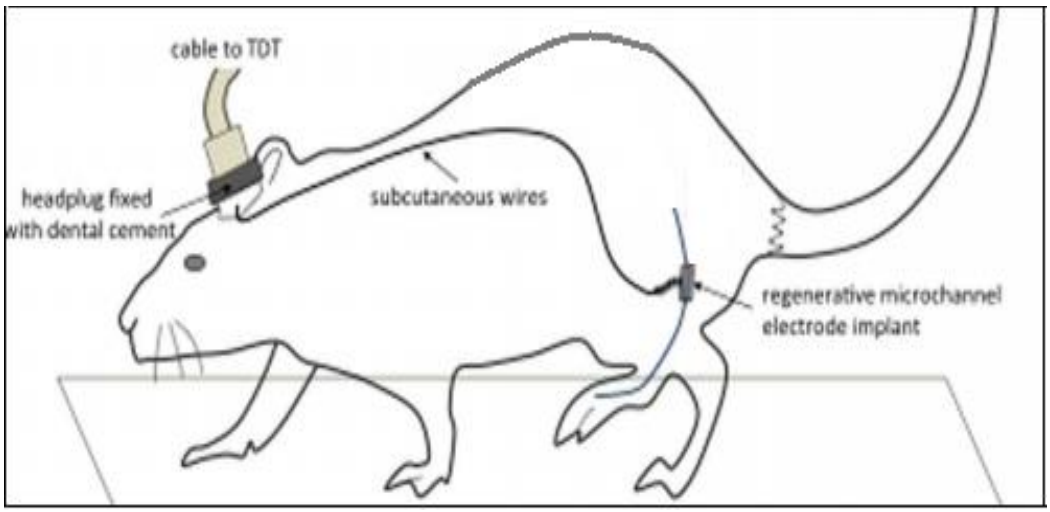

Figure 4.3: Schematic view of the animal model for μPNI on sciatic nerve

CHAPTER V

FUTURE RESEARCH

In recent years, many scientific and technological efforts have been devoted to develop hybrid bionic systems that link, via neural interfaces, the human nervous system with electronic and/or robotic prostheses, with the main aim of restoring motor and sensory functions in patients with spinal cord injuries, brain injuries, or degenerative diseases. Several designs, such as cuff electrodes, shaft electrodes, longitudinal intra fascicular electrodes (LIFE), regenerative sieve, and microchannel electrodes demonstrated selective recording and stimulation. However, the devices have limited electrode sites and recordings can only be obtained from the limited number of nerve fascicles. Moreover, they require advanced micro fabrication techniques that not all labs have access to and it makes the devices very expensive. In the present study, we designed 64-electrodes in the microchannel device. The μPNI has been successfully implanted in the rat sciatic nerves.This device was simply handcrafted, nocleanroom facility and micro machined fabrication technique was needed.Our future goal is to increases the number of electrodes inside the microchannel for it to receive more axon signals from the regenerated peripheral nerve. Currently we are fabricating 128 electrodes device. This will help to provide reliable connections, and better signal resolution for more functionality on neural prosthetics. Since increasing the number of electrodes means increasing the number of wires, the device would require more space for implementation. Therefore, another goal for future work is to keep the

device as compact as possible to prevent it from becoming a bulky device which will help maintain a minimally invasive implementation process.

The combination of these future goals will allow more control in neural prosthetic devices with the use of a larger number of neural signals while keeping the device's weight and size to a minimum.

CHAPTER VI

CONCLUSION

This research work describes in detail the design, testing of 64-electrode μPNIand successfully implanted in*Lewis* rats sciatic nerves. This device was simply handcrafted and developed with low cost components. Being constructed from commercially available material and not required micromachining techniques. Hear we developed 64-electrode μPNI device for recording nerve signals. We used PDMS microchannel stacks as components of the μPNI. PDMS has been widely used as a major material of the implantable devices for both research and clinical purposes [62-67], due to its easy fabrication technique and biocompatibility. To achieve translational capabilities, PDMS could be replaced by biodegradable materials, such as PCL, PLGA, and PGA [68-73]. After the nerve regeneration, the biodegradable microchannel will be dissolved to give the structures as close to a natural nerve as possible. Each biodegradable material needs to be tested for its own biocompatibility and degradation rate in the peripheral nerve model. The number of PDMS microchannel stacks can be easily modified that encourage the greatest amount of regeneration of neural tissue. Microchannel with a diameter 120 μm can be used to isolate individual axons by restricting the space allotted for regeneration. Other axons would thereby be forced to enter other microchannel.

