Rapid prototyping of flexible intrafascicular electrode arrays by picosecond laser structuring

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Abstract

Objective. Interfacing the peripheral nervous system can be performed with a large variety of electrode arrays. However, stimulating and recording a nerve while having a reasonable amount of channels limits the number of available systems. Translational research towards human clinical trial requires device safety and biocompatibility but would benefit from design flexibility in the development process to individualize probes. Approach. We selected established medical grade implant materials like precious metals and Parylene C to develop a rapid prototyping process for novel intrafascicular electrode arrays using a picosecond laser structuring. A design for a rodent animal model was developed in conjunction with an intrafascicular implantation strategy. Electrode characterization and optimization was performed first in saline solution in vitro before performance and biocompatibility were validated in sciatic nerves of rats in chronic implantation. Main results. The novel fabrication process proved to be suitable for prototyping and building intrafascicular electrode arrays. Electrochemical properties of the electrode sites were enhanced and tested for long-term stability. Chronic implantation in the sciatic nerve of rats showed good biocompatibility, selectivity and stable stimulation thresholds. Significance. Established medical grade materials can be used for intrafascicular nerve electrode arrays when laser structuring defines structure size in the micro-scale. Design flexibility reduces re-design cycle time and material certificates are beneficial support for safety studies on the way to clinical trials.

Keywords: peripheral nerve, interface, electrode, Parylene C, laser fabrication, neural prostheses

(Some figures may appear in colour only in the online journal)
1. Introduction

Ongoing research in biomedical engineering gave rise to a multitude of implantable electrode arrays and their numerous applications. A classification of these electrode arrays can be done by their intended implantation site or the mechanical properties of the substrate. The first approach leads to surface, extraneural and penetrating electrode arrays [1]. The second approach further classifies into flexible and non-flexible arrays [2]. Well known examples for flexible substrates are silicone rubber and polyimide, non-flexible substrates are usually fabricated from silicon wafers. Silicone rubbers have traditionally been used as encapsulants and substrates in precision-machined devices, while they are now also used as substrate materials for laser fabricated electrode arrays [3, 4]. Polyimides were investigated for application in cochlea implants and were later on used in implantable, high-density electrode arrays due to their compatibility with standard microelectromechanical (MEMS) processes [5, 6]. Silicon based microelectrodes have been in application since 1969, mainly used for measurements in the brain cortex [7].

Concerning flexible electrode arrays, silicone rubber offers large stretchability and a high degree of freedom for the designer, if used with laser fabrication of metal foils. However, this leads to relative thick arrays of several 100 µm which are mainly used for surface applications [4] or as a carrier for stiffer penetrating elements [8]. Cleanroom fabricated polyimide electrodes are only minimally stretchable but offer a high degree of flexibility, while offering a thickness of about 10 µm [9, 10]. A drawback of this technology is the need for several lithography masks and sophisticated process technology, which contradicts the need for multiple design iterations during development of the electrode arrays. Moreover, there is no medical-grade polyimide available that has records for long-term human implantation. During the recent years, research in ultra-flexible electrodes based on biocompatible substrate materials has been conducted. However, flexibility goes hand in hand with utilizing very thin substrate layers and thus limited application in vivo due to low mechanical stability [11, 12]. The intended application has to be taken into consideration, while choosing the right type of electrode array and the right fabrication method.

This study proceeds with the development of intrafascicular electrode arrays for peripheral nerves. Ultrashort pulse lasers in the picosecond (ps) and femtosecond (fs) range offer new possibilities in high-resolution metal cutting and subsequent thinning due to cold ablation [13]. This allows to combine the advantages of laser fabricated and lithography based electrode arrays. Both, the variability in the design process and the tenacity of the substrate, are necessary to have a feasible implantation procedure in a peripheral nerve. A comparable electrode array, called the TIME electrode (transverse intrafascicular multichannel electrode), has been fabricated in a lithographic process with polyimide and implanted in human to treat phantom limb pain and to restore sensory feedback in prosthesis control [14, 15]. Due to the absence of a double-sided fabrication process the electrodes were folded, which resulted in an arrow shaped structure of about 20 µm thickness. Next to increasing the integration density, the transversal penetration of the nerve was simplified. Two pilot studies have been carried out to gain first insight in the new technology and limitations of the utilization of Parylene C in combination with ablated metal foils [16, 17].

Parylene C, in contrast to other thin film polymers used as substrates, has the advantage of being classified for chronic implantation by the United States Pharmacopoeia (USP class VI). However, due to a relatively low Young’s modulus of 2.7GPa small filament electrodes cannot withstand much force. This challenge has been addressed by coating biodegradable layers like silk or maltose to enhance the robustness, which is, however, not suitable for intraneural electrodes due to an increase in thickness of several 100 µm [18, 19]. Thus, in this work we propose alternatively to increase the stiffness with the embedded metal layer as well as adding a guidance directly to the array. To address the limited capability of plain metal to transfer charge, the possibilities to increase the active surface by laser hatching as well as the electrochemical deposition of nanostructures were investigated [20]. Ultrashort pulse lasers offer the possibility to structure a metal surface by arranging sub-micron ripples, which develop on a material due to surface plasmon interference (SPI) of the incident laser light, to form interference patterns [21, 22]. All these research items were integrated in a novel fabrication process, electrochemically investigated in vitro and transferred into rodent animal models for acute and chronic in vivo experiments.

