Long-term usability and bio-integration of polyimide-based intra-neural stimulating electrodes

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Stimulation of peripheral nerves has transiently restored lost sensation and has the potential to alleviate motor deficits. However, incomplete characterisation of the long-term usability and bio-integration of intra-neural implants has restricted their use for clinical applications. Here, we conducted a longitudinal assessment of the selectivity, stability, functionality, and biocompatibility of polyimide-based intra-neural implants that were inserted in the sciatic nerve of twenty-three healthy adult rats for up to six months. We found that the stimulation threshold and impedance of the electrodes increased moderately during the first four weeks after implantation, and then remained stable over the following five months. The time course of these adaptations correlated with the progressive development of a fibrotic capsule around the implants. The selectivity of the electrodes enabled the preferential recruitment of extensor and flexor muscles of the ankle. Despite the foreign body reaction, this selectivity remained stable over time. These functional properties supported the development of control algorithms that modulated the forces produced by ankle extensor and flexor muscles with high precision. The comprehensive characterization of the implant encapsulation revealed hyper-cellularity, increased microvascular density, Wallerian degeneration, and infiltration of macrophages within the endoneurial space early after implantation. Over time, the amount of macrophages markedly decreased, and a layer of multinucleated giant cells surrounded by a capsule of fibrotic tissue developed around the implant, causing an enlargement of the diameter of the nerve. However, the density of nerve fibers above and below the inserted implant remained unaffected. Upon removal of the implant, we did not detect alteration of skilled leg movements and only observed mild tissue reaction. Our study characterized the interplay between the development of foreign body responses and changes in the electrical properties of actively used intra-neural electrodes, highlighting functional stability of polyimide-based implants over more than six months. These results are essential for refining and validating these implants and open a realistic pathway for long-term clinical applications in humans.

Keywords: intra-neural electrode, selectivity, stability, biocompatibility, control.

1. Introduction

The ability to communicate bi-directionally with the peripheral nervous system has opened promising perspectives to control neuroprostheses, restore lost sensation, and alleviate motor deficits resulting from neurological disorders [1, 2]. For example, peripheral nerve stimulation has been successfully used in humans to alleviate foot drop after neuromotor disorders [3-7], to treat bladder dysfunction [8, 9], and to reanimate paralysed muscles after spinal cord injury [10-13].

In all these applications, the stimulation was applied using an epi-neural cuff electrode [14]. While this type of interface is suitable when a simple and non-selective control of the stimulation is required, more advanced neuroprostheses rely on the ability to target specific fiber bundles within the nerve in order to modulate their recruitment in real-time using application-specific control strategies. In general, for a desired outcome, the type of neural interface is chosen based on the trade-off between invasiveness, selectivity, and reliability [1, 15]. To this respect, intra-neural electrodes are the most appropriate implants since they provide superior selectivity of recording and stimulation, increased signal-to-noise ratio of recordings, and lower threshold of activation than extra-neural implants [16, 17]. For instance, intra-neural stimulation of the median and ulnar nerves re-established graded sensory feedback that improved the control of a hand prosthesis after amputation in humans [18, 19]. Despite this success, clinical studies using intra-neural implants have remained investigational and restricted to short-term implantation periods.

Several limiting factors have prevented the deployment of intra-neural implants for extended durations in human patients. First, these implants are markedly more invasive compared to epi-neural cuffs [20-23]. Second, the anatomical and functional consequences of the immediate penetration into and long term implantation within the host tissue have been solely characterized on passive implants without cabled connections [24, 25]. Third, little is known about the stability and selectivity of intra-neural implants to enable functional applications over extended time.

To remedy these limitations, we evaluated the selectivity, stability, functionality, and biocompatibility of a polyimide-based intra-neural implant over a period of 6 months. Polyimide is a common material used in thin film technology for in vivo applications that allows versatile microfabrication of electrode implants with varying shape and geometry [25-27]. Polyimide-based intra-neural implants have emerged as a viable alternative to rigid silicon-based penetrating microelectrode arrays [19, 28-32] for clinical applications.

Previous studies in acute animal preparations showed that polyimide-based intra-neural implants enable the preferential activation of specific groups of muscles that are innervated by different branches of the same stimulated nerve [17, 25, 26, 33]. However, the long-term stability of this selectivity has not been investigated. Independently, the foreign body response to polyimide-based intra-neural implants has been evaluated using passive, untethered implants over periods of up to 3 months [24, 25, 34, 35]. Taken together, these studies described the formation of a mild fibrotic encapsulation around the implant that developed in parallel to inflammatory responses, but had no sustained impact on the density of nerve fibers, axonal conduction velocity, or locomotor capacities of the implanted animals.

While these studies have provided critical information that supported preliminary clinical applications, the results have all been obtained in different groups of animals, focusing on specific

modalities, and at discrete time-points. Therefore, the interactions and inter-dependencies between foreign body reactions and electrical properties over time have not been studied systematically and conjointly in longitudinal studies. To date, no chronic and realistic studies on intra-neural thin-film electrodes have been carried out that would allow to understand the impact of cabled implants and regular stimulation on electrode function and implant bio-integration.

Here, we studied the selectivity, stability, functionality, and biocompatibility of the SELINE polyimide-based intra-neural implant [25, 27] that was inserted into the sciatic nerve of 28 healthy rats for durations of up to 6 months. After a period of adaptation that approximatively lasted one-month, the electrodes exhibited stable and selective responses to charge delivery that persisted over the remaining duration of the experiments. This selectivity supported the development of real-time stimulation paradigms that precisely controlled the forces produced at the ankle. In the same rats, we characterized the development of foreign body reaction over time, and showed that they tightly correlated with concomitant changes in the electrical properties of the electrodes. The removal of the implant did not cause detectable motor impairments and did not further harm the tissue. These results uncover relationships between actively used implants and neural tissue responses in rats and demonstrate the stability of intra-neural electrodes for reliable long-term interfacing with the peripheral nervous system. Our study assessed for the first time intra-neural thin-film electrode performance and in vivo biocompatibility in a chronic rat model. These unprecedented findings obtained in ecological settings provide a framework for the safe and long-term implantations of polyimide-based intra-neural electrodes in clinical applications.

2. Material and Methods

2.1 Animals

All animal procedures and experiments were approved by the Veterinarian Offices of the Cantons of Vaud and Geneva, Switzerland. A total of 28 adult female Lewis rats (LEW-ORlj, Charles River Laboratories, France) with initial weight of ~200 g were implanted with a SELINE electrode [25] into their left sciatic nerve (**Fig. 1A**), with different indwelling times up to 6 months (**Fig. 1B** and table **T1**). Animals were housed three by three on a 12-hour light-dark cycle with food and water ad libitum and were given social time three times a week.