The μPNI has been developed and successfully implanted in the rat sciatic nerves. The neural signal were collected from the rat sciatic nerve using TDT signal acquisition

system.Currently, we are fabricating 128-electrodes device for receiving more axon signals from the regenerated peripheral nerve. This will help to provide reliable connection, and better signal resolution for more functionality on neural prosthetics.

AAMI	Association for the Advancement of Medical Instrumentation
CNR	Cuff nerve diameter ratio
EMG	Electromyography
FINE	Flat interface nerve electrodes
LIFE	Longitudinal intra fascicular electrode
MEMS	Micro electro mechanical system
PCL	Polycaprolactone
PDMS	Polydimethylsiloxane
PGA	poly(glycolic acid)
PLGA	Poly(lactic-co-glycolic acid)
PNS	Peripheral nervous system
REMI	Regenerative multi electrode interface
TDT	Tucker- Davis Technologies
TF-FINE	Thin-film longitudinal intrafascicular electrode
TMR	Targeted muscle reinnervation
UV	Ultraviolet
μPNI	Micro channel Peripheral nervous interfaces

Table 4.1: Table of Acronyms

REFERENCES

[1] K. Ziegler-Graham, E. J. MacKenzie, and P. L. Ephraim et al., "Estimating the prevalence of limb loss in the United States: 2005 to 2050," Arch. Phys. Med. Rehabil., vol. 89, no. 3, pp. 422–429, Mar. 2008.

[2] P. F. Adams, G. E. Hendershot, and M. A. Marano, "Current estimates from the National Health Interview Survey, 1996," Vital Health Stat., vol. 10, no. 200, pp. 1–203, Oct. 1999.

[3] T. R. Dillingham, L. E. Pezzin, and E. J. MacKenzie, "Limb amputation and limb deficiency: Epidemiology and recent trends in the United States," South Med. J., vol. 95, no. 8, pp. 875–883, Aug. 2002.

[5] R. J. Vetter, J. C. Williams, and J. F. Hetke et al., "Chronic neural recording using silicon-substrate microelectrode arrays implanted in cerebral cortex," IEEE Trans. Biomed. Eng., vol. 51, no. 6, pp. 896–904, Jun. 2004.

[6] X. Liu, D. B. McCreery, and R. R. Carter et al., "Stability of the interface between neural tissue and chronically implanted intracortical microelectrodes," IEEE Trans. Rehabil. Eng., vol. 7, no. 3, pp. 315–326, Sep. 1999.

[7] J. B. Hijjawi, T. A. Kuiken, and R. D. Lipschutz et al., "Improved myoelectric prosthesis control accomplished using multiple nerve transfers," Plast. Reconstr. Surg., vol. 118, no. 7, pp. 1573–1578, Dec. 2006.

[8]T. A. Kuiken, P. D. Marasco, and B. A. Lock et al., "Redirection of cutaneous sensation from the hand to the chest skin of human amputees with targeted reinnervation," Proc. Nat. Acad. Sci. USA, vol. 104, no. 50, pp. 20061–20066, Dec. 11, 2007.

[9] L. A. Miller, K. A. Stubblefield, and R. D. Lipschutz et al., "Improved myoelectric prosthesis control using targeted reinnervation surgery: A case series," IEEE Trans. Neural Syst. Rehabil. Eng., vol. 16, no. 1, pp. 46–50, Feb. 2008.

