Development and characterization of novel microelectrode arrays for neurophysiology

Ph.D. theses

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1. INTRODUCTION

Due to the finite conductivity of the extracellular space of the neural tissue, ionic currents corresponding to neural activity give rise to a potential field, which can be measured with electrodes, in vivo. Using high density arrays of electrodes, the information content of the signals gained by such measurements can be vastly increased. The technology of microelectromechanical systems (MEMS) allows us to batch-fabricate such probes in a precise, reproducible manner. In spite of the broad spectrum of the available types of microelectrode arrays (MEAs) to date, several ongoing research projects focus on the construction, improvement and utilization of these devices.

The impedance of the electrodes can be a crucial factor, affecting signal-to-noise ratio. Decreasing the effective surface area of an electrode results in increased impedance. Since the geometric areas of the microelectrodes are limited, various electrode materials of high roughness factor, e.g. carbon nanotubes, conductive polymers and metal deposits with sponge-like structure have been developed. However, the durability of such materials during implantation and signal recording procedures are not yet thoroughly understood.

Typical materials of the MEMS industry, such as Si, SiO_2, SiN_x, noble metals, etc. are highly biocompatible. Yet extracellular probes made of inert and non-toxic materials can also trigger the foreign body response of the immune system. The stiffness of silicon, in contrast to the flexibility of the neural tissue, is not advantageous in this aspect. Implants made of flexible materials provide smoother coupling with the soft tissue, they can follow small motions and pulsations of the brain, hence cause less disturbance in their environment. For these reasons, the dominance of silicon-based MEAs has been decreasing recently, and polymer-based devices made of polyimide, SU-8 or Parylene C are getting more and more into focus.

This work comprises four different novelties related to neural MEAs. It includes various developments and their functional characterization with acute in vivo measurements in rat cerebrum.

2. SPECIFIC AIMS

- The shaft length of silicon-based probes, manufactured using standard MEMS technology can be increased from the common millimeter scale to a much greater one, up to several centimeters in order to reach deep-brain structures. We created probes with length of 1.5-7 cm and thickness and width of 200-400 μm, suitable for the penetration of the meninges (including the dura mater) and precise targeting. The scope of our experiments was to investigate the functionality of such devices during acute in vivo experiments, including their suitability for unit activity detection.

- In order to increase signal-to-noise ratio of neurophysiological measurements, low impedance electrodes can be created with high surface area deposits. Platinized platinum (Pt/Pt), due to its relatively simple deposition procedure and high roughness factor, would be an excellent candidate for such purposes, but its mechanical stability is claimed to be poor. We have developed low-impedance Pt/Pt microelectrodes and tested their compatibility with acute in vivo implantations and extracellular recordings.

- Polymer-based MEAs are frequently used for electrocorticography (ECoG). In this case, the electrodes are placed onto the surface of the brain tissue. Furthermore, they are also utilized as intracerebral implants, in when they function as extracellular MEAs. We aimed to create and test a construction between these two variations: a row of electrodes with protruding sites, which can slightly penetrate into the tissue and record signals from below the surface of the brain.

- We intended to perform simultaneous ECoG and extracellular laminar recordings with a novel, flexible electrode array system, containing an implantable shaft and a sheet electrode array, which can be placed onto the brain

surface. The concept can be interpreted as an all-flexible “thumbtack” neural MEA, which had been successfully used for recording field potentials, multiple unit and SUA in behaving and anaesthetized humans.

3. METHODS

3.1. Methods related to in vivo recordings with silicon-based probes of extreme shaft length

3.1.1. Microtechnology

The silicon-based MEAs were microfabricated at the Department of Microtechnology, Institute for Technical Physics and Materials Science, Hungarian Academy of Sciences, from 4-inch single-crystal silicon wafers, using well-characterized processes of the MEMS industry, such as thermal growth of SiO_2, Low pressure chemical vapor deposition (LPCVD) of SiN_x, 15/270 nm TiO_x/Pt metal layer patterning with lift-off technique utilizing Al and positive photoresist for sacrificial layer. The insulator (SiO_2 and SiN_x) layers were patterned with photolithography followed by selective wet chemical etching. The three-dimensional shape of the devices was formed with deep reactive ion etching (DRIE). Shaft dimensions ranged from thickness of 200-380 μm, width of 206-400 μm, length of 15-70 mm. Tetrodes and linear electrode array types were created.

