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

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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 and compound motor action potential 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*.

Keywords

peripheral nerve interfaces; nerve cuff electrodes; nerve health; electrode design; axon morphology; composite electrode; chronic cat

1. 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) [1–6]. Stimulating currents are delivered to the neural targets in these systems by electrodes that are inserted into the muscle tissue near the nerve [7, 8], sutured to the muscle at the nerve entry point [9], or placed around the nerves [10, 11]. 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 [9]. 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 [10] have been deployed in both the upper and lower extremities and maintained stable activation thresholds for several years [12–14]. More recently, NCEs have also been deployed for sensory restoration in amputees [15–17]. In these subjects, NCEs have restored natural perception of touch in the phantom limb at different locations allowing for improved ability to grasp objects [16] with stable functional responses for over 2 years [17]. There are numerous other applications of NCEs in neuroprostheses as well. These include nerve block for pain and spasticity [18–20], bladder control restoration [21, 22], and several others [23–25].

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 a novel NCE design. One requirement for novel neural interface applications is that an NCE must be able to 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 [26–28] with reduced 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 has been demonstrated with motor response to stimulation in human subjects at several locations [29, 30]. 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 [16]. As well, sensory locations from each contact and stimulation thresholds remained stable for multiple years [17].

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 [31–33], several areas of the brachial plexus [34], and others [35], 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 [31, 32] where hip flexion and external rotation can cause the femoral nerve to be sharply angled at 80–90° underneath the inguinal ligament [36, 37]. 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 [38]. 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 [39], pudendal [40], and superior gluteal [41] nerves in the pelvis and gluteal space [42], as well as the axillary nerve in the axilla [34] and several areas of the brachial plexus, which is closely related to several bony structures against which it may be compressed [38, 43]. Clinical experience has also indicated that NCE bulk should be minimized when implanting NCEs in superficial locations [17].

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 and be manufactured using a microfabrication ready process to allow for this method of achieving high contact density [44, 45].

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 C-FINE (figure 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 [46]. This design enables the construction of NCEs with high contact densities, either by hand or by microfabrication techniques [44, 45] for increased spatial resolution. To ensure safety and nerve health, the C-FINE was designed to keep transient circumferential pressure less than the acute [47, 48] and sustained [49, 50] 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 [51, 52]. 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 [11, 53–55]. Cats were monitored for any physiological or electrophysiological changes and nerve histology was examined for any changes in nerve and axon morphology.

2. Methods

2.1. Electrode design and manufacture

The C-FINE (figure 1) is constructed using a laminar manufacturing process which layers a stiff polymer bar between two layers of flexible silicone sheeting [56]. 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 7 mm 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 [57]) 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^{-1} [58]. The stiffness at the center of a rectangular beam follows from [59]:

$$\frac{F}{d} = \frac{4E_{\text{bend}}wh^3}{L^3} \quad (1)$$

The stiffness is defined here as the force, F , required causing a deformation, d , and is governed by the bending modulus, E_{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^{-1} . While these calculations guided the decision towards stiffening bar material and thickness, pressure exerted at different deformations was verified by balloon inflation testing. The *in vitro* and *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 [10, 11].

For *in vitro* testing, 16 C-FINEs were manufactured by Ardiem Medical (Indiana, PA) of size 10 mm $\times$ 1 mm $\times$ 7 mm (width $\times$ height $\times$ depth). For *in vivo* testing, C-FINE shells without wires were manufactured in the Class 100 clean-room space of the Case Western Reserve University (CWRU) Microfabrication Laboratory (MFL) as described elsewhere [56]. 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 [56]. 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 [54], 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 [58, 60]. 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 [29, 33]).

2.2. 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 [61]. 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 [62]. This is supported by research showing the threshold circumferential pressure for acute nerve damage to be 50 mmHg [47, 48], 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 [49, 50]. 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). 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 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. 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 d. 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 CWRU Institutional Animal Care and Use Committees (IACUC).

2.4. Implantation and behavior monitoring

The cats were induced with Ketamine (10 mg kg^{-1} , 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 are sized such that their inside luminal area was larger than the cross sectional area of the target nerves as described elsewhere [29, 30]. 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 hyper-extension 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, 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.

2.5. 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) [63]. Supramaximal stimulation was delivered by a custom, MATLAB-programmable external stimulator developed at CWRU, proximal (approximately 1 cm) and distal (approximately 1 cm) to the C-FINE shell with a stainless steel electrode at a pulse amplitude of 2 mA and a pulse width of $100 \mu\text{s}$. EMG recordings were made with pairs of mono-polar 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 supra-maximal stimulus did not saturate the amplifiers. Data was 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 [64].

