Directed stimulation with interfascicular interfaces for peripheral nerve stimulation

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Abstract

Objective. Computational models have shown that directional electrical contacts placed within the epineurium, between the fascicles, and not penetrating the perineurium, can achieve selectivity levels similar to point source contacts placed within the fascicle. The objective of this study is to test, in a murine model, the hypothesis that directed interfascicular contacts are selective.

Approach. Multiple interfascicular electrodes with directional contacts, exposed on a single face, were implanted in the sciatic nerves of 32 rabbits. Fine-wire intramuscular wire electrodes were implanted to measure electromyographic (EMG) activity from medial and lateral gastrocnemius, soleus, and tibialis anterior muscles.

Main results. The recruitment data demonstrated that directed interfascicular interfaces, which do not penetrate the perineurium, selectively activate different axon populations.

Significance. Interfascicular interfaces that are inside the nerve, but do not penetrate the perineurium are an alternative to intrafascicular interfaces and may offer additional selectivity compared to extraneural approaches.

1. Introduction

Neuroprostheses are devices used for the restoration of motor, sensory, and cognitive deficits in individuals with neurologic injury or disease. Electrical currents can elicit a response in nerves that are faithfully transmitted to their respective end organs, which respond as if the signal was generated naturally by the body, enabling artificial control of paralyzed musculature [1]. Developing interfacing technologies with the peripheral nerve can be challenging since an effective neuroprosthesis should selectively stimulate desired axons without causing changes to the natural neurological function of the nerve.

The peripheral nerve is a non-homogeneous structure where the axons are protected by three neural tissue layers, the epineurium, perineurium and the endoneurium. The innermost layer, the endoneurium contains Schwann cells, fibroblasts and other components that surround all the axons [2]. The axons, Schwann cells and the endoneurial components are enclosed by a multi-layered cellular perineurial membrane to form a nerve fascicle. The perineurium consists of collagen and squamous cells that are interconnected by tight junctions (zonulae adhaerentes) to prevent paracellular transport [3–6]. The innermost perineurial cells functionally contribute to the blood–nerve barrier (BNB), like the blood–brain barrier in the central nervous system, regulating the passage of nutrients to the endoneurial micro-environment. Important functions of the perineurium are to maintain a positive intrafascicular pressure, relative to the endoneurium and regulate the local environment by acting as a diffusion barrier to several substances. It also plays a major role in peripheral nerve function and metabolism [7, 8]. In contrast to the highly structured and tight perineurium, the epineurium, the outermost tissue, is a loosely organized collagenous tissue whose main function is generally thought to maintain the structural integrity of the nerve [9, 10]. The epineurium primarily consists of longitudinal collagen fibers interspersed with...
a few elastin fibers [11, 12] and surrounds the nerve to fill the spaces between fascicles.

Neural interfaces can be implanted at different locations within the nerve to achieve excitation of individual axons. Extrafascicular electrodes place contacts around the nerve, outside the epineurium. These interfaces have been used clinically for fascicle-selective stimulation [13, 14]. Although they require the nerve to be freed from the surrounding tissues, extrafascicular electrodes are generally simple to implant [15] and have shown selective stimulation in multiple long-term clinical trials for motor and sensory restoration [16–18]. Most pre-clinical and clinical studies with extrafascicular electrodes have demonstrated functional selectivity [16, 19–21], however, they have limited access to axons in the center of larger nerves, such as the femoral or median nerves. Modeling studies have investigated approaches such as development of threshold–current stimulation algorithms, increased number of contacts, and modification of waveforms to improve selectivity [22–24]. Field-manipulation has been used in some studies to obtain some subfascicular selectivity upon reshaping of the nerve [25]. The human nerve, however, has many more fascicles than the pre-clinical animal models [26]. Even when reshaped, it is still likely that there will be several fascicles that are not at the surface, or the nerve would need to be reshaped to an unrealistic width for the anatomical space constraints.

