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Epileptic seizure recordings of a non-human primate using carbon nanotube microelectrodes on implantable silicon shanks

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Abstract— **In order to assess the signal to noise ratio and spatial selectivity of nanostructured microelectrodes, our consortium built an original assembly of surface macroelectrodes and deep microelectrode arrays. Acute and chronic assessments were achieved based on rodent and non human primate model. Eventually, we used this device to monitor by association of the surface and deep microelectrodes nominal behavior of the animals and induced epileptic seizure.**

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I. Introduction

In recent years, brain-computer-interfaces (BCI) have been developed to allow patients with motor handicaps to interact with their environment. BCI systems require three basic functionalities: brain activity recording, signal processing and transfer of commands to exterior actuators (e.g. computer, mechanical prosthesis, etc.). The best results are achieved using microwires[1] or multielectrodes array similar to a Fakir's carpet[2]. This technology reaches the limits of neuronal resolution but is quite invasive. The penetration of these electrodes in the grey matter induces an important reactive gliosis which insulates the electrodes and triggers neuronal depletion around the implant, consequently this reduces the system life-time to only a few months[3].

In recent years, more and more articles describe the advantages of carbon nanotube and their likelihood to be good electrodes candidates. From in-vitro studies which analysis the specific interface of neural cells with CNT, Cellot *et al.* demonstrated that the action potentials measured on MEA covered by CNT are differs from the usual shapes due to the adhesion of cells on these materials[4]. Moreover during chronic in-vivo recordings[5], we also chose to compare two kinds of electrode materials, titanium nitride (TiN) known to be used for in vivo applications and carbon nanotubes (CNTs) which were reported to be a good substrate for neural culture and electrophysiology recording in different in vitro studies[6]. To limit the reactive gliosis on the electrodes, we demonstrated that the use of nanostructurated surfaces especially with carbon nanotubes did not trigger inflammatory reaction. Moreover Keefer *et al*[7] showed that the recorded current through these nanostructurated electrodes was higher during acute recording session. It is all to the point the nanostructuration may improve the signal to noise ratio.

In this study, we developed an implantable multi-electrode array to record epileptic seizures in the sensorimotor cortex. The device is composed of an original assembly of silicon shank that hosts the nanostructurated electrodes and platinum surface electrodes. To solve the technical challenge of connector housing and aging in-vivo, we developed a modular watertight packaging.

In this paper, we present a chronic assessment of this system. The recording of the microelectrodes confirm that nanostructurated microelectrodes can be embedded in an implantable recording system.

This technology presents similarity with the ones of the Imtek[8] developed in the framework of the Neuroprobes project but as far as we know it is the first fabrication an in-vivo assessment of carbon nanotubes embedded on silicon shanks. This consortium also reported on the biocompatibility of the silicon shanks in a recent communication[9].

II. Material & Methods

A. Implant description

The implant includes 64 microelectrodes and 8 macroscopic cortical electrodes.

The 40µm diameter microelectrodes are patterned on a 50µm thick silicon shanks, spaced every 100µm between 1.5 and 3mm deep.

The silicon microelectrodes are connected to a flexible ribbon and an Anisotropic Conductive Film to offer flexibility and to adapt the shank position to the pace of the brain. The flex were designed by MXM Neuromedics and subcontracted to Cicorel, see Fig. 1. To avoid the aging of the connectors, we chose to mount the connectors on a removable Printed Circuit Board. This trick allows a very compact assembly on the head of the animal. This PCB is shown on Fig. 2 (left).

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Fig. 1: assembly of the flex ribbon with the silicon shanks

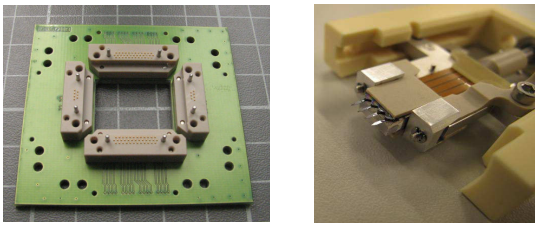

Fig. 2: left: Printed Circuit Board bearing the connectors and the pogo pins microdrive, right: adapted for the silicon shank insertion

The electrical connection is obtained thanks to pogo pins. The implantation procedure includes the insertion of the silicon shank thanks to a dedicated microdrive, see Fig. 2(right).

