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PAPER

Q-PINE: A quick to implant peripheral intraneural electrode

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Abstract

Objective. The implantation of intraneural electrodes in amputees has been observed to be effective in providing subjects with sensory feedback. However, this implantation is challenging and time consuming. Surgeons must be especially trained to execute the implantation. Therefore, we aimed at developing a novel peripheral intraneural electrode and insertion mechanism, which could drastically reduce the overall implantation time while achieving a high neural selectivity. **Approach.** A new insertion method based on hollow microneedles was developed to realize the prompt and effective simultaneous implantation of up to 14 active sites in a transversal manner. Each needle guided two Pt/Ir microwires through the nervous tissue. After the insertion, the microneedles were released, leaving behind the microwires. Each microwire had one active site, which was coated with poly-3,4-ethylenedioxythiophene (PEDOT) to enhance the electrochemical properties. The active sites were characterized by evaluating the impedance, charge storage capacity, and maximum injectable charge. Twelve quick to implant peripheral intraneural electrodes (Q-PINES) were implanted in four pig sciatic nerves to evaluate the implantation time and neural selectivity. We compared the stimulation of the sciatic nerve with that of its branches. **Main results.** The average surgical access time was 23 min. The insertion time for 12 electrodes was 6.7 min (std. ± 1.6 min). The overall implantation time was reduced by 40.3 min compared to the previously reported values. The Q-PINE system demonstrated a satisfactory performance during *in vitro* and *in vivo* characterization. The electrochemical results showed that the PEDOT coating successfully increased the electrochemical parameters of the active sites. **Significance.** With an average impedance of 1.7 k Ω , a maximum charge level of 76.2 nC could be achieved per active site. EMG recruitment curves showed that 46% of the active sites exhibited selective stimulation of four out of six muscles. The histological analysis indicated that the microwires successfully penetrated the nerve and single fascicles.

1. Introduction

Several groups have used intraneural electrodes to deliver electrical current to peripheral nerves to restore sensory feedback or record neural signals. The transversally intrafascicular multichannel electrode (TIME), developed by (Boretius *et al* 2010),

allowed the restoration of sensory feedback for up to six months in upper limb (Petrini *et al* 2019b; Raspopovic *et al* 2014), and lower limb amputees (Petrini *et al* 2019c). In 2011, with current delivered intraneurally using a longitudinal intrafascicular electrode (LIFE), two blindfolded participants could distinguish objects (Horch *et al* 2011). In 2017, Utah

slanted electrode arrays (USEAs) were intraneurally inserted in the nerves of two participants to control a virtual robotic hand and induce cutaneous sensations on the phantom hand of the participants. In addition, epineural electrodes have demonstrated notable success in upper and lower limb amputees. Specifically, the FINE, implanted around the median and ulnar nerve, could provide sensory feedback for up to one year (Tan *et al* 2014).

Compared to epineural electrodes, the use of intraneural electrodes is desirable, since the associated active sites (ASs) are smaller and closer to the target fascicles. Consequently, the electrical current can be delivered with a considerably higher precision (Oddo *et al* 2016, Raspopovic 2017), and the target fascicles can be activated individually with a high precision, thereby increasing the selectivity of such electrodes. However, such electrodes are more difficult to implant and more likely to cause neural damages owing to the penetration of the peripheral nerve during the implantation (Di Pino *et al* 2014).

For this reason, several improvements have been recently made with respect to the biocompatibility and long term performance of intraneural electrodes such as the TIME or LIFE (Boehler *et al* 2017, Pena *et al* 2017, Wurth *et al* 2017, Čvančara *et al* 2020).

Specifically, Teflon insulated Pt90/Ir10 microwires (LIFE), as well as polyimide electrodes (tfLIFE and TIME), have demonstrated excellent performance in terms of the biocompatibility. LIFEs, for example, were implanted and used successfully in animals for six months and in humans for one month (Lefurge *et al* 1991, Lawrence *et al* 2003).

TIMEs were implanted in animals and humans and used for up to 6 months (Petrini *et al* 2019b; Wurth *et al* 2017). Nevertheless, the limitations of intraneural electrodes in terms of the implantation time and simplicity have not been addressed. A prompt implantation procedure is of significance to enhance the safety and wellbeing of the patients. Cheng *et al* reported that with each increase of half an hour in the surgical time, the likelihood of complications increased by 14% (Cheng *et al* 2018). In 2014, Di Pino *et al* reported that 90 min were required by a skilled surgeon to implant four tfLIFEs-4 in humans. Moreover, the electrodes were implanted with a success rate of 66% (two of six electrodes broke during the implantation). The electrode breakage led to an increased implantation time. Such a complex and time consuming surgical procedure could limit the widespread use of intraneural electrodes in future clinical applications.

Recently, attempted TIME implants in transradial (Petrini *et al* 2019b) and transfemoral (Petrini *et al* 2019a; 2019c) amputees required 11 and 4 h, respectively. In these studies, two different surgeons implanted the TIMEs, and the findings indicated that

the transversal insertion time of thin film electrodes depends significantly on the surgeons' training.

To promote clinical applications, the implantation time and success rate for such neural implants should be increased. In particular, in our previous studies (D'Anna *et al* 2017, Micera *et al* 2010, Petrini *et al* 2019a; 2019b, Raspopovic *et al* 2014), the median, ulnar and sciatic nerves were required to be microdissected to ensure the correct insertion of the TIMEs. This procedure increased the duration and complexity of the surgery. The chainlink connection between the guidance needle and thicker polyimide structure was noted to be a vulnerable aspect of the implantation procedure (Di Pino *et al* 2014).

Many research groups have attempted to address the problem of the considerable implantation time. Recent studies attempted to exploit different insertion devices (IDs). For instance, Felix *et al* (2013) developed silicon-based stiffeners, which allowed the insertion of soft neural electrodes in nervous tissues. Yim *et al* (2018) presented a 3D printed spring charged ID. Other researchers (Etemadi *et al* 2016) embedded Pt microwires in gelatin based solutions to form a stiff insertion support for soft microwires. However, all these methods were designed for the CNS. In the context of the PNS, a pressure gun developed by Rousche and Normann (1992) is still in use at present to insert the USEA in central and peripheral nerves. To our knowledge, this device has yet not been tested with other electrode types. Finally, when developing a novel intraneural electrode such as the quick to implant peripheral intraneural electrode (Q-PINE), it is essential to consider the use of medical grade materials to facilitate a possible translational to human implants (Stieglitz 2020).

Therefore, we developed a Q-PINE, together with an ID (figure 1), which could drastically reduce the insertion time, and thus, the implantation complexity, while maintaining a high neural selectivity. This manuscript describes the design, development, and *in vitro* and *in vivo* testing of the Q-PINE system.

2. Materials and methods

The Q-PINE and ID (figure 1(A)) were designed to enable prompt insertion of the microwires, which acted as the ASs. The ID was used to guide the Q-PINE under the nerve, and it helped push several microneedles through the nerve simultaneously. Every microneedle contained two microwires (i.e. ASs). Once the Q-PINE was inserted into the nerve, its structure was fixed to the nerve. Subsequently, the Q-PINE was released from the ID and attached to the neurostimulator. Consequently, *in vitro* and *in vivo* validations were performed. The Q-PINE was evaluated in terms of its electrochemical functionality, ease of implantation, and neural selectivity performance.

