ANATOMICALLY-VERSATILE PERIPHERAL NERVE ELECTRODES PRESERVE NERVE HEALTH, RECRUIT SELECTIVELY, AND STABILIZE QUICKLY

BY

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DEDICATION

For my friends and family, who have tirelessly supported me, guided me, and encouraged my graduate education.
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<tbody>
<tr>
<td>ADLs</td>
<td>Activities of daily living</td>
</tr>
<tr>
<td>AISA</td>
<td>American Spinal Injury Association</td>
</tr>
<tr>
<td>C-FINE</td>
<td>Composite flat interface nerve electrode</td>
</tr>
<tr>
<td>CMAP</td>
<td>Compound muscle action potential</td>
</tr>
<tr>
<td>CNR</td>
<td>Cuff-to-Nerve ratio</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CV</td>
<td>Conduction velocity</td>
</tr>
<tr>
<td>CWRU</td>
<td>Case Western Reserve University</td>
</tr>
<tr>
<td>DAQ</td>
<td>Data acquisition</td>
</tr>
<tr>
<td>APW</td>
<td>Pulse width difference between primary and secondary recruited muscles</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>ES</td>
<td>Electrical stimulation</td>
</tr>
<tr>
<td>$f_{\text{stim}}$</td>
<td>Frequency of delivery of stimulation pulses ($1 / \text{IPI}$)</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and drug administration</td>
</tr>
<tr>
<td>FINE</td>
<td>Flat interface nerve electrode</td>
</tr>
<tr>
<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
</tr>
<tr>
<td>IDE</td>
<td>Investigational device exemption</td>
</tr>
<tr>
<td>IPG</td>
<td>Implanted pulse generator</td>
</tr>
<tr>
<td>IPI</td>
<td>Interpulse interval ($1 / f_{\text{stim}}$)</td>
</tr>
<tr>
<td>IRB</td>
<td>Institutional Review Board</td>
</tr>
<tr>
<td>IST-16</td>
<td>16-channel implanted stimulator-telemeter</td>
</tr>
<tr>
<td>LCn</td>
<td>Left contact n</td>
</tr>
<tr>
<td>LIFE</td>
<td>Longitudinal intrafascicular electrode</td>
</tr>
<tr>
<td>M&lt;sub&gt;30&lt;/sub&gt;</td>
<td>Moment after 30 minutes of fatigue testing</td>
</tr>
<tr>
<td>MFL</td>
<td>Microfabrication Laboratory</td>
</tr>
<tr>
<td>MMT</td>
<td>Manual muscle test</td>
</tr>
<tr>
<td>MNCV</td>
<td>Motor nerve conduction velocity</td>
</tr>
<tr>
<td>NCE</td>
<td>Nerve cuff electrode</td>
</tr>
<tr>
<td>NCS</td>
<td>Nerve conduction studies</td>
</tr>
<tr>
<td>PA</td>
<td>Pulse amplitude</td>
</tr>
<tr>
<td>PFA</td>
<td>Paraformaldehyde</td>
</tr>
<tr>
<td>PI</td>
<td>Proportional-Integral</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>--------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td>PID</td>
<td>Proportional-Integral-Derivative</td>
</tr>
<tr>
<td>PNS</td>
<td>Peripheral nervous system</td>
</tr>
<tr>
<td>PW</td>
<td>Pulse width</td>
</tr>
<tr>
<td>RCn</td>
<td>Right contact n</td>
</tr>
<tr>
<td>RF</td>
<td>Rectus femoris</td>
</tr>
<tr>
<td>Sart</td>
<td>Sartorius</td>
</tr>
<tr>
<td>SCI</td>
<td>Spinal cord injury</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>sMMT</td>
<td>Stimulated manual muscle test</td>
</tr>
<tr>
<td>SOPS</td>
<td>Sum of phase-shifted sinusoids</td>
</tr>
<tr>
<td>SNAP</td>
<td>Sensory nerve action potential</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal-to-Noise ratio</td>
</tr>
<tr>
<td>SPINE</td>
<td>Slowly penetrating interfascicular nerve electrode</td>
</tr>
<tr>
<td>T_{0.135}</td>
<td>Time before knee extension moment reaches 0.135 Nm/kg body weight</td>
</tr>
<tr>
<td>T_{50}</td>
<td>Time before knee extension moment reaches 50% of its initial value</td>
</tr>
<tr>
<td>TIME</td>
<td>Transverse intrafascicular electrode</td>
</tr>
<tr>
<td>UMN</td>
<td>Upper motor neuron</td>
</tr>
<tr>
<td>USEA</td>
<td>Utah slanted electrode array</td>
</tr>
<tr>
<td>VA</td>
<td>Department of Veterans Affairs</td>
</tr>
<tr>
<td>VM</td>
<td>Vastus medialis</td>
</tr>
<tr>
<td>VI</td>
<td>Vastus intermedius</td>
</tr>
<tr>
<td>VL</td>
<td>Vastus lateralis</td>
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Anatomically-Versatile Peripheral Nerve Electrodes Preserve Nerve Health, Recruit Selectively, and Stabilize Quickly

ABSTRACT

By

MAX J FREEBERG

Peripheral nerve cuff electrodes (NCEs) have been deployed in neuroprostheses restoring or modulating motor, sensory, and autonomic functions. They address myriad pathologies including stroke, spinal cord injury, amputation, seizure, chronic pain. As these applications encompass more indications, NCEs may be deployed in more anatomically-challenging locations while still delivering selective and stable stimulation and preserving the health of the implanted nerves.

A novel class of reshaping electrodes with patterned regions of stiffness enable implantation in a widening range of anatomical locations. Patterning stiff regions and flexible regions of the electrode enables nerve reshaping while accommodating anatomical constraints of various implant locations ranging from peripheral nerves to spinal and autonomic plexi.

We introduce the composite flat interface nerve electrode (C-FINE), flexible and small enough to be suitable for implantation near joints or other constrained locations. Benchtop testing verified the C-FINE does not exert ischemia-inducing pressure on nerves, even in the face of potential nerve swelling. Animal testing verified safety of C-FINE shells...
implanted on peripheral nerves for 3 months through a combination of nerve conduction studies and quantitative histology.

Classically, this benchtop and animal testing followed by chronic observation of neuroprosthesis function have served as a proxy for directly measuring nerve health. We implanted the first-in-man C-FINEs on the proximal femoral nerves near the inguinal ligament of a man with cervical spinal cord injury. Over the first year of implantation we established the safety of C-FINEs in this anatomically constrained location directly via clinical electrodiagnostics. Future NCE designs can use these clinical results as a baseline for expected changes in a well-functioning neuroprosthesis.

Previous NCEs have not been able to selectively activate hip flexors and knee extensors when implanted at the proximal femoral nerve. With 8 contacts and the potential to gently reshape the nerve, the C-FINEs selectively separated these functions and also recruited independent populations of knee extensors. We further established the reliability of intraoperative predictions of C-FINE performance through chronic use, and the expected time-course of NCE stabilization using electromyography intraoperatively and postoperatively. We find that the C-FINEs are selective at this location and their function stabilizes quickly.
CHAPTER 1

Motivation, background, and hypotheses

1.1 Motivation and overview

The peripheral nervous system (PNS) offers a unique window into the control and monitoring of the human body. There are numerous anatomical locations to interface with the PNS which can profoundly impact numerous functions. Neuroprostheses interfacing with the PNS have proven useful for stimulation, recording, and modulation neural activity to address numerous indications, including upper motor neuron (UMN) injury, epilepsy, extremity amputation, chronic pain, sleep apnea, and others.

Stimulation of peripheral nerves’ motor axons permits restoration of motor control in the setting of stroke, spinal cord injury (SCI), and multiple sclerosis. This has resulted in restoration of breathing [1], standing [2], and arm and hand function [3]. Stimulation of motor fibers can be further exploited to maintain a patent airway in individuals with sleep apnea [4], [5] and prevent aspiration pneumonia [6], [7]. Stimulation of sensory fibers has proven effective at restoring sensation in amputees and improving ability to grasp objects [8]–[10]. A variant of nerve stimulation, electrical nerve block, has been deployed for treating chronic pain [11]–[14], and improving bladder voiding and spasticity [15]–[17] following injury to the central nervous system.

Recording from peripheral sensory fibers can be employed in closed-loop prosthesis control [18], [19] following amputation. This allows for intact feedback from
the muscle to enhance prosthesis function. Recording from motor axons can also be used to control prostheses based on intended movement [20]–[23].

As novel interventions require new nerve cuff electrodes (NCE) to be deployed in new anatomical locations, it is crucial to increase confidence in their ability to preserve nerve health, recruit axons selectivity, and perform stably. Broadly, the goal of this project is to provide guidance about the deployment of NCEs in neuroprostheses. Specifically, we propose methods to track peripheral nerve health following NCE implantation and provide baseline results. We also provide guidance about NCE optimization intraoperatively and the timeline of rehabilitation following implantation. Because NCE contact density is important for selectivity, but redundant channels are functionally unnecessary, intraoperative contact optimization becomes more important as the spatial resolution within the electrode increases.

We achieve these goals with a novel, non-penetrating NCE, the compliant flat interface nerve electrode (C-FINE). This NCE was designed for selective stimulation at more proximal and anatomically constrained locations with access to numerous multipurpose fascicles. We introduce the C-FINE and explore the benchtop and chronic animal testing of C-FINE safety. We translate 8-contact C-FINEs to a standing neuroprosthesis in its first-in-man implantation. We exploit the C-FINEs’ selectivity to implement fatigue-delaying paradigms which can generate moments for longer lengths of time than otherwise possible.
1.2 Background

1.2.1 Nerve-based electrodes

There are numerous locations from which to electrically stimulate the peripheral nervous system. In general, more invasive electrodes, such as penetrating nerve electrodes enjoy the most intimate access to peripheral nerve axons and the highest potential for selective activation or recording of those axons. Proximity is crucial for recording neural activity, given the small amplitude of the signal [24] and surrounding sources of noise [25].

Noninvasive electrodes placed on the surface of the body can elicit muscle contractions, help ease pain, or be used for recording signals such as ECG or EEG. However, their resolution is low and donning and doffing the electrodes is cumbersome [26]. In the case of surface electrodes used in motor neuroprostheses, they may not elicit a full contraction of a muscle or, at high currents, activate pain fibers causing discomfort. Organ-based electrodes aim to stimulate the nerve while anchored to the effector organ. In the case of motor neuroprostheses these include electrodes penetrating the muscle (intramuscular) and sutured to its surface (epimysial). They can be employed to activate small nerve branches or to target larger nerves to produce strong contractions of individual muscles and have been used for long-term restoration of standing and transfers [27], [28]. However, the chronic stability of muscle-based electrodes depends on several factors, such as the changing distance between the electrode and the target nerve as the muscle contracts, and generally require approximately 6 weeks of immobility prior to use allow encapsulation to prevent electrode movement [29].

Nerve-based electrodes can stimulate the entire nerve or a desired portion of it. There are two classes of nerve-based electrodes. Penetrating electrodes breach the
epineurium and potentially the perineurium while non-penetrating electrodes are located outside, but in close proximity to, the epineurium. *Interfascicular* electrodes penetrate the epineurium but not perineurium [30]. Examples include the slowly penetrating interfascicular nerve electrode (SPINE) which applies a small force to penetrate the epineurium while fascicles are pushed out of the way [31], and the multigroove electrode [32] which requires a surgeon to dissect out fascicles to place into discrete compartments within the electrode. *Intrafascicular* electrodes pierce the perineurium for intimate access to axons. Examples include the longitudinal intrafascicular electrode (LIFE), transverse intrafascicular multichannel electrode TIME [33], and Utah slanted electrode array (USEA) [34], [35]. Acutely, these electrodes promise high selectivity for recording and stimulation of axon populations within fascicles. However, they have limited subchronic (less than 1 month duration) evidence of function in clinical trials. The LIFE and USEA have been implanted in the peripheral nerves of amputees but only for a period of up to a month [36], [37] while the TIME has been implanted [38], [39] in an amputee for several months. In animals, these electrodes can cause behavioral deficits which may be temporary [40].

Non-penetrating nerve-based electrodes provide an ideal tradeoff between invasiveness and selectivity, especially for stimulating the nerve. Classically, these electrodes have been round in cross section. Examples include the Case Western Reserve University (CWRU) self-sizing spiral and the Huntington helix [41]. A newer class of NCEs, the flat interface nerve electrode (FINE) aims to match or gently reshape the naturally oblong configuration of peripheral nerves [42]. A flatter nerve places a higher proportion of fascicles at its edges for electrical access from outside the nerve. While NCEs
may not match the selectivity of penetrating electrodes, they have proven selective enough for functional use in sensory [8]–[10] and motor [2], [3], [43], [44] neuroprostheses. They can be employed in applications where the nerve must remain healthy for long periods of time post-implantation and have a long record of stable function over periods exceeding a decade [45]. They also have advantages over muscle-based intramuscular or epimysial electrodes in implantable motor system neuroprostheses [29], [46], including strong contractions with access to all fascicles in a nerve [2], chronically-stable recruitment, and low failure rates [2], [47]. NCEs can also improve the surgical efficiency of implanted neuroprostheses since multiple muscular targets can be accessed through a single incision with a selective multicontact electrode.

1.2.2  Local anatomical considerations for nerve-based electrodes

Because the indications for interfacing with the PNS are numerous, the location of the interface can vary greatly. Potential locations for electrode implantation include more distal nerves, proximal nerve trunks, autonomic plexi, spinal roots, and small nerves serving visceral organs, among others. As NCEs increase in selectivity through shape [42] and increasing contact density, they become functionally suited to implantation in diverse anatomical locations. Beyond this, NCE development requires consideration of anatomy of the nerve, its fascicles, and surrounding tissues to maintain a healthy, stable interface with the nerve.

Nerves near joints, [48]–[50], at the brachial plexus [51], [52], and others locations [53] can bend significantly during movement. An NCE which is flexible along its length is able to match the nerve’s pliability along its length. Nerves which are in volumetrically
constrained regions [51], [54]–[56] or which are superficial [10] require an NCE as small as possible. Researchers must also consider a safe surgical approach which does not endanger nearby sensitive structures, such as blood vessels.

One challenging implantation location of interest to this report is the proximal femoral nerve trunk. In this region, the femoral nerve is vulnerable to compression and stretching because of the proximity of the inguinal ligament [48], [49]. When the hips are flexed and rotated the femoral nerve can become sharply angled at 80-90° underneath the ligament [57], [58].

1.2.3 Electrodiagnostics and the sequelae of peripheral nerve injury

Two scales have classically graded the pathology and prognosis of peripheral nerve injuries, dividing them into either 3 [59] or 5 categories [60]. The mildest form of nerve injury, neurapraxia, generally occurs from compression. The axons and myelin of peripheral nerves are sensitive to ischemia, and pressures of 20-30 mmHg can cause neuropathy as seen in carpal tunnel syndrome [61], [62]. Neurapraxia involves disruption of the myelin and thus impacts conduction through the affected region although the axons are still intact. Neurapraxia is generally temporary, with recovery occurring over 2-12 weeks [63].

Axonotmesis involves disruption of axons. This is generally secondary to traumatic crush or stretch injury but can also be caused by ischemia. Axonotmesis ranges in severity based on the amount of structural damage. It may impact the axons alone or it may disrupt the endoneurium and perineurium [63], [64]. Because the axon is severed, Wallerian degeneration occurs and the axon dies back to the cell body over the 5-12 days following
injury [65]. When axons regenerate following injury, they must grow distally from the cell body, relying on other structures of the nerve to guide them to muscle or sensory organs. The more intact the surrounding perineurium and endoneurium, the more likely axons are to recovery, with more mild axonotmesis (perineurium intact) recovering in 2-6 months and more severe axonotmesis recovering in 2-18 months. However, reliability and timeframe of recovery is dependent on axon length and estimated clinically to proceed at approximately 1 mm/day [66].

In neurotmesis, the nerve, including its surrounding structures, is completely interrupted. Because there is no support structure for axonal regrowth, surgery is required for axonal regeneration to occur [67], and recovery may not be complete and can take years.

Electrodiagnostic assessments of nerve health include nerve conduction studies (NCS) of the peripheral nerves and electromyography (EMG) of the muscle(s) they innervate. The NCS grades motor and sensory nerve conduction through a segment of nerve based on conduction velocity (CV) and the amplitude and area of their evoked responses. Sensory nerve conduction is measured by evoked potentials recorded from sensory branches of a nerve, the sensory nerve action potential (SNAP). Muscle nerve conduction is measured at a muscle innervated by the nerve through compound muscle action potential (CMAP) amplitude, area, and CV. EMG examination of muscles can determine if weakness is neurogenic or myopathic and can localize the neuropathy to the upper motor neuron or peripheral nerve [64].

Because of the different pathologies of nerve injuries, changes in NCS and EMG are expected at different times following an injury. Testing can determine whether the pathology occurred from demyelination (neurapraxia) or loss of a population of axons
(axonotmesis). Studies in the first week following injury typically establish nerve continuity. Because of the delay for Wallerian degeneration to occur, stimulation distal to a lesion may appear normal for 7 to 11 days [60]. At this point, a lack of response cannot distinguish a neurapraxic conduction block from axon discontinuity. From about 1-3 weeks, studies can distinguish neurapraxia from axonotmesis affecting a large number of axons. If a large number of axons are disrupted at this point, distal CMAP amplitude drops because Wallerian degeneration will have occurred [68]. This would not occur in a neurapraxia [63]. EMG of affected muscles is slower to show changes, with spontaneous fibrillation potentials developing 2-6 weeks after denervation and continuing until axons regenerate. NCS and EMG studies several months after injury can assess the degree of axonal regeneration [69].

1.2.4 Current methods for ensuring nerve health

A classic metric to ensure NCE safety originates from nerve repair studies. Nerves grafted with stiff cylindrical cuffs healed best when the ratio between the cuff’s inner diameter and the nerve’s outer diameter (the “cuff-to-nerve ratio,” or CNR) was at least 1.5. This is thought to allow for adequate circulation during transient swelling [70].

Later studies identified a more universal measure of blood flow and predicted nerve health. These found that pressures of 20 mmHg begin to limit intraneural blood flow and pressures of 50 mmHg fully compromise blood flow [71]. These pressures are supported by carpal tunnel studies showing permanent nerve changes with sustained pressures above 30 mmHg [61], [62]. These pressures provide design guidelines when
creating a novel NCE design and encourage NCEs which match the compliance of surrounding tissues.

After implantation, nerve health is generally assumed based on neuroprosthesis function and stimulation charge threshold, the minimum charge required to elicit a response \([47], [72]\). Charge thresholds are an indication of nerve-cuff interactions. However, they are not sensitive to mild axonotmesis. In the perioperative period, they may not distinguish whether a lack of stimulation response is due to damage of the nerve or temporary changes at the interface, such as air bubbles \([47]\). With these methods there is no way to determine if a decrease in stimulated moment is caused by deconditioning, changes in nerve health, or issues at the nerve-cuff interface. Because of the lack of standardized nerve health monitoring, information is lacking about which changes are normal and which are cause for concern, making it difficult to decide when to revise an NCE perioperatively.

1.2.5 Selective nerve stimulation

In the case of motor neuroprostheses, selective stimulation of antagonistic muscles is crucial to control the combined moment at a joint and resulting movement \([73]\). Selectively activating independent populations of agonist muscle units may allow for a reduction in stimulation duty cycle and resulting delay in muscle fatigue during isometric contractions \([74]–[76]\). Sensory neuroprostheses require selective stimulation as well to evoke percepts at various locations and separate them from motor responses \([9]\).

There are several approaches to determining the selectivity of an NCE before chronic deployment in a neuroprostheses. Studies of fascicular anatomy have traced the path of motor and sensory fibers from nerve to end-organ \([50], [77]–[79]\). These
painstaking studies provide guidance about the general organization of a given nerve. Combined with computational modeling studies, they provide an estimate of the chances that a given NCE design and contact layout is capable of independently activating separate populations of axons [80], [81]. The required NCE design and layout can be manufactured and further verified through a series of temporary, intraoperative experiments [44]. These types of studies, for instance, have shown that 4-contact spirals at the proximal femoral nerves cannot reliably separate activation of hip flexors and knee extensors [82], leading 4-contact spirals to be implanted distal to nerve branches to hip flexors [2]. However, modeling and intraoperative experiments that 8-contact reshaping electrodes, such as the C-FINE, would be better suited and selective enough for standing and stepping neuroprostheses in this region [44], [80].

1.2.6 Upper motor neuron injury clinical impacts and interventions

One intervention of particular interest is the restoration of motor function to individuals with UMN injury, such as SCI. In the US alone, SCI affects 236,000-327,000 individuals [83], [84] with 12,000-20,000 new cases annually [85], [86]. The functional impacts of SCI strongly depend on location and completeness of the injury but can include muscle deconditioning, osteopenia, thrombosis, urinary tract infections, and pressure ulcers [87].

Neuroprostheses utilizing electrical stimulation (ES) have helped restore various functions to individuals with SCI, based on injury level. For those with higher cervical injuries, ES can improve activities of daily living (ADLs) by increasing hand pinch and grasp [88]. For those with volitional control over their proximal upper extremities ES
enables standing and transfers, and can improve cardiovascular and skin health [27], [89]–[91].

Many efforts have focused on improving standing duration [2], [74]. Systems utilizing muscle-based electrodes have provided stronger hip and knee extension for standing [92], [93] than systems stimulating through surface electrodes. Systems deploying nerve-based electrodes have improved upon those results with even stronger knee extension [2]. Novel stimulation techniques also show promise for improving standing duration through the delay of fatigue [74], although implementing these stimulation paradigms in current neuroprostheses has been challenging.

1.3 Specific aims & hypotheses

This report introduces the C-FINE, a new neural interface selective and flexible enough for implantation on proximal nerve trunks and in challenging locations near joints or in confined areas. We hypothesized that this electrode would be sufficiently selective and pliable enough to deploy functionally and preserve nerve health even in these restricted locations. The specific aims in this report allowed us to test this hypothesis, first verifying C-FINE safety in the fore- and hind-limbs of cats before implanting C-FINEs on the proximal femoral nerves subjected to large bending movements around the inguinal ligament in a human volunteer. Previous chronically implanted electrodes have not been sufficiently selective to implant at this location as part of a standing neuroprosthesis so special attention is also paid to both the selectivity of 8-contact C-FINEs as well as its effects on nerve health.
1.3.1 **AIM 1: Verify the safety of C-FINEs in a chronic animal model.**

The C-FINE was designed with patterned regions of stiffness allowing for safe implantation in novel anatomical locations while allowing the electrode to match or gently reshape the target nerve to enhance selectivity. It is flexible for implantation near joints where nerves bend and small for implantation in volumetrically constrained locations. Proof-of-concept bench testing verified the circumferential pressure exerted by the C-FINE during inflation was less than both the transient [94], [95] and sustained [61], [62] pressure thresholds for compromised intrafascicular blood flow that might lead to nerve damage.

Here we tested the chronic safety of the C-FINE in a feline model at the well-established sciatic nerve and in the forelimbs. We proposed forelimb implantation as a challenge to the flexibility and small size of the C-FINE. This location is close to highly mobile joints with little loose connective tissue which might accommodate extra NCE volume. Because the main effects we examined would be attributable to the mechanical design the C-FINE itself, we implanted 12 C-FINE shells, without contacts or lead wires in the fore- (n = 6) and hind- (n = 6) limbs of 3 cats. The cats were monitored for 3 months at which point we could determine the motor nerve conduction through the implanted nerves and quantify histology of the implanted nerves for any signs of damage.

**Hypothesis 1.1:** C-FINE shells chronically implanted around fore- and hind-limb nerves in cats will not significantly alter motor nerve conduction.

**Hypothesis 1.2:** C-FINE shells chronically implanted around fore- and hind-limb nerves in cats will not significantly change histology quantified by axon density or relative myelin thickness.
AIM 2: Monitor the health of femoral nerves chronically implanted and stimulated with C-FINEs through clinical electrodiagnostics.

Because NCEs maintain intimate contact with peripheral nerves, changes in NCE design, implant locations, surgical access, and rehabilitation programs have myriad potential sequelae for nerve health. The effects of these changes are classically anticipated through benchtop or animal studies. The impact of the NCE on human subjects is then typically evaluated through the proxy of neuroprosthesis function. Chronic clinical electrodiagnostic testing of peripheral nerves implanted with a novel NCE ensures healthy nerves, not adversely impacted by design changes. A lack of chronic clinical reporting has the potential to diminish trust in the long-term safety of NCEs.

We evaluated neurophysiologic changes longitudinally during one year of implantation and exercise using a standing neuroprosthesis employing the first-in-man 8-contact C-FINEs. We tracked motor and sensory nerve conduction, any changes at the muscle indicative of denervation, and effects of exercise on the quadriceps. This has implications for the C-FINE and suggests a general model for electrodiagnostic evaluation of nerve health following implantation of a nerve-based electrode in the PNS.

Hypothesis 2.1: C-FINEs on the proximal femoral nerve trunk will not cause significant detrimental effects to nerve health as measured by clinical nerve conduction studies and EMG examination.

Hypothesis 2.2: Electrodiagnostics will reflect increased muscle fiber recruitment following rehabilitation and stimulated exercise with C-FINEs.
1.3.3 **AIM 3:** Quantify the selectivity of muscle recruitment through each contact of C-FINEs on human femoral nerves and track stability from implant through chronic use.

Intraoperative evaluation of long-term NCE performance is necessary to optimize NCE placement, choose contacts to connect to an implanted stimulator, and guide postoperative recovery and exercise. However, the extent and time frame of changes to motor responses of NCEs from implantation through chronic use are unclear, especially in the first several weeks postoperatively, the “perioperative” period. This is primarily because intraoperative and postoperative monitoring techniques are usually different. Using the same measurement techniques at each time point, we compared intraoperative, perioperative and chronic performance of 8-contact C-FINEs on the proximal femoral nerves. We evaluated whether NCEs placed in this proximal location would be capable of separately activating knee extensors and hip flexors. We further explored whether distinct populations of knee extensors could be recruited independently. We compared perioperative and chronic function of each contact to intraoperative function and determined over what timeframe the recruitment of each C-FINEs contact stabilizes.

**Hypothesis 3.1:** The C-FINE will be able to separately stimulate functionally distinct groups of muscles innervated by the femoral nerve generating knee extension moments sufficient for standing.

**Hypothesis 3.2:** The optimal contact for a given function will remain the optimal contact from implant through 6 months chronic use.
1.4 Organization of dissertation

Chapter 2 introduces the anatomically-versatile C-FINE, which can reshape the nerve while remaining flexible along its length with minimal volume so that it can be both selective and amenable to implantation near joints or in confined locations. This chapter covers the C-FINE’s design, benchtop design verification of internal pressure, and safety testing in a chronic cat model.

Chapter 3 provides guidance for the chronic monitoring of nerve health following implantation of an NCE on motor or sensory peripheral nerves. Specifically, this chapter chronicles the nerve health for 1 year postoperatively after the first-in-man implant of C-FINEs on the femoral nerve in close proximity to the inguinal ligament. It further tracks the effects of rehabilitation and exercise with C-FINEs.

Chapter 4 documents changes in selectivity following implantation of a flat NCE and the time-course over which they may occur. As selectivity of NCEs increase, stability becomes increasingly important as smaller physical shifts in an NCE can lead to changes in the stimulated responses. We detail the selectivity of 8-contact C-FINEs on the proximal femoral nerve trunk and the stability of their responses for 6 months postoperatively with a focus on perioperative stability in the weeks following implantation.

Chapter 5 exploits the selectivity of 8-contact C-FINEs to implement fatigue delaying paradigms. It further introduces closed-loop control of advanced stimulation paradigms and insights into an optimal contact-cycling pulse width.
Chapter 6 summarizes the contributions of this report and details their significance in the context of current neuroprostheses and future studies. It also summarizes the limitations of these studies and suggest future work which would build upon the results in this report.
CHAPTER 2

The design and chronic tissue response of a composite nerve electrode with patterned stiffness

The content of this chapter is based on the following published manuscript:


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2.1 Abstract

Objective. As neural interfaces demonstrate success in chronic applications, a novel class of reshaping electrodes with patterned regions of stiffness will enable application to a widening range of anatomical locations. Patterning stiff regions and flexible regions of the electrode enables nerve reshaping while accommodating anatomical constraints of various implant locations ranging from peripheral nerves to spinal and autonomic plexi.

Approach. Introduced is a new composite electrode enabling patterning of regions of various electrode mechanical properties. The initial demonstration of the composite’s capability is the composite flat interface nerve electrode (C-FINE). The C-FINE is constructed from a sandwich of patterned PEEK within layers of pliable silicone. The shape of the PEEK provides a desired pattern of stiffness: stiff across the width of the nerve to reshape the nerve, but flexible along its length to allow for bending with the nerve. This is particularly important in anatomical locations near joints or organs, and in constrained compartments. We tested pressure and volume design constraints in vitro to verify that the
C-FINE can attain a safe cuff-to-nerve ratio (CNR) without impeding intraneural blood flow. We measured nerve function as well as nerve and axonal morphology following 3-month implantation of the C-FINE without wires on feline peripheral nerves in anatomically constrained areas near mobile joints and major blood vessels in both the hind and fore limbs.

**Main results.** *In vitro* inflation tests showed effective CNRs (1.93 +/- 0.06) that exceeded the industry safety standard of 1.5 at an internal pressure of 20 mmHg. This is less than the 30 mmHg shown to induce loss of conduction or compromise blood flow. Implanted cats showed no changes in physiology or electrophysiology. Behavioral signs were normal suggesting healthy nerves. Motor nerve conduction velocity (MNCV) and compound motor action potential (CMAP) did not change significantly between implant and explant (p > 0.15 for all measures). Axonal density and myelin sheath thickness was not significantly different within the electrode compared to sections greater than 2 cm proximal to implanted cuffs (p > 0.14 for all measures).

**Significance.** We present the design and verification of a novel nerve cuff electrode, the C-FINE. Laminar manufacturing processes allow C-FINE stiffness to be configured for specific applications. Here, the central region in the configuration tested is stiff to reshape or conform to the target nerve, while edges are highly flexible to bend along its length. The C-FINE occupies less volume than other NCEs, making it suitable for implantation in highly mobile locations near joints. Design constraints during simulated transient swelling
were verified in vitro. Maintenance of nerve health in various challenging anatomical locations (sciatic and median/ulnar nerves) was verified in a chronic feline model in vivo.