2.2 Chronic experimental model

The experimental model is graphically summarized in **Fig. 1A**. All surgical interventions were performed under aseptic conditions and full anaesthesia with isoflurane in oxygen enriched air (1 - 2 %). Animals were placed on a heating pad to prevent hypothermia during surgery. Analgesic medication (Buprenorphine (Temgesic), ESSEX Chemie AG, 0.01–0.05mg per kg, subcutaneous) and antibiotics (Amoxicillin (Clamoxyl), Pfizer AG, 0.5 ml/kg, subcutaneous) were administered during 5 days post-surgery.

Intra-neural electrode implantation with connector on backstage. The detailed implantation procedure for the SELINE electrode has been described previously [25]. Briefly, two longitudinal skin incisions of about 2 cm were made over the lower lumbar spine for the fixation of the backstage and at the level of the mid-thigh for access to the sciatic nerve. The sciatic nerve was carefully exposed and freed from surrounding tissues. Then, the 3D printed pedestal was sutured via its five suture-holes to the fascia of

the back-muscles (multifidus muscle and gluteus superficialis) through a 2x2 cm piece of surgical mesh (Mersilene mesh, Ethicon Inc., NJ) to favour the stabilization by fibrotic healing. The wires (CZ1187, Cooner Wire Corp. CA), leading from the nano-strip connector (NPD-18-DD-GS, Omnetics Connector Corp, MN) incorporated in the pedestal to the printed circuit board (PBC), were passed subcutaneously and under the gluteus maximus muscle with a plastic tunnel guide until the opening leading to the sciatic nerve. From here, the needle was obliquely inserted into the sciatic nerve about 0.5 cm above bifurcation of tibial and peroneal branches. The wires were subcutaneously placed in a stress-release loop to allow for leg movements without pulling on the implant via the cables.

Intramuscular electrodes implantation with connector on head-stage. To record electromyography (EMG) activity, bipolar electrodes were implanted into the following four muscles of the left hindlimb: tibialis anterior (TA), soleus (SOL), gastrocnemius medialis (GM), plantaris (PL). Recording electrodes were fabricated by exposing a 0.5 mm notch in the insulation of the implanted Teflon-coated stainless steel wires (AS632, Cooner Wire Corp., CA). A common ground electrode was created by removing 1 cm of insulation at the distal extremity of one wire, which was then subcutaneously placed over the left shoulder. All wires were subcutaneously tunnelled to a single percutaneous amphenol connector (Omnetics Connector Corp., MN) that was fixated with dental cement to the skull of the rat.

Surgical interventions were identical for all animals, except for the acute group, which did not receive intramuscular electrodes and whose animals were euthanized immediately after testing the stimulation performance of the electrodes. After surgery, all rats were placed in an incubator until full recovery from anaesthesia. An additional recovery period of minimum five days was given before the start of the experiments. From there, both percutaneous interfaces at the back and at the head were cleaned regularly with saline or antiseptic spray (Bepanthen Plus, Bayer, Germany) as preventive treatment for infections.

2.3 Chronic electrode characterization

Weekly muscle recruitment curves were performed by injecting increasing amounts of charge into each of the channels of the intra-neural implants while recording the obtained compound muscle action potential (CMAP) from each of the implanted muscles. A custom-made stimulation protocol (Matlab, The MathWorks Inc, MA) prompted a stimulator unit (IZ2H, Tucker Davis Technologies, FL) to deliver biphasic cathodic-first current pulses (1 Hz) of 40 to 80 μ s duration and increasing current intensities ranging from sub-threshold to a saturation value of the recorded CMAPs for the channel being tested. The EMG activity was amplified (1000x, Differential AC amplifier, AM systems, WA), filtered (10 – 2000 Hz bandpass) and acquired into the TDT RZ2 system (Tucker Davis Technologies, FL) at 25 kHz for offline analysis. For every channel, three repetitions of each current step were performed and the average peak-to-peak amplitude of the evoked CMAP of each muscle was used to analyse the relationship between stimulation-evoked muscle activity and stimulation intensity via the computation of muscle recruitment curves (**Fig. 2A**). At the end of the experiment, the recorded CMAPs were normalized to their maximal amplitude obtained throughout the duration of the experiment (up to 6 months). These data were then used to assess the stability of the selectivity in muscle recruitment for every channel, as well as the stability of the electrical properties of the implants over time (**Fig. 2A**). Selectivity for every muscle was assessed by calculating a selectivity index (SI) as the ratio between the normalized CMAP of a target muscle i , and the sum of the normalized CMAPs elicited in all muscles recorded [17, 36]:

$$SI_i = \frac{CMA P_i}{\sum_{j=1}^n CMA P_j},$$

For a given channel, the SI for muscle i was comprised between 0 and 1, with 0 indicating absence of selectivity and 1 demonstrating exclusive activation of muscle i without activating other muscles. A channel was considered selective for one of the tested muscles when the SI for this muscle was higher than 0.6. In the rat sciatic nerve, SOL, GM, and PL muscles are innervated by the tibial fascicle, while the TA muscle is innervated by the peroneal fascicle (**Fig. 2A**). As such, inter-fascicular selectivity (tibial vs. peroneal nerve stimulation) and intra-fascicular selectivity (SOL vs. PL vs. GM activation) could be assessed in this experimental setup.

To assess the stability of the implant in terms of electrical properties, for each channel, the threshold charge, defined as the amount of charge necessary to elicit 10% of a maximal muscle activation, was used to assess changes in stimulation efficiency from week to week. Further, impedance between each channel (intra-neural) and the implant's ground (extra-neural) was measured at 1 kHz in vivo on a weekly basis. The stability over time in both metrics was assessed via pair-wise comparisons between all weeks (Kruskal-Wallis test) and further underlined by fitting an exponential decay function to the median time derivatives of each metric. Finally, we counted the number of functional channels per implant according whether muscle activity was elicited within the charge injection limits defined by cyclic voltammetry [26, 37].

2.4 Control of movement amplitude

We assessed the possibility of functionally exploiting the obtained selectivity and performed amplitude and frequency modulations of the stimulation for selective channels. Rats were lightly sedated with Dormitor (Dorbene, Graeb, DE, 0.0025 – 0.005 ml/kg) to isolate the effect of the stimulation protocol from possible movements artefacts, and were then placed at the edge of a small table with the legs hanging (**Fig. 3A**). The tail was pulled up with a string and fixated to an artificial ceiling in order to maintain the lower trunk straight and to ensure that legs were free from movement constraints. Joint kinematics of the left hind-limb were recorded using the high speed motion capture system Vicon (Vicon Motion Systems Ltd., UK) combining 12 infrared cameras (200Hz). Small reflective markers were positioned on the left hind-limb joints iliac (crest), greater trochanter (hip), lateral condyle (knee), lateral malleolus (ankle), and the distal end of the fifth metatarsal (MTP). The kinematic data (200 Hz) and EMG signals (2 kHz) were recorded simultaneously during stimulation protocols of either increasing amplitude (pulse width at 40 μ s and frequency at 1Hz) or increasing frequency (pulse width at 40 μ s and amplitude at value with highest SI obtained). For each step during either modulation, we computed the ankle joint angle using the cosine law

$$\odot = \cos^{-1} \frac{A \cdot B}{||A|| \cdot ||B||} * \frac{180}{\pi},$$

where A and B are equal to the length of the segment between the knee and ankle markers and the ankle and foot markers respectively.

