Polymeric C-shaped Cuff Electrode for Recording of Peripheral Nerve Signal

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\textsuperscript{a} Institute of Microelectronics (IME), Agency for Science, Technology and Research (A*STAR), 117685, Singapore

\textsuperscript{b} Singapore Institute for Neurotechnology (SINAPSE), National University of Singapore, 117456, Singapore

\textsuperscript{c} Department of Electrical and Computer Engineering, National University of Singapore, 117576, Singapore

\textsuperscript{d} Department of Biomedical Engineering, School of Medicine, Johns Hopkins University, Baltimore, MD 21205, USA

\textsuperscript{\ast}Correspondence information: Ning Xue, PhD, Institute of Microelectronics (IME), Agency for Science, Technology and Research (A*STAR), 11 Science Park Rd, 117685, Singapore

Email address: xuen@ime.a-star.edu.sg

Telephone number:+65-83587155
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Abstract

We reported a polyimide-based C-shaped neural interface electrode to record peripheral nerve signal from the rat sciatic nerve. The neural interface electrode consisted of four recording probes protruded from the C-shaped frame, and two reference electrode sites on the C-shaped frame. Thanks to the unique bendable protruding structure of the recording probes, electrode sites were able to adapt to and maintain close contact to the nerve without restricting nerve movement. Gold, gold-carbon nanotube (CNT) composite, and platinum were chosen as the electrode materials. The electrodes were characterized by
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electrochemical impedance spectroscopy (EIS) analysis in phosphate buffer solution (PBS) (pH 7.4). The results showed that the gold-CNT electrode (area of 8500 \( \mu \text{m}^2 \)) had an average impedance of 11.1 k\( \Omega \), approximately 15-fold and 3-fold lower than gold and platinum, respectively. An in vivo nerve signal recording test was also performed using the three electrodes. The magnitude of the compound nerve action potentials (CNAPs), the latency of the onset and the duration of the CNAPs were measured and analysed under various stimulus currents. The results of the CNAPs magnitude and the signal-to-noise ratio (SNR) indicated a proportional relationship to the stimulus current beyond the threshold current of 0.3 mA. Our electrode has the capability to measure nerve signals less than 5 \( \mu \text{V} \).

*Keywords:* Polyimide C-shaped electrode; gold-carbon nanotube; electrochemical impedance spectroscopy (EIS); nerve signal recording; MEMS
1. Introduction

Peripheral nerve prosthetics is a rapidly growing new research field that focuses on the development of artificial devices for the restoration of motor and sensory functions in patients suffering from injuries of the peripheral nerve system. These systems can significantly improve the quality of life of those patients with diverse neurological disabilities as complement of traditional therapeutic approaches. Frequent applications of peripheral nerve prostheses include pain relief [1],[2], walk rehabilitation by activation of muscles involved in gait [3],[4], and, more recently, control of hand prostheses in amputees [5],[6]. Commonly, electrodes are placed in segments with remaining functionality of injured or transacted nerves serving as a nerve interface to enable a communication between the device and the nervous system [7],[8]. The nerve interface is then connected to either stimulator or recording devices and several types of control units, providing functionality to the device. These system act in open-loop; however, next generation of neuroprosthetic devices will provide both recording ad stimulating capabilities to achieve a bidirectional closed-loop communication [9]-[11].

Neural electrodes are the key components of the peripheral nerve prosthetics and, as such, they are the main focus of research in this field. Electrodes for neural stimulation transduce the external commands for motor or sensory controls into nerve signals by applying a specific pattern of electrical current. The provided stimulation should remain below the charge-carrying capacity of the electrode to avoid an irreversible electrochemical process and nerve damage [12],[13]. Electrodes for neural recording are effectively "tap-wiring" neural signals, which are then processed and used as feedback signal to control the artificial prosthesis. These electrodes should excel in their electrochemical properties
including retaining low impedance over long periods of time and withstanding erosion in the physiological environment. New designs incorporate a high contact density in one device to provide high spatial resolution and selectivity for nerve signals [14],[15].

The ideal nerve electrode should allow a long-term multi-channel interface with the nerve system, which basically means stable electrochemical properties and minimal nerve damage. Unfortunately, no solution has been found so far leading to numerous types of electrodes designs and a variety of electrode materials. Based on nerve invasiveness, electrode designs can be classified as follows: (1) extraneural electrodes, such as cuff electrodes or similar (i.e. flat-interface electrodes (FINE)), which encircle nerve surfaces [16],[17]; (2) interfascicular electrodes, which place the active contacts in between nerve bundles [18]; (3) intrafascicular electrodes, such as the longitudinal or transverse intrafascicular electrodes (LIFE or TIME, respectively [19-21]), which penetrate the epineurium so the active contacts are inside the nerve fascicles; and (4) regenerative sieve electrodes, which are placed between two terminal ends in a transected nerve allowing axonal outgrowth through them in touch with the active contacts. The higher is the invasiveness, the more selective communication with individual nerve fascicles is possible, but the more serious is the nerve damage [14]. The use of biocompatible materials for electrode fabrication, such as gold, platinum, platinium black, and platinium/iridium (Pt/Ir) for the contact sites as well as silicon and polymers as substrate materials, attempts to optimize the nerve-electrode interaction in these designs [22].