2.2 Introduction

Implanted neuroprostheses employing electrical stimulation have been shown effective in restoring basic upper or lower extremity motor function to individuals paralyzed by cervical or thoracic level spinal cord injury (SCI) [2], [90], [96]–[99]. Stimulating currents are delivered to the neural targets in these systems by electrodes that are inserted into the muscle tissue near the nerve [100], [101], sutured to the muscle at the nerve entry point [46], or placed around the nerves [42], [102]. Non-penetrating nerve cuff electrodes (NCEs) that maintain intimate contact with the epineurium require lower stimulating currents to activate the neural targets than muscle based electrodes, and exhibit more stable recruitment properties and lower incidents of failure due to movement during muscle contractions [46]. If placed proximal to the branch points of a nerve innervating a muscle with multiple motor points, they can more completely activate the muscle than a single intramuscular or epimysial electrode. Moreover, if placed on a nerve trunk proximal to branches to multiple muscles, they can potentially activate all distal muscles with a single electrode implanted through a single surgical incision. Spiral NCEs [102] have been deployed in both the upper and lower extremities and maintained stable activation thresholds for several years [45], [47], [72], [103]. More recently, NCEs have also been deployed for sensory restoration in amputees [8]–[10]. In these subjects, NCEs have restored natural perception of touch in the phantom limb at different locations allowing for improved ability to grasp objects [9] with stable functional responses for over 2 years [10],
There are numerous other applications of NCEs in neuroprostheses as well. These include nerve block for pain and spasticity [11]–[13], bladder control restoration [15], [17], and several others [5], [7], [23].

As neural interfaces target new applications, a novel class of reshaping NCEs with patterned regions of stiffness will allow for safe and functional implantation in new anatomical locations. Patterning the NCE with stiff and flexible regions allows for either matching or reshaping target nerves to maximize stimulation selectivity while not adversely impacting nerve health in anatomically constrained locations. There are potential locations for NCE implantation near joints, in constrained locations, spinal plexi, autonomic plexi, and others which would benefit from an improved NCE design. One requirement for novel neural interface applications is that an NCE must be selective enough to stimulate target fascicles while avoiding undesired ones. In motor neuroprostheses, highly selective NCEs can be deployed on a proximal nerve innervating several muscles to isolate the prime mover of a desired motion while avoiding antagonists [104]–[106] with minimal surgical dissection and potential trauma. By reshaping the nerve into a more oblong cross-section, the Flat Interface Nerve Electrode (FINE) increased the surface area available to external stimulation and thus increased space available for contact placement around the nerve, and consequently the selectivity of the electrode. The selectivity of the FINE was demonstrated with motor response to stimulation in human subjects at several locations [43], [44]. This was further demonstrated in chronic upper extremity sensory restoration applications. In upper limb amputees, each contact of 8 contact FINEs produced either a sensation at a unique location or a unique sensation [9]. Sensory locations from
each contact and stimulation thresholds continue to remain stable after years post-implantation [10], [45].

Another requirement for implanting an NCE for novel targets is that it must maintain nerve health and create a stable electrical interface with the target nerve by operating within the mechanical and volumetric requirements of the implant site without breaching the epineurium. There are several areas which can present nerve health concerns for NCE implantation due to nerve movement and the tight confines of surrounding anatomy. Nerves in regions near joints, such as the groin [48]–[50], several areas of the brachial plexus [51], and others [53], can experience large deviations and bending during limb movement. For instance, in the groin, the femoral nerve is vulnerable to injuries at the inguinal ligament [48], [49] where hip flexion and external rotation can cause the femoral nerve to be sharply angled at 80-90° underneath the inguinal ligament [57], [58]. The brachial plexus can be bent and stretched during shoulder abduction and external rotation because it is tethered at the prevertebral fascia and at the axillary sheath [52]. These areas require an NCE that is flexible along its length to mimic the target nerve’s axial (along the length of the nerve) pliability. These and other locations are also often volumetrically constrained with little loose connective tissue so an NCE should be as thin as possible to avoid adding unnecessary bulk to an already tight area. Examples include the sciatic [107], pudendal [54], and superior gluteal [55] nerves in the pelvis and gluteal space [56], as well as the axillary nerve in the axilla [51] and several areas of the brachial plexus, which is closely related to several bony structures against which it may be compressed [52], [108]. Clinical experience has also indicated that NCE bulk should be minimized when implanting NCEs in superficial locations [10].
Meeting both selectivity and safety requirements of implantation at new target locations requires a novel class of non-penetrating, reshaping NCE. This design must be capable of adapting to various target nerves to achieve long-term, stable clinical selectivity by either matching the shape or reshaping a target nerve within its fascial plane. It must also offer mechanical flexibility to adapt to potential implant locations and their surrounding tissues. In particular, it should be flexible along the nerve axis to allow for bending of the nerve. An oblong NCE shape is ideally suited for bending if proper materials are used in its construction. The NCE must also occupy as little volume as possible to reduce pressure on the target nerve, especially during transient swelling and in volumetrically-constrained compartments. To account for future selectivity and location needs, the design should also allow for adaptation to different patterns of nerve reshaping.

**Figure 2.1: Diagrams and pictures of C-FINEs.**
Left: Diagrams of an open C-FINE from a side view (top) and from above (bottom) and of a closed C-FINE from a side view (middle). Diagram shows definitions of dimensions as well as contacts over PEEK stiffening bar (yellow bar in middle of cuff) flanked by thin, flexible regions of silicone (light gray). Right: Examples pictures of the C-FINE, open (top) as well as closed (bottom), viewed from above.
and be manufactured using a microfabrication ready process to allow for this method of achieving high contact density [109], [110].

Here we report on the design of a new composite electrode enabling patterning regions of varying stiffness as well as its in-vitro mechanical testing and chronic in-vivo safety testing. The initial demonstration of the composite’s capability is the composite flat interface nerve electrode (C-FINE), which reshapes nerves into more oblong cross sections while minimizing electrode volume and stiffness along the length of the nerve. We achieved this with a compliant, composite manufacturing process and a configuration with a varying stiffness profile along the length of the nerve and minimal material volume. The Composite Flat Interface Nerve Electrode (C-FINE) (Figure 2.1) consists of a narrow stiffening bar laminated between two wider flexible layers, allowing the nerve to bend along its length without impingement. The hinge and suture closure minimize material volume [111]. This design enables the construction of NCEs with high contact densities, either by hand or by microfabrication techniques [109], [110] for increased spatial resolution. To ensure safety and nerve health, the C-FINE was designed to keep transient circumferential pressure less than the acute (60 mmHg) [94], [95] and sustained (20 mmHg) [61], [62] pressure thresholds for nerve damage and compromised intrafascicular arterial blood flow. Chronic safety of the C-FINE was determined in a feline model with properly sized C-FINE shells, without wires, installed in anatomically constrained regions of both the fore and hind limbs for 3 months. To mimic nerve health difficulties that may be seen in certain proximal implant locations, NCEs were implanted in close proximity to highly mobile joints. In the forelimb, this is compounded by a lack of loose connective tissue to accommodate the extra volume of a NCE. In fact, the forelimb is clinically and
experimentally known as a potential location of osteofascial compartment syndrome at the humerus, radius/ulna, and caudal ante-brachium [112], [113]. The sciatic nerve was chosen as the neural target in the hind limbs because of its extensive use in previous NCE development work and its well understood fascicular structure [42], [73], [114], [115]. Cats were monitored for any physiological or electrophysiological changes and nerve histology was examined for any changes in nerve and axon morphology.

2.3 Methods

2.3.1 Electrode design and manufacture

The C-FINE (Figure 2.1) is constructed using a laminar manufacturing process which layers a stiff polymer bar between two layers of flexible silicone sheeting [116]. 125 µm thick layers of silicone form the top and bottom surfaces of the electrode, which sandwich the inner electrode assembly. The inner assembly, held together with silicone adhesive, comprises platinum foil electrode contacts and PFA-insulated stainless steel lead wires, in addition to a 125 µm thick layer of unrestricted PEEK-Optima™ polymer (Invibio Biomaterials Solutions, Conshohocken, PA). This thin layer of PEEK polymer increases the electrode’s wall stiffness and nerve reshaping ability along the width of the nerve while remaining flexible along its length and maintaining minimum electrode material and volume.

The stiffening bar runs the full width of the C-FINE for any given size. Its depth (2.3 mm) was chosen to match the anticipated size required for placement of stimulating contacts while leaving an equal amount (2.3 mm) of flexible region flanking the stiffening bar along the length of the nerve. The completed C-FINEs were 7mm deep along the length
of the nerve. PEEK was chosen as the material of choice for the stiffening bar because of its relatively high flexural modulus (4 GPa [117]) among unrestricted polymers. The thickness of the stiffening bar (125 µm) was chosen to approximate the stiffness of previous reshaping electrodes, in which a 0.9 mm thick FINE has a stiffness of 66.2 N/m [118]. The stiffness at the center of a rectangular beam follows from [119]:

\[
\frac{F}{d} = \frac{4E_{\text{bend}}wh^3}{L^3}
\]

Eq. 2.1

The stiffness is defined here as the force, \( F \), required causing a deformation, \( d \), and is governed by the bending modulus, \( E_{\text{bend}} \), of the stiffening bar as well as its depth, \( w \), thickness, \( h \), and width, \( L \). Thus, a 125 µm thick, 2.3 mm deep, 10 mm wide bar of PEEK is expected to produce a stiffness of 71.9 N/m. While these calculations guided the decision towards stiffening bar material and thickness, pressure exerted at different deformations was verified by balloon inflation testing. The \textit{in vitro} and \textit{in vivo} studies exclusively used C-FINEs with 125 µm thick PEEK stiffening bars.

Certain areas of the electrode contain only a single 125 µm layer of silicone and serve as more flexible hinge zones, while the areas of the electrode containing the stiff PEEK bars form the nerve-reshaping side walls of the electrode. Thus, the center area of the electrode, where the electrode contacts are located, has the stiffness required to reshape the nerve, while the areas of the electrode proximal and distal to the electrode contacts are much more flexible and mimic the nerve’s compliance. A double suture closure mechanism significantly reduces material volume over other closure mechanisms using extra lengths of sheet silicone or molded silicone [42], [102].

For \textit{in-vitro} testing, 16 C-FINEs were manufactured by Ardiem Medical (Indiana, PA) of size 10 mm x 1 mm x 7 mm (width x height x depth). For \textit{in-vivo} testing, C-FINE
shells without wires were manufactured in the Class 100 cleanroom space of the Case Western Reserve University (CWRU) Microfabrication Laboratory (MFL) as described elsewhere [116]. Briefly, the manufacture involves a laminar process where the stiffening bar is enclosed by silicone sheets. To construct a C-FINE shell, a silicone sheet cut to the size of double the cuff width and depth plus the hinge heights is placed on a diagram of an open C-FINE. Uncured silicone is used to adhere the PEEK stiffening bar to the silicone sheet and to adhere the outer sheets of silicone (sized to the width by depth of the C-FINE) to the PEEK bar. The completed C-FINE shell is then cured. Details, including contact placement in recording electrodes, are described in detail elsewhere [116]. For testing in cats, the C-FINE shells were manufactured without platinum contacts or lead wires because the C-FINEs were not intended for electrical stimulation. The primary goal here was to isolate and quantify any effects of this new NCE design on nerve morphology and health, rather than changes attributable to lead tethering or other factors such as animals tugging on them [114], which could confound the results. C-FINE shell sizes available for animal implantation included 3 mm, 4 mm, 5 mm, 6 mm, or 7 mm wide—all 1 mm high and 7 mm deep. A standard 1 mm NCE height was chosen to avoid reshaping a nerve to a size smaller than a nerve’s largest fascicles [118], [120]. For human implantation, C-FINE sizes will depend on the expected area range of the target nerve (e.g., available sizes for the human femoral nerve span a range of area from 10 mm² to 22.5 mm², based on cadaveric studies and intraoperative testing [44], [50]).
2.3.2 *Inflation testing*

One measure of safety originates from nerve repair studies. Nerves grafted together with the aid of stiff cylindrical cuffs regenerated best when the nerve stumps were enveloped in a cylindrical cuff with a ratio between the cuff’s inner diameter and the nerve’s outer diameter (the “cuff-to-nerve ratio” or CNR) of at least 1.5. This allows for ample blood flow even during transient nerve swelling [70]. A more relevant measure predicting nerve damage involves applied pressure. The threshold for compromising intrafascicular arterial blood flow is 50 mmHg and arterial blood flow begins to be limited around 20 mmHg [71]. This is supported by research showing the threshold circumferential pressure for acute nerve damage to be 50 mmHg [94], [95], which coincides with the threshold for intrafascicular arterial blood flow compromise. Sustained pressures in excess of 30 mmHg have been correlated with reversible symptoms of carpal tunnel syndrome, indicating that no permanent neural damage occurs at these pressures [61], [62]. Therefore, strong evidence supports establishing the maximum transient circumferential pressure at 50 mmHg, and chronically, less than 20 mmHg. In order to adapt the cylindrical nerve cuff CNR standard of 1.5 to an elongated cuff design, the C-FINE has been designed such that it assumes an effective CNR > 1.5 at an internal cuff pressure of 20 mmHg or less. Effective CNR is calculated by first measuring the cross-sectional area of the electrode’s lumen, then calculating the diameter of a circle that would enclose that cross-sectional area, and dividing it by the diameter of the largest nerve around which the electrode would be implanted. C-FINE size selection is based on choosing height and width combination which does not constrict the nerve at implant (cross sectional area of the nerve is less than the inside luminal area of the C-FINE).
For the purpose of inflation testing, each electrode was sized 10 mm in width by 1 mm in height by 7 mm in depth. Each electrode was placed around a nerve-simulating balloon connected to a water column inflation apparatus. By increasing the water level in the column, the pressure level within the inflation balloon was increased from 0 mmHg to 60 mmHg, in increments of 10 mmHg. At each of the seven pressure levels, and for each electrode, the electrode’s outer width and outer height was measured and recorded (Figure 2.2). Outer width and outer height measurements were then converted to the corresponding inner width and inner height values by subtracting the thickness of the electrode walls (1.27 mm subtracted for height and 1.40 mm subtracted for width). Using common ellipse area formulae and the inner width and inner height measurement data, each electrode’s enclosed area was calculated at each of the seven pressure levels. The electrodes’ enclosed areas were then converted into their “effective diameters,” which was defined to be the diameter of a circle that encloses an identical area. The effective diameter at each pressure level was then divided by the effective diameter of the electrode in its resting configuration, which is the diameter of the largest circular nerve around which the

Figure 2.2: Example of in vitro testing of C-FINEs with balloon at 30 mmHg. This example shows the setup with rulers to measure C-FINE dimensions at different balloon pressures. Views shown focused on the C-FINE from the side to measure height (top) and from above to measure width (bottom).
electrode would be implanted. This yielded a CNR value for each electrode at each pressure level. These data were then aggregated, and 16-sample mean and standard deviation were calculated for each pressure level.

2.3.3 Animals

After confirming pressure and CNR constraints were met in vitro, we verified the chronic safety of the C-FINE in tightly-confined spaces in 3 young adult domestic shorthaired cats implanted with un-instrumented C-FINE shells for periods of 84, 88, and 90 days. Cats were kept in a common housing area together and allowed to freely roam after recovering from anesthesia following implantation. All procedures were performed in accordance with the Case Western Reserve University (CWRU) Institutional Animal Care and Use Committees (IACUC).

2.3.4 Implantation and behavior monitoring

The cats were induced with Ketamine (10mg/kg, IM). Once sedated a cephalic vein catheter was placed for IV access and the cats were intubated with the appropriate size tube and placed on an ADS1000 for Isoflurane anesthesia. All procedures were performed under sterile surgical protocols.

The target nerves were exposed and measured using a sterile tape ruler and a custom measuring tool to provide an estimate of nerve width and height to determine the area of a rectangle that would circumscribe the cross section of the nerve. C-FINE shells were sized such that their inside luminal area was larger than the cross sectional area of the target nerves as described elsewhere [43], [44]. C-FINE shells were implanted on 4 nerves in
each cat for a grand total of 12 cuffed nerves: the distal sciatic nerves bilaterally (six total) proximal to the branches to the tibial and common peroneal nerves, and either the median (four total) or ulnar (two total) nerves depending on the safety of surgical access in close proximity to the brachial artery. On the sciatic nerves, shells were 5 mm (1 nerves), 6 mm (3 nerves) or 7 mm (2 nerve) wide. On the median and ulnar nerves, shells were 3 mm (2 nerves), 4 mm (2 nerves), or 5 mm (2 nerves) wide.

The cats were monitored three times per week for any signs of pain, weakness, or lameness. For the sciatic nerves, motor deficits were carefully monitored by noting any hyperextension of the tarsus, knuckling, walking on the hock of the leg, difficulty squatting (e.g., while using a litter box) or trotting, and leg dragging or limpness. Sensation involving the sciatic nerves was further monitored by testing withdrawal to foot pressure. For the median and ulnar nerves, motor deficits were monitored by noting any limp or difficulty trotting.

Figure 2.3: Surgical pictures of C-FINE implant, explant, and cross section.
Examples show implant on cat sciatic nerve (top) as well as the same cuff at explant (middle) and a cross section after fixation (bottom).
forelimb claw extraction and retraction, and kneading. Sensation was further monitored by testing withdrawal to pressure at the pad of the forelimb paws at the first digit or fifth digit for the median or ulnar nerves, respectively.

2.3.5 Electrophysiology

Motor nerve conduction velocity (MNCV) was measured through the region of nerve where C-FINE shells were implanted. This was carried out at both implant and explant procedures by stimulating the nerve and recording resulting muscle twitches by needle electromyography (EMG) [121]. Supramaximal stimulation was delivered by a custom, MATLAB-programmable external stimulator developed at CWRU, proximal (approximately 1cm) and distal (approximately 1cm) to the C-FINE shell with a stainless steel electrode at a pulse amplitude of 2mA and a pulse width of 100µs. EMG recordings were made with pairs of monopolar EMG electrodes, amplified 325-fold through a differential pre-amplifier (B&L Engineering, Tustin, CA) with a pass band of 12-2975 Hz. CED 1902 isolated programmable amplifiers (Cambridge Electronic Design, Cambridge, UK) further amplified the signal such that EMG response to supramaximal stimulus did not saturate the amplifiers. Data were collected through a National Instruments BNC-6259 A/D DAQ board (National Instruments, Webster, TX) to a laptop computer. The distance between stimulation electrodes was recorded as were EMG recordings to determine the difference in time to muscle twitch at the different stimulation locations. Compound muscle action potential (CMAP) amplitude was measured with the same needle EMG setup and calculated as the peak to peak amplitude of the voltage change over the first 20 ms of the CMAP waveform [122].
2.3.6  Tissue fixation and examination

After 3-month period of implantation, the cuffed regions of the nerves were exposed for repeat electrophysiology testing. Under isoflurane sedation, cats were perfused with 4% paraformaldehyde (PFA) through a catheter inserted through the left ventricle of the heart and extended just past the aortic valve. The cuffed nerve along with any encapsulation tissue contained within the C-FINE shell was grossly dissected for plastic embedding and evaluation of axonal morphology (Figure 2.3 and Figure 2.5). The encapsulation tissue surrounding the C-FINE shell was separately dissected out for paraffin embedding and evaluation of morphology and quantification of encapsulation tissue outside the shell and to note any effects on surrounding tissues, such as blood vessels (Figure 2.3 and Figure 2.6).

For the sections of nerves with implanted C-FINE shells, histological blocks were taken following perfusion in 4% PFA for at least 24 hours. These blocks were washed in phosphate buffered saline for 24 hours and sectioned transversely into 0.5mm blocks. These were fixed in Karnovsky’s fixative (1% PFA, 1.25% glutaraldehyde in 0.1M phosphate buffer) [123] for two hours and post-fixed in OsO₄ (1% in 0.1M phosphate buffer). The blocks were dehydrated in increasing ethanol concentrations followed by increasing propylene oxide concentrations and embedded in Spurr’s resin [124]. Embedded blocks were sectioned at 1µm and stained with Toluidine Blue for examination under light microscopy. Histological sections were examined for features indicative of nerve damage, including changes in axon density, myelination of axons (e.g., the shape of the myelin sheath), and the ratio of the inner axonal diameter to the total outer diameter including the myelin sheath, the g-ratio. Changes in these features have been reported in nerve damage.
as well as regeneration following damage [125]. Histology of sections of nerve under the implanted C-FINE shells (both near the center and at the distal edge of the C-FINE shell) and as far as possible distal to the implanted C-FINE shells were compared to sections of nerve approximately 2-5cm proximal to the C-FINE shell. Care was taken to sample the nerve more than 1cm proximal to the proximal edge of the C-FINE shell since previous studies show that crush injuries can cause Wallerian degeneration for several millimeters proximal to the injury [126].

To examine the surrounding encapsulation and impact on surrounding tissues such as nearby arteries, sections of the approximately 2cm length of tissue surrounding the C-FINE shell and nerve (including nearby nerves and arteries, where applicable) were fixed in 4% PFA for at least 24 hours. These sections were embedded in paraffin and sectioned at 6µm and stained with Masson’s Trichrome to examine connective tissue. These sections were examined under light microscopy at 40x magnification. Tissue was examined for the thickness, density, and maturity of encapsulation tissue over the middle of the C-FINE shell compared to the edges of the C-FINE shell, which are less stiff and expected to produce less of an inflammatory response than the central region under the stiffening bar.

2.3.7 Axonal morphology

Images were acquired using AxioVision imaging software (Carl Zeiss, Inc., Thornwood, NY). Whole-nerve images were obtained under 40x or 100x light microscopy and stitched together. For analysis of axon and myelin morphology, nerve slices were examined under 400x magnification in light microscopy. Nerve images were sampled at the edges and in the middle of each fascicle. Changes in axon density, shape of the myelin
sheath, and ratio of myelin thickness to axon diameter are characteristic of nerve damage and regeneration [125]. Myelin thickness, axon diameter distributions, and axon density were quantified across each nerve for sections proximal to the implanted C-FINE shell and underneath the C-FINE shell. Healthy nerve should have consistent, densely packed axons, with myelin thickness proportional to axon diameter [127]. Decreased axon density may indicate either axonal loss or swelling. Decrease in myelin thickness may indicate a demyelination followed by re-myelination. Changes in axon diameter distributions may indicate a preferential loss in one type of fiber (large vs. small). Axons clustering within a single Schwann cell can indicate axon regeneration following denervation [118], [125], [128], [129]. A paired, single-tail, student’s t-test was used to quantify significance of changes in these morphological measures across hind or fore limb nerves comparing sections underneath the C-FINE shell to those proximal to the C-FINE shell.

2.4 Results

2.4.1 In-vitro C-FINE characterization

Pressure testing with all 16 samples showed that the C-FINE will assume a rounder shape and then stretch to accommodate a slowly inflating balloon at pressures ranging from 10 to 60 mmHg (Figure 2.4) At 0 mmHg applied internal pressure, the bulk of the folded-over deflated balloon material propped the electrode walls open. As a result, electrode cross-section data recorded at the 0 mmHg pressure level are not indicative of the normal resting state of the C-FINE at 0 mmHg. At 10 mmHg, the C-FINE attained an effective CNR of 1.82 ± 0.05, which is more than 6 standard deviations above, and ostensibly safer than, the industry standard CNR of 1.5. At 50 mmHg applied pressure, the threshold for
intrafascicular arterial blood flow compromise, the C-FINE achieved an effective CNR of 2.12 ± 0.05. These data indicate that as pressure increases inside the C-FINE, its cross sectional area expands and could possibly accommodate the acute changes in tissue and fluid volume expected from post-operative swelling or other condition.

The C-FINE’s laminar construction and PEEK stiffening bars allowed for a NCE of minimal volume that is still capable of gently reshaping target nerves. Computer modeling in SOLIDWORKS 3D CAD (Dassault Systèmes SOLIDWORKS Corp., Waltham, Massachusetts) of the C-FINE confirmed the minimal volume of this NCE compared to other designs. For instance, a 10 mm x 1 mm C-FINE offers a greater than 10% reduction in volume compared to similarly sized spiral NCE (a 4 mm diameter spiral

**Figure 2.4:** Effective CNR at increasing pressure inside 16 C-FINEs (10 mm x 1 mm x 7 mm, width x height x depth).

Solid line indicates CNR with error bars given for ± SD. Dashed line shows 1.5 CNR reference. Diagrams underneath solid line express approximate shape of expanded cuff (black ovals) compared to original shape of C-FINE (gray rectangles). As balloon pressure was increased from 10 to 60 mmHg, the cuff deformed (became more round in shape, up to approximately 20 mmHg) and then stretched under higher pressures.
with two wraps). A reduction in volume compared to a spiral NCE is impressive because the circular shape of the spiral produces a smaller circumference around a given nerve than a more oblong shape, like that of the C-FINE. Moreover, on more oblong nerves a larger spiral (for instance, 6 to 10 mm for the femoral nerve [82]) would likely have to be used, further increasing the volumetric advantage of the C-FINE. A 10 mm x 1 mm C-FINE also offers over an 80% volume reduction compared to the original molded FINE (10.1 mm³ for the C-FINE compared to 50.9 mm³ for the FINE [10], [44]).

Table 2.1: Electrophysiology results from implant compared to explant.

Values given as mean ± SEM (n = 6 nerves for both upper and lower extremity). No significant difference between implant and explant conditions using a 1-tailed, paired, student’s t-test (p > 0.05).

<table>
<thead>
<tr>
<th>Nerve</th>
<th>MNCV (m/s)</th>
<th>CMAP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Implant</td>
<td>Explant</td>
</tr>
<tr>
<td>Sciatic (6)</td>
<td>83.04 ± 6.05</td>
<td>84.55 ± 5.12</td>
</tr>
<tr>
<td>Median (4) and Ulnar (2)</td>
<td>73.66 ± 5.38</td>
<td>72.31 ± 4.41</td>
</tr>
</tbody>
</table>

2.4.2 Cat physiology and electrophysiology

The three cats, each implanted with bilateral hind and fore limb C-FINE shells, resumed normal behavior following recovery from anesthesia, 4 to 8 hours postoperatively. From this point through the course of the 3-month implant period, the cats showed no adverse physiological impact from implantation. Specifically, they showed no signs of pain, weakness, or lameness or changes in behaviors. From the period several hours after surgery and during the entire period while the cats were implanted, there were no signs of any ill effects from the C-FINE shells in terms of any difficulty trotting or gait abnormalities as described elsewhere [130], any other signs of weakness or decreased toe movement, or any decrease in sensation in the relevant dermatomes.
MNCVs and CMAP amplitudes were measured via EMG through the C-FINE shells at implant and explant procedures by stimulating approximately 1 cm proximal and distal to the nerve and recording muscle twitch responses [131]. Pooled MNCV and CMAP amplitudes are shown in Table 2.1. All recorded MNCV and CMAP amplitudes were within normal reported limits for cats [121], [132]. One-tailed, paired samples t-test showed no significant difference in MNCV or CMAP amplitudes at implant compared to explant for either the sciatic nerves alone (n = 6: p = 0.33 and p = 0.26, respectively), or the median and ulnar nerves combined (n = 6: p = 0.38 and p = 0.15, respectively).

Table 2.2: Quantified axonal morphology proximal to cuff vs. underneath cuff.
Values given as mean ± SEM (n = 6 nerves for both upper and lower extremity). No significant difference between proximal, control, nerve and nerve underneath C-FINE shells using a 1-tailed, paired, student’s t-test (p > 0.05).

<table>
<thead>
<tr>
<th>Nerve</th>
<th>Density (count/mm²)</th>
<th>g-Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Underneath C-FINE</td>
</tr>
<tr>
<td>Sciatic (n=6)</td>
<td>8091 ± 426</td>
<td>8167 ± 510</td>
</tr>
<tr>
<td>Median (n=4) and Ulnar (n=2)</td>
<td>8990 ± 268</td>
<td>8741 ± 462</td>
</tr>
</tbody>
</table>

2.4.3 Nerve reshaping and axonal morphology

Morphological examination of the whole nerve proximal to, underneath, and distal to the C-FINE shells show that the C-FINE had reshaped the nerves to a more oblong cross-section in the forelimb (Figure 2.5 and Figure 2.7) and maintained naturally-oblong sciatic nerve cross-section in the hind-limb. This was more notable in the fore rather than hind limbs, where the sciatic nerve has a more oblong cross-section in-situ. Reshaping occurred in the forelimb with a single-fascicle nerve (Figure 2.5) as well as with a multi-fascicled nerve (Figure 2.7). This reshaping occurred in hind and fore limb without adverse changes to nerve health.
Histological analysis showed healthy nerve containing densely packed axons with diameters following an expected bimodal distribution and well myelinated. An example of a 400x magnified section of sciatic nerve underneath an implanted C-FINE shell (Figure 2.8) reflects that the axons: 1) are densely and evenly packed, 2) both large and small diameter, and 3) possess a myelin sheath proportional to axon diameter, with large caliber axons being more thickly myelinated than smaller caliber axons. These observations were
quantified across the hind (n = 6) and fore limb (n = 6) nerves by comparing axonal morphology proximal to the implanted C-FINE shell with morphology underneath the C-FINE shell. The axon density and g-ratio (the ratio of the inner diameter of the axon to the diameter of the axon plus myelin sheath) across the 6 hind and 6 fore limb nerves are shown in Table 2.2. The mean axon densities and g-ratios across nerves are consistently within normal ranges for cats [128]. A one-tailed, paired samples t-test showed no significant difference in axon density or g-ratio proximal to compared to underneath the C-FINE shell for either the sciatic nerves alone (n = 6: p = 0.37 and p = 0.36, respectively), or the median and ulnar nerves combined (n = 6: p = 0.26 and p = 0.14, respectively).