2.5 Closed-loop control of movement-evoked traction force

To assess the controllability of the observed modulation of movement amplitude with stimulation frequency, we built a proportional-integral controller (PI) that adjusted the stimulation frequency in closed loop based on real-time recordings of the traction force that was produced by the ankle

movement upon stimulation through one channel. The real time control platform was implemented in a multithreaded C++ code (Visual Studio 2012, Microsoft, WA) as an adaptation from a real-time control structure previously developed [38]. Electrical stimulation was delivered with an IZ2H stimulator (Tucker Davis Technologies, FL) and real-time readouts of the evoked traction force were recorded through the Vicon system via a force plate (2 kHz, HE6X6, AMTI, MA). The data flow was imported into the C++ environment via Ethernet using the DataStream SDK software. The recorded force signal was filtered online using least mean squares adaptive filters and compared to a defined target force (either constant step function, for the characterization of the controller, or a sinusoidal function to assess controllability).

The rat foot tip was attached with a string to the three-dimensional force plate while verifying no initial pulling was exerted from the leg hanging position over the string to the force plate. The controller adjusted the frequency of stimulation based on the error between the evoked traction force and the target force as follows:

$$f_{t+1} = f_t + k_p e_t + k_i \int_{t-20}^t e_t dt,$$

where f_t is the frequency of stimulation at frame t , k_p and k_i the coefficients for the proportional and integral parts respectively of the controller, and e_t the error between the produced force and the target force at frame t .

Characterization. We characterized the controller in a series of experiments, during which we put step functions of height 0.2 N and width 3.5 s as target force and varied the coefficients for the proportional and integral parts of the controller (**Fig. S1**). Initial overshoot (% of target force), rise time (s) and sum of squared errors between target force and evoked traction force were measured to assess the performance of each variant of coefficients. A Kruskal-Wallis test was used to assess differences between the variants across rats and channels.

Closed-loop control of stimulation frequency. We performed an additional set of experiments, to demonstrate the controllability of the produced muscle activations in real time. In each trial, the stimulation frequency was updated continuously based on the difference between recorded and target force, which in this case was a sinus function (wavelength 3 seconds, 3 repetitions). Values for the proportional (k_p) and the integral (k_i) corrections of the controller were obtained from the characterization experiment, and were kept constant during each trial. To measure performance, we assessed correlation between the evoked and the target force throughout the three repetitions. We further computed the sum of squared errors between evoked and target signal and analysed the distributions of frequencies used to reproduce the target force during each repetition of the sinus function.

2.6 Impact on fine motor control

Previously obtained electrophysiological and walking track data has shown that the implantation of polyimide based implants does not alter nerve conduction velocities or stepping patterns [25, 34]. To complement this data, 5 rats were trained daily during two weeks to perform a skilled locomotor task, consisting in walking over a horizontal ladder with irregularly spaced rungs (**Fig. 7A**). Rats were tested prior to surgery, and every five days post-surgery during one month. After this period, we explanted the implant from the nerve and tested leg motor control capacities of these rats on the ladder task during an additional month. Each session consisted in high-resolution kinematic recording (Vicon system) of

five trials (runs). Performance per animal was computed as the percentage of missed steps across these five runs. A Kruskal-Wallis test was used to assess differences in performance between the different evaluations time-points before and after the implantation. One month after the removal, all rats were euthanized, perfused and their sciatic nerves were dissected for histological analyses to further assess the impact of the explant surgery on the nerves.

2.7 Perfusion and tissue handling

At the end of each experimental procedure, rats were terminally anaesthetized with an overdose of pentobarbital and trans-cardially perfused with Ringer's solution containing 100000 IU/L heparin and 0.25 % NaNO₂ followed by 4% phosphate buffered paraformaldehyde (pH 7.3) containing 5% sucrose. The implanted sciatic nerve was harvested alongside with the contralateral control nerve and fixed overnight in the same fixative solution. The nerves were washed in PBS 0.1M, routinely processed, embedded in paraffin blocks, and each sample was entirely sectioned (transversal cross sections) on a microtome (Hyrax M25, Microm, DE) at 4 µm thickness. Three tissue sections in a ribbon of ten were selected and mounted 3 by 3 onto coated glass slides (Superfrost Ultra Plus, Thermo Fisher Scientific, MA), yielding tissue slices of approximatively every 40 µm between glass slides. Mounted tissue samples were dried overnight at 37°C and then stored at +4°C until use. Unless otherwise stated, the tissue reaction to the implants was assessed at the level of the implant (due to the oblique insertion, we selected slices with the implant visible in the tibial fascicle to ensure comparability between animals) and compared to tissue samples proximal and distal to the implant (≥ 0.75 mm above and below entry/exit of electrode into the nerve) as well as to control tissue (intact contralateral nerve). The tissue at implant level of two animals (one in group P0 and one in group P165) could not be used for histology or immunohistochemistry as cutting on microtome resulted in torn tissue slices that were improper for analysis.

2.8 Histology and immunohistochemistry

Hematoxylin and Eosin (H&E) and Sirius red (SR) staining were performed with the automatic Tissue-Tek Prisma & Coverslipper HQ^{plus} machine (Sakura, NE). For immunohistochemistry, slides were dewaxed and placed 20 minutes in a citrate buffer bath (pH 6) at 95°C for antigen retrieval, followed by 1h blocking of non-specific sites in bovine serum albumin (BSA 1% diluted in PBS 0.1M). Selected sections were then processed for immunohistochemical labelling against myelin (myelin basic protein MBP, 1:200, Abcam), axons (anti-beta III tubuline TUJ1, 1:200 Abcam), macrophages (anti protein CD68 1:400 Serotec), DNA/RNA of cells (4', 6-diamino-2-phenylindole DAPI, 1.5 µg/mL, Vector), and fibroblasts (fibroblast specific protein FSP1/S100A4, 1:200, MerckMillipore). The following secondary antibodies were used: goat anti-rabbit IgG Alexa488, goat anti-rat IgG Alexa555, goat anti-mouse IgG Alexa555 (ThermoFisher Scientific). Briefly, after blocking, sections were incubated overnight in a primary antibody solution at 4°C, followed by rinses in PBS 0.1M. Sections were then incubated once more for 1h in a solution of appropriate secondary antibody (Life Technologies) diluted 1:400 in PBS 0.1M and BSA 1%, followed by additional rinses. Slides for fluorescence microscopy were mounted with a DAPI-containing medium (Vectashield, Vector Laboratories). All stained slides were cover-slipped for microscopy.

2.9 Image acquisition, selection, and processing

Images of slices stained with H&E and SR were acquired with an optical microscope at 20x magnification (Olympus slide scanner VS120-L100, Olympus Corp., EU) while immunohistochemically stained tissue was imaged with a fluorescence microscope at 40x magnification (Leica TCS SPE, Leica Microsystems, DE).