[10] D. K. Leventhal and D. M. Durand, "Chronic measurement of the stimulation selectivity of the flat interface nerve electrode," IEEE Trans. Biomed. Eng., vol. 51, no. 9, pp. 1649–1658, Sep. 2004

[11] z. Durand, H. J. Park, and B. Wodlinger, "Models of the peripheral nerves for detection and control of neural activity," in Proc. IEEE Eng. Med. Biol. Soc. Conf., 2009, vol. 2009, pp. 3326–3329,2009,

[12] L. R. Hochberg, M. D. Serruya, and G. M. Friehs et al., "Neuronal ensemble control of prosthetic devices by a human with tetraplegia," Nature, vol. 442, no. 7099, pp. 164–171, Jul. 13, 2006.

[13] R. A. Normann, "Technology insight: Future neuroprosthetic therapies for disorders of the nervous system," Nat. Clin. Pract. Neurol., vol. 3, no. 8, pp. 444–452, Aug. 2007.

[14] N. A. Fitzsimmons, W. Drake, and T. L. Hanson et al., "Primate reaching cued by multichannel spatiotemporal cortical microstimulation," J. Neurosci., vol. 27, no. 21, pp. 5593–5602, May 23, 2007.

[15] G. S. Dhillon, S. M. Lawrence, and D. T. Hutchinson et al., "Residual function in peripheral nerve stumps of amputees: Implications for neural control of artificial limbs," J. Hand Surg. Am., vol. 29, no. 4, pp. 605–615, Jul. 2004.

[16] S. Micera, L. Citi, and J. Rigosa et al., "Decoding information from neural signals recorded using intraneural electrodes: Toward the development of a neurocontrolled hand prosthesis," Proc. IEEE, vol. 98, no. 3, pp. 407–417, Mar. 2010.

[17] S. Raspopovic, M. Capogrosso, and S. Micera, "A computational model for the stimulation of rat sciatic nerve using a transverse intrafascicular multichannel electrode," IEEE Trans. Neural Syst. Rehabil. Eng., vol. 19, no. 4, pp. 333–344, Aug. 2011.

[18] R. Biran, D. C. Martin, and P. A. Tresco, "Neuronal cell loss accompanies the brain tissue response to chronically implanted silicon microelectrode arrays," Exp. Neurol., vol. 195, no. 1, pp. 115–126, Sep. 2005.

[19] R. Biran, D. C. Martin, and P. A. Tresco, "The brain tissue response to implanted silicon microelectrode arrays is increased when the device is tethered to the skull," J. Biomed. Mater. Res. A, vol. 82, no. 1, pp. 169–178, Jul. 2007.

[20] B. K. Leung, R. Biran, and C. J. Underwood et al., "Characterization of microglial attachment and cytokine release on biomaterials of differing surface chemistry," Biomaterials, vol. 29, no. 23, pp. 3289–3297, Aug. 2008.

[21] J. C. Williams, J. A. Hippensteel, and J. Dilgen et al., "Complex impedance spectroscopy for monitoring tissue responses to inserted neural implants," J. Neural Eng., vol. 4, no. 4, pp. 410–423, Dec. 2007

[22]K. Garde, E. W. Keefer, and B. Botterman et al., "Early interfaced neural activity from chronic amputated nerves," Front. Neuroeng., vol. 2, no. 5, pp. 1–11, 2009.

[23]Dhillon GS, Lawrence SM, Hutchinson DT, Horch KW (2004) Residual function in peripheral nerve stumps of amputees: implications for neural control of artificial limbs. J Hand Surg Am 29: 605-615.

[24] Kuiken TA, Li G, Lock BA, Lipschutz RD, Miller LA, Stubblefield KA, et al. (2009) Targeted Muscle Reinnervation for Real-Time Myoelectric Control of Multifunction Artificial Arms. JAMA 301: 619–628.

[25] Rossini PM, Micera S, Benvenuto A, Carpaneto J, Cavallo G, et al. (2010) Double nerve intraneural interface implant on a human amputee for robotic hand control. ClinNeurophysiol 121: 777-783.

