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ECoG recordings of a non-human primate using carbon nanotubes electrodes on a flexible polyimide implant

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Abstract— **In order to understand the behavior of carbon nanotubes in-vivo, we develop an original flexible microelectrode array. We place on it two types of electrodes, carbon nanotubes (CNTs) and titanium nitride (TiN). We used this device to monitor electrocorticogram of a non-human primate during one year. Our results demonstrate the biocompatibility of our CNT. We also show the magnitude of the ECoG is greater using CNT electrodes than TiN electrodes when performing long term in-vivo assessments.**

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.). First applications of BCI have been achieved with electroencephalographic (EEG) signals[1] and led to commercial systems (e.g. Gtec, Micromed). But EEG collects brain signals through the skull and the scalp which imposes intrinsic limitations in the spatial and temporal resolution of the signal. The resolution of EEG recording is in the range of 3 cm^2. On the other side of the resolution scale, one can find microelectrodes experiments. The investigators use either microwire[3] or multielectrodes array looking like Fakir's karpet[4]. 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 insulate the electrodes and neuronal depletion around the implant, typically this reduces the system life-time to only a few months[5].

An alternative solution is to process electrocorticographic signals (ECoG) recorded directly at the surface of the cortex. Contrary to EEG based BCI, this technique allows us to avoid attenuations due to the skull and the scalp. Thus the spatial resolution is improved to less than 1cm and the temporal resolution to few milliseconds[6],[7],[8]. Moreover, ECoG implants are less invasive than microelectrode arrays and are more stable[9].

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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[10]. Moreover during acute in-vivo recording, Keefer *et al.* showed that the recorded current through these electrodes was higher hence a better signal to noise ratio[11]. 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[12],[13].

In this study, we developed an implantable multi-electrode array to record ECoG based on a flexible polyimide template in order to limit the mechanical lesions generated by the pulsations of the brain.

In this paper, we present the first results on flexible cortical CNTs multi-electrodes array chronically implanted in a non-human primate. ECoG signals were collected for one year and show encouraging results for future applications of CNTs as an implantable electrode material.

II. Material & Methods

A. Implant description

The 32-electrode implant was made on a 2cm x 4cm flexible 120µm-thick polyimide support. A copper layer previously structured to define conducting lines; contacting pads and the electrode contact areas were included in a sandwich of polyimide layers. The coverlays were then etched by laser ablation to open access to pads and electrodes areas. 2 x 16 electrodes made of silicon with a surface of 1mm² covered of TiN and CNTs were alternatively disposed in the contact areas by pick and place technique with a pitch of 2mm to define a total sensing area of 1.2cm .by 1.2cm [14].

A counter electrode in TiN of 10mm² was also placed at the extremity of the matrix and connected to a platinum wire to insure its potential's stability. Finally, pads were bonded to a QTE connector (Samtec®), which was packaged in a PEEK housing equipped with a removable cap to protect it from the surrounding environment between each recording sessions.

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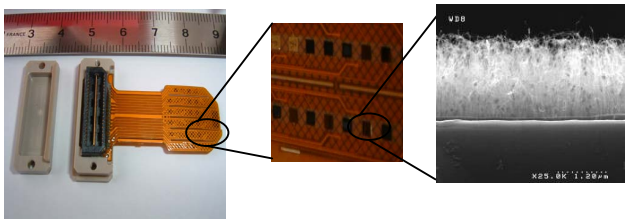

Fig. 1: Description of the assembly of CNT electrodes on the flexible substrate

On Fig. 1, one can see the flexible polyimide stacking, in the middle the silicon based electrodes and eventually on the right a SEM picture of the carbon nanotubes.

B. Carbon nanotubes growth

Carbon nanotubes were grown by chemical vapor deposition (CVD) technique, using a protocol which was reported in details by Dijon[15], briefly described as follows. First, we deposited a 3 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 600°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. Finally, 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.

C. Sterilization

Surgical instruments were steam sterilized in an autoclave for an hour and a half at 130°C under 2bar of pressure, while the implant was sterilized by ethylene oxide exposure for fifteen hours and placed in a sealed package until the operation.

D. Surgical operation

Chronicle implantation of cortical 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 then placed in a stereotaxic frame before the incision of the scalp. A 2cm x 2cm craniotomy was conducted to position the sensitive area of the implant directly in contact with the cortex, under the dura mater, in the central area which mainly cover 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. Finally, the bone flap was placed back on top of the implant and was sealed with dental cement, maintaining the implant in position.

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

E. Signal acquisition

Brain signals were monitored weekly during one years, starting one week after the implantation. We used the electronic chain, BioMEA©, developed by the CEA and described in a previous paper[16] able to acquire electrophysiological signals at a sampling frequency of 13kHz.

During each session the monkey was placed in a primate chair equipped with a head holder to restrain nose-down inflexions of 15° relative to the stereotaxic plane and a harness to avoid full rotation around the vertical axis. These precautions are necessary to insure the monkey's security and to prevent any accident during the recordings.