Determination of cross sectional area of i) nerve fascicles, ii) capsule, and iii) the foreign body giant cells layer carried manually out on H&E or SR sections using open source software Fiji for the area measurements (ImageJ, NIH, MD). Blood vessels were also counted manually on H&E sections in the entire tibial fascicle. All manual quantifications were performed twice to ensure robustness. Collagen deposition was quantified on SR stained sections (**Fig. S2**) and the number of cell nuclei in the nerve fascicles was determined on H&E sections (**Fig. S3**), both with custom-written routines in Fiji-ImageJ, detailed in the supplementary material. 3D reconstructions of fascicles, capsule, and the implant were performed by manually contouring the different components on the images obtained from H&E stained serial sections (400 μm between sections) and subsequently aligning them in Neurolucida software (MBF Bioscience, Williston, VT, USA) for 3D reconstruction.

Macrophage presence in the different compartments of the nerve cross-section (extra-fascicular space, local endoneurial environment, and distant endoneurial environment) as well as the fiber density and their distance to the implant were quantified with two other custom written routines in Fiji (**Fig. S4** and **Fig. S5** respectively and Supplementary methods).

For the quantification of any metric in images obtained from immunohistochemistry, 3 consecutive sections per animal were analysed. Due to the oblique insertion, at the implant level, we selected slices with the implant visible in the tibial fascicle to ensure comparability between animals. This fascicle was then referred to as local endoneurial environment, as opposed to distant endoneurial environment for fascicles in which the implant was not present in the cross-section being analysed. All images displayed in figures of the paper have been adjusted for brightness and contrast in accordance with guidelines for accurate data representation of this journal.

2.10 Statistical analyses

Whenever possible (at least 5 data points per group), we employed a non-parametric Kruskal-Wallis test to assess differences between groups. To determine time-dependent impact of the implant on the host tissue, we conducted Spearman's correlation analysis between all the metrics that were quantified across groups.

3. Results

A SELINE implant was inserted into the sciatic nerve of 5 rats for a duration of 6 months. We performed weekly measurements and functional experiments in order to assess the functional stability of the implants for chronic applications and to determine their bio-integration within the implanted nerve (**Fig. 1A**). In addition, six groups of rats ($n = 3$ rats per group) underwent the same surgical procedures, but were sacrificed at earlier time-points in order to evaluate the relationships between the developing foreign body reaction and the electrical properties of the electrodes during the first month after implantation (**Fig. 1B** and **Table 1**). One group of rats was not stimulated, while the other groups received the same stimulation protocols as the rats implanted for 6 months.

3.1 Stability and selectivity of polyimide-based intra-neural implants

Post-mortem dissection revealed that all the implants had remained secured within the sciatic nerve over the entire duration of the experiment. None of the rats had to be terminated prematurely. During intra-operative testing, all the channels of all implants elicited movement. Weekly evaluations showed that 60% of channels were functional at 3-month post-implantation, and that 25 out of 100 channels were functional at 6-month post-implantation (**Fig. S6**), corresponding to about 12 years in humans [39].

To characterize the selectivity of implants, we conducted weekly measurements of the relationships between the amount of injected charge in each channel of the implant and the amplitude of compound muscle action potentials (CMAPs) elicited into flexor and extensor muscles of the ankle (**Fig. 2A**). We thus obtained recruitment curves, which are illustrated for two representative channels in **Fig. 2A**. To quantify the selectivity of muscle activation, we calculated a selectivity index (SI), which expressed the relative activation of a given muscle compared to all the recorded muscles (**Fig. 2A**). Immediately post-implantation, approximatively 80% of all channels exhibited a selectivity index above 0.6 (**Fig. 2B**). Channels classified as non-selective ($SI < 0.6$) were likely located between fascicles in the extra-fascicular space. About 30% of the selective channels activated a single extensor muscle, thus displaying intra-fascicular selectivity. The selectivity index of channels engaging extensor versus flexor muscles remained stable over time, even at 6-month post-implantation (**Fig. 2B**).

To evaluate the stability of electrical properties, we measured the stimulation threshold charge and impedance of all channels over the entire course of the experiment. We found that both electrical properties progressively increased during the first month post-implantation and then remained stable over the subsequent months (multiple comparison Kruskal-Wallis, $p < 0.05$, **Fig. 2C**). A similar increase in threshold charges was observed in the short-term groups (**Fig. S7**).

3.2 Functionality of polyimide-based intra-neural implants

We then exploited the selective channels to evaluate the usability of the implant for functional applications. Rats were lightly sedated and then positioned with the legs in the air (**Fig. 3B**). We measured the changes in leg kinematics and muscle activity when applying stimulation of increasing amplitude or frequency through the channels (**Fig. 3B**). Stimulation at low amplitude elicited a dorsiflexion or a plantarflexion of the ankle, depending on the selectivity of the selected channel. Increase in stimulation amplitude resulted in co-contraction of ankle flexors and extensors, which blocked the foot in a fixed position (**Fig. 3B**). On the contrary, we found a robust linear relationship between the stimulation frequency and the movement of the ankle in dorsiflexion and plantarflexion (**Fig. 3C**).

This result suggested that the stimulation frequency could be exploited to control the movement of the ankle. To test this possibility, we built a proportional-integral (PI) controller that adjusted the stimulation frequency in order to minimize the discrepancy between the traction force produced by the movements of the foot and a desired force (**Fig. 3A**). We used a step function (3.5 sec, 0.2 N) to calibrate the proportional and integral coefficients of the controller (k_p and k_i respectively) for the control loop feedback mechanism (**Fig. S1**). The optimal coefficients of the controller were consistent across rats (**Fig. S1C**). We used these coefficients to adjust the stimulation frequency in closed-loop in order to control the traction force in real-time. The controller was able to reproduce a sinusoidal force with high fidelity (**Fig. 3D**, $R^2 = 0.85$, $n = 4$ rats with 2 or 3 channels/rat). To maintain a minimal error between the desired

and recorded traction forces, the PI controller gradually increased the range of stimulation frequencies (**Fig. 3D**).

3.3 Assessment of the developing foreign body response

We conducted a series of macroscopic and microscopic evaluations on the explanted nerves in order to assess the physical and biological consequences of the implant on the sciatic nerve.

3.3.1 Macroscopic observations

Dissection of the implanted nerves revealed the presence of hemorrhages in the acute group, which were due to the interruption of superficial blood vessels on the nerve surface during implantation (**Fig. S8**). With the exception of the acute and short term groups (up to 7 days post-implantation), all the explanted nerves displayed an excess of connective tissue at the entry and exit sites of the implant in the nerve. The overall shape of the nerves remained preserved, although chronically implanted nerves (6 months) displayed an enlargement around the site of implantation.