3.1.2. In vivo measurements

The probes were tested on Wistar rats. Animal care and experiments were performed in compliance with Animal Care Regulations of the Institute of Cognitive Neuroscience and Psychology of the Hungarian Academy of Sciences and order 243/1988 of the Hungarian Government, which is an adaptation of directive 86/609/EGK of the European Committee Council. The animals were initially anesthetized via intraperitoneal injection of a mixture of 37.5 mg/ml ketamine and 5 mg/ml xylazine at 0.3 ml/100 g body weight injection volume. The sleeping state was maintained by intramuscular updates of the same solution (0.3 ml / hour). The animals were mounted in a stereotactic frame, restraining their heads. After shaving the scalp, it was incised in order to gain access to the skull. Soft tissues, including the periosteum covering the skull were removed with a scalpel. The bone was cleaned with 1% hyperol

solution. Animal preparation procedures described hitherto were employed for in vivo operations corresponding to other topics of the dissertation, the ones comprising platinized platinum electrodes and polymer-based MEAs. Crainotomy window was opened from -1 mm to -5 mm anteroposterior (AP), from 1 mm to 4 mm mediolateral (ML) in reference to the bregma. Each probe, attached to the micromanipulator arm of the stereotactic device was slowly inserted into the cerebrum of an animal without removing or incising the dura mater.

3.1.3. Signal acquisition and data processing

Signals were recorded using a two-stage amplifier and a data acquisition system with a gain of 1000, 20 kHz sampling rate and 16 bit resolution. Edit 4.5 software of Neuroscan was used for off-line signal visualization, filtering and analysis. The Klusters free software was used for clustering unit activities.

3.2. Methods related to in vivo durability tests of platinized platinum electrodes

3.2.1 Silicon probes

Silicon-based probes were employed for the experiments of this thesis. In the midline of the single, 7 mm long, 280 μm wide, 80 μm thick probe shafts 24 sputter-deposited platinum electrodes had been manufactured with geometric areas of 30 μm × 30 μm.

3.2.2 Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) measurements were performed in lactated Ringer's solution, using an Ag/AgCl reference electrode and a counter electrode of platinum wire with relatively high surface area. The probe signal was sinusoidal, 25 mV RMS. A Reference 600 instrument was used as potentiostat and Gamry Framework 6.02 and Echem Analyst 6.02 software were used for experimental control, data collection and analysis. Experiments were performed in a Faraday cage.

3.2.3. Cyclic voltammetry

Cyclic voltammetry (CV) allowed sensitive measurements of the surface area of the electrodes. Curves were obtained with a PAR 283 potentiostat, in a three compartment electrochemical cell. 0.5 M H_2SO_4 solution has been deoxygenated with argon of

99.9995 purity and was used as electrolyte, isolated from air. A hydrogen electrode and a platinum sheet were used as reference and counter electrodes, respectively. The real surface areas of the recording sites were computed from the CV curves and by dividing these data with their projected footprint area (geometric surface area), the roughness factor of the electrodes were determined.

3.2.4. Electrolytic deposition of platinum

In order to obtain electrodes with large effective area, Pt/Pt layers were electrochemically deposited using a PAR 283 potentiostat and a solution of 1 g $PtCl_4 \times 2HCl \times 6\ H_2O$ + 2 cm^3 conc. HCl + 200 cm^3 H_2O. Potentiostatic rather than galvanostatic deposition was chosen, since it yields a more controllable process and a more homogenous deposit. Parameters such as potential and time have been set to 100 mV (vs. reversible hydrogen electrode) and 10 minutes, respectively. During preliminary experiments, several other depositing parameters were also tested before these were chosen. The 24-channel MEAs have been modified the following way. Platinum was electrochemically deposited onto every second (2^{nd}, 4^{th}, etc.) channel simultaneously using the above described plating method, while odd-numbered (1^{st}, 3^{rd}, etc.) channels were only immersed in the plating solution and no voltage was applied on them (these sites were used as controls in this study). Odd-numbered and even-numbered channels will be referred to as thin-film Pt and Pt/Pt electrodes, respectively.

3.2.5. In vivo measurements

The durability of the electrodes was tested via subjecting such prepared MEAs to in vivo measurement sessions, performed in rat cerebrum. Probes were implanted and used for extracellular recording twelve times. Before every third implantation, a cleaning process was performed in 0.5 M H_2SO_4, with the application of ±1.5 V DC voltage, for 3 seconds, 5 times, in reference to a Pt electrode with much larger surface area.