The chair was then placed in an electrophysiology room shielded to limit external electromagnetic perturbations. In order to reduce electrical noise, the implant was connected to the BioMEA acquisition card, placed close to the monkey with a 1m shielded cable. However, the BioMEA power supply was placed outside the electrophysiological room.

Spontaneous brain activities were then recorded for one hour prior to anaesthetize the monkey with a dose of 10mg/kg of ketamine to monitor the evolution of brain activity during 300s after the injection.

F. Signal Processing

Data were analyzed using Spike2© software.

After a DC remove, the signals were analyzed in the frequency domain calculated by Fast Fourier Transform (FFT) algorithm. This was done over a 15 seconds time frame divided in segments of 1.263 seconds (16384 points) in order to apply a Hamming's window to limit calculus artifacts and set the spectrum resolution at 0.79Hz.

III. RESULTS & DISCUSSION

A. Carbon nanotubes characterization

SEM analysis (Fig. 2) indicates that CNTs layer were around 2µm thick with CNTs diameters ranging between 20 and 40nm.

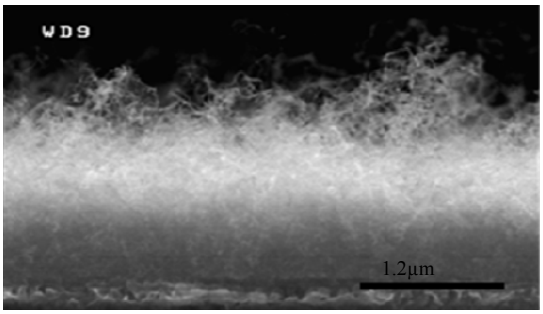

Fig. 2 Scanning electronic microscopy picture of carbon nanotubes

B. Electrocorticogram recording

Brain activity was recorded weekly during one year. Fig. 3 represents typical ECoG signals after filtration. We chose

a sequence synchronized with a lower limb contraction. We observed that signals represented as sonogram are richer from CNTs electrodes than these recorded from TiN electrodes. This observation was consistent over the course of the study, even though the signal may have varied from time to time in amplitude (between 50µV and 200µV) and in noise (between 20µV and 50µV) depending on the experimental conditions.

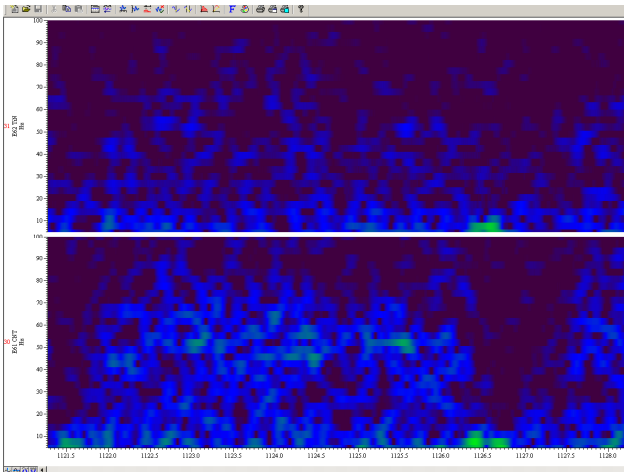

Fig. 3: Typical ECoG recordings represented in Sonogram with Spike 2, top picture is the sonogram of TiN electrodes and bottom picture is the sonogram of CNT electrodes (x: time in seconds, y: frequency in Hz).

To identify the information content of the signal, we analyzed the spectrum of each electrode to compare the intensity in the three different bands of interest between 5 and 45Hz: Alpha (8-13Hz), Beta (15-20Hz), and the low Gamma-band (25-45Hz). Fig. 4 shows the average spectrum of the different electrodes made of CNT and TiN between 5 and 300Hz.

The data can be processed to minimize the artifacts caused by head and/or cable movements as well as the 50Hz-noise generated by an AC power line and the environmental noise using a CAR (Common average reduction) but this subtraction is not relevant if the signals are two different with each other. As the electrodes are in contact with the motor cortex but also on blood vessels, it is not realistic to hope very consistent. The artifacts of the 50Hz power supply are visible and we decided not to suppress them by a rejection.

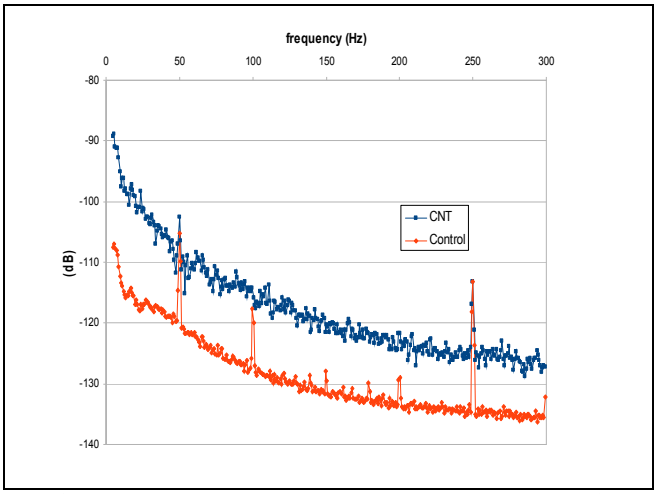

Fig. 4: Comparison of the power spectra of ECoG measured with CNTs electrode in blue and TiN electrode in red.