2.4.4 Morphology of cuff encapsulation

Encapsulation at explant appeared minimal for C-FINE shells on the sciatic nerves and unremarkable for C-FINE shells implanted on the median and ulnar nerves, given the

Figure 2.6: Masson’s Trichrome stained tissue adjacent to cuff. Samples located approximately within the rectangular region outlined in image of nerve cross section (Figure 2.5 Left, bottom). Each pair of images shows large, stitched area as well as magnified section of encapsulation tissue outside the cuff and near the brachial artery. Magnified areas show areas of mature encapsulation (dense blue-stained collagen) which is thicker in the middle of the cuff. Black areas caused by artifact.
potential for increased pressures in the cat forelimb [112], [113]. This finding was reaffirmed by histology and with magnified images taken during nerve dissection. Masson’s Trichrome stained sections of the tissue surrounding C-FINE shell implants show dense, mature, encapsulation tissue around the outside of the C-FINE shells in some instances (Figure 2.6). The thickness of the encapsulation also decreases at the proximal and distal ends compared to the stiffer portion of the C-FINE shell in the middle. In many cases in the hind-limb, there was no visible encapsulation around the C-FINE shells on the sciatic nerve (an example from explant can be seen in 2.3, Middle). Because of how thin this layer of encapsulation was around many nerves, Masson’s Trichrome stained sections were not able to be used to reliably quantify encapsulation thickness across all nerves and

![Figure 2.7: Example gross and magnified reshaping of multi-fascicle nerve.
Top: Gross anatomy including cross section (left) of ulnar nerve underneath distal end of stiffening bar in the middle section of the cuff and segment of ulnar nerve distal to cuff (right). Bottom: Histological sections taken roughly at lines shown in images at the top showing fascicle layouts underneath cuff (left) and approximately 2cm distal to cuff (right).](image)
2.5 Discussion

In order to match the selectivity and mechanical requirements of novel NCE targets, we have introduced a new composite electrode enabling patterning of regions of various mechanical properties. This composite electrode can be adapted to the different mechanical properties at various implant locations, ranging from peripheral nerves anywhere along their length to spinal and autonomic nerve plexuses. In this case, we have specifically demonstrated the composite’s capability with the composite flat interface nerve electrode (C-FINE). The C-FINE was designed to maintain the selectivity of a FINE by gently reshaping the nerve to align its fascicles while minimizing the bulk and stiffness of the NCE along the length of the nerve. One key design feature of the C-FINE that allows for this is regionally patterned stiffness achieved with a stiffening bar in the middle. The
stiffening bar must be able to achieve reshaping without the risk of applying dangerously high pressures to the nerve in the event of swelling. \textit{In vitro} we verified that the C-FINE would not pose a risk to a swelling nerve with balloon inflation tests. In fact, the CNR exceeds 1.5 at pressures as low as 10 mmHg. Accommodating a larger area was achieved by the C-FINE assuming a more circular cross-section and also by stretching of the silicone sheeting. As balloon pressure was increased from 0 to 20 mmHg, the C-FINEs initially deformed into a more circular cross section to accommodate a larger area. Above 20 mmHg through 60 mmHg, the C-FINEs accommodated a larger area primarily by stretching (diagrams of the C-FINE shapes at different pressures are shown in Figure 2.4). One limitation of the pressure testing of the C-FINE is that the size of the electrode was not able to be accurately measured at 0 mmHg due to the width of the folded-over deflated balloon being larger than the internal volume of the C-FINE lumen. This slightly propped open the C-FINE when the balloon was fully deflated due to the relatively small opening size.

In the future, we plan to exploit the composite design of the C-FINE to fabricate high-density NCEs. Because there is a polymer stiffening bar where the contacts are located, we will explore microfabricating high-density electrode contacts and lead traces [109], [110] directly on the PEEK stiffening bars. This can allow for the deposition of a higher number of highly flexible contacts and leads within a C-FINE of a given width, allowing for potentially increased selectivity. Future studies will also target novel implant locations. These locations include nerves of varying sizes. While this study examined C-FINEs up to 10 mm in width, the sciatic nerve demands NCE designs which could exceed 30 mm [78] in width. Wider C-FINEs, with proportionately longer stiffening bars, are expected to be less stiff since stiffness is inversely proportional to the cube of beam length.
It may be desirable to exert consistent pressure across a wide range of nerve widths. Since beam thickness and length, respectively, have cubic and inversely cubic effects on stiffness, the beam thickness to achieve a desired stiffness could be calculated as a function of length. Thus, C-FINEs could be constructed that exhibit a consistent stiffness across a wide range of sizes. Alternatively, a stiffer or reinforced polymer in the same thickness could be utilized. The C-FINEs in this study were designed to be implanted in humans at widths greater than or equal to 10 mm—the size used in \textit{in vitro} testing. However, C-FINE shells implanted in cats in this study were all narrower than this (3 to 7 mm). This makes them roughly 2 to 11 times as stiff as the C-FINEs that will be deployed in human subjects. The decreased stiffness of human-sized devices adds a safety factor against any potential impact on nerve health from implanting C-FINEs in humans.

The PEEK bar can be patterned into any arbitrary shape with laser cutting techniques. This allows for the option to design the stiffness of the electrode into arbitrary patterns dependent on the application need. For example, it is possible to have a wider stiff area in the center of the electrode and tapering at the hinge and closure ends. Serpentine patterns, open center beams, and other designs to accommodate tissue needs are possible.

Several closure mechanisms have been used in past NCE designs. Examples include NCE closures relying on elastic forces \cite{102}, a button and post mechanism \cite{134}, adhesive or cured polymer \cite{135}, and surface tension \cite{136}. The NCE must hold itself in intimate proximity to the nerve during the period after implantation but before the body has formed a layer of encapsulation around the NCE, securing it near the nerve. Furthermore, a reshaping electrode requires a closure strong enough to exert pressure on the nerve required for reshaping. A mechanism relying on weaker forces, such as surface
tension, would be insufficient for a reshaping electrode. Many closure mechanisms which are strong are also bulky, stiff, or both. In order to minimize size and closure stiffness while securely holding the C-FINE securely around the target nerve, a suture closure was chosen. Limitation of the suture closure includes the increased implant time involved with tying, as well as the potential for sutures to be improperly tied. In fact, during the course of this study, one of the sutures on one C-FINE shells opened after it was implanted. This was not noticed until explant of the C-FINE shell 3 months later. A well trained surgeon minimizes the risk of this happening in human implants. Another consideration is the material of the suture. This study used braided 4-0 polyester suture that is known to cause increased inflammatory response [137], which may enhance suture closure but could promote encapsulation around theuffed nerve. On the Masson’s Trichrome stained sections of tissue flanking C-FINE shells, there is evidence of significant dense scar tissue surrounding the sutures in some of the C-FINE shells. During explant and gross dissection, the suture end of the C-FINE shell was difficult to remove as it was well adhered to surrounding tissue. The suture can also be seen inside the luminal area, adjacent to the nerve (Figure 2.3, bottom). While the suture did not cause morphological changes within the nerve, revisions were made to avoid any suture within the inside of the C-FINE’s lumen. For the human grade production of C-FINEs, sutures were embedded between two layers of silicone to avoid promotion of inflammation between the nerve and the C-FINE. The type of suture was also changed to a monofilament to avoid additional inflammatory response.

While the C-FINEs that were subjected to balloon testing were manufactured by a contract manufacturer (Ardiem Medical, Indiana, PA), the C-FINE shells that were implanted in cats for about 3 months were manufactured in a clean room at CWRU and
were manufactured without platinum contacts or leads. It is unlikely that the lack of inclusion of platinum foil contacts would have a significant impact on the mechanic properties of the C-FINE or on chronic nerve health. However, leads can cause a NCE to become tethered and thus pull on the nerve if they are not given enough slack or if percutaneous leads are forcibly pulled. In previous studies the inclusion of percutaneous leads had a significant effect on potential changes in nerve morphology. This is especially true for cats which are prone to pulling on and damaging percutaneous leads [114]. Moreover, the number of leads and their stiffness changes with their number, and the main innovation here is the C-FINE cuff design rather than a novel lead design. We intend this NCE design to be used with different numbers of contacts depending on application, and even with microfabrication allowing for high density printed contacts and leads [109], [110]. For this reason, and to avoid confounding these results with changes due to leads being pulled, we have focused on the primary mechanical and material design improvements of this NCE, involving the flexibility and low volume of the C-FINE cuff itself. As such, only C-FINE shells were implanted. Previous studies have demonstrated that NCE shells, by themselves, can produce detectable nerve health changes if they are sufficiently stiff and narrow [118]. No such observations were evident of the C-FINEs in our study.

NCE implants on the median and ulnar nerves of cats are not well established compared to implants on the sciatic nerve. While a spiral NCE has previously been implanted in the fore limb of 1 cat [102], these nerves are generally not a site of NCE implant. This area is tightly confined with little loose connective tissue and with the ulnar and median nerves flanking the brachial artery. Due to this confinement, the forelimb is a
potential location of osteofascial compartment syndrome at the humerus, radius/ulna, and caudal ante-brachium [112], [113]. A future study exploring the impact of different NCE designs on nerve health in such a constrained area could involve implantation of different NCE designs in contralateral limbs. Implanting contralateral limbs of the same animal is helpful because attempting to quantitatively compare axon densities and g-ratios between this study with future NCE design studies can be problematic due the effects of inconsistent fixation on myelin sheath morphology [138], [139].

Axonal morphology was very well preserved in the nerves with implanted C-FINE shells. This is especially important in the fore limbs of the cat which is spatially constrained, with little fat or loose connective tissue to accommodate an implant. As well, due to the length of the limb, the electrode is necessarily close to highly mobile joints. In all cats, there were no behavioral changes or signs of weakness, change in gait, or pain from shortly after surgical implant through the remainder of the three months while the cats were implanted with the C-FINE shells. Moreover, MNCV and CMAP amplitudes measured through the region with the implanted C-FINE shell were not significantly different when measured during implant compared to explant and were within normal ranges for cats [121], [132].

The most sensitive measure of nerve health following explant of the C-FINE shells is histological changes in axonal morphology, including quantification of axon density and g-ratio of the nerves proximal to, underneath, and distal to the C-FINE shells. Axonal histology is very sensitive to both damage of nerve as well as damage followed by regeneration, even at levels that are not physiologically obvious [118]. For instance, edema is frequently seen in nerve compression, even with pressures as low as 10 mmHg for 2
hours, with extensive myelination changes requiring about 30 mmHg of applied pressure for 2 hours [140]. The g-ratio and density of all nerves was not significantly changed underneath the C-FINE shells compared to proximal to them with the exception of one forelimb nerve. In the case of this nerve, surgical error during implantation caused a bleed of the brachial artery which required pressure to be held constantly on the nerve for approximately one hour. Sections of this nerve were taken proximal to, underneath, and every millimeter distal to the center of the C-FINE shell for 5mm, approximately 2cm distal to the distal edge of the C-FINE shell. By about 2 mm distal to the distal edge of the C-FINE shell the morphology of the nerve was normal. There were also only very minor signs of regeneration of the nerve distal to the C-FINE shell (Figure 2.9). The main finding in the sections underneath the C-FINE shell was edema with a decrease in axon density compared to proximal sections. This was accompanied by occasional large, thinly myelinated axons, and occasional evidence of myelin debris. Aside from these histological changes, there were no functional deficits or pain noted and MNCVs and CMAPs were not significantly changed through this region of this nerve. This indicates that any damage was not severe and that there was no significant Wallerian degeneration of this nerve. Future studies should further explore using immunohistochemistry to determine different etiologies of possible nerve damage or signs of regeneration.

Overall, these behavioral, functional, and morphological results indicate there was no significant change caused by chronic implantation of these C-FINE shells, with the exception of one instance of surgical error. This is especially important in the forelimb

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1 For a background and discussion of some of these methods, see Appendix A
where the C-FINE was able to replicate the reshaping properties of the original FINE while being amenable to placement around nerves which are in tightly-confined areas [112], [113] near joint spaces, sensitive structures, and prone to frequent movement. These changes in the C-FINE enable consideration of more implant locations for NCEs. For instance, implantation of a NCE on the femoral nerve near the inguinal ligament allows for potential control of both hip flexion and knee extension. However, in this region, the femoral nerve is particularly vulnerable to compressive and stretch injuries at the inguinal ligament [48]. This has been suspected as the location of compressive injuries during spine and gynecological procedures, likely due to patient positioning [48], [49] which can cause the femoral nerve to be sharply angled at 80-90° underneath the inguinal ligament [57], [58]. As such an NCE must be as flexible as possible along the length of the nerve to avoid impingement as the nerve bends, and as thin as possible to avoid adding unnecessary bulk to an already tight area. There are many such areas which benefit from a regionally compliant NCE, including several areas of the brachial plexus [51], and others [53]. The brachial plexus can be bent and stretched during shoulder abduction and external rotation because it is tethered at the prevertebral fascia and at the axillary sheath [52]. This requires

Figure 2.9: Sample histology of nerve subjected to over 1 hour of compression during implant.

During implant, a bleed of the brachial artery required the nerve and brachial artery to be held under compression for about one hour. Samples show relatively normal histology distal to implanted cuff. Underneath the cuff there is evidence of swelling and mild demyelination of some larger fibers.
an NCE which is able to match the mechanics of the brachial plexus along its length. As well, the brachial plexus is closely related to several bony structures against which it may be compressed [52], [108]. This necessitates an NCE which is not only flexible along the nerve but also as small as possible in volume, approximating as much as possible a film on the nerve. Moreover, not only does the increased flexibility and decreased volume allow for placement of C-FINEs in tight areas, it may also decrease stimulation charge thresholds and help to maintain a stable interface for stimulation, owing to less overall impact on nerve health. Stability of the nerve-electrode interface is crucial for long term functional restoration so that responses to stimulation are predictable over time.

The preliminary results from these in vitro and animal studies were sufficient to get approval from the FDA to deploy the C-FINE for chronic implantation in humans in two investigational device exemptions (IDEs G110043 and G040214). Twelve C-FINEs have since been chronically implanted in 5 human subjects for periods now exceeding 18 months. One subject received 8-contact C-FINEs on bilateral proximal femoral nerves as a part of a motor system neuroprosthesis for standing after SCI (March, 2016); one trans-humeral and one trans-radial amputee each received 16-contact C-FINEs on each the median and ulnar nerves for sensory restoration (April 2016 and June 2017), and two trans-tibial amputees received C-FINEs on each of the tibial, common peroneal, and distal or proximal sciatic nerves above the knee as part of a sensory system neuroprosthesis (May and December, 2016). At the time of this report, all subjects have exhibited stable stimulation charge thresholds and motor or sensory recruitment properties. The detailed analysis of the chronic clinical, technical and functional performance of the C-FINEs in
humans will be reported following completion of those studies and is further detailed for
the first-in-man C-FINEs in Chapters 3 and 4.

2.6 Conclusion

We have presented a novel approach to manufacturing a non-penetrating NCE with
patterned regions of stiffness applicable to a widening range of target applications. We
have focused on a design which reshapes peripheral nerves while applying little pressure
along their length and minimizes NCE volume, the composite flat interface nerve electrode
(C-FINE). We have rigorously tested the C-FINE’s volume and pressure design constraints
*in vitro* and C-FINE shells’ mechanical and biological safety in a feline model. *In vitro*
inflation tests resulted in effective CNRs (1.93 +/- 0.06) that exceeded the industry safety
standard of 1.5 at 20 mmHg, consistently less than the sustained 30 mmHg known to
compromise blood flow. Cats implanted with C-FINE shells for 3 months showed no
behavioral signs of neuropathy, no significant changes in electrophysiology (p > 0.15 for
all MNCV and CMAP measures), and no significant change in axonal morphology (p >
0.14 for axon densities and g-ratios). These data support the chronic mechanical and
biological safety of the new NCE configuration.

The C-FINE offers the potential advantages of improved selectivity by gently
reshaping nerves and providing high contact density for improved spatial resolution
through microfabrication, while simultaneously minimizing material volume and
maximizing flexibility along the length of the nerve. This regionally patterned stiffness was
achieved with a polymer stiffening bar in the middle of the C-FINE shell and low volume
was aided by the use of a suture closure. These design changes allow the C-FINE to be
implanted in very tightly confined areas with little loose connective tissue and near highly mobile joints. This concept is supported by behavioral, functional, and morphological results indicating that proper implantation of C-FINE shells does not cause adverse effects to nerve health. Preliminary results from the in vitro and chronic in vivo studies supported approval from the FDA to move forward and chronically implant C-FINEs in human subjects.
CHAPTER 3

Chronic nerve health following implantation of femoral nerve cuff electrodes

The content of this chapter is based on the following manuscript submitted to the Annals of Clinical and Translational Neurology:

Chronic nerve health after implantation of femoral nerve cuff electrodes, Manuscript submitted for publication.

3.1 Abstract

Objective. Peripheral nerve stimulation with implanted nerve cuff electrodes can restore standing, stepping and other functions to individuals with spinal cord injury. We performed the first study to evaluate the electrodiagnostic changes due to electrode implantation acutely, chronic presence on the nerve peri- and post-operatively, and long-term delivery of electrical stimulation.

Methods. A man with bilateral lower extremity paralysis secondary to cervical spinal cord injury sustained 6 years prior to enrollment received an implanted standing neuroprosthesis including composite flat interface nerve electrodes (C-FINEs) placed on the proximal femoral nerves bilaterally near the inguinal ligament. Electromyography quantified neurophysiology preoperatively, intraoperatively, and through 1-year postoperatively. Stimulation charge thresholds and evoked knee extension moments quantified neuroprosthesis function over the same time intervals.
Results: Femoral compound motor unit action potentials increased 31% in amplitude and 34% in area while evoked knee extension moments increased significantly (p<0.01) by 79% over 1 year of rehabilitation with standing and quadriceps exercises. Charge thresholds were low and stable, averaging 19.7 nC±6.2 (standard error). Changes in saphenous nerve action potentials and needle electromyography suggested minor nerve irritation perioperatively.

Interpretation: This is the first human trial reporting both acute and chronic neurophysiologic changes due to application of and stimulation through nerve cuff electrodes. Electrodiagnostic tests indicated generally healthy nerves with strengthened responses following stimulated exercise. Temporary electrodiagnostic changes suggest minor nerve irritation intra- and perioperatively, not continuing chronically nor impacting neuroprosthesis function. These clinical outcomes follow chronic implantation of a neuroprosthesis that allowed the subject to stand, and indicate that it is possible to safely implant electrodes on the proximal femoral nerve close to the inguinal ligament. The research suggests the electrodiagnostic findings that can be expected from implanting nerve cuff electrodes and their time-course for resolution, which may be applicable to prostheses modulating other peripheral nerves and functions.

3.2 Introduction

Electrical stimulation of peripheral nerves can restore or modulate functions of the somatic and autonomic peripheral nervous system. Somatic interfaces can modulate several functions ranging from prostheses restoring movement following central
neurological injury [2], [72] to those restoring sensation following extremity amputation [8], [9]. Surgically implanted neuroprostheses can restore function to individuals with spinal cord injury (SCI, abbreviations in Table 3.1) by eliciting contractions of the otherwise paralyzed muscles via electrical stimulation of peripheral motor nerves [2], [90], [96]–[99]. Non-penetrating nerve cuff electrodes (NCEs) maintain intimate contact with the epineurium and have advantages over other electrode designs [29], [46], including strong, stable recruitment, and improved functional outcomes [2], [47]. NCEs on nerves in the upper [8]–[10], [72] and lower [45], [47] extremities have been reported functionally reliable for more than 11 years post-implantation [45] in terms of stimulation threshold and functional output over time [47], [72]. To date, the effects of both implantation and chronic use of NCEs on neurophysiology and muscle innervation have not been examined with standard clinical measures, including both nerve conduction studies (NCS) and needle electromyography (EMG). This dearth of neurophysiological outcomes and lack of baseline electrodiagnostic changes in well-functioning neuroprostheses make it difficult to gauge the impact of novel NCE designs, implant locations, surgical approaches, and rehabilitation paradigms on nerve health.

Table 3.1: Abbreviations in chapter. Abbreviations are listed in alphabetical order.

<table>
<thead>
<tr>
<th>Abbrev.</th>
<th>Definition</th>
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<tr>
<td>C-FINE</td>
<td>Composite flat interface nerve electrode</td>
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<td>CMAP</td>
<td>Compound muscle action potential</td>
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<td>EMG</td>
<td>Electromyography</td>
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<tr>
<td>IPG</td>
<td>Implanted pulse generator</td>
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<tr>
<td>MNCV</td>
<td>Motor nerve conduction velocity</td>
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<td>NCE</td>
<td>Nerve cuff electrode</td>
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<td>NCS</td>
<td>Nerve conduction studies</td>
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<td>RF</td>
<td>Rectus femoris</td>
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<tr>
<td>Sart</td>
<td>Sartorius</td>
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<tr>
<td>SCI</td>
<td>Spinal cord injury</td>
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<tr>
<td>SNAP</td>
<td>Sensory nerve action potential</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>VI</td>
<td>Vastus intermedius</td>
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<tr>
<td>VL</td>
<td>Vastus lateralis</td>
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<td>VM</td>
<td>Vastus medialis</td>
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As NCE designs include higher contact densities [42] they are able to achieve sufficient selectivity to be implanted on proximal nerve trunks [44]. The flat interface nerve electrode (FINE) gently reshapes peripheral nerves to increase accessible surface area for multiple contacts, allowing for better access to distinct populations of nerve fascicles [42]. The 8-contact FINE is sufficiently selective to separate hip flexors from knee extensors [44] when implanted proximal to the first branch off the femoral nerve, approximately 1-2 cm distal to the inguinal ligament [50]. The inguinal ligament is a possible site of femoral nerve entrapments, especially during hip flexion and rotation [48], [49], [57], [58]. The composite flat interface electrode (C-FINE, Figure 3.1a) [141] was developed to be a small and flexible reshaping NCE to accommodate sharp bending of the nerve in such locations. Yet, the acute and chronic consequences of the C-FINE on neurophysiology, especially in novel implant locations (e.g., close to the inguinal ligament) have not been established.

Numerous elements of a neuroprosthesis, such as NCE design, lead routing, implant location, and surgical approaches, may affect nerve health. Neurophysiological and electrodiagnostic changes can also be caused by intraoperative nerve manipulation, perioperative positioning [57] and swelling [142], and nerve traction. These changes can range from temporary demyelination and neurapraxia to varying degrees of axonotmesis [63], with a wide range of prognoses [64]. The neurophysiologic consequences of neuroprosthesis design changes have been extrapolated from benchtop and animal models [102], [118], [141] and then tracked chronically through observations of neuroprosthesis performance [2], [10]. With myriad potential etiologies and sequelae, it is crucial to grade neurophysiological changes following NCE implantation using well-established
electrodiagnostic studies in chronic implant recipients. This is especially important in subjects with SCI who have limited clinical exams at baseline.

We performed the first human trial evaluating the short and long-term health of nerves implanted and stimulated with NCEs through electrodiagnostic studies preoperatively, intraoperatively, and postoperatively through one year. We tracked femoral motor and saphenous sensory NCS and needle EMG, NCE stimulation charge thresholds, and tetanic knee moments, before and after initiating an exercise program with stimulation. We report the electrophysiological and clinical outcomes of the first-in-man deployment of bilateral 8-contact C-FINEs on the proximal femoral nerve trunks of one subject with SCI (Figure 3.2).
Methods

A combination of electrodiagnostics and metrics of neuroprosthesis functional performance was employed to quantify femoral nerve health and nerve-cuff interactions at seven time-points over a 1-year period postoperatively (Figure 3.3). Measures of neuroprosthesis performance included stimulation charge thresholds and tetanic knee extension moments. NCS and needle EMG systematically compared neuromuscular function to preoperative baseline levels. For the first 6 months postoperatively, percutaneous leads allowed access to all 16 contacts across bilateral C-FINEs. After this period, percutaneous leads were removed and 3 contacts per C-FINE selective for isolated knee extension were connected to an implanted pulse generator (IPG) that delivered stimulation through these 6 contacts. Electrodiagnostic studies with surface stimulation

Figure 3.2: Implant diagram and C-FINE recipient standing using implanted neuroprosthesis.

A: Lead routing showing C-FINEs (purple) and connectors (green) to either percutaneous leads (orange) or an IPG (blue) for percutaneous or standing phases, respectively. B and C: Front and side views of the subject standing from his wheelchair with the IPG with a physical therapist providing contact to guard but not support. 93.1 +/- 2.1% of body weight was supported through his legs.

3.3 Methods

A combination of electrodiagnostics and metrics of neuroprosthesis functional performance was employed to quantify femoral nerve health and nerve-cuff interactions at seven time-points over a 1-year period postoperatively (Figure 3.3). Measures of neuroprosthesis performance included stimulation charge thresholds and tetanic knee extension moments. NCS and needle EMG systematically compared neuromuscular function to preoperative baseline levels. For the first 6 months postoperatively, percutaneous leads allowed access to all 16 contacts across bilateral C-FINEs. After this period, percutaneous leads were removed and 3 contacts per C-FINE selective for isolated knee extension were connected to an implanted pulse generator (IPG) that delivered stimulation through these 6 contacts. Electrodiagnostic studies with surface stimulation
interrogated the whole nerve. All procedures took place at the Cleveland VA Medical Center in Cleveland, OH. The subject consented prior to participation to all study related procedures as approved by the local institutional review board under investigational device exemption IDE#G040214 from US FDA for chronic human implantation of the C-FINE.

3.3.1 C-FINE Implantation

The C-FINE (Figure 3.1a) is a NCE designed to match the naturally oblong cross section of many peripheral nerves [50], [78] and gently reshape them into a flatter cross section. A flattened architecture allows for isolated stimulation of functionally distinct fascicles and the placement of more stimulating contacts around a nerve [42]. The C-FINE employs graduated stiffness to reshape the nerve along the middle of the C-FINE while remaining highly flexible along the length of the nerve to accommodate nerve bending [141]. We implanted 8-contact C-FINEs bilaterally on the proximal femoral nerves of a male volunteer (age 25, 5-years post-injury) with SCI (C5 American Spinal Injury
Association impairment scale C). The C-FINEs had 4 platinum-iridium contacts evenly distributed on each of the top and bottom interior surfaces of the electrode for a total of 8 contacts.

Lower density (i.e. 4-contact) NCEs do not have the resolution to separately activate distinct functional groups of fascicles [82]. The anticipated selectivity of 8-contact C-FINEs [44] justified implanting the devices proximal to branches to both the knee extensors and hip flexors on the common nerve trunk. Because of the potential of the femoral nerve at this site to move beneath the inguinal ligament during hip flexion[48], [49], [57], [58], a surgeon verified that the C-FINEs did not get compressed or pulled underneath the ligament with each hip flexed to near 90°. A redundant loop for strain relief (Figure 3.1b) was left in the distal leads close to the C-FINEs prior to subcutaneous tunneling to proximal connector sites in the abdomen. The strain relief loop reduced the potential for transmitting tethering forces to the devices or nerves during hip movement.

Intraoperatively, an external stimulator elicited twitch contractions via stimulation through the C-FINE contacts. Monopolar EMG needles inserted into muscles innervated by the femoral nerve (Figure 3.1d), recorded the evoked EMG intraoperatively, measuring compound muscle action potential (CMAP) amplitudes and latencies. Following EMG recording, the leads from the C-FINEs were first passed to abdominal spring-and-pin connectors[143]. Helical wound percutaneous leads[144], [145] connected to each NCE allowed access to all 8 individual contacts. These percutaneous leads were then tunneled subcutaneously and individually to exit sites on the proximal anterolateral thighs (Figure 3.2a). The subject cleaned, monitored, and re-dressed the percutaneous interfaces for the
next 6 months, during which time he experienced only minor complications such as an occasional mild erythema (white arrow in Figure 3.1c).

Intraoperatively and 3 weeks post-implantation, twitch responses to single pulses of stimulation were collected to avoid tetanic contractions and minimize risk of NCE movement. Following this 3 weeks period, we initiated exercise paradigms with tetanic stimulation. Following the 6-month percutaneous phase, the subject received a fully implanted standing neuroprosthesis. Percutaneous leads were removed and 6 out of 16 C-FINE contacts for knee extension bilaterally were connected to an IPG, along with 5 additional intramuscular electrodes for hip and trunk extension bilaterally.

3.3.2 Electrodiagnostic Testing

Before implantation and throughout the 1-year follow up period, a neurologist subspecializing in electrodiagnostics and neuromuscular diseases conducted all electrodiagnostic testing. Two weeks prior to implantation, NCS and needle EMG studies evaluated baseline nerve health. Preoperative NCS of bilateral lower extremities quantified saphenous, sural, and superficial peroneal sensory nerve action potentials (SNAPs), and peroneal, tibial, and femoral CMAPs. Needle EMG was performed on 9 muscles innervated by these nerves. Intraoperative testing consisted of collecting CMAPs of muscles innervated by the femoral nerve (Figure 3.1d), and postoperative testing focused on NCS of the femoral and saphenous nerves, and needle EMG of the femoral nerve innervated muscles. CMAP measurements with the subject sitting (hip flexed to 90°) and supine ruled out femoral nerve compression during hip flexion. For the purposes of this study where the NCEs were placed on the femoral nerves, postsurgical testing focused on the femoral motor
nerve responses, and its terminal nerve branch, the saphenous sensory nerve. It was helpful to track the saphenous studies, since sensory responses are three orders of magnitude smaller than motor responses, and hence more susceptible to axonal or demyelinative nerve damage[146].

Preoperatively, and postoperatively at 2, 4, 6, and 12 months, surface stimulation elicited femoral CMAPs while recording over the rectus femoris. Surface stimulation was contraindicated intraoperatively and during the first 3 weeks postoperatively due to potential incision site complications. Intraoperatively and at all postoperative time-points, CMAPs (blue shading in Figure 3.3) were recorded with pairs of monopolar EMG needles in response to stimulation delivered through the C-FINEs. During the 6-month percutaneous period, the externalized leads to the C-FINE contacts were connected to a custom-designed current-controlled stimulator[147] that delivered monopolar, charge-balanced, biphasic pulses with pulse amplitude ranging from 0.1 to 2.0 mA and pulse widths ranging from 1 to 255 µs. Twitch stimulation involved stimulating with a single pulse with at least 1 second between pulses. Tetanic stimulation delivered a train of pulses at 20 Hz.

Three weeks postoperatively, custom pre-programmed stimulation paradigms [99], [148] were initiated to recondition the knee extensor musculature. The exercise paradigm generated contractions of quadriceps muscles to build knee extension strength with high load and low repetition exercises with progressively increasing ankle weights while sitting. To build endurance, the stimulation paradigms utilized long duration and low load exercises. These exercise programs continued throughout the 6-month percutaneous phase. After 6 months, the standing phase began with implantation of the IPG[149], [150],
connected to a subset of 3 C-FINE contacts bilaterally, and with the insertion of additional intramuscular electrodes to activate hip and trunk extensor muscles necessary for standing, including bilateral quadratus lumborum, erector spinae, gluteus maximus, and posterior portion of adductor magnus as well as unilateral hamstring (right) and gluteus medius (left). The subject continued to build knee extension strength and endurance with a take-home standing rehabilitation program, which he began to use approximately 6 weeks after IPG installation (8 months after C-FINE implantation).