Polyimide is a common substrate material with demonstrated biocompatibility and, furthermore, polyimide cuff electrodes are considered so far the best compromise between invasiveness and signal quality[23]-[25]. Due to the variation in the nerve sizes, cuff
electrodes have to be customized for each specific purpose. The nerve size varies not only between individuals of the same specie but also along time in the same subject. Furthermore, interaction with a foreign body such as the electrode will produce tissue inflammation, scarring formation, and other chronic changes that will affect the nerve diameter. For this reason, cuff electrodes need to be designed 1.3 to 1.5 times larger in diameter than the target nerve, in order to account for these changes and prevent nerve constriction. This has the consequence of a loser contact and poorer signal quality.

Previously, we presented a new cuff design that attempts to overcome two major problems: invasiveness and electrode contact [26]. Now, we developed a non-invasive, multi-channel, polyimide-based flexible C-shaped neural interface electrode device with protruding metal contacts for signal recording and we demonstrated its use in rat nerves. The C-shaped design permits the dynamic adaptation of the nerve interface to the chronic changes in the nerve and allows the use in a range of nerve sizes. The electrode-to-nerve contact remains consistent by using protruding bendable probes on the device’s ring frame.

2. Experiment

2.1 Design of C-shaped electrode

The device is based on a polyimide-metal-polyimide sandwich structure with exposed electrode contacts and pads. The different elements of the device are designed prior to fabrication to adapt to the target nerve (in this case, the sciatic nerve of an adult Sprague Dawley rat). These elements design criteria are the followings (see fig. 1a): (a) a two-dimensional (2D) ring frame that encircles the nerve. The inner diameter of the ring was designed to be slightly larger than the nerve diameter; (b) four or more working probes that protrude from the inner edge of the ring frame. The working probes need to be sufficiently
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bendable to have close contact to the nerve wall; (e) reference electrodes in the outer part of the ring frame where an eventual contact with the nerve is minimized; (d) a polyimide tab at the end of each C-ring to facilitate manipulation and opening of the ring frame for nerve placement; (e) a ribbon link between the beginning of the ring frame and the connection pad to prevent tensions of the connecting wires to the nerve, which might cause nerve lesions.

Taken into account these design considerations, we designed the electrode as shown in Fig. 1a. The device consisted of four bendable triangular sharp contact probes (area 8500 um² each) axisymmetrically around the polyimide frame, and two reference electrodes sitting on top of the frame. The inner diameter of the ring was 1.3 mm, while the outer diameter was 2.5 mm, so it could fit to a nerve diameter of 1.0-1.3 mm. The polyimide ring could be opened up by applying a twisting force on the two separated tabs. The ribbon cable was 1.5 mm wide and 8.5 mm long. The pad openings on the end of the ribbon cable were designed to adapt to the connection of a commercial flexible printed circuit (FPC) connector for further extending to the data acquisition system and the computer display.

2.2 Fabrication of C-shaped electrode

MEMS technology was employed to fabricate the multi-channel polyimide C-shaped nerve electrode interface. The fabrication started with an 8-inch silicon wafer. The fabrication process flow is demonstrated in Fig. 1b. First, a 1-µm-thick Al layer was deposited onto the silicon substrate as a sacrificial layer (Fig. 1b(i)). A 6-µm-thick polyimide film (PI-2611, HD microsystem) was then spin-coated and hard cured under 300 ºC for 30 minutes at a 4 ºC/min ramping rate. This was followed by a 200-nm-thick Al
deposition as a hard mask. After the first lithography step, the bottom polyimide structure was patterned on the thin Al surface. Afterwards, the exposed polyimide was etched by a reactive plasma etching (RIE) process (O₂ gas flow 50 sccm and CF₄ gas flow 10 sccm, RF power 150W). Next, the remaining thin Al layer was removed as shown in Fig. 1b(ii). Layers of Ti (20nm)/Au (300 nm) or Ti (20nm)/Au (300nm)/Pt (200nm) metal electrodes, traces and pads were subsequently deposited and patterned by a metal evaporation and lift-off process (Fig. 1b(iii)). Next, a second 6-μm-thick polyimide layer was coated and fully cured at 350 °C for 30 minutes. According to the same process as the first polyimide layer patterning, the second polyimide layer was etched and the structure was formed (Fig. 1b(iv)). Finally, the polyimide chip was released from Al sacrificial etching using an electrochemical anodic dissolution process (Fig. 1b(v), also see section 2.3).

2.3 Polyimide chip releasing

Anodic dissolution, an electrochemical process often used for metal refining and metal etching, was used for polyimide chip releasing. To begin, a copper wire extension was attached to the silicon substrate and bonded with silver conductive glue. The bonding area was subsequently encapsulated by non-conductive glue to prevent corrosion during the anodic dissolution process. Next, 0.5 M NaCl was added into a glass beaker as an electrolyte. Platinum was used as an inert electrode connected to the cathode, whereas the aluminium sacrificial layer on the chip was connected to the anode through the bonded copper wire. As voltage is applied to the two electrodes, the aluminium electrode undergoes oxidation and is dissolved into electrolyte in the form of Al³⁺ or Al₂O₃. H₂ gas is released from the Pt surface due to H⁺ reduction. The voltage was set below 1 V to avoid O₂
formation at the anode. During the process, the exposed aluminium was dissolved in less than 5 minutes. However, the unexposed aluminium underneath the polyimide chip took much longer time to dissolve even though the magnetic stirrer was adapted to expedite ion exchange between the silicon surface and the solution. The entire chip releasing process depends on the unexposed aluminium area. In our case, it took approximate 5 hours. Lastly, the released chip was rinsed with 5% HCl acid to remove the suspended Al₂O₃ on the device surface.

