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In vitro and *In vivo* Electrochemical Characterization of a Microfabricated Neural Probe

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Abstract—**The electrochemical behavior of neural implants with 50 µm-diameter platinum electrodes was tested during acute implantations in the motor cortex of anesthetized rats. Custom Ag|AgCl reference electrodes were prepared that could be co-implanted with the probes. The results obtained *in vivo* are compared with *in vitro* measurements performed in buffered saline solution (PBS) with and without the addition of bovine serum albumin (BSA). The presence of BSA clearly altered the performance of the electrodes which was studied by means of cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), voltage transient measurements (VT) and monitoring of the open circuit potential (OCP). We found that hydrogen gas evolved at 1.22 A/cm^2 in BSA-free PBS whereas in BSA-containing PBS it occurred already at 0.51 A/cm^2.**

Keywords: Neural probe, Electrochemical Characterization, *In vivo*, *In vitro*.

I. Introduction

THE electrical performance of neural probes for electrical recording and stimulation is usually determined *in vitro* using model solutions having ion concentrations similar to the interstitial and cerebrospinal media. These test solutions usually comprise sodium chloride in physiological concentration with admixtures of buffers such as phosphate or carbonate salts [1]. The *in vivo* biochemical conditions, however, are more complex than that. The abundant presence of brain- and blood-specific proteins with an affinity to adsorb onto metal surfaces can largely alter the electrochemical behavior of implantable electrodes [2]. To allow a more realistic description of the electrode-tissue interface, it is important to perform *in vivo* electrochemical measurements and find *in vitro* models that better mimic the *in vivo* environment.

In this paper, we explore the electrochemical behavior of microfabricated Pt-based neural probes *in vivo* in rat brains and compare the results with the *in vitro* results performed in pure PBS and BSA-containing PBS solutions. Electrochemical impedance spectroscopy was used to determine the impedance at the electrode-electrolyte and electrode-tissue interface. Cyclic voltammetry *in vitro* and *in vivo* allowed a qualitative assessment of the potentiodynamic behavior of the electrodes and also gave insights into the voltage-dependent effects of protein adsorption. In addition, we performed *in vitro* voltage transient measurements as a means to determine the influence of protein adsorption on voltage excursions and thus on the maximum charge injection capability of the electrodes.

II. Procedures And Materials

A. Device Fabrication

In this study we used neural probes containing platinum contacts of 50 µm diameter arranged in a 2x5 array with a pitch of 100 µm. The probes were developed for simultaneous recording and stimulation of the brain cortex. For the fabrication we used 8-inch silicon wafers with 250 nm thermal oxide on both front- and backside. Platinum interconnects and electrode sites were patterned by means of lift-off. 1 µm thick Parylene C (Specialty Coating Systems, USA) was deposited as biocompatible insulation material. The electrode sites and bondpads were opened by reactive ion etching (RIE). With a two-step deep RIE on front- and backside, through-wafer groves were etched to define the probe outline and thickness. In a final step 100 nm tantalum pentoxide were sputter-deposited on the wafer backside as a protective insulation layer.

The devices were released and thoroughly cleaned in 80°C hot photoresist stripper (Microstrip 2001, Fujifilm) then rinsed in isopropyl alcohol and deionized water and finally wire-bonded onto custom PCBs. Connectivity to external instrumentation was established using flat-cables inserted into FCP connectors (Omron, Electronic Components, Europe) which were mounted on both the probe PCBs and on a switchboard.

