Biostability Assessment of Flexible Parylene C-based Implantable Sensor in Wireless Chronic Neural Recording

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Biostability assessment of flexible Parylene C-based implantable sensor in wireless chronic neural recording

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Abstract

The stability of polymer-based sensors in a biological environment remains a challenge, as delamination and swelling often compromise mechanical and electrical capability. We have developed a neural implant based on Parylene C, a biocompatible flexible polymer, with PEDOT-nanostructured gold patterns to record the brain electrical activity. Here, we show first evidence of device biostability through *in vitro* soaking tests in artificial brain environment and *in vivo* recording in mice. Our results indicate that after over the six months trial, more than 75% of the *in vitro* electrodes have stable impedance, and the implanted sensors in mice were able to accurately record signals from mice hippocampi. None of the implants presented with signs of Parylene degradation or metal corrosion. Overall, the devices are promising candidates for reliable, chronically implanted sensors in the biomedical field.

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Keywords: implantable sensor ; biostability ; chronic recording ; Parylene C ; neuroprosthesis

1. Introduction

Implantable flexible polymer-based sensors are expanding in the biomedical field, because they are able to adapt to the inherently soft tissues, promoting body acceptance in the long run. However, the body can be an aggressive environment for many types of materials and devices in contact with tissues or body fluids in the long term. Implant unstability stems either from the host response, that is the local and system response of the living systems to the

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material, or from material response to said living systems [1]. Prolonged contact in physiological fluids tends to affect the device in the form of corrosion of electrical sites, membrane biofouling, swelling and delamination of the passivation layer [2]. In the case of chronically-implanted neuroprosthesis, implants must be able to record and/or stimulate neural tissues over long periods of time without adversely affecting body fluids, tissues and organs [3].

Parylene C is a flexible, highly biocompatible polymer that has gained attention over the years in the biomedical field. Parylene C is currently investigated in thick layers (~20-25µm) as a flexible substrate for a wide range of biomedical devices, especially for neuroprosthetic applications [4], [5]. However, the biostability of Parylene-based devices still lacks crucial perspective. In order to achieve such hindsight, *in vitro* appropriate models and *in vivo* accurate experiments need to be intensely pursued.

We have developed a Parylene C-based neural probe with nanostructured gold electrodes to record the brain activity. In this study, we intend to demonstrate preliminary evidence of the biostability of our Parylene implant for chronic neural applications, via *in vitro* soaking tests in artificial brain environment and prolonged *in vivo* wireless recordings in mouse brain.

2. Materials and methods

2.1. Neuroprosthesis fabrication

In previous studies, we conceived a flexible neuroprosthetic device made of Parylene C [5] (Fig 1a). This polymer presents with outstanding biocompatibility (USP Class VI), excellent chemical inertness and low Young's modulus. The implant is patterned via lift-off with 40µm–diameter gold electrodes for the recording of neural activity. To improve signal quality, the electrodes are nanostructured with electrochemically-deposited conductive polymer PEDOT [6]. Because the probe as such tends to buckle on the surface of the brain during its implantation in the cortex, the shank is backed with a resorbable silk fibroin coating [7]. Silk fibroin acts as a stiff support that enables insertion in brain tissues, before degrading itself harmlessly at a tunable rate.

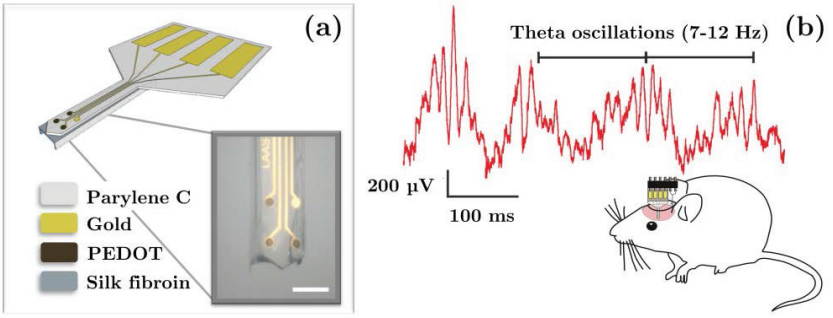

Fig 1. (a) Schematic representation (not to scale) and microscopic image (scale bar is 200 µm) of a Parylene C-based neural probe with three PEDOT electrodes and one gold electrode as control. The shank is supported on a resorbable silk coating to allow insertion in the brain. (b) Typical brain activity recorded from a freely-moving mouse discovering a new environment (acquisition cage). The Theta oscillations (7-12 Hz) are representative of an active behavior during exploration.