2.4 Device packaging

The pad opening of the C-shaped electrode was designed to match a FPC connector (FH19SC-6S-0.5SH, Hirose Electric Co. Ltd). As demonstrated in Fig.1c, the left end of the chip was inserted into the FPC together with a filling spacer. The black moving lid was then closed to clamp the neural interface and also to create a tight electrical connection. Next, a FPC cable was bonded to the other end of the FPC connector by conductive silver epoxy. Finally, a group of wires was soldered to the FPC cable for further wire extension.

2.5 Carbon nanotube (CNT) coating

The active contact surface area of Au electrode can be increased by roughening the Au surface with CNT coating [27]. CNT powder (0.5–2.0 μm long and < 8 nm diameter) was purchased from Cheap Tubes Inc. Similar to the experiment setup for device releasing, Au-CNT nano-composite electroplating coating was performed in a Au electrolyte bath (TSG-250, Transene) with a mixture of CNT (1mg/mL) using Au wire as the anode and our device as the cathode. The two electrodes were connected to a function generator (TG1010,
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10MHz D.D.S., Thurlby Thandar Instruments Ltd., UK) with a monophasic voltage pulse (1.2 V, 10 Hz, 50% duty cycle) of 1 min. During the cycles, the multi-layer CNT was deposited and covered with Au particles [28].

2.6 Electrochemical measurements

To study the signal recording capability and charge transport property of the C-shaped electrode neural device, electrochemical impedance spectroscopy (EIS) measurement was conducted at room temperature using a potentiostat/galvanostat (Autolab PGSTATC302, Metrohm Autolab B.V., Netherlands) in phosphate buffered saline solution (PBS, 5mL, 0.1M). PBS was used to mimic the physiological environment. The experiments were performed in a three-electrode cell system with a Pt wire as the counter electrode, an Ag-AgCl pellet (3M KCl) as the reference electrode and the metal probe of the C-shaped chip as the working electrode. For the EIS, the electrochemical impedance magnitude and phase measurement was taken with a frequency range between 0.5 Hz and 500 kHz using a 10 mV AC signal.

2.7 Accelerated aging testing

Elevated temperature was used to accelerate the aging process in polymers. In this experiment, three polyimide Au chips were chosen. The ring structure of the chips were immersed in a custom-made container with PBS (500mL, 0.1M). The container has soaked in a water bath at 80 °C for 4 weeks. The PBS was refreshed every 1 week.

2.8 Device implantation and in vivo experiment setup
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The surgical procedures were performed in compliance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the National University of Singapore. Female Sprague Dawley rats (8 weeks, 200-300 g in weight) were anesthetized with pentobarbital sodium (50 mg/kg). The surgical site on the right leg was shaved and sterilized. Then, a skin incision was created along the femoral axis of the right leg. The sciatic nerve was subsequently exposed, isolated and freed from the surrounding tissues from the sciatic notch to the knee. The C-shaped electrode was opened by twisting the two tabs above the ring using tweezers, followed by placement of the ring structure onto the nerve. The two protruding tabs were then bonded with biocompatible ultraviolet (UV) glue through 1 min UV hard curing. During the surgery, the exposed nerve area was moistened with PBS to keep the nerve healthy.

A concentric Pt bipolar stimulating electrode was placed in the proximal segment of the sciatic nerve, close to the spinal cord. A schematic diagram of the open-loop nervous stimulation and recording system is described in Fig. 1d. The electrode site was about 3 cm distance distal to the stimulating electrode. A monophasic pulse stimulus was then applied through the Pt stimulating electrode via a potable pulse current stimulator (Model DS3, Digitimer Ltd., UK). The duration of the pulse was 20 μs with a repetition rate of 1 Hz. The current magnitude varied from 0.2 mA to 1 mA. The four-channel C-shaped electrode was connected to a portable wideband filter/amplifier (1-5000 Hz, gain: 10,000) (USB-ME32-FAI, Multichannel systems, Germany) and sampled at 20 kHz. Finally, the signal was processed and analysed offline using custom-made algorithms in Matlab (MathWorks, Inc. USA).
3. Results and discussion

3.1 Micro fabrication results

The photomicroscopy image of the completely fabricated C-shaped electrode chip released from the silicon substrate is shown in Fig. 2a. The four probes were axisymmetrically located around the inner ring frame. The released chips had high product yield (>90%) and very low initial stress. The thickness of the polyimide substrate (~12 μm) allows flexible properties. This permits the bending of parts that contact the nerve minimizing the risk of nerve damage.