B. Electrochemical Methods

The electrochemical characterization of the probes involved *in vitro* and *in vivo* cyclic voltammetry, electrochemical impedance spectroscopy and *in vitro* voltage transient measurements. CV with a sweep rate of 100 mV/s was used *in vivo* and *in vitro* to determine the onset potentials for hydrogen and oxygen evolution while EIS with an excitation signal of 10 mV_{rms} and frequencies between 1 Hz and 100 kHz was employed *in vivo* and *in vitro* to

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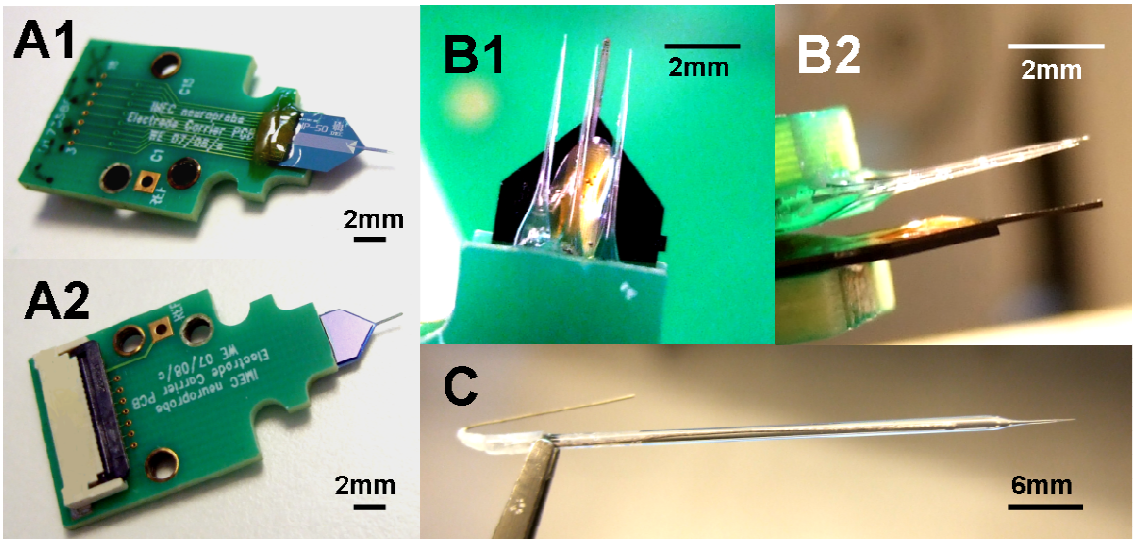

Fig. 1: A1, A2: Pictures of a packaged probe with FCP connector on the PCB backside. B1, B2: Pictures showing three custom reference electrodes assembled face-to-face with a probe. C: Picture of a custom reference electrode.

investigate the impedance of the electrode-electrolyte and electrode-tissue interface. VT was used *in vitro* to apply biphasic, charge-balanced current pulses and measure the electrode polarization response in presence and absence of proteins. From this information, current densities for electrochemically safe stimulation, i.e. without the occurrence of water hydrolysis, were estimated.

All *in vitro* and *in vivo* measurements were performed in a three-electrode configuration using a potentiostat/ galvanostat combined with a frequency response analyzer and a fast sampling ADC module that allowed sampling rates up to 10MHz (Autolab PGSTAT302N, ECO Chemie B.V., NL). A Faraday cage connected to the potentiostat ground was used both *in vitro* and *in vivo*.

C. In vitro setup

In vitro experiments were performed in two model fluids for the interstitial medium: phosphate buffered saline (PBS; 0.15M NaCl, 0.032M Na_2HPO_4, 0.0080M KH_2PO_4; pH=7.4) and PBS of same composition as above supplemented with bovine serum albumin (1.5% w/v). The latter medium allowed us to study the influence of protein adsorption on the electrode performance. In all cases, a coiled Pt wire served as large-area auxiliary electrode and a commercial double-junction Ag|AgCl electrode (Radiometer Analytical, France) was used as reference.