220-390 g Wistar rats (a total of 12) have been prepared for surgery with the previously described methods of in vivo recordings with silicon-based probes of extreme shaft length. Craniotomy was performed from -4.0 mm to -1.0 mm AP, from 1.0 mm to 4.0 mm ML in reference to the bregma. The dura mater was left intact. In order to reduce the number of animals used, a probe was implanted three times into a rat, at different locations. Implantations were targeted at AP/ML stereotactic locations

of -3.6 mm/2 mm; -3.6 mm/3.6 mm; -3 mm/2.8 mm, which allowed us to reach the hippocampus and the thalamus with 4-5 mm deep implantations in reference to the surface of the brain for the recordings. In order to avoid large blood vessels, a maximal deviation of 0.15 mm from original target locations was in some cases necessary. Recordings were performed for about one hour, with the previously described recording and signal processing system which was employed for in vivo recordings with silicon-based probes of extreme shaft length. After 12 implantations, thermal noise measurements were carried out, in vitro, in lactated Ringer's solution, with the same setup.

Electrode impedance values were measured before and after recordings at 1 kHz, using an EASI-1 BAK model, with sine wave current of less than 500 nA. The reference electrode for both in vivo recordings and impedance measurements was a pointed platinum wire located beneath the skin posterior to the scalp.

3.3. Methods related to polymer-based microelectrode arrays with protruding sensor sites

3.3.1. Design and fabrication

The microfabricated component of the device was manufactured on an oxidized Si substrate wafer. It consists of a bottom PI layer, a middle metal (TiO_x + Pt) layer and a top SU-8 layer. We have chosen this polymer composition in order to exploit the preferential features of the materials: PI sufficiently adheres to the substrate, yet it can be peeled off easily without the necessity of a sacrificial layer, while SU-8 can be conveniently patterned by photolithography, which makes it more suitable for forming the top layer. Two photolithographic masks with resolution of 1 μm define the layout.

24 electrodes were arranged linearly in a single, 1.15 mm long row. Arrow-shaped recording sites were designed on the edges of microscopic spikes. The sites can be approximated with isosceles triangles with base lengths of 22 μm and heights of 20 μm.

Characterization of the electrode impedances was performed in vitro, in physiological saline, by EIS with the apparatus which had been used for the characterization of high surface area deposits.

3.3.2. In vivo measurements

For in vivo testing, rats were anesthetized and prepared for stereotactic operation with the previously described methods of in vivo recordings with silicon-based probes of extreme shaft length. Craniotomy was performed from -1.0 mm to -4.0 mm AP, from 1.0 mm to 5.0 mm ML in reference to the bregma. The MEAs were mounted on a micromanipulator. The microdevice was adjusted into a transverse plane, its longitudinal axis was perpendicular to the surface of the neocortex. The dura mater was removed at the target location. The spiky tips, containing the electrodes were submerged into the tissue and brain signals were recorded with the previously described recording and signal processing system which was employed for in vivo recordings with silicon-based probes of extreme shaft length.. This procedure was performed at several locations in the craniotomy window.

3.4. Methods related to polymer-based microelectrode arrays with protruding sensor sites

3.4.1 Fabrication

In order to realize the device according to our fourth specific aim, the same rapid and cost-effective process flow was utilized, which had been successfully employed for the construction of the electrode array with protruding sites. The processes resulted in a PI (bottom insulator) – TiO_x/Pt (conductive) – SU-8 (top insulator) layer structure. Two components were constructed: one containing the ECoG electrodes (8 channels) and one for the laminar extracellular microelectrode array (16 channels). They were assembled perpendicularly and fixed together with a drop of epoxy resin, at their backsides, avoiding the electrodes.