Closer proximity of the electrode to the target axons facilitates increased selective stimulation in the peripheral nerves [27, 28]. Intraneural electrodes that place contacts directly next to the axons by penetrating through the perineurium are called intrafascicular electrodes. These electrodes can provide increased access to the interior of the nerve, but the disruption to the perineurium may affect the integrity of the BNB. Complications due to changes in the endoneurial environment include increased endoneurial pressure, nerve fiber compression, variable shifts in thresholds and loss of axons [29, 30]. These events have particularly been observed in larger nerve fibers [4, 31, 32] and in chronically implanted intrafascicular devices [30, 33, 34]. Surgical techniques required to implant these electrodes could be intense and time-consuming, since it may require additional manipulation of the nerve beyond its exposure [35, 36].

Intrafascicular electrodes are placed outside the fascicles, within the epineurial layer, thereby leaving the perineurium intact. This concept was first introduced in the design of the slowly penetrating interfascicular electrode (SPINE) which reshaped the nerve slowly and implanted contacts within the epineurium, around the fascicles [37]. Another interfascicular approach involved the development of a multigroove electrode, which essentially served as a cuff electrode for each fascicle of the nerve. The fascicles of the nerve were surgically isolated by dissecting the epineurium, and implanted within a groove [38]. In another study, a glass rod was implanted within the epineurium with channels positioned over the two fascicles of the sciatic nerve [39]. All these interfaces contained the electrical contacts within passive elements to create an insulating plane shielding them from non-target fascicles. Interfascicular interfaces offer the potential to selectively access axons in a compromise between extrafascular and intrafascicular approaches.

Computational modeling studies have indicated the advantage of implanting an electrode with a directed contact that is un-insulated only on one face, but without an insulating plane, close to the fascicles [40]. This type of electrode produces a directed asymmetric field, unlike a point source contact (contact with all faces un-insulated). The current study tests this approach by constructing a directed contact on a wire by removing a small portion of one face of the insulation (figure 1). It was hypothesized that the directed field produced by such a contact, implanted within the epineurium and outside the fascicles will produce selective recruitment of axons.

2. Methods

2.1. Experimental procedure

Thirty-two New Zealand rabbits weighing between 2.6 and 3.4 kg were anesthetized initially with ketamine and xylazine via intraperitoneal injection and then intubated and ventilated on isoflurane and oxygen. When the animal did not react to toe pinch and other reflexes, the dorsal surface over the popliteal fossa was shaved and prepared for surgery to expose the sciatic nerve. Once the nerve was visible, the surrounding muscles were separated and the nerve was traced proximal to the branching point of the common peroneal (CP)/tibial nerves. The lateral and the medial gastrocnemius muscles were also exposed. Electromyogram (EMG) activity was measured by implanting bipolar intramuscular electrodes in the bellies of lateral and medial gastrocnemius, soleus and the tibialis anterior (TA) muscles.

The nerve and the area around it were bathed with physiological saline throughout the course of the experiment to prevent dehydration. The incision site was closed following electrode implantation to prevent nerve desiccation due to exposure. Blood oxygenation was monitored with a pulse oximeter. ECG leads were attached to monitor cardiac activity. Body temperature was maintained with a circulating water heating pad placed under the animal. An intravenous line (IV) in one of the superficial veins in the ear and drip of 5% dextrose with sodium bicarbonate was maintained throughout the experiment. All animal procedures were approved by the Institutional Animal Care and Use Committee of Case Western Reserve University.
2.2. Design of the interfascicular electrodes

2.2.1. Interfascicular wire electrode

The interfascicular electrodes were fabricated with a single-stranded 35 N LT® wire (Fort Wayne Metals, IN) coated with ethylene tetrafluoroethylene. The outer diameter of the bare wire was 101.6 µm, coated with a 25 µm thick insulation. One face of the wire was un-insulated with a scalpel under a microscope by approximately 100 µm in length to create a directed contact (figure 1). The proximal tip (implanted side) of the wire was insulated with cyanoacrylate. Approximately 1 cm length including the de-insulated portion was implanted longitudinally into the epineurium of the nerve. The total length of the insulated wire, approximately 32 cm, was routed out of the site, and connected to a stimulator.