Fig. 2b shows a photo image after the device packaging. To isolate the metal pad opening of the device from the aqueous environment in the following in vivo experiment, the zone of the FPC connector was encapsulated by a biocompatible UV epoxy with low liquid permeability. Six wires were connected to the signal preamplifier with four working probes and two reference electrodes. Au-CNT coatings on the six electrodes were subsequently employed. As shown in Fig. 2c and d, the surface of the pure gold electrode was smooth and shiny, whereas the Au-CNT coated electrode surface looked dark due to light scattering because of its roughness.

The scanning electron microscopy (SEM) images in Fig 2e-g present the surface of one working probe made of Au, Au-CNT and platinum materials, respectively. As seen distinctly, the Au and Pt surface were reflective and smooth compared to the Au-CNT surface. The inset of Fig. 2f displays a close look of the morphology of the coated Au-CNT structure. The Au-CNT composite has a polycrystal flower feature, which increases the conductive surface exceptionally [28].

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3.2 Electrochemical characterization

Impedance characterization of the electrode-electrolyte interface is of importance to quantify the functionality of the electrode on both neural signal recording and stimulation. The neural signal is in the range of micro-volts and is highly sensitive to noise arising from the surrounding electrolyte media or muscle movement. The Johnson's voltage noise [19] is proportional to the real part of the interface impedance, expressed as

$$ V = 4kT \cdot Re[Z] $$

In the above formula, $k$ is the Boltzmann's constant and $T$ is the media temperature. From here it can be seen that a low impedance improves the signal to noise ratio (SNR). Additionally, low impedance leads to low output voltage at given stimulus current during stimulation activity, avoiding irreversible damage of the tissue and the electrodes.

Interface impedance is commonly characterized by electrochemical impedance spectroscopy (EIS). The adapted lumped equivalent circuit model proposed by Franks et al [29] consists of the electrical components of non-faradic constant phase angle impedance ($Z_{CPA}$), faradic impedance (sum of charge transfer resistance $R_{ct}$ and mass diffusion Warburg impedance ($Z_w$)) and the solution spreading resistance ($R_s$) (Fig. 3a).

The constant phase angle impedance ($Z_{CPA}$) presents the non-faradic double-layer capacitance resulting from the charge distribution between the electrode surface and the Outer Helmhotz Plane in the solution, implying the electrodes ability to redistribute the charge and cause charge flow in the electrolyte when voltage is applied. By considering the
inhomogeneous capacitor surface, $n$ (0≤$n$≤1) is introduced. $Z_{CPA}$ is given by the following equation:

$$Z_{CPA}(\omega) = 1/(j\omega)^nC_{dl}$$

(2)

where $C_{dl}$ is the empirical parameter, which is an electrode material dependant parameter in units of $F \cdot s^{n-1}/cm^2$.

Faradic impedance reveals the processes of reduction and oxidation at the interface where electrons are transferred between the electrode and the electrolyte. The $R_{ct}$ results from the electron transfer rate at the interface and $Z_w$ is determined from the mass diffusion, which is considered negligible for commonly used materials within the considered electrophysiological frequency [30]. Finally, $R_s$ implies the resistance of current spreading from the working electrode to a counter electrode in the electrolyte.

Fig. 3b shows the experimental setup for the electrochemical measurement by EIS. The EIS measurements were performed on ten electrode sites (area of 8,500 μm²) (n=10) from different samples for each of the three materials (Au, Au-CNT and Pt). Means and standard derivations were then calculated and listed in Table 1. The normalized mean values of the three materials are given in Fig. 3c.

Based on the equivalent interface circuit (Fig. 3a), the measured data were fitted by Autolab FRA Program (Metrohm Autolab B.V., Netherlands) with an electrophysiological frequency range between 100 Hz and 2 KHz. The extracted parameters of $Z_{CPA}(C_{dl}, n)$, $R_{ct}$, $R_s$ and impedance magnitude are summarized in table 1.

The relatively small impedance value (168 kΩ @1KHz) of the Au electrode is attributable to its large electrode area from the C-shaped pattern design. A 1 minute long
deposition of Au-CNT deposition resulted in a 15-fold decrease of the impedance. The impedance values of the Pt electrode were between the Au-CNT and Au, which indicates that this type of electrode can also perform well for signal recording. The same trend can be observed from the data of the non-faradic double-layer capacitance ($C_{dl}$). From table 1, the tremendous increment of double-layer capacitance ($C_{dl}$) in Au-CNT model contributes to the decrease of its electrochemical impedance, also implying the enhancement of the charge storage capability. The values of $n$ for Au and Pt materials are close to 1, implying a pure capacitor property of non-faradic charge redistribution during current flow in the electrolyte, whereas $n=0.76$ for Au-CNT material can be explained by its inhomogeneous capacitor surface from a composite of Au and CNT.

3.3 In vitro long-term stability test

Body implanted material should not deteriorate during its shelf life. Polyimide is a well-known biocompatible material for implantation. B. Rubehn, et. al. [31] has demonstrated that polyimide is stable in PBS at body temperature and even at 60 °C over the course of 20 months in terms of its chemical and mechanical properties. However, the stability of the adhesion between metal and polyimide still needs to be investigated. The effective area of the metal electrode would increase if tissue fluid penetrated in between the two layers. This is unacceptable and will result in inaccurate nerve signal recording.