[26] Agnew SP, Schultz AE, Dumanian GA, Kuiken TA (2012) Targeted reinnervation in the transfemoral amputee: a preliminary study of surgical technique. PlastReconstrSurg 129: 187-194.

[27] Badia J, Boretius T, Andreu D, Azevedo-Coste C, Stieglitz T, et al. (2011) Comparative analysis of transverse intrafascicular multichannel, longitudinal intrafascicular and multipolar cuff electrodes for the selective stimulation of nerve fascicles. J Neural Eng 8: 036023.

[28] Tyler DJ, Durand DM (2002) Functionally selective peripheral nerve stimulation with a flat interface nerve electrode. IEEE Trans Neural SystRehabilEng 10:294-303.

[29] A Grill WM, Mortimer JT (2000) Neural and connective tissue response to long term implantation of multiple contact nerve cuff electrodes. J Biomed Mater Res 50: 215-226

[30] Serra J, Bostock H, Navarro X (2010) Microneurography in rats: a minimally invasive method to record single C-fiber action potentials from peripheral nerves in vivo. Neurosci Lett 470: 168-174.54

[31] Lawrence SM, Dhillon GS, Jensen W, Yoshida K, Horch KW (2004) Acute peripheral nerve recording characteristics of polymer-based longitudinal intrafascicular electrodes. IEEE Trans Neural SystRehabilEng 12: 345-348.

[32] Lawrence SM, Dhillon GS, Jensen W, Yoshida K, Horch KW (2004) Acute peripheral nerve recording characteristics of polymer-based longitudinal intrafascicular electrodes. IEEE Trans Neural SystRehabilEng 12: 345-348

[33] Navarro X, Calvet S, Rodríguez FJ, Stieglitz T, Blau C, et al. (1998) Stimulation and recording from regenerated peripheral nerves through polyimide sieve electrodes. J PeripherNervSyst 3: 91-101.

[34] Ceballos D, Valero-Cabré A, Valderrama E, Schüttler M, Stieglitz T, et al. (2002) Morphologic and functional evaluation of peripheral nerve fibers regenerated through polyimide sieve electrodes over long-term implantation. J Biomed Mater Res 60: 517-528.

[35] Lago N, Ceballos D, Rodríguez FJ, Stieglitz T, Navarro X (2005) Long term assessment of axonal regeneration through polyimide regenerative electrodes to interface the peripheral nerve. Biomaterials 26: 2021-2031.

[36] Castro J, Negredo P, Avendaño C (2008) Fiber composition of the rat sciatic nerve and its modification during regeneration through a sieve electrode. Brain Res 1190: 65-77.

[37] Cho SH, Lu HM, Cauller L, Romero Ortega MI, Lee JB, et al. (2008) Biocompatible SU-8-Based Microprobes for Recording Neural Spike Signals from Regenerated Peripheral Nerve Fibers. IEEE Sensors Journal 8: 1830-1836

[38] Garde K, Keefer E, Botterman B, Galvan P, Romero MI (2009) Early interfaced neural activity from chronic amputated nerves. Front Neuroeng 2: 5.20.

[39] FitzGerald JJ, Lago N, Benmerah S, Serra J, Watling CP, et al. (2012) A regenerative microchannel neural interface for recording from and stimulating peripheral axons in vivo. J Neural Eng 9: 016010

[40]Cho, Sung-Hoon, et al. "Biocompatible SU-8-based microprobes for recording neural spike siznals from regenerated peripheral nerve fibers." *Ieee Sensors Journal* 8.11 (2008): 1830-1836.

[41] Gore RK, Choi Y, Bellamkonda R, English A (2015) Functional recordings from awake, behaving rodents through a microchannel based regenerative neural interface. J Neural Eng 12: 016017

[42] Choi Y, Gore RK, English AW, Bellamkonda RV (2012) Multilumen PDMS scaffolds for peripheral nerve repair and interface. In: the 40th Neural Interfaces Conference, Salt Lake City, UT, 2012.