2.2.2. Longitudinal interfascicular contacts

Multiple wires were individually implanted in the epineurium along the length of the nerve.
Figure 2. (A) Multiple interfascicular contacts. (B) Contacts inserted in the rabbit sciatic nerve. Longitudinal contacts: each electrode had a directed contact and an insulated tip. Each contact was individually inserted into the epineurium proximal to the branching point.

(longitudinally) at locations proximal to the branching point of the common trunk of the sciatic nerve (figure 2). Generally, two contacts were implanted between the CP and tibial branches that run together in the common trunk of the sciatic. Two more contacts were implanted one each on the medial and lateral side of the nerve. Depending on the diameter of the nerve, one to two additional contacts were implanted between these contacts for a total of 6–7 contacts. In two experiments, 8 and 10 contacts were implanted because the nerves were larger in size.

2.2.3. Transverse interfascicular contacts
Multiple contacts between the fascicles were also implanted in a transverse configuration (figure 3). We achieved this by using the structure of the flat interface nerve electrode (FINE) \cite{41, 42} to stabilize the interfascicular electrodes. The structure of the FINE was fabricated with polydimethylsiloxane \cite{43, 44} and was approximately 4 mm wide and 1 mm high internally, based on the dimensions of the rabbit sciatic nerve. Five interfascicular wire electrodes with directional contacts were inserted through the top of the cuff. Each electrode wire was fed through a small-gauge hypodermic needle inserted at regular intervals along the width of the cuff. This device was implanted on the nerve proximal to the CP/tibial branching point and the wires were adjusted from the outside, if, upon visual inspection, they were not in the epineurium.
2.2.4. Single directed contact
The effects of the orientation of a single directional contact (figure 1) were tested by implanting a single wire electrode in the epineurium, outside the fascicle. This electrode was inserted longitudinally in between the fascicles. After recording a set of recruitment curves, the orientation of the contact was changed by holding a portion of the wire outside the nerve and rotating it as close as possible to an angle between 60° and 90°. It was not exact, so we do not report results in terms of the angle. For each trial, there were at least four unique rotation positions and as many as eight. Each of the orientations was a new position and tested for independent recruitment properties. The electrode external to the nerve was marked at regular intervals around the circumference to identify the orientation of the contact as it was rotated. The recruitment pattern was measured at each orientation. After a set of recruitment curves, the interface was explanted and re-implanted near the same location. At explant, viability of the interface was visually inspected and was tested by measuring conductivity at the contact to ensure intact insulation at the tip and around the contact. Upon viability testing, it was re-implanted into the nerve proximal to the branching point. Each new implant was treated as a separate trial (table 1).

Table 1. Interfascicular trials. The total number of trials and animals for each type of interfascicular interface implanted. Each implant is treated as an independent trial and implants were performed on both nerves of the animal.

| Type of interface          | Number of trials | Number of animals |
|----------------------------|------------------|-------------------|
| Single interfascicular wire| 32               | 14                |
| Longitudinal multiple contacts | 34          | 14                |
| Transverse multiple contacts | 34            | 12                |

2.3. Instrumentation
A custom battery-powered, computer-controlled stimulator (Crishtronics, Cleveland, OH) delivered
Figure 4. Experimental set up. A computer-controlled stimulator delivered monopolar biphasic pulses to the interfascicular electrodes. EMG signals were low pass filtered at 500 Hz and sampled at 2400 Hz from four muscles.

charge balanced, biphasic rectangular pulses with an amplitude range of 0.02–5 mA (resolution 0.02 mA) and a pulse width range of 10–500 µs (figure 4). CED amplifiers (Model 1902, Cambridge Electronic Design, Cambridge, UK) with a gain of 90–990, performed the amplification, AC coupling, and low pass filtering (at 500 Hz) of the EMG signals. The amplifier gains were set to prevent saturation and adjusted to maximize the size of supra-maximal twitch response from each muscle. A 32-bit, PCMCIA A/D card (National Instruments, Austin, TX) acquired the signals at 2.4 kHz to a computer.