3.3.3 **EMG Evaluating Neuroprosthesis Performance**

Prior to stimulation, a surface reference electrode (2” x 4”, Nicolet-VIASYS, Madison, WI) was placed over the greater trochanter of the contralateral leg. The neuromuscular subspecialist placed monopolar EMG needle electrodes (Nicolet 27 to 28 gauge needles, 13 mm – 50 mm length (Natus Medical Inc., Pleasanton, CA) approximately 2 cm apart into knee and hip extensor muscles innervated by the femoral nerve (Figure 3.1d). EMG was recorded independently and simultaneously from the three heads of the vasti (vastus medialis, VM; vastus intermedius, VI; vastus lateralis, VL), rectus femoris (RF), and sartorius (Sart) muscles.

Stimulation delivered through each contact on the C-FINEs at all measurement intervals elicited twitch EMG responses. The signal from each pair of monopolar needle EMG electrodes was amplified, filtered, and sampled as described for intraoperative NCE evaluation [43]. Custom MATLAB (MathWorks, Natick, Massachusetts) code was developed to interface programmable EMG amplifiers with the computer-controlled stimulator. EMG responses from each muscle were rectified, integrated over the duration
of the M-wave (from 3 ms through up to 30 ms after stimulation), and normalized by the maximum twitch for the muscle following supramaximal stimulation on all contacts. Dividing the distance between the C-FINE and the recording needles for each muscle by the onset latency of the CMAP for that muscle determined motor nerve conduction velocities (MNCVs). The onset latency was defined as the time between stimulation and M-wave onset, with a 0.5 ms offset to approximate neuromuscular junction delay [151], [152]. The MNCVs averaged across all of the muscles provided the conduction velocity for the femoral nerve.

Calculation of the stimulation threshold charges follows:

\[ Q_\theta = I \times PW_\theta \]  \hspace{1cm} \text{Eq. 3.1}

The current, \( I \) (either 0.8 mA or 1.4 mA) applied through each contact was the minimum current able to reach supramaximal stimulation by the maximum pulse width of 255 \( \mu s \). Current was adjusted on a contact-by-contact basis, but remained consistent for each contact current across all time-points to eliminate a potential source of variability in charge threshold calculations [153], [154]. \( PW_\theta \) was defined as the pulse width at which CMAP amplitude reached 10% of its supramaximal stimulation value. Charge threshold stability based on EMG was tracked for all 16 contacts during the 6 month percutaneous phase, and then derived from the moment measurements for the 6 contacts connected to the IPG at 9 months and 1 year postoperatively.

### 3.3.4 Surrogate measures of muscle strength

Starting at 3 weeks postoperatively, and for all remaining time-points, a 6 degree-of-freedom load cell (JR3 Inc., Woodland, CA) measured isometric, tetanic knee extension
moments with the knee fixed at 20° flexion and with the distal thigh fixed to the seat of a robotic dynamometer (Biodex, Shirley, NY). Stimulation was delivered through each contact at 20 Hz for approximately 3 seconds with at least 15 seconds of rest between repeated bursts to avoid fatigue, as described for previous standing neuroprosthesis studies [47]. We determined whether there was a significant linear relationship between knee moment and time-point in the study by fitting a linear regression to the yearlong mean moment data for each leg and using a t-test on the null hypothesis that slope of the fitted line is 0, which would indicate no change as a result of stimulated exercise.

As a secondary measure of quadriceps strength and reconditioning, thigh circumference was measured at all postoperative time-points and compared to a pre-exercise baseline. The same individual consistently measured circumference at a site 15 cm proximal to the site of attachment of the quadriceps tendon to the patella. By assuming the thigh was approximately circular in cross section, a squared ratio of circumferences provided an estimated ratio of area.

3.4 Results

At preoperative baseline, the C-FINE volunteer had very limited volitional control of his lower extremity muscles. Manual muscle testing showed the following: volitional hip flexion was 3/5 (strength against gravity) on the right, and was 2/5 (strength parallel to gravity) on the left; and knee extension was 2/5 bilaterally. Preoperative needle EMG showed limited activation with otherwise normal motor unit potential morphology and recruitment patterns in the bilateral tensor fascia lata and right flexor digitorum longus muscles. No other tested muscle in the bilateral lower extremities showed volitional
activity: sartorius, rectus femoris, vastus lateralis, vastus medialis, tibialis anterior, medial gastrocnemius, nor extensor hallucis longus. Preoperatively, no tested muscle revealed electrophysiologic evidence for active or chronic denervation. At baseline, the subject reported light touch sensation over the saphenous dermatome reduced to roughly 50% of normal. Likewise, sensation was decreased to light touch and absent to pinprick throughout the lumbar and sacral dermatomes.

Preoperative NCS in the lower extremities revealed essentially normal bilateral sensory nerve (saphenous, superficial peroneal and sural) and motor nerve (femoral, peroneal and tibial) responses. The left saphenous sensory response showed a decreased amplitude, but was otherwise normal. In conjunction, preoperative needle exam and NCS were consistent with a central etiology, such as SCI, for his clinical weakness.

3.4.1  Motor nerve conduction studies

C-FINE stimulation with recording at all heads of the quadriceps revealed consistent, symmetric, and normal MNCV > 41 m/s, (p < 0.05) throughout the 24-week percutaneous phase of the study (Figure 3.4a). Although there was an apparent decrease in MNCV from intraoperative measurements compared to 1 week postoperatively, this drop was not statistically significant (p = 0.3 on the left and p = 0.07 on the right). There was no statistically significant difference between the MNCVs on the right as compared to the left (p = 0.06). Femoral nerve latencies elicited by surface stimulation at the groin and measured at the rectus femoris were recorded preoperatively and again at 2, 4, 6, and 12 months. These were always within normal limits (< 6 ms).
CMAPs showed increasing amplitudes and areas over the course of this 1-year study. There was no significant decrease (paired t-test, p = 0.06 on the right and p = 0.85 on the left) in the CMAP amplitudes and areas secondary to hip flexion (Figure 3.4b and 3.4c). The increase in CMAP amplitude and area occurred after the subject started his rehabilitation exercise program 3 weeks postoperatively. CMAP amplitudes and areas, obtained through surface stimulation, were above baseline at all time-points except one, 2 months postoperatively, when amplitudes averaged 9.6% and areas 18.2% below baseline bilaterally. This time point correlated with an increase in the stimulation threshold (discussed below), which limited the ability for supramaximal stimulation. In contrast, when stimulating through the C-FINEs, CMAP amplitudes at 2 months essentially equaled (+1.5%) baseline C-FINE stimulation. By 6 months postoperatively, with regular exercise, CMAP amplitudes and areas elicited by surface stimulation increased by an average of 31% and 34%, respectively. From 6 months to 1 year postoperatively CMAP values were stable and unchanged by the standing exercises initiated around 8 months postoperatively.

3.4.2 Sensory nerve conduction studies

Saphenous SNAP amplitudes were normal in the right lower extremity, and borderline below normal in the left lower extremity. Bilateral saphenous sensory responses were stable at 3 weeks postoperatively, but absent by 2 months (Figure 3.4d). The right saphenous SNAP started to recover by the 1 year test date; however, the left saphenous response remained absent until this time point. Subjectively, the subject did not notice any
Figure 3.4: Summary of femoral motor and sensory nerve conduction studies. Shading indicates the preoperative period when baseline measurements were recorded. A: Femoral MNCV. Mean intraoperative MNCV and postoperative MNCV compared to normal velocities. Error bars represent SEM between velocities to the heads of quadriceps muscles. B: CMAP Amplitudes: Postoperative with surface and C-FINE stimulation compared to baselines with surface stimulation elicited 2 weeks preoperatively and minimum normal values. CMAPs also compared with subject sitting (hips flexed 90°) and supine. Error bars represent standard deviations between 3 consistent trials. C: CMAP Areas collected similarly to CMAP amplitudes and at the same time points. D: SNAP Amplitudes. Postoperative amplitudes compared to baseline elicited 2 weeks preoperatively and minimum normal amplitudes.
clinical change in his lower extremity sensation; however, his sensation was already compromised in the setting of his SCI. When obtained, saphenous SNAPs demonstrated velocities greater than 41 m/s, ranging from 42 to 51 m/s. Return of right saphenous SNAP by the 1 year test date suggests a regeneration rate of roughly 2-3 mm/day from the time of surgical manipulation which is consistent with other studies for distal peripheral nerve axonal regeneration [60], [155].

3.4.3 Needle EMG

Preoperatively, needle EMG showed normal insertional activity, without any spontaneous activity. At 1, 3, and 9 weeks postoperatively, rare fibrillation potentials were seen in the following muscles: sartorius and VM on the right; and sartorius, RF, and VL on the left (Table 3.2). As expected, more proximal muscles (sartorius, RF) showed signs of active denervation initially, and as the proximal muscles improved, the distal ones showed rare fibrillation potentials. No fibrillation potentials were seen at or after 15 weeks postoperatively, indicating that nerve irritation, inflammation, or damage did not occur after the intraoperative or perioperative periods.

3.4.4 Stimulation charge thresholds

Stimulation charge thresholds were calculated for each available C-FINE channel at

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<td><strong>Muscle</strong></td>
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Table 3.2: Locations and time-points of active denervation.
First column indicates distance from C-FINEs to where EMG needle typically inserted in muscle. Yellow (right leg) and blue (left leg) boxes indicate rare fibrillation potentials at a specific time-point.
all time-points (Figure 3.5). Fifteen out of 16 contacts had charge threshold below 100 nC at every time-point and the remaining contact’s charge threshold only exceeded 100 nC for a single measurement. The mean of the charge thresholds was lowest at implantation at 9.7 nC ± 1.7 (SEM) over all contacts. These rose postoperatively, and peaked around 2 months at 45.8 nC ± 6.9 (SEM). At the end of the 6-month percutaneous phase, thresholds across all 16 contacts averaged 27.8 nC ± 4.4 (SEM). At 6 months the mean threshold across the 6 contacts selected to be connected to the IPG was 28.1 nC ± 9.2 (SEM), and at 1 year it was 19.7 nC ± 6.2 (SEM), revealing that thresholds continued to decline as expected. Most of this drop occurred by 9 months after which values appeared to plateau. By 1 year, the thresholds appeared to be stable and were not expected to decline further [72]. These charge thresholds and the time course of their changes are consistent with previous NCE studies [47], [72].

3.4.5 **Surrogate markers for muscle strength**

From the initiation of tetanic stimulation (3 weeks postoperatively) through 1 year of neuroprosthesis use for exercise and standing, maximum single-contact tetanic moment for the 6 contacts connected to the IPG increased by an average of 79% ± 16 (SEM) (Figure 3.6). Linear regression t-tests determined a significant increase in moment during this study on both the left (p < 0.01) and right (p < 0.02) legs. The initial average moment of 0.42 Nm/kg per contact on the left increased by 110% to produce an average moment of 0.89 Nm/kg per contact. The initial average moment of 0.49 Nm/kg per contact on the right increased by 44% to produce an average moment of 0.70 Nm/kg per contact.
Over the first 5 months of stimulated exercise, thigh circumference increased 11% and 12% on the right and left side, respectively. With the addition of stimulated standing exercise following installation of the IPG and hip/trunk electrodes, thigh circumference increased an additional 6% on the right and 8% on the left by 1 year. This resulted in total circumference gains of 18% and 21% on the right and left thighs, respectively. Thigh cross-sectional areas derived from these circumference measurements, increased by 39% on the right and 47% on the left, suggesting increases in force generating capacity with reconditioning exercise and standing [156], [157].

3.5 Discussion

This study reports the neurophysiological results secondary to implantation of C-FINEs on bilateral femoral nerves as part of a standing neuroprosthesis in a subject with

Figure 3.5: Stimulation charge thresholds from implantation through 1 year postoperatively.
Thresholds defined by charge when rectified, integrated, normalized CMAPs (solid lines) or moments (dashed lines) reach 10% of maximum recorded value for a given muscle. Error bars on median measure represent SEM. Reduction in data points at 9 months and 1 year due to removal of percutaneous leads and 3 out of 8 contacts bilaterally being connected to the IPG.

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3.5 Discussion

This study reports the neurophysiological results secondary to implantation of C-FINEs on bilateral femoral nerves as part of a standing neuroprosthesis in a subject with
Figure 3.6: Normalized knee moments over 1 year on each of the selected knee extensor selective C-FINE contacts at 0.8 mA, 255 µs. These contacts were chosen because they were able to achieve strong knee extension moments with insignificant hip flexion. Error bars indicate standard deviation between contacts. Dashed line represents linear best-fit to mean tetanic moments. Based on linear regression t-testing, an increase in moment over 1 year is significant (p < 0.01 on the left and p < 0.02 on the right).

cervical SCI. While a larger number of NCEs across more subjects and different nerve sites will help bolster these results in the future, there are several important implications from this study. Our findings represent baseline electrodiagnostic measures and changes that can be reasonably expected over 1 year of implantation and chronic use of C-FINEs on peripheral nerves.

There are multiple possible etiologies for the mild electrodiagnostic changes seen on EMG testing. Nerve manipulation during dissection and NCE placement can cause neurapraxia or mild axonotmesis. C-FINE movement along the nerve in the perioperative period, prior to stabilization with encapsulation tissue, could also cause nerve irritation. Fibrillation potentials seen with needle EMG started approximately 1 week after surgery
and cleared between 9 to 15 weeks postoperatively. This time course is consistent with neurapraxia or mild axonotmesis occurring during NCE implantation.

On NCS with surface stimulation, CMAP amplitudes and areas decreased by less than 20%, at 2 months. Stimulation through the nerve cuff resulted in CMAPs similar to baseline around the 1-2-month timeframe. In association, there was a significant increase in the stimulation charge threshold at this point. The raised threshold limited the ability to reach a supramaximal response, especially through surface stimulation. Increases in stimulation charge thresholds in the first several months postoperatively is often attributed to inflammation between electrode and nerve and is well established [10], [47], [72]. A similar increase in charge thresholds was observed for stimulation through the C-FINEs in this study, which rose almost five-fold over the first 2 months postoperatively. However, over the last 10 months of this study, thresholds consistently declined and stabilized as expected.

NCS also revealed saphenous SNAP loss and partial recovery between 6 months to 1 year. Combined with the presence of temporary mild fibrillation potentials, these findings are consistent with mild axonal loss and regeneration [63]. The rate of recovery for the right saphenous SNAP is consistent with established axonal regeneration rates [63], [64] and support temporary, perioperative irritation. Postsurgical neurapraxia tends to have a positive prognosis [158] and these results show a positive functional outcome in this subject. Moreover, the subject reported no appreciable changes in his preserved sensation on the dermatomes served by the nerve at any time during the study, suggesting that the changes may be sub-clinical. Additionally, SNAPs were small on the left side preoperatively indicating there may have been previous traumatic impact to this nerve. All
other studies indicated that the C-FINEs had a positive impact on the rehabilitation potential for the muscles innervated by the femoral nerves, and improved functional status. We recommend that electrodiagnostic studies be used to track neurophysiological changes due to an implanted NCE, since this data will allow for clinical determinations about NCE viability and nerve health.

Despite mild changes in neurophysiology, the standing neuroprosthesis deployed in this study was clinically successful. After 3 months of training, this subject was able to stand in excess of 30 minutes (Figure 3.1), limited by orthostatic hypotension or shortness of breath secondary to being wheelchair dependent for the previous 5 years. Quadriceps fatigue and weakness did not limit his ability for sustained standing. Force plate measurements [2] indicated that 93.1 +/- 2.1% of his body weight was supported by his legs while standing. Standing times greater than 30 minutes are far in excess of the typical standing times of implanted neuroprosthesis recipients which are on the order of 10 minutes, even after a year of training [2], [159]. These standing times are largely attributable to the performance of the C-FINEs. Each of the three individual contacts within each C-FINE generated four times the 0.135 Nm/kg of knee extension [160]–[162] required for quiet standing. Furthermore, each contact, on average, generated twice the 0.4 Nm/kg [162], [163] required for a sit-to-stand transition, and even higher moments were generated when stimulating through multiple contacts on a C-FINE simultaneously.

After 1 year of various quadriceps-focused exercises, notable increases or improvements were noted in muscle bulk, strength generated with stimulation, and electrodiagnostic measures. Bilaterally CMAPs increased an average of 31%, and isometric moments increased an average of 79%. Amplitudes and areas of femoral CMAPs
increased during the initial 6 months of exercise with stimulation through the NCE. However, there was no appreciable increase from 6 months to 1 year, despite significant increases in elicited knee extension moment and thigh circumference indicating muscle hypertrophy. Notably, CMAP amplitudes, areas, and duration remained unchanged with hip flexion, indicating that the C-FINE was flexible enough to accommodate nerve bending in proximity to the inguinal ligament.

Limitations of this study included session-to-session variability in isometric moment measurements, which were attributed to several factors. First, there was inconsistency with day-to-day differences in muscle fatigue. This unpredictability is exaggerated in individuals with SCI, especially during stimulation induced contractions of the paralyzed muscles [164]. Additionally, there may be methodological or technical sources of variability, such as the uncertainty repeated positioning of the knee during dynamometer measurements of knee moment. Isometric knee extension moments were measured at 20° of knee flexion to reduce the risk of injury during tetanic contractions with the knee flexed due to the length-tension properties and moment arm of the quadriceps. At this angle isometric knee extension moments are highly sensitive to changes in knee angle. At 20° of knee flexion, moment is about 38% of maximum (occurring around 80° knee flexion). If the knee is flexed to 30°, moment rises to roughly 51% of maximum, and if the knee is extended to 10°, the moment falls to 29% of maximum [165]. Thus, changes in 10° from the target knee angle of 20° of flexion can result in a rise of 34% or drop of 23%2. Despite the multiple sources of variability, increase in knee moment during the study were

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2 For more information about day-to-day variance of isometric moments at 20°, see Appendix B.
statistically significant ($p < 0.01$) and averaged 79% across both legs. These gains are large enough to be clinically significant as well. Although not statistically significant, the moment gains were much larger in the left leg (110%) than the right (44%). At baseline, there was more atrophy and weakness in his left lower extremity, possibly due to asymmetric disuse and deconditioning resulting in him favoring his right leg. The C-FINE stimulation and rehabilitation paradigms may have compensated for the asymmetry and allowed him to use both his legs equally, so that by the end of the study weight bearing on his legs during standing with the neuroprosthesis was more symmetric.

Another limitation is that all 16 contacts of the C-FINEs were only accessible during the 6-month percutaneous phase, after which 6 out of the 16 contacts were chosen for connection to an IPG for future use during exercise and standing. This meant that moment could not be tracked with stimulation through all 16 contacts after 6 months. However, the 6 contacts were specifically selected based on their ability to strongly activate and isolate distinct populations of knee extensors. Furthermore, with sufficient yet safe stimulation charge through a single contact of the nerve cuff, it is likely possible to depolarize the entire nerve. Similarly, stimulation charge thresholds were only tracked through these 6 contacts after the percutaneous phase ended, and charge thresholds at 9 months and 1 year were derived from moment rather than EMG measurements. However, it is unlikely that this change in methodology would impact the outcome as the link between CMAP areas and moment generation are well established.

CMAP amplitudes elicited by supramaximal stimulation through all 8 contacts of a C-FINE are larger than amplitudes elicited by surface stimulation. However, the opposite relation holds for CMAP areas. This is likely due to limitations in the stimulator used in
this study which requires 1ms between deliveries of stimulation on different contacts. Over 8 contacts, a 7ms delay develops from stimulation on the first through eighth contacts. This can cause excitation of some populations of motor units while others are in their refractory period, resulting in CMAP dispersion over time and reduced CMAP areas. When charge required for supramaximal stimulation increases these effects are exaggerated because stimulation of all motor units relies more heavily on charge delivered by multiple contacts which are temporally out-of-phase.

It is not surprising that EMG will be able to measure electrodiagnostic changes in the setting of surgery with nerve manipulation and NCE implantation. However, it is remarkable that the EMG findings are relatively minor and transient, with minimal clinical relevance. It is also crucial to balance these findings with the marked clinical benefits and restored function obtained with these neuroprostheses. While saphenous SNAP and needle EMG in muscles innervated by the femoral nerve showed temporary, subclinical changes, they reflect little functional impact. In fact, this standing neuroprosthesis helped this subject achieve long standing times shortly after full system implantation. This research lays the groundwork for determining which electrodiagnostic findings may be expected, and the time-course over which they may resolve. Observing EMG changes significantly beyond our current findings may suggest that NCEs are causing ongoing irritation or nerve compromise. Based on the severity of the changes, the clinical course and medical management of implant recipients can be adjusted, and NCEs removed or replaced accordingly. While we reached these conclusions with a motor neuroprosthesis, they can be further applied to prostheses modulating other somatic nerves and functions in the PNS, such as pain and sensation.
3.6 Conclusion

This is the first human trial reporting the year-long electrophysiological changes and rehabilitative improvements in a subject able to exercise his lower extremities and stand 6 years after SCI. The robust and increasing femoral motor responses, in conjunction with the minor EMG signs of neurapraxia and irritation perioperatively, establish that the C-FINEs were safely implanted on the proximal femoral nerve trunks above branches to hip flexors. This is the first human trial reporting nerve health following chronic NCE implantation this far proximally on the femoral nerve. Hip flexion and the proximity of the C-FINEs to the inguinal ligaments did not result in entrapment mononeuropathy. Hence, this protocol and NCE design may be extrapolated to other locations near highly mobile joints and possible sites of nerve entrapment to generalize the results.
CHAPTER 4

Intraoperative stimulated responses predict chronic performance of 8-contact composite flat interface nerve electrodes on human femoral nerves

The content of this chapter is based on the following manuscript being prepared for resubmission:


4.1 Abstract

Background. Selective peripheral nerve cuff electrodes (NCEs) in motor system neuroprostheses can generate isolated muscle movements and enhance surgical efficiency by accessing multiple muscles from a single location on a proximal nerve trunk. Intraoperative evaluation of NCE selectivity and performance is necessary to determine connections to implanted pulse generators (IPGs) and for determining post-implant capability. Intraoperative decisions can be critical when it is assumed that the intraoperative response represents the chronic NCE system performance. Yet, perioperative changes to motor responses of NCEs have not been documented over intervals of days or weeks. Knowing how recruitment properties of NCEs change over time is necessary to predict chronic performance based on intraoperative observations, optimize placement relative to nerve branches to antagonistic muscles, select the most desirable subset of contacts to connect to an IPG, and guide post-operative recovery and exercise programs.
Methods. 8-Contact composite flat interface nerve electrodes (C-FINEs) were implanted bilaterally on the proximal femoral nerves of a male volunteer (age 25, 5-years post-injury) with SCI (C5 ASIA Impairment Scale C) involving complete loss of volitional knee extension. This allowed for access to knee extensors and hip flexors with a single NCE bilaterally. Intraoperatively and at 5 time points postoperatively over 6 months, charge thresholds, isometric twitch EMG, and moment recruitment selectivity at the knee and hip were measured via temporary percutaneous leads connected to all 16 contacts.

Results. Mean charge threshold was lowest during implantation averaging 9.7 nC ± 1.7 (SEM), peaked around 2 months at 45.8 nC ± 6.9 (SEM), and then declined over the following 4 months to 27.8 nC ± 4.4 (SEM). Five contacts in each C-FINE were selective for knee extension and 3 generated hip flexion. Three of the 5 knee extension contacts activated independent populations of quadriceps motor units with an average maximum moment of 0.83 Nm/kg. Recruitment patterns stabilized 1 to 3 weeks postoperatively and did not change thereafter. Fourteen of 16 contacts recruited the same muscles 6-months postoperatively as they did at implantation. Changes in recruitment in the remaining 2 contacts only involved muscles activated by adjacent contacts.

Conclusions. The C-FINEs in this first-in-man application were sufficiently selective to separate knee extension from hip flexion and to isolate 3 independent populations of knee extensors, each at levels sufficient for standing (> 0.135 Nm/kg). Intraoperative measurements predicted chronic outcomes for 87.5% of contacts and recruitment on all contacts stabilized within 3 weeks. Intraoperative measurements can guide channel
selection and determine the viability of proximal NCE placement. Exercise and rehabilitation with these NCEs can commence as early as 3 weeks postoperatively.

### 4.2 Introduction

Surgically implanted neuroprostheses can restore motor function to individuals with spinal cord injury (SCI) by exciting the peripheral motor nerves via electrical stimulation to elicit contractions of the appropriate paralyzed muscles [2], [90], [96]–[99]. Non-penetrating nerve cuff electrodes (NCEs) that maintain intimate contact with the epineurium have advantages over muscle-based intramuscular or epimysial electrodes in implantable motor system neuroprostheses [29], [46], including strong and chronically-stable recruitment, improved functional outcomes, accelerated progress through rehabilitation and low failure rates [2], [47]. NCEs can also improve the surgical efficiency of clinical deployment of implanted neuroprostheses since multiple muscular targets can be accessed through a single incision with a selective multicontact electrode, compared to several incisions for multiple muscle-based electrodes. These advantages grow as NCE designs evolve to include higher contact densities to improve selectivity and activate functionally distinct muscles or individual fascicles within a nerve [42], and are constructed to be appropriate for anatomically constricted locations typical of proximal nerve trunks with high fascicle counts serving multiple muscles [141]. However, closer contact placement increases the chance of changes in recruitment properties with minor changes of the relative position of the NCE to the underlying fascicles. It is also unclear to what extent recruitment properties can be expected to change from implantation through the first
several weeks postoperatively, and when in this perioperative period responses can be expected to fully stabilize.

Stimulation charge thresholds are an indicator of nerve-cuff interactions. Stable stimulation thresholds and consistent primary function of each contact are important to implementing a motor system neuroprosthesis. The thresholds of multicontact peripheral nerve interfaces stabilize over the course of weeks or months after implantation as healing and encapsulation progress and inflammation resolves [2], [10], [72]. Thresholds and recruitment properties then remain consistent over time for reliable chronic neuroprosthesis performance [9], [47]. However, the methods to assess recruitment properties and selectivity often differ from implantation to follow-up, making the extent of any acute changes to selectivity difficult to appreciate. Intraoperatively, recruitment selectivity can be efficiently quantified through needle electromyography (EMG) without the need for additional transducers in the surgical field [43], [44], [82], while postoperatively recruitment is more often evaluated by observation or measurement of resulting joint moments with a dynamometer or custom mechanical instrumentation [47], [103]. Thus, it is unclear if channel selection can be optimized based on intraoperative measurements and if viability of NCE placement on a proximal nerve trunk can be verified intraoperatively. It is also unclear how long of a recovery period is required after NCE implantation to ensure stable responses.

The long-term clinical utility of a neuroprosthesis depends on the consistency and stability of stimulated responses. Numerous studies have demonstrated the functional stability of NCEs for the period of about two months to several years following implantation for both motor [47], [72], [103] and sensory [9], [10] neuroprostheses in both
the upper and lower extremities. However, the most likely time for recruitment properties to change is during the first several weeks post-implantation, before the electrode has been encapsulated and the chances of movement of the stimulating contact relative to the nerve is greatest. For example, recruitment selectivity during the first two months after implantation of intramuscular electrodes do not reliably predict long-term functional responses [29]. There is limited data regarding the stability of NCEs during the immediate perioperative period to indicate the extent to which selectivity changes from implantation through chronic use or the time course over which responses stabilize. One study [72] compared stability intraoperatively and at 5 weeks through 2 years postoperatively of 3 contacts on a 4-contact spiral NCE on the radial nerve. However, perioperative stability has not been explored in more challenging implant locations, or with reshaping NCEs with higher contact densities. The proximal femoral nerve near the inguinal ligament, for example, may be a significantly more challenging testbed for NCE stability because of the nerve’s potential for bending and moving as the hip flexes and extends [48], [49], [57], which may result in stresses on an NCE not seen elsewhere that may change its recruitment properties. Reshaping electrodes are not round in cross section [42], [141] and may respond differently to external forces than a round NCE. Furthermore, previous studies do not detail changes in the responses of an NCE in a motor neuroprostheses in the first week to 5 weeks postoperatively to elucidate when responses stabilize.

Predicting the recruitment on each contact of an NCE becomes more important as demands for higher selectivity increase. NCEs with a higher number of contacts improve selectivity. However, implanted pulse generators (IPG) are often limited in the number of output channels available for stimulation. The function of some contacts on a high density
NCE may be redundant, and connecting duplicate channels to an IPG may be inefficient and preclude assignment to muscles that may further extend or enhance functional outcome. Intraoperative testing of stimulation can identify the most useful channels to be attached to the IPG, but only if the recruitment and selectivity intraoperatively represent the chronic performance of the electrode. By understanding the extent of recruitment changes on the contacts of an NCE, decisions can be made at implantation to optimize the assignment of the relatively limited number of output channels of currently available IPGs.

Predicting chronic recruitment of NCE contacts also implies that intraoperative measurements can help determine the viability of proximal NCE placement. This can determine whether an electrode will be placed proximally on a nerve trunk or if selectivity limitations require implantation distal to antagonist branches. Understanding the time course over which responses stabilize can also help guide the postoperative recovery after NCE implantation and helps determine when a recipient can begin exercising and/or how long to restrict physical activity that may affect encapsulation and electrode performance.

Lower extremity neuroprostheses for standing and stepping after paralysis are a good example of the importance of understanding the acute stability of stimulated responses of multicontact NCEs, and predicting chronic performance from intraoperative observations. The proximal femoral nerve trunk, near the inguinal ligament, contains fascicles innervating the uniarticular quadriceps muscles (vastus intermedius, medialis

**Figure 4.1:** 8-contact composite flat interface nerve electrode (C-FINE).  
*Left:* C-FINE in open configuration.  
*Right:* C-FINE in closed configuration with sutures installed.
and lateralis) for knee extension, as well as several strong hip flexors (sartorius and rectus femoris). Activating the knee extensors without hip flexion is important for stance phase of gait, and to achieve and maintain a neutral, upright standing posture that maximizes the body weight supported by the legs and minimizes the effort exerted by the upper extremities to remain erect. Conversely, hip flexion without knee extension is required during swing phase of gait to ensure foot-floor clearance and effective forward progression without tripping. A NCE on the proximal femoral nerve can elicit contractions of both the hip flexor and knee extensor muscles, but to optimize clinical outcome it must generate those actions selectively and independently of one another.

Previously, activation of hip flexors has been eliminated in standing neuroprostheses by locating multicontact spiral NCEs on the femoral nerve distal to the branches serving the sartorius and rectus femoris [2]. While adequate for standing, such an approach would be less effective in a neuroprosthesis to achieve effective stepping motions. More recently, simulation studies [80] and intraoperative testing [44] has shown that an 8-contact FINE on the proximal femoral nerve is capable of separately and independently activating knee extensors and hip flexors with a single NCE. Beyond selectively separating movements of different joints from one another, the ability to isolate individual synergists acting at a single joint, such as the vasti, also allows for implementation of advanced stimulation paradigms that involve rotating between the muscles to allow them to rest and offset the effects of fatigue [103].