2. Materials and methods

2.1. Electrode fabrication

2.1.1. Design considerations. Whereas the initial electrode design was inspired by the TIME electrodes, the task of rethinking the implantation strategy arose due to deviating material parameters. Thin film polyimide electrodes like LIFE and TIME have a thickness of roughly 10 µm and can thus be folded, forming an arrow-like tip which can incorporate a needle with suture, to be easily pulled through a nerve [23, 24]. Parylene C based ps laser fabricated intrafascicular electrodes cannot be fabricated this thin due to the lower Young’s modulus compared to polyimide as well as due to use of a metal foil instead of evaporated metal layers. The general thickness of these electrodes imposes the necessity of changing the implantation strategy. Due to the design freedom the tip of the electrode can be extended to form an elongation to be used as suture material (figure 1(A)). Inspired by the TIME electrodes, two electrode arrays can be fabricated at the same time and stay connected via a bridge of Parylene C. After placing a loop of medical suture inside the electrodes can be folded alongside the symmetric axis to form an arrow-like structure. Along the sharpened tip, the two single-sided electrodes can be pulled through the nerve, forming a double-sided electrode array (figure 1(B)). The possibility to freely change the thickness and layout of the metal foil with a ps laser allows to terminate the electrode tip in a thin metal beam. In contrast to thin film metals a conductive wire can be resistance welded on top of it. In this case a medical grade MP35N wire is sharpened and resistance welded to the tip, replacing the demand for a surgical needle (figure 1(C)).
2.1.2. Fabrication process. All intrafascicular electrode arrays were fabricated with a passively mode-locked Nd:YVO4 picosecond laser (Rapid 10, Coherent Inc., Santa Clara, CA). Through internal frequency tripling the native wavelength is converted to 355 nm. Movement and focusing of the 12 µm laser spot is implemented by a two axis deflection unit with an integrated f-theta lens (Superscan II, Raylase AG, Wesslingen, Germany) inside a process chamber.

Two types of intrafascicular electrode arrays were fabricated in this setup; electrode arrays with a silicone rubber interlayer in a single- and double-sided configuration (figure 2 left column) and electrodes that only consist out of Parylene C and a metal foil electrodes (figure 2 right column). All fabrication routes started with the lamination of adhesive polyimide tape (No. 5413, 3M Co., St.Paul, MN) on a 2" by 2" mechanical carrier (96% pure alumina ceramic substrate) (figure 2(1)).

For single- and double-sided electrodes the deviations in the individual routes are minor. Making use of the ‘Gorham process’ [25] a 10 µm layer of Parylene C (Dimer DPX-C, Coater PDS 2010, Speciality Coating Systems Inc., Indianapolis, IN) was deposited on top of the carrier structure (figure 2(2, left)). To offer adhesion for the metal foil a 10 µm thick layer of n-heptane-diluted MED-1000 silicone adhesive (NuSil Technology LLC, Carpinteria, CA) was spin-coated on top of the Parylene C (figure 2(3, left)). With 5 min of curing, if needed, the structures that later on form the backside contacts were lasered and ablated (figure 2(4, left)). Afterwards a 25 µm thick metal foil (Pt90/Ir10, Goodfellow Cambridge Ltd, Huntington, United Kingdom) was laminated (figure 2(5, left)). Applying a hatching pattern with the ps laser allowed to thin down the metal to 10 µm at desired areas before patterning the interconnection sites, conducting tracks and electrodes (figures 2(6, left) and (7, left)). Excess material was peeled off and the remaining electrode array again covered with Parylene C (figures 2(8, left) and (9, left)). Afterwards laser hatching was used to open as well as roughen the electrode sites and the contact pads for MicroFlex Interconnects and resistance welding (figure 2(10, left)) [26]. At his step some electrodes were also subjected to structured laser interference patterning (SLIP), by continuously hatching across the electrode surface with 0°, 4° and 135° angled lines of 8 µm spacing, at the minimal possible power level necessary for cold ablation [21]. The intrafascicular electrode arrays
were released by laser cutting the outer perimeters through the Parylene C and subsequent peeling from the release layer (figures 2(11, left) and (12, left)).

Electrode arrays consisting of only metal and Parylene C follow an altered fabrication route (all following steps: figure 2 right column). A 100 µm thick MED-1000 silicone adhesive was spin-coated as a release layer (figure 2(2, right)). After the layer was cured for a week to decrease the adhesion properties a 25 µm thick metal foil (Pt90/Ir10) was laminated on top and transferred to the ps laser (figure 2(3, right)). There, a mirrored version of the electrode array design was hatched in the metal foil before coating it with a 10 µm thick layer of Parylene C (figures 2(4, right) and (5, right)). Next, the boundaries of the metal foil were laser cut and flipped with the Parylene C facing downwards (figures 2(6, right) and (7, right)). By applying a hatch pattern the metal layer was thinned down to 10 µm, before transferring the electrode design by ablating the metal not completely through to the Parylene C (figure 2(8, right)). The excess metal could be peeled whereas the intended structures adhered to the Parylene C layer. Afterwards a second Parylene C layer was deposited on top, completely encasing the complete electrode array (figure 2(9, right)). With hatching the electrode sites were opened, releasing the array was achieved by laser cutting completely through the two layers of Parylene C (figures 2(10, right) and (11, right)). To decrease the adhesion of the silicone adhesive ethanol was applied, so that the array could be peeled off (figure 2(12, right)).