2.4. Metrics and data analysis
An automated data collection algorithm (written in MATLAB- Mathworks, Inc., Natick, MA) used a binary search routine to generate pulse amplitude modulated recruitment curves [45]. The algorithm varied pulse amplitude to generate twitch recruitment curves. The twitch response was defined as the integrated and rectified EMG response between 3 and 18 ms following the stimulation pulse. The limits of 3–18 ms were chosen to eliminate stimulation artifact and any reflexive contribution to the EMG signal, respectively. Five twitch responses were averaged together for each point on the recruitment curve. Twitch frequency of 4 Hz was used to minimize the experiment time without fusing the muscle twitches. For each animal, pulse-amplitude-modulated EMG response recruitment curves were acquired. The pulse amplitude was varied between 0 to 2 mA with a fixed pulse width of 50 or 100 µs.

During each experiment, the largest quantified EMG response of each muscle across all trials was defined as that muscle’s maximum activation. The response of the muscle to a given stimulus value was quantified as the percent activation that muscle exhibited relative to its maximum response observed across all trials.

2.4.1. Axon population selectivity
EMG recruitment trajectories were analyzed to measure axon selectivity [19, 25, 37]. The analysis was divided into three different stages: (a) EMG recruitment trajectories, (b) overlap of the EMG recruitment trajectories, and (c) overall selectivity of the interface.

2.4.2. EMG recruitment trajectories
The EMG recruitment space was defined in the four-dimensional space, each dimension being the EMG activation of a muscle: TA, soleus (Sol), medial gastrocnemius (MG), and lateral gastrocnemius (LG). Each trace represented the muscle recruitments of a contact. The EMG data was interpolated to get an even spacing of points for the recruitment trajectory. Every vector point on the trace was defined as the activation of each muscle at a stimulation amplitude for that particular channel:

$$\text{EMG}_{r}(q) = \text{EMG}_\text{TA}(q)\hat{i} + \text{EMG}_\text{Soleus}(q)\hat{j} + \text{EMG}_\text{MG}(q)\hat{k} + \text{EMG}_\text{LG}(q)\hat{l}$$  (1)

$\text{EMG}_{r}(q)$ is the EMG activation of muscle ‘$a$’ at charge ‘$q$’ for a channel/orientation ‘$r$’. An example two-muscle EMG recruitment trajectory generated from the recruitment of the muscles is illustrated (figure 5). Contacts 1 and 2 are functionally different, except for a portion of their traces near the origin. This portion is the overlapped region and is represented by the dashed circled area. The next section describes the calculation of the percentage of overlap between traces.

2.4.3. Overlap of the EMG recruitment trajectories
Overlap and selectivity used in this study was previously published [19, 20, 25, 37]. Briefly, overlap was defined as the percentage of one channel’s trajectory that is equivalent to another channel’s trajectory. An overlap region was calculated by the set of
Figure 5. Conceptual plot of an EMG trajectory. A typical EMG recruitment space with two muscles is shown. Contacts 1 and 2 are represented by the pink and black traces, respectively. Each point on the trace is a function of the EMG activation of the two muscles at a stimulus amplitude. Contacts 1 and 2 are functionally unique except for a portion indicated by the circled region. This region represents the overlap between the two contacts. The overlap of contact 1 with contact 2 is approximately 30%. The overlap of contact 2 with contact 1 is approximately 10%. Beyond this overlapped region, the contacts are functionally different. Overlap in multiple dimensions is determined by calculating the Euclidean distance of each point on Contact 1’s trace to all the other points on Contact 2’s trace and repeating it for all the points on both the traces.

points where the trajectories are within the 95% confidence interval of each other. The 95% confidence intervals were determined by the measured standard deviations of the twitch responses and the student-t distribution.

2.4.4. Calculation of overall selectivity

The overall selectivity ($\Lambda$) was defined as the percentage of contact pairs that overlapped less than 50%. The overlap value of a contact with every other contact was tabulated to form an overlap matrix. This matrix was generated for each trial to determine independence of individual orientations/channels. Each entry in this matrix, $O_{ij}$, represented the percentage of overlap of the groups of axons recruited by the two channels/orientations defined as the $i$th recruitment with the $j$th recruitment. The diagonal values were ’1’ which represented a complete overlap of that channel with itself. The contact pairs, $O_{ij}, O_{ji}$ that had less than 50% overlap for each were considered selective. Traces of channels that did not overlap with one another were considered to recruit two different sets of axons and the corresponding channels were considered to have selective recruitments. The traces that did overlap were considered to recruit axon populations that could not be distinguished apart. In such a case, the recruitments of the corresponding channels were not considered independent. This was used to calculate the overall selectivity of the interface.