3.3.2 Encapsulation of polyimide-based intra-neural implants

We stained serial cross-sections of the implanted nerves with Hematoxylin and Eosin in order to assess the general morphology of the nerve. Comparison of acutely (**Fig. 4A**) and chronically (**Fig. 4B**) implanted nerves revealed that the size of the nerve increased in the region surrounding the implant. This local enlargement derived from a capsule mainly composed of collagen and fibroblasts that enveloped the implant (**Fig. S9**). To quantify this enlargement, we measured the diameter of the nerve along its length (**Fig. 4C**), the cross-sectional area of each fascicule, and the area of the capsule surrounding the implant in each group of animals. We found that the local enlargement of the nerve was primarily due to the progressive growth of this capsule around the implant (Spearman's $R = 1$, $p < 0.0001$, **Fig. 4D**). The capsule developed during the first 3 to 4 weeks after implantation, and then remained stable during the subsequent months post-implantation (**Fig. 4D**). 3D reconstructions of the fascicles, the implant and the surrounding capsule illustrated the important contribution of the fibrotic capsule to the enlargement of the nerve (**Fig. 4C**).

We then quantified the components of the capsule and of the local endoneurial environment (**Fig. 4E**). For this, we stained nerve cross-section with Sirius Red in order to measure the area of the tight encapsulating layer of multinucleated giant cells around the implants, and the density of collagen in the local endoneurial environment. We also assessed the level of cellularity and microvascular density on Hematoxylin and Eosin stained sections. We found aggregates of multinucleated giant cells around and in direct contact with the surface of the implant as early as seven days post-implantation. This layer increased gradually during the first four weeks post-implantation. At the chronic time-point, the size of this layer was considerably reduced (**Fig. 4E**). The amount of collagen relative to the cross-sectional area of the local endoneurial environment did not increase during the first four weeks post-implantation. While the size of neither nerve nor capsule changed between 4 weeks and 6 months post-implantation, the density of collagen was increased at the chronic stage (**Fig. 4E**). The cellularity in the local endoneurial environment increased considerably, starting at 7 days post implantation (**Fig. 4E**). In turn, the endoneurial microvascular density increased as early as 1.5 days post-implantation. Both cellularity and microvascular density remained elevated at the chronic stage. Spearman's correlation analysis

revealed that the growth of the capsule was significantly correlated with the amounts of vasculature ($R = 1$, $p < 0.0001$), collagen ($R = 0.997$, $p < 0.0001$), and cellularity ($R = 0.78$, $p < 0.0001$) measured over time.

3.3.3 Inflammatory response to polyimide-based intra-neural implants

The insertion of the implant into the nerve triggered an inflammation that aimed to eliminate the debris caused by the penetration and to engage repair mechanisms. To visualize the spatiotemporal dynamics of this inflammation, we stained nerve cross-sections against the monocyte/macrophage marker CD68 and evaluated the inflammation in the different nerve compartments (**Fig. 5**). Macrophages migrated from the vascular system, and infiltrated the extra-fascicular space early after implantation (**Fig. 5A**). The macrophages were first recruited at the site of injury in the local endoneurial environment. Evaluations at 17 days post implantation showed that both the local and the distant endoneurial environments were densely populated with macrophages (**Fig. 5B**), indicating a multifocal infiltration throughout the endoneurial environment. While inflammatory cells were still present in the chronic stage, their distribution was restricted to the local endoneurial environment (**Fig. 5C**). To measure these inflammatory responses, we calculated the relative amount of fluorescence with respect to the total area of the different nerve compartments (**Fig. 5D**).

To visualize the spatiotemporal pattern of macrophage infiltration in the local endoneurial environment, we further quantified the area of each detected fluorescent particle as a function of its distance from the implant (**Fig. 5E**). This analysis revealed a progressive accumulation of small particles into large clusters in the early phase of inflammation. Even though the presence of macrophages persisted in the chronic stage, the mean area of macrophage clusters returned to near-normal values. Moreover, their presence was more concentrated around the implant.

3.3.4 Impact of the implant on fiber density

Finally, we assessed the impact of the implant on the fiber density of the nerve and the fiber organization around the implant. To visualize the fibers, we stained nerve cross-sections with antibodies against axons (TUJ1) and myelin (MBP). The penetration of the implant induced an immediate change in the morphology of the fibers. Close inspection showed that the axons adopted a bended shape indicating that the fibers had moved aside from the implant during the insertion. Indeed, we did not detect intra-neural hemorrhage, suggesting that the fibers were not severed during implantation (**Fig. 6A**). In the sub-acute stage, the nerve cross-sections exhibited swollen axons and spheroid formation in close vicinity of the implant (**Fig. 6B**). At the early stage, a zone void of axons appeared around the electrode, which corresponded to the ongoing formation of a capsule (**Fig. 6C**). From this time point to the chronic stage, this zone progressively expanded (**Fig. 6D-E**). Axons that were mainly non-myelinated gradually reappeared within this zone, with some axons present as close as 30 to 50 μm from the implant (**Fig. 6D-F**).

We used a semi-automatic algorithm to estimate the density of myelinated fibers in the entire nerve cross-section both proximal and distal to the implant (**Fig. 5S**), and normalized the measured density also to contralateral control counts obtained from non-implanted nerves (**Fig. 6G**). At each time point examined, the ratios of proximal to distal measurements as well as distal to control measurements were close to one, suggesting that no substantial change in number of myelinated fibers had occurred after implantation. Despite the development of the capsule, we consistently observed an increase in detected axons at the implant level that was highest at 4 weeks post implantation, and had remained substantially increased in the chronic stage (**Fig. 6H**). We further measured the distance between the

implant and the closest 20% of axons and myelin (**Fig. 6I**). Both axons and myelin moved away from the implant during the first month post-implantation. Albeit in the chronic group the mean distance of the closest 20% of fibers resembled the distance from the implant in the acute group, the myelin had not moved closer, suggesting that most of the regenerating axons did not bear myelin.

3.3.5 Stimulating through a tethered implant does not exacerbate the foreign body reaction

Previous studies have been looking at passive and untethered intra-neural electrodes to assess the induced foreign body reaction of such implants. To evaluate the potential impact of providing stimulation to the nerve, we created an additional control group that was implanted with a tethered implant and EMG electrodes but through which no stimulation was provided. Even though the number of animals per group did not allow us to statistically assess whether such differences were present, we obtained very similar results in all metrics examined for the animals in the groups 28a (active) and 28p (passive).

3.4 Impact of implantation and implant removal on fine motor control

We finally assessed the impact of the insertion and removal of the implant on leg motor control capacities (**Fig. 7**). For this, we conducted kinematic analyses during locomotion along the irregularly spaced rungs of a horizontal ladder. We calculated the percent of missed steps of the leg with the implant during evaluations conducted before implantation, at regular intervals after implantation, and during one month after the removal of the implant. We did not detect significant alteration of locomotor performance after insertion or removal of the intra-neural implant at any of the test time-points ($p > 0.05$, Kruskal-Wallis test) (**Fig. 7B**). Evaluation of the nerve tissue one month after removal (**Fig. 7C**) showed the expected signs of scarring, mainly reflected by enhanced collagen deposition and increased cellularity at the passage site of the implant. Immunohistochemical labelling against macrophage/monocyte marker CD68 revealed the sparse presence of macrophages along the passage site of the implant. These signs of inflammation were extremely small when comparing with the presence of inflammatory cells in the nerves that were implanted during one month and in which the implant was not removed (groups 28a and 28p).