All electrode types were interconnected via a ceramic adaptor to a cable; the double-sided arrays were additionally resistance welded with a 75 µm diameter MP35N wire at the tip to facilitate implantation into the nerve (figure 2(13)).

2.1.3. Imaging of electrode samples. The fabricated samples were optically characterized with a scanning electron microscopy (SEM) system (Phenom PRO Desktop SEM, Phenom-World BV, Eindhoven, Netherlands) in a configuration with 10kV acceleration voltage. Hatched and SLIP electrodes were also investigated with a Zeiss Auriga 60 (Carl Zeiss AG, Oberkochern, Germany) focused ion beam setup (FIB) with integrated SEM. A focused gallium ion beam was used to create a cross section in the metal surface, before cross sectional images were taken via SEM.

2.2. Mechanical characterization

A T-pull test and a peel test were performed [27] to investigate the adhesion between the different material interfaces inside the intrafascicular electrode array. Both tests were performed with a bond tester (type 4000 with WP10kg measurement cartridge, Dage Holdings Ltd, Buckinghamshire, United Kingdom). Samples for adhesion measurements of two Parylene C layers were prepared by depositing two layers with an individual thickness of 8 µm, with a small strip of Polyimide tape between them at one end. Utilizing the ps laser system the samples were cut in stripes of 28 mm in length and 5 mm in width. One layer was fixated in a chuck, the second layer was clamped and pulled away in an 180° angle. Adhesion was calculated with (1), in which $F_A$ is the applied average force while delamination and $b$ the width of the samples [28]:

$$ P_{180} = 2 \times F_A / b. $$

For the investigation of the adhesion between Pt90/Ir10 and Parylene C metal samples were hatched and cut to measure 28 mm in length and 5 mm in width. One series of the samples were hatched with 2 µm deep lines of 29 µm spacing and an inclination of 90° and 45° towards each other. For further investigation on the effect of the roughness of the Pt90/Ir10, two rectangular hatch patterns with a spacing of 288 µm between the lines and a varying depth of 2 µm and 5 µm were fabricated, others remained unhatched. After a deposition of a 8 µm thick Parylene C layer the samples were submitted to a 90° peel-test. Adhesion was calculated with (2), in which $F_A$ is the applied average force while delamination and $b$ the width of the samples [28]:

$$ P_{90} = F_A / b. $$

2.3. Electrochemical characterization

Electrode arrays were investigated with electrochemical impedance spectroscopy (EIS). Measurements ranged between 100kHz to 1 Hz at a sinusoidal excitation amplitude of 10 mV with a potentiostat and a frequency analyzer (Solartron 1260&1287, Solartron Analytical, Farnborough, United Kingdom) in a three-electrode configuration. As working electrode (WE), a large-area platinum counter electrode (CE) and as reference electrode (RE) Ag/AgCl (3M KCL) were placed in phosphate buffered saline solution (PBS). Analysis of the data was performed with the software Zplot v2.8 (Scribner Associates Inc., Southern Pines, NC). An electrochemical cleaning step, using cyclic voltammetry (CV) (100 times with 300 mV s⁻¹ from −0.6 V to 0.8 versus Ag/AgCl in PBS), was performed before starting the measurements.

Pulse testing was done in a two-electrode setup (WE: front or back side electrodes; CE: 1 cm² Pt/Ir) in PBS with a PlexStim Electrical Stimulator system (Plexon Inc., Dallas, TX) [29]. Stimulus was a rectangular, symmetrical, charged balanced pulse (cathodic first, pulse width: 200 µs with 10 µs interpulse delay, repetition frequency: 200 Hz). The voltage response across the phase boundary ($V_{ph}$) was recorded, while varying the amplitude of the current. Due to an open circuit potential (OCP) of 300 mV the safe charge injection limit was reached at a potential of $V_{ph} = −900$ mV (with a cathodic water window of −600 mV in PBS).

Platinum with nanorough surface was deposited to improve electrochemical behavior of the electrodes, thus gaining a better insight of the enlargement of the real surface by ps laser hatching. Details of the deposition process can be found in [20].

2.4. In vivo evaluation

2.4.1. Implantation procedure. Experiments were conducted implanting Parylene C based electrodes in the rat (Sprague-Dawley, 250 ± 20 g) sciatic nerve. Implants were made at
the distal half of the thigh, where the sciatic nerve divides into the tibial, peroneal and sural branches, but they are still bundled together and enclosed by a common epineurium. All procedures were performed under ketamine and xylazine anaesthesia (90/10 mg kg\(^{-1}\) i.p.) and in compliance to protocols approved by the Ethical Committee of the Universitat Autònoma de Barcelona, Spain in accordance with the European Communities Council Directive 2010/63/EU.

The implantation procedure was based on already established methods with the TIME electrode [24]. In the case of Parylene C electrodes, the MP35N guiding wire (figure 1(C)) was added to help penetration in the nerve. Thus, the MP35N thin wire was used to pierce the nerve branches and pull the flexible electrode through the sciatic nerve until the correct positioning. All the process was monitored under a dissection microscope to ensure that the electrode active sites were located inside the nerve tissue. Then, the electrode was fixed to the closest muscles with a 10-0 suture stitch to prevent motion. The PCB of the connected electrodes was placed and fixed on the gluteus muscles while the silicone wire was placed subcutaneously, leaving the Omnetics connector fixed over the skin with a plastic base on the back of the animals at the hip level. After implant, animals were left to recover in warm pads and then housed in plastic cages at 22 ± 2 °C under a 12:12 h light cycle with free access to food and tap water with amitriptyline.