Accelerating test in 80 °C was performed for 4 weeks. Three samples were taken out from PBS every week and immediately performed the EIS measurement. The rate of aging is increased by a factor [32]:
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\[ f = 2^{(T - T_{ref})/10} \]  

(3)

where \( T \) is an elevated temperature used to accelerate the aging effect, \( T_{ref} \) is the reference body temperature (37°C). Then, \( f' \) is calculated to be 19.7 at 80 °C water bath. So, four weeks aging test at 80 °C is equivalent to immersion of device in PBS at body temperature of 37 °C for 18 months. The unit impedance results at frequency of 1 KHz are given in Fig. 4.

The result reveals that the impedance magnitude was gradually increased by 21% at the first three weeks and dropped by 30% at the end of the fourth week, indicating diffusion of PBS into polyimide layer. The impedance increment may be attributed to salt crystallization on Au surface. These results indicate that the C-shaped electrode chip is stable in physiological environment for at least 12 months.

3.4 Nerve signal recording

Nerve recording was tested using an Au C-shaped electrode chip in the sciatic nerve of an adult rat. The in vivo nerve signal recording setup is shown in Fig. 5a-c. During placement of the electrode around the nerve, the four probes satisfactorily bent to adapt to the nerve circumference (see Fig.5d). The nerve was then stimulated at 1 Hz using a concentric Pt bipolar electrode (Fig. 5c). The stimulus current was increased from 0.2 mA by the step of 0.1 mA. The evoked nerve response signal was recorded, averaged and denoised (bandpass filtering: 300–2000 Hz) over one minute (60 stimulus pulse repetition). The threshold stimulus current was measured to be 0.3 mA. Above this point, a robust nerve signal, namely compound nerve action potential (CNAP), was consistently observed.
in all four channels (Fig.5e,f) after de-noising. The CNAP amplitude and, consequently, the signal to noise ratio (SNR) of the raw data increased with the stimulation strength, particularly from 0.4 mA to 0.5 mA (see Fig. 5f). The peak amplitude of the CNAP at the threshold current of 0.3 mA was measured to be 40 μV. The measurements (peak magnitude of the CNAPs, the latency to onset, duration of CNAPs and the SNR of the raw data) are listed in Table 2. As the stimulation current increased, the latency to onset and the duration of the CNAPs varied correspondingly: the latency to onset decreased from 3.2 to 2.0 ms and duration of CNAP increased from 5 to 16 ms as the stimulation increased from 0.3 to 0.6 mA. This trend can be explained by more rapid changing of neurons membrane potential at high current stimulus and it would require longer time for membrane to return to its rest potential.

As noticed from Fig. 5f, the recorded nerve signals in the channel 1-3 had similar waveform profiles and magnitudes of CNAPs, indicating identical subfascicles response to the Pt electrode stimulation. The signal from channel 4 presented lower CNAP peak magnitude, which could mean that fewer nerve fibres adjacent to this channel were activated by the stimulation current.

Our results were consistent with those similar studies published in the past decades using different peripheral neural electrodes. C. Krarup et. al. [33] recorded signals in the range of 3-10 μV using a traditional cuff electrode based on silicon rubber substrate and implanted around the hindlimb nerve of adult cats. Using polyanide transverse intrafascicular multichannel electrodes (TIME), J. Badia, et. al. [34] reported an amplitude in the CNAPs ranging from 7 μV to 15 μV in rat sciatic nerves. A. Ramachandran [35] et.
al. presented the sieve electrode is capable to measure the CNAPs signals as small as 5 μV, below which the signal may not be extracted from the background noise, using sieve electrode implanted in transected sciatic nerves of rats. From our recording (as seen from the 0.3 mA stimulation in Fig. 5f), we can draw conclusion that the Au electrode is capable to record signal much less than 5 μV.

In vivo neural signal recordings using the Pt and Au-CNT electrodes were also performed on another two rats. The implantation conditions (i.e. positions of Pt bipolar stimulating electrode and C-shaped electrode) were the same as the Au electrode test. The threshold current was calculated to be 0.3 mA for both Pt and Au-CNT electrodes. Sample recordings of the nerve response from Pt and Au-CNT electrodes at this stimulation current are shown in Fig. 6. The raw data before noise cancelation was used to compare the SNRs among the three electrodes materials. As expected, lower impedance values produced a better SNR values (SNR values 0.87, 1.0064, and 1.23 for Au, Pt and Au-CNT, respectively). The SNRs from the raw data was also enhanced by averaging of 60 stimulus pulse repetition. These results cannot, however, be use to quantitatively compare the performance of the three materials as recording electrode for the following reasons: (1) the stimulating contact area of the Pt bipolar electrode may not be identical among the three surgeries. Thus the charge injection via Pt electrode would alter; even when the same current was applied as the previous test. (2) The implantation process of each device would unavoidably lead the probes to have a different contact area with the nerve. (3) During surgery, the nerve suffers from dehydration and its vitality would be degraded over time.
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The time slot from commencement of surgery to nerve signal recording cannot be controlled identically among three surgeries.