The overall selectivity $\Lambda$ was calculated by:

$$\text{Selectivity} = \Lambda = \frac{\text{Total number of independent contact pairs}}{\text{Total number of possible combinations}}$$

$$0 \leq \Lambda \leq 1.$$  

3. Results

3.1. Multiple interfascicular contacts

An overlap matrix (table 2) was generated from the four-dimensional analysis of the traces of a trial with six longitudinally inserted interfascicular contacts and the overall selectivity was calculated to be, $\Lambda = 15/15 = 1$.

In a trial with five contacts implanted in the transverse configuration, four of the five contacts were able to selectively recruit different groups of axons (table 3). The overall selectivity value of this interface was calculated to be $\Lambda = 6/10 = 0.6$. The grayed areas in the table represent pairs with significant overlap ($\geq 0.5$). All the overlapped entries corresponded to Channel 5, making it a redundant channel. When Channel 5 was not considered, the overall
Table 2. Overlap matrix for six longitudinal interfascicular contacts. The overlap matrix of a trial containing six interfascicular contacts inserted longitudinally was generated. Each entry in the table represents the percentage of overlap one channel has with the other. Diagonal entries of ‘1’ represent functional equivalency due to a complete overlap of one channel with itself.

|       | Ch 1 | Ch 2 | Ch 3 | Ch 4 | Ch 5 | Ch 6 |
|-------|------|------|------|------|------|------|
| Channel 1 | 1.0  | 0.2  | 0.4  | 0.3  | 0.2  | 0.2  |
| Channel 2 | 0.1  | 1.0  | 0.2  | 0.1  | 0.1  | 0.1  |
| Channel 3 | 0.1  | 0.1  | 1.0  | 0.3  | 0.2  | 0.1  |
| Channel 4 | 0.1  | 0.0  | 0.3  | 1.0  | 0.1  | 0.0  |
| Channel 5 | 0.1  | 0.2  | 0.3  | 0.1  | 1.0  | 0.1  |
| Channel 6 | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  | 1.0  |

Table 3. Overlap matrix for transverse multiple contacts. The overlap table for transversally implanted multiple contacts was calculated. The grayed areas represent entries that have a significant degree of overlap. The function of Channel 5 could be reproduced by other channels in the interface.

|       | Channel 1 | Channel 2 | Channel 3 | Channel 4 | Channel 5 |
|-------|-----------|-----------|-----------|-----------|-----------|
| Channel 1 | 1.0       | 0.4       | 0.1       | 0.4       | 0.5       |
| Channel 2 | 0.3       | 1.0       | 0.3       | 0.4       | 0.5       |
| Channel 3 | 0.3       | 0.3       | 1.0       | 0.4       | 0.5       |
| Channel 4 | 0.2       | 0.3       | 0.3       | 1.0       | 0.5       |
| Channel 5 | 0.6       | 0.5       | 0.6       | 0.6       | 1.0       |

Table 4. Overlap matrix for a single interfascicular electrode. A value of 1.0 indicates complete overlap, such as orientation 1 has a complete overlap with orientation 1. Two orientations ‘i’ and ‘j’ are independent of each other, when the sum of their overlap values, \( O_{ij} + O_{ji} < 1 \). In this case, all the orientations are independent of each other.

|       | Orientation 1 | Orientation 2 | Orientation 3 | Orientation 4 |
|-------|---------------|---------------|---------------|---------------|
| Orientation 1 | 1.0           | 0.3           | 0.3           | 0.3           |
| Orientation 2 | 0.2           | 1.0           | 0.3           | 0.3           |
| Orientation 3 | 0.3           | 0.2           | 1.0           | 0.2           |
| Orientation 4 | 0.3           | 0.4           | 0.3           | 1.0           |

selectivity value of the matrix with Channels 1–4 was \( \Lambda = 6/6 = 1.0 \).