4. Discussion

We studied the selectivity, stability, functionality and biocompatibility of polyimide-based intra-neural implants that were inserted into the sciatic nerve of rats for extensive durations. We found that after a period of approximatively one month during which a foreign body reaction developed around the implant, the electrodes maintained stable and selective responses to charge delivery that enabled the precise control of ankle movements in anesthetized rats. We discuss these results with a particular emphasis on the stability and usability of polyimide-based intra-neural implants, the implication of the acute and chronic biological responses to the implant insertion, and the potential of this technology for long-term clinical applications.

Stability and usability of polyimide-based intra-neural implants

We found that the SELINE electrodes exhibit remarkably stable responses to charge delivery that supported robust stimulation selectivity over extended periods of time notwithstanding the development of an encapsulating layer around the implant, in particular during the first month after insertion. Encapsulation of intra-cortical implants has been linked to the decline of recording electrode functionality [40-42]. Likewise, experimental and modelling studies showed that the accumulation of multi-nucleated giant cells around the implant alter the path of current flow [43, 44]. We confirmed these findings in the context of peripheral nerve stimulation. We found a tight relationship between the thickness of the encapsulating layer around the implant and the graded increases in stimulation thresholds and electrode impedance (**Fig. 8**).

The biological response to the insertion of the implant was particularly pronounced during the first month after implantation. During this period, the electrodes displayed a logical increase in impedance, which led to an augmentation of stimulation thresholds for the majority of the electrodes, stabilizing around 20nC. Such thresholds have been shown to be extremely usable in human applications [18] and are about one magnitude lower than epi-neural electrodes in similar applications [45]. Further, we were able to exploit the implants for long-term functional applications. Two factors contributed to maintaining implant functionality. First, inter-fascicular selectivity was preserved under a certain range of stimulation currents. Second, we were able to leverage this selectivity through the modulation of stimulation frequency. Indeed, we found a linear relationship between the stimulation frequency and hind-limb kinematics, which we used to control a range of forces with high precision in anesthetized rats. Previous studies similarly showed that the stimulation frequency was the most reliable control parameter to modulate limb kinematics or sensory information using intra-neural or epidural spinal cord stimulation protocols [18, 46-49]. This high degree of controllability over extended durations opens a realistic pathway to utilize intra-neural implants to alleviate motor deficits and restore graded sensation for a range of clinical applications.

Immediate and chronic biological responses to the implant insertion

The characterization of the immediate mechanical consequence and long-term biological response to implant insertions plays an important role in optimizing implant design and validating their usability for clinical applications [50]. Here, we conducted comprehensive anatomical evaluations to characterize the mechanical impact, inflammation and foreign body reaction following insertion of the SELINE implant, and propose methods to mitigate these detrimental responses.

We observed a succession of inter-connected changes over the course of the implantation. During the first hours after insertion, the nerve fibers displayed minimal mechanical displacements in the immediate vicinity of the implant, but without noticeable signs of damage. As early as one day after the insertion, axonal swelling and myelin breakdown became apparent. Previous studies linked these changes to Wallerian degeneration [51, 52]. We found that during the first week after implantation, the macrophages emerging from the extra-fascicular space had migrated into the endoneurial space. The role of these macrophages is to scavenge and remove myelin and axonal debris [51-53]. During the same period, a capsule developed around the implant, forming an approximately 100 μm broad zone of enhanced cellularity, mainly composed of monocytes and fibroblasts. This capsule increased in size until one month post-implantation, consistent with the one-month period of adjustments in the electrical properties of the implants (**Fig. 8A**). At extended time-points post-implantation, the capsule combined two adherent layers. The first layer consisted of an accumulation of densely packed multinucleated giant

cells in close vicinity of the implant (approximately 25 μm thickness). The second layer (about 100-150 μm thickness) was composed of a fibrotic network surrounding the multinucleated giant cells (**Fig. 8B**). The size and structure of this capsule was comparable to the capsule that forms around implants inserted into the cerebral cortex [42]. Despite the persistence of inflammatory signs in the chronic stage, the amount of macrophages was substantially reduced compared to early time-points, and remained confined to the proximity of the implant. The evolution of this inflammatory process replicates Wallerian degeneration associated responses that have been described previously [54-59].

The astroglial foreign body reaction that spontaneously forms around the tip of electrodes inserted in the brain repels nerve fibers, thus preventing central axons from sprouting into the capsule [60]. For this reason, the capsule has been termed the dead zone [40]. In contrast, we found the presence of small diameter unmyelinated fibers scattered throughout the second layer of the capsule, starting a few weeks post-implantation. These results suggest that the fibrotic network and sustained foreign body reaction triggered by the implant do not prevent regenerative sprouting of peripheral nerve axons. These axons may arise from two mechanisms. First, these thin axons may represent ongoing attempts of damaged axons to regenerate through the capsule. Second, this local sprouting may result from the continuing micro-motion of the tethered implant, which may trigger a recurrent cycle of inflammatory reaction and regenerative sprouting. We detected an increased microvascular density and the presence of potentially pro-regenerative macrophages that have both been linked to regeneration in the peripheral nervous system [61-66].

These biological responses led to an increase in impedance of the electrodes and in threshold charges during the first month post-implantation in all the tested rats. These pathophysiological reactions may be linked to the mechanical insult due to the insertion of the implant, to the foreign body response, the repeated delivery of current, or a mixture of all these mechanisms. To estimate the role of charge delivery, we conducted anatomical evaluations in a group of rats that never received electrical stimulation through the implant. This group of rats exhibited identical biological responses compared to rats that received stimulations, suggesting that electrical stimulation is not a major contributor in the development of the foreign body reaction. Consequently, we propose that the foreign body reaction was primarily linked to the insertion-associated trauma together with the mechanical and chemical mismatch between the implant and the nerve. Many strategies may mitigate the mechanical impact of the implant insertion. First, computational modelling may help identifying optimal conditions for surgical insertion of the implants. Second, the geometry of the implant plays a significant role in defining the amount of damage during insertion. Here, we have exploited computational modelling of tissue deformation to various geometries of the entering implant tip [67] to design implants that minimize penetration damage. The limited damage observed a few hours after implantation confirmed that minimal mechanical damage resulted from the insertion. Shape memory alloys that change properties with temperature may provide an alternative material to design implants minimizing penetration damage [68]. Third, various strategies are explored to reduce the chemical and mechanical mismatches between tissue and implant, which both play a significant role in triggering foreign body reaction responses [69]. Chemically, surface modification of substrate materials [41, 70] and organic materials [71] may aid reducing the inflammation and granulomatous reaction, respectively. Mechanically, previous studies showed that reducing the mechanical mismatch between the neural structures and the implants minimize foreign body reaction. For example, soft spinal implants that matched the mechanical properties of the targeted tissue showed remarkable bio-integration in the central nervous system [72].