2.6. 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 (figures 3 and 6). 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 (figures 3 and 5.2).

For the sections of nerves with implanted C-FINE shells, histological blocks were taken following perfusion in 4% PFA for at least 24 h. These blocks were washed in phosphate buffered saline for 24 h and sectioned transversely into 0.5 mm blocks. These were fixed in Karnovsky's fixative (1% PFA, 1.25% glutaraldehyde in 0.1 M phosphate buffer) [65] for 2 h and post-fixed in OsO₄ (1% in 0.1 M phosphate buffer). The blocks were dehydrated in increasing ethanol concentrations followed by increasing propylene oxide concentrations and embedded in Spurr's resin [66]. Embedded blocks were sectioned at 1 μ m 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 [67]. 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 2–5 cm proximal to the C-FINE shell. Care was made to sample the nerve more than 1 cm 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 [68].

To examine the surrounding encapsulation and impact on surrounding tissues such as nearby arteries, sections of the approximately 2 cm 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 h. These sections were embedded in paraffin and sectioned at 6 μ m and stained

with Masson's Trichrome to examine connective tissue. These sections were examined under light microscopy at 40× 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.

2.7. Axonal morphology

Images were acquired using AxioVision imaging software (Carl Zeiss, Inc., Thornwood, NY). Whole-nerve images were obtained under 40× or 100× light microscopy and stitched together. For analysis of axon and myelin morphology, nerve slices were examined under 400× 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 [67]. 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 [69]. 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 versus small). Axons clustering within a single Schwann cell can indicate axon regeneration following denervation [58, 67, 70, 71]. 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.

3. Results

3.1. In vitro C-FINE characterization

Pressure testing with all 16 samples showed that the C-FINE will assume a more rounded shape and then stretch to accommodate a slowly inflating balloon at pressures ranging from 10 to 60 mmHg (figure 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 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 expanded 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 × 1 mm C-FINE offers a greater than 10% reduction in volume compared to similarly sized spiral NCE (a 4 mm diameter spiral 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, 6–10 mm for the femoral nerve [72]) would likely have to be used, further increasing the volumetric advantage of the C-FINE. A 10 mm × 1 mm C-FINE also offers over an 80% volume reduction compared to the current clinically reported FINE (10.1 mm³ for the C-FINE compared to 50.9 mm³ for the FINE [17, 29]).

3.2. 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, 4–8 h 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 [73], 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 [74]. Pooled MNCV and CMAP amplitudes are shown in table 1. All recorded MNCV and CMAP amplitudes were within normal reported limits for cats [63, 75]. 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).

3.3. 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 (figures 5 and 6) 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 5) as well as with a multi-fascicled nerve (figure 6). 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 400× magnified section of sciatic nerve underneath an implanted C-FINE shell (figure 7) 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. The mean axon densities and g -ratios across nerves are consistently within normal ranges for cats [70]. 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).

3.4. 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 potential for increased pressures in the cat forelimb [51, 52]. 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 5). As well, the thickness of the encapsulation 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 figure 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 implanted C-FINE shells due to either lack of encapsulation tissue or tearing of tissue during slicing and placement of paraffin sections onto slides.

4. 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 electrode 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 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. *In vitro* we were able to verify 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 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 [44, 45] 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 [76] 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 (equation (1)). 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 [77] 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 *in vitro* testing. However, C-FINE shells implanted in cats in this study were all narrower than this (3–7 mm). This makes them roughly 2–11 times as stiff as the C-FINES that will be deployed in human subjects. This increased stiffness 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 [10], a button and post mechanism [78], adhesive or cured polymer [79], and surface tension [80]. 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 [81], which may enhance suture closure but could promote encapsulation around the cuffed 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 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 [54]. 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 [44, 45]. 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 [58]. 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 [10], 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 [51, 52]. 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 [82, 83].

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 [63, 75]. 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 [58]. For instance, edema is frequently seen in nerve compression, even with pressures as low as 10 mmHg for 2 h, with extensive myelination changes requiring about 30 mmHg of applied pressure for 2 h [84]. 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 was the likely cause of this change. During implantation, surgical error caused a bleed of the brachial artery which required pressure to be held constantly on the nerve for approximately 1 h. Sections of this nerve were taken proximal to, underneath, and every millimeter distal to the center of the C-FINE shell for 5 mm, approximately 2 cm 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 8). 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.