Furthermore, to evaluate the long-term biocompatibility of these new electrodes, non-connected devices without wires were also implanted in other animals following the same procedure and nerve function tests were performed for up to twelve months. These non-connected devices were chosen to avoid forces and tensions on the nerve due to the PCB and wires of the functional electrodes to evaluate possible nerve damage due to the implanted electrode only.

Three months post implantation for both functional and nonfunctional electrode groups and twelve months post implantation for the nonfunctional electrode groups, animals were deeply anesthetized with an overdose of pentobarbital and perfused transcardially with 4% PFA in phosphate buffer (PB). After the perfusion, the sciatic nerve segment including the distal part of the nerve was kept in 3% glutaraldehyde-3% (PB). After the perfusion, the sciatic nerve segment including the proximal sciatic nerve and the distal half of the thigh was postfixed in 2% OsO\(_4\) for 2 h, dehydrated through ethanol series and embedded in epon resin. Semithin sections (0.5 μm thick) were stained with toluidine blue and examined by light microscopy. The number of myelinated fibers in the distal tibial nerve was counted in images taken with a BX51 light microscope and a DP50 digital camera (Olympus K.K., Tokyo, Japan) at 100× chosen by systematic random sampling of squares representing at least 30% of the nerve cross-sectional area. The cross-sectional area of the tibial nerve was measured at 4× and the total number of myelinated fibers estimated.

2.4.2. Nerve function evaluation. The functional properties of the nerves that had been implanted were evaluated by means of nerve conduction, algesimetry and locomotion tests from 2 weeks up to 12 months after the implant of the non-connected devices. Nerve conduction tests were performed by stimulating the sciatic nerve proximally with single electrical pulses and recording the compound muscle action potentials (CMAPs) of tibialis anterior (TA), gastrocnemius medialis (GM) and plantar interossei (PL) muscles as previously described [30, 31]. The nociceptive threshold to mechanical stimuli was evaluated by means of an electronic Von Frey algesimeter (Biöseb, Chaville, France) following the same protocol than in [32]. Briefly, rats were placed on a wire net platform in plastic chambers, and a metal tip applied to the sole of the hindpaw until the rat withdrew the paw in response to the stimulus. Finally, the walking track test was performed to assess locomotor function after the implant. The plantar surface of the hindpaws was painted with black ink and the rat was left to walk on top of a white paper along a corridor [33]. The print length, the distance between the 1st and 5th toes and between the 2nd and 4th toes were measured to calculate the sciatic functional index (SFI) [34].

2.4.3. Histological evaluation. To identify the location of the electrode inside the nerve, paraffin embedded nerve segments were cut in transverse sections (10 μm thick), mounted in silane-coated slides and dried overnight. The sections were deparaffinized and a standard luxol fast blue (LFB) staining was performed overnight to visualize myelin in nerve samples and then a hematoxylin-eosin (HE) staining was performed in the same nerve slices. Then, samples were dehydrated and mounted with DPX (Sigma-Aldrich, St. Louis, MO).

In order to evaluate the potential damage to the implanted nerves, distal nerve segments were postfixed in 2% OsO\(_4\) for 2 h, dehydrated through ethanol series and embedded in epon resin. Semithin sections (0.5 μm thick) were stained with toluidine blue and examined by light microscopy. The number of myelinated fibers in the distal tibial nerve was counted in images taken with a BX51 light microscope and a DP50 digital camera (Olympus K.K., Tokyo, Japan) at 100× chosen by systematic random sampling of squares representing at least 30% of the nerve cross-sectional area. The cross-sectional area of the tibial nerve was measured at 4× and the total number of myelinated fibers estimated.

2.4.4. Electrode functionality evaluation. To assess the electrode nerve stimulation capabilities over time, monophasic rectangular voltage pulses with a width of 20 μs and amplitude from 5 to 100 V were delivered through each one of the different electrode active sites against a small needle electrode placed subcutaneously in the back of the animal. Simultaneously, electromyographic (EMG) signals were recorded from TA, GM and PL muscles, which are innervated by different fascicles or subfascicles of the sciatic nerve [35] using needle recording electrodes placed in each muscle. Signals were amplified (P511 AC; Grass; WestWarwick, RI) by 200× or 1000×, and digitized with a PowerLab recording system (PowerLab16SP, ADInstruments, Bella Vista, Australia). The same experiment was repeated in five implanted rats at 0, 7, 15, 30, 45 and 60 days post implant. The amplitude of the CMAP was measured from baseline to the positive peak and normalized to the maximum CMAP amplitude obtained in each experiment. The EMG data was used to calculate the stimulation threshold at which a 5% of maximal muscle response was achieved. Moreover, the selectivity index (SI) was also calculated to quantify the activation of a single muscle among the set of three muscles (TA, GM, PL) when stimulating by each one of the active sites, as previously described [30, 36]. Thus, the maximum CMAP obtained for one active site (as) was used to calculate the maximum selectivity index (SIas max) for each muscle and each electrode and the mean of the maximum SIas (SIas max) was plot. Besides, the selectivity
index for the device (Sld) was also calculated following the described formulae by [30].

2.4.5. Statistics. Results are expressed as mean ± SEM. Statistical analysis of mean were performed using one or two-way ANOVA followed by Tukey post hoc test for differences between groups or time-points using GraphPad Prism software. Differences among groups or times were considered significant when \( p < 0.05 \).