D. In vivo setup

The *in vivo* electrochemical experiments required the fabrication of custom Ag|AgCl reference electrodes small enough to minimize tissue disruption when co-implanted with the probes. These reference electrodes were prepared from quartz glass capillaries (Sutter Instruments, USA) with an outer diameter of 1 mm and an inner diameter of 0.5 mm. With a micropipette laser-puller (Model P-2000, Sutter Instrument, USA) pipettes were pulled to a tip length of ~ 6 mm and orifice opening of ~ 3 µm. For the reference electrolyte, a 1.5% w/w agar gel was prepared in 3M KCl: 0.6g agar were soaked in 20g 3M KCl under stirring at 50°C for 1h and then heated up to 200°C under further addition of 20g KCl. After the mixture clarified, it was quickly injected into the pipettes through the large opening with the help of a syringe. Thin silver wires were chloridized in 3M KCl (Auto Chloridizer, HSE, Germany) generating a thin AgCl layer. They were carefully inserted into the agar-filled micropipettes. The large pipette openings were sealed with small sheets of parafilm to prevent dehydration of the agar. A ready-prepared micro-reference electrode is shown in Fig. 1 C. The reference electrodes were stored in 3M KCl till implantation.

For each implantation, three micro-reference electrodes were glued onto a PCB of the same type as the probe PCB. The PCBs were then screwed face-to-face onto the stereotaxtic frame (Fig. 1 B1, B2). The probe-pipette distance was around 2 mm, whereas the pipette-pipette distance was roughly 1 mm.

E. Animal surgery

All *in vivo* measurements were performed in the motor cortex of in total four anesthetized rats at following coordinates with respect to the probe tip: 2 mm posterior and 1.4 mm lateral to Bregma and 1.5 to 1.8 mm subdural). Experiments were carried out in accordance with protocols approved by the local University animal ethics committee and in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC). During the experiments the rats were anesthetized with urethane (1.3 g/kg, i.p.) and placed in a stereotactic frame equipped with micromanipulators to precisely control the implant position.

After resection of the dura mater, the assembly was inserted perpendicularly to the surface of the cortex by a micromanipulator with a constant speed of 10 µm/s to a depth of 1500 µm. A metal clamp attached to the skin of the animal served as counter electrode during the experiments.

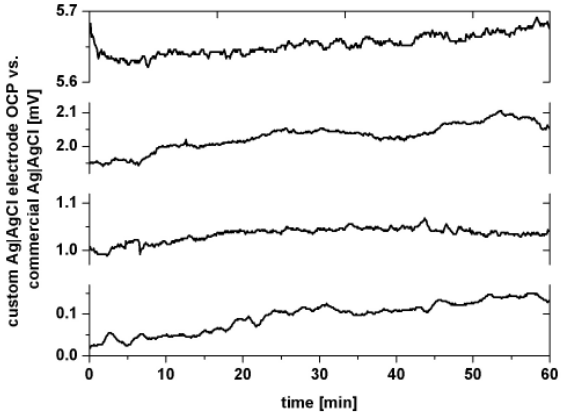

Fig. 2: OCP over time for various custom Ag|AgCl micro-reference electrodes measured in 3M KCl versus a commercial Ag|AgCl electrode.

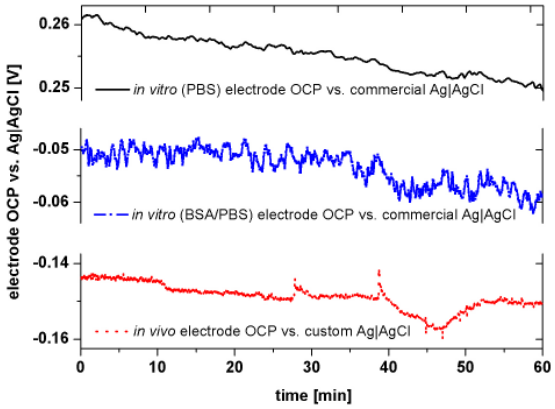

Fig. 3: Pt electrode OCPs monitored over time in vitro in plain PBS (top), BSA-containing PBS (center) and in vivo (bottom).

III. Results And Discussion

A. Stability of the custom reference electrode OCP

The reliability of the custom Ag|AgCl electrodes was determined by monitoring their OCP over time in 3M KCl against a commercial Ag|AgCl reference electrode. In Fig. 2, OCP traces of four freshly prepared custom reference electrodes measured in 3M KCl are plotted over time. Their average drift ranged from 10.3±0.1 nV/s to 33.9±0.6 nV/s. The observed DC offset of few millivolts was considered acceptable for the envisaged purpose.