4. Conclusion

A novel C-shaped type neural interface electrode has been designed and fabricated, consisting of a polyimide-metal-polyimide sandwich structure. Its four bendable protruding probes can fit different nerves size without sacrificing electrode nerve contact by adjusting the bending angle of the probes. Three electrode materials (Au, Au-CNT and Pt) were selected and the EIS measurement shows that the impedance of Au-CNT is 15 and 3 times lower than Au and Pt, demonstrating that Au-CNT would tremendously reduce the signal to noise ratio and thus increase the signal recording resolution. Long-term stability test of C-shaped neural electrode was performed in vitro by means of accelerated aging test. The EIS results indicate that the chip can be stable in at least 12 months at physiological temperature 37 °C. In vivo experiments were also performed using Au, Pt and Au-CNT electrode devices. The evoked four-channel compound nerve action potentials (CNAPs) were recorded and analysed. The minimum recordable signal of electrode was less than 5 μV.

Acknowledgements

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Figure Captions

Fig. 1. Schematics of (a) device design, (b) device fabrication, (c) device packaging and (d) device implantation and *in vivo* test setup.

Fig. 2. Photography of the fabricated C-shaped electrode: (a) device after releasing from silicon substrate, (b) device packaging, one probe with (c) Au surface, (d) Au-CNT surface. SEM image of one probe (e) Au electrode, (f) Au-CNT electrode, inset is magnified Au-CNT image, showing irregularly rough surface, (g) Pt electrode.

Fig. 3. EIS measurement on C-shaped electrode. (a) Lumped equivalent circuit model of electrode-electrolyte interface; (b) EIS measurement system setup; (c) EIS measurement results of three materials: Au, Au-CNT, Pt electrode.

Fig. 4. EIS measurement over 4 weeks on three C-shaped Au electrodes.

Fig. 5. (a) Overview of the system setup for in vivo rat sciatic nerve signal recording. Photography of rat surgery (b) before chip implantation and (c) after chip implantation. (d) Photomicroscopy image of the implanted chip into nerve. (e)-(f) Nerve response to different stimulus current by using Pt bipolar electrode. (e) Averaged nerve response over
60 stimulation events (repetition rate 1 s). (f) Magnification of the nerve response period from 0.09 s to 0.1 s.

Fig. 6. Averaged nerve signal recording results of (a) Pt and (b) Au-CNT electrode before noise cancellation algorithm at 0.3 mA stimulus current, over 60 stimulation events (repetition rate 1 s).
Table Captions

Table 1. Summary of the fitted parameters results from the interface equivalent circuit of the three electrode materials.

Table 2. Comparison among magnitude of CNAPs, latency to the onset, duration of CNAPs and SNR among different current stimulus.
**Table 1.** Summary of the fitted parameters results from the interface equivalent circuit of the three electrode materials.

<table>
<thead>
<tr>
<th></th>
<th>$C_{dl}$ (μF·s$^{n-1}$/cm²)</th>
<th>$n$</th>
<th>$R_{ct}$ (Ω·cm²)</th>
<th>$R_s$ (Ω·cm²)</th>
<th>Normalized impedance magnitude (Ω·cm²@1KHz) (± std. dev.)</th>
<th>Impedance magnitude of one electrode ($kΩ @1$KHz) (± std. dev.)</th>
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<tr>
<td>Au electrode</td>
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<td>0.39</td>
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<td>168(±14.9)</td>
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<td>Au-CNT electrode</td>
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<td>0.77</td>
<td>51</td>
<td>0.94(±0.088)</td>
<td>11.1(±1.04)</td>
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<tr>
<td>Pt electrode</td>
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<td>0.91</td>
<td>1.83</td>
<td>2690</td>
<td>3.02(±0.43)</td>
<td>35.5(±5.06)</td>
</tr>
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<table>
<thead>
<tr>
<th>Current Stimulus</th>
<th>Magnitude of CNAPs (µV)</th>
<th>Latency to the onset (ms)</th>
<th>Duration of CNAPs (ms)</th>
<th>SNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 mA stimulus</td>
<td>40</td>
<td>3.2</td>
<td>5</td>
<td>0.87</td>
</tr>
<tr>
<td>0.4 mA stimulus</td>
<td>70</td>
<td>3.0</td>
<td>8</td>
<td>2.8</td>
</tr>
<tr>
<td>0.5 mA stimulus</td>
<td>600</td>
<td>2.2</td>
<td>13</td>
<td>10-20</td>
</tr>
<tr>
<td>0.6 mA stimulus</td>
<td>1400</td>
<td>2.0</td>
<td>16</td>
<td>30-50</td>
</tr>
</tbody>
</table>
Fig. 1. Schematics of (a) device design, (b) device fabrication, (c) device packaging and (d) device implantation and *in vivo* test setup.
Fig. 2. Photography of the fabricated C-shaped electrode: (a) device after releasing from silicon substrate, (b) device packaging, one probe with (c) Au surface, (d) Au-CNT surface. SEM image of one probe (e) Au electrode, (f) Au-CNT electrode; inset is magnified Au-CNT image, showing irregularly rough surface, (g) Pt electrode.
Fig. 3. EIS measurement on C-shaped electrode. (a) Lumped equivalent circuit model of electrode-electrolyte interface; (b) EIS measurement system setup; (c) EIS measurement results of three materials: Au, Au-CNT, Pt electrode.
Table 1. Summary of the fitted parameters results from the interface equivalent circuit of the three electrode materials.