3. Results

3.1. In vitro characterization

3.1.1. Sample fabrication. All electrode arrays were fabricated following the proposed fabrication route. The arrays featured a ground electrode outside the nerve and 4–6 active sites for intraneural implantation (figures 3(A) and (B) version with 6 electrode contacts). However, the 80 µm electrode contacts showed small deviations in actual geometry due to the use of a mechanical deflection system for the laser movement (figure 3(C)). An optical observable roughening of the surface could be achieved with normal hatching patterns (figure 3(D)). The metal surface showed a wave structure that represent the laser path as well as a grainy roughening on the surface. With SLIP patterning the grains were formed multiple times over and interacted forming an interference pattern on the metal surface (figure 3(E)). Close up a very rough pattern with deep pores could be observed (figure 3(F)). Cross section views revealed a clear difference in the penetration depth of the created structures for hatched and SLIP electrodes. Whereas the hatched surfaces exhibit a distinct 3D surface enlargement, the penetration depth of the grains is in the range of several 10 nm (figure 4(A)). Pores of the SLIP pattern were observed to penetrate the metal surface for at least 1.5 µm (figures 4(B), (C)). However, while running the ps laser beam across the surface a faint light was observed on the backside, implying some pores might penetrate deeper into the bulk.

3.1.2. Mechanical measurements. The mean adhesion of Parylene C on bare, untreated P90/Ir10 foil was found to be roughly 60 mN cm\(^{-1}\). However, between two 10 µm thick layers of Parylene C a mean adhesion of 4400 mN cm\(^{-1}\) was
measured. Applying the hatch pattern with 2 µm depth lowered the mean adhesion to 47 mN cm$^{-1}$, whereas an increase of depth to 5 µm led to an increase in mean adhesion to 3053 mN cm$^{-1}$. Narrowing the hatching pattern and providing crossing points of 4 µm depth led to an increase in mean adhesion to 4012 mN cm$^{-1}$ (figure 5(A)).

### 3.1.3. Electrochemical measurements

The fabricated electrodes showed the anticipated high pass behavior. This correlates to a $R||C-R$ electrode model which can be fitted to all electrodes.

Access resistance $R_A$ of the peeled electrodes was measured to be 5540 Ω (standard deviation (std.) 541 Ω) whereas for the peeled electrodes 4762 Ω (std. 47 Ω) were determined. At 1 kHz the impedance magnitudes were measured to 55.5 kΩ (std. 12.5 kΩ) and 43.8 kΩ (std. 3081 Ω) respectively. After coating the electrodes according to [20] the impedance magnitude at 1 kHz dropped to 7.6 kΩ (std. 941 Ω) for the peeled and 5.8 kΩ (std. 182 Ω) for the hatched electrodes. Without coating, both types featured a cutoff frequency of over 50 kHz. Coating the electrodes lowered the cutoff frequency to 1 kHz. SLIP electrodes yielded an $R_A$ of 4105 Ω (std. 177 Ω) and an impedance magnitude at 1 kHz of 18.7 kΩ (std. 632 Ω) (figure 5(B)).

Over the phase boundary of a peeled electrode a mean charge of 2.97 nC per phase (maximum charge injection capacity ($Q_{\text{max}}^{\text{inj}}$) resulted in 59.64 µC cm$^{-2}$) could be delivered before reaching the cathodic water window. Hatched electrodes were able to support a mean charge of 9.93 nC ($Q_{\text{max}}^{\text{inj}}$: 197.63 µC cm$^{-2}$). With the coating the mean delivered charge of peeled and hatched 80 µm Pt/Ir electrodes, both pure and coated with platinum nanostructures, as well as SLIP roughened electrodes. (C) Long-term pulse testing of a hatched (non-coated) 80 µm Pt/Ir electrode. (D) Complete assembly of intrafascicular Parylene C based electrode used for the measurements.

### 3.2. In vivo tests

#### 3.2.1. In vivo biocompatibility

To evaluate functional changes in the nerves with implanted electrodes, neurophysiological tests were performed at different time points. In electrophysiological tests, there were no differences in the CMAP amplitude (figure 6(A)) and latency to the onset of the response of TA, GM or PL muscles between the implanted and the contralateral intact limb of the rats during the 12 months follow-up. Regarding
pain assessment, pain threshold of animals implanted with both connected and non-connected devices remained around 30 g throughout all the studied time points. Besides, no alterations in the locomotion pattern were found during the year of implantation at any of the implanted animals.

Evaluation of the distal segment of nerves implanted with both non-connected and connected electrodes showed no morphological alterations, differences in the total number of myelinated fibers (figure 6(B)) neither signs of degeneration after three or twelve months of implant for the non-connected devices or after three months for the connected electrodes.

The histological analysis showed that at the segment where the electrodes were implanted, there were no signs of nerve degeneration and that the fascicular architecture of the nerve was preserved (figures 6(C) and (D)) after 3 or 12 months of implant. Besides, no important changes in the matrix accumulation around the electrode seem to occur from 3 months onward for both connected and non-connected devices. Thus, the main matrix deposition surrounding the electrodes occurs during the first weeks after the implantation.

3.2.2. In vivo electrodes functionality. The threshold of stimulation needed to elicit a 5% of the total muscle response showed significant changes over time (figure 7(A)) and two different phases could be observed over time. During the first weeks of implant, an increase in the stimulation threshold was observed compared to acute tests; then, a plateau was reached from 2 weeks to 2 months of implant.