2.2 In vitro ageing of Parylene C implants

Soaking tests are often carried out to evaluate implants degradation over time [8]. Four Parylene C implants with each four PEDOT-modified gold electrodes are soaked in Artificial Cerebral Spinal Fluid (ACSF) for a period of six months. Each device is bonded with anisotropic conductive film to a long flexible cable, and glued for isolation (Polytech EP630). The devices are soaked in ACSF (25mL) in plastic cups, and the lid is sealed to prevent evaporation. Every week, the samples are retrieved from the oven and let cool at room temperature. Electrochemical Impedance Spectroscopy (EIS, 10 Hz-10 kHz) is then performed directly in the soaking medium. SEM images are taken at different times to evaluate morphology evolution.

2.3 Chronic wireless recordings in mice brain

In vitro ageing is a model that unfortunately does not take into account tissue density, liquid renewal and biological components. Therefore, preliminary trials *in vivo* are an essential step towards implantation in humans.

In this study, device biostability is assessed by recording the hippocampal brain waves produced by five freely-moving mice discovering a new environment, during a period of maximum 6 months. The Parylene implants are integrated with a wireless packaging so that the animals are free to move in the acquisition cage. For each mouse, a Parylene C probe is implanted in the CA1 region of the hippocampus, while a reference electrode (microscrew with silver wire) is screwed to the occipital bone. The whole apparatus is fixed in dental ciment.

The hippocampus is a region of brain involved in both memory consolidation and spatial navigation. The activation of specific pyramidal cells known as “place cells” in the context of spatial navigation leads to a very characteristic brain signal composed of Theta oscillations at around 7-12 Hz (Fig 1b). During the recording sessions, each mouse is connected to a headstage, mounted with a wireless battery on top (MultiChannel System). The animal is then transferred to the acquisition cage, composed of a carton box with litter, where it stays for five minutes. During this time, the animal discovers its new environment, and its brain activity is recorded.

3. Results and discussion

3.1 In vitro evolution of Parylene implants

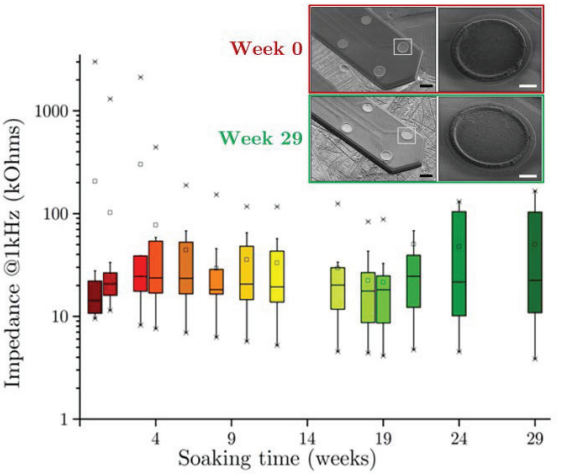

Fig 2. Box plot and SEM images showing impedance and morphology evolution measured at 1 kHz at different soaking times in ACSF for 16 PEDOT-modified electrodes. No significant sign of alteration of neither the Parylene substrate nor the PEDOT nanostructuration are observed, correlated with stable impedance measurement over time. Black scale bars is 50 µm, white scale bar is 10 µm.

Fig 2 displays the statistics of impedance evolution of all the electrodes soaked during this study. To allow comparison, impedance is taken at 1 kHz, frequency often used in electrophysiology as a reference because it corresponds to the frequency of neuron spikes. The graph shows that the impedance median of all 16 electrodes lies between 10 and 25 k Ω at 1 kHz, and 90% of all impedance values are below 100 k Ω at all times. Most of the outliers, represented as outside crosses, are above one order of magnitude compared to the impedance median, and are probably linked to flawed electrodes after Clean Room fabrication (problems in passivation etching, for instance) or faulty connectic or packaging.

As a matter of fact, the impedance of the electrodes on 3 out of 4 soaked implants were either at stable value, that is to say within 10% of their initial value, or even decreased. Scanning microscope images of the four implants showed no significant sign of Parylene delamination or swelling (Fig 2). The passivation layer remains well-retained on the surface, and the device sides appear unaltered. The presence of few aggregates on the Parylene surface is most likely due to precipitations of ACSF ions on the devices. Besides, from a macroscopic point of view, the PEDOT coatings seem indifferent to the wet ageing experiment. Indeed, it appears that some residues first found on the electrode sites might have dissolved in the soaking medium, which could contribute to the slight reduction in impedance value observed with the EIS measurement.