[43] Choi Y, Park S, Chung Y, Gore RK, English AW, et al. (2014) PDMS microchannel scaffolds for neural interfaces with the peripheral nervous system. In: The 27th IEEEInternational Conference on Micro Electro Mechanical Systems (MEMS 2014), San Francisco, CA.

[44] Choi Y, Shafqat F, Heo H, Bellamkonda RV (2012) Development of micro channel nerve grafts and their application in peripheral nerve regeneration. In: 2012 International Annual Symposium on Regenerative Rehabilitation, Pittsburgh, PA

[45] Gore RK, Choi Y, English AW, Bellamkonda RV (2012) Peripheral neural recordings in awake and behaving rats after sciatic regeneration through a microchannel based neural nterface. In: Neuroscience 2012, Society for Neuroscience 42nd annual meeting, New Orleans, LA.

[46] Hossain R, Kim B, Pankratz R, Ajam A, Park S, et al. (2015) Handcrafted multilayer PDMS microchannel scaffolds for peripheral nerve regeneration. Biomed Microdevices 17: 109.

[47] Ajam, Ali, et al. "Handcrafted Microwire Regenerative Peripheral Nerve Interfaces with Wireless Neural Recording and Stimulation Capabilities."*International Journal of Sensor Networks and Data Communications* 2016 (2016).

[48] KurstjensGaM, Borau A, Rodriguez A, Rijkhoff NJM, Sinkjaer T (2005) Intraoperative recording of electroneurographic signals from cuff electrodes on extradural sacral roots in spinal cord injured patients. The Journal of Urology 174: 1482–1487.

[49] Stöver T, Lenarz T (2009) Biomaterials in cochlear implants. GMS Curr Top Otorhinolaryngol Head Neck Surg 8: Doc10.

[50] Malcolm RK, Edwards KL, Kiser P, Romano J, Smith TJ (2010) Advances in microbicide vaginal rings. Antiviral Res 88 Suppl 1: S30-39.

[51] Grill WM, Craggs MD, Foreman RD, Ludlow CL, Buller JL (2001) Emerging clinical applications of electrical stimulation: opportunities for restoration of function. J Rehabil Res Dev 38: 641-653.

[52] Martens FM, Heesakkers JP (2011) Clinical results of a brindley procedure: sacral anterior root stimulation in combination with a rhizotomy of the dorsal roots. AdvUrol 2011: 709708.

[53] Andriot M, Degroot JV, Meek J, Meeks R, Gerlach E, et al (2007) Silicones in Industrial Applications. Inorganic Polymers, RD Jaeger and M. Gleria, Edsed: Nova Science Publishers pp. 61-161.

[54] Sivak WN, Bliley JM, Marra KG (2014) Polymeric Biomaterials for Nerve Regeneration: Fabrication and Implantation of a Biodegradable Nerve Guide. In: Axon Growth and Regeneration A. J. Murray, Ed ed: Springer pp. 139-148.

[55] Liu JJ, Wang CY, Wang JG, Ruan HJ, Fan CY (2011) Peripheral nerve regeneration using composite poly (lactic acid-caprolactone)/nerve growth factor conduits prepared by coaxial electrospinning. Journal of Biomedical Materials Research Part A 96A: 13-20.

[56] Nectow AR, Marra KG, Kaplan DL (2012) Biomaterials for the development of peripheral nerve guidance conduits. Tissue Eng Part B Rev 18: 40-50.

[57] Kehoe S, Zhang XF, Boyd D (2012) FDA approved guidance conduits and wraps for peripheral nerve injury: a review of materials and efficacy. Injury 43: 553-572
.
[58] Mobasseri A, Faroni A, Minogue BM, Downes S, Terenghi G, et al. (2015) Polymer scaffolds with preferential parallel grooves enhance nerve regeneration. Tissue Eng Part A 21: 1152-1162.

[59] Sterne GD, Brown RA, Green CJ, Terenghi G (1997) Neurotrophin-3 delivered locally via fibronectin mats enhances peripheral nerve regeneration. Eur J Neurosci 9: 1388-1396.