The purpose of this study is to document the acute and chronic selectivity and stability of the first-in-man implantation of the composite flat interface nerve electrode (C-FINE, Figure 4.1) on the proximal femoral nerve near the inguinal ligament in humans.
This is also the first long-term implantation of an NCE on the femoral nerve proximal to branches to hip flexors. We quantify the selectivity at the level of individual muscles via EMG intraoperatively and postoperatively, and at the level of individual joints via moment recordings perioperatively over the course of weeks, and chronically for up to six months. We further document the stability of charge thresholds, the primary muscle(s) recruited and resulting “functional stability” of recruitment order when stimulating through each contact. Consistent recruitment of the most selective knee extension and hip flexion contacts from implantation through 6 months would indicate that intraoperative measurements could optimize channel selection and verify C-FINE placement. Stable recruitment of each contact within the first month would indicate that exercise with the C-FINE could begin sooner than in previous studies [2], [47] that assumed a 6-week recovery period based only on the time course of the biological healing process.

4.3 Methods

We tracked the stability of muscle recruitment (“functional stability”), threshold (“threshold stability”), and moments over time. EMG and moment recruitment on every C-FINE contact were recorded at 6 time points over a 6-month period, starting with EMG recorded during the implantation surgery and ending at implant of a permanent stimulator (Figure 4.2). All procedures took place at the Louis Stokes Cleveland Veterans Affairs Medical Center (LSCVAMC) in Cleveland, OH. The FDA and LSCVAMC Institutional Review Board (IRB) approved the investigation of the chronically implanted C-FINEs under the investigational device exemption IDE#G040214 and the subject consented prior to participation.
EMG was recorded at all 6 time points during 6-month percutaneous phase, including during implantation. Moment was collected at all post-operative time points but was restricted to twitch moments before the third week postoperatively. Percutaneous phase ended when a 16-channel IPG, full standing neuroprosthesis system was implanted. This required a reduction in the number of contacts for knee extension from 8 to 3 bilaterally to allow stimulus channels to be assigned to intramuscular electrodes for hip and trunk extension. 

**Figure 4.2: Experimental timeline.**

EMG was recorded at all 6 time points during 6-month percutaneous phase, including during implantation. Moment was collected at all post-operative time points but was restricted to twitch moments before the third week postoperatively. Percutaneous phase ended when a 16-channel IPG, full standing neuroprosthesis system was implanted. This required a reduction in the number of contacts for knee extension from 8 to 3 bilaterally to allow stimulus channels to be assigned to intramuscular electrodes for hip and trunk extension. 

**4.3.1 Subject and C-FINE Implantation**

We implanted 8-Contact C-FINEs (Figure 4.1) on the proximal bilateral femoral nerves of a male volunteer (age 25, 5-years post-injury) with SCI (C5 AIS C) involving complete loss of volitional knee extension, as verified by clinical needle EMG examination performed two weeks prior to implantation. The C-FINEs had 4 platinum-iridium (PtIr) contacts evenly distributed on each of the top and bottom interior surfaces of the electrode for 8 total contacts, inner lumen dimensions of 10 mm x 1.5 mm. The surgeons exposed the femoral nerves bilaterally and implanted the C-FINEs at the femoral triangle.

Previous 4-contact NCE implantation usually required dissection and placement distal to the femoral nerve branches innervating the hip flexors [2]. The anticipated selectivity of 8-contact C-FINEs [44] justified locating the devices proximal to branches to both the knee extensors and hip flexors on the common nerve trunk. Because of the potential of the femoral nerve to move beneath the inguinal ligament during hip flexion [48], [49], [57], [58], each hip was flexed to near 90° while a surgeon verified that the C-
FINEs did not get pulled against or underneath the ligament. The C-FINEs were positioned with their leads running distally to create a redundant loop for strain relief (Figure 4.3, left) before being passed subcutaneously to proximal connector sites in the abdomen. The strain relief loop reduces the potential for transmitting tethering forces to the devices and nerves during hip movement.

The leads from the C-FINEs were connected to an external stimulator and EMG needles were inserted into muscles innervated by the femoral nerve to record the evoked EMG intraoperatively (Section 2.4). Following EMG recording, the leads from the C-FINEs were passed to spring-and-pin connectors [143] in abdominal connector sites. Helically wound percutaneous leads [144], [145] were connected to each NCE to allow access to all 8 individual contacts within each device. The percutaneous leads were tunneled subcutaneously to a common exit site on the proximal anterolateral thigh. Percutaneous leads were tunneled individually to the exit site and separated by approximately 1 cm (Figure 4.3, right). The percutaneous interfaces were maintained chronically for 6 months and the subject was trained to clean, monitor and re-dress the exit sites independently. During and following implantation, only twitch stimulation was used for 3 weeks to minimize perioperative movement. Tetanic stimulation and take-home exercise were initiated after 3 weeks post implantation.
4.3.2 Percutaneous Stimulation

The 6-month percutaneous phase allowed access to all C-FINE contacts to explore their selectivity and stability. For percutaneous stimulation, a surface return electrode was placed over the left lower quadrant of the abdomen. This position was chosen as it will be the site of the implanted stimulator, and best replicates the expected current flow through the body with the implanted stimulation system.

To stimulate through the C-FINEs, the percutaneous leads were connected to a custom-designed current-controlled stimulator [147] which delivered monopolar, charge-balanced, biphasic pulses with pulse amplitude ranging from 0.1 to 2.0 mA and pulse widths ranging from 1 to 255 µs. Pulse width modulated recruitment curves were collected with either a binary search sampling method as described elsewhere [44] or a proprietary method which samples from the space of available pulse amplitudes and widths\(^3\). Twitch recruitment involved stimulating with a single pulse at a time with at least 1 second between pulses. Tetanic stimulation delivered a train of pulses at 20 Hz.

Three weeks post-surgery, temporal patterns of stimulation [99], [148] reconditioned the strength and endurance of knee extensors and hip flexors. Stimulated contractions elicited via the C-FINE contacts selective for the quadriceps muscles built knee extension strength with high load, low repetition exercises with progressively increasing ankle weights while sitting. High duty-cycle, low load cyclic exercises while supine built endurance. Similar reconditioning paradigms exercised the hip flexors by utilizing C-FINE contacts selective for hip flexion. The subject continued the program

\(^3\) For further details on this “pulse space” sampling method, see Appendix C
throughout the remainder of the 6-month percutaneous phase. After 6 months, an IPG was connected to a subset of the C-FINE contacts [149], [150] and additional intramuscular electrodes were inserted to activate the hip and trunk extensor muscles for standing.

4.3.3 Electromyography (EMG) Recording

Stimulation separately on each contact of the C-FINEs in each leg separately at all measurement intervals elicited twitch EMG responses. Prior to stimulation, a surface reference electrode (2” x 4”, Nicolet-VIASYS, Madison, WI) was placed over the greater trochanter of the contralateral leg. A neurologist specializing in neuromuscular medicine placed monopolar EMG needle electrodes (Nicolet 27 to 28 gauge needles, 13 mm – 50 mm length, Natus Medical Inc., Pleasanton, CA) approximately 1 cm apart into primary knee extensors and hip extensors innervated by the femoral nerve. EMG was recorded independently from the three heads of the vasti (vastus medialis, Figure 4.4: EMG and knee moment recording schematic.

EMG and moment are recorded simultaneously with muscles instrumented with monopolar EMG needles at the approximate locations shown while the subject sits in dynamometer with knee fixed at 20° flexion with distal thigh fixed in place. Anatomy modified from [166].

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VM; vastus intermedius, VI; vastus lateralis, VL), rectus femoris (RF), and sartorius (Sart).

Each pair of EMG needle electrodes was AC-coupled to a differential amplifier (Figure 4.4) with a gain of 325 and passband of 12-2975 Hz (B&L Engineering, Tustin, CA). Programmable amplifiers (1902, Cambridge Electronic Design, CED, Cambridge, UK) further low-pass filtered \( f_{LPF} = 1 \text{ kHz} \) and amplified the signal for a total-system gain of 1155. An A/D DAQ board (BNC-6259, National Instruments, Webster, TX) sampled the signal at 2.5 kHz. Custom MATLAB (MathWorks, Natick, Massachusetts) code interfaced with the programmable amplifiers and the custom, computer-controlled stimulator. EMG responses of each muscle were rectified, integrated over the duration of the M-wave (from 3 ms through up to 30 ms after stimulation), and normalized by the maximum twitch for the muscle following supramaximal stimulation on all contacts.

4.3.4 Moment Recording

At all post-operative measurement intervals, isometric knee twitch moments were measured simultaneously with EMG recordings. The knee was fixed at 20° flexion with the distal thigh fixed to the seat of a robotic dynamometer (Biodex, Shirley, NY). To best mimic subject positioning during intraoperative measurements, the subject reclined as close to supine as possible. EMG needles were inserted in target muscles and EMG recorded as previously described. Tetanic moment was also measured from week 3 onward. All twitch \( f_{stim} \leq 1 \text{ Hz} \) and tetanic \( f_{stim} = 20 \text{ Hz} \) moments were recorded using a 6 degree-of-freedom load cell (JR3 Inc., Woodland, CA).

Six months postoperatively, isometric hip flexion moments were measured with the subject reclining as close to supine as possible. Accurate measurement of hip flexion
required locking the knee in constant extension to avoid changes in leg center of mass during stimulation. C-FINE contacts selective for knee extension without hip flexion were identified. Lack of any measureable effect on hip moment with these contacts was verified by both stimulated EMG and stimulated manual muscle testing (sMMT) performed by a trained and experienced physical therapist. Minimal stimulation through these contacts was used to maintain the knee in extension while the hip was fixed at 35° flexion in the dynamometer. To measure hip flexion generated by the remaining contacts, stimulation was delivered through each contact at 20 Hz for approximately 3 seconds as described elsewhere [47]. Pulse widths were chosen to cover evenly the range of available pulse widths of the external stimulator (50 µs, 100 µs, 150 µs, 200 µs, and 255 µs). Isometric knee moments collected at the same visit at matching pulse amplitudes and widths were used to generate recruitment curves in hip vs. knee moment space.

4.3.5 Evaluation of C-FINE Thresholds, Selectivity, and Stability

At each time point recruitment charge thresholds and recruitment order of muscles for each contact were determined from rectified, integrated, and normalized EMG. Stimulation threshold charges were calculated as follows.

\[ Q_{\theta} = I \times PW_{\theta} \quad \text{Eq. 4.1} \]

The current, \( I \) (either 0.8 mA or 1.4 mA) applied through each contact was chosen to be the minimum current able to reach saturation of the EMG recruitment curves by the maximum pulse width of 255 µs. For each contact, the same current was applied across all time points since changes in current would generate a different threshold charge [153],
Charge threshold stability was tracked over 6 months.

The first muscle(s) recruited for each contact was defined by the first muscle or muscles to generate at least 50% of its maximum value before the onset (<10%) of another muscle, as indicated by EMG. Muscle recruitment order was used to estimate rough estimates of fascicular maps with the assumption that fascicles, or groups of fascicles, which are activated at lower charges are likely closest to the stimulating contact. The first muscle recruited was also used to determine the functional stability of the C-FINEs over 6 months postoperatively compared to intraoperative values. The “primary function” of each contact represents the primary joint(s) at which stimulation through the contact generates moment. This was divided into hip flexion, knee extension, or both (mixed). The primary function of each contact was determined with EMG and verified by grading contractions generating moment at the knee and at the hip with the sMMT.

Subtle changes in charge thresholds over time necessitate a substantial difference in activation charge between different muscles. At minimum, the separation between activating antagonists must be greater than the minimum charge difference of an IPG. In this study, the minimum step in charge was achieved by increasing pulse width by 1 µs. Thus, to measure robust separation of muscle recruitment and quantify stability over time, the separation of primary and secondary recruitment curves was defined as follows.

$$\Delta PW = PW_{P50} - PW_{S10}$$  \hspace{1cm} \text{Eq. 4.2}

$PW$ indicates the pulse width when either the primary muscle(s) recruited achieved 50% muscle activation via EMG ($PW_{P50}$) or when the secondary muscle(s) recruited achieved 10% muscle activation via EMG ($PW_{S10}$). Because tracking stability is a primary outcome
of this study, the secondary muscle(s) recruited could be either an agonist or antagonist of the first muscle(s). Additionally, at all time points, the “primary” muscle was consistently defined as the first muscle activated at 6 months by a given contact, even if different muscles were activated first intraoperatively. Thus, \( \Delta PW \) could be negative intraoperatively if intraoperative measurements did not predict chronic performance.

Selectivity for either synergistic muscles or different heads of a single muscle was examined using moment measurements and overlap techniques described elsewhere [103]. Stimulation was applied through pairs of contacts in the C-FINE with the second contact stimulated within the refractory period (< 2 ms) of the axons activated by the first [167], [168]. If stimulation through both contacts activates the same motor units, they will not generate additional moment when stimulating in pairs compared to individually. Conversely, if they activate independent populations of motor units, the moment generated by stimulation through both contacts together should equal the linear sum of the moment generated by stimulation through each contact individually. Overlap between contacts was defined here as follows from [74].

\[
O_{i,j} = \frac{M_i + M_j - M_{i+j}}{M_{i+j}}
\]  

\text{Eq. 4.3}

\( M_i \) is the moment generated when contact \( i \) is stimulated at pulse width \( PW_i \) and \( M_{i+j} \) is the moment generated when contact \( i \) and \( j \) are stimulated together at their respective pulse widths, \( PW_i \) and \( PW_j \), within 2 ms of each other. To identify the subset of contacts selective exclusively for knee extension, all contacts that generated hip flexion before producing measurable knee extension were eliminated. Among remaining contacts, knee
extensors with low overlap with each other were selected for use in the standing neuroprosthesis.

4.4 Results

4.4.1 Stimulation thresholds are stable over 6 months

Charge thresholds (Figure 4.5) were consistent with those reported for previous NCE implants [2], [47], [72]. Fifteen out of 16 contacts had charge threshold below 100 nC at all time points and the remaining contact’s charge threshold only exceeded 100 nC for a single measurement at 1 month post-surgery. The mean of the charge thresholds also follow an expected trend where the lowest thresholds, averaging 9.7 nC ± 1.7 (SEM) over all contacts, were seen during implantation. These rose postoperatively, and peaked around 2 months at 45.8 nC ± 6.9 (SEM) before declining to 27.8 nC ± 4.4 (SEM) at 6 months.

![Stimulation Charge Thresholds](image)

**Figure 4.5:** Stimulation thresholds over time.
Charge eliciting above-threshold EMG on each contact at each of 6 time points intraoperatively and postoperatively. Error bars on median across all contacts represent SEM. Each contact tested with consistent pulse amplitudes of 0.8 mA or 1.4 mA over time.
4.4.2 EMG shows that C-FINEs selectively recruit knee extensor and hip flexor muscles

Contact 6 of the left C-FINE was selective for vastus intermedius and vastus lateralis intraoperatively and through 6 months postoperatively (Figure 4.6). By the strictest measure [44] this contact was not selective for an individual muscle since both VI and VL were recruited together. However, this contact was functionally selective for a population of uniarticular knee extensors and remained selective for this specific population throughout the study. Of note, the slope of the recruitment curves was steep compared to previous studies [44], [47], [72]. These muscles were maximally recruited before any other muscle was activated significantly (>10% [43]).

The first muscle(s) recruited and the primary joint function of all 16 C-FINE contacts across the right and left legs are summarized in Table 4.1 intraoperatively, at 1 week, and at 3 weeks through 6 months postoperatively. Muscle recruitment order did not change after 3 weeks for any contact. The EMG-based results show that 3 contacts on each

![Figure 4.6: Example EMG Recruitment over Time for Left C-FINE Contact 6. VM, vastus medialis; VI, vastus intermedius; VL, vastus lateralis; RF, rectus femoris; Sart, sartorius. EMG recruitment collected intraoperatively at day 0 (left) and at 3 weeks (middle), and 6 months (right) postoperatively. Shaded areas indicate ±1 standard deviation at each point. Pulse amplitude is 0.8 mA across all time points. Primary function (VI & VL) remains the same over time. Threshold increases in first 3 weeks and is further increased at 6 months.](image-url)
side elicited flexion withdrawal reflex activity and were likely nearest to sensory fascicles.
As verified by sMMT and moment measurements, these 3 contacts function as hip flexors.
Twitch EMG recruitment data also show 2 contacts that exclusively recruited the vasti on
both sides (2 and 3 on the right, and 6 and 7 on the left) at all time points. One additional
contact (4 on the right) recruited rectus femoris along with vastus lateralis, and another
contact (3 on the left) selectively recruited vastus medialis at chronic time points, but not
intraoperatively. The remaining 2 contacts on each side recruited a combination of a hip
flexor (sartorius) and a mixed knee extensor and hip flexor (rectus femoris). Moment
recordings (Figure 4.8), however, indicated that these 2 contacts on each side primarily
generated knee extension with very little (< 0.035 Nm/kg) hip flexion.

The data from Table 4.1 were used to create rough estimates of the fascicular
anatomy of the proximal femoral nerves in this subject (Figure 4.7). These qualitative
renderings estimate where fiber populations may sit relative to one another, but not

Table 4.1: Primary EMG recruitment and function for each contact.
Reflex, a delayed (roughly 30 ms to 50 ms after the M-waves) hip flexor response; VM, vastus
medialis; VI, vastus intermedius; VL, vastus lateralis; Vasti, all 3 vasti; RF, rectus femoris; Sart,
sartorius. Orange shows contacts which had significant functional changes from implant compared
to chronic recruitment. Light orange shows a contact with a change that would not be functionally
significant. Primary function indicates the main function of each contact chronically based on EMG
and confirmed with sMMT.

<table>
<thead>
<tr>
<th>Right</th>
<th>First Muscle(s) Recruited</th>
<th>Primary Function</th>
<th>Left</th>
<th>First Muscle(s) Recruited</th>
<th>Primary Function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 Weeks</td>
<td>1 Week</td>
<td>3+ Weeks</td>
<td></td>
<td>0 Weeks</td>
</tr>
<tr>
<td>1</td>
<td>Reflex</td>
<td>Reflex</td>
<td>Reflex</td>
<td>Hip</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>VM</td>
<td>VM</td>
<td>VM</td>
<td>Knee</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>VI, VM, VM, VL, VM, VL</td>
<td>Knee</td>
<td>3</td>
<td>Sart, VM, VM</td>
<td>Knee</td>
</tr>
<tr>
<td>4</td>
<td>RF, VL</td>
<td>RF, VL</td>
<td>RF, VL</td>
<td>Mixed</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>RF, Sart, RF, Sart, RF, Sart</td>
<td>Mixed</td>
<td>5</td>
<td>RF, Sart</td>
<td>RF, Sart</td>
</tr>
<tr>
<td>6</td>
<td>RF, RF, VI, VI, VL</td>
<td>Knee</td>
<td>6</td>
<td>VI, VL, VI, VL, RF, VI, VL</td>
<td>Knee</td>
</tr>
<tr>
<td>7</td>
<td>Reflex</td>
<td>Reflex</td>
<td>Reflex</td>
<td>Hip</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>Reflex</td>
<td>Reflex</td>
<td>Reflex</td>
<td>Hip</td>
<td>8</td>
</tr>
</tbody>
</table>
absolute location nor size of fascicles within the nerve. The C-FINE and nerve cross sections in this figure are arranged horizontally as they are in the subject’s legs, with sutures facing medially bilaterally. The maps span data obtained intraoperatively, at 1 week, and at 3 weeks through 6 months postoperatively.

4.4.3 Intraoperative measurements predict chronic function of 88% of C-FINE contacts

Changes in the first muscle(s) recruited intraoperatively compared to 1 week and 3 weeks through 6 months postoperatively are summarized in Table 4.1. Possible corresponding shifts in fascicular anatomy that could have caused these changes are illustrated schematically in Figure 4.7. However, any changes in the anatomical location of the fascicles within the C-FINEs over time could not be verified in this study.

Across all time points and the 14 contacts stable intraoperatively through chronic use, ΔPW was significantly (p < 0.05 throughout) higher than 1 µs and averaged 76 µs.

![Figure 4.7: EMG-Estimated Fascicular Anatomy.](image)

Anatomy predicted by EMG recruitment responses to stimulation on each contact. C-FINEs rotated as they are implanted in subject with contact numbers indicated on bottom row. Blue circles in C-FINEs indicate suture side of electrodes. Color code for fascicle groupings at bottom of figure.
There was a large range in ΔPW across all contacts from 7 to 223 µs. ΔPW was larger for contacts on the medial aspect of the C-FINEs compared to those on the lateral aspect (Table 4.2). This is attributable to the clustering of fibers to different muscles on the lateral side of the femoral nerve in this subject (Figure 4.7) making spillover more likely. Additionally, this spillover often involved agonist muscles (such as another head of the vasti), artificially lowering the ΔPW of many contacts from a functional standpoint.

On the 2 contacts which shifted primary muscle recruitment perioperatively, ΔPW involved negative values and thus was not significantly greater than 1 µs (p = 0.10 on the left and p = 0.48 on the right). When including only chronic time points at 3 weeks or longer, ΔPW was significantly (p < 0.05) greater than 1 µs across all 16 contacts, indicating stability on 14 contacts perioperatively and stability on all 16 contacts between 1 and 3 weeks postoperatively.

It was not feasible to record hip and knee moment intraoperatively. Based on EMG, the 2 contacts that had significant shifts in recruitment would likely have been strong knee

### Table 4.2: ΔPW across all contacts and every visit

Results show mean ± SEM across either all visits or across only visits following the perioperative period (after the 1-week time point and onward). Shading indicates when ΔPW is not significantly greater than 1 µs. ΔPW is significantly (p < 0.05) greater than 1 µs for all non-shaded cells and for all contacts from 3 weeks onward.

<table>
<thead>
<tr>
<th>Right</th>
<th>ΔPW (µs) Over Time Period</th>
<th>Left</th>
<th>ΔPW (µs) Over Time Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Visits</td>
<td>3+ Weeks</td>
<td>All Visits</td>
</tr>
<tr>
<td>1</td>
<td>116 ± 23</td>
<td>124 ± 36</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>124 ± 27</td>
<td>97 ± 29</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>21 ± 9</td>
<td>31 ± 8</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>17 ± 4</td>
<td>18 ± 5</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>26 ± 5</td>
<td>20 ± 6</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>0 ± 12</td>
<td>16 ± 4</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>95 ± 18</td>
<td>92 ± 23</td>
<td>7</td>
</tr>
<tr>
<td>8</td>
<td>147 ± 29</td>
<td>160 ± 44</td>
<td>8</td>
</tr>
</tbody>
</table>

There was a large range in ΔPW across all contacts from 7 to 223 µs. ΔPW was larger for contacts on the medial aspect of the C-FINEs compared to those on the lateral aspect (Table 4.2). This is attributable to the clustering of fibers to different muscles on the lateral side of the femoral nerve in this subject (Figure 4.7) making spillover more likely. Additionally, this spillover often involved agonist muscles (such as another head of the vasti), artificially lowering the ΔPW of many contacts from a functional standpoint.
extensors intraoperatively, but they may have caused minor hip flexion. On the right C-
FINE, the 6th contact shifted from rectus femoris to vastus intermedius and lateralis. On the
left C-FINE, the 3rd contact shifted from sartorius and vastus medialis to vastus medialis
alone. Thus, both became more knee selective, but both would have had a strong knee
extensor component intraoperatively as well. Any changes in recruitment order involved
becoming selective for, or losing selectivity for muscles recruited by adjacent contacts. For
instance, contact 6 on the right C-FINE became selective for vastus intermedius and vastus
lateralis which, at implant, were recruited by contact 3 (directly across from contact 6) and
contact 4 (diagonally across from contact 6), respectively. Contact 3 on the left C-FINE
stopped recruiting sartorius alongside vastus medialis and sartorius was consistently
recruited by contacts 4 and 5 (next to and diagonally across from contact 3, respectively).

4.4.4 C-FINEs channels selectively generate functional knee extension or hip flexion
moments

Recruitment curves in weight-normalized, isometric hip and knee moment space at
a single time point (6 months postoperatively) are shown in Figure 4.8. Hip and knee
moments were measured independently at the same pulse amplitudes and pulse widths as
described in Section 2.4. Moments are shown for 1 contact on each side selective for hip
flexion, as well as for 3 contacts on each side selective for knee extension. At knee
extension moments below 0.51 Nm/kg, there was no measureable hip flexion on any of the
knee extensor selective contacts. Moreover, only 1 knee extensor selective contact on each
leg elicited measureable hip flexion before knee extension moments saturated.

For this study, functionally detrimental hip flexion during standing was defined as
0.035 Nm/kg, 10% of the 0.35 Nm/kg hip extension per leg required for the sit-to-stand
transition [161]. This amount of hip flexion would be easily overcome by stronger hip extensors. Selective knee extension without detrimental hip flexion was verified with measurements of isometric moments at the knees and hips. Individually, the 6 contacts selected to be connected to the IPG generated an average of 0.83 Nm/kg knee extension with hip flexion significantly (p < 0.05) below 0.035 Nm/kg hip flexion from every contact with average hip flexion of 0.008 Nm/kg. Standing requires 0.135 Nm/kg of knee extension [160]–[162] while the sit-to-stand transition requires 0.4 Nm/kg [162], [163]. These 6 knee extensor selective contacts all exceeded both these values with no hip flexion.

The contacts primarily responsible for eliciting hip flexion also generated knee flexion which is useful during stepping. Slow gait requires about 0.25 Nm/kg and normal gait requires about 0.4 Nm/kg of hip flexion [169], [170]. One contact on the right C-FINE achieved hip flexion of 0.49 Nm/kg with 0.12 Nm/kg of knee flexion. Hip flexion was significantly (p < 0.05) greater than 0.25 Nm/kg with completely absent resultant knee extension (p < 0.05). One contact on the left C-FINE achieved hip flexion of just over 0.25
Nm/kg with 0.17 Nm/kg of knee flexion. Hip flexion was not significantly greater than 0.25 Nm/kg (p = 0.39). However, resultant knee extension was completely absent (p < 0.05). Thus, while hip flexion generated on the right would be sufficient for walking, the hip flexion on the left would potentially need to be supplemented with stimulation of other muscles or a compensatory gain pattern. Additionally, there are functional implications because hip flexion moment was at least partially augment by reflexive activity (detailed in Section 4.5).

4.4.5 Each C-FINE isolated 3 independent populations of knee extensors

Each implanted C-FINE had 5 contacts which were selective for knee extensors and all generated insignificant hip flexion moments (<0.035 Nm/kg). It is unnecessary to connect contacts with redundant functions to an implanted neuroprosthesis. However, EMG results (Table 4.1) showed that, across these 5 contacts in each C-FINE, separate knee extensor muscles were recruited, including distinct vasti, as well as rectus femoris. By stimulating each of these 5 contacts alone and in pairs, 3 contacts per C-FINE were identified that produced significant knee extension moments with low overlap (Table 4.3) with one another, according to Eq. 4.3. The tetanic isometric moments of each of these contacts, activated both individually and in combination, are shown in Figure 4.9. Stimulation combinations include all pairs of contacts and all 3 contacts together.

Of the 3 low-overlap knee extension contacts on each C-FINE, stimulation through all pairs of contacts generated significantly (p < 0.01) higher moments than stimulation through a single contact by an average of 80% across all contacts and pairs. This was true even at the relatively high moment of 0.3 Nm/kg generated via stimulation through
individual contacts. On the right, stimulation through 3 contacts generated significantly higher moment than any pair of contacts. On the left, stimulation through 3 contacts generated significantly higher moment than 2 of the pairs but the increase in moment was not significant compared to the pair of non-adjacent contacts (5 & 7, p = 0.07) (Figure 4.9). This confirms that substantial independent motor unit pools were recruited by individual contacts.

Table 4.3 quantifies the amount of overlap between sets of contacts according to Eq. 4.3. Between pairs of contacts, overlap never exceeded 23%. Contacts separated by another contact (2 and 4 on the right and 5 and 7 on the left) had very low (<1%) overlap whereas contact pairs without another contact in-between averaged 15%. These overlaps are all less than the amount of overlap (25%) previously shown low enough to maintain quiet standing with fatigue-delaying paradigms [74]. This was achieved with moments for individual contacts that each, independently, produced more than twice the moment.
required for quiet standing (0.135 Nm/kg). Moreover, any pair of these contacts bilaterally generated the knee extension moment required for the sit-to-stand transition (0.4 Nm/kg) with little activation of muscles activated by the third contact on each side. With all 3 contacts together, overlap was 21% on the right and 29% on the left. At these stimulation intensities on all 3 contacts, the right leg generated more than double the knee extension moment required for the sit-to-stand transition.

### 4.5 Discussion

These data indicate that chronically implanted C-FINEs on the bilateral proximal femoral nerves produce stable and consistent charge thresholds across all contacts, indicating stable and effective nerve-cuff interactions. During this 6-month percutaneous study, 1 selective hip flexion contact and 3 selective knee extension contacts were chosen as ideal and non-redundant for a standing and stepping neuroprosthesis. The 3 selective knee extensor contacts provided low overlap with each other while individually generating double the moment required to maintain standing [160]–[162]. Between pairs of contacts, overlap did not exceed 23%, below the 25% overlap which has previously been employed to achieve standing in excess of 10 minutes with multiple fatigue delaying stimulation paradigms [74]. These data were confirmed by repeated EMG and moment measurements and reaffirmed that an 8-contact C-FINE is selective enough to separate hip flexion from

### Table 4.3: Overlap ratio of contact pairs and all 3 contacts per leg.

Overlap ranges from 0 to 100% with 0 indicating no overlap and 100% indicating redundant function on multiple contacts.

<table>
<thead>
<tr>
<th>Right Contacts</th>
<th>Overlap (%)</th>
<th>Left Contacts</th>
<th>Overlap (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 + 4</td>
<td>1</td>
<td>3 + 5</td>
<td>23</td>
</tr>
<tr>
<td>2 + 6</td>
<td>1</td>
<td>3 + 7</td>
<td>17</td>
</tr>
<tr>
<td>4 + 6</td>
<td>19</td>
<td>5 + 7</td>
<td>1</td>
</tr>
<tr>
<td>2 + 4 + 6</td>
<td>21</td>
<td>3 + 5 + 7</td>
<td>29</td>
</tr>
</tbody>
</table>
knee extension, and to isolate 3 distinct populations of knee extensor motor units when implanted at the proximal femoral nerve. The functional response at all 16 contacts had stabilized after 1 to 3 weeks postoperatively. The primary muscle(s) recruited has remained the same for 14 out of 16 contacts from implantation through 6 months of follow-up. Moreover, intraoperative measurements would have predicted contact 3 on the right and contact 6 on the left as ideal for knee extension, if limited to 1 contact per C-FINE. These remained strong and selective knee extensors through 6 months (Figure 4.6 for left C-FINE). This suggests that long-term performance of implanted NCEs can be predicted at the time of implantation, allowing for optimization of IPG connections without the need for a percutaneous phase.