Potential for long-term clinical applications

The limited understanding of the dynamic interactions between neural tissue and implant functionality has hindered the dissemination of penetrating peripheral nerve implants in clinical applications. Despite the diversity of intra-neural implants with various levels of selectivity, these implants have only been used for research studies in humans. Here, we conducted a comprehensive characterization of polyimide-based implants to quantify the potential of these electrodes for clinical applications. Based on our results in rats, we anticipate that this type of implants has the potential to remain functional for extended periods of time in humans. Indeed, the size mismatch between the implant and the targeted nerve is substantially larger in rats compared to humans. Consequently, the impact of motion and tethering forces are substantially larger in rats compared to humans, adding challenges for the resistance of the electrodes, wires, and connectors, which are often among the reasons for the loss of functional channels over the time of implantation. Moreover, rats exhibit more pronounced inflammatory responses and fibrotic reactions compared to humans [73]. The present results cannot be directly extrapolated to the nerve environment of humans. Yet, the milder foreign body reaction and lower impedance of intra-neural electrodes reported in larger animal models and humans suggest that the electrode stability observed in rats over 6 months could translate into implants that remain functional and selective for periods ranging from years to decades in humans [39]. Moreover, we show that the removal of the implant from the nerve in the chronic stage did not cause detectable alteration of fine motor control capacities in our rats. Indeed, anatomical examination revealed that the removal of implant did not damage the neural tissue beyond an expected but non-impairing scar formation. The possibility to remove and exchange non-functional implants is critical for clinical settings. The present results open realistic perspectives for long-term clinical applications, including motor prostheses to alleviate motor deficits of the upper and lower limbs as well as sensory prostheses to restore lost sensation.

5. Conclusion

We characterized the electrical and functional properties of polyimide-based intra-neural implants and their bio-integration in the peripheral nerve environment over extended durations in rodents. These parallel assessments provided a comprehensive and integrated understanding of the interplay between the properties of the implant and the biological responses to the chronic implantation. This knowledge is essential to refine and validate implants for long-term human applications. We showed that despite the unavoidable foreign body reaction, the electrodes remained functional and selective for the entire duration of the experiments, supporting high-precision control over ankle movements. Our results provide a comprehensive assessment of polyimide-based intra-neural electrode functionality, stability, and bio-integration and open a realistic pathway to refine and disseminate such implants in clinical applications in humans.

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Figure captions

Fig. 1 Experimental setup for chronic assessment of functional polyimide-based intra-neural implants.

(A) The self-opening intra-neural polyimide-based multi-channel electrode is implanted into the rat sciatic nerve proximal to the separation of the different fascicles. The connector of the implant is embedded in a percutaneous 3D-printed pedestal (back-plug) that is sutured to the muscles on the back at the sacral spinal cord level. EMG activity of ankle muscles (via percutaneous connector mounted on the head (head-plug)) and kinematics of hind-limb movements (via a motion capture system with reflective markers) are recorded during different experimental protocols. PCB: printed circuit board. (B) Experimental timelines for the chronic group (n=5) and the acute to long-term groups (n=23). 0 to 28 refer to the days post implantation, indicating the different groups. 28a: active electrodes implanted during 28 days, 28p: passive electrodes implanted during 28 days. At 30 days post-implantation, 5 animals had the implant removed and were tested during another month. All other groups had n=3 animals in the non-chronic group.

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Fig. 3 Functional experiments demonstrate controllability of intra-neural PNS.

(A) The effect of stimulation through different channels was recorded under the form of hind limb kinematics, EMG, and movement-related traction force. (B) Increase in stimulation amplitude resulted in muscle co-contraction, preventing the modulation of ankle joint movement. (C) Modulation of stimulation frequency allowed precise control of ankle joint kinematics. Linear relationship between stimulation frequency and the extent of the produced movement was obtained for both ankle flexion and extension across animals (mean $\pm$ sem). (D) Real-time closed loop modulation of stimulation frequency demonstrated high precision control of movement-elicited traction force. The controller compensated for fatigue by applying a statistically significantly higher range of frequencies (one representative example in the middle) to keep the error between target and recorded signals low (n=4 animals with 2x2 and 2x3 channels each).

Fig. 4 Characterization of implant encapsulation. (A) H&E staining of nerve cross-section in the acute phase (0 days post implantation). No haemorrhage is present. (B) H&E staining of nerve cross-section in the chronic phase. Note the same scale bar on both images. The local endoneurial environment (here tibial fascicle) in the chronic phase is substantially enlarged when compared to the acute phase. Dashed lines indicate the implant encapsulation, characterized by a layer of hyper-cellularity (white dot) and a layer of collagen fibers and fibroblasts parallel to the implant (black dot). (C) This trend for nerve enlargement was consistently observed across animals in the chronic group. 3D reconstructions of the fascicles, the implant, and the capsule for one representative animal from this group illustrate the implant encapsulation in the fascicular enlargement. (D) The formation and remodelling of the capsule correlated significantly with the observed nerve enlargement throughout the time-points of this experiment. (E) Top: Insets of Sirius Red stained sections for different time-points revealed the appearance of an epithelioid layer of foreign body giant cells tightly around the implant that was quantified across animals. Bottom: Quantification of different components in the local endoneurial environment revealed increased collagen deposition at the chronic stage and a substantial increase in cellularity (7 days post-implantation) and a gradual densification of microvasculature over the first four weeks post-implantation. Both increases were sustained at the chronic stage. Legend: Empty arrowheads - bent nerve fibers, full arrowheads - part of implant, white full circles - hyper-cellularity, black full circles - parallel collagen fibers and fibroblast, stars - multinucleated giant cells.

Fig. 5 Inflammation. Immunohistochemical labelling against CD68 showed presence of macrophages at different time-points after implantation. (A) Acute (0 days post-implantation). (B) Mid (17 days post-implantation). (C) Chronic (165 days post-implantation). (D) Quantification of CD68+ objects in distinct nerve compartments (extra-fascicular space, local endoneurial environment, distant endoneurial environment) was expressed as area of fluorescence normalized to the analysed area of the respective nerve compartment and confirmed the qualitative observations. Macrophage infiltration started from the extra-fascicular space (endogenous blood vessels), then migrated towards the implant, and at 17 days post implantation was found widely distributed throughout the nerve. (E) Expression of mean particle size (area of CD68+ object) as function of mean particle distance from the implant for each group further confirmed the progressive accumulation in larger clusters near the implant over time. Although macrophages persisted at the chronic stage, they were less distributed and did not appear as big clusters. Red line differentiates qualitatively particles from clusters based on the average surface area of detected macrophages ($\pm 80 \mu\text{m}$). Legend: le - local endoneurial environment, de - distant endoneurial environment, e - extra-fascicular space, bv - blood vessel, p.c. - posterior cutaneous branch (excluded from analysis), scale bar on insets is $25 \mu\text{m}$.