The overall axonal selectivity for 34 trials of longitudinally inserted contacts was 0.85 ± 0.2 with an average total number of 6.53 ± 1.03 contacts tested in each trial. The average selectivity for the 34 trials inserted in a transverse configuration was 0.73 ± 0.3 for an average of 5 contacts in each trial.

3.2. Single interfascicular wire
The overlap matrix (table 4) from a trial of a single directed contact implanted between two fascicles (figure 6) was generated. The overlap matrix generated by four orientations indicated that the overlap between all the orientations was less than 50%. This suggests that there was no significant overlap in the axon groups recruited by all the orientations. The overall selectivity of the interface was calculated to be, \( \Lambda = 6/6 = 1.0 \).

EMG recruitment trajectories were analyzed for the tibial (recruiting MG/Soleus) and CP fascicles (recruiting TA) (figure 7). These trajectories were analyzed until the first muscle reached its maximum value for that orientation.

Pulse amplitude (0–2 mA, pulse width: 100 µs) modulated recruitment curves generated by the four different orientations (1–4) were also analyzed (figure 8). Each orientation had a different order of muscle recruitment.

For other single wire electrodes trials, we generated data from multiple orientations (table 5). EMG trajectories were similarly analyzed across 32 trials for single wire electrodes. The average axonal selectivity per multiple orientations with a single wire was, \( \Lambda = 0.8 \pm 0.3 \) with an average number of selective orientations per wire being 4.75 ± 0.8. The summary of the average overall selectivity and the average number of orientations/contacts is in table 5.

4. Discussion
We show that interfascicular electrodes with directed contacts can produce selective recruitment of axons. Multiple directional contacts, and a single contact implanted within the epineurium produced different, selective recruitments.

Interfascicular interfaces have been explored in the past with varying degrees of success [37–39]. These interfaces are attractive to study because they have the potential to provide the desired proximity to the axons without disruption of the perineurium. Trauma to the perineurium can lead to a cascade of events triggering the encapsulation response of the tissue. Injury and compression of the perineurium chronically can lead to formation of fibrosis which
Figure 6. Histological cross section of a rabbit sciatic nerve (toluidine blue stained). Two fascicles were seen at the site of the implant of a single directed contact. The approximate position of the wire electrode is indicated by the green circle.

Figure 7. EMG recruitment trajectories. The EMG recruitment trajectories of four different orientations from a single interfascicular electrode (varying pulse amplitude, PW: 100 µs) in three dimensions (of the four) were plotted. These trajectories were limited to the maximal activation of the first muscle. Orientations 1–4 of a single wire electrode are represented. Orientation 1 is initially selective to TA then recruits Soleus. Orientation 2 is selective to MG. Orientation 3 recruits both the MG and TA. Orientation 4 is also selective to MG, but to a lower degree.
Figure 8. Single interfascicular contact recruitment curves. The muscle recruitment curves from four different orientations of the single interfascicular wire are shown in the plots. Figures (A)–(D) refer to Orientations 1–4 respectively. Each line on the plot represents a muscle, as indicated by the legend in the middle. A single wire was able to produce four different recruitment curves.

Table 5. Summary of the overall selectivity obtained with each interface.

| Type of interface                        | Number of trials | Average overall selectivity (Λ) | Average number of orientations/contacts |
|-----------------------------------------|------------------|---------------------------------|----------------------------------------|
| Single interfascicular wire             | 32               | 0.8 ± 0.3                       | 4.75 ± 0.8                             |
| Longitudinal multiple contacts          | 34               | 0.85 ± 0.2                      | 6.53 ± 1.03                            |
| Transverse multiple contacts            | 34               | 0.73 ± 0.3                      | 5                                      |

may cause tethering at the affected site, resulting in a superimposed traction injury [35, 36, 46]. Intrascicular and extraneurial interfaces have been well studied extensively over the years. Recently, interfascicular interfaces were called the ‘least common in literature’ [47]. Our study explores the space between the two types of interfaces and fills the gap in the literature of peripheral nerve interfaces.