2.1. Electrode design considerations

The Q-PINE system design specifications (table 1) were set to realize a straightforward insertion procedure and reduce the implantation time compared to that of other intraneural electrode to facilitate future translation activities of intraneural electrodes. Furthermore, a high spatial neural selectivity was required to be ensured to selectively activate the target nerve fibers. To this end, a stable mechanical fixation of the electrode to the nerve was required. In addition, the Q-PINE was required to be made of medical grade materials or materials already successfully evaluated in long term experiments to ensure a high biocompatibility, and therefore, a long term implantation period. The main goal was to combine the advantages of the existing electrodes within one system, i.e. the Q-PINE. An ID, similar to the one reported for the USEA (Rousche and Normann 1992) along with the silicone structure of the FINEs (Tan *et al* 2014) was expected to enable a prompt and repeatable insertion. A FINE like silicone fixating portion (SFP) was expected to enable a stronger mechanical fixation to the nerve (figure 1(A)). To achieve a high selectivity, horizontal and vertical AS distributions were desired to enable a selective stimulation of the nerve fibers (figure 1(B)). Finally, the use of Pt/Ir wires coated with Teflon, as proposed by Horch *et al* (2011), along with medical grade materials for the SFP was expected to ensure a high biocompatibility.

2.2. Validation and verification process

To validate and verify the realization of the design specifications (Table 1), several evaluations were performed. To ensure the required electrical functioning of the Q-PINE, an electrochemical characterization of the electrodes was performed. In acute *in vivo*

experiments, the surgical access and implantation time were recorded. The neural selectivity was evaluated by determining the muscular recruitment curves based on the electromyographic (EMG) signals recorded while stimulating the sciatic nerve. The compound muscle action potentials (CMAP) and selectivity indexes with respect to the stimulated muscles were calculated following Veraart *et al* (1993). In addition, the charge injection necessary to elicit selectively single muscle groups was analyzed. The stimulation possibilities in the sciatic nerve and its branching nerves were compared to verify the performance of the Q-PINE. Finally, a histological analysis was performed to clarify whether the implanted electrode penetrated the target areas. The mechanical stability of the implant was not quantified.

2.3. Manufacturing process

The Q-PINE consisted of an outer part composed of two main elements. The first main part corresponded to the supporting platform (SP, see figure 1(A)). The SP was 3D printed (ProJet MJP 3600, 3D systems, US). A hollow microneedle with an o.d. and i.d. of 130 μm and 75 μm , respectively, could be inserted (RI.MOS Medical Products, ITA) in the SP. Each microneedle contained two 25 μm thick Pt90/Ir10 microwires (Advent Materials, UK). The microwires were insulated using a 5 μm thick Teflon sheet and deinsulated at a specific height, which represented the ASs. The second main part was an SFP (figure 1(A)), to provide mechanical stability by encapsulating the nerve similar to the FINE (Tan *et al* 2014). The SP and SFP were held together through a hook system, which was part of the SP. During the insertion, the microneedles were pushed through the nerve. The electrode was closed using a second SFP. The

Table 1. Design specifications intended to be replicated in the Q-PINE design to optimize the implantation time, neural selectivity, mechanical fixture to nervous tissues and biocompatibility. ID = Insertion Device, SFP = Silicone Fixating Portion.

Q-PINE	USEA	TIME	LIFE	FINE	Q-PINE
Easy to implant	ID & SFP	ID	–	–	Silicone structure
Neural selectivity	Longitudinal & vertical AS distribution	Longitudinal & vertical AS distribution	Vertical AS distribution	–	–
Mechanically stable	SFP	–	–	–	Silicone structure
Biocompatibility	Teflon insulated Pt/Ir wires & medical grade silicone	–	–	Teflon insulated Pt/Ir wires	Medical -grade silicone

microneedles were removed, and the ASs contacted the nerve. The ASs were distributed at two different heights. Two electrodes with different numbers of ASs were available. Therefore, either five or seven microneedles were placed adjacent to one another. Q-PINES with 10 or 14 ASs were developed.

Following Schiefer *et al* (2010), the shape of the SFP horizontally distributed the fascicles and flattened the nerve. Each second microneedle was displaced in a two row pattern to ensure a feasible a PCB routing (figure 1(A)). The SP hook system ensured that both the SFPs remained in place, holding the electrode in position (figure 1(B)).

A poly-3,4-ethylenedioxythiophene (PEDOT) coating was applied to each AS to increase the maximum injectable charge (max Q-inj). Two microwires exited the flat side of each microneedle. The out-pointing ends of the microwires were deinsulated and soldered to a custom designed PCB (Cad Line, ITA). The PCB was fixed to the SP by using ultraviolet (UV) curable glue (Bondic, US). The microneedles were temporarily fixed to the PCB and SP and released upon insertion. The needles could only be removed in one direction (figure 1(C)) because of the barrier created by the UV glue. From the PCB, lead wires (Farnell, UK) were connected to a 20 pin connector (TFM-110-01-L-D; SAMTEC, US), which was connected by a neurostimulator (Trucker-Davis Technologies, US).

Two oval holes in the SP (figure 2(1A)) allowed the SP to be fixed to a 3D printed ID. During the surgical procedure, the electrodes could be released by inserting tweezers in between the SP and ID. This step prevented the surgeon from touching the electrode structure during the insertion, thereby decreasing the risk of damaging the electrode. Using the ID, the Q-PINE was inserted from beyond the nerve by applying force to the SP. Once the needles transversally penetrated the nerve, a second SFP was pushed over the needles and the SP hook system. The needles were removed from the opposite side of the insertion location. A total of 10–14 microwires remained inside the nerve and pointed to the bottom part of the electrode (figure 1(C4)).

Kwik-Sil silicone adhesive (World Precision Instruments LLC, US) was applied to mechanically

fix the Pt/Ir wires exiting from the bottom of the Q-PINE (figure 1(C5)).

2.3.1. Assembly of the basic structure

The following procedures were implemented to produce one electrode, and the same processes were applied for the 10 and 14 AS models. The basic structure was composed of two general parts, which were assembled at the end of the procedure. Part A: The SP, ID, and two molds for the SFP were 3D printed using VisiJet M3 Crystal (3D-Systems, US). Under steering, the parts were placed in sunflower oil (50 °C) for 60 min to remove the residual support resin. The oil residuals were removed by cleaning the parts with a test tube brush and soapy water. Next, the parts were airdried. At this point, the ID was considered to be prepared. The SFP was created by filling each mold with 0.6 ml of Med-2000 (Nusil Technology, US). Microscope glass slides (VWR International LLC, US) sized 75 mm × 25 mm were used to cover and close the molds. The glass slides were pressed against the molds using 100 mm plastic clamps. The molds were baked for 60 min at 40 °C in a ventilated oven. Subsequently, the clamps were opened, and the glass slides were removed. A smooth cornering of the molds was implemented to reduce the tissue damage caused by the SFP. The silicone was removed from the molds by using flat tweezers (RS Components, UK).

