Plastic neuronal probes for implantation in cortical and subcortical areas of the rat brain

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Abstract: We discuss the fabrication of flexible implantable probes for recording neuronal activity in the rat brain. We fabricated such probes bearing 12 platinum electrodes, using polyimide as a substrate and SU-8 as an insulation layer. The fabrication process was simplified through the use of laser ablation to define the probe outline. The probes showed not only good mechanical flexibility but also the required stiffness for implantation. Histology results and electrical recordings of neuronal activity lend support to the idea that the combination of polyimide and SU-8 represents a good choice of materials for the fabrication of implantable neuronal probes.

Keywords: bioelectronics; implantable electrodes; hippocampus; cortex; *in vivo* recordings; flexible microarrays.

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1 Introduction

The computational power of neuronal networks arises from the complex interactions and cooperation of a large number of neurons embedded in a functional network. Thus, a direct investigation of the temporal dynamics of neuronal populations (and structures) must be addressed by the simultaneous observation of multiple neurons and their networks. The last decade has seen the emergence of a new type of electrode that provides recordings of the activities of populations of neurons (local field potentials – LFPs) and single neurons (unitary activity). These silicon-based probes with multiple recording sites make it possible to collect signals at the sub-millimetre scale from small volumes of tissue [1–3]. The increase in the number of recording sites (metal sites) per probe is driven by the need to record as much information as possible without increasing the volume of the implant. The signals recorded from these probes are weak and need to be preamplified using silicon CMOS circuitry in the vicinity of the recording sites. These probes were designed to be implantable chronically in an animal able to move freely to realise behaviour recordings. However, the technology of silicon probes suffers from several limitations, which slow down the process of furthering our understanding of brain function. The probes create a tissue scar that, with time, impairs the quality of the recordings of neuronal signals. The rigid nature of the probes cannot compensate for small brain movements and is thus not ideal for extended *in vivo* recording. Therefore, the probes always induce an inflammatory reaction in the brain because of the mechanical or biological mismatch between probe and tissue. These factors degrade the electrical characteristic of the recordings with time [4]. Moreover, the probes can easily break while they get inserted into the brain or while they are implanted in the region of interest.

A strategy to decrease the mismatching between the mechanical properties of implanted probes and tissue involves the use of flexible materials. Probes based on

polymers such as polyimide [5–7], parylene [8] and SU-8 [9,10] have been developed to establish a better brain–device interface for acute and chronic experiments. Several groups have used such materials as a mechanical support both for the metal electrodes as well as for electrical insulation [11,12]. Such probes were even integrated with microfluidic channels for drug delivery [13]. Strategies to further improve the biocompatibility of these probes have been developed and involve coatings of polyethylene glycol (PEG) [14], benzocyclobutene [15], polyethyleneimine [16] or poly(3,4-ethylenedioxythiophene) [17]. Furthermore, the biocompatibility and biodegradability of PEG have been exploited to temporarily increase the stiffness of the probes and ease their implantation. This material is solid at room temperature and can be dissolved when in contact with tissue [8].

The development of plastic probes is very much the subject of the ongoing research, aiming to fabricate probes with long-term implantation capacity, high-quality signal recording and facile packaging. In this paper, we describe the fabrication of flexible probes based on a polyimide substrate, platinum electrodes and an SU-8 insulation layer. We describe the fabrication of such probes and their *in vivo* evaluation in recording neuronal activity in the rat brain. The innovation was in the laser cutting of the probes, which simplified their fabrication, and in the combination of the polyimide with SU-8, which improved their stiffness.