Characterization of electrode impedances was performed by EIS in physiological saline. Impedance reduction was only performed on the sites of the implantable (extracellular) electrode array. The electrolytic deposition procedure was identical to the one developed earlier, detailed at the section titled "*3.2. Methods related to in vivo durability tests of platinized platinum electrodes"*

3.4.2. In vivo characterization

We performed electrophysiological recordings in the cerebrum of rats in order to test the functionality of the MEAs. A total of 5 Wistar rats, weighing 270-400 g have

been anesthetized and prepared for stereotactic operation as described previously at the section titled *"3.1. Methods related to in vivo recordings with silicon-based probes of extreme shaft length"*. Craniotomy was performed from -1.0 mm to -6.0 mm AP, from 2.0 mm to 7.0 mm ML in reference to the bregma. The implantation of the extracellular MEAs were targeted at the stereotactic location of -3.36 mm AP, 5.5 mm ML, perpendicularly to the brain surface, which allowed us to perform laminar measurements in the barrel cortex and reach into the hippocampus. The dura mater was incised above the target location in order to achieve a smooth implantation. The probes were handled and implanted with a forceps, attached to a moving arm of the stereotactic apparatus. The signal recordings were carried out using a 32-channel Intan RDH-2000 amplifier system connected to a computer via USB 2.0, sampling with a frequency of 20 kHz. After the recording sessions, the probes were removed from the brain and cleaned. We soaked them in an aqueous solution of 10 mg/ml Terg-A-Zyme for 10-15 minutes. 3-4 times during this period and after it we removed the probes from the solution to rinse with distilled water.

4. RESULTS

4.1. Results obtained with silicon-based probes with extreme shaft length

The probes tolerated the implantation through the intact dura mater without bending or breaking. Good quality single and multiple unit activities were recorded from the cortex and thalamus with all 5 probes under investigation.

With a 3 cm long, 400 μm wide, 200 μm thick tetrode for example, a total of 35 cells were recorded. Two or three clusters could be separated in general from the recorded unit activity at one recording position. The mean peak-to-peak amplitude of the averaged action potentials of the cells was 128.9 ± 54.3 μV and the number of spikes in a sorted unit cluster was 1452 ± 1829 on the average. Clear refractory periods (1-2 ms) visible on the autocorrelograms of the separated clusters were signs of appropriate spike sorting. In most cases the action potential waveforms of the same unit could be detected on several of the four channels simultaneously. The majority of the neurons were excitatory pyramidal cells (n = 13) and thalamocortical cells (n = 21) with wide spikes (half-amplitude duration: 319 ± 69.4 μs, range: 208.5 – 452 μs), except for one neuron with narrow action potentials (164.5 μs) probably recorded from the nucleus reticularis thalami (nRt). The nRt neuron fired bursts containing 6-10 action potentials, while the recorded cortical cells discharged mostly single spikes, spike doublets or triplets.

4.2. Results and discussion concerning in vivo durability tests of platinized platinum electrodes

4.2.1. Impedance and roughness factor

Data obtained from CV indicate that the electrochemical cell configuration and plating parameters allowed formation of platinum deposits with average roughness factor of 950. The electrolytic Pt deposition reduced the impedance of the electrodes (in

physiological saline) with several orders of magnitude in the frequency range of 0.1 Hz - 1 kHz. At 1 kHz, EIS yielded 552 ± 151 kΩ and 38.7 ± 2.25 kΩ on the average for thin-film Pt and Pt/Pt electrode impedances, respectively.

4.2.2. Durability

Averaging impedance values at 1 kHz, obtained after in vivo recordings, merged from measurements with 69 electrodes of 3 probes, yielded 616 ± 129 kΩ for thin-film Pt and 112 ± 36 kΩ for Pt/Pt electrodes. These are different from those values, which could be extracted from the EIS measurements in lactated Ringer's solution. The difference between in vitro and in vivo impedance is not surprising, since impedance depends not only on electrode, but on solution properties as well. As a result of the 12 implants, the effective surface / geometric area ratio dropped from 950 to 330 in case of Pt/Pt electrodes, and from 1.0 to 0.85 in case of thin-film Pt electrodes.

4.3. Results and discussion concerning the development and characterization of a novel polymer-based microelectrode arrays with protruding sensor sites

4.3.1. The realized device

The microfabrication process flow was carried out with high yield. Failures, such as imperfections in the pattern of the conductive layer, or tearing the foils while peeling them off of the substrate occurred with a prevalence of less than 10%. To my knowledge, this device was the first microfabricated neural electrode array with layer composition of PI – TiO_x/Pt - SU-8 (bottom insulator – conductor – top insulator). The advantage of the novel composition is that during the contour etching step it does not require an additional masking layer (e.g. aluminium) to be formed onto the top of the MEMS component, since the top SU-8 layer (which can be directly patterned with photolithography) and the platinum layer have both proven to be suitable for masking the dry etching process, thus defining the contour.