Part B: Road runner copper wires with a thickness of 148 µm (insulated with 12 µm of polyurethane) were used as the lead wires. Approximately 50 µm of the insulation was removed on both the ends by using a soldering iron. Leadfree SAC305 (RS Components, UK) was used to solder the wires to the double layered PCBs made of FR4. The wires were soldered to the PCB before assembling the PCB, SP, and SFP. The PCB had drillings corresponding to the 120 µm holes in the SP (figure 2(2A)). The conductivity between the PCB pads was verified using a Fluke 117 multimeter (Fluke, US). Assembly: To assemble Parts A and B, silicone glue Med-1131 (Nusil Technology, US) was equally distributed on the even part of one SFP. Subsequently, the SFP was unified with the SP by inserting the SP hook system into the SFP

holes. To ensure alignment, the needles were inserted while penetrating the SP and SFP before the assembly (figure 2(2A)). The bottom of the PCB was covered with Med-1131 and placed on the top side of the SP by using the previously inserted needles as guidance. This step ensured the correct alignment among the holes in the PCB, SP, and SFP. This assembled basic structure (PCB, SFP, and SP) was pressed using 100 mm plastic clamps and baked for 30 min at 40 °C until the Med-1131 was completely cured.

2.3.2. Material choices

To ensure a high biocompatibility of the Q-PINE, the SP was printed using Visijet Crystal 3 M, which is a medically graded material (USP Class-VI). The SFP was molded using Nusil Med-2000 (ISO-10 993-5 certified). The SFP was fixed to the SP using the medically graded Nusil Med-1131. The microwires used as the ASs were previously used in human experiments and demonstrated a high biocompatibility (Horch et al 2011, Nicolelis 2019). The PCB was covered with UV curable glue to ensure the correct fixation of the

microneedles. Since the UV glue (Bondic, US) was not biocompatible, it was covered with Nusil Med-1131. PEDOT, which was used to coat the ASs and has demonstrated promising results for long term implants (Yang *et al* 2015, Feron *et al* 2018). The non-biocompatible materials that contacted with tissue were the lead wires and SAMTEC connectors, which were not coated with silicone. Table 2 highlights the materials used in assembling the Q-PINE.

2.3.3. Active site preparation

The ASs were prepared by cutting Pt/Ir microwires in 17.5 mm long pieces by using a laser cutter (Universal Laser System, US). Each microwire represented one AS and was deinsulated twice. The tip was deinsulated to ensure that the microwire contacted the PCB. The middle region of the microwire was deinsulated to create the AS. Among the 20 microwires, 10 microwires had a 'short' active site, and the remaining microwires had a 'long' active site (figure 1(A)). The center of a 0.5 mm thick stainless steel support sized 80 by 60 mm (figure 2(1B)) was covered with a 0.07 mm wide Kapton tape (RS Components, UK). Using two alignment screws, the stainless steel support was fixed to a custom made polyethylene support (figure 2(1B)). The polyethylene support was aligned with the rectangular cutting board of the laser cutters. Due to the repeated removal and placement of the stainless steel support in the laser cutter, this step was essential to ensure a high precision when performing the laser cutting of the microwires. Pieces sized 0.5×1 mm were cut out of the Kapton tape covering the stainless steel support. The intensity of the laser was set as 15%, velocity was set as 100%, and the pulses per inch (PPI) was set as 1000. The PPI remained constant for all the procedures. The now loose Kapton squares were removed using fine tweezers. The open 'windows' were cleaned using ethanol and colored (figure 2(1B), alignment squares) using a waterproof pen. The Kapton tape was removed, leaving behind two colored alignment squares (figure 2(1B)). The stainless steel support was covered with ultrathin biadhesive tape (RS Components, UK). Under a stereoscope (SMZ2500 NICON, JPN), 20 microwires were horizontally placed between two alignment squares. The biadhesive tape ensured that the microwires remained in a straight orientation. All the 20 wires were covered with Kapton tape. From the bottom up, the layers were as follows: polyethylene, stainless steel, biadhesive, Pt/Ir wires, and Kapton tape. The polyethylene support was placed and aligned with the laser cutter. The ASs and one end of the microwire were deinsulated two times, with an intensity and velocity of 15% and 100%, respectively. Next, the Kapton tape was cleaned using a microtissue soaked in ethanol. This procedure was performed twice. Subsequently, the same location was cut three times at an intensity and velocity of 1% and 2%, respectively. A final cut

was performed at an intensity and velocity of 1% and 2%, respectively. The microwires were cut on both ends to obtain 10.7 mm long pieces. Next, longitudinal cuts were performed along the outer side of all the alignment squares, at a laser intensity and velocity of 1% and 1%, respectively. This step was performed twice. The stainless steel support was removed from the polyethylene support and placed in distilled water in an ultrasonic bath for two minutes to remove possible Teflon residuals on the deinsulated locations. The stainless steel support was placed in an ethanol bath to loosen the Kapton tape. Using a stereoscope, the Kapton tape was carefully removed, and the microwires were lifted off the tape by using flat tweezers. These microwires were placed on an aluminum metal support covered with conductive double sided carbon tape. Subsequently, the ASs were observed using a scanning electron microscope (ZEISS, DE). The AS length and quality were determined. Overall, the lengths of 30 deinsulated ASs were measured using the SEM.

2.3.4. Coating of active sites

As reported by Cogan (2008), intraneural ASs are usually coated to compensate for the small geometric surface area (GSA). In our case, a PEDOT coating was applied to optimize the electrochemical parameters of the ASs. The coating was expected to decrease the impedance and increase the maximum charge storage capacity and the max Q-inj of the ASs. A 0.01 M EDOT solution was synthesized using 0.1% (w/v) 3,4-ethylene dioxythiophene (Merck, DE) and 0.2% (w/v) poly (styrene sulfonate) sodium salt (Merck, DE). The protocol corresponded to (Cui and Zhou 2007). To dip the wires into the monomeric solution, the wires were temporarily soldered to a custom made PCB (Cad Line, ITA), which was fixed to a microscope glass slide (figure 2(2B)). The EDOT was polymerized in a three electrode setup including an Ag/AgCl electrode as the reference. A large area Pt mesh electrode was used as a counter electrode, and the AS was considered as the working electrode. For each AS, a galvanostatic deposition charge of 5.1 mC cm^{-2} was applied using a potentiostat (Gamry Reference 600, US). The ASs were rinsed with distilled water, air dried, removed from the custom PCB, and placed on the support for SEM acquisition. The length of 30 ASs was measured using the SEM.

2.3.5. Electrode characterization

Electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and voltage transient measurements (VTm) were performed for 30 noncoated and 30 PEDOT-coated microwires. All the measurements were performed using the aforementioned three electrode setup. The PCB microscope glass support was used to characterize the microwires before assembling the electrodes. Phosphate buffer solution (PBS, 80 ml) was purged for 20 min with nitrogen

Table 2. Electrode component materials and their medical grade certification (if available).