A. Silicon microfabrication

The silicon shanks devices were fabricated from 8-inch (200mm) MEMS wafer processing technology at CEA/LETI. The process flow is based on double-side polished Silicon On Insulator (SOI) wafer patterning, to create free-standing 50µm thick silicon cantilever shanks that incorporate regularly spaced CNT electrodes (40µm diameter) to record neuronal activity. The electrodes are spaced every 200µm on the shank so that the depth of insertion of the electrodes is between 1.6mm and 3mm which allows the recording several layer of the grey matter -especially from III to V.

The fabrication starts with a thermal oxidation process performed at 1050°C and resulting in a 0.5µm thick silicon oxide (SiO_2) layer covering the SOI wafer both sides. For the backside etching a hard mask of 7µm thick low-stress silicon oxide layer was then deposited onto the SOI backside at 240°C by Plasma Enhanced Chemical Vapor Deposition (PECVD) method. Then, 20nm thick Titanium (Ti) and 200nm Titanium Nitride (TiN) layers were sputtered onto the wafer front side, followed by a 20nm thick Chromium (Cr) layer to cover the TiN. A front side photolithography process and argon ion etching was performed to pattern the electrodes. After resist stripping, a 1.5µm thick low-stress PECVD SiO2 layer was deposited onto the wafer front-side to insulate the electrical lines. Afterwards, the SiO2 layer was locally patterned to open the electrode. The thickness of this insulation layer is adapted to the height of the carbon nanotubes. Then there is no mechanical stress on the carbon nanotubes during insertion of the shank. After the Cr protective layer is chemically etched, a 5nm thick Nickel catalyst layer was deposited on the front-side by physical vapor deposition (PVD) and patterned by photolithography and argon ion etching. Front-side photolithography was then performed to define the shape of the silicon shanks (outlines and trenches). This step was followed by Reactive Ion Etching (RIE) etching of the top-side 1.5µm SiO2 oxide layer and Deep RIE etching of the 50µm thick SOI silicon active layer down to the Buried OXide Layer, removed afterwards by BHF etching. Eventually a double photolithography - Deep RIE etching step was performed at the back side to release the cantilever shanks and separate the chips, before the CNT growth onto the recording sites. Fig. 3 shows a SEM picture of the silicon shank after release and after CNT growth.

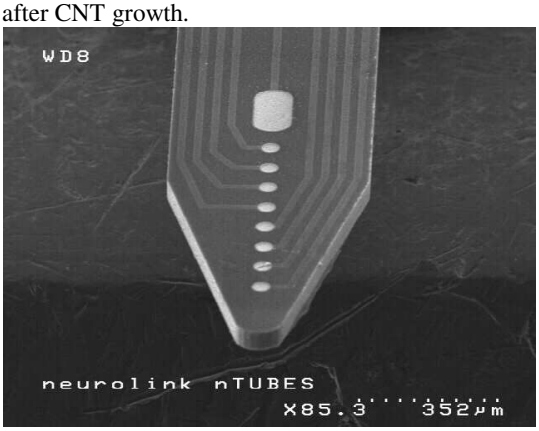

Fig. 3: Scanning Electron Microscope pictures of a silicon shank, featuring 8 nanostructurated recording sites and a counter electrode.

B. Carbon nanotubes growth

Carbon nanotubes were grown by chemical vapor deposition (CVD) technique, using a protocol which was reported in details by Dijon[10], introduced in the silicon microfabrication and briefly recalled as follows. First, we deposited a 5 nm thick layer of nickel on TiN samples to catalyze the carbon nanotubes synthesis. Growth step took place in a CVD reactor heated up to 650°C under a reductive atmosphere of hydrogen (H_2, 5slm) in which we introduced a gas mixture of acetylene and helium for 10 minutes at a pressure of 3.10^3 Pa. Eventually, the reactor was cooled down under H_2 flow until the temperature reached 300°C.

CNTs were then analyzed by scanning electronic microscopy (SEM) and Raman spectroscopy to characterize their degree of graphitization, Fig. 4 is zoom image of the electrode on the silicon shank. The diameter of the tubes is typically 30nm.