Regarding the stimulation selectivity of the electrodes no significant differences were observed from acute stimulation at the implant day to chronic stimulation over 2 months of implant (figure 7(B)), with a SI for each muscle around 0.55 during all the follow-up. The SI for the device was also maintained stable (figure 7(C)). These results proved that parylene electrodes are able to selectively stimulate different axonal subpopulations of the sciatic nerve, depending on the active site used and the intensity of stimulation.

4. Discussion

In this work we describe a new method for the fabrication of a novel intrafascicular electrode array. Even though the individual electrode sites are rather small, a precise fabrication over several transfer steps during the process is possible. Utilizing ps laser technology the adhesion of the individual
Coating the electrodes gave insights on the difference in active area of the two processes. At 1 kHz the peeled electrodes have a 1.27 times higher impedance compared to the hatched electrodes. This factor changed to 1.31 after the coating. In terms of $Q$\textsubscript{inj} max, a factor of 3.34, which dropped to 1.21 after the coating, was measured. The same investigation on SLIP electrodes hints towards a surface enlargement by a factor of 3, which is about 15% higher compared to Green et al [21], however small changes in diameter strongly influences the electrochemical characteristics of microelectrodes [29]. Considering widespread use of iridium oxide covered microelectrodes a comparison needs to be addressed. Over the phase boundary of such a 80 $\mu$m electrode results a $Q$\textsubscript{inj} max of 2.3 mC cm$^{-2}$ [37], which is over 10 times larger than the hatched electrodes (3 times for the coated electrodes), but the impedances can be roughly matched by coating a hatched electrode. However, the hatched electrodes have a 3–4 times larger $Q$\textsubscript{inj} max compared to bare platinum electrodes [38] and show a good long-term stability during pulse testing. A future shift to SLIP electrodes might further improve application. The nanorough Pt coating has already been investigated for long-term stability over 240 million pulses and in vitro cytotoxicity [20].

Furthermore, parylene-based electrodes have shown good biocompatibility and safety after long-term implantation in an in vivo rat model. As shown by the electromyography studies, no changes in the nerve function were observed. Besides, no signs of pain or locomotion alterations were found after 1 year follow-up. These results agree to those shown with similar electrodes designs [24] or with other intrafascicular electrodes [39]. Moreover, histological analysis revealed no evidence of nerve damage due to the devices implanted.

However, tissue accumulation surrounding the implanted devices was observed after months of implant in both connected and non-connected electrodes, as reported for other neural electrodes [39, 40]. The increase of threshold stimulation necessary to induce a response in the first two weeks indicate that matrix accumulation around the implanted device mainly occur during the first weeks after implantation, similar to other studies [41]. The later stabilization implies that after 4–8 weeks there is no more tissue deposition around the electrodes, although changes in its cellular nature may occur [42]. Such tissue deposition surrounding the electrodes explain, among other facts, the increase in the voltage needed to stimulate nerve fibers, as the distance between the active sites and the nervous tissue is widened. Nevertheless, the electrodes showed a stable SI of stimulation, without significant changes over time, indicating stable position in the implanted nerve. Thus, parylene-based electrodes meet the needs for chronic implants in animals or even in humans [43]. However, further studies are needed to elucidate if these new Parylene C electrodes are able to record neural signals after long-term implantation.

5. Conclusions

A novel fabrication process and implantation technique for thin intrafascicular Parylene C based electrode arrays was implemented and used to create arrays for a small animal model. During chronic implantation the electrode arrays exhibited good biocompatibility and functionality. A design change for including more electrode sites and for adaptation to larger nerve diameters has to be made to prove feasibility in a larger animal model and ultimately the application in human patients. The published fabrication process allows for rapid prototyping of novel biocompatible electrode arrays.

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Figure 7. (A) Threshold of stimulation to achieve 5% of the maximal muscular response over 8 weeks of follow-up for GM, TA and PL muscles. (B) Maximal selectivity index for GM, TA and PL muscles after 8 weeks of implant. (C) Progression of device selectivity index over time. $p < 0.05$, two-way ANOVA.

layers can be adjusted to each other, forming a mechanical stable system. Disparities in the peeled and hatched electrodes were observable optically and by electrochemical measurements. Larger standard deviations reflect the disadvantageous manual peeling of the small contacts in comparison to a touch-free direct hatching. The fabrication process now allows for quick design changes which are only limited by the maximum work area of the deflection unit.

This factor changed to 1.31 after the coating. In terms of $Q$\textsubscript{inj} max, a factor of 3.34, which dropped to 1.21 after the coating, was measured. The same investigation on SLIP electrodes hints towards a surface enlargement by a factor of 3, which is about 15% higher compared to Green et al [21], however small changes in diameter strongly influences the electrochemical characteristics of microelectrodes [29]. Considering widespread use of iridium oxide covered microelectrodes a comparison needs to be addressed. Over the phase boundary of such a 80 $\mu$m electrode results a $Q$\textsubscript{inj} max of 2.3 mC cm$^{-2}$ [37], which is over 10 times larger than the hatched electrodes (3 times for the coated electrodes), but the impedances can be roughly matched by coating a hatched electrode. However, the hatched electrodes have a 3–4 times larger $Q$\textsubscript{inj} max compared to bare platinum electrodes [38] and show a good long-term stability during pulse testing. A future shift to SLIP electrodes might further improve application. The nanorough Pt coating has already been investigated for long-term stability over 240 million pulses and in vitro cytotoxicity [20].