We expect that there would be some variability of recruitment selectivity between C-FINE recipients due to anatomical variations in fascicular anatomy [50]. However, 8-contact reshaping NCEs would generally be expected to be able to separate knee extensors and hip flexors [44], [80]. This study shows the first evidence of this selectivity in 2 chronically implanted C-FINEs in a human subject. More subjects were not included in this initial study for several reasons. The implant location is novel for a long-term NCE implant and this represents the first implantation of C-FINEs in man. Moreover, this study primarily explored NCE stability in the days, weeks, and months postoperatively. With current IPG channel limitations, this required a 6-month percutaneous phase along with 5 time-consuming postoperative visits from the C-FINE recipient. The percutaneous leads produced only intermittent, mild erythema in this volunteer (Figure 4.3, right) that resolved following cleaning with antiseptic ointment. However, indwelling leads do present a minor risk of infection and require cleaning and bandaging making implanted stimulators
preferable. Based on the conclusions from this study, a repeat study with a time-consuming testing schedule and indwelling percutaneous leads may not be necessary for future implantation of C-FINEs on the femoral nerve.

The contacts shown to be selective for isolated and independent knee extensor muscles (2, 4, and 6 on the right and 3, 5, and 7 on the left) were identified by stimulating all pairs of contacts at different pulse widths [103] and finding the subset which, as a group, produced the lowest overlap. The adjacent pairs of contacts in these subsets sit diagonally to each other, thus maximizing the distance between them. This geometric consideration provides another insight when optimizing the selectivity and chronic performance of a NCE for a motor system neuroprosthesis at the time of implant surgery.

Delivering stimulating current via the 8-contact C-FINE was also able to produce hip flexion moments on both legs sufficient for slow gait (0.25 Nm/kg) and moment on the right leg sufficient for normal cadence gait (0.4 Nm/kg) [169], [170]. However, the strongest hip flexion in this subject was primarily generated by stimulating sensory fascicles causing a flexion withdrawal reflex. These reflexes are known to habituate with repeated activation over time [171] making them less than ideal for use for a stepping neuroprosthesis. This was verified to be the case in this subject as hip flexion moments decreased after repeated stimulation at a rate unlikely to be related to fatigue.

Clinically significant hip flexion moments were not generated by direct activation of sartorius and RF, which together generated about 0.02 Nm/kg of hip flexion. Both simulation [80] and intraoperative [44] data suggest that an 8-contact C-FINE is capable of selectively activating sartorius. EMG results in this study also suggest that at least some motor units in sartorius were strongly recruited bilaterally. Lack of significant hip flexion
moment from sartorius in the presence of measurable EMG recordings may be due to normalization of the EMG signals to the maximum value, which may only represent a small portion of the muscle. The C-FINEs were implanted as far proximal as feasible while remaining distal to the inguinal ligament. However, it is possible that some branches to portions of sartorius were not contained within the C-FINEs due to anatomical variations from person to person [50]. Guaranteeing access to the innervation of sartorius and other hip flexors might require either implantation proximal to the inguinal ligament or the use of multiple NCEs. Lastly, it is possible that the bulk of fibers to sartorius were co-activated with sensory fibers. This would suggest that either the fibers to sartorius are surrounded on both sides by sensory fibers or that they may not lay at the nerve’s epineurium where this non-penetrating NCE could selectively activate them, although this intermingling of fibers is uncommon in the femoral nerve [50]. In the case of non-surface fascicles, either a stiffer reshaping electrode which further elongates the nerve, or an epineurium-penetrating electrode, such as the slowly penetrating interfascicular nerve electrode (SPINE) [31] would be required for selective activation.

Inconsistencies between EMG and moment measurements demonstrate the need for an intraoperative algorithm incorporating several measurements. Measuring moments intraoperatively with either load cells or sMMT would verify the clinical significance of any EMG results and obviate the source of this artefact. The sensitivity to low stimulation currents also indicates that a contact is close to a target group of fascicles and this sensitivity is important in selecting contacts. Lastly, NCE geometry itself may indicate which contacts to connect: those separated spatially are more likely to activate different populations of axons.
The generation of selective knee extension and hip flexion was achieved here with monopolar stimulation and a distant return. While increasing contact density and reshaping of nerves allows for increased selectivity with NCEs [42], [80], multipolar stimulation paradigms are another method to improve the resolution of axonal recruitment [81]. Modeling suggests that benefits of high contact density and multipolar stimulation on selectivity are additive [172], [173]. Employing multipolar stimulation could further improve selectivity for unique knee extensor channels in future neuroprosthesis recipients. It is also possible that the improved selectivity due to multipolar stimulation would have also allowed for clinically meaningful activation of sartorius in this subject. Unfortunately, the IPG implanted after the 6-month percutaneous phase is only capable of monopolar stimulation.

The chronic “stability” of the new C-FINE neural interface was defined in this study in terms of “charge threshold stability”, which has widely been used in the literature [47], [72], [174], and “functional stability” of the recruitment order and joint moment exhibited by stimulating through each contact. We tracked these measures of stability from implantation through 6 months postoperatively. Stability during the first 5-6 weeks postoperatively is not well described anywhere in the literature, especially regarding the stability of recruitment order and function of NCE contacts in humans. The C-FINE stimulation charge thresholds rose after implantation to peak at 2 months and then continued to decline for the next 4 months. This time course is consistent with previous NCE studies [10], [72] and likely indicates the maturation of encapsulation tissue or resolution of swelling and the inflammatory processes over time. While thresholds have
consistently declined, it is not expected that they will return to intraoperative levels, since these were prior to encapsulation [43], [44].

There are several possible reasons for the significant rise in stimulation charge thresholds in the first several weeks. The likely primary cause of increased thresholds postoperatively compared to intraoperatively is encapsulation tissue which can form between the nerve and NCE [141]. The proximal femoral nerve is a difficult implant location owing to its proximity to the inguinal ligament and nerve movement during hip flexion and rotation. Increased NCE movement relative to the nerve could increase the formation of encapsulation tissue. A loose NCE could also result in larger threshold fluctuations before encapsulation tissue has matured. Further, manipulating a nerve during surgery or perioperative swelling carries a risk of a temporary neurapraxia which can affect nerve conduction for weeks to several months postoperatively [67], [175]. Thresholds declined between 2 and 4 months, consistent with the time course of recovery defining neurapraxia.

A key aim of this study was to understand the consistency of the first muscle(s) recruited by each contact as well as the time course of any functional changes. To help visualize any fascicular shifts, we created fascicle maps based on the recruitment order of the muscles observed for each contact. There are numerous potential nerve and fascicle structural changes which can affect recruitment [176]. We assumed that the primary cause of a change in recruitment order was a change in the physical relationship between a contact and the underlying fascicles. While we cannot infer the absolute location of fascicles or verify the fascicular anatomy in this subject, the predicted anatomical maps presented here can be considered a crude approximation of where groups of fascicles are located relative
to one another based on the measured stimulated output [42]. The gross organization of estimated fascicles is consistent with findings from cadaveric studies [50], which reported medial sensory fibers and lateral hip flexors. These features are shared across the right and left legs of this subject. These fascicles are expected to be near the edge of the femoral nerve trunk since their branches are generally the first to exit [50].

Out of 16 contacts, 14 maintained the same primary recruitment order and function from implantation through 6 months postoperatively. Furthermore, after only 1 to 3 weeks, all contacts assumed the primary and secondary muscle recruitment order that they maintained through 6 months postoperatively. The 1 to 3-week stabilization of the recruitment curves indicates that the C-FINE is securely encapsulated and unlikely to be dislodged by movement or exercise. This indicates that exercise might be initiated as early as 3 weeks after NCE implantation as opposed to the 6 weeks assumed by previous studies [2], [47]. These data suggest the importance of exploiting intraoperative testing to optimize neuroprosthesis implantation, and imply that intraoperative performance may be a strong indicator of chronic behavior. That is, a C-FINE could be implanted and a small subset of the multiple contacts confidently connected to an IPG with a limited number of independent output channels. However, our experience indicates that EMG testing alone may not be sufficient to optimize the down-selection of C-FINE contacts. Reflexive activity can easily be missed when relying on twitch EMG recording alone, especially if the evoked reflexes do not activate muscles instrumented with recording electrodes or are dampened by anesthesia. Intraoperative sMMT or moment recording is imperative to avoid such false negative during intraoperative decision-making.
While these functional stability results are promising, similar data comparing recruitment intraoperatively and postoperatively is necessary to verify that they apply to other NCE designs or locations, such as the sciatic, tibial, or fibular nerves above the knee. Nevertheless, the proximal femoral nerve is uniquely challenging testbed for NCE stability because of the large excursions it experiences and the stresses that can be applied by the inguinal ligament during hip flexion that are not present in other anatomical locations. These factors have the potential to dislodge the NCE and ultimately alter recruitment patterns acutely or chronically with hip movement. Given the minimal recruitment changes seen in this study, it is likely that recruitment would be as, if not more, stable in less challenging locations distant from moving joins, such as the posterior thigh between the hip and knee.

4.6 Conclusion

We present the first-in-man deployment of C-FINEs and the first long-term implantation of NCEs at the proximal femoral nerve trunk with access to both hip flexors and knee extensors. The 8-contact C-FINEs were sufficiently selective to separate knee extension from hip flexion with 5 out of 8 contacts on each C-FINE selective for knee extension (hip flexion moments significantly below 0.035 Nm/kg). The C-FINEs could selectively activate 3 independent populations of knee extensors with an average maximum moment of 0.83 Nm/kg per contact, sufficient for the sit-to-stand transition (> 0.4 Nm/kg). These data indicate that 8-contact C-FINEs implanted at the proximal femoral nerve can be sufficiently selective for use in standing neuroprostheses.
We also established the stability of recruitment order of NCE contacts. The C-FINEs demonstrated threshold and functional stability despite close proximity to the inguinal ligament. The charge thresholds averaged 9.7 nC ± 1.7 (SEM) at implant, 45.8 nC ± 6.9 (SEM) at 2 months, and 27.8 nC ± 4.4 (SEM) at 6 months. These were consistent with levels from previous NCE implants. Functionally, stimulated responses from 14 out of 16 contacts were the same at implant as they were 6 months postoperatively, with consistently significant separation of recruitment curves. Any changes in recruitment order involved muscles activated by adjacent contacts and responses on all contacts had stabilized by 1 to 3 weeks postoperatively. These stability data indicate that intraoperative measurements can guide channel selection and determine the viability of proximal NCE placement. Exercise and rehabilitation with these NCEs can commence as early as 3 weeks postoperatively.
CHAPTER 5

Open- and closed-loop advanced stimulation paradigms offset the effects of fatigue

5.1 Abstract

Surgically implanted neuroprostheses can restore motor function to individuals with paralysis from upper motor neuron dysfunction by exciting peripheral motor nerves via electrical stimulation. Current standing neuroprostheses employ constant, supramaximal stimulation resulting in rapid fatigue. Improving standing times gives patients greater clinical benefit from weight bearing exercises and functional benefits to activities of daily living. Advanced stimulation paradigms, such as carousel and sum of phase-shifted sinusoids (SOPS), reduce stimulation duty cycle and have shown promise in delaying fatigue and being deployed during standing [74]. This study explores these advanced stimulation paradigms and exploits the selectivity of 8-contact composite flat interface nerve electrodes to implement these paradigms with 3 independent populations of knee extensor motor units. We further introduce closed-loop control of carousel stimulation in the recipient of a standing neuroprosthesis. The data reinforce the fatigue-delaying potential of carousel and SOPS paradigms and their feasibility during standing, as well as demonstrate the ability of closed-loop carousel stimulation to generate significantly higher moments than supramaximal constant stimulation after 16 minutes. The data also support the use of shorter cycling periods on the order of seconds rather than periods on the order of minutes. These results have implications for expected fatigue-
delaying benefits of deploying advanced stimulation paradigms in standing neuroprostheses and provide insight into optimal paradigm parameters and potential closed-loop control methods.

5.2 Introduction

Surgically implanted neuroprostheses can restore motor function to individuals with spinal cord injury (SCI), stroke, and multiple sclerosis by exciting the peripheral motor nerves via electrical stimulation (ES) to elicit contractions of the appropriate paralyzed muscles [2], [90], [96]–[99]. This intervention promises several health benefits, including improved vascularity in stimulated tissues [177], positive cardiovascular effects [178], increase in lean muscle mass [179], decreased blood glucose [180], reduction of pressure ulcers [181], increase in bone density [182], and restored participation in activities of daily living (ADLs) [27], [183].

Current standing neuroprostheses employ constant, supramaximal stimulation [2], [46], [184] resulting in rapid fatigue. This fatigue has several physiological mechanisms including alterations in excitability, loss of metabolites and buildup of waste products, and calcium movement [185], [186]. Beyond these reasons, central nervous system injury increases fatigability through a conversion of muscle to fast glycolytic fibers [187], [188], which begins soon after injury and increases markedly over the first 10 months post-injury [189]. Moreover, ES recruits muscle fibers in the reverse order of physiological recruitment. Instead of recruiting fatigue resistant fibers first and fast-fatiguing fibers for maximal force, ES preferentially recruits fast-fatiguing fibers with larger diameters prior to smaller diameter slow twitch motor units [190]. Worse still, tetanic contractions
resulting from constant stimulation exceeding 50% of maximum [191] actually diminish the natural increase in blood flow observed during physiological contractions and needed for adequate delivery of metabolites and removal of waste products [192], [193]. As a result of these factors, standing time with neuroprostheses are inconsistent between subjects, ranging from minutes to hours [159].

Offsetting the effects of fatigue would allow longer, safer, more consistent use of standing neuroprostheses for ADLs, and the clinical benefits of longer periods of weight bearing which improve with increased intensity and duration [194]. Previous attempts at delaying fatigue through various methods of duty-cycle reduction have shown promise. These methods generally involve sharing a load between independent populations of motor units over time. Such strategies have shown promise in improving FES biking distances [195], and that duty cycles of less than 25% markedly reduce fatigue over 30 minutes [196]. Alternating populations allows for the muscle to reestablish circulation and may have a pumping effect in the proximal vasculature, improving tissue oxygenation and replenishing stores of glycogen and ATP and reducing acidosis [185], [186], [197]. Previous work demonstrated the feasibility of implementing fatigue-delaying paradigms to delay fatigue with duty cycles of 25% and 33%, although implementing the patterns in standing was inconsistent [74]. Two such paradigms are carousel and sum of phase-shifted sinusoids (SOPS). Carousel stimulation cycles stimulation through independent motor populations, activating one population at a time while the others rest [198]. SOPS works in a similar fashion except all populations contribute some fraction of their motor units over time, tracking phase-shifted sinusoidal moments which sum to a constant moment greater than the contribution of any individual population [74].
Implementing these paradigms requires sufficiently selective stimulation to activate independent populations of synergistic motor units. More numerous independent populations allows for either a greater reduction of duty cycle for carousel stimulation or greater increase in summed moment for SOPS. The selectivity of the FINE has been demonstrated with motor responses to stimulation in human subjects at several locations [43], [44]. In Chapter 4, we quantified the high selectivity of 8-contact composite flat interface nerve electrodes (C-FINEs) implanted on the proximal femoral nerves and showed that they can activate several independent populations of knee extensor motor units.

This study exploits the selectivity of 8-contact C-FINEs to access 3 independent populations of knee extensors, and utilizes them to explore the potential to delay fatigue in a chronically implanted recipient of a standing neuroprosthesis. We implement carousel and SOPS paradigms, show their feasibility in standing, and compare their fatigue resistance to each other and standard constant stimulation. This report also describes the implementation of a new, closed-loop method to control carousel stimulation using feedback to adjust stimulus parameters to maintain a constant knee moment. This closed-loop method shows great promise for offsetting the effects of fatigue and maintaining moment output for longer than clinical standing patterns using constant, unchanging stimulation. Finally, we explore the effects of various cycle periods (i.e., time to stimulate through every contact once) on fatigue resistance.
5.3 Methods

This study implemented various fatigue delaying stimulation paradigms in a subject with previously-verified selective stimulation of multiple quadriceps motor unit populations. Initial implementation of these paradigms occurred over a 7-month period. Following this period of exercise, calibration and controller development, we tested the fatigue resistance of the paradigms during 7 multi-day visits over a 10-month period. All procedures took place at the Louis Stokes Cleveland VA Medical Center in Cleveland, OH. The FDA and hospital Institutional Review Board approved the investigation of the chronically implanted C-FINEs under the investigational device exemption IDE#G040214 and the subject consented prior to participation.

5.3.1 Subject and standing system

8-Contact C-FINEs were implanted bilaterally on the proximal femoral nerves of a male volunteer (age 25, 5-years post-injury) with SCI (C5 AIS C) involving loss of volitional knee extension. The C-FINEs had 4 platinum-iridium (PtIr) contacts evenly distributed on each of the top and bottom interior surfaces of the electrode for 8 total contacts, inner lumen dimensions of 10 mm x 1.5 mm. Section 4.3.1 further details the C-FINE and their implantation in this subject.

For 6 months postoperatively, percutaneous leads allowed access to all 16 C-FINE contacts. During this time, exercise via C-FINE stimulation reconditioned strength and endurance of knee extensor muscles as detailed in Section 4.3.2. Chapter 4 details the optimization and selection of the 3 lowest-overlap knee-specific contacts in each C-FINE. Briefly, selectivity for knee extension was confirmed
with moment and electromyography (EMG) measurements, which reduced the number of appropriate contacts to 10. These contacts were further evaluated for their overlap with each other using moment measurements during near-simultaneous stimulation [74], [103]. At the end of the percutaneous phase, our optimization resulted in the selection of the 3 contacts on each C-FINE that most independently activated independent populations of knee extensor motor units, each beyond levels sufficient for standing, 0.135 Nm/kg [160]–[162].

The implant phase began with the installation of an implanted pulse generator (IPG) [149], [150]. We connected the right C-FINE’s optimized contacts (2, 4, and 6) to the IPG. On the left, a failure at one contact’s connector site prohibited using preferred contact 7. Instead, we connected contacts 3, 5, and 6 to the IPG. Additional intramuscular electrodes activated hip and trunk extensors. With this subset of contacts connected to the IPG, the subject verified that he could stand stably using low-overlap stimulation parameters on only 1 contact per C-FINE. These contacts were then used to initially implement advanced stimulation paradigms. During the following year of the implant phase, the subject completed standing training and repeated fatigue testing.

5.3.2 Moment recording and stimulation paradigms overview

Isometric knee extension moments were measured with the knee fixed at 20° flexion with the distal thigh fixed to the seat of a robotic dynamometer (Biodex, Shirley, NY). To minimize variability, the same practitioner measured knee flexion angle for all tests. A 6 degree-of-freedom load cell (JR3 Inc., Woodland, CA) recorded moment which was subsequently normalized by the subject’s bodyweight. Moment signals were all
filtered by a 1-second moving average filter. For fatigue testing and calculation of fatigue times, the moment signals were further filtered by a 20-second moving average filter since the primary outcome of this study is offsetting the effects of fatigue over a long period of time rather than optimal paradigm tuning.

We implemented carousel and SOPS paradigms without moment feedback (open-loop) and carousel stimulation with moment feedback (closed-loop). Table 5.1 provides details about paradigm parameters. All paradigms utilized 20 Hz tetanic contractions at varying charges on each contact. Figure 5.1 shows examples of pulse widths and resulting individual and combined moments used during the SOPS advanced stimulation paradigms. Figure 5.2 provides examples of the net joint moments and their variability, or “ripple,” during fatigue testing with each paradigm. The ripple index, which should be minimized for stable, comfortable and consistent operation, was defined as the ratio of peak-to-peak moment variations over one period to the average moment at the same time for each paradigm. For “constant” and “standing” patterns this was calculated over 1 minute. We

Table 5.1: Parameters defining take-home and advanced stimulation paradigms.
The parameters defining key aspects of each paradigm discussed in this chapter are outlined below. “T” indicates length of cycle period. The factors we explored with each set of paradigms that most significantly affects fatigue are highlighted in gray. In this chapter, paradigms with “long” cycling periods are highlighted in blue while those with “short” cycling periods are highlighted in green.

<table>
<thead>
<tr>
<th>Class</th>
<th>Paradigm Name</th>
<th>Target Moment per Contact (Nm)</th>
<th>Full Paradigm Moment (Nm)</th>
<th>Duty Cycle</th>
<th>Period (s)</th>
<th>Fatigue Trials (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Take-Home</td>
<td>Standing</td>
<td>Supramaximal</td>
<td>Supramaximal</td>
<td>100%</td>
<td>∞</td>
<td>2</td>
</tr>
<tr>
<td>Open-Loop</td>
<td>Constant</td>
<td>10</td>
<td>30</td>
<td>100%</td>
<td>∞</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Carousel</td>
<td>10</td>
<td>10</td>
<td>33%</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>SOPS</td>
<td>10</td>
<td>15</td>
<td>50%</td>
<td>60</td>
<td>2</td>
</tr>
<tr>
<td>Closed-Loop</td>
<td>PI, T = 90s</td>
<td>15</td>
<td>15</td>
<td>33%</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>PI, T = 30s</td>
<td>15</td>
<td>15</td>
<td>33%</td>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>PI, T = 15s</td>
<td>15</td>
<td>15</td>
<td>33%</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>PI, T = 6s</td>
<td>15</td>
<td>15</td>
<td>33%</td>
<td>6</td>
<td>2</td>
</tr>
</tbody>
</table>

118
compared the fatigability of open-loop carousel to SOPS paradigms, as well as to a moment-matched “constant” stimulation pattern and compared closed-loop carousel stimulation to the subject’s heuristically defined take-home “standing” pattern. Common outcomes included time to reach 0.135 Nm/kg ($t_{0.135}$), the moment required to maintain quiet standing [160]–[162], time before moment declines to 50% of peak moment ($t_{50}$), and moment after 30 minutes of fatigue testing ($M_{30}$).

5.3.3  Open-loop carousel and SOPS

Both carousel and SOPS paradigms aim to delay the onset of fatigue by increasing the duty cycle for independent populations of motor units, which should allow for them to rest and recover and improve circulation to those relaxed parts of the muscles.

Carousel reduces duty cycle by stimulating through 1 contact at a time [198] and regularly cycling between contacts. This can be implemented with a ramp to ease transitions between contacts [74], although a ramp was not used in this study because creating a ramp requires a reliable recruitment curve, which may change as muscles fatigue.
Three contacts per C-FINE offered sufficiently low overlap with one another to use during carousel stimulation. The resultant moment is the same as that of each individual contact. Stimulus pulse widths were always tuned to values that generated 10 Nm of moment independently for each contact. In contrast, SOPS stimulation achieves constant moment output by generating phase-shifted sinusoidal moments on different contacts [74]. An example is shown in Figure 5.1 using SOPS with 3 contacts. In this study, we targeted evenly phase-shifted minute-long sinusoidal moment traces ranging from 0 to 10 Nm. Pulse widths for each contact were derived by inverting recruitment curves for each contact to estimate the values which would generate these sinusoidal profiles.

Carousel stimulation reduces duty cycle in direct proportion to the number of contacts ($n$) used (1:$n$-1, or in the case of 3 contacts 1:2 “On” vs. “Off”), while the net knee moment is constant. The resultant moment is the same as that of the individual contacts. SOPS, on the other hand, results in a duty cycle of 1:1. However, the net resulting moment is larger than that generated by any individual contact. Because phase-shifted sinusoids

\[ \text{Figure 5.2: Examples of moments generated by stimulation paradigms.} \]
Moment roughly 10 minutes into a fatigue test. Shading indicates full range of data from both legs. Ripple can be appreciated in all of the patterns, but is smallest in open-loop carousel stimulation.
alone cancel out, the resulting moment is a sun of the offset moment generated by each contact according to the following equation [74].

\[ M = \frac{mn}{2} \]

\textbf{Eq. 5.1}

Where \( M \) is the total moment generated by the paradigm, \( m \) is the target peak moment of 1 contact’s sinusoidal moment, and \( n \) is the number of contacts. This increase only holds true if the peak output is the same across all contacts and the overlap between them is low. In this implementation, 3 contacts were used in SOPS resulting in a duty cycle of 50\% with 15 Nm expected paradigm moment. Previous work has implemented carousel and SOPS with short cycling periods of 2 seconds to reduce ripple between contacts [74]. However, other work has indicated that periods longer than this can effectively delay fatigue [199]. In this study we employed a total cycle time of 60 seconds for both paradigms resulting in single-contact periods of 20 seconds for carousel stimulation and 60 seconds for SOPS.

During each testing session, pulse amplitude was fixed at 0.8 mA and the pulse width on each contact was tuned to generate a tetanic moment of 10 Nm while stimulating through each contact individually. Low overlap at these pulse widths was confirmed by stimulating near-simultaneously through pairs of contacts and all 3 contacts at the tuned pulse widths as detailed in \textbf{Chapter 4}. The open-loop advanced stimulation paradigms were tuned based on these pulse widths for each contact.

\subsection*{5.3.4 Constant and standing paradigms}

The “constant” paradigm involved stimulation through all 3 contacts on each C-FINE simultaneously at the levels established to reach 10 Nm on each contact. Thus, if
there was no overlap between contacts, this pattern was expected to initially generate 30 Nm of knee extension.

Clinical standing paradigms in neuroprostheses use constant supramaximal stimulation of knee extensors to ensure that the knees don’t buckle [2], [46], [184]. This pattern of stimulation doesn’t require selective stimulation of independent knee extensor populations or feedback about moment or knee angle. Amplitude for this “standing” pattern was fixed at 0.8 mA on each contact and pulse width was chosen from moment recruitment curves to achieve maximum moment for a given contact.

5.3.5 Closed-loop control of carousel stimulation

Muscle groups fatigue and recover at different rates depending on their muscle fiber type makeup and muscle length [200]. As a result, the performance of even well-tuned open-loop paradigms will degrade over time. With supramaximal stimulation this degradation is masked because the knee is locked in extension. With paradigms which aim for lower target moments, this degradation can manifest in knee buckling. A closed-loop controller can adjust to changes in moment output and respond by increasing stimulation charge. This ensures constant moment between the contacts used in these paradigms and constant moment over time.

Proportional-Integral-Derivative (PID) control is a well-established control algorithm and well suited to maintaining knee extension moment at a set level. PID controllers are easily tuned and their results are reproducible, although they do not directly take into account delay in a system [201], [202]. Even though there is an electromechanical delay from the delivery of stimulation to the generation of muscle force and joint moment
[203], [204], PID control to modulate pulse width was utilized because the primary goal was to compare fatigue with different paradigms rather than investigate the ideal moment-matching controller. Because rapid changes in moment are not expected during a fatigue test, the derivative gain was set to 0 and a PI controller [205], [206] was employed for simplicity (Figure 5.3). The PI controller was tuned by with a step response to supramaximal stimulation on 1 channel and applied to modulate pulse width on all 3 channels. Figure 5.4 shows an example of PI-controlled carousel with a 15-second period.

Because the standing pattern produced moments exceeding 0.135 Nm/kg at the end of 30-minutes fatigue tests, the PI controller used a target moment of 15 Nm or 0.28 Nm/kg, more than double standing requirements. The performance with PI-controlled carousel stimulation was graded by the amount of time before the controller was unable to maintain the target moment at 255 μs, the maximum pulse width available through the IPG. PI-controlled carousel fatigue tests were compared across both legs of the subjects and at multiple time points to mitigate some effects of training and fatigue which may have been present at a single visit. Cycle period of intermittent stimulation affects fatigue but the optimal period is unclear [199]. We explored the interactions of cycle period with fatigue by comparing closed-loop carousel stimulation at periods of 90, 30, 15, and 6 seconds.

**Figure 5.3: PI Controller Diagram.**
Diagram outlines function of PI controller and integration with stimulation paradigms. Each of 3 contacts has its own PI controller and controller in use switches every 1/3 of a period.
representing a fixed 1:2 duty cycle with “On” times of 30, 10, 5 and 2 seconds and “Off” times of 60, 20, 10 and 4 seconds.

5.3.6 Standing and fatigue testing

To be functionally relevant, these fatigue delaying paradigms must generate moments sufficient and stable enough to support a subject’s body weight through the feet. The subject positioned his feet on a set of force plates (AMTI, Watertown MA) measuring right and left leg forces. He performed the sit-to-stand transition with his take-home clinical “standing” pattern then maintained quiet standing with either his take-home pattern or one of the advanced stimulation paradigms. He used a walker wider than the width of the force
plates so the force plates recorded only forces through his feet rather than those transmitted through the walker.

Fatigue testing sessions typically lasted 30 minutes and this study generally limited comparisons between paradigms to 30 minutes even if strong moments could be maintained beyond that time. Fatigue tests comparing PID controlled carousel stimulation with different cycle periods were stopped shortly after the controller failed to maintain target moment regardless of fatigue test duration. All fatigue test results were averaged across both legs.

The effects of fatigue in response long tetanic contractions can be long lasting. Decreases in stimulated moment producing capability largely recover in approximately 18 hours, but have been reported to occasionally take longer [207]. Due to practical time constraints limiting access to the subject, the long term effects of fatigue were minimized.

**Figure 5.5:** Rested and fatigued ripple indices for different stimulation paradigms. All results averaged over both legs. “Rested” is sampled at beginning of fatigue test after reaching peak moment, “Early Fatigue” after 15 minutes and “Later Fatigue” after 28 minutes. “Averaged” results represent the mean over multiple time points during fatigue test (gray bars). Error bars indicate standard deviation across legs and multiple time points during fatigue test.
by allowing at least 18 hours between testing sessions. Furthermore, we did not conduct fatigue testing within the first 7 months of rehabilitation with C-FINEs to allow for reconditioning exercise to stabilize endurance, and fatigue tests comparing different paradigms were spread across multiple subject visits to account for other sources of day-to-day variability [208], [209].