[60]Ajam, Ali, et al. "Handcrafted Microwire Regenerative Peripheral Nerve Interfaces with Wireless Neural Recording and Stimulation Capabilities."*International Journal of Sensor Networks and Data Communications* 2016 (2016).

[61] Hossain, Ridwan, et al. "Handcrafted multilayer PDMS microchannel scaffolds for peripheral nerve regeneration." *Biomedical microdevices* 17.6 (2015): 1-9.

[62] KurstjensGaM, Borau A, Rodriguez A, Rijkhoff NJM, Sinkjaer T (2005) Intraoperative recording of electroneurographic signals from cuff electrodes on extradural sacral roots in spinal cord injured patients. The Journal of Urology 174: 1482–1487.

[63]Stöver T, Lenarz T (2009) Biomaterials in cochlear implants. GMS Curr Top Otorhinolaryngol Head Neck Surg 8: Doc10.

[64]Malcolm RK, Edwards KL, Kiser P, Romano J, Smith TJ (2010) Advances in microbicide vaginal rings. Antiviral Res 88 Suppl 1: S30-39.

[65] Grill WM, Craggs MD, Foreman RD, Ludlow CL, Buller JL (2001) Emerging clinical applications of electrical stimulation: opportunities for restoration of function. J Rehabil Res Dev 38: 641-653.

[66] Martens FM, Heesakkers JP (2011) Clinical results of a brindley procedure: sacral anterior root stimulation in combination with a rhizotomy of the dorsal roots. AdvUrol 2011: 709708.

[67]Andriot M, Degroot JV, Meek J, Meeks R, Gerlach E, et al (2007) Silicones in Industrial Applications. Inorganic Polymers, RD Jaeger and M. Gleria, Edsed: Nova Science Publishers pp. 61-161.

[68] Sivak WN, Bliley JM, Marra KG (2014) Polymeric Biomaterials for Nerve Regeneration: Fabrication and Implantation of a Biodegradable Nerve Guide. In: Axon Growth and Regeneration A. J. Murray, Ed ed: Springer pp. 139-148.

[69] Liu JJ, Wang CY, Wang JG, Ruan HJ, Fan CY (2011) Peripheral nerve regeneration using composite poly (lactic acid-caprolactone)/nerve growth factor conduits prepared by coaxial electrospinning. Journal of Biomedical Materials Research Part A 96A: 13-20.

[70] Nectow AR, Marra KG, Kaplan DL (2012) Biomaterials for the development of peripheral nerve guidance conduits. Tissue Eng Part B Rev 18: 40-50.

[71] Kehoe S, Zhang XF, Boyd D (2012) FDA approved guidance conduits and wraps for peripheral nerve injury: a review of materials and efficacy. Injury 43: 553-572.

[72] Mobasseri A, Faroni A, Minogue BM, Downes S, Terenghi G, et al. (2015) Polymer scaffolds with preferential parallel grooves enhance nerve regeneration. Tissue Eng Part A 21: 1152-1162.

[73] Sterne GD, Brown RA, Green CJ, Terenghi G (1997) Neurotrophin-3 delivered locally via fibronectin mats enhances peripheral nerve regeneration. Eur J Neurosci 9: 1388 1396.

BIOGRAPHICAL SKETCH

ManisankarChennamsetti was born on June 27, 1992. He has done his Bachelors of Technology in Electronics and Communications from JNT University, India in 2013 and his Master's degree from the University of Texas Rio Grande Valley in August 2016. Currently he is Research Assistant in department of Electrical Engineering. His main research on Regenerative Peripheral nerve interface that facilitate a direct connection between the nervous system and external technologies suchas limb prosthetics or data acquisition system for future processing. Upon Graduation from the University of Texas Rio Grande Valley, ManisankarChennamsetti is looking forward to being Ph.D program in the United States.

Permanent Address: 2-21/A Ravipadu Post, Pedanandipadu Mandal, Guntur Dist, AP-522015

Permanent email Address: manisankar114@gmail.com