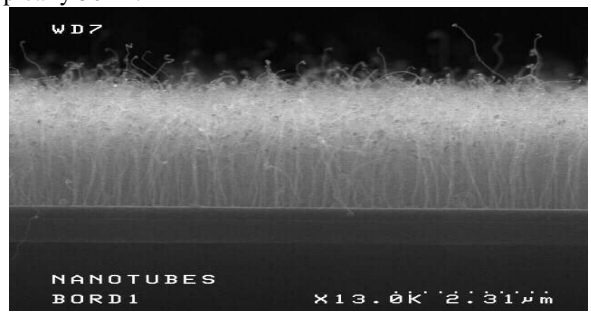

Fig. 4: SEM picture of carbon nanotubes grown by the CEA of the silicon shanks

C. *Sterilization*

Surgical instruments were steam sterilized in an autoclave at 121°C under 2bar of pressure during 20 minutes, while the implant was sterilized by ethylene oxide exposure for fifteen hours and placed in a sealed package until the operation.

D. *Surgical operation*

Acute and chronic implantation of electrodes was performed in accordance with European Committee Council directive 86/609/EEC, and can be summarized as follow:

Prior to the operation, a monkey (macaca fascicularis) was anaesthetized with a ketamine (imalgene©) injection dose of 20mg/kg. The anesthesia was maintained by injecting 5mg/kg of ketamine every 30 minutes during the operation. The primate was placed in a stereotaxic frame before the incision of the scalp. A 2cm x 2cm craniotomy was conducted to position the active part of the implant directly, in the motor region (pre-rolandic gyrus) and a portion of the post-rolandic primary sensory region, while the implant connector was placed on the skull in a watertight housing.

Post operative antibiotics therapy was provided by Amoxicillin/Clavulinic Acid (22 mg / kg, intramuscular) over the course of the study to prevent infection.

E. *Chronic signal acquisition*

Brain signals were monitored weekly during one month, starting one week after the implantation. During each session, the monkey was placed in a primate chair equipped with a head holder to restrain the movement of the head of the monkey. The chair was then placed in an electrophysiology room shielded to limit external electromagnetic perturbations. The MicroMED recording system was placed outside the shielded room and linked with a 1m shielded cable.

F. *Signal Processing*

Data were analyzed using MicroMed Sync Plus using a 1600 Hz HFF (high frequency filter); 200 Hz LFF (low frequency filter) and 50Hz notch filter were used during the cortical recordings. The recording speed was 20 cm/s. The signal scale is 200µV/cm. As the seizure can also occur of the reference electrode, we used a differential representation, which also subtracts the ambient noise.

III. RESULTS & DISCUSSION

A. *Electrodes impedance spectroscopy*

The behavior of the electrodes have to be assessed in frequency, to this aim with used a galvanostat supplied by Biologic. The electrodes were dipped on a PBS solution and a 1mm disk of platinum was used for the counter electrodes. The galvanic mode was used to control the current density at the interface between the electrode and the electrolyte. On Fig. 5, the phase is plotted in red of CNT microelectrodes and in purple for platinum cortical macroelectrodes. The impedance module is plotted in blue for CNT microelectrodes and in green for platinum cortical macroelectrodes.

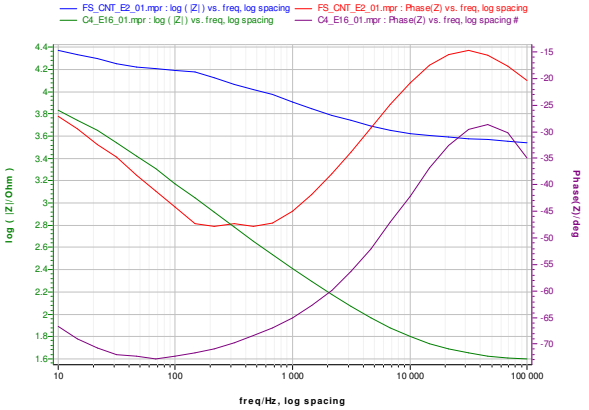

Fig. 5: Comparison of the impedance spectroscopy of surface electrodes and CNT microelectrodes