The EIS curves, including an average impedance magnitude of 797 kΩ at 1 kHz, show that SU-8 and PI layers insulate the metal wires properly, otherwise abnormally low impedances should have been measured. Also, there was no observable sign of insulation failure after seven days of soaking.

4.3.2. In vivo recordings

In vivo experiments were performed as a proof of concept for the MEA. The microdevice came into contact with the brain surface without causing visible bleeding in all cases, the arrow-shaped sites could visibly penetrate into the neural tissue. An oscillation of approximately 1.6 Hz was visible on all of the sensor channels due to the anesthesia. Comparing them to each other, a divergence was observable in the waveforms, showing the spatial variation of the LFP in the cortex along the 1.15 mm length of the array. The microdevice has some limitations and disadvantages. One of these is the necessity of the removal of the dura mater. Otherwise, the soft and flexible sites were not able to enter into the tissue medium. This is not a serious issue during acute recordings, but if such a device was intended to be used chronically, it would be a severe disadvantage due to the risk of drying and infections. A further limitation derives from the one-dimensional geometry of the electrode array. In order to upgrade the concept to create a two-dimensional MEA with protruding sensor sites, multiple one-dimensional arrays are to be integrated together or a more subtle microfabrication technology is to be developed.

4.4. Results and discussion concerning the development and characterization of a polymer-based multimodal microelectrode system

4.4.1. The realized device

The microtechnological and assembly processes resulted in the unique, three-dimensional probe geometry, consistent with our design. The attachment of the shank (containing the extracellular electrodes) to the ECoG component was sufficient, the connection remained intact in all cases during the in vitro and in vivo tests. The mechanical robustness of the lead was adequate, failures only occurred as a result of extreme pulling forces. Our overall experience was that these flexible tools do not require so much care during handling compared to the more brittle silicon-based extracellular MEAs.

The original, sputtered thin-film Pt extracellular electrodes (with geometric area of 707 μm^2) had an average impedance magnitude of 559.5±148.4 kΩ at 1 kHz. We decided to reduce this value in order to obtain a better signal-to-noise ratio during

measurements. The electrolytic deposition of platinum yielded a Pt/Pt layer of high roughness factor, hence the average impedance magnitude at 1 kHz reduced to 27.6±8 kΩ. As expected, the ECoG sites of larger (31400 μm^2) geometric area had much lower original impedances: 18.6±0.5 kΩ on the average.

4.4.2. In vivo recordings

Similarly to the in vivo signals obtained with electrode array with protruding sensor sites, a synchronous slow wave oscillation with up- and downstates can be seen on all channels, indicating slow-wave sleep (SWS). LFP has a positive peak during upstates on the brain surface and in the outer cortical layers, while in the deeper layers the LFP polarity of the waves is reversed. This phenomenon could be observed regarding the ECoG channels and the channels of the implanted component located in the cortex. Elevated activity in higher frequency domains of the LFP signals on the lower channels indicate that the tip of the implanted shank reaches down into the hippocampus, as expected.

In the cortex, high intensity of multiunit activity could be observed within the upstate periods. In the hippocampus, unit activities do not follow the oscillation closely, which meets our expectations, since slow waves are supposedly generated by neocortical and thalamic oscillators.

5. CONCLUSIONS

- Not only local field potential changes, but activities of single cells can be detected acutely in the extracellular space of the central nervous system of a rat using silicon-based microelectrode arrays fabricated with MEMS technology, with shaft length ranging from 15-70 mm, thickness and width ranging from 200 to 400 μm. Such probes are suitable for implantation through the intact dura mater.

- Low impedance extracellular neural microelectrodes can be realized with electrochemical deposition of high surface area platinum, furthermore, the deposition parameters can be chosen so that the electrodes with lowered impedances will be able to withstand 12 acute implantation-recording sessions.

- Following the removal of the dura mater of rats, a microelectrode array with a row of protruding sensor sites can be inserted underneath the cortical surface, allowing recordings of local field potential changes. The array can be constructed with an SU-8 – TiO_x/Pt – polyimide layer structure, which can be realized with the utilization of MEMS technology.

- Simultaneous electrocorticographic and laminar extracellular recordings can be obtained acutely in the central nervous system of rats with a multimodal, flexible, polymer-based microelectrode array constructed with an SU-8 – TiO_x/Pt – polyimide layer structure.

6. LIST OF PUBLICATIONS

6.1. Papers connected to the theses

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