2 Materials and methods

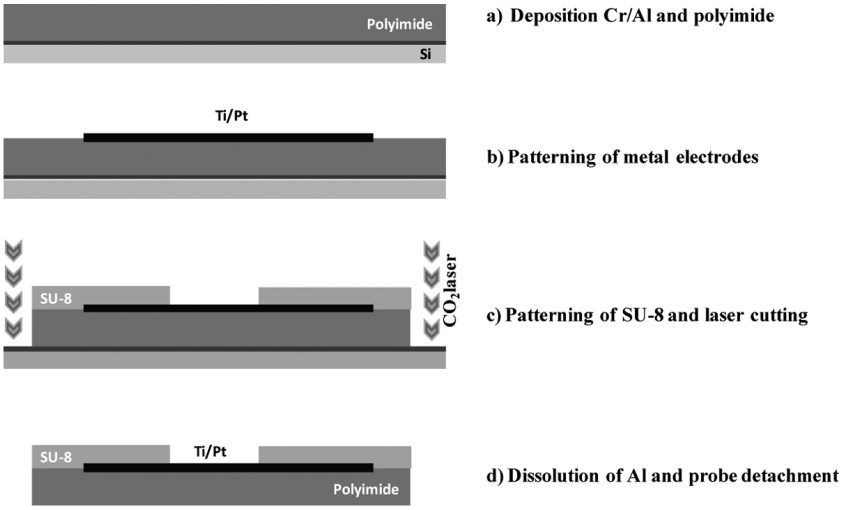
2.1 Probe design and fabrication

The fabrication of the polyimide-based probes involved the deposition and patterning of alternating layers of polyimide, platinum (Pt) and SU-8, and largely followed processes described in the literature [12]. In short, the Pt electrodes were deposited on the polyimide, patterned by lift-off, and covered with an SU-8 layer. For ease of fabrication, the probes were built on a silicon substrate, and were released using anodic dissolution of a sacrificial aluminium layer at the end of the fabrication process.

The fabrication process, displayed in Figure 1, began with the evaporation of 100 nm of chrome (Cr) and 500 nm of aluminium (Al) onto an *n*-doped silicon wafer. To make the polyimide layer, a PI2611 solution from HD Microsystems was spin-coated on top of this Cr/Al sacrificial layer. The final thickness of the polyimide was adjusted to 20 μm by coating with two layers of PI2611. Two soft bakes at 90°C for 90 s and at 180°C for 90 s were carried out after the first and second polyimide precursor applications, respectively. Finally, the film was cured for 1 h at 350°C in a YES oven under nitrogen flow with the heating/cooling ramp rate recommended by HD Microsystems. Subsequently, an S1813 (Shipley Microposit photoresist) layer was deposited on the polyimide surface, and exposed and developed according to the manufacturer's specifications. A 10 nm layer of titanium (Ti), used as an adhesion promoter, and a 20 nm layer of Pt were evaporated onto the wafer. Stripping the photoresist with 1165 stripper solution completed the metal patterning. A 10 μm thick layer of SU-8 2015 (MicroChem Corp.) was subsequently applied to the whole wafer. It was spin-coated and soft-baked at 65°C for 2 min and then at 95°C for 7 min, and then was exposed with 150 mJ/cm^{-2} using an ABM contact aligner. The substrate was post-baked at 65°C for 2 min, and at 95°C for 4 min.

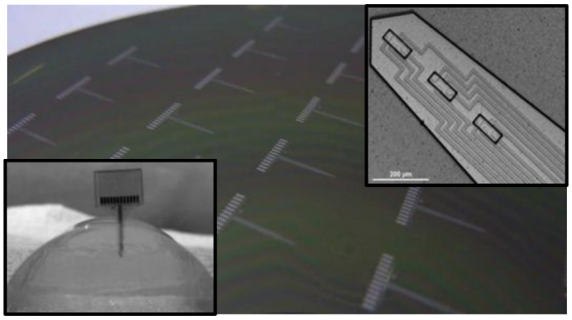
Development in SU-8 developer solution opened windows over the Pt electrodes and their pads.

Figure 1 Process flow diagram for the fabrication of probes



The definition of the probe outline in the polyimide layer was performed by laser ablation. A CO_2 laser engraving/cutting tool (Universal Laser Systems VersaLaser) made a precision cut of the polyimide layer. The probes were then detached from the wafer by anodic metal dissolution of the sacrificial layer. The wafer was immersed in a salt solution (2 M NaCl) at RT with a large platinum counter electrode [18]. A constant positive voltage (1.2 V) was applied to the aluminium layer, which dissolved, releasing the polyimide probes. Finally, the probes were rinsed in DI water to remove the salt marks. The probes were 1 cm long and 30 μm thick. The metal electrodes were placed on a 5 mm long single shank. They had a recording area of 20×20 μm^2 or 10×20 μm^2 and were assembled in a tetrode configuration (see Figure 2). The interconnect part of the probe contained 12 pads with an area of 800×200 μm^2, with a 200 μm electrode-to-electrode spacing. Insulated metal wires were soldered directly on the pads and connected to an Omnetics connector.