Electrode Components	Material	In contact with tissue	Conform to ISO
Supporting Portion (SP)	Visijet Crystal 3 M	Yes	—
PCB	FR4	No	—
Material Fixing PCB to SP	Nusil Med-1131	Yes	ISO-10 993-5
Material Covering PCB	Nusil Med-1131	Yes	ISO-10 993-5
Needles	Stainless Steel	Yes	—
Material Fixing Needles	BONDIC	No	—
Microwires	PTFE	Yes	—
Active Sites	PEDOT	Yes	—
Ag-Glue Pt/Ir-PCB	QUICK CHIP SMD291AX10	No	—
SFP	Nusil Med-2000	Yes	ISO-10 993-5
SAMTEC TFM-110-01-L-D	Vectra E-130i	Yes	—
Lead Wires	PU (BS4520 Conform)	Yes	—
Solder Lead Wires-PCB	Lead Free Solder	No	—

to remove oxygen. The PCB microscope glass support was dipped into the solution only as far as the ASs in the solution. A sinusoidal 5 mV RMS AC wave was delivered to measure the impedance in the frequency range of 10 Hz to 100 kHz. The impedance was measured at a physiologically reasonable frequency of 1 kHz (Neto *et al* 2018). CV was performed to evaluate the cathodic charge storage capacity (CSCc) of the developed ASs. MATLAB functions were used to calculate the time integral of the cathodal CSC. Cycling was performed with a scan rate of 1 V s^{-1} , between -0.6 and 0.8 V as the potential limits (Venkatraman *et al* 2011). It was necessary to stay in the voltage range to prevent the oxidation or reduction of water. The VTm were performed by injecting rectangular, biphasic, cathodic first current pulses. The pulse width was fixed as 500 μs , according to the in vivo stimulation parameters. The current amplitude ranged from 10 to 500 μA . The resulting VTm data were analyzed in MATLAB to determine the highest current possible at which the reduction limit of -0.6 V was not exceeded by the electrode potential. The max Q-inj was calculated using MATLAB. In general, the max Q-inj is defined as the maximum charge that can be injected in a current controlled stimulation pulse without damaging the AS material or coating (Cogan 2008). The calculation method has been described previously by Cisnal *et al* (2018).

2.3.6. Assembly of the final electrode

After the microwires were coated and characterized, they were removed from the PCB microscope glass support and placed in long and short AS pairs. Only the ASs with a low impedance ($<3.5 \text{ k}\Omega$) and high max Q-inj ($>26.5 \text{ nC AS}^{-1}$) were used. To better distinguish the long and short ASs, the long wires were marked using a waterproof pen. One long microwire and one short active microwire were inserted into the flat $75 \mu\text{m}$ hole of each needle (figure 2(3B)). The wires were required to emerge exactly 1.5 mm from the needles to ensure the required AS height. A length of 0.5 mm was Teflon insulated, and the residual

outer end (1 mm) was deinsulated. This 'GND' part (figure 2(1B)) was soldered to the PCB in a subsequent process. Once the microwires were inserted, they were bent at 90° . Subsequently, the needles were placed in a vertical position (figure 2(1C)) to prevent the microwires from slipping back out of the needle. The needles were inserted in Part A (figure 2(1C)). The flat part of the needle was aligned with the SP to implement the desired height of the ASs. This step was performed under the SMZ2500. The microwires exiting the flat needle part were aligned over the corresponding PCB pad according to the mapping scheme (figure 2(2C)). Uv glue was used to cover the flat needle exit and the outpointing microwires. For curing, the glue was exposed to UV light for 30 s. Ablestik 8–1LMI (Henkel, DE) was deposited to place the microwires and PCB pads in contact (figure 2(3C)). The final assembly was baked for 110 min at 120°C . The top part of the final assembly was covered with UV glue and consequently cured to deinsulate all the electronic parts. Finally, the UV glue was covered with a thin layer of Nusil Med-1131 through dipcoating. The lead wires were soldered to the 20 pin connector, which was later connected to the neurostimulator. The final assembled electrode was connected to the ID, by pushing the pins of the ID through the oval holes of the SP (figure 2(4C)). The electrode was composed of Part A, which included five or seven needles (Part B).

2.4. Animal preparation

The protocol for all the animal studies (no. 76/2014 PR) was approved by the Italian Ministry of Health and was in accordance with the Italian law (D.lgs. 26/2014). Four 32–38 kg (3–4 months old) male farm pigs were included in this study. The animals were premedicated with Zolet[®] (10 mg/kg). Next, the animals were anesthetized using Propofol (2 mg kg^{-1} intravenously) and maintained under 1%–2% isoflurane in air enriched by 50% oxygen. Additionally, the animals received an infusion of 500 ml NaCl (0.9%) solution to prevent dehydration. The animals

were placed and maintained in a lateral position. The oxygen saturation, arterial pressure, and heart rate were constantly monitored.

2.5. Experimental setup

The start and end time of each surgical process (implantation time) and the electrode insertion time (insertion time) were recorded to determine the insertion time for each electrode. The CMAPs were recorded and analyzed to assess the neural selectivity of the Q-PINES. First, the maximum contraction amplitude for each muscle was obtained by separately stimulating the sciatic, peroneal, and tibial nerve, using cuff electrodes (Cortec, GER). We hypothesized that the cuff electrodes would produce the maximum muscle activation (Kundu *et al* 2014). The cuff electrodes were implanted to identify the maximal muscle contraction and later explanted. Subsequently, the Q-PINES were implanted and characterized. The EMG response of six muscles of the right hind legs was measured using EMG surface electrodes (Elettromedicali, ITA). Furthermore, the selectivity index was calculated online. All the results were normalized to the EMG maxima recorded during the stimulation performed prior to the Q-PINE insertion. To validate the functioning of the experimental setup (figure 3), the normalized EMG was plotted online. The steps are explained in the following text.

2.5.1. Q-PINE implantation process.

An approximately 17 cm long cut was performed between the patella of the right hindlimb and origin of the tail. A sternum spreader was inserted and opened to gain access to the biceps femoris and gluteus superficialis. The connective tissue was removed to gain access to the sciatic nerve and its bifurcation (tibial and peroneal nerve). Two Vycril 4–0 sutures (Johnson & Johnson, US) were used to lift the nerve and create a ‘bridge’ to make space under the nerve. Using the ID, the Q-PINE was guided under the nerve. The needles of the Q-PINE were inserted into the nerve using the ID (figure 1(C1)). Without releasing the ID, the structure was closed, pushing the additional SFP over the needles (figure 1(C2)). The additional SFP was held in place by the SP hook system (figure 1(C3)). The needles were removed, thereby releasing the microwires, which emerged from the additional SFP (figure 1(C4)). Kwik-Sil silicone adhesive was applied to deinsulate the wire tips and fix the microwires mechanically (figure 1(C5)). After letting the glue dry for approximately 1 min, the Q-PINE was released from the ID by inserting flat tweezers in the cavity between the ID and Q-PINE (figure 2(4C)).

Subsequently, the sutures were released, the ID and sternum spreader were removed, and the electrodes were connected to the neurostimulator. The animation in supplementary video 1 (available online at stacks.iop.org/JNE/17/066008/mmedia) shows the

insertion and characterization method of the Q-PINES. The overall implantation process was considered to start when the surgeon incised the skin to access the sciatic nerve. The process ended as soon as the electrode was connected to the neurostimulator. The time was recorded using a stopwatch. Three Q-PINES were implanted in each animal. We evaluated the surgical access time, insertion time of the Q-PINE, and the overall implantation time (surgical access time and insertion time). To compare our results with those of Di Pino *et al* (2014), who reported the implantation time for four tFLIFEs (90 min), we calculated the implantation time of four Q-PINES by multiplying the average insertion time of one Q-PINE by four and adding this value to the average surgical access time. One 14 AS electrode was implanted in the sciatic nerve, and two 10 AS electrodes were implanted in the tibial and peroneal nerve (figures 4(A) and (B)). This configuration allowed the sciatic and branch nerve stimulations to be compared. All the Q-PINE implantation procedures (figure 1(C)) were performed by a surgical technician assisted by engineers.