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 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 [51, 52] 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 [31]. This has been suspected as the location of compressive injuries during spine and gynecological procedures, likely due to patient positioning [31, 32] which can cause the femoral nerve to be sharply angled at 80–90° underneath the inguinal ligament [36, 37]. 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 [34], and others [35]. 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 [38]. This requires 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 [38, 43]. 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). Seven C-FINEs have since been chronically implanted in 3 human subjects. 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 amputee received 16-contact C-FINEs on each the median and ulnar nerves for sensory restoration (April 2016), and one transtibial amputee received C-FINEs on each of the tibial, common peroneal, and distal sciatic nerves above the knee as part of a sensory system neuroprosthesis (May, 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.

5. 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 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.

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Figure 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.

Figure 2.

Example of *in vitro* testing of C-FINEs with balloon at 30 mm Hg. 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).

Figure 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).

Figure 4.

Effective CNR at increasing pressure inside 16 C-FINEs (10 mm × 1 mm × 7 mm, width × height × depth). Solid line indicates CNR with error bars given for $\pm$ 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 mm Hg, the cuff deformed (became more round in shape, up to approximately 20 mm Hg) and then stretched under higher pressures.

Figure 5.

Examples of gross and histological morphology at implant and explant at locations proximal to, underneath, and distal to implanted C-FINE shell on a single-fascicle nerve. P: Proximal, C₁ through C₃: Underneath cuff with C₁ located under the stiffening bar and C₂ and C₃ distal to it, D: Distal to cuff. 5.1: Gross tissue appearance at implant (left) and explant (middle) and a cross section through cuffed median nerve and surrounding structures (brachial artery and ulnar nerve) at the level of C₁. Blue coloring in cross section is due to a labeling dye which is washed away prior to histology. 5.2: Masson's Trichrome stained tissue adjacent to cuff located approximately within the rectangular region outlined in image of nerve cross section (5.1, right). Samples shown are all at the level of the cuff ranging from underneath stiffening bar (C₁) to the distal edge of the cuff (C₃). Each pair of images shows large, stitched area as well as magnified section of encapsulation tissue outside the cuff and

near the brachial artery. 5.3: Implanted nerve histology at multiple magnifications proximal to (P, top), underneath (C₁, middle) and distal to (D, bottom) the implanted C-FINE shell. On lowest magnification, note the oblong shape of the fascicle underneath the cuff, compared to the round fascicle proximally and distally. On middle magnification, note the even, densely and consistently populated axons within the fascicle. On highest magnification, note the densely populated axons with a range of axon diameters (large and small) with myelin sheath thickness proportional to axon diameter (this is clearly observed in the higher magnification in figure 7).

Figure 6. 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 2 cm distal to cuff (right).

Figure 7.

Example histology underneath cuff on a sciatic nerve. Note densely and evenly packed axons of both large and small diameters. Also note that relatively small axons are thinly myelinated while larger axons have a thicker myelin sheath.

Figure 8.

Sample histology of nerve subjected to over 1 h 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 1 h. Samples show relatively normal histology distal to implanted cuff. Underneath the cuff there is evidence of swelling and mild demyelination of some larger fibers.

Table 1

Electrophysiology results from implant compared to explant. Values given as mean $\pm$ 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 ($p > 0.05$).

Nerve	MNCV (m s^{-1})		CMAP (mV)	
	Implant	Explant	Implant	Explant
Sciatic (6)	83.04 $\pm$ 6.05	84.55 $\pm$ 5.12	15.97 $\pm$ 0.86	15.20 $\pm$ 0.99
Median (4) and Ulnar (2)	73.66 $\pm$ 5.38	72.31 $\pm$ 4.41	14.82 $\pm$ 1.23	15.32 $\pm$ 0.98

Table 2

Quantified axonal morphology proximal to cuff compared to underneath cuff. Values given as mean $\pm$ 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 ($p > 0.05$).

Nerve	Density (count mm ⁻²)		<i>g</i> -ratio	
	Proximal	Underneath C-FINE	Proximal	Underneath C-FINE
Sciatic ($n = 6$)	8091 $\pm$ 426	8167 $\pm$ 510	0.63 $\pm$ 0.02	0.62 $\pm$ 0.03
Median ($n = 4$) and ulnar ($n = 2$)	8990 $\pm$ 268	8741 $\pm$ 462	0.62 $\pm$ 0.02	0.59 $\pm$ 0.04