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Soft polymer sensor for recording surface cortical activity in freely moving rodents

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ABSTRACT

A surface sensor has been developed in order to record the cortical activity in animal models for neuroscience research. The device is made of gold on Cyclic Olefin Polymer (COP) and Cyclic Olefin Copolymer (COC) soft polymers using microsystems technology. The resulting system is flexible, customizable, biocompatible, and fulfills reproducibility standards during the manufacturing process. Impedances are in the range of 100 kΩ, thus making the system suitable for recording field potentials on the cortical surface. This paper explains the implementation process together with the implantation procedures and the results obtained in behaving rats. Results show that COP-based sensors are preferable to COC due to their higher flexibility and ease of manipulation. The versatility of COP polymers offers the possibility to adapt the electrode array to the cortical surface thus increasing surface of contact with the brain tissue. To validate the current approach, 8-contact sensors were implanted bilaterally over the motor cortices of awake, free moving rats. Cortical activity was evaluated on free moving animals and under the effect of different drugs. The quality and features of the recordings was in accordance with that of the current state of art systems thus demonstrating the feasibility of using COP-based systems for electrophysiological recordings in experimental investigation.

1. Introduction

Most of our knowledge about brain function and how neural processes mediate in motor and cognitive performance comes from information obtained from electrophysiological recordings [1]. In the treatment of some diseases like Parkinson's, schizophrenia or epilepsy some therapies involve the stimulation and recording of deep brain structures. These techniques constitute a valuable opportunity not only to treat these diseases but also to investigate about their origin and evolution. The implantation of deep brain stimulators and subdural arrays of electrodes has allowed obtaining the activity of several structures of human brain. Nevertheless, and due to ethical issues, it is not possible to record from other brain areas apart from the therapeutic targets. Alternatively, and in order to investigate the physiology/pathophysiology of the mammalian brain, approximations based in the use of animal models must be used.

There are two main modalities for obtaining electrophysiological recordings [2]. Records can reach structures deep in the brain, or they may be restricted to the cortical surface. In this second case, the method most frequently used involves making small craniotomies in which screws are set, soldered to conductive wires and connected to the recording system. This is a suitable option for its simplicity in implementation and low cost, at the same time it is not overly invasive or painful for the animal. However, there are situations where it is required to record electrophysiological activity of large regions of the cortical surface with a good spatial resolution. In these scenarios screw-based approaches cannot be used. Implementations using electrode arrays can be a good alternative for covering the areas of interest. Inspired in the systems used for patients with epilepsy, there are some commercial solutions that could be adapted for sizable animals (eg. in monkeys) [3]. In recent years, different solutions have been proposed for the modeling of large-scale neuronal networks in mouse models using needle-type electrode clusters for spatial mapping technique [4]. Choi et al. reported a new method for high-resolution EEG mapping in freely behaving mice using a nanofrabricated microelectrode [5] and McCall et at. proposed the implementation of nano-fabricated

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optometric devices including very interesting characteristics such as wirelessly powered microscale, inorganic light-emitting diodes (μ-ILEDs) and multimodal sensors [6].

Nevertheless these solutions are too sophisticated and likewise commercial ones, they are often too expensive or offer a limited variety of geometries. As a result, they are hardly applicable in approaches that involve the use of a substantial number of animals such as those aimed at studying the pathophysiology of certain diseases, the application of genetic therapies or the development or testing of new therapeutic drugs. In order to respond these actual needs, Ledochowitsch et al. and Rubehn et al. employed parylene and polyimide polymers as a substrate for the development of multichannel ECoG-electrode array offering good physical properties and measures in animal model [7,8]. In the present work, the design and testing process of sensors based on Cyclic Olefin Polymer (COP) and Cyclic Olefin Copolymer (COC) soft polymers is described and applied to implants in small animal models (rodent). In recent decades developments in the field of bioMEMS and advances in micro and nanoscience have opened a window of opportunity to minimize and implement mechatronic systems that can lead to biomedical applications. The micro-fabrication of these sensors allows a wider cortical surface coverage, increases the degree of integration of the systems and also provides a high versatility for the design of customized layouts suitable for specific applications In addition, it also eases the surgery and system implantation processes, what results in a better recovery of the animals with a gain in the quality of recordings.

2. Material and methods

2.1. Design and manufacture of sensor

The materials used as a substrate of the sensor were the Cyclic Olefin Polymer (COP) (TOPAS 6013 F08-52-3 101.6 μm thick purchased from TOPAS Advanced Polymers) and Cyclic Olefin Copolymer (COC) (Zeonor mcs-foil 005 188 μm thick purchased from Microfluidic ChipShop). They are very similar amorphous thermoplastic polymers, with the exception that COPs use a single type of monomer during formulation. COCs are fabricated through copolymerization of cyclic olefin with α-olefin or ethylene; whereas COPs are developed via cyclic olefin ring-opening metathesis polymerization followed by an hydrogenation [9,10]. These soft polymers have previously been used in micromanufacturing processes, since they are flexible (Young's Modulus of 1.7–3.0 GPa and 2.6–3.2 GPa respectively) and are easily adaptable (can be cut with simple desktop scissors) after production. In addition, both material are high transparency, low specific gravity, low water absorbency and also have excellent biocompatibility properties [11,12].