The power spectra are computed as 10*log(FFT(V)) on fifteen seconds. The red curve is a little bit smoother but this representation brings out that the CNT electrodes collect more signal than the TiN electrodes. Systematically, the signal was found more powerful, the gain differs from on day to another in the range of 5 to 18 dB. The consistency of the signal was checked using EMG (ElectroMyoGram) synchronized with video. Consequently the signal to noise ratio is increased. As the signal to noise ratio for in-vivo electrodes is low –in the range of 3- this difference in signal magnitude induce a longer lifetime for these CNTs electrodes.

IV. Conclusion

Data analysis comparison over time is complex because signal quality is greatly dependent on the monkey's behavior and experimental parameters (cable connection, environmental noises, etc.). However it remains clear that one year after the implantation, our flexible device is still able to record neuronal signals. We also showed that nanostructuration with CNTs is an effective way to improve signal quality by increasing signal over noise ratio. This result to our knowledge is original since only in-vitro or acute results on CNT electrodes were published to date. We did also note the absence of epileptic activity which could be linked to brain irritation. We observed no acute reactive gliosis process which may have encapsulated the electrodes and would have made impossible the signal acquisitions.

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REFERENCES

[1] [1] A. P. Georgopoulos , J. T. Lurito, M. Petrides, A. B. Schwartz , and J. T. Massey, "Mental rotation of the neuronal population vector," Science , vol. 243, pp. 234-236, 1989.

[2] [2] A. B. Schwartz et al, Brain-Controlled Interfaces: Review Movement Restoration with Neural Prosthetics -. Neuron 2006

[3] [3] Nicolelis, M.A. et al. (1995) Sensorimotor encoding by synchronous neural ensemble activity at multiple levels of the somatosensory system. Science 268, 1353–1358.

[4] 4 Selim Suner, Matthew R. Fellows, Carlos Vargas-Irwin, Gordon Kenji Nakata, and John P. Donoghue, Reliability of Signals From a Chronically Implanted, Silicon-Based Electrode Array in Non-Human Primate Primary Motor Cortex, IEEE transactions on neural systems and rehabilitation engineering, vol. 13, no. 4, December 2005

[5] [5] R. J. Vetter, J. C. Williams, J. F. Hetke, E. A. Nunamaker, and D. R. Kipke, "Chronic neural recording using silicon-substrate microelectrode arrays implanted in cerebral cortex," IEEE Transaction on Biomedical Engineering, vol. 51, no. 6, p. 9, 2004.

[6] [6] E. C. Leuthardt, G. Schalk, J. R. Wolpaw, J. G. Ojemann, and D. W. Moran, "A brain–computer interface using electrocorticographic signals in humans," J. Neural Eng., vol. 1, pp. 63-71, 2004

[7] [7] W. J. Freeman, M. D. Holmes, B. C. Burke, and S. Vanhatalo, "Spatial spectra of scalp EEG and EMG from awake humans," Clin. Neurophysiol. vol. 114, pp. 1053–1068, 2003

[8] [8] G. Furtscheller, B. Graimann, J. E. Huggins, S. P. Levine, and L. A. Schuh, "Spatiotemporal patterns of beta desynchronization and gamma synchronization in corticographic data during selfpaced movement," Clin. Neurophysiol., vol. 114, pp. 1226–1236, 2003.

[9] [9] E. Margalit *et al.*, "Visual and electrical evoked response recorded from subdural electrodes implanted above the visual cortex in normal dogs under two methods of anesthesia," J. Neurosci. Methods, vol. 123, pp. 129–137, 2003.

[10] [10] Cellot et al., Carbon nanotubes might improve neuronal performance by favouring electrical shortcuts, Nature Nanotechnology, 21 December 2008

[11] [11] Keefer E.W., Botterman B.R., Romero M.I., Rossi A. F. and Gross G.W.. Carbon nanotubes coating improves neuronal recordings. Nature nanotechnology, vol. 3, July 2008.

[12] [12] T.Gabay *et al*.,"Electro-chemical and biological properties of carbon nanotubed multi-electrode arrays," Nanotechnology, vol. 18, no. 24, 2007.

[13] [13] K. Wang, H. A. Fishman, H. Dai, J. S. Harris, "Neural Stimulation with a carbon nanotube microelectrode array," Nano. Lett., vol.-,no. 9, pp. 2043-2048, 2006.

[14] [14] F Sauter-Starace, O. Bibari, C. Chabrol, C. Pudda, Patent EN 08/03975, Implantable probes.

[15] [15] J. Dijon *et al*., "Towards a low-cost high-quality carbon-nanotube field-emission display," Journal of the Society for Information Display , vol. 12, no. 4, pp. 373-378, Dec. 2004

[16] [16] G. Charvet; O. Billoint.; L. Rousseau, B. Yvert, "BioMEA: A 256-Channel MEA System with Integrated Electronics," in Proc. 29th Annu. EMBS Conf., 2007, pp. 171-174