B. *Recordings of epileptic seizure*

The epilepsy session was planed four weeks after implantation. Unfortunately, at this stage, the shank with platinum electrodes was no longer inserted in the brain of the monkey. Consequently, we could not compare nanostructured and flat microelectrodes as shown in our previous communication (EMBS 2009) but only deep and surface electrodes. This damage was explained by repeated local cares (3 times a week). Spontaneous brain activities were then recorded for one hour prior to injection of penicillin (volume 5µl, 2500 Units, flow rate: 1µl/min). Using an electrode holder attached to a stereotactic frame, a micro-catheter was inserted intracortically in the right motor cortex. As shown on **Fig. 6**, the injection was careful positioned in the center of the electrode array, so as to have a distance to source equal in all sides. The outlet of the micro-catheter was located 2mm under the dura mater. The epileptic activity of penicillin injection was observed in approximately 6 minutes. EEG recordings were performed for 90 minutes. To control the epileptic syndrome we proceed to a diazepam injection. Markers were placed on the recording each time clinical evidences were observed. Video control is used to compare recordings events with clinical evidence of the seizure.

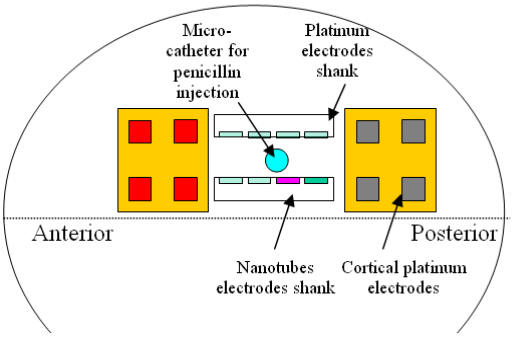

Fig. 6: Schematic top view of the monkey head

Penicillin Epileptic model is one of the most useful acute models in the field of experimental epilepsy studies. This model is also essential for analysis of the spread and synchronicity of epileptogenic activity[11]. It allows obtaining EEG records as in acute partial seizure by application penicillin to cortical surface. Penicillin-induced epileptic activity begins focally, but then spread in a dose dependant way[12]. This characteristic can be used as a tool in electrode characterization allowing the comparison of recording quality of different electrodes materials using a highly reproducible brain activity. Electrodes can be disposed surrounding the injection site and signal acquisition can be made from this very clear controlled brain source.

On Fig. 7, we represent the differential recording of the deep and surface electrode. The data shown represent a silicon shank in the sensorimotor cortex, parallel to the anteroposterior plane, the array of surface electrode is placed 5mm ahead.

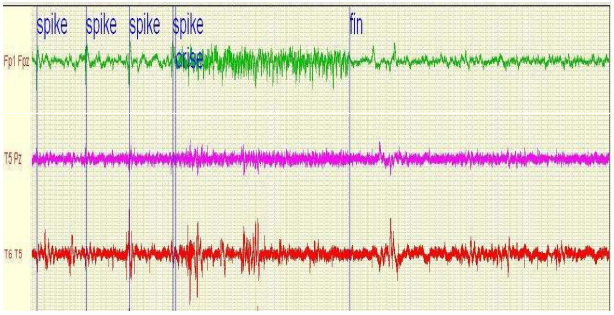

Fig. 7: Spatiotemporal characterization of a seizure, Example of epileptic seizure preceded by 4 interictal events: in green micro recording with deep electrodes close to the epileptic spot, in pink microrecording far from the epileptic spot and in red cortical macroelectrodes.

On this snapshot, one can clearly see for cortical spikes which correspond to early contraction of the arm of the monkey, followed by a 45 seconds long seizure. The disposition of the electrodes coupled to the differential representation shows that the system can record locally the onset of the seizure. On the selected deep microelectrodes (in green), the events are measured with a signal to noise ratio of 6 compared to the surface electrode which SNR is 2.

Consequently, even if the implantation highlighted new technical challenges, we demonstrated to this system can be used to monitor the brain cortical activity combining deep grey matter recording and cortical recording.

IV. Conclusion

Using epileptic seizure gives an accurate and clinically observable evidence of brain activity. Data analysis comparison over time is easy, even if the epileptic model might be dangerous if not perfectly controlled.

One month after the implantation, our flexible device is still able to record surface and deep neuronal signals. We also showed that nanostructuration with Carbon nanotubes can be a good candidate for in-vivo electrodes. This result to our knowledge is original since only in-vitro or acute results on CNT electrodes were published to date.

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