Fig. 6 Impact of intra-neural electrode on fibers. Immunohistochemical labelling against tubulin contained in axons (Tuj1), myelin (MBP) and cell nuclei (dapi). Cross section of implanted sciatic nerve (A) 2 hours after implantation, (B) 36 hours after implantation, (C) 7 days post implantation, (D) 17 days post implantation, (E) 28 days after implantation, and (F) 165 days after implantation. (G) Fiber density proximal and distal to the implant did not differ and was constant across time (top) and was also comparable to control fiber counts (bottom). (H) At the implant level, the number of fibers increased progressively and was highest after 4 weeks. I) Over time, the distance between the implant and the closest 20% of fibers increased until 17 days post-implantation. Albeit at the chronic stage, the distance of the closest 20% of axons had returned to values similar to the acute implantation (top), this was not the case for myelin (bottom), suggesting that regenerating but unmyelinated fibers had occupied the fibrotic zone around the implant. Dotted line indicates reference lines (in G and H a ratio of 1, in I the values from the control group (0 days post implantation)).

Fig. 7 Impact of implantation and removal on fine motor control. (A) The task consisted in the rats (n=5) running over a ladder with irregularly spaced rungs. Performance was measured by recording the hind-limb kinematics with a motion capture system based on 5 infrared markers placed on the hind limb joints and then computing the percentage of missed steps. Animals were trained daily for two weeks before the pre-implantation recording that was subsequently considered as baseline performance. (B) Performance in running over this ladder was not significantly affected by neither the implantation nor the removal of the intra-neural implant at any time-point. (C) The explanted nerves showed signs of scarring 30 days post removal, mainly reflected by enhanced collagen deposition at the passage site of the implant (Sirius Red (SR) staining and inset 1, within dotted boundaries). Increased cellularity (H&E staining, inset 2, within dotted boundaries) at the passage site of the implant was also found in all animals, presumably due to the accumulation of fibroblasts secreting collagen. Immunohistochemical labelling against macrophage/monocyte marker CD68 (green, inset 3, within dashed boundaries; full line designates fascicle boundary) revealed the presence of some macrophages along the passage site of the implant. Quantification of macrophage presence showed that the amount of inflammation was substantially lower than in nerves where the implant had remained inside.

Fig. 8 Summary of observations and schematic of possible response of nerve tissue to implant. (A) Summary of mean observations (linear interpolation between 28 and 165 days post-implantation for all foreign body reaction related metrics) shows the covariation of the electrodes electrical properties and the developing foreign body response over time of implantation. Spearman's correlation analysis revealed that the growth of the capsule was significantly correlated with the amounts of vasculature ($R = 1$, $p < 0.0001$), collagen ($R = 0.997$, $p < 0.0001$), and cellularity ($R = 0.78$, $p < 0.0001$) measured over time. Both changes from week to week in impedance and threshold charge were highly anti-correlated to fascicle size ($R = -1$), capsule size ($R = -1$), microvascular density ($R = -0.997$), and cellularity ($R = -0.78$) ($p > 0.0001$). (B) Illustration of possible mechanisms of peripheral nerve tissue response to insertion and chronic implantation of polyimide-based, intra-neural implant.

TABLE 1 - Groups of animals participating in the study

Group	n	Duration (day)	Stimulation protocol	Other
1 - Acute	3	0	In surgery	
2 - Subacute	3	1.5	In surgery	
3 - Early	3	7	In surgery, weekly	
4 - Mid	3	17	In surgery, weekly	
5 - Late	3	28	In surgery, weekly	
6 - Late	3	28	never	
6 - Late	5	28	never	Implant removal after 30 days, Impact on fine motor control
7 - Chronic	5	165	In surgery, weekly	Experiments with frequency control

Figure 1
A CHRONIC EXPERIMENTAL MODEL

B TIMELINE

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Figure 2**A ASSESSMENT OF MUSCLE RECRUITMENT PROPERTIES** **B SELECTIVITY** **C STABILITY OF ELECTRICAL PROPERTIES**

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Figure 3

A EXPERIMENTAL SETUP

B HIGH STIMULATION AMPLITUDE REDUCES SELECTIVITY

C STIMULATION FREQUENCY MODULATES ANKLE JOINT ANGLE

D REAL TIME CLOSED-LOOP CONTROL OF TRACTION FORCE

Structure of PI-controller

Fig. 3 Functional experiments demonstrate controllability of intra-neural PNS. (A) The effect of stimulation through different channels was recorded under the form of hind limb kinematics, EMG, and movement-related traction force. (B) Increase in stimulation amplitude resulted in muscle co-contraction, preventing the modulation of ankle joint movement. (C) Modulation of stimulation frequency allowed precise control of ankle joint kinematics. Linear relationship between stimulation frequency and the extent of the produced movement was obtained for both ankle flexion and extension across animals (mean $\pm$ sem). (D) Real-time closed loop modulation of stimulation frequency demonstrated high precision control of movement-elicited traction force. The controller compensated for fatigue by applying a statistically significantly higher range of frequencies (one representative example in the middle) to keep the error between target and recorded signals low ($n=4$ animals with 2x2 and 2x3 channels each).

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A ACUTE (0 days post implantation)

B MID (17 days post implantation)

C CHRONIC (165 days post implantation)

D QUANTIFICATION OF MACROPHAGES

Extra-fascicular space (e)

Local endoneurial environment (le)

Distant endoneurial environment (de)

E Spatio-temporal dynamics of macrophage clusters in local endoneurial environment (le)

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Figure 6**A ACUTE (0 days p. i.)** **D MID (17 days p. i.)** **B SUB-ACUTE (1.5 day p. i.)** **E LATE (28 days p. i.)** **C EARLY (7 days p. i.)** **F CHRONIC (165 days p. i.)** **G FIBER DENSITY** **H AXON DISTRIBUTION AROUND IMPLANT** **I DISTANCE FROM IMPLANT**

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Figure 8

A SUMMARY OF OBSERVATIONS OVER TIME

B ILLUSTRATION OF POSSIBLE RESPONSES OF NERVE TISSUE TO IMPLANT

Fig. 8 Summary of observations and schematic of possible response of nerve tissue to implant. (A) Summary of mean observations (linear interpolation between 28 and 165 days post-implantation for all foreign body reaction related metrics) shows the covariation of the electrodes electrical properties and the developing foreign body response over time of implantation. Spearman's correlation analysis revealed that the growth of the capsule was significantly correlated with the amounts of vasculature ($R = 1$, $p < 0.0001$), collagen ($R = 0.997$, $p < 0.0001$), and cellularity ($R = 0.78$, $p < 0.0001$) measured over time. Both changes from week to week in impedance and threshold charge were highly anti-correlated to fascicle size ($R = -1$), capsule size ($R = -1$), microvascular density ($R = -0.997$), and cellularity ($R = -0.78$) ($p > 0.0001$). (B) Illustration of possible mechanisms of peripheral nerve tissue response to insertion and chronic implantation of polyimide-based, intra-neural implant.