B. Stability of the Pt electrode OCP

The *in vivo* open circuit potential of the Pt electrodes was monitored immediately after implantation (Fig. 3 bottom). The voltages were shifted in average with 0.4-0.5 V towards more negative values than the OCPs obtained *in vitro* in plain PBS (Fig. 3 top). Similar observations could be made *in vitro* with electrodes immersed in the BSA-containing PBS solution (Fig. 3 center). Here, a voltage drop of about 0.1-0.3 V compared to the plain PBS solution could be observed. The OCP reduction is due to the adsorption of charged proteins. In the case of albumin, which at pH=7.4 has a net negative charge, this leads to a charge redistribution at the electrode surface and thus to a change in electrode potential.

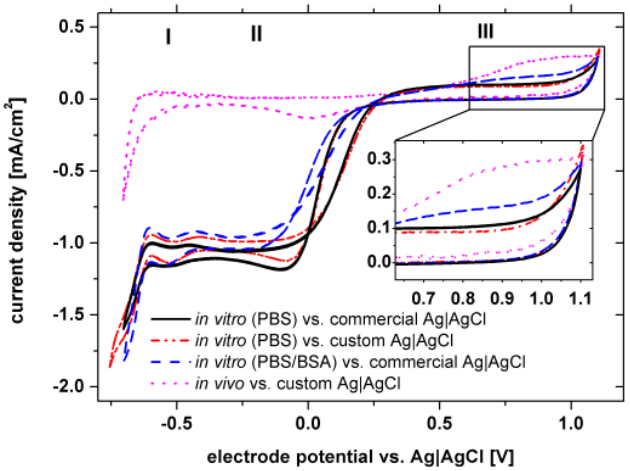

Fig. 4: Comparison of CVs of Pt electrodes measured *in vitro* in plain PBS using a custom and commercial reference electrode, in BSA-containing PBS and *in vivo*. Inset graph of the anodic region 0.6-1.1 V.

C. Cyclic voltammetry

Fig. 4 shows cyclic voltammograms of electrodes measured *in vitro* in plain PBS versus a commercial and a custom Ag|AgCl electrode. Also shown are CVs obtained in BSA-containing PBS solution measured versus a commercial Ag|AgCl electrode and *in vivo* measured versus a custom Ag|AgCl electrode. In all cases, the limits for H_2 and O_2 gas evolution were -0.6 V and 1.1 V, respectively.

In plain PBS, the voltammetric response obtained using the commercial reference electrode corresponds well with the one obtained with the custom reference electrode which indicates a reliable performance of the latter one. In the voltammograms one can distinguish three regions: a hydrogen adsorption and desorption region (I), then a flat potential region typical for the capacitive behavior of the double layer (II) followed by oxidation reactions (III). The large current step at ~ 0 V originates from the reduction of dissolved oxygen present in the solution.

The CV obtained in the albumin-containing PBS shows an increased current signal in the anodic potential region (inset Fig. 4) which can be attributed to protein adsorption and oxidation at the electrode surface [5].

Looking at the *in vivo* CV,the contribution of dissolved oxygen was found to be smaller than *in vitro* which indicates that oxygen adsorption onto the electrode surface is blocked by the presence of an adsorbed protein layer. This layer also inhibits the hydrogen layer formation [4] and leads to a less pronounced occurrence of peaks in the hydrogen region. The large current signal in the anodic region could be an indication that the protein content and/or the concentration of anions such as phosphate or carbonate are higher *in vivo* than in the BSA-containing solution.

D. EIS

The measured impedance magnitude *in vivo* was larger

Table 1: Average values for impedance magnitude and phase angle measured at 1 kHz *in vitro* in plain PBS and BSA-containing PBS and *in vivo*. Values are averaged for 3 Pt electrodes each.