5.4 Results

5.4.1 Advanced stimulation paradigms produce more ripple than constant stimulation

Despite tuning pulse widths immediately prior to fatigue testing, there was significantly (p < 0.015) more ripple in the moment of both open-loop carousel and SOPS paradigms compared to constant stimulation (Figure 5.2). Even when an open-loop cyclic paradigm was well-tuned initially, differing rates of fatigue between separate contacts often caused the development of moment ripple during fatigue testing. This was most evident

![Figure 5.6: Weight supported through subject’s legs while standing with different open-loop stimulation paradigms.](image)

**Left:** Proportion of weight through both legs with take-home standing pattern compared to standing with carousel or SOPS patterns. Error bars indicate standard deviation in one standing session. **Right:** cyclic weight shifting between right and left legs occurred during standing with SOPS.
with SOPS stimulation (Figure 5.5). Eventually, as all of the contacts fatigue, this effect diminishes slightly and the ripple decreases as does the moment elicited by each contact.

Closed-loop carousel stimulation caused significantly more ripple than the standing pattern (Figure 5.5). The quality of ripple differed between open-loop and closed-loop paradigms. The ripple in open-loop paradigms was largely attributed to different moments elicited through different contacts. Closed-loop paradigms matched moment between contacts well but exhibited significant ripple while controlling the moment of individual contacts. However, this effect diminished significantly at higher pulse widths in later fatigue testing, at which point ripple from contact switching is evident (Figure 5.4).

**Figure 5.7:** Averaged fatigue tests of open-loop stimulation paradigms. All traces are averaged over both legs with shading indicating full range of data from both legs. Moments are normalized by subject’s weight and shown on a log-scale ranging from 0.05 to 1 Nm/kg. Horizontal dotted line indicates 0.135 Nm/kg.
5.4.2 Force plate data confirm weight supported through legs when using advanced paradigms to stand

While open-loop advanced stimulation paradigms produce significant ripple in joint moment, the mass of the body acts as a damper when these paradigms are used during standing. One another, the subject can shift weight from one leg to the other as needed (Figure 5.6, Right). The upper extremities of males are estimated to account for roughly 11% of total body mass [210], [211] and are naturally supported, at least partially, by the walker. Using his take-home standing pattern, he supported an average of 94 ± 6% of his bodyweight on his legs. With carousel stimulation the weight supported averaged 91 ± 4% and with SOPS it averaged 93 ± 4% (Figure 5.6, Right).

5.4.3 Open-Loop SOPS delays fatigue compared to constant stimulation

Figure 5.7 compiles fatigue trials across all stimulation paradigms averaged over both legs and several visits and Figure 5.8 summarizes the outcomes of those trials. Averaged across both legs, $M_{30}$ was significantly ($p = .04$, 1-tailed t-test) higher for SOPS than carousel and constant stimulation. Although $t_{0.135}$ and $t_{50}$ appear higher for SOPS than carousel or constant stimulation this difference was not significant ($p = 0.06$). Other
measures indicate that SOPS and carousel appeared to delay fatigue compared to constant stimulation but with the limited number of trials, these differences did not reach statistical significance. Constant stimulation had a \( t_{0.135} \) of \( 5.4 \pm 0.4 \) (SEM) minutes while \( t_{0.135} \) for carousel stimulation was \( 7.4 \pm 1.1 \) (SEM) minutes and \( 22.3 \pm 3.6 \) (SEM) minutes for SOPS. Constant stimulation initially develops higher moment than SOPS or carousel paradigms but rapidly fatigued to 24% of its initial moment over the first 10 minutes. The \( t_{50} \) further suggested that advanced paradigms could delay fatigue compared to constant stimulation, taking \( 2.9 \pm 0.3 \) (SEM) minutes for constant stimulation but \( 10.8 \pm 1.9 \) (SEM) for carousel and \( 22.6 \pm 5.4 \) (SEM) minutes for SOPS.

**Figure 5.9:** Averaged fatigue tests of closed-loop stimulation paradigms.
All traces are averaged over both legs with shading indicating full range of data from both legs. Moments normalized by subject’s weight and shown on a log-scale ranging from 0.05 to 1 Nm/kg. 6-second-period PI carousel controller is shown. Horizontal dotted line indicates 0.135 Nm/kg.
5.4.4 Closed-Loop carousel outperforms open-loop and take-home paradigms

PI-controlled stimulation is shown only with a period of 6 seconds in Figure 5.9 since this period outperformed longer periods (Section 5.4.5). The PI-controlled carousel is compared to only the standing pattern since these patterns are presumably able to recruit nearly all of the quadriceps motor units whereas the open-loop patterns recruit only a subset of this population. $M_{30}$ is significantly ($p < 0.04$, 1-tailed t-test) higher for closed-loop carousel (cycling period of 6 seconds) than for the take-home standing pattern (Figure 5.10). Since the PI-controlled carousel never dropped below 0.135 Nm/kg or to 50% of its initial moment these measures were not tested for statistical significance.

It has been proposed that ATP breakdown and work done are proportional to the force-time integral [212], [213], which is proportional to the moment-time integral in a fixed, isometric contraction. The take-home standing pattern initially produces significantly ($p = 0.01$) higher energy contraction than PI-controlled carousel. However, from 16 through 30 minutes, closed-loop carousel stimulation sustains significantly ($p = 0.02$ at 16 minutes) higher energy contraction than standing-pattern stimulation with the difference widening over time (Figure 5.11). Over the full course of 30 minutes fatigue
trials, closed-loop carousel appeared to do more work than the standing paradigm, but the result was not statistically significant ($p = 0.29$) by 30 minutes.

5.4.5 Shorter cyclic stimulation periods outperform longer periods

PI-controlled carousel stimulation appeared to maintain target moment for longer with shorter cycling periods (Figure 5.12). Cycling periods of 6 seconds (2 seconds per contact) appeared to outperform longer periods, but the difference was not statistically significant. When paired, PI-controlled carousel patterns with a period of 15 seconds or shorter (15 or 6 seconds) held target moments for significantly ($p = 0.02$) longer than those with a period of 30 seconds or longer (30 or 90 seconds, Figure 5.13). On average, the shorter-period paradigms maintained moment 80% longer than longer-period paradigms.

5.5 Discussion

Previous work indicated the potential benefits of implementing carousel and SOPS stimulation in standing neuroprostheses and inconsistently deployed these paradigms during standing [74]. The present study bolsters the feasibility of deploying advanced
stimulation paradigms in standing neuroprostheses to offset the effects of fatigue in a yearlong study following 1 subject. These data suggest that open-loop advanced stimulation paradigms have the potential to delay fatigue although this was only shown statistically for SOPS owing to the low number of repeated trials feasible here. Open-loop advanced stimulation paradigms could be deployed with hardware available in current standing neuroprostheses. However, they still face difficulties with proper tuning and degradation of tuned performance over time as muscles fatigue. The effect of muscle fibers fatiguing at different rates is evident during control of closed-loop stimulation which attempts to maintain a constant moment (Figure 5.4). During fatigue testing with closed-loop paradigms, the pulse widths increased by different amounts on each contact which is not solely explained by unique recruitment curve slopes on different contacts.

NCE selectivity is crucial to implement advanced stimulation paradigms. Recruiting strong independent populations of motor units allows for the biggest reduction in duty cycle for carousel or the biggest increase in paradigm moment for SOPS. Based on

Figure 5.12: PI-Controlled carousel fatigue testing with varying periods. Fatigue testing stopped by controller being unable to reach intended moment or after 30 minutes. PI controller results averaged over both legs at multiple time points to compare efficacy of 6 vs. 15 vs. 30 vs. 90 second periods. Dotted lines indicate when each controller fails to meet target moment. Shading indicates full range of data from both legs.
pre-test tuning to approximately 10 Nm per contact and assuming 0 overlap between contacts, we expected 0.56 Nm/kg peak moment for constant stimulation, 0.21 Nm/kg peak for carousel, and 0.37 Nm/kg for SOPS. The peak moments of the paradigms, averaged between trials on both legs was 0.48 Nm/kg for constant stimulation, 0.26 Ng/kg for carousel, and 0.35 Nm/kg for SOPS. The peak paradigm moments from SOPS and constant stimulation indicate some overlap (Eq. 4.3) was present in the constant stimulation (16.7%) and present but lower in SOPS (5.7%). The peak paradigm moments of carousel stimulation illustrate potentiation between tuning and early fatigue testing.

The ripple with open-loop advanced stimulation paradigms was significant compared to constant stimulation. However, it did not preclude the subject from standing with advanced stimulation paradigms. Closed-loop carousel has potential to greatly reduce ripple caused by unequal moments elicited through each contact. This form of ripple is crucial to address if shorter cycling periods are superior to longer ones, as our data suggest. Part of the ripple in closed-loop carousel stimulation owes to the controller not taking contraction delays into account. Step function responses to supramaximal stimulation indicated approximately 160 ms delay from stimulation to measured moment generation. Dead-time compensation can be integrated into PI controllers through various methods.

Figure 5.13: Short- vs. long- period torque maintenance.
Times in minutes until controller fails to maintain target torque. Error bars represent SEM. Shorter period (6s or 15s) carousel stimulation maintains moment significantly longer (p = 0.015) than longer period (30s or 90s) carousel stimulation. 2 trials at each period (8 total trials).
such as smith predictors [201], [202]. Despite significant ripple, the primary outcome for this study was maintaining moment longer than supramaximal constant stimulation. Moreover, quiet standing without knee buckling was achieved with both carousel and SOPS.

Although low-ripple closed-loop carousel stimulation was not achieved in this study, closed-loop carousel outperformed all other paradigms tested in this study in terms of moment maintained after 30 minutes. Closed-loop control of advanced stimulation paradigms could be applied to standing neuroprostheses using mechanomyography. Mechanomyography muscle contraction sensors proven capable of replicating dynamometer measurements in electrically stimulated muscles in individuals with SCI [214]. Feedback could also be provided by instrumentation measuring knee angle to detect knee buckling or overextension. Adding this instrumentation may allow these paradigms to realize their full potential for clinical benefits of longer weight bearing [194] and functional benefits of longer standing times [27], [183].

The limitations to this study primarily include the variability of fatigue testing with human subjects and time constraints associated with performing repeated fatigue testing. A high number of repeated tests is expensive in terms of time since each test precludes repeated fatigue testing for the remainder of the day. Tetanic moments can vary for several reasons day-to-day and visit-to-visit for myriad reasons. From day to day, imprecise fixation of knee angle (discussed in Appendix B) and day-to-day fatigue exacerbated by the multi-day effects of low frequency stimulation [207]. We partially mitigated these factors through several protocols details. One experienced practitioner fixed the knee was fixed at 20° to minimize variation; at least 18 hours commenced between fatigue tests on
a given leg; and fatigue tests were averaged between both legs. Between visits, the subject underwent reconditioning exercises at home, which can increase fatigue resistance through changes of fast-glycolytic fibers into fast-oxidative fibers [208], [209]. Strength can be affected by several other factors, such as nutrition and general health. We aimed to mitigate the effects of these factors by testing paradigms at multiple time-points and averaging results over time, and by initiating fatigue testing after standing rehabilitation had begun—7 months after initiating exercise with C-FINEs—to minimize the initial increase in strength and fatigue resistance associated with early rehabilitation.

Caution should be employed when making generalizations about the physiology of delaying fatigue based on the closed-loop carousel results. While open-loop paradigms work with a consistent subset of quadriceps motor units, closed-loop patterns increase recruited populations over time. Thus, open-loop paradigms delay fatigue solely by resting populations of motor units. When a duty cycle provides insufficient rest to motor units, moment is no longer maintained. Closed-loop paradigms offset the effects of fatigue both by resting motor unit populations and by spatially recruiting additional motor units when the duty cycle is inadequate to ensure recovery from fatigue. The combination of recovery and spatial recruitment strategies may outperform either approach individually. Spatial recruitment plateaus in effectiveness when stimulation charges are increased to the points where activated motor unit populations are no longer independent. At this point, fatigue is offset solely by the recovery mechanism, which is less effective because high charges introduce overlap between contacts, effectively increasing the duty cycle. This makes closed-loop patterns valuable for functional moment maintenance and improved blood
flow, but they may have fewer physiological implications for studying the delay of fatigue and its mechanisms.

Future work should focus on repeating these fatigue tests across more subjects, secondary measures of fatigue, tuning advanced stimulation paradigm parameters, such as cycling-period length, and implementing closed-loop control in standing neuroprostheses. Paradigm parameters can be further tuned in animal models which avoid much of the inherent variability and time limitations of human subjects. While we found that short periods for cycling advanced stimulation paradigms are superior to longer ones, periods shorter than 6 seconds may offset the effects of fatigue further. Repeating fatigue tests across subjects will further establish the value of fatigue-delaying paradigms. It will also help address some of the inherent variability in fatigue testing with human subjects. For instance, previous work indicated similar fatigue delays between carousel and SOPS [74] compared to constant stimulation. In this study, SOPS appears to delay fatigue significantly longer than carousel or constant stimulation. Part of this may be explained by the higher paradigm moment available to SOPS compared to carousel. The $t_{50}$ for SOPS appeared to be twice as large as for carousel, however this was not significantly significant. Testing these paradigms in more subjects may shed more light on the inherent performance of SOPS compared to carousel.

The subject in this study was able to stand with his take-home standing pattern for 30 minutes and was limited by orthostatic and cardiovascular symptoms of fatigue. When standing with advanced stimulation paradigms, his breathing appeared less labored and he was subjectively less light-headed. These effects were not quantified in this study; however, they would be expected given the lower duty cycle and potential pumping actions.
of the advanced stimulation paradigms which could allow greater return of blood systemically. Future work should explore these secondary measures of fatigue, which can affect standing times and perceived effort during standing.

5.6 Conclusion

This study builds upon previous exploration of carousel and SOPS paradigms by exploiting the selectivity of 8-contact C-FINEs to implement these paradigms and introduce closed-loop control of carousel in a standing neuroprosthesis recipient. The data reinforce the potential for open-loop stimulation paradigms to delay fatigue compared to constant stimulation and demonstrate these paradigms’ success during quiet standing. We further demonstrate that PI-controlled carousel stimulation maintains moment significantly longer than open-loop advanced and take-home standing patterns. These data suggest that cycle periods of several seconds offset the effects of fatigue longer than those an order of magnitude longer, although this should be born out with more subjects. Future work should include repeated testing of these paradigms in other subjects, measuring blood flow, lactate and other indicators of fatigue, optimizing paradigm parameters in animal models, and implementation of closed-loop control during standing through proxy measures of knee moment or angle.
CHAPTER 6

Discussion, conclusions, and future directions

6.1 Discussion

The work in this report aims to reduce the perceived invasiveness of NCEs properly designed for their anatomical surroundings by documenting the expected clinical findings pre-, intra-, peri-, and postoperatively to capture both acute and chronic changes. We do this as we introduce and functionally characterize the C-FINE, an NCE designed to be amenable to implantation in a variety of locations, such as near joints or in constrained fascial compartments. The 8-contact C-FINE deployed in this report also demonstrated high enough selectivity to enable implantation on the femoral nerve proximal to hip flexors and knee extensors while maintaining the ability to activate each muscle group separately for stepping and standing, respectively.

We verified C-FINE safety with an animal model in an atypical implant location. While previous work assumed chronic nerve health based on observed neuroprosthesis performance, we proposed additional guidelines for using electrodiagnostics to monitor the chronic health of nerve following NCE implantation. Our rigorous NCE testing and clinical monitoring peri- and postoperatively should instill confidence in the safety of implanted NCEs.

C-FINEs stabilized rapidly on the femoral nerves and chronic responses were generally predictable based on intraoperative responses. However, we provide the first evidence of perioperative changes of NCE recruitment using the same methodology.
intraoperatively and postoperatively. These changes were found to be minor, allaying concerns that the electrodes could change their positions relative to the underlying fascicles or shift relative to the nerve. Although they suggest that a higher contact density makes electrode function more prone to subtle shifts in recruitment, such shifts were observed to be relatively inconsequential and readily mitigated by intelligent selection of the subset of contacts to connect to an IPG.

6.1.1  AIM 1: Verify the safety of C-FINEs in a chronic animal model.

Hypothesis 1.1: C-FINE shells chronically implanted around fore- and hind-limb nerves in cats will not significantly alter motor nerve conduction.

We implanted twelve C-FINE shells in the upper (n = 6) and lower (n = 6) extremities of 3 cats for 3 months. We observed behavior and movement regularly throughout this period and noticed no discernable changes due to the NCEs, although this was not rigorously quantified. Motor nerve conduction through the area of the C-FINE shells was quantified through NCV and CMAP amplitudes prior to implant of the C-FINE shells and again at explant. NCV and CMAP at explant were not significantly different than those at implant (p > 0.15). Functional and electrodiagnostic measures indicate that there was no significant impairment of conduction through the implanted sections of nerves. These findings support the stated hypothesis.
Hypothesis 1.2: C-FINE shells chronically implanted around fore- and hind-limb nerves in cats will not significantly change histology quantified by axon density or relative myelin thickness.

The nerves implanted with C-FINE shells were harvested from the cats after 3 months. Sections of nerve taken proximally to the implant were compared to those underneath the cuff. The histology of these sections was quantified for 2 features of denervation and reinnervation. In the setting of nerve damage, there is often inflammation and potential Wallerian degeneration. If the axons are damaged but able to reinnervate, their myelin sheath does not reform as thickly. Thus, two measures quantified axonal damage: axon density throughout the nerve and the ratio of the axon’s inner diameter to the diameter of the whole fiber, including myelin (the g-ratio). Throughout the nerves there were no significant ($p > 0.14$) differences between either g-ratios or axon densities within or distal to the C-FINE the shells compared to proximally. These finding support the above hypothesis and suggest that the C-FINE design is compatible with physically constrained anatomical locations, as well as that the nerve is highly tolerant to chronic C-FINE implantation.

6.1.2 AIM 2: Monitor the health of femoral nerves chronically implanted and stimulated with C-FINEs through clinical electrodiagnostics

Hypothesis 2.1: C-FINEs on the proximal femoral nerve trunk will not cause significant detrimental effects to nerve health as measured by clinical nerve conduction studies and EMG examination.

Measures of motor nerve conduction showed no significant detrimental effects over the course of this study. MNCVs were always significantly ($p < 0.05$) above 41 m/s and
CMAP amplitudes were always significantly (p < 0.01) above 4 mV. Moreover, CMAP amplitudes and areas were not significantly changed with hips flexed to 90° compared to 0° (by paired two-tailed t-test across all time points, p = 0.06 on the right and p = 0.85 on the left). However, there were temporary appearances of fibrillation potentials in several muscles and sensory responses at the saphenous nerve were unmeasurable bilaterally from 2 to 6 months indicating intra- or peri-operative irritation which eventually resolved. The data generally support this hypothesis and suggest that motor nerve function in terms of its electrophysiology is generally unaltered by the presence of the C-FINE.

**Hypothesis 2.2: Electrodiagnostics will reflect increased muscle fiber recruitment following rehabilitation and stimulated exercise with C-FINEs.**

Axonal damage and dieback would be expected to reduce the amplitude of CMAPs as fewer muscles fibers are recruited by the femoral nerve. In the first two months postoperatively, the appearance of rare fibrillation potentials on needle EMG indicate minor nerve irritation occurring perioperatively. However, these fibrillation potentials resolve and, over the year of this study with rehabilitation and exercise, CMAP amplitudes elicited through the C-FINEs rose significantly (p < 0.02) by 16% averaged across both legs. This corresponded to tetanic moments increasing significantly (p < 0.01) an average of 79% across both legs. Together with measureable increases in thigh circumference and cross-sectional area, these improvements in moment generating capacity reinforce the efficacy of the C-FINE to recondition paralyzed muscles and indicate there was not a significant loss of motor fibers over this yearlong study. Motor fascicles and their associated muscle fibers remained viable and responsive to classic exercise routines.
6.1.3 **AIM 3:** *Quantify the selectivity of muscle recruitment through each contact of C-FINEs on human femoral nerves and track stability from implant through chronic use.*

**Hypothesis 3.1:** The C-FINE will be able to separately stimulate functionally distinct groups of muscles innervated by the femoral nerve generating knee extension moments sufficient for standing.

Previous work [44] defined selectivity on a per-muscle basis, regardless of function. For this report, we considered contact selectivity at the level of the joint. Thus, if multiple knee extensors were recruited together without hip flexors, that contact would be considered selective for a population of knee extensors. Each C-FINE contained contacts which independently generated hip flexion or knee extension. This was verified by collecting knee extension and hip flexion moment produced by each contact. Ten out of 16 contacts generated strong knee extension. The 6 best knee extension contacts individually generated an average extension moment of 0.83 Nm/kg with hip flexion significantly (p < 0.05) below potentially detrimental levels. These knee extension moments are well in excess of the 0.135 Nm/kg required for quiet standing. Among these 6 contacts, both EMG and moment-based refractory-period overlap methods [103] determined that multiple independent populations of knee extensor motor units were recruited which generated significantly (p < 0.05) more moment in pairs compared to individually at levels of 0.3 Nm/kg. This further confirms that the motor unit pools recruited by each individual contact were independent and non-overlapping. Contacts on each C-FINE generated hip flexion sufficient for slow walking (0.25 Nm/kg) with absent knee extension (p < 0.05). However, this was not significantly above 0.25 Nm/kg on the left (p = 0.39), and the strong hip flexion generated by C-FINE stimulation was primarily reflexive. EMG indicated that sartorius
was recruited alongside rectus femoris, although this did not generate strong hip flexion moment (~0.02 Nm/kg). These findings only partially support the hypothesis that functionally distinct (knee extension vs. hip flexion) groups of motor units can be isolated by the C-FINE on the proximal femoral nerve. While clearly distinct and independent motor unit pools in the quadriceps complex could be isolated and maintained over time, direct activation of individual hip flexor muscles and separation of hip flexors from knee extensors were not fully demonstrated.

**Hypothesis 3.2: The optimal contact for a given function will remain the optimal contact from implant through 6 months chronic use.**

The EMG-determined function was the same intraoperatively and postoperatively for 14 out of 16 C-FINE contacts with recruitment curves always significantly (p < 0.05) separated by at least 1 µs. The remaining 2 contacts changed muscle recruitment (recruitment curve separation was not always greater than 1 µs, p > 0.1). However, all changes were subtle, involving recruitment of muscles that adjacent contacts recruited, and all 16 contacts stabilized by 1 to 3 weeks postoperatively. In general, this makes it difficult to choose 1 contact to connect to an IPG at implant. However, if two strong knee extension contacts were selected on each C-FINE, at least one would be a strong knee extensor chronically. Moreover, intraoperative EMG indicated 1 best contact for knee extension on each C-FINE (#3 on the right and #6 on the left) which did remain strong and selective for knee extension. However, EMG alone should not be used for intraoperative decision making. Moment generating capacities must also be assessed, at a minimum through stimulated manual muscle testing. Similarly, avoiding reflexes in important
intraoperatively, which may be difficult due to anesthesia [215]. With these additional measures, the large number of contacts in a high density NCE can be narrowed intraoperatively for connection to an implanted pulse generator. These findings support the hypothesis if additional information besides individual stimulated responses are included in the decision making process.

6.2 Limitations

There are several limitations common to the human studies in this report. Foremost is that we report results from the implantation of a neuroprosthesis in only 1 recipient. Repeating nerve health, selectivity, and stability tests in future C-FINE recipients will bolster the results in this report. The fatigue testing can be replicated in a subset of existing [74] and future neuroprosthesis recipients whose implants have access to independent populations of agonist motor units.

Another limitation which affects many of the chapters in this report is the session-to-session and day-to-day variability in isometric moment recording at 20° knee flexion. This knee angle was chosen to minimize risk of damage to an insensate joint during isometric contraction. However, maximum moment varies with small perturbations away from 20° and measurement techniques are imprecise. These variations were minimized by employing the same researcher to set knee angle across all experiments.

There are additional limitations unique to each chapter and are summarized below.

In Chapter 2, we implanted C-FINE shells without any lead wires or contacts. This does not replicate implementation in a neuroprosthesis. Lead wires, especially could cause
effects on nerve health as they can tether or otherwise pull on the nerve. However, they were excluded to focus testing on the shape and composition of the C-FINEs rather than lead design.

During the balloon pressure testing, the setup was unable to accurately measure 0 mmHg because the bulk of the folded-up deflated balloon was larger than the C-FINE opening. As a result, pressure results at 0 mmHg are inaccurate.

In Chapter 3, all 16 contacts of the C-FINEs were accessible only during the 6-month percutaneous phase, after which 6/16 contacts were chosen for stimulation through an IPG. As a result, moment and thresholds could not be tracked with stimulation through all 16 contacts after 6 months. However, the 6 contacts were specifically selected based on their ability to strongly activate and isolate distinct populations of knee extensors.

In Chapter 4, differences between EMG recordings and hip moment measurements highlight a limitation with EMG as a proxy for moment. The EMG results are normalized and the needles capture the activity of only 1 part of each muscle. Thus, what appears to be a large contraction by normalized EMG may not be clinically significant. For this reason, EMG was limited to determining stability of the C-FINE, a task at which selective muscle recording excels. Additionally, hip flexion was primarily elicited via reflexive activity. This is not important for a standing neuroprosthesis, however, the habituation of hip flexion with repeated stimulation would be problematic for reciprocal stepping of any appreciable distance. Measurement of moment and of any withdrawal reflexes
intraoperatively would lend more confidence that NCE implantation can be guided by intraoperative measurements.

In Chapter 5, the C-FINE recipient was able to stand for approximately 30 minutes and standing times were limited by shortness of breath and symptoms of orthostatic hypotension. Because he could generate knee extension exceeding 0.135 Nm/kg for longer than 30 minutes, the target moment of 0.135 Nm/kg was less clinically meaningful. The 15 Nm that the closed-loop controller aimed to match was arbitrarily chosen for dynamometer testing to simulate operation of the moment maintenance during standing. However, this value was not selected specifically for its relevance for standing with this subject and the results of the tests may not translate directly to a functional outcome. These studies were included to demonstrate the potential benefits of the degree of selectivity afforded by the 8-contact C-FINE. Much additional work is required to translate the fatigue delaying paradigms to functional activities such as standing before their clinical value can be determined. Additionally, because the closed-loop trials were designed to match an arbitrary and pre-determined knee moment, the effects from visit-to-visit and day-to-day variations in moment may be exaggerated. It is likely that matching a moment of 13 or 14 Nm may be sustainable for much longer than matching one at 15 Nm. Yet the preliminary results of this study support the feasibility and suggest potential benefits of closed loop control, and in so doing warrant further research and development directed toward translating it to functional activities such as standing.
6.3 Implications

This report introduces the C-FINE and validates its chronic safety. The C-FINE was designed for implantation in challenging anatomical locations and unlocks several new potential implant locations as targets for a reshaping electrode. These include nerves near joints and in tightly confined areas. This implication is supported both by in-vitro bench testing, chronic animal testing and clinical testing in a human recipient.

In its first human trial, C-FINEs were deployed on the femoral nerve near the hip and close to the inguinal ligament. This alone is significant because of both the novelty of the C-FINE design and the challenging nature of the implant location. This is also the first report to document chronic nerve health electrodiagnostics following implantation of NCEs. These indicated generally healthy nerve but also showed what minor changes could be expected perioperatively in future well-functioning neuroprostheses.

Not only did the C-FINEs maintain nerve health in this challenging location, but they stabilized rapidly – within 3 weeks in this case. We documented their stability in the perioperative period and the weeks postoperatively. We present the first study showing changes in this period of time and that these changes tend to be minor—involving functions of adjacent contacts. These results imply that rehabilitation and reconditioning exercise might be able to be initiated sooner than commonly thought post-implant without compromising recruitment selectivity.

The unprecedented chronic selectivity of the C-FINEs is attributable to it being a reshaping electrode and to its contact density. It is the first electrode to separately activate hip flexion and knee extension on the proximal nerve. Moreover, the C-FINEs were selective enough to activate distinct populations of knee extensors at this location. We
exploited the selectivity of the C-FINEs to demonstrate the promise of fatigue-delaying paradigms. Closed-loop implementation of carousel stimulation successfully maintained higher moments after 30 minutes than even supramaximal stimulation. We use this paradigm to present evidence that shorter cycling periods on the order of seconds rather than minutes are better at offsetting the effects of fatigue. These results have implications for deploying advanced stimulation paradigms in standing neuroprostheses and provide insight into optimal paradigm parameters and potential closed-loop control methods.

While we characterized the C-FINE’s function in a standing motor neuroprosthesis, the C-FINE was designed as an “anatomically-versatile” nerve-based electrode. The implications of its impact on nerve health and speed of stabilization are wide-ranging to various types of current and future neuroprostheses. It is important to note that the particular application (standing) and NCE location (proximal thigh) should be thought of as testbeds for the electrode design and to translate the outcomes of chronic animal studies to advance the functionality of neuroprosthetic interventions. Generalizing the findings of this study will require repetition in humans and verification at each new neural target or clinical application, but the results we observed were consistent from leg-to-leg in the first human C-FINE recipient and within four limbs and three animals in the chronic cat study, lending confidence to extrapolating them to broader indications.

6.4 Future work

The C-FINEs implanted chronically in cats (Chapter 2) were 2 to 11 times stiffer than those that have or that we anticipate will be implanted in humans. This is because stiffness is a function of the length of the stiffening bar and the devices constructed to meet
the dimensions of the nerves of the cat are smaller than those we expect to implant on larger human nerves. While there is established work about the maximum healthy pressures one can exert on a nerve, the minimum pressure needed to reshape a nerve into a more oblong configuration is still unclear. The connective tissue sheaths have an elastic modulus between 2-11 MPa [216]–[218], while the axoplasm approximates a fluid [219]. The reshaping pressure also depends on the surrounding tissues which can exert pressure of their own in addition to pressure exerted by the NCE. The interactions between device dimensions, stiffening bar design, and pressures to align fascicles without compromising blood flow remain to be further investigated.

The human studies in this report should be repeated across other potential neuroprosthesis recipients and at other anatomical locations. We report results of nerve health, selectivity, and stability at the proximal femoral nerve, but other locations may provide faster stabilization or new challenges. The proximal sciatic nerve innervates the hip extensors and ankle musculature and may be an appropriate neural target for a highly selective NCE. When the hip is flexed with knee extended, the sciatic nerve stretches 2-3 cm [220]. These factors provide additional challenges to C-FINE design, selectivity and stability. Novel locations such as the proximal sciatic nerve warrant in-depth analysis to inform electrode configuration and intraoperative testing to verify acute selectivity. A proximal sciatic location would also warrant repeated chronic nerve health monitoring following NCE installation because of the extensive excursion of the nerve, proximity to the bony prominences of the pelvis, and exposure to pressures and shear stresses during activities like sitting and transfers unexperienced at a femoral nerve location.
In Chapter 4 we proposed that intraoperative measurements can guide channel selection in neuroprostheses with the caveat that EMG measurements alone are insufficient. We recommend supplementing these measurements with moment measurements, at least including stimulated manual muscle testing. It’s also crucial to determine any reflexive activity intraoperatively. It is established that isoflurane depresses spinal reflexes to some extent [221], [222]. Some studies report that nociceptive withdrawal reflexes are greatly diminished under gas anesthesia [215]. However, clinical experience has previously indicated that it is possible to generate withdrawal reflexes in response to electrical stimulation under isoflurane anesthesia. Future neuroprosthesis implantation surgeries can clarify how much withdrawal reflexes are damped under isoflurane and what role the depth of anesthesia plays.