In this study a total of 45 different geometries were designed. In such designs, the distribution and the number of contacts (8, 16 or 32) varied. We also evaluated different contact diameters to study the surface/impedance ratio. In Fig. 1A, examples of sensor design are shown. All the paths were 100 μm wide with a distance of 100 μm between them. The diameters of the tip of the electrodes in contact with the cortex between 100 and 150 μm separated by 1 mm. For the socket, the paths were 800 μm wide and at least 1.8 mm length with 475 μm of separation. Fig. 1 shows some schematics of the geometries designed together with photographs of the recording contacts, the sensor-to-socket connections and a whole view of a finished device.

The process of micro-fabrication of surface sensors consists of several stages shown in Fig. 2. On the first step, the electrodes and the sensor tracks are defined. In order to do so, micro-fabrications techniques based on a photolithography process are used, ending up with a deposit and a lift-off process. EVG 620 exposure with positive resist s1818 is used for the photolithography process. A 200 nm-thick gold thin film onto a 15 nm titanium adhesion layer was deposited by RF sputtering process onto COC and COP substrates [13].

During the next stage of production, the tracks are isolated, exposing only the electrodes. In order to modify the hydrophobic properties of the polymers, a surface activation is performed by oxygen plasma using a Thierry Pico 5 Liters machine [13]. After the activation, a second photolithography process is done with negative resist SU-8 [14]. That isolates the sensor at the same time that provides high biocompatibility. The resist layer employed has a thickness of 3.6 μm (Fig. 1B).

The next step of the process is the sensor assembly: firstly the desired morphology is cut, then a mini-connector is attached and finally the whole device is consolidated by epoxy resin. Thus, by using a scalpel and with the aid of a microscope (Dino-Lite Pro AM4113T), the sensor is cut into the desired shape. Mini-connectors Plexon A11365-001 were used for the assembly. These connectors are standard connectors used in neuroscience research and match with the electrophysiological recording system in the Laboratory of Clinical Neurophysiology of CIMA. The electrical connection is accomplished by tin soldering (Fig. 1C). Soldering of the socket connector, carried out with an AD 2700 with an iron handpiece Cartridge 2010-009 from JBC, can be performed on the same or in a perpendicular plane to that of the sensor, depending on the experimental needs. Ground and reference wire both ended in stainless steel screws are then soldered to the connector. Finally, in order to isolate and strengthen the assembly welds, epoxy resist is applied to cover the connector-sensor connection (Fig. 1D).

Final verification and measure of impedances of each sensor contacts is done by using a NanoZ device (Neuralynx Inc.); a computer-based system for multichannel electrode impedance measurement. This system measures impedance in the 1 kΩ to ~100 MΩ range, for frequencies from 1 Hz to 4986 Hz, with 1 kΩ display resolution and ± 1% accuracy. Impedances were measured at 1000 Hz (sinusoidal waveform, current 1 nA RMS (max), bias 50 pA (typical)) by immersing the sensor into 0.9% NaCl solution and using the ground screw as counter electrode.

2.2. In vivo testing of the sensor

In order to check the suitability of the deployed system, sensors were implanted in two male Wistar rats. Recordings were carried out along different behavioral states including wakefulness, physiological sleep and under the effect of several drugs. Animals were allowed to freely move during the recording process.

Both, surgeries and recordings were carried out in the Laboratory of Clinical Neurophysiology of CIMA, following procedures approved by the Ethics Committee for Animal Experimentation of the University of Navarra protocols.

For the implantation process, we selected geometries with eight 125 μm in diameter electrodes disposed linearly and separated by 1 mm. Due to the flexibility of the employed substrate, it allows arranging the sensor parallel to the brain surface whereas the socket remains perpendicular to the scalp, thus allowing an easy connection of the recording equipment. The geometry of the sensor was selected due to its suitability for proceeding with a bilateral recording of the primary motor cortex activity on its antero-posterior axis. 8-contact sensors were implanted over each hemisphere thus resulting in a 16-channel configuration set up (Figs. 3 and 4). For the sterilization, the electrode was maintained in 70% ethanol for 2 h previous to implantation.

Briefly, chirurgical procedures were performed under inhalatory anesthesia (1–2% isoflurane, 0.8–1.5 L/min). A cutaneous incision was made to expose the skull. Then, bilateral craniotomies over the