

Fabrication and testing of microelectrodes for small-field cortical surface recordings

Joseph Kitzmiller · David Beversdorf · Derek Hansford

© Springer Science + Business Media, Inc. 2006

Abstract A microfabrication approach to produce a microelectrode array that is suitable for use with human patients has been developed. The device is comprised of materials that are consistent with those of clinically used macroelectrodes (platinum electrode contacts suspended within a biomedical grade polydimethylsiloxane, PDMS). Photolithography, metal deposition, wire bonding, and PDMS encapsulation were used to fabricate the device. Cytotoxicity testing with both mammalian and human cortical cells suggests that the device is suitable for use with human patients and implementation of the device in animal studies revealed that reliable evoked potentials could be acquired with the designed spatial resolution.

Keywords Surface microelectrodes · Cortical column potential measurement · Platinum lift-off

1. Introduction

Both penetrating and non-penetrating electrode arrays have been developed and utilized by scientists to investigate the electrical activity of the cortex. Penetrating microelectrode arrays have been used extensively in animal studies but their use with humans has been limited (Burmeister, 2002; Hoppelmann, 2001; Waren, 2001). As a result of the associated potential risks, studies utilizing penetrating microelectrode arrays with humans have only involved areas of cortex that are destined for surgical resection (Bechtereva, 2000; Oya, 2002; Schwartz, 2000; Williamson, 1993).

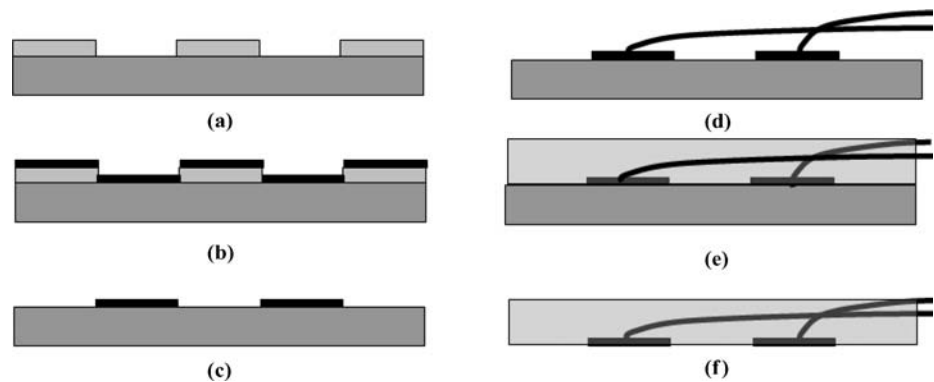
Clinically, non-penetrating surface macroelectrode arrays are used subdurally to help localize a seizure focus in preparation for epilepsy surgery (e.g. Radionics Cortical Grid Electrodes distributed by Integra Neuroscience, Plainboro, NJ). However, no studies investigating the use of non-penetrating subdural surface microelectrode arrays with human patients have been reported. Such studies have presumably not been performed due to a lack of biocompatible microelectrodes for human use. The aim of our study is to develop a non-penetrating surface microelectrode array potentially suitable for use with human patients, which will allow investigators to study areas of cortex that penetrating electrodes cannot study. Investigators would be able to examine all portions of exposed cortex, even those not destined for surgical resection. This paper reports the development of an electrode array and testing for this eventual purpose.

2. Surface microelectrode array design and fabrication

The design of our microelectrode array was restricted to materials acceptable for human use. Therefore, we chose to fabricate our device using the same materials that comprise commercially available surface macroelectrodes (Cortical strip electrode, Cat. Nr. 16166-2, Integra NeuroSciences, Plainsboro NJ). Platinum electrode contacts suspended within a flexible biomedical-grade polydimethylsiloxane (PDMS) were selected. Due to limitations of designs that result from stresses from the addition of metals to PDMS microstructures (e.g. Maghribi, 2002), a process was desired in which the metal processing was performed prior to incorporation of the PDMS matrix into the process. The dimensions and spacing of the microelectrode contacts were chosen as 200-micron squares with bi-directional pitch of 400 microns to

J. Kitzmiller · D. Beversdorf · D. Hansford (✉)
The Ohio State University, Columbus, Ohio, USA
e-mail: hansford.4@osu.edu

Fig. 1 Fabrication of microelectrode array: (a) patterning of photoresist; (b) deposition of Pt; (c) dissolution of photoresist; (d) bonding of wires from pads to connector; (e) pouring and curing of PDMS; (f) packaging of array



allow sufficient spatial resolution for activity measurement at the cortical column level. Cortical columns are the functioning units on the surface of the cortex in humans and other mammals with a geometric arrangement on the order of 300 to 600 microns (Mountcastle, 1997).