<table>
<thead>
<tr>
<th>Material</th>
<th>$C_{dl}$ ($\mu F$)</th>
<th>$n$</th>
<th>$R_{ct}$ ($\Omega \cdot \text{cm}^2$)</th>
<th>$R_s$ ($\Omega \text{cm}^2$)</th>
<th>Normalized impedance magnitude ($\Omega \cdot \text{cm}^2$@1KHz)</th>
<th>Normalized impedance magnitude (± std. dev.)</th>
<th>Impedance magnitude of one electrode ($k\Omega$@1KHz)</th>
<th>Impedance magnitude of one electrode (± std. dev.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au electrode</td>
<td>27.6</td>
<td>0.90</td>
<td>0.39</td>
<td>28600</td>
<td>14.31(±1.27)</td>
<td>168(±14.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Au-CNT electrode</td>
<td>4788</td>
<td>0.76</td>
<td>0.77</td>
<td>51</td>
<td>0.94(±0.088)</td>
<td>11.1(±1.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pt electrode</td>
<td>163</td>
<td>0.91</td>
<td>1.83</td>
<td>2690</td>
<td>3.02(±0.43)</td>
<td>35.5(±5.06)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Comparison among magnitude of CNAPs, latency to the onset, duration of CNAPs and SNR among different current stimulus.

<table>
<thead>
<tr>
<th>Current (mA)</th>
<th>Magnitude of CNAPs (µV)</th>
<th>Latency to the onset (ms)</th>
<th>Duration of CNAPs (ms)</th>
<th>SNR</th>
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Fig. 3. EIS measurement on C-shaped electrode. (a) Lumped equivalent circuit model of electrode-electrolyte interface; (b) EIS measurement system setup; (c) EIS measurement results of three materials: Au, Au-CNT, Pt electrode.
Fig. 4. EIS measurement over 4 weeks on three C-shape Au electrodes.
Fig. 5. (a) Overview of the system setup for in vivo rat sciatic nerve signal recording. Photography of rat surgery (b) before chip implantation and (c) after chip implantation. (d) Photomicroscopy image of the implanted chip into nerve. (e)-(f) Nerve response to different stimulus current by using Pt bipolar electrode. (e) Averaged nerve response over 60 stimulation events (repetition rate 1 s). (f) Magnification of the nerve response period from 0.09 s to 0.1 s.
Fig. 6. Averaged nerve signal recording results of (a) Pt and (b) Au-CNT electrode before noise cancellation algorithm at 0.3 mA stimulus current, over 60 stimulation events (repetition rate 1 s).
Ning Xue received the B.S. and M.S. degree in electrical engineering from Dalian University of Technology, Dalian, China, in 2005 and 2008 respectively. He obtained Ph.D. degree in the Department of Electrical Engineering at University of Texas at Dallas, (U.T. Dallas) in 2012, where he worked on RF MEMS and pressure sensor. He was a research engineer in Agiltron, Inc, developing MEMS optical devices. He is currently holding a Scientist position in Miniaturized Medical Device (MMD) programme in the Institute of Microelectronics (IME), Singapore since 2013. His research interests include neural prosthetics, MEMS fabrication, Microfluidic device, BioMEMS for wireless implantable application, pressure sensors, and optical and RF MEMS systems.

Tao Sun was awarded B. Eng and M.S. in State Key Lab of Advanced Welding and Joining by Harbin Institute of Technology in 2002 and 2004, respectively. He obtained his Ph.D. degree in mechanical engineering from the University of Hong Kong in 2010. Before joining the Institute of Microelectronics (IME), Singapore, in Nov. 2010, he mainly focused on developing bioactive and biocompatible composite coatings for medical applications of Ti, Ti-6Al-4V and NiTi shape memory alloys. Since April 2011, he has served as a Scientist for miniaturized medical device program at IME, and also involved in the neural probe project and optical coherence tomography project as a co-project leader. He has authored and co-authored more than 30 journal and international conference papers. His current research interests include biomaterials, bio-MEMS and drug release.

Wei Mong Tsang received the B.Eng. (first class Honors) and M.Phil. degrees in electronic engineering from the Chinese University of Hong Kong, Hong Kong, in 2000 and 2002, respectively, and the Ph.D. degree in electrical and electronic engineering from the University of Surrey, Guildford, U.K., in 2007. He was a Postdoctoral Associate in the Research Laboratory of Electronic, Massachusetts Institute of Technology (MIT), Cambridge, USA. In 2012, he joined the Institute of Microelectronics (IME), Singapore as a Scientist at IME, Singapore. His research interests include the bio-microelectromechanical systems, neural prosthetics, electron field emission, and nanotechnology. Dr. Tsang is a member of the Materials Research Society. He was the recipient of E.W.Müller “Outstanding Young Scientist Award” from the International Field Emission Society in 2006.