Modeling studies compared the field distribution and selectivity of a point-source contact (insulation removed on all sides) and a directional contact (insulation removed only on one face (figure 1)) implanted in the epineurium [40]. The directional contacts provided higher selectivity than the point source contacts. Modeling also predicted higher selectivity for directional contacts situated adjacent to the perineurium, outside the fascicles. Our study verified this experimentally by implanting multiple channels with a directional contact within the epineurium of the nerve, outside the fascicles, proximal to the branching point. Multiple electrodes, with contacts oriented in different directions, distributed around the fascicles, increased the probability of selectively recruiting different groups of axons within the fascicles.

Selectivity, in this study was analyzed by determining the overlap between axon populations,
recruited by each orientation/contact in the interface [19, 25, 37]. This analysis was used to determine the independence of each contact. The primary assumption in this methodology was that two different populations of axons would produce non-overlapping EMG trajectories. Overlapping trajectories were conservatively interpreted to be produced by axon populations that could not be distinguished apart. It is possible that these overlapping trajectories are produced by independent groups of axons. However, this cannot be proven without physiologically tracing individual axons that each orientation recruited. Therefore, two overlapped traces were not considered to be unique recruitments.

Increased number of contacts distributed around the fascicles provided a significant number of contacts with unique recruitments. Over 80% of the contacts inserted longitudinally selectively recruited different groups of axons. Nearly 75% of the transversally implanted contacts also produced unique recruitments. The 20%–25% redundancy in the interfaces can be attributed to contacts positioned close to each other, or not being located next to a fascicle, though we did not rigorously address this correlation. The number of unique recruitments is expected to reduce with an increased number of contacts, distributed in the same vicinity. Ten contacts distributed in a space will produce more overlap than two contacts distributed in the same space. Although, this may affect the overall selectivity value, the interface with the ten contacts will allow recruitment of axons from multiple locations and the choice of contacts that have unique recruitments.

The reshaping capabilities of the FINE were utilized in the design of the transverse interface. The structure of the FINE was chosen to bring the deeper fascicles to the surface and implant the directional contacts between the fascicles. The transverse configuration was utilized to distribute the contacts along the width of the nerve. Interestingly, the selectivity of the transverse configuration was lower than the longitudinal configuration. This probably had to do with challenges in the accurate placement of a contact close to the fascicle. Fascicles within a nerve are known to divide and fuse throughout the length of the nerve, turning at angles and exchanging fibers between fascicles [48]. Contacts implanted in a lateral-medial orientation might not be adjacent to a fascicle, if one of the fascicles is directly above the other. This problem can be circumvented by having contacts of different lengths embedded offset to each other. Longitudinally inserted contacts were threaded along the length of the fascicle to be situated against most of the fascicles, increasing selectivity.

The importance of using a directional contact was further studied by investigating the effects of its orientation. A single directional contact was implanted between fascicles and rotated between recruitments. The change in the orientation of the contact resulted in selective recruitment of axons. The recruitment curves obtained from one orientation were observed to be different from the other orientations, indicating the importance of field distribution around the perineurium. This phenomenon was observed even in two fascicle models (figure 6). An overall selectivity of ’1′ in a two-fascicle model with four orientations implied that every orientation recruited portions of at least one of the fascicles without significant overlap. This indicated sub-fascicle selectivity with a single directional contact. This interface, in addition, was also able to recruit muscles selectively. It was possible to selectively activate MG and TA that are recruited by the tibial and the CP fascicles, respectively. Further, MG, LG and soleus muscles that are commonly innervated by the same fascicle (tibial) could also be separated by this interface.

Analysis of muscle recruitment data suggested that the TA and the MG muscles were the first to attain threshold most often. The gastrocnemii and TA muscles consist of ‘fast-twitch’ fibers primarily [49], that are usually innervated by axons with higher conduction velocities [50]. Anatomy of the fascicles recruiting these muscles is also responsible for their preferential recruitments. The TA is recruited by the CP fascicle, which is the smaller of the two fascicles. The threshold for activation of a smaller fascicle is generally lower than a larger fascicle [51]. This is because the size of the perineurium is directly proportional to the size of the fascicle. The thicker perineurium of a larger fascicle provides increased resistance, increasing the amount of current necessary to activate that fascicle [52]. MG is thought to be recruited by larger diameter axons than those that innervate LG and soleus, which reduces its activation threshold [53]. This effect, however, is smaller than the effect of the size of the CP and tibial fascicles.