The fabrication process for the microelectrode array consisted of standard silicon processing, photolithography, a chemical lift-off technique, wire bonding, and PDMS encapsulation and release. A schematic of the microelectrode array fabrication methodology is shown in Figure 1.

The platinum electrode contacts were first patterned using a lift-off process on a silicon substrate. Shipley 1813 photoresist was applied to HMDS-coated silicon wafers (Wafernet, Inc., San Jose, CA) via spin coating to achieve a uniform thickness of 1.3 microns. The thickness was subsequently verified via profilometry. The photoresist was exposed to G-line radiation through a transparency mask and developed. The patterned photoresist was then exposed to a chemical bath of chlorobenzene for 4 minutes to toughen the edges of the features. An electron beam evaporator was then used to deposit a 200-nanometer layer of 99.9% platinum metal. The wafer with patterned resist and platinum coating was then placed in a 10 minute acetone bath to remove the patterned photoresist leaving only the array of platinum electrode contacts on the silicon substrate.

The next objective was to electrically connect each platinum electrode contact to a separate bonding pad connecting to an insulated wire for interfacing with the data acquisition computer. The wires chosen for wire bonding were 30-micron diameter aluminum wires and the wires chosen for interfacing with the data acquisition equipment were 150-micron diameter silver coated aluminum wires. The ends of the interfacing wires were stripped and crimped, as shown in Figure 4, so that a flat bonding surface was available to facilitate wire wedge bonding. The wire bonding was performed using a Kuliche and Softe Autobonder, which utilizes ultrasonic vibration in order to create the heat necessary for bonding. Electrical tape (1/2" width) was used to hold the interfacing wires (150-micron diameter silver coated aluminum wires) in place while the wire bonding was performed. The electrical tape was kept at least 2 inches away from the plat-

inum pads in order to ensure that the adhesive residue did not contaminate the future final product. There was some difficulty in spatially arranging high numbers of wires in such a manner that they did not electrically interact with each other for more than 16 electrodes. For future practical application, the use of printed circuitry will allow the connection of higher numbers of electrodes for more functional arrays.

After wire bonding, the final step of the fabrication process was to encapsulate the platinum contacts, wire bonds, and the interfacing wires within a flexible PDMS material. The biomedical grade PDMS chosen was Silastic[®] MDX4-4210 Medical Grade Elastomer (Dow Corning). A 10:1 (by weight) mixture of biomedical grade linear PDMS and crosslinking agent was manually mixed in a glass container with a 1/8" stainless steel mixing rod for several minutes until a homogeneous mixture was achieved. The PDMS mixture was then poured on top of the silicon substrate, platinum contacts, wire bonds, and insulated interfacing wires and allowed to cure for 72 hours in a dessicator at room temperature. The purpose of the vacuum dessicator was to remove any residual gas within the PDMS resin due to the stirring and reaction of the crosslinking agent. In order to facilitate easy removal of the encapsulating PDMS from the silicon substrate, a monolayer of hexamethyl disilazane (HMDS) was applied to the substrate (Si wafer, platinum pads, wire bonds, and interfacing wires) prior to the pouring of the PDMS.

After sufficient curing of the PDMS mixture, the final product (platinum pads, bonded wire, interfacing wires, and encapsulating PDMS) is gently peeled off the substrate and mechanically cut using a stainless steel razor blade into the final desired 1 cm × 1 cm square for easy handling and placement. The thickness of the final microelectrode array was near 1 cm.