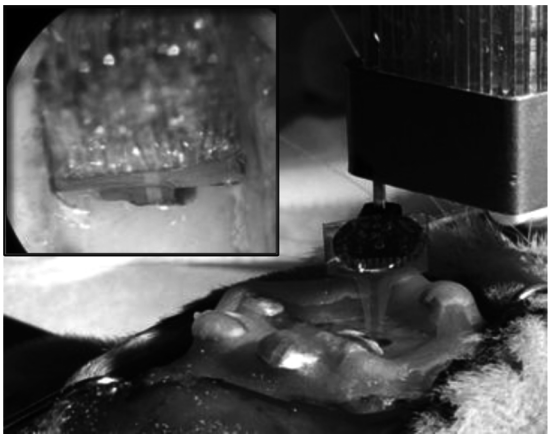
Figure 2 Probes before laser cutting and detachment from the carrier wafer. Insets show the outline of the shank of a probe (upper right) and a probe implanted into agarose gel (lower left) (see online version for colours)



2.2 Histology

All the protocols have been approved by the Institutional Animal Care and Use Committee of INSERM. A probe was attached to a carrier, which was placed on a stereotaxic holder to control precisely the implantation. A male Wistar rat (378 g) (Charles River, MA) was anaesthetised with an O_2-isoflurane mixture (0.6 L/min). The skull of the animal was immobilised in a stereotaxic apparatus and a warmed cushion placed under the animal to keep its temperature constant at 37°C. A 2×3 mm^2 craniotomy was performed in the right hemisphere above the somatosensory cortex and the dorsal hippocampus, centred at the stereotaxic value of −3 mm in the anterio-posterior axis and −2 mm in the medio-lateral axis, relative to Bregma. The dura mater was removed and the electrode was slowly lowered to 3.5 mm from the surface of the brain. The probe was then securely maintained in its final position by fixing its carrier to the skull with the help of metallic pins cemented to the skull. Finally, a plastic cover was fixed with dental cement to seven stainless steel support screws implanted in the skull (shown in Figure 3), to protect the implanted device. The rat was then kept in its home cage with food and water *ad libitum* in controlled conditions of temperature and light.

Figure 3 Polyimide probe implantation into the rat brain. The inset shows the implanted probe during electrical recording



After 14 days, the animal was deeply anaesthetised by a 4 ml intraperitoneal injection of 7% chloral hydrate, and then perfused via the aorta with 1 L of 4% paraformaldehyde solution (PFA) in a 0.12 M phosphate buffer (PB). The brain was extracted and placed in a 4% PFA solution for overnight postfixation. It was then placed for 24 h in 20% sucrose in 0.12 M PB solution and frozen at −80º. The implanted hemisphere was cut in 40 µm sagittal slices using a cryostat. To identify both the track of the probe in the brain and the possible tissue reaction to polyimide, some sections were stained with Nissl coloration (Cresyl violet) and some other sections around the microelectrode track were taken for anti-Glial Fibrillary Acidic Protein (GFAP) immunochemistry as described later. The slices were mounted on SuperFrost slides, stained, then dehydrated and protected by cover slips with Eukitt (Fisher Scientific, Electron Microscopy Sciences).

The second batch of freely floating sections was first rinsed for 30 min in 0.02 M potassium PBS (KPBS: 16 mm K_2HPO_4, 3.5 mm KH_2PO_4, 150 mm NaCl, pH 7.4),

incubated for 30 min in 1% hydrogen peroxide to block endogenous peroxidases, and rinsed in KPBS. The slices were then incubated for 1 h at RT in KPBS containing 0.3% Triton X-100 (KPBS–Triton) and 3% normal Goat serum (NGS, Vector Laboratories), and overnight at RT in GFAP monoclonal antibody (Rabbit anti-GFAP) diluted 1 : 1000 in KPBS–Triton containing 1% NGS. After these steps, sections were rinsed for 30 min in KPBS and incubated for 1 h at RT in biotinylated goat anti-rabbit IgG (Vector Laboratories) diluted 1 : 200 in KPBS containing 3% NGS. Sections were then rinsed in KPBS, incubated for 1 h at RT with an avidin–biotin–peroxidase complex solution prepared according to the manufacturer's recommendations (Vector Laboratories). After several rinses in KPBS, the slices were processed for 15 min in 0.04% 3–3-diaminobenzidine-HCl and 0.006% hydrogen peroxide diluted in KPBS. They were then rinsed in KPBS, mounted on SuperFrost slides, dried, dehydrated and coverslipped with Eukitt.