Ignacio Delgado-Martinez was born in Pamplona, Spain, in 1977. He received the medical degree from the University of Cantabria, Spain, in 2002. In 2006, he received the M.D.-Ph.D degree from the University of Göttingen, Germany for his thesis work in synaptic transmission at the Max-Planck Institute. In 2008, he joined the Department of Neurosurgery of the University Hospital Charité of Berlin, Germany, where he worked as neurosurgeon. In parallel, he continued his research in the Max-Delbrück Center of Berlin. Since 2012, he is research fellow of the Singapore Institute for Neurotechnology (SINAPSE). His current research interests include the design of new neuroprosthetic approaches for the clinical treatment of peripheral nerve injuries, the use of computer games as a cognitive rehabilitation tool, and the development of tools for robotic neurosurgery.
**Sanghoon Lee** received the B.S. degree at the department of electronic material engineering in Kwangwoon University, Seoul, Korea in 2009 and his M.Eng. degree at the department of electrical and computer engineering (ECE) in National University of Singapore (NUS) in 2013. The research topic of M.Eng. was the development of new functional superparamagnetic nanoparticles and the applications to various cancer diagnosis/treatment modalities such as glioblastoma and liver cancer in nanomedicine. He is currently pursuing his Ph.D. under the supervision of Prof. Vincent C. Lee at the department of ECE in NUS as well as Prof. Shih-Cheng Yen in Singapore Institute for Neurotechnology (SINAPSE). His current research efforts are focused on the development of MEMS based neural prostheses.

**Swathi Sheshadri** received the B.E. degree from the department of Instrumentation Technology at the Visvesvaraya Technological University, Karnataka, India in 2011 and is presently pursuing her M.Sc. degree from the department of Electrical and Computer Engineering (ECE) in National University of Singapore (NUS). Her research topic of M.Sc. was Neural Decoding of Peripheral Nerve Signals. She is currently also working as a research staff under the supervision of Prof. Shih-Cheng Yen at Singapore Institute for Neurotechnology (SINAPSE). Her current research efforts focused on signal processing for neural prosthetic applications.

**Zhuolin Xiang** received his B.Eng. degree from the Department of Information and Electronics, Beijing Institute of Technology, Beijing, China in 2011. He is now a Ph.D student in Electrical and Computer Engineering, NUS. His research interests focus mainly on BioMEMS Devices for drug delivery and neural interfacing.

**Srinivas Merugu** completed Master of Science degree from Institute for Micromanufacturing at LaTech University in 2002 and Ph.D. in electrical engineering from University of Utah in 2008. He has worked under Solzbacher for three more years as a post-doctoral researcher, with research on wafer level fabrication of Utah Electrode Arrays (UEA). He is currently working as a Scientist in MEMS Integration group at Institute of Microelectronics. His research interests are in Bio-MEMS especially in Neuroprosthetics and Micro-Total Analysis systems. He has more than twelve years experience in MEMS fabrication processes. He has developed fabrication processes for diverse MEMS devices such as carbon nanotube based DNA sensor, adaptive stiffness neural electrodes, microvalves based and aluminum nitride based devices such as checkerboard oscillator and hydrophone using Smart SOI wafers. He is also interested in Aluminum-Nitride based MEMS devices such as Resonators and PMUTs.

**Yuandong Gu** received his Ph.D. in Pharmaceutics and M.E.E in Electrical Engineering, both from the University of Minnesota, Twin Cities, USA in 2003. He is the Technical Director of Miniaturised Medical Devices (MMD) and Sensors & Actuators Microsystems (SAM) programmes at the A*STAR Institute of Microelectronics (IME) since 2013. He spent 10 years in the Honeywell Sensors and Wireless Lab as a principal research scientist before joining A*STAR Institute of Microelectronics. His industrial research covers broad areas include chemical and biosensors, ultrasound, chip-level thermal management, chip-level vacuum systems, and deeply miniaturized medical equipments.
Shih-Cheng Yen received the B.S.E. and Ph.D. degrees from the Department of Bioengineering, University of Pennsylvania, Philadelphia, in 1993 and 1998, respectively. He is currently an Assistant Professor with the Department of Electrical and Computer Engineering, National University of Singapore, Singapore. His research interests include neural coding, mechanisms underlying computation in the visual system, visual psychophysics, and biological models of the visual system. Prof. Yen has won several awards, including the Solomon R. Pollack Award for Best Ph.D. Dissertation in 1998, the Philadelphia Young Engineer Award in 1993, the Rose Prize for Outstanding Undergraduate Research, University of Pennsylvania, in 1993, and the NASSAU Award for Undergraduate Research, University of Pennsylvania, in 1993.

Nitish V Thakor received B.Tech. degree in electrical engineering from Indian Institute of Technology, Bombay, India, in 1974 and the Ph.D. degree in electrical and computer engineering from the University of Wisconsin, Madison, in 1981. He served on the faculty of Electrical Engineering and Computer Science of the Northwestern University, Evanston, IL, between 1981 and 1983, and since then he has been with the Johns Hopkins University, School of Medicine, Baltimore, MD, where he is currently serving as a Professor of Biomedical Engineering. He has established a Center for Neuroengineering at the Johns Hopkins University with the aim of carrying out interdisciplinary and collaborative engineering research for basic and clinical neurosciences. He is also the director in Singapore Institute for Neurotechnology (SINAPSE). He is actively interested in developing international scientific programs, collaborative exchanges, tutorials, and conferences on neuroengineering and medical microsystems.