The work in Chapter 5 should be refined in animal models and repeated across other neuroprosthesis recipients. Animal work can focus on optimizing paradigm parameters to delay fatigue. Repeating fatigues tests across subjects will further establish the value of these fatigue-delaying paradigms. It will also help address some of the inherent variability in fatigue testing with human subjects. Some of the work in this report could be pooled with a previous fatigue-delaying study [74]. However, no previous work explored the promising closed-loop control of advanced stimulation paradigms. Better methods of tuning the PI controller should be addressed in future work, and the addition of delay compensation should also be investigated to improve controller-induced ripple. Future work should also monitor secondary measures of fatigue, such as blood return and buildup of waste products. This would be especially interesting if closed-loop carousel stimulation is implemented during standing using a proxy feedback for moment, such as
mechanomyography [214]. Lastly, fatigue delaying paradigms should be explored in other activities, such as stimulation-generated over-ground or stationary cycling.

6.5 Conclusions

The studies in this report have both narrow and widely applicable conclusions. We successfully introduced the C-FINE and showed its chronic safety in difficult implant locations in cats. We then showed its anatomic versatility by implanting 8-Contact C-FINEs at a novel location on the proximal femoral nerve. Despite proximity to the inguinal ligament and access to several muscles, these C-FINEs had minimal impact on nerve health and proved selective enough to generate independent knee extension and hip flexion. The functional response of 88% of the C-FINE contacts was predictable intraoperatively and the function of each contact in the C-FINEs stabilized within 3 weeks, despite implantation in an area subject to repeated bending stresses. More broadly, we proposed electrodiagnostic methods to track nerve health chronically which can increase confidence in the safety of NCEs, and identified a timeline indicating that rehabilitation with NCEs can begin 3 weeks after implantation. We also exploited the selectivity of the C-FINEs to demonstrate the feasibility of delaying fatigue with advanced stimulation paradigms. Closed-loop control of the carousel stimulation paradigm was most successful, capable of generating moments exceeding supramaximal stimulation after 30 minutes. These paradigms can be implemented in new as well as existing motor neuroprostheses utilizing multicontact NCEs, provided that they activate independent populations of agonist muscles. Overall, we provide general guidance for NCE deployment, along with 18 months of repeated testing and consistently good clinical results from the first-in-man C-FINEs in
a standing neuroprosthesis, which should reduce the perceived risks of NCE implantation and help guide decision-making intra- and peri-operatively.
APPENDIX A

Histological and immunohistochemical assessment of peripheral nerve damage and regeneration

A.1 Cellular mechanisms of peripheral nerve regeneration

Section 1.2.3 details the etiology and prognosis of peripheral nerve injury, ranging from neurapraxia to neurotmesis. Following these types of injuries, nerve regeneration can occur through different methods, although these mechanisms share key cells and cellular components. Two key cell types involved in nerve repair are Schwann Cells (SCs) and macrophages. SCs are the primary support cell of the PNS, myelinating axons and releasing Nerve Growth Factor (NGF) [223]. The SCs can recruit macrophages responsible for clearing debris and modulating the activity of SCs [224]. Since these mechanisms repair existing architecture of the peripheral nerve, the speed and degree of restored function depend on the extent of damage to the axon, its myelin, and surrounding structures in the endoneurium and perineurium.

Compressive injuries can damage nerves via ischemia following compressed blood vessels with potential contributions from venous stasis and intraneural edema [223]. This type of injury primarily impacts the SCs and myelin sheath of axons, leaving other structures intact. SCs proliferate and dedifferentiate into an immature state. They cease forming myelin and activate systems which break down myelin debris and help preserve axon viability [225]. Following active injury and myelin clearance, mature SCs remyelinate the axons.
Following axonotmesis of up to roughly a quarter of axons, axons are repaired by collateral branching of the intact axons, which sprout to denervated muscles. Sprouts can originate from nodes of Ranvier or the nerve terminal. They begin growing 4 days following injury and continue for roughly 3-6 months.

Following more complete axonal loss, regeneration requires more extensive debris clearance and SC proliferation. When SCs are no longer contacting axons, they halt myelin synthesis and produce neurotrophic factors, such as NGF and ciliary neurotrophic factor (CNTF) to help produce a new population of SCs [226]. SCs also recruit macrophages to clear myelin debris [224], recover cholesterol, and further enhance SC proliferation [227] along endoneurial tubes, if they remain. These tubes are called bands of Büngner, and they guide axons from their proximal location to their target organs [228]. A lack of endoneurial tubes impedes axonal regrowth. Additionally, if the tube is not populated by an axon within 4 months, it will shrink, further impeding recovery [229].

A.2 Current histological methods

Histological methods assessing peripheral nerve damage and regeneration have been the gold standard for most research. They involve chemical fixation of tissue and embedding in paraffin or resin, depending on the thickness of sections. Masson’s trichrome staining can visualize extracellular matrix and surrounding collagen. Osmium tetroxide (OsO₄) preserves the lipids in myelin and allows its visualization, classically with toluidine blue staining. These techniques were applied in Chapter 2 to monitor for nerve damage or regeneration. Newer staining procedures combine staining for nuclei, collagen, and myelin to identify these components simultaneously [230]. These methods allow for visualization
of changes in myelination, axon density and diameter, as well as active and former inflammation.

Following compressive injury, nerve morphology is preserved. However, chronic compression injuries lead to thinner myelination and decreased intermodal length. SC proliferation increases the number of Schmidt-Lanterman incisures (SLIs), pockets of cytoplasm left behind following remyelination [223].

Following axonotmesis, missing axons and/or edema decreases axon density and myelin debris is present. Some active inflammatory processes indicative of nerve damage and repair can be visualized with conventional histological staining techniques. Macrophages are foamy-appearing as they degrade myelin and it is possible to determine which caliber of axons are being degraded from visual inspection of histological samples [231]. The tissue surrounding the nerve may also show hypercellularity from the inflammatory response. As collateral branching occurs, abnormal clustering of axons within the same band of Büngner often can be observed [118]. As axons regenerate, their myelin sheath is thinner resulting in a decreased ratio of myelin to axon diameter (g-ratio), which can also be determined histologically [125], [129].

A.3 Current immunohistochemical methods

Histological fixation and staining techniques permit the quantification of neurapraxia and axonotmesis, but only give a qualitative sense of axon sprouting and regrowth. Immunohistochemistry (IHC) techniques can target proteins specific to different cell types, cell activities, growth factors, and tissue structures. This is especially important when grading the extent of ongoing peripheral nerve damage and regeneration [228].
Several relevant cell types can be distinguished via IHC techniques (Table A.1). Neurons can be identified by antibodies to neurofilaments (NF-200) and neurotubules (β-III tubulin [232]), SCs by antibodies to glial fibrillary acid protein (GFAP) and S-100β, and macrophages by antibodies to MAC387 [231]. Key structures of the axon can be identified as well, such as neurotubules, neurofilaments, and cytoskeleton proteins.

Axonal regrowth can be quantified with antibodies to various axonal products, including, protein gene product 9.5 (PGP9.5) [233], neurofilaments, and S-100β [234]. High counts of SCs are a positive marker of nerve regeneration. Beyond general axonal recovery, proteins expressed by axons differ with fiber maturity, size, and myelination. Their quantification offers deeper insight into nerve regeneration. The extent and maturity of axonal regeneration can be determined by quantifying the number of axons staining for antibodies to growth associated protein 43 (GAP-43) versus NF-200. GAP-43 is prominent in regenerated or immature axons while NF-200 is prominent in mature, thickly myelinated axons [235]. Large neurofilament content generally correlates with larger axon diameters

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen</th>
<th>Cell Type(s), Structure(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGP9.5</td>
<td>Protein Gene Product 9.5</td>
<td>Neurons</td>
</tr>
<tr>
<td>NF-200</td>
<td>Neuronal 200 kD intermediate filament</td>
<td>Neurons (mature)</td>
</tr>
<tr>
<td>GAP-43</td>
<td>Growth associated protein 43</td>
<td>Neurons (immature)</td>
</tr>
<tr>
<td>IB4</td>
<td>Isolectin B4</td>
<td>Neurons (unmyelinated, sensory)</td>
</tr>
<tr>
<td>CGRP</td>
<td>Calcitonin gene-related peptide</td>
<td>Neurons (unmyelinated, sensory)</td>
</tr>
<tr>
<td>PanNF</td>
<td>Neurofilaments</td>
<td>Neurons (neurofilaments)</td>
</tr>
<tr>
<td>β-III tubulin</td>
<td>β-tubulin isotype 3</td>
<td>Neurons (neurotubules)</td>
</tr>
<tr>
<td>Po</td>
<td>Glycoprotein Po</td>
<td>Myelin</td>
</tr>
<tr>
<td>MBP</td>
<td>Myelin basic protein</td>
<td>Myelin</td>
</tr>
<tr>
<td>GFAP</td>
<td>Gial fibrillary acid protein</td>
<td>Schwann Cells</td>
</tr>
<tr>
<td>S-100β</td>
<td>β-chain of S-100 dimer protein</td>
<td>Schwann Cells</td>
</tr>
<tr>
<td>MAC387</td>
<td>Calprotectin protein</td>
<td>Macrophages</td>
</tr>
<tr>
<td>Laminin</td>
<td>Laminin</td>
<td>Extracellular matrix, basal lamina</td>
</tr>
</tbody>
</table>
Studies have quantified the amount of large fibers with antibodies to axonal-neurofilaments (PanNF) to classify axons by fiber diameter [238]. Other studies have used antibodies to calcitonin gene-related peptide (CGRP) and isolectin B4 (IB4) to selectively quantify unmyelinated sensory fibers [239]. Others have stained for antibodies to myelin basic protein (MBP) or to Glycoprotein Po (Po) to quantify the myelination of axons in nerve regeneration [232]. Cytoskeletal components can offer further insight into damage of structures supporting the axons. For instance, laminin is a component of the extracellular matrix and the membrane supporting SC proliferation and axon regeneration. Quantifying laminin offers a prediction of potential nerve regeneration [240], [241].

A.4 Nerve quantification in applied studies

When combined, histological and IHC methods can quantify both nerve damage and regeneration and predict the potential for functional recovery based on surrounding structures and the inflammatory response. From histological quantification, one can determine the number of axons and cells and their morphology in an area of regeneration. From IHC, one can quantify the relative amount of regeneration in that area. This provides deep insights into nerve damage and regeneration when developing novel peripheral nerve interfaces with varying levels of risk to nerve function, including those which penetrate and/or sever the nerve. A combined analysis has been deployed in several studies to track potential nerve damage and regeneration following chronic implantation of both penetrating and regenerative peripheral nerve interfaces [231], [239], [241].

One such study quantified the effects of chronic implantation of a penetrating interface, the Utah slanted electrode array (USEA), chronically implanted in cats [231].
This study quantified fiber density, g-ratio, and fiber distribution with histological methods. Some of these measures were significantly impacted by the implantation of the USEA. Following axonal damage and inflammatory response, they further identified hypercellularity and myelin degradation. To assess axonal and inflammatory effects of theUSEA in the nerve, IHC methods visualized macrophages at the electrode interface, severed axons outside their normal fascicular structure, and actively regenerating axons around the electrode. Together, the addition of IHC techniques helped identify the diversion of axons around the electrode and regeneration of axons severed by the electrode.

A chronic study in rats compared nerve grafts containing SCs expressing various neurotrophic factors [239]. They used histological and IHC methods to quantify graft morphology, fascicular organization, regenerated fiber diameter, proportion of small sensory fibers, myelination, and g-ratio. These changes were reflected in locomotor function of the implanted rats and relied on IHC methods to quantify.

Unlike the responses to penetrating and regenerative peripheral nerve interfaces, the C-FINE shells implanted in Chapter 2 histologically showed preserved axon densities and g-ratios with generally healthy appearing nerve without notable cellular infiltrate. This indicates that the C-FINE shells had minimal impact on nerve health. While IHC methods could have been used to further verify minimal foreign body response, the minimal or absent damage caused by C-FINE shells essentially obviated the need for advanced IHC quantification of nerve regeneration and inflammatory response. Had the impact been greater than minimal, specific IHC methods may have been indicated to definitively identify the source of irritation and progress of regeneration.
APPENDIX B

Day-to-Day variance in isometric moments at “20°”

B.1 Introduction

When measuring absolute isometric moments, variance in moments between experiments can be attributable to several factors affecting day-to-day moment recording. First, there is inconsistent muscle fatigue. The potential for fatigue is exaggerated in individuals with SCI, especially during electrical stimulation of paralyzed muscles [164]. Moreover, even with exercise and rehabilitation the muscles of neuroprosthesis recipients are not well conditioned compared to those under volitional control. The subject is then asked to participate in a multiday visit consisting of 8-hour days of rehabilitation, standing, cycling, and isometric fatigue testing. We expect this contributes significantly to day-to-day moment variations. Additionally, there are technical limitations resulting in imperfect measurement of knee angle. Consistent knee angle is important for recording isometric moment. In this report, isometric knee extension moments were measured at 20° of knee flexion to reduce the risk of injury to an insensate knee joint during tetanic contractions. However, at this angle, isometric knee extension moments are highly sensitive to changes in knee angle. At 20° of knee flexion, moment is about 38% of maximum possible moment, which occurs around 80° knee flexion. If the knee is flexed to 30°, moment rises to roughly 51% of maximum, and if the knee is extended to 10°, the moment falls to 29% of maximum [165]. Thus, changes in 10° from the target knee angle of 20° of flexion can result in a rise from perfectly-measured “20° isometric moment” of 34% or drop of 23%. To reduce
variation, the same practitioner verified subject positioning and knee angle for every experiment. Variance in isometric moments is typically assessed with repeated contractions elicited within minutes of one another. Here, we briefly explore the magnitude of day-to-day changes in isometric moments.

### B.2 Methods

During a multiday visit at approximately 18 months into rehabilitation with C-FINEs, we elicited supramaximal tetanic (20 Hz) contractions on 6 contacts of the C-FINEs. We fixed the knee at 20° flexion in a robotic dynamometer (Biodex, Shirley, NY) and recorded moment with a 6 degree-of-freedom load cell (JR3 Inc., Woodland, CA). On each day, each contraction was held for 3 seconds with 15 seconds rest in-between and stimulation through each contact repeated 3 times.

Standard deviations across all trials (right and left legs) were averaged together by taking the square root of the averaged variance across those trials. The variance between contacts is expected and repeatable since they activate different populations of axons and was not included in this averaged standard deviation.

### B.3 Results and conclusions

The standard deviation across all contacts (Figure B.1) ranged from 3% to 14.5% of the average moment elicited by each contact. Across all contacts, the standard deviation was 11.4% of the average moment generated. The largest day-to-day change in moment on any contact was 27% and occurred between the second and third days.
We recorded the lowest average moments on day 2 of this 3-day experiment. On this day the subject had been fatigued throughout the previous day. Anecdotally, his knees began to buckle during standing much more quickly on day 2 than they typically do. This suggests that fatigue may play a strong role in day-to-day moment variations. However, knee angle cannot be discounted and may have played a role in the rebound of moment from day 2 to 3 alongside recovery from fatigue. While less applicable to standing, isokinetic contractions may be less variable than isometric moments [242]. These results indicate that longer periods of rest may be necessary between fatigue testing and that fewer fatigue tests should be performed at a visit. Consistency of absolute isometric moment can be improved with more reliable fixation of knee angle.

Figure B.1: Day-to-Day isometric moment variations
Error bars indicate standard deviation of average isometric moment across 3 consecutive days of single visit. LCn and RCn indicate left and right C-FINE contact, respectively.
C.1 Abstract

This paper describes a method to efficiently sample EMG recruitment space over a wide range of pulse amplitude ($PA$) and pulse width ($PW$). A gradient based search method is developed to find high information areas of a recruitment surface. This search method is first examined in the context of simulated EMG recruitment data and its ability to sample and subsequently fit Gompertz-Function-inspired surfaces to it. The search method is then used to determine parameters when stimulating through an 8 contact flat interface nerve electrode (FINE). The recorded EMG recruitment data are then used to validate the Gompertz surface fitting method as well as the search method.

C.2 Introduction

The aim of a functional electrical stimulation (FES) system is to restore or improve the function of individuals with neurological compromise, especially those with upper motor neuron (UMN) involvement. FES has been shown to be effective in restoring basic lower extremity motor function in individuals with stroke or spinal cord injury [89]. In the
Past, stimulation at the femoral nerve using the flat interface nerve electrode (FINE) has been shown to be effective in recruiting muscles in a selective fashion [44]. This muscle selectivity is important to stimulate the proper muscles during different phases of gait and also to delay fatigue by alternating stimulation of functionally synergistic muscles [2]. To test the efficacy of nerve cuffs prior to implantation, intraoperative testing has been performed on patients while recording recruitment of muscles using electromyography (EMG) [44]. As the number of contacts on a cuff increases, stimulation can better activate the desired muscles without recruiting unwanted ones that would be counter-productive to the intended movement [80], but it also becomes more time consuming to discover the best stimulation parameters—in this case, pulse amplitude \((PA)\) and pulse width \((PW)\)—to achieve this selectivity. Thus, it is important to intelligently choose which parameter sets to use in intraoperative testing as the number of stimulating contacts increases.

Previous methods to find nerve stimulation parameters generally focus on recording EMG recruitment along one dimension at a time (often modulating \(PW\) at a set \(PA\)) [82]. Here, we examine search methods of stimulation parameters across the full space of both \(PA\) and \(PW\). As well, we explore the validity of fitting Gompertz-Function-inspired surfaces to EMG response data to predict muscle responses to any set of stimulation parameters. These Gompertz surfaces are tested by fitting and validating using separate EMG datasets elicited after bilateral stimulation of proximal femoral nerves. Together, these methods can gather data efficiently to allow for more effective testing of nerve cuffs to be used for restoration of function after UMN injury.
C.3 Methods

The process we explore for efficiently determining nerve stimulation parameters starts with simulating EMG recruitment surfaces. These surfaces are used to test the efficiency of methods to collect data points which provide information about the shape of the surfaces. These points are then validated against the simulated dataset by fitting a surface to the sampled points and calculating the coefficient of determination ($R^2$) over the full simulated space (Matlab, Natick, MA). After determining the efficiency of this process, the search method was used to characterize EMG recruitment over $PA$ and $PW$ during the acute application of a FINE to the bilateral proximal femoral nerves. To validate the surface fitting procedure and thus the search method described below, the EMG recruitment data were randomly divided into separate datasets for surface fitting and testing.

C.3.1 Surface creation

The primary set of simulated EMG recruitment surfaces were modeled as an analogy to a Gompertz function. The Gompertz function is often used to model population growth, for instance, in tumors [243]. It is appealing to use this function to describe the recruitment of a population of axons. As well, the Gompertz function was chosen since it has been shown to fit human EMG recruitment data well along a single dimension (pulse amplitude modulation or pulse width modulation) [244]. The Gompertz surfaces were created based on 5 parameters: $A$, to control overall amplitude, $B$ to control shifting of the curve along $PA$ or $PW$, and three variables ($C_{PA}$, $C_{PW}$, $C_{PAPW}$) to control the rate of rise in each the direction of $PA$, $PW$, and the product of $PA$ and $PW$. The form of the surface follows:
The 5 parameters used in Eq. C.1 were varied independently of each other for each surface created based on separate independent uniform distributions. Some parameters could vary from approximately ten-fold (for instance, $A$) to one-hundred-fold ($C_{PA}$) or more ($B$) so that the surfaces produced would rise at values of $(PA, PW)$ that covered the full space available. The parameters controlling rise were all negative, as was $B$, while $A$ was always positive. The function used a sum of parameters in the second exponent as this was required to control rise of the sigmoid independently along $PA$, $PW$, and their product across the space of $PA$ and $PW$.

To ensure robust behavior with expected EMG signals, noise was added to all simulated surfaces. The added noise was Gaussian with a mean of zero and a standard deviation ranging from 0.01 to 0.2 of the maximum simulated EMG value. To ensure further robustness, search and fit methods were also tested against surfaces that were created only with the assumption that they must be monotonically increasing in $PA$ and $PW$. These surfaces were created by randomly increasing recruitment with each step in $PA$ or $PW$.

C.3.2 Search methods

The pulse space search method aims to isolate areas with a high gradient and to cover all values between a predetermined high (0.9) and low (0.1) thresholds, which are normalized to the highest point on the surface. Thus, density of search points positively correlates to the gradient of the surface. In addition, if there is a large change in recruitment...
values between any two $PA$, $PW$ pairs, the algorithm will explore the recruitment values at $PA$ and $PW$ values between the original $PA$, $PW$ pair.

The search is conducted as follows. First the value of the surface at maximum $PA$ and $PW$ is found. Second, the search starts at the highest value in $PA$ and the lowest value in $PW$. If the surface at this point is below lower threshold, a recruitment curve is searched along $PW$ at the current value in $PA$. The rate of movement along this curve depends on the gradient found from the previous two sampled points such that points are sampled more closely when gradients are higher. This local search stops when all muscles’ EMG data is above upper threshold. Conversely, if the lower threshold was not passed, $PA$ is lowered until the criterion is met and then a curve is found along $PW$ at a lower $PA$. Third, $PA$ is again lowered for another search along $PW$. This search begins at the $PW$ where upper threshold was passed on the previous curve (it is assumed that at lower $PA$ the point will likely be below upper threshold) and occurs both in increasing and decreasing directions to pass upper and lower thresholds, respectively. This repeats until two curves are produced. Fourth, the local searches are repeated in the opposite direction (starting at high $PW$ and low $PA$ to create curves that search along $PW$). **Figure C.1**

**Figure C.1:** Example of Pulse Space Search Method and Resulting Surface Fit on Simulated EMG Recruitment Data.

Blue dots show sampled points in the simulated EMG recruitment surface. Note that the pulse space search method sampled few points in the plateau of this surface.
illustrates an example of the results of this process and shows that points are closer together where the surface rises steeply.

The pulse space search method was compared to a method of collecting samples consisting of a predefined grid over the space of $PA$ and $PW$. This method requires minimal computational overhead but is unable to increase resolution in specific areas as needed.

C.3.3 Surface fitting

Search methods were evaluated by how well they provided points to fit a surface to the simulated EMG data. This was compared using the calculated $R^2$ between the simulated surface and the fitted surface across every defined point (all $PA$ and all $PW$) in the recruitment surface space.

During each fit iteration, the $R^2$ value for the points sampled by the algorithm was calculated. If this $R^2$ value was low, the fit was rerun with randomized initial guess parameters. Through initial testing, it was clear that some surfaces (such as surfaces only assuming random monotonic increase in recruitment) required more control over the dependence of recruitment surface on $PA$ and/or $PW$. Thus, four more parameters were introduced including a parameter on each of $PA$ and $PW$ to control the power to which each value was raised and a parameter on each of $PA$ and $PW$ to control a shift in the value of each variable. Figure C.1 shows an example of a surface fitted using this method.

C.3.4 EMG recording

An 8 contact FINE was used to validate the pulse space search method. Two FINEs were implanted bilaterally around the proximal femoral nerves of a volunteer with motor-
complete spinal cord injury. The nerve cuff electrode was connected to a stimulator which could deliver a monopolar, charge-balanced, biphasic stimulus independently to each contact with a range of $PA$ from 0.1 mA to 5 mA and $PW$ from 1 $\mu$s to 255 $\mu$s. Stimulus pulses were delivered 3 times at each $(PA, PW)$ point. EMG signals from rectus femoris, vastus lateralis, vastus intermedius, vastus medialis, and sartorius were recorded with needle electrodes. The EMG signal was amplified and low pass filtered at 1,000 Hz and sampled at 2,500 Hz. The signal was then rectified and integrated from 3 ms to 40 ms, and this value was normalized by the maximum EMG recording for a given channel. The pulse space search method was utilized to determine which points in $PA$ and $PW$ to sample.

Informed consent was acquired prior to all experimental procedures and all human testing protocols were approved by the Institutional Review Board of MetroHealth Medical Center, Cleveland, OH.

C.3.5 Surface fitting validation

75 EMG Recruitment surfaces were collected using the pulse space search method (5 muscles over 15 contacts). These surfaces were randomly divided into separate data sets so that one was used to fit surfaces to the recruitment data and the other set was used to verify the fit. As well, these 75 surfaces were placed into three groups based on the ratio of the normalized mean to the standard deviation of the signal at a given point (3 samples were taken at each point). This is noted here as low, medium, and high SNR groups.

Goodness of fit was determined using coefficient of determination ($R^2$) of both datasets and also by examining what proportion of surfaces correctly predicted the $PW$ at which EMG threshold (determined as 10% of maximal recruitment) was passed at $PA$ of
0.8 mA and 1.4 mA. The $PW$ threshold predictions were deemed correct if they fell within the range of $PW$ that was found during implantation when binary search along $PW$ at these amplitudes was carried out on the same muscle and contact.

C.4 Results

C.4.1 Fitting surfaces with different sampling methods

Figure C.2 provides an overview of the data initially collected when sampling from and then fitting surfaces to simulated EMG data. Using the pulse space search method on 1 surface at a time achieves a high value of $R^2$ with much fewer points than a search on 12 surfaces at a time. This is expected since there are fewer high-information areas in space to sample. However, this difference disappears quickly as number of surfaces increases such that searching 12 surfaces at a time only requires a small increase in sample size compared to 6 surfaces at a time. Also of note is that a predefined grid method for choosing points is consistently less reliable at fitting a surface with a given number of samples. This is obvious when examining the worst fits from both methods: the 2.5th percentile using the grid method is generally quite low.

Based on these simulated surfaces, using the pulse space search method, about 15 samples can fit a single Gompertz surface with 0.025 and 0.05 standard deviation noise. To classify the recruitment for 12 surfaces at once (stimulating 12 muscles with one electrode), this search method needs about 45 samples. The grid method reaches a plateau at about the same number of samples as the search method on 12 surfaces. However, the mean of the $R^2$ value is about 0.05 lower and the 2.5th percentile is up to 0.5 lower. The grid method also never reaches the same $R^2$ values that the search method does, likely due
to imprecision during surface fitting. It is also of note that this search and fit method is relatively robust to simulated noise (Figure C.2 A and B).

C.4.2 Efficient nerve stimulation parameter selection

During implantation, the pulse space search method was able to characterize EMG recruitment surfaces well using an average of 58 sample points (a range of 29 to 103 over all contacts). This required approximately 1 minute of time to characterize each channel. As well, this could have been achieved with fewer points, since extra sampling was required for validation datasets.

C.4.3 Validation of fitting recruitment surfaces

To validate the previous results which used simulated recruitment surfaces, EMG data from the subject with bilateral proximal femoral nerve cuffs were fit and validated (Figure C.3). The goodness of fit was dependent on the quality of the recorded EMG signal from the muscle being stimulated. For this reason, the 75 recruitment

![Figure C.2: Goodness of Fit of Simulated EMG Recruitment Surfaces Using Space Search and Grid-Based Method.](image)

Coefficient of determination ($R^2$) mean (solid lines) and 2.5th and 97.5th percentiles (dashed lines) vs. sample number. Results are shown for noise added with a standard deviation of 2.5% of signal (a) and 5% of signal (b).
surfaces fit in this study were divided into three groups based on sum of the ratio of normalized mean to standard deviation at each point (SNR in Figure C.3). While a relationship between $R^2$ and SNR is noted, differences are not significant. It is believed they would be with more subjects.

The best $R^2$ values are over 0.8 for the dataset being fit, and over 0.75 for the validation dataset. This does drop as SNR drops, especially in the validation set. This same trend is not noted when the fitted Gompertz surfaces are used to predict the $PW$ at which threshold (10% of maximum) occurs. This is likely due to the Low SNR group of surfaces having high recruitment only at higher $PA$, $PW$ values (thus the average mean over the sample collected is lower and so SNR is lower). This results in the $PW$ range of thresholds being larger and thus easier to predict. There may be a difference between threshold prediction results at $PA$ of 0.8 mA and 1.4 mA but this is not significant in these results.

C.5 Discussion

Based on the initial analysis of fitting simulated muscle recruitment surfaces, fitting a surface to adaptively-chosen (as in the pulse

Figure C.3: Evaluation of Gompertz Surfaces Fit to Experimental EMG Recruitment Data.

EMG data were divided into equally sized groups of 25 surfaces based on the ratio of the normalized mean to standard deviation of signal at a given point (low, medium, high SNR groups). Goodness of fit was evaluated by mean $R^2$ value (a) of both the fit and validation data sets and proportion of predictions of threshold $PW$ (10% of maximum) that fall within the binary searches (b) at different values of $PA$. Error bars represent standard error of the mean.
space search) points presents a promising method of characterizing muscle recruitment as it relates to changes in PA and PW. As well, the pulse space search method works quickly and provides samples to fit recruitment surfaces well. The sampling method is much more efficient at acquiring recruitment data than previous methods involving modulation of a single stimulation parameter at a time. In the amount of time that previous methods characterized two to three curves along PW, this method characterizes a full surface over PA and PW. The method will be faster if validation datasets are not required. In the future, it may also be beneficial to examine possible benefits of weighting certain areas in the PA, PW space more highly than others. These areas would ideally be found by examining many recruitment surfaces and finding areas of significantly elevated or decreased information. As well, future comparison to other sequential sampling methods, such as particle filters, could be fruitful. Surface fitting appears promising but could be improved. More time allowed for computation iterations can give better fitting results. Assuming that recruitment should monotonically increase over PW and PA, it may be possible to weight areas that are presumed to be known (such as high or low plateaus). This may provide more reliable fitting in fewer iterations by ensuring the proper surface shape even if only a few points in the high or low plateaus are sampled.

The analysis performed on EMG data provides strong evidence that recruitment surfaces can be well characterized using Gompertz surfaces. Muscles that are highly recruited by a given contact are fit very well. However, there remains concern with poorer fitting of less-strongly recruited muscles with lower SNRs. In the future, running similar surface fitting validation with results from a patient with a chronically-implanted nerve
electrode (where thousands of data points could be obtained at one time) could be very useful to further confirm the validity of these methods.
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