The perineurium is the main component of the nerve that maintains the integrity of the axons and their environment. In addition to its physiological properties, its unique electrical properties can be taken advantage of for electrode design applications. The effect of the perineurium depends on its relative resistance, which is a function of its thickness. Due to its large resistivity with respect to the other neural tissues, the perineurium causes bending of field lines around fascicles [54]. This shunting can cause the current from an extraneural source to be directed away from a larger, more resistive fascicle toward a smaller, less resistive fascicle [51]. Modeling studies have predicted that this effect could be avoided by implanting directional contacts adjacent to the fascicles [55].

Interfascicular interfaces designed so far have used versions of directional contacts [37–39]. The SPINE used silicone ‘beams’ to shield the contacts and confine the electric field to a smaller region. The multigroove electrode had contacts within a groove, kept insulated from the rest of the interface. The
fabrication and the implant of these interfaces were rigorous requiring several processing steps [38]. Directional contacts, investigated in this study were easier to fabricate and implant. Once the nerve was exposed, it was not necessary to separate it from the surrounding connective tissue to insert the longitudinal contacts. This reduced the invasiveness of the procedure and protected the nerve’s physiological condition.

We did not confirm histologically that the transverse configuration did not penetrate the perineurium in our study. However, it is a reasonable assumption that the wires did not go through the fascicles. Studies have reported difficulties with penetrating the fascicular space despite deliberate attempts to do so [36, 56]. Pneumatic/high-speed inserters have been designed to break through the tough barriers of the perineurium [57, 58]. In addition, sharp/needle electrodes are typically used for intrafascicular interfaces to reduce insertion forces. Our implant used blunt wires on the FINE which was implanted on the nerve without any additional force.

Extraneural and intrafascicular approaches have demonstrated selectivity through decades of development through chronic pre-clinical and clinical trials. This study demonstrates encouraging results from an initial feasibility study with directed-interfascicular interfaces tested in various configurations (longitudinal and transverse). These designs can be taken further by developing on concepts that target ease of manufacturability and/or implant. Recommendations for further development of interface designs based on the results of this study are (a) central insulated core with contacts along the face of the core. This design is inspired by the selectivity results of the single directional contact rotated several times for multiple orientations. This microfabricated device could be longitudinally inserted along the nerve, between the fascicles. This would essentially provide multiple directional contacts distributed along the length of a multi-fasciculated, large nerve. (b) Another potential design is a high density FINE containing several interfascicular and extraneural contacts. The interfascicular and extraneural contacts would be offset from each other to be able to access different portions of the fascicles.

The preference of the type of interfascicular design would depend on several factors including anatomical location of implant, microfabrication of the implant, scope of the study, etc. From our analysis, we anticipate the longitudinal configuration to be advantageous due to the ease of surgical implantation and the ability to be in closer anatomical proximity to axons in a multi-fasciculated nerve. Even with the reshaping that the FINE offers, it is difficult to access axons in the middle of a densely populated human nerve. A ‘shot-like’ approach where several contacts are longitudinally implanted in the epineurium would greatly reduce the need to separate the nerve from its surrounding fascia and could be done in a minimally invasive manner.

This study was performed in a rabbit sciatic nerve which has 2–3 fascicles in the popliteal fossa region. Pre-clinical studies need to be performed with microfabricated devices in multi-fasciculated animal models. Future work with computationally generated complex algorithms with field-shaping studies can provide comprehensive manipulation of the currents enabling desired functional response.

5. Conclusions

In conclusion, this study investigated the feasibility of an interfascicular interface with a directed contact design. We have demonstrated that interfascicular interfaces are able to selectively activate axon populations and muscles without penetrating the perineurium. This study provides the motivation to investigate further design and development of these interfaces, potentially using novel polymers for electrode materials to reduce mechanical mismatch that could occur on a chronic basis. Chronic studies are also necessary to test the viability of these interfaces on a long-term basis.

Data availability statement

The data that support the findings of this study are available upon reasonable request from the authors.

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