3. Testing and evaluation

Characterization during the different steps of the fabrication process included profilometry and optical microscopy. Optical microscopy revealed that the desired geometry and spacing of the electrode configuration was maintained

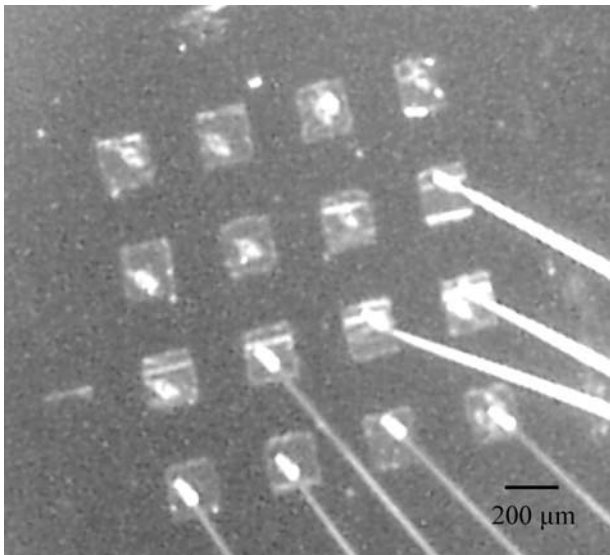


Fig. 2 Optical microscopy (100 \times) of intact wire bonds to individual 200-micron square platinum electrode contacts on a silicon substrate

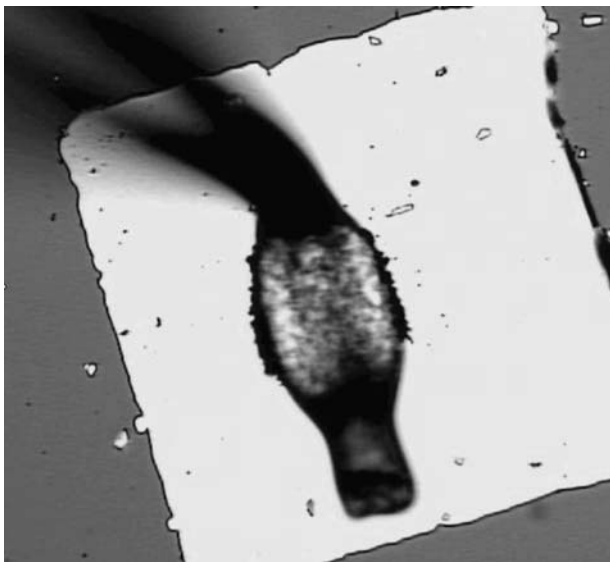


Fig. 3 Optical microscopy (500 \times) of intact wire bonds to individual 200-micron square platinum electrode contacts on a silicon substrate

throughout the photolithography process. Optical microscopy of intact wire bonds to the individual platinum electrode contacts is shown in Figures 2 and 3. Optical microscopy of a working prototype of the surface microelectrode array, shown in Figure 4, revealed also that the desired geometry and spacing of the electrode contacts were maintained throughout the entire fabrication process. Electrical integrity testing, conducted using a microprobe station, revealed that electrical resistance across each electrode and interfacing wire was 0.574 ± 0.0082 ohms.

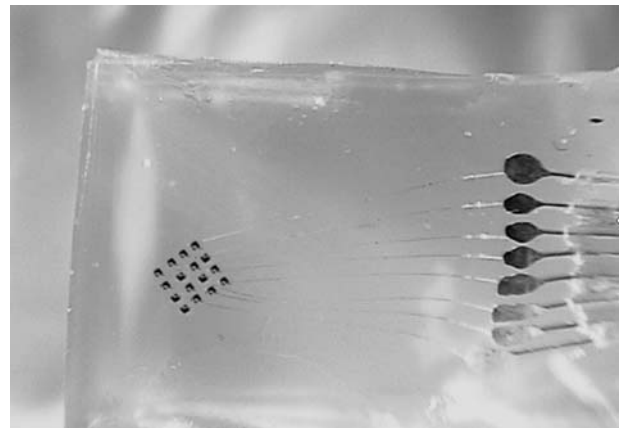


Fig. 4 Optical microscopy (50 \times) showing a prototype of the surface microelectrode array. Eight of the 200-micron platinum square electrode contacts (*left*) are wire bonded to the bonding pads of the interfacing wires (*right*) and suspended within a biomedical grade PDMS