2.5.2. Stimulation and EMG recording.

During the experiments, all the parameters were controlled using a custom made real time graphical user interface (GUI) in MATLAB. In the GUI, the amplitude, pulse width, and frequency could be defined for each individual AS (figure 3). Incremental amplitude ramps were introduced for each electrode contact. Each amplitude was applied for one second, followed by a one second pause. The 3 Hz stimulation allowed the averaging of the CMAP responses in each step of the ramp. In accordance with Badia *et al* (2011); Kundu *et al* (2014); Wurth *et al* (2017), the amplitude modulation was considered to characterize the muscle responses pertaining to the Q-PINES. An amplitude range of 30–1500 μ A was considered. As in the work of Kundu *et al* (2014), the amplitude steps were 10 or 20 μ A. Supplementary video 2 shows one of the characterization procedures *in vivo*. *EMG recordings.* To record the resulting bipolar EMG signals, ten 27 mm, monopolar EMG surface electrodes were implanted on six muscles: m. tibialis anterior (TA), m. fibularis anterior (FiAnt), m. fibularis posterior (FiPost), m. extensor digitum longus (ExDL), m. soleus (SOL) and m. gastrocnemius (GM). The muscles were exposed by removing the skin between the knee and ankle to ensure correct electrode placement. The individual muscles were superficially stimulated through hook electrodes to find the most excitatory motor point. The EMG surface electrodes were fixed longitudinally to the muscle fibers, as the EMG signal was the greatest in this orientation. The EMG signals were amplified ($\times 1000$) and bandpass filtered at 10–250 Hz. A bandstop at 50 Hz was applied to remove the hum frequencies, by using the PZ5-EMG-Preamplifier (Trucker-Davis Technologies,

US). The PZ2-Processing unit (Trucker-Davis Technologies, US) forwarded the data to the TDT workstation, in which the recorded EMG data were further processed (figure 3). *Analysis of EMG recruitment.* The CMAPs were calculated in a 2–9 ms time window after the stimulation onset. This time window helps exclude the possible reflex responses (Kai and Nakabayashi 2013, Kundu *et al* 2014). For every stimulus and muscle, three EMG maximum peak responses (3 Hz stimulation) were averaged (average peak response) (Kundu *et al* 2014, Wurth *et al* 2017). The sciatic, tibial, and peroneal nerves were stimulated. During the stimulation, the GUI plotted the resulting CMAP and stimulus transmitted by the IZ2. The average peak response for the 3 Hz stimulation and each muscle was saved in a MATLAB matrix. After each ramp, the maximum average peak response (using the cuffs) for each muscle was considered to be the 100% excitation level. In this manner, the future average peak response recordings were normalized. Three Q-PINES were inserted into the sciatic, peroneal, and tibial nerves. The individual Q-PINE ASs were stimulated while plotting the nonnormalized average peak responses, normalized average peak responses, injected stimuli and selectivity index curve. The maximum excitation values obtained prior to the insertion of the Q-PINES were used for all further EMG normalizations. For each muscle, a selectivity index was calculated online. The calculation method was established by Veraart *et al* (1993) and has been widely applied (Boretius *et al* 2010, Badia *et al* 2011, Kundu *et al* 2014, Wurth *et al* 2017). Therefore, only a brief description of the methods is presented. The selectivity indexes $SI_{m,j}(I)$ for each muscle j were calculated as follows:

$$SI_{m,j}(I) = \frac{EMG(I)_{RL,j}}{\sum_{i=1}^N EMG(I)_{RL,i}} \text{ where } N = 5$$

$EMG(I)_{RL,j}$ is the average peak response, where I denotes the stimulation amplitude, and RL denotes the recruitment level in percentage points. A selectivity index of 1 indicates that only muscle j was activated. $SI_{m,j}(I)$ was plotted with respect to the activation level for all the Q-PINE ASs, along with the corresponding recruitment curves. Only $EMG(I)_{RL,j} \geq 30\%$ was considered to compute the selectivity index (Bao *et al* 2009). The resulting $SI_{m,j}$ was evaluated for each of the Q-PINES ASs. The sciatic nerve and branch stimulations (tibial and peroneal nerve) for the Q-PINES were compared. Confusion matrices were plotted for all the Q-PINES to evaluate the selectivity. A value of $SI_{m,j} > 0.4$ was considered to corresponding to a selective Q-PINE (see discussions). *Statistics (max Q-inj and $SI_{m,j}$).* The average injected charge and standard deviation for the 12 Q-PINES implanted in four pigs was analyzed. The max Q-inj was calculated for each electrode and animal. Furthermore, the average $SI_{m,j}$ and

standard deviation for all the implanted electrodes were calculated.

2.5.3. Assessment of the AS locations in the nerve.

After each experimental session, the animals were euthanized according to protocol. The nerves with the Q-PINES still inserted were cut and explanted. Six Q-PINES, obtained from pig nrs 2 and 4 were analyzed under a digital microscope (HIROX, JAP) by using flat tweezers. Precision scissors were used to cut through the microwires, separating the additional SFP from the implanted nerve. The insertion of the ASs was evaluated visually. When no active sites were observed to exit the nerve after removing the additional SFP, the sites were considered to be inside the nerve (inserted) or under the nerve (not inserted). By gently removing the nerve from the electrode, the wires that the penetrated the nerve could be observed. The microwires that did not penetrate the nerve usually remained flattened between the SFP connected to the SP and the nerve (figure 4(C), yellow line). The other implanted nerves (pig nrs 1 and 3) were straightened on a cork sheet. The nerves were placed overnight in a 4% phosphate buffered paraformaldehyde (pH 7.3) solution containing 5% sucrose. The microwires were cut using precision scissors, and the electrode was removed. The microwires were removed to prevent any damage to the microtome cutting blade. The nerves were rinsed in 0.1 M PBS, routinely processed, and embedded in a paraffin block (Wurth *et al* 2017). Transversal cross-sections at every 50 μm were cut using a microtome, with a thickness of 10 μm . The sections were mounted on microscope glass slides and analyzed under a microscope (Laica DMi8, DE) to evaluate any tissue penetration by the needles/microwires.

3. Results

3.1. Implantation procedure

Four 14 AS Q-PINES, and eight 10 AS Q-PINES were implanted in four pigs to evaluate the implantation time and neural selectivity. We implanted three Q-PINES in each pig. The implantation time was considered to be the sum of the surgical access time and the time taken to insert three Q-PINES into the nerve (insertion time). The average surgical access time for the four implants was 23 min. The average electrode insertion time was 6.5 min (std. ± 1 min) and 6.9 min (std. ± 2.2 min) for the 14 and 10 AS Q-PINES, respectively. The average implantation time for all the Q-PINES was thus 6.7 min (std. ± 1.6 min). The overall average implantation time of the three Q-PINES was 43.1 min. Di Pino *et al* (2014) reported an implantation time of 90 min for four tFLIFEs; in contrast, we recorded an implantation time of 49.8 min for four Q-PINES. Therefore, we could implant our

Table 3. Dimensions of 30 deinsulated and 30 PEDOT-coated ASs.

	de-insulated	PEDOT
AS length [μm]	150.6	131
std $\pm$	13.8	18
rel. %	9	14

Table 4. Statistics for ASs impedance, charge storage capacity, and max Q-inj before and after PEDOT coating.

	de-insulated	std $\pm$	PEDOT	std $\pm$
Impedance [$\text{k}\Omega$]	64.5	16.2	1.7	0.4
CSCc [mC/cm^2]	6.6	1.4	28	7.6
max Q-inj [nC]	17.1	3.1	76.2	26.3
max Q-inj [mC/cm^2]	0.14	0.03	0.74	0.3

electrodes in a 40 min shorter period compared to that of Di Pino *et al* (2014).