Furthermore, parylene-based electrodes have shown good biocompatibility and safety after long-term implantation in an in vivo rat model. As shown by the electromyography studies, no changes in the nerve function were observed. Besides, no signs of pain or locomotion alterations were found after 1 year follow-up. These results agree to those shown with similar electrodes designs [24] or with other intrafascicular electrodes [39]. Moreover, histological analysis revealed no evidence of nerve damage due to the devices implanted.

However, tissue accumulation surrounding the implanted devices was observed after months of implant in both connected and non-connected electrodes, as reported for other neural electrodes [39, 40]. The increase of threshold stimulation necessary to induce a response in the first two weeks indicate that matrix accumulation around the implanted device mainly occur during the first weeks after implantation, similar to other studies [41]. The later stabilization implies that after 4–8 weeks there is no more tissue deposition around the electrodes, although changes in its cellular nature may occur [42]. Such tissue deposition surrounding the electrodes explain, among other facts, the increase in the voltage needed to stimulate nerve fibers, as the distance between the active sites and the nervous tissue is widened. Nevertheless, the electrodes showed a stable SI of stimulation, without significant changes over time, indicating stable position in the implanted nerve. Thus, parylene-based electrodes meet the needs for chronic implants in animals or even in humans [43]. However, further studies are needed to elucidate if these new Parylene C electrodes are able to record neural signals after long-term implantation.

5. Conclusions

A novel fabrication process and implantation technique for thin intrafascicular Parylene C based electrode arrays was implemented and used to create arrays for a small animal model. During chronic implantation the electrode arrays exhibited good biocompatibility and functionality. A design change for including more electrode sites and for adaptation to larger nerve diameters has to be made to prove feasibility in a larger animal model and ultimately the application in human patients. The published fabrication process allows for rapid prototyping of novel biocompatible electrode arrays.

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**References**

[1] Navarro X, Krueger T B, Lago N, Micera S, Stieglitz T and Darío P 2005 A critical review of interfaces with the peripheral nervous system for the control of neuroprostheses and hybrid bionic systems J. Peripher. Nerv. Syst. 10 229–58

[2] Ordonez J, Schuettler M, Boehler C, Boretti T and Stieglitz T 2012 Thin films and microelectrode arrays for neuroprosthetics MRS Bull. 37 590–8

[3] Donaldson P E K 1991 Aspects of silicone rubber as an encapsulant for neurological prostheses Med. Biol. Eng. Comput. 29 34–9

[4] Schuettler M, Stiess S, King B V and Suaning G J 2005 Fabrication of implantable microelectrode arrays by laser cutting of silicone rubber and platinum foil J. Neural Eng. 2 S121–8

[5] Shamma-Donoghue S A, May G A, Cotter N E, White R L and Simmons F B 1982 Thin-film multielectrode arrays for a cochlear prosthesis IEEE Trans. Electron Devices 29 136–44

[6] Stieglitz T, Beutel H and Meyer J-U 1997 A flexible, lightweight multichannel sieve electrode with integrated cables for interfacing regenerating peripheral nerves Sensors Actuators A 60 240–3

[7] Wise K D, Angell J B and Starr A 1970 An integrated-circuit approach to extracerebral microelectrodes IEEE Trans. Biomed. Eng. 17 238–47

[8] Tyler D J and Durand D M 1997 A slowly penetrating interfascicular nerve electrode for selective activation of peripheral nerves IEEE Trans. Rehabil. Eng. 5 51–61

[9] Stieglitz T, Beutel H, Schuettler M and Meyer J-U 2000 Micromachined, polycrystalline devices for flexible neural interfaces Biomed. Microdevices 2 283–94

[10] Lacour S P, Wagner S, Huang Z and Suo Z 2003 Stretchable gold conductors on elastomeric substrates Appl. Phys. Lett. 82 2404–6

[11] Kaltenbrunner M et al 2013 An ultra-lightweight design for imperceptible plastic electronics Nature 499 458–63

[12] Wagner S and Bauer S 2012 Materials for stretchable electronics MRS Bull. 37 207–13

[13] Nebel A, Herrmann T, Henrich B and Knappe R 2005 Fast micromachining using picosecond lasers Lasers and Applications in Science and Engineering (SPIE Proc.) ed J T Schriempf (San Jose, CA, 22 January 2005) pp 87–98

[14] Raspopovic S et al 2014 Restoring natural sensory feedback in real-time bidirectional hand prostheses Sci. Transl. Med. 6 222ra19

[15] Oddo C M et al 2016 Intraneural stimulation elicits discrimination of textural features by artificial fingertip in intact and amputee humans Elife 5 e09148

[16] Mueller M, Boehler C, Jaeger J, Asplund M and Stieglitz T 2016 A double-sided fabrication process for intrafascicular Parylene C based electrode arrays Conf. Proc. IEEE Engineering in Medicine and Biology Society vol 2016 pp 2798–801

[17] Mueller M, Ulloa M, Schuettler M and Stieglitz T 2015 Development of a single-sided Parylene C based intrafascicular multichannel electrode for peripheral nerves Proc. of the 7th Internat. IEEE/EMBS Conf. on Neural Engineering (NER) vol 2015 pp 537–40

[18] Wu F, Tien L W, Chen F, Berke J D, Kaplan D L and Yoon E 2015 Silk-backed structural optimization of high-density flexible intracortical neural probes J. Microelectromech. Syst. 24 62–9