Cytotoxic evaluation of the surface microelectrode array utilized optical and fluorescent microscopy and Laser Scanning Cytometry (LSC). Mammalian cortical cells (harvested rat pup cortical cells, embryonic day 18) and human cortical cells (obtained from America Tissue Culture Collection, cell line CRL-10442 and designation HCN-1A) were prepared according to specifications (Misulis, 1994) and cultured onto glass slides (control) and the surfaces of the microelectrode arrays (experimental). Fluorescent microscopy of the experimental surfaces, Figure 5, and the control surfaces provided a qualitative analysis of the cytotoxicity of the device surface. After 96 hours, cells were stained with FURAII, a calcium dependant fluorescent stain taken-up only by viable cells, and evaluated with fluorescent microscopy. Viable cells were observed on both the control and experimental surfaces. Additionally, the appearance of dendritic and/or axonic growth

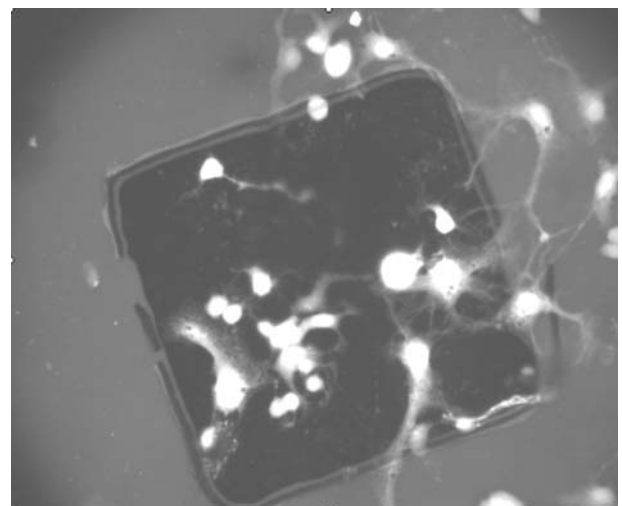


Fig. 5 Fluorescent microscopy (100 \times) of rat cortical cells growing on the microelectrode array surface (FURAII stain at 96 hours)

was observed in the fluorescent microscopy evaluation of cells on both the experimental and control surfaces. These qualitative results suggest that the microelectrode and control surfaces were not toxic to the human and mammal cells (Black, 1999).

In order to conduct a quantitative analysis of cytotoxicity, laser scanning cytometry was used to count and compare the numbers of viable cells on the glass slides (control) and the microelectrode array surfaces (experimental). Cells were subsequently stained with Propidium Iodide, which stains only the nucleus of viable cells, to facilitate cell counting with the LSC. With identical scan areas for all runs, the following results were obtained: The cell count mean for the experimental surface was 3460 ± 117 cells, and the mean cell count for the control surface was 3086 ± 47 cells. The 95% confidence intervals were (3266, 3694) and (2992, 3188) for the experimental and control surfaces respectively. The quantitative analysis statistics demonstrate that the number of viable cells on the experimental surfaces is not less than the number of viable cells on the control surfaces. Therefore, the microelectrode array surface is not toxic to the mammalian cortical cells based on quantitative analysis.

Implementation of the surface microelectrode array consisted of collecting Visual Evoked Potentials (VEPs) from the subdural occipital cortex of a pig under anesthesia (Misulis, 1994). The microelectrode array was placed on the occipital cortex near the midline (Eckhorn, 2001; Karamanlidis, 1972) and interfaced with the Biologic[®] Data Acquisition equipment (Misulis, 1994). A Biologic[®] LED visual stimulation board was presented to each eye individually at mid-line gaze, while the visual evoked potentials were collected using the surface microelectrode array.

During data collection, the computer triggered the visual stimulator to phase-lock the stimulus to each sweep. The analog signals were sent from the cortical microelectrodes to the switchbox and finally to the computer for further amplification and frequency filtering. Signal filtering (60 Hz and resonant 60 Hz) was used to remove electrical noise and a signal-capturing window of 250 milliseconds was chosen. This window is long enough to ensure the capture of a typical evoked visual potential waveform (Misulis, 1994). A P100 potential is typical of Visual Evoked Potentials (VEPs) and has a positive peak with a latency of approximately 100 milliseconds after stimuli presentation. The amplified signal was then sampled two times per millisecond and converted into a digital signal, and stored in memory for signal averaging. Gain of the amplifier was set at 20,000, with the high filter set at 100 Hz and the low filter set at 1.0 Hz. At least 100 sweeps were captured for signal averaging for each potential.