2.3 Electrical recordings

A male Wistar rat (500 g) (Charles River, MA) was anaesthetised with a ketamine/xylazine mixture (2 : 1). Additional doses were given through the experiment. The probe implantation took place as described earlier. The probe was inserted in the somatosensory cortex. Two miniature stainless steel screws were driven into the skull above the cerebellum and served as ground and reference electrodes, respectively. The probe was connected to an HST head stage (Plexon), which was connected to a multi-channel Digital Lynx 10S acquisition system (Neuralynx). The neurophysiological signals were amplified (1000×), band-pass-filtered (1 Hz–5 kHz) and acquired continuously at 32 kHz on the 64-channel Neuralynx system (16-bit resolution). The neurophysiological data were explored using NeuroScope [19].

3 Result and discussion

Polyimide has been successfully used owing to its good dielectric properties as an encapsulation layer in microelectronic devices, and as a non-toxic material for *in vivo* applications [20,21]. Its mechanical flexibility is believed to help minimise the damage created by micromotions of the implant with respect to the soft biological tissue. Implantable probes can be fabricated according to the process described in Figure 1. Frequently, Pt is used to make electrodes for biological applications owing to its resistance to corrosion and its good charge delivery capacity. Pt microelectrodes can be easily patterned on top of polyimide by lithographic lift-off. Finally, the use of a photo-imageable insulation layer such as SU-8 simplifies the definition of the active electrode area and pads. This layer also serves to improve the stiffness of the probes, making easier their manipulation during the implantation into the brain, as well as facilitating their interconnection to external electronics. The fabrication can be carried out using standard lithographic processes, resulting in several probes on a single carrier wafer (Figure 2). Details of the shank are shown in the inset.

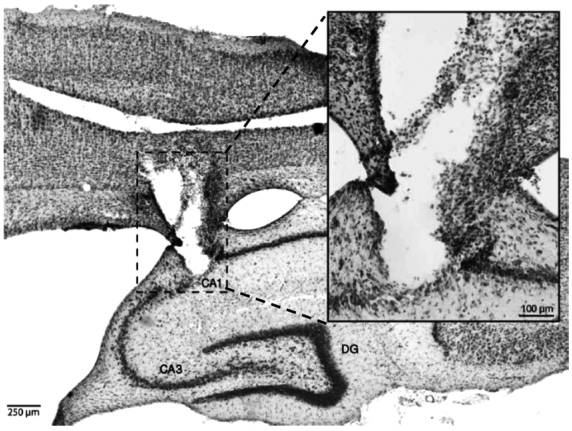
An issue with the fabrication of probes based on a polyimide substrate is the definition of their outline, which allows the probes to be separated from each other. This is usually done by means of reactive ion etching or wet etching [22]. We found that etching of polyimide resulted in a residual layer near the substrate that was not easily

removable and kept the probes from separating from each other. We, therefore, used a CO_2 laser ablation process to define the final probe outline. The final step in the fabrication of the probe involves separation from the carrier wafer. Anodic metal dissolution permits the detachment of the probes through the use of an Al sacrificial layer [18].

To test their ability to be implanted, the probes were inserted into an agarose gel with 0.8% weight/volume (w/v). This gel mimics the stiffness of the dura matter, which is the most difficult layer of the brain to penetrate, and was used as a model for preliminary evaluation of the probes. The inset of Figure 2 shows a probe successfully implanted in this gel. It should be mentioned that after the probes were detached from the silicon wafer, they tended to bend a bit, possibly owing to internal stress. However, the polyimide probes with SU-8 layer showed enough rigidity to penetrate the agarose gel despite their slightly bent shape.