3.2. Active site dimensions

The ASs were coated with PEDOT to improve the electrochemical properties. Using the SEM, the length of 30 deinsulated and 30 PEDOT-coated ASs was measured (table 3).

Examples of deinsulated and PEDOT-coated ASs are shown in figure 5(A1)–(A3). As shown in A2, the AS dimension was not reduced. Comparing the results shown in figure 5(A2) and (A3), varying deinsulation could be observed. Material alteration or the presence of Teflon residues could be observed on the AS surface, as shown in A3. With a difference of $19.6 \mu\text{m}$ (values of $150.6 \mu\text{m}$ and $131 \mu\text{m}$ for the noncoated and PEDOT-coated ASs, respectively), the coated ASs were smaller than the deinsulated ASs (figure 5(A3)). After depositing PEDOT, only a length of $131 \mu\text{m}$ was coated, corresponding to a GSA of 0.01 mm^2 .

3.3. Electrochemical performance

The EIS, CV, and VTm analyses of 30 deinsulated and 30 PEDOT-coated ASs were performed to verify the quality of the coating procedure. The results showed that the PEDOT coating successfully enhanced the electrochemical properties.

According to the EIS results (figure 5(B)), the impedance significant decreased from $64.5 \text{ k}\Omega$ (deinsulated) to $1.7 \text{ k}\Omega$ (PEDOT). Furthermore, a decrease in the phase occurred (figure 5(B)), demonstrating that behavior of PEDOT coated ASs was more resistive than that of the noncoated ASs.

After the coating, the CSCc increased from 6.6 mC cm^{-2} to 28 mC cm^{-2} (table 4). The reasonably high std. of 7.6 mC cm^{-2} (27%) confirms that the deinsulation method led to notable variations in the length. The CV results before and after the PEDOT coating are shown in figure 5(C). A significant increase in the CSCc can be observed.

The max Q-inj per AS (sample analysis results shown in figure 5(D)) increased from 17.1 to

76.2 (tot. 59.1 nC) from the noncoated case to the PEDOT coating case, corresponding to an increase of approximately five times, with values of 0.14 and 0.74 mC cm^{-2} , respectively. The std. of 26.3 nC per AS (34%) indicates the occurrence of a variation in the deinsulation method.

3.4. Selectivity

Among the 136 implanted ASs, 46% were selective, with $SI_{m,j} > 0.4$ (figure 6). In particular, 31% of the ASs did not elicit any muscular activity, and 21% of the ASs activated the EMG but were not selective. With a std. of ± 15 , the variation in the selectivity among the four implants was reasonably low. Four out of the six muscles could be selectively stimulated. In pig nr 1, the Q-PINEs could selectively elicit three muscles (GM, SOL, FiPost) in the sciatic nerve and branches. Moreover, 41 of 63 selective active sites elicited the GM. The ExDL was elicited only by the peroneal implant in pig nr 2. The TA and FiAnt could not be stimulated selectively. The implants in pig nrs 1 and 2 (59% selectivity) were more effective than those in 3 and 4 (33% selectivity). The results for the sciatic and branched stimulations were comparable (43% and 49%, respectively). The sciatic implants elicited the GM, SOL, FiPost, and the branched implants could additionally elicit the ExDL. In terms of the implanted Q-PINEs, on average, 1.3 muscles could be elicited per electrode.

The recruitment, selectivity index curves, and confusion matrix examples for pig nr 1 are shown in figures 6(B)–(D). The data for AS 6 of the peroneal implant is shown in B1 to D1. B2–D2 and B3–D3 show the results for AS 5 of the tibial implant and AS9 of the sciatic implant, respectively. The confusion matrix of AS9 confirms the data reported in A1, with 7 out of the 14 ASs stimulated selectively. The sciatic implant mostly elicited the GM and SOL. The peroneal implant mostly activated the FiPost, and the tibial implant elicited the GM and SOL.

Of the 12 implanted electrodes, the peroneal electrode in pig nr 3 and tibial electrode in pig nr 4 did not elicit any muscle activity (not shown). This finding was confirmed by a low percentage of the selective ASs, 35% and 40%, for pig nrs 3 and 4 when stimulating the branches, respectively (figure 6(A)).

The results for the sciatic nerve and branch implants were similar, with the selective ASs being 43 and 49%, respectively.

The average $SI_{m,j}$ of all the selective ASs are shown in figure 6(D1)–(D3). All the implants exhibited a similar selectivity. The average standard deviation for all the implants was low (0.06). The peroneal and tibial implants of pig nrs 1 and 4 did not show any selectivity, respectively.

3.5. Charge injection

With an average Q-inj of 218 nC and a std. $\pm$ of 44 nC , the tibial implants exhibited the highest consistency

among one another. In the case of the peroneal implant in pig 2, extremely high charge injection values (751.3 nC) were required to ensure selectivity. This charge level significantly exceeded the safe max Q-inj of 76.2 nC AS⁻¹ (see discussions). In summary, a charge of 326 nC was applied to stimulate individual muscles selectively. A comparison of the used Q-inj during the experiments is shown in figure 7.

3.6. Histological analysis

The nerves of pig nrs 1 and 3 were transected and used to perform histological analysis (figure 4(D)). The red arrows indicate the penetration locations of two needles. Overall, six Q-PINES from pig nrs 2 and 4 were extracted and analyzed using a microscope. Visual inspection showed that 54 of the 68 Pt Ir⁻¹ microwires (79%) entered the implanted nerves and exited the nerves on the opposite side (figure 4(C)). The nerves of pig nrs 1 and 3 were transected and used to perform histological analysis. Unfortunately, no visible traces of the Q-PINE insertion could be detected.

3.7. Design specifications

The ID and needle mechanism to release the Q-PINE ASs led to shorter implantation times. The SFP helped align the Q-PINES in the middle of the implanted nerves. The histological data confirmed

that the Q-PINE ASs were inserted in single fascicles. The AS distributions could not be recorded. However, it was observed that the Q-PINES could selectively stimulate different nerve fibers, eliciting single muscles. The mechanical fixation of the Q-PINES could only be tested visually during the insertion and was not quantified. Due to the nature of the acute implantation of the Q-PINES no conclusions could be derived regarding the biocompatibility of the Q-PINE materials.