3.2 In vivo evolution of Parylene implants

Spectral representation of a recording session is depicted on Fig 3, for both stable and unstable electrodes over time. For some electrodes, it appears that a faulty contact results in electrode unstability at certain given time during the trial. Overall, the number of recording electrodes *in vivo* in this trial varies depending on the timing, but at any given moment, between 70 and 90% of all recorded electrodes is indeed functional. It is important to notice that a number of electrodes (~8%) were not operating from the first day of recording, consistent with probable faulty contacts or direct mechanical damage during insertion. Extra attention should be paid on packaging repeatability to avoid failures in the future.

The alteration of electrode impedance before and after implantation can be observed through EIS measurement and morphology assessment via SEM images (Fig 4). After 30 weeks implantation in a mouse brain, the impedance

of both gold and PEDOT-modified electrodes in increased by roughly a factor of two at 1 kHz. This raise in impedance is not significant in the context of electrophysiology, and could be linked to a variety of parameters, among which a slight aggregation of biological tissues on electrode sites, or a mechanical trauma during probe retraction. Besides, SEM images of the implanted devices show no apparent sign of either Parylene delamination or PEDOT alteration, consistent with the *in vitro* results.

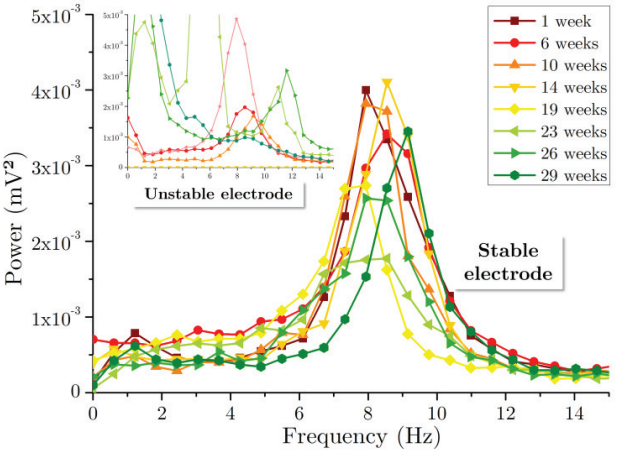

Fig 3. Examples of stable and unstable recordings from electrodes implanted in the same mouse. The stable signal still displays a noticeable Theta oscillation with rather stable amplitude. On the other hand, after 14 weeks, the unstable electrode has lost the ability to accurately record brain activity (random power amplitudes in the spectral representation).

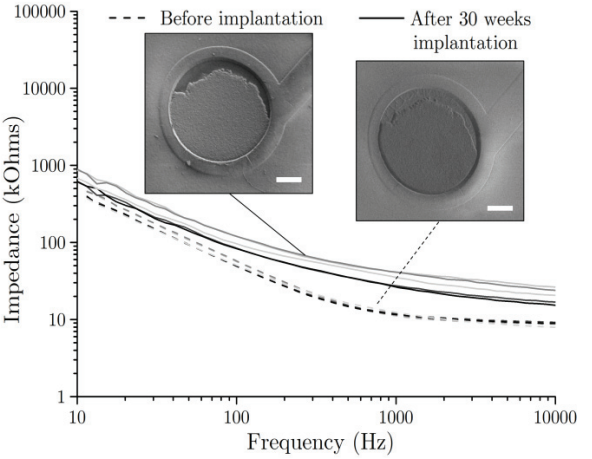

Fig 4. EIS evolution before and after 30 weeks implantation in mouse brain for PEDOT-modified electrodes, along with a representative SEM image before and after implantation (scale bar is 10 µm).

4. Conclusion

In this study, we intended to give a preliminary assessment of the stability of Parylene-based neural probes for possible chronic purposes, through both *in vitro* and *in vivo* models. Parylene devices were soaked in artificial brain physiological fluid for a period of 6 months, during which both morphology and electrical properties were monitored. No significant sign of alteration of neither the Parylene substrate nor the PEDOT nanostructuration were observed, correlated to stable impedance measurement over time. Besides, five mice were implanted with each a Parylene implant, and wireless recordings from freely-moving animals were gathered for up to 6 months. Evolution of neural recordings quality could not be tied to possible material degradation over time, but the morphology and impedance comparison before and after implantation were encouraging, with once again no sign of Parylene degradation in time. The next step in line towards biostability assessment should now involve the evaluation of tissue reaction to our devices in the long run via immunohistochemical analysis.

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