	PBS	BSA/ PBS	*In vivo*
Magnitude [kOhm]	266±10	331±7	776±17
Phase angle [- degrees]	81.5±0.1	82.6±1.0	69.9±3.9

than *in vitro* for all frequencies which is in accordance to general observations made for electrode-tissue interfaces. Table 1 summarizes the values for magnitude and phase angle at 1 kHz. The *in vivo* phase angle is smaller than the ones obtained in both *in vitro* fluids suggesting a less capacitive behavior. Comparing these values is, however, intriguing, considering the fact that the OCP values in all three cases differed from each other.

E. In vitro voltage transient measurements

Symmetric, cathodic-first, biphasic, rectangular current pulses with 400 µs pulse width and 100 Hz frequency were applied in plain PBS (Fig. 5 left column) and BSA-containing PBS (Fig. 5 right column). The maximum current density was determined from the onset of hydrogen evolution determined from the first derivative of the voltage response waveform (dV/dt). H_2 evolution is characterized by a leveling-off at the end of the cathodic voltage response. By contrast, capacitive charging of the double layer is represented by a relatively linear voltage rise. Gas evolution can thus be identified as a sudden decrease of dV/dt before the onset of the anodic pulse (Fig. 5 bottom). We found that in plain PBS hydrogen evolution was observable at a current density of 1.22 A/cm^2 which lies way above the limit found in BSA-containing PBS which was 0.51 A/cm^2.

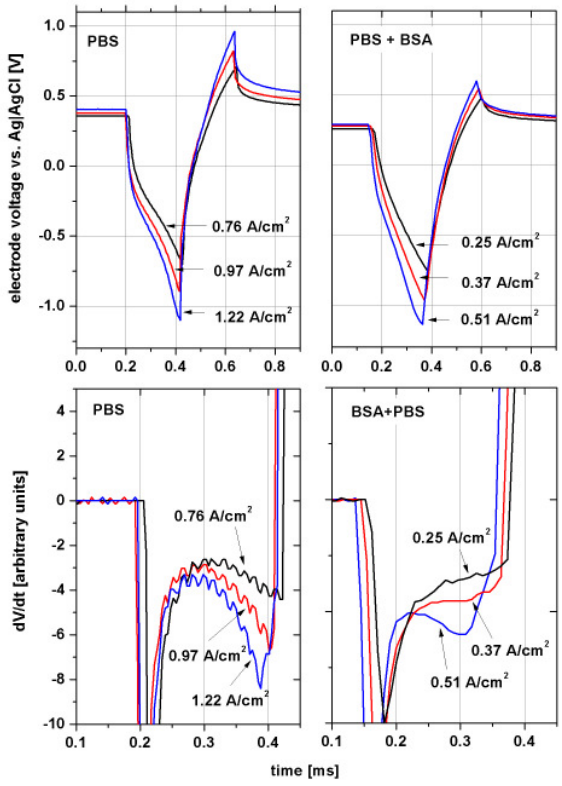

Fig. 5: Voltage transient measurements performed in plain PBS (left column) and BSA-containing PBS (right column). The top graphs show the voltage response in each case. The bottom graphs show the first derivative of the cathodic part of the voltage waveforms.

IV. Conclusions

In this study we prepared custom Ag|AgCl reference electrodes that could be co-implanted with microfabricated neural probes in order to perform *in vivo* electrochemical measurements. The advantage of using Ag|AgCl instead of plain platinum or stainless steel lies in the higher stability and reliability of the former one. The half-cell potential of the Ag|AgCl|KCl system within the glass pipettes remains largely undisturbed by the surrounding medium or tissue and is thus more stable over time. In the case of platinum or stainless steel, adsobtion of biofilms onto the metal surface can alter their half-cell potential and therefore limit their use as reference electrodes.

The experimental results suggest that in order to better predict realistic behavior of electrodes *in vivo* it is important to use *in vitro* model fluids that do not only take into account the ionicity and buffering capacity of neural tissue but also the protein content. We found that the presence of BSA not only increased the electrode impedance but also drastically reduced the current density for hydrogen gas evolution. It became clear that safe limits for stimulation largely depend on the medium used to test the probes. Further investigations are necessary to also determine the *in vivo* charge injection limits and identify the role of various proteins under different stimulation conditions.

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