4. Discussion

The Q-PINE was designed to reduce the time and complexity of the implantation procedure to promote the clinical application of intraneural electrodes. Before implantation, the electrochemical properties were evaluated *in vitro*. The implantation time complexity and neural selectivity were characterized *in vivo*. The materials that were expected to contact the tissue were required to be biocompatible and non-immunogenic. Therefore, the extensively examined Teflon-coated Pt/Ir microwires (Dhillon *et al* 2004, Cogan 2008, Micera *et al* 2010, Horch *et al* 2011, Venkatraman *et al* 2011) were used as the electrodes. Due to its biocompatible properties (Asplund *et al* 2009) and desirable electrochemical properties (Wilks *et al* 2009), PEDOT was used to coat the ASs.

The other parts of the electrode were either medically graded or covered by medically graded silicone (see table 2). During this study, only acute experiments were performed. Therefore, we did not investigate aspects such as the porousness of the materials used for the assembly or the reaction of the tissues involved in the implantation. Moreover, only visual inspections of the mechanical stability of the Q-PINE implant were performed. Future work will be focused on realizing a mechanical quantification and characterization.

4.1. In vitro characterization

The microwires were successfully deinsulated using the laser cutter (figure 5(A)). However, a high variability in the AS length was observed, and the average AS size decreased after the PEDOT coating, indicated that the deinsulation method was not highly precise. Material alterations or the presence of Teflon residues (figure 5(A3)) likely occurred at the deinsulation location. The imprecisions could be a result of a noncontinuous but pulse-like cutting procedure. However, the PPI value was already set as the

Figure 7. Average injected charge (Sel_{mj} > 0.4) for 12 Q-PINs implanted in four pigs.

maximum value. Another explanation can be that the ventilation of the laser cutting area led to temperature variations during the deinsulation process, which led to the Teflon residues being left behind. Finally, the microwires, as acquired, were coated with four Teflon sheets, which likely also influenced the cutting procedure. When the laser intensity was increased, the area of the deinsulation was perfectly clean. However, the deinsulation length was extremely large (300–400 μm).

Moreover, we could not verify whether all the wires were deinsulated over the complete circumference, as several SEM sessions would have been required to be performed for the same wires. To prevent damaging the extremely fragile microwires owing to the tweezers, we manipulated the microwires as little as possible. Therefore, more than two SEM sessions were not performed for a wire (before and after coating). In future trials, the wires could be mounted on a support that can retain the wires in the air during the SEM analysis. Moreover, the SEM platform can be flipped 180° to obtain a complete picture. Nevertheless, because the GND part of the wires must remain in contact with the conductive carbon tape to prevent sample charging, the procedure is highly delicate. New methods must be developed to properly deinsulate the Teflon-coated Pt/Ir microwires with such a small target length. So far, only Pena *et al* (2017) have reported a length of not less than 1 mm for longitudinally deinsulated ASs on Pt/Ir microwires, performing heat deinsulation. Other researchers (Lind *et al* 2010, Venkatraman *et al* 2011, Etemadi *et al* 2016) mostly cut the microwires and used the exposed tip as the AS. With a GSA of 0.01 mm² (PEDOT-coated ASs), the Q-PINs AS exhibit a reasonable range compared to that for other neural electrodes. The LIFE electrode (Pena *et al* 2017) had a 1 mm long and 24 μm thick Pt/Ir AS, leading to a GSA of $\sim 0.075 \text{ mm}^2$. As

a comparison, the TIME presented by Boretius *et al* (2010) had a GSA of 0.003 mm². A newer version of the TIME (Stieglitz *et al* 2017) had a diameter of 80 μm , and thus, a GSA of 0.005 mm².

The PEDOT coating enhanced the electrochemical properties of the developed ASs (figure 5). A PEDOT deposition charge 5.1 mC cm⁻² was not enough to achieve a sufficient max Q-inj for the developed ASs. During the *in vivo* characterization, charges between 166 and 538 nC per AS were necessary to selectively stimulate the hindlimb muscles of the pigs. The electrochemical characterization of our ASs showed that a max Q-inj of 76.2 nC could be applied before the AS would be damaged. To resolve this issue, higher deposition charges should be applied. Nevertheless, although higher deposition charges can increase the max Q-inj, they can decrease the coating stability (Friedel *et al* 2009, Venkatraman *et al* 2011). In this study, we preferred to have a stable thinner PEDOT coating, even though it reduced the stimulation possibilities. Further experiments must be performed to increase charges while ensuring a high deposition stability.

In Venkatraman *et al* (2011), PEDOT-coated Pt/Ir wires were reported to have a CSCc of 123 mC cm⁻² and a max Q-inj of 2.92 mC cm⁻². In this work, we obtained a CSCc of 28 mC cm⁻² and a max Q-inj of 0.74 mC cm⁻². With a max Q-inj of 76.2 nC per AS, these values could be improved. Following Cogan (2008), after PEDOT coating, the CSCs should increase by a factor of approximately 15. In our case, the CSCs increased by a factor of 4.2. Several factors could be responsible for this phenomenon. The low CSCc and max Q-inj could be attributed to the insufficient deposition charge or extremely short deposition time. Another issue was that the ASs were likely not measured in a 3D manner but only from one side. To analyze the microwires from several sides, the wires would have to be extensively manipulated. To prevent excessive damage to the wires, this step was not performed.

Furthermore, the material alterations during deinsulation or the presence Teflon residues on the ASs likely led to a low CSCc and max Q-inj. The Teflon residues potentially created an interlayer between the AS material (Pt/Ir) and the PEDOT.

Finally, the rather high scanning rate of 1 V s⁻¹ (Venkatraman *et al* 2011) likely influenced the resulting CSCc. As reported by Cogan (2008), under high scanning rates, the observation of the detailed morphological characteristics of the AS surface and analysis of the CSCs become challenging. However, since Venkatraman *et al* (2011) characterized the same type of microwires, we applied a scan rate of 1 V s⁻¹ to enable a comparison with the results of Venkatraman *et al* (2011).

Further research is necessary to investigate these aspects. To optimize the ASs, a more effective way of

removing the Teflon coating should be established, and the PEDOT deposition must be examined under different deposition parameters.

To realize the large scale production of the Q-PINE, new methods must be developed to properly deinsulate the Teflon-coated Pt/Ir microwires with small target lengths. In this case, the PEDOT application can be realized without additional issues. The insertion of the microwires into the microneedles can likely be performed using an automated micromanipulator, thereby increasing the precision of the AS height during the Q-PINE assembly.

4.2. In vivo characterization

4.2.1. Implantation strategy.

The insertion was considered to be sufficiently easy as the implantation time (surgical access and insertion) was lower than that reported by Di Pino *et al* and the electrode did not break during the process. With an average implantation time of 43.1 min for four Q-PINES, we successfully reduced the implantation time. Di Pino *et al* (2014) reported an implantation time of 90 min. In total, we reduced the implantation time by 40.3 min. Moreover, the complexity reduction was demonstrated as the implantation was not performed by a trained neurosurgeon. Finally, 10 out of the 12 implanted Q-PINES selectively elicited the individual muscles, leading to an implantation success rate of 83.4% compared to the value of 66% for the tLIFE4s implanted by (Di Pino *et al* 2014). This finding further shows that the surgery times reported by Petrini *et al* (2019a; 2019b; 2019c) can be reduced by implanting Q-PINES. For electrodes such as the USEA (Rousche and Normann 1992) or LIFE (Kundu *et al* 2014), results regarding the implantation time were not available, and thus, comparisons with this work could not be performed.