Four VEPs were collected from each eye, with the initial two potentials collected during left eye stimulation, the middle four potentials during right eye stimulation, and the final two potentials during left eye stimulation. Results for each

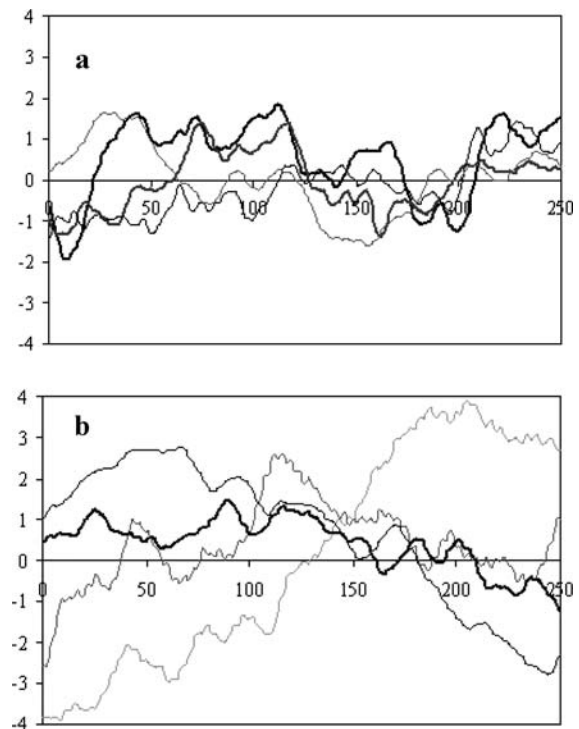


Fig. 6 Four potentials recorded from a domestic pig during (a) left eye stimulation and (b) right eye stimulation using the surface microelectrode array. Voltage (mV) is plotted against time (msec). Visual stimulation occurred at time = 0

potential from a single electrode are shown for each eye in Figure 6 and potentials averaged within each eye are shown in Figure 7. For the left eye, all four potentials revealed a delayed P100-like potential, and the latency of the peak at P100 for each run ranged from 112 to 119 milliseconds, with the averaged signal latency of 114.5 milliseconds. The observed P100s were slightly later than 100 ms due to anesthesia and use of an LED visual stimulator (Kitzmilller, 2004). With the microelectrode array in the same position, visual stimuli were presented to the right eye and the activity was recorded. P100-like activity was not reliably detected during the right eye stimulation.

This collection of the small-field cortical surface potentials suggests that electrode examined in the microelectrode array may have detected activity specific to the ocular dominance column dominant for left eye activity. The reason for the pattern of eye stimulation (left eye, right eye, left eye) during microelectrode recording was to demonstrate that the P100-like activity recorded could be reproduced during subsequent left eye stimulation and that the absence of P100-like activity during right eye stimulation was not a result of failure of the collection equipment after the initial left eye recordings. In order to demonstrate that failure to detect activity from the right eye did not result from impaired function of the visual pathways from the right eye, 1500-micron penetrating microelectrodes were subsequently implanted in

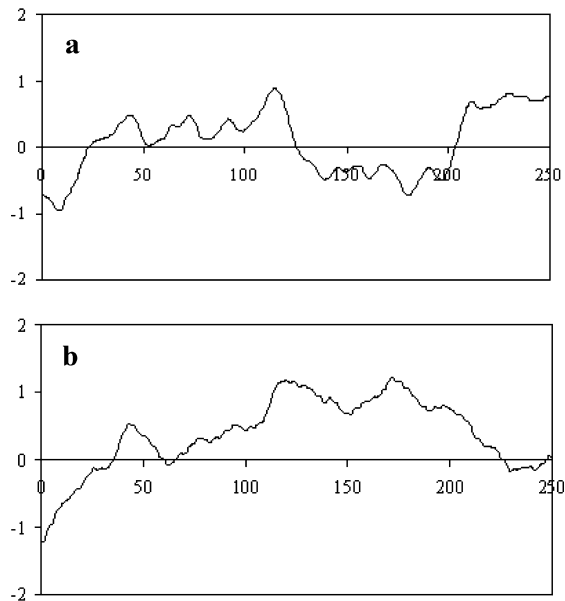


Fig. 7 Averages of four potentials recorded from a domestic pig during (a) left eye stimulation and (b) right eye stimulation using the surface microelectrode array. Voltage (mV) is plotted against time (msec). Visual stimulation occurred at time = 0

the same cortical region, and identical stimuli, signal detection, and signal averaging parameters demonstrated robust activity from electrodes detecting left and right eye activity, respectively. Therefore, since stimulation from both eyes could elicit activity at the occipital cortex with penetrating electrodes, the failure to detect surface activity from the right eye was not simply the result of impaired function of the visual pathway of the right eye in this mammal and may be the direct result of the fact that the electrode contact of the microelectrode array was directly over a cortical column that was specific for the left eye only.