[19] Xiang Z, Yen S-C, Xue N, Sun T, Tsang W M, Zhang S, Liao L D, Thakor N V and Lee C 2014 Ultra-thin flexible polyimide neural probe embedded in a dissolvable maltose-coated microencrole J. Micromech. Microeng. 24 65015

[20] Boehler C, Stieglitz T and Asplund M 2015 Nanostructured platinum grass electrodes that enable superior impedance reduction for neural microelectrodes Biomaterials 67 346–53

[21] Green R A, Matteucci P B, Dodds C W D, Palmer J, Dueck W F, Hassarati R T, Byrnes-Preston J P, Lovell N H and Suaning G J 2014 Laser patterning of platinum electrodes for safe neurostimulation J. Neural Eng. 11 56017

[22] Baudenbacher A, Ignatovich F, Bruyant A, Huang C, Des Colas Francis G, Weerber J-C, Aures X, Wiederrecht G P and Novotny L 2007 Surface plasma interference excited by tightly focused laser beams Opt. Lett. 32 2535

[23] Lago N, Udina E, Ramachandran A and Navarro X 2007 Neurobiological assessment of regenerative electrodes for bidirectional interfacing injured peripheral nerves IEEE Trans. Biomed. Eng. 54 1129–37

[24] Badia J, Boretti T, Pascual-Font A, Udina E, Stieglitz T and Navarro X 2011 Biocompatibility of chronically implanted transverse intrafascicular multichannel electrode (TIME) in the rat sciatic nerve IEEE Trans. Biomed. Eng. 58 2324–32

[25] Gorham W F 1966 A new, general synthetic method for the preparation of linear poly-p-xylenelines J. Polym. Sci. A 4 3027–39

[26] Meyer J-U, Stieglitz T, Scholz O, Haberer W and Beutel H 2001 High density interconnects and flexible hybrid assemblies for active biomedical implants IEEE Trans. Adv. Packag. 24 366–74

[27] ASTM D1876-08 2008 Standard Test Method for Peel Resistance of Adhesives (T-Peel Test) (West Conshohocken, PA: ASTM International) (https://doi.org/10.1520/D1876-08)

[28] Packham D E 2005 Handbook of Adhesion 2nd edn (New York: Wiley) pp 311–5

[29] Cogan S F 2008 Neural stimulation and recording electrodes Annu. Rev. Biomed. Eng. 10 275–309

[30] Badia J, Boretti T, Andreu D, Azvedo-Coste C, Stieglitz T and Navarro X 2011 Comparative analysis of transverse intrafascicular multichannel, longitudinal intrafascicular and multilobar cuff electrodes for the selective stimulation of nerve fascicles J. Neural Eng. 8 36023

[31] Citrone A, Del Valle J, Santos D, Badia J, Filippeschi C, Micera S, Navarro X and Bossi S 2015 A three-dimensional self-opening intraneural peripheral interface (SELINE) J. Neural Eng. 12 16016

[32] Santos D, Wieringa P, Moroni L, Navarro X and Valle J D 2016 PEOT/PBT guides enhance nerve regeneration in long gap defects Adv. Healthc. Mater. 6 160029

[33] Navarro X 2016 Functional evaluation of peripheral nerve regeneration and target reinnervation in animal models: a critical overview Eur. J. Neurosci. 43 271–86
[34] de Medinaceli L, Freed W J and Wyatt R J 1982 An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks Exp. Neurol. 77 634–43
[35] Badia J, Pascual-Font A, Vivo M, Udina E and Navarro X 2010 Topographical distribution of motor fascicles in the sciatic-tibial nerve of the rat Muscle Nerve 42 192–201
[36] Veraart C, Grill W M and Mortimer J T 1993 Selective control of muscle activation with a multipolar nerve cuff electrode IEEE Trans. Biomed. Eng. 40 640–53
[37] Boretius T, Yoshida K, Badia J, Harreby K, Kundu A, Navarro X, Jensen W and Stieglitz T 2012 A transverse intrafascicular multichannel electrode (TIME) to treat phantom limb pain—towards human clinical trials Proc. IEEE RAS EMBS Int. Conf. Biomedical Robotics and Biomechatronics vol 2012 pp 282–7
[38] Donaldson N d N and Donaldson P E K 1986 Performance of platinum stimulating electrodes mapped on the limitvoltage plane Med. Biol. Eng. Comput. 24 431–8
[39] Lago N, Yoshida K, Koch K P and Navarro X 2007 Assessment of biocompatibility of chronically implanted polyimide and platinum intrafascicular electrodes IEEE Trans. Biomed. Eng. 54 281–90
[40] Christensen M B, Pearce S M, Ledbetter N M, Warren D J, Clark G A and Tresco P A 2014 The foreign body response to the Utah slant electrode array in the cat sciatic nerve Acta Biomater. 10 4650–60
[41] Würth S et al 2017 Long-term usability and bio-integration of polyimide-based intra-neural stimulating electrodes Biomaterials 122 114–29
[42] del Valle J, de La Oliva N, Muller M, Stieglitz T and Navarro X 2015 Biocompatibility evaluation of Parylene C and polyimide as substrates for peripheral nerve interfaces Proc. of the 7th Int. IEEE/EMBS Conf. on Neural Engineering (NER) vol 2015 pp 442–5
[43] Micera S 2016 Staying in touch: toward the restoration of sensory feedback in hand prostheses using peripheral neural stimulation IEEE Pulse 7 16–9