4.2.2. Selectivity.

In this study, we considered a value of $SI_{m,j} > 0.4$. In other studies (Boretius *et al* 2010, Badia *et al* 2011, Kundu *et al* 2014), higher indexes have been assumed. We believe that we achieved a slightly lower selectivity because of the considerably larger active sites in our case (Oddo *et al* 2016, Raspopovic 2017). The TIMEs reported in previous studies (Kundu *et al* 2014, Petrini *et al* 2019a; 2019b; 2019c) had an AS diameter of 80 μm , corresponding to a GSA of 0.005 mm^2 . In the current study, the GSA was two times larger, with a value of 0.01 mm^2 . Smaller active sites can be realized in the future by improving the deinsulation process or replacing the Pt/Ir active sites with a polyimide structure such as in the TIME. To enable accurate characterization of the EMG data, the sciatic nerve and branch nerves were stimulated prior to the Q-PINE insertion to obtain a 100% EMG amplitude. We used a 2–9 ms window to exclude the muscular reflex signals, according to

the previously reported ranges (Kai and Nakabayashi 2013). The difference was negligible and assumed to be caused by the differences in the acquisition systems. The Q-PINES mostly activated the GM. Since the GM is the biggest muscle and has the most fascicles, this finding is reasonable (Wank *et al* 2006). The TA and FiAnt were not elicited by the Q-PINES, likely because of the small size of the peroneal nerve. Furthermore, this aspect was likely influenced by the distance between the AS pairs. In the Q-PINE, the interneedle distance was 750 μm , which is considerably large compared to that of the USEA, with an inter-AS distance of 400 μm (Branner *et al* 2001). One of the strengths of the USEA is the large number of active sites compared to that in the Q-PINE (96 and 12, respectively). Therefore, future considerations can include increasing the number of ASs in the Q-PINE to increase the selectivity. An advantage of the Q-PINE is the use of soft microwires; in contrast, the USEA is made of stiff, silicon-based needles, which potentially lead to more notable tissue damage. In terms of the implants, the results showed that the last two implants (pig nrs 3 and 4) exhibited a lower selectivity than that for the former implants. Only 33% of the ASs were selective compared to those in the case of the first two implants (59%). Since the surgical method or electrode production process was identical, this phenomenon likely occurred because of the variable anatomy of the pigs. Although the weight and age of all the pigs were the same, the nerve location and dimensions were likely different (Reinoso-Barbero *et al* 2014), which likely considerably influenced our results. We demonstrated that each Q-PINE could elicit 1.3 muscles on average. In the past, TIMEs and tLIFEs were implanted in the median nerve of pigs (Kundu *et al* 2014) eliciting 2.2 and 1.1 muscles per AS, respectively. With a value intermediate to the selectivity of the TIME and tLIFEs, our results can be considered to be reasonable. However, to enable a direct comparison, the Q-PINE would need to be implanted into the median nerve. An extensive anatomical study of a pig's sciatic nerve could help optimize the future Q-PINE variant. The confusion matrices (figure 6(D)) indicated that each electrode mostly elicited one muscle, and this phenomenon was also noted by Kundu *et al* (2014). Finally, the results of the Q-PINES inserted in the sciatic nerve and branches were comparable (figure 6(A)). This finding indicates that a single implant in the sciatic nerve could be sufficient to selectively stimulate the hind-limb muscles.

4.2.3. Charge injection.

Under a charge of 326 nC, the average current necessary to selectively stimulate the muscles was significantly higher compared to the value of 49.4 nC (TIME) reported by (Kundu *et al* 2014). In addition, in the case of the Q-PINES, the max Q-inj

value of 76.2 nC was exceeded during the experiments (figure 7). Therefore, we believe that the AS and PEDOT coating were damaged during the *in vivo* experiments (Cogan 2008). A detailed study regarding the AS deinsulation method and PEDOT coating is necessary in this case. Possible solutions include applying a laser with a higher precision or removing the insulation by using other heat sources.

4.2.4. Histological electrode placement.

The insertion location of certain microwires could be determined through a histological analysis. The findings confirmed that the microwires could be inserted through the sciatic nerves of the pigs and several fascicles could be penetrated simultaneously. We have observed that the microwires are extremely rigid to be cut by the cutting blade of the microtome. To our knowledge, a histological analysis on Pt/Ir microwires has not been yet performed. Therefore, in this study, six electrodes were manually removed from the implanted nerves to evaluate the insertion of the microwires. Overall, 79% of the microwires penetrated the nervous tissue, and 46% of these ASs were selective. It is considered that the movement caused by the separation of the nerve and electrode displaced the microwires, and thus, the AS locations could not be conclusively identified.

A significant advantage of the Q-PINE structure is that the 3D AS distribution is similar to that of the USEA, which cannot be realized using TIMES or tLIFEs. Additionally, the Q-PINE allows the implantation of soft Pt/Ir microwires, whose biocompatibility has been noted to be higher than that of rigid materials such as silicon, which is used for the USEA (Lefurge *et al* 1991, Lawrence *et al* 2003, Christensen *et al* 2016). A clear disadvantage is that the histological analysis could not be performed due to the rigidity of the Pt/Ir microwires. This problem could be resolved by substituting the microwires with materials such as polyimide. A fairer comparison can be realized after conducting long term investigations of Q-PINES.

5. Conclusion

Our acute experiments confirmed that the Q-PINE can be rapidly and easily implanted. Even though the AS dimensions were large, a reasonable selectivity could be attained. We believe that it is necessary to further adapt the Q-PINE by substituting the Pt/Ir wires with polyimide thin film electrodes. In addition, the neural selectivity can be further increased by understanding the histology of the pig's sciatic nerve. Further investigations should be performed before chronically implanting the electrode. The ASs of the Q-PINE could be distributed according to the sciatic fascicle distribution.

Finally, to adapt the Q-PINE for long term implants, the lead wires and connector should be

replaced with those made of biocompatible materials. If possible, the FR4 PCB and BONDIC could also be replaced.

The Q-PINE is an interesting approach to promote clinical exploitation. If noted to be successful in long term implants, the characteristics of the Q-PINE in terms of the reduced surgery time could facilitate its clinical usability. The recovery time from anesthesia could also be reduced. Moreover, the surgeons would not have to invest considerable time in the surgery room, thereby reducing the risk of errors.

In conclusion, we believe that the Q-PINE is a valuable solution to reduce the implantation time and increase the overall clinical exploitability of intraneural peripheral neuromodulation. In this regard, the current approach could also be used for other applications such as vision restoration (Gaillet *et al* 2020) or vagus nerve stimulation for bioelectronic medicine (Vallone *et al* 2020).

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Author contributions

I.S. designed the study, developed the mapping software, performed the experiments, performed the histological analysis, analyzed the data, prepared the figures, and wrote the paper. F.M.P. designed the study, performed the experiments, and reviewed the paper. T.N. and A.G. performed the experiments and analyzed the data. A.M.P. performed the experiments and developed the mapping software. F.B. and K.G. performed the experiments. M.M.O. performed the histological analysis. S.R. designed the study and performed the experiments. S.M. designed the study, supervised the experiments and reviewed the paper. F.R. and all the other authors read, commented on, and approved the manuscript. All the authors authorized the submission of the manuscript.

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