4. Conclusion

Microfabrication was used to construct a microelectrode array comprised of 200-micron square platinum electrodes, with bi-directional pitch of 400 microns, suspended within a biomedical grade PDMS. Characterization and electrical integrity testing of the device demonstrated a robust design. Results of the qualitative and quantitative cytotoxicity analysis suggest that the device is suitable for use with human patients. Implementation of the microelectrode array with a

pig animal model demonstrated that evoked small-field cortical potentials could be collected and analysis of the VEPs collected suggest that the microelectrode array may be capable of collecting electrical activity specific to the cortical columns.

Acknowledgements Most of the fabrication and characterization work was performed at the Ohio MicroMD facility. The testing and evaluation work was conducted at The Ohio State University departments of Neuroscience and Veterinarian Science laboratories. Financial support included NIDA, the Ohio MicroMD MicroGrant program, and the Ohio State University Alumni Grant program. The authors gratefully acknowledge the contribution of the following people: James Burnes (contribution of wire bonding expertise), Nicholas Ferrell (contribution of characterization and LSC analysis expertise), Dr. Karl Obreitan (contribution of cell culture expertise), Linda Fortin (contributing assistance and data acquisition expertise during the animal study), and Dr. Valerie Bergdall-Costell (performing the surgical aspects of the animal experiments).

References

- J.J. Burmeister, F. Pomerleau, M. Palmer, B. Day, P. Huettl, and G. Gerhardt, *Journal of Neuroscience Methods* **119**, 163 (2002).
- B. Heppelmann, M. Pawlak, and R.F. Schmidt, *Experimental Brain Research* **141**, 501 (2001).
- D.J. Waren, E. Fernandez, and R.A. Norman, *Neuroscience* **105**, 19 (2001).
- N. Bechtereva and Y. Abdullaev, *International Journal of Psychophysiology* **37**, (2000).
- H. Oya, H. Kawasaki, M.A. Howard III, and R. Adolphs, *Journal of Neuroscience* **22**, 9502 (2002).
- T.H. Schwartz, M.M. Haglund, E. Lettich, and G.A. Ojemann, *Journal of Cognitive Neuroscience* **12**, 803 (2000).
- P.D. Williamson, J.A. French, V.M. Thadani, J.H. Kim, R.A. Novelly, S.S. Spencer, D.D. Spencer, and R.H. Mattson, *Annals of Neurology* **34**, 781 (1993).
- M. Maghribi, J. Hamilton, D. Polla, K. Rose, T. Wilson, and P. Krulvitch, Stretchable micro-electrode array. In: *Proc. 2nd Intnat'l IEEE-EMBS Conf on Microtech in Med & Biology*. pp. 80–83 (2002).
- V.B. Mountcastle, *Brain* **120**, 701 (1997).
- K.E. Misulis, *Spelmann's Evoked Potential Primer: visual, auditory, and somatosensory evoked potentials in clinical diagnosis*. (Butterworth-Heinemann, Newton, MA 1994).
- J. Black, *Biological Performance of Materials: Fundamentals of Biocompatibility* (Marcel Dekker Inc., New York, 1999).
- R. Eckhorn, A. Stett, T. Schanze, F. Gekeler, H. Schwahn, E. Zrenner, M. Wilms, M. Eger, and L. Hesse, *Ophthalmologie* **98**, 369 (2001).
- A.N. Karamanlidis and J. Magras, *Brain Research* **44**, 127 (1972).
- J. Kitzmiller, Design, engineering, and evaluation of a novel microgrid electrode array to monitor the electrical activity on the surface of the cerebral cortex. (Electronic Theses and Dissertations Center, June 2004), www.ohiolink.edu/etd/