After the successful implantation in agarose gel, a probe was mounted on a carrier for *in vivo* evaluation. An adult rat was anaesthetised and placed into a stereotaxic frame. A craniotomy was performed in the skull and the probe was lowered into the brain as illustrated in Figure 3. The probe was inserted 3.5 mm deep to penetrate the cerebral cortex matter and reach the hippocampus. It was left implanted for 14 days, before histology was performed to visualise the track of the probe as well as to check its biocompatibility. To identify the track of the probe in the brain, sections were stained with Nissl colouration. The Nissl solution is an acidophilus colourant, which binds preferentially to acidic components such as the Nissl bodies present in large amount in neuronal cytoplasm. In the brain, the Nissl solution stains almost exclusively the neurons. This colouration reveals the anatomical neuronal background, allowing us to determine the general histological characteristics of the tissue, which in turn help to identify the track of the microelectrode through the brain. Figure 4 shows the *post-hoc* histology of the region of the brain where the probe was implanted. The track of the probe could be followed through the cortex, and the probe reaches a final depth in the CA1 region of the hippocampus. It can also be seen that the probe did not penetrate all the way to 3.5 mm (the estimated depth of implantation), owing to its bent shape.

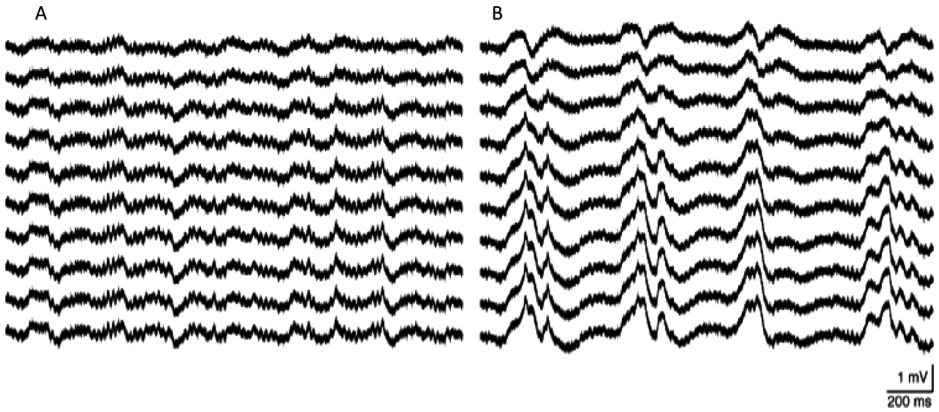
Figure 4 Nissl colouration of a brain slice around the placement of the probe. The main frame shows the final depth reached by the implanted microelectrode (dashed box), corresponding to the CA1 region of the dorsal hippocampus. The inset shows the area of the dashed box in more detail



GFAP staining (not shown here) was used to identify an eventual glial reaction (gliosis), since astrocytes and microglia are generally involved in the immunological response of the brain tissue owing to the presence of a foreign body. The preliminary immunochemistry results reveal the presence of a small glial response, which appears to be minor and localised around the microelectrode. Altogether, we can say that the polyimide probe was implanted for 14 days and induced a low inflammatory response, which is an encouraging result for obtaining good-quality–long-term recording of the neuronal activity.

To assess the ability of the probes to record neuronal activity *in vivo*, a probe was inserted through the somatosensory cortex of an anaesthetised rat. The probe recorded LFPs through different layers, showing a typical pattern of slow activity that is recorded under anaesthesia (Figure 5(A)) [23]. These electrodes allowed us to follow with good fidelity the fluctuations of the LFPs under control conditions (Figure 5(A)). They also allowed us to monitor increased network activity resulting from the action of 100 nmol bicuculline, deposited at the surface of the cortex. Bicuculline is a $GABA_A$ receptor antagonist, which triggers sharp-wave events in the LFPs (Figure 5(B)). These results show that the probes fabricated here are able to record neuronal activity across different electrode sites in the rat brain.

Figure 5 Cortical LFP recordings: (A) Ten channel recordings of LFPs in the cortex of an anaesthetised rat. Note the slow fluctuations of the LFPs in the different cortical layers (top: deeper layers, bottom: upper layers); (B) Bicuculline (100 nmol) was deposited at the surface of the cortex and triggered sharp-wave events through all the cortical layers



4 Conclusions

In conclusion, we fabricated plastic neuronal probes with 12 platinum electrodes in a tetrode configuration for implantation in the cortical and subcortical areas of the rat brain. The fabrication process was simplified through the use of laser ablation to define the final probe outline. Histology results after a 14-day implantation show only a small glial response around the microelectrode. Good-quality recordings of neuronal activity were achieved during slow brain activity under anaesthesia, and during increased activity resulting from the action of bicuculline. These results lend support to the idea that the combination of polyimide and SU-8 represents a good choice of materials for the

fabrication of implantable neuronal interfaces. The probes showed not only good mechanical flexibility but also the required stiffness for implantation.

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