Journal of the Arkansas Academy of Science

Volume 48 Article 37

1994

Multisite Microprobes for Electrochemical Recordings in Biological Dynamics

G. Sreenivas
University of Arkansas, Fayetteville

S. S. Ang
University of Arkansas, Fayetteville

R. M. Ranade
University of Arkansas, Fayetteville

A. S. Salian
University of Arkansas, Fayetteville

W. D. Brown
University of Arkansas, Fayetteville

Multisite Microprobes for Electrochemical Recordings in Biological Dynamics

G. Sreenivas, S.S. Ang, R.M. Ranade, A.S. Salian and W.D. Brown
Department of Electrical Engineering
University of Arkansas
Fayetteville, AR 72701

Abstract

For over 30 years, techniques have been developed that allow for the microscale (10-30 μm) measurement of chemical signals with high temporal resolution (1-200 Hz). Such measurements, called *in vivo* electrochemical recordings, allow for the direct determination of neurotransmitter molecules and related compounds in biological systems. Multiple recordings, simultaneously performed at different, closely spaced, well defined locations throughout a three-dimensional tissue volume in the brain, are of interest in neuroscience. Developments in microelectronic techniques enable the fabrication of multi-electrode microprobes for recording extracellular action potentials generated by individual neurons simultaneously. A high-yield microfabrication process has been successfully developed for the fabrication of a novel semiconductor-based, four-site silicon microprobe that involves a three-mask process and standard UV photolithography. A plasma process has been developed for dry etching of the gold electrodes and conducting lines. The electrochemical behavior of the microprobe is investigated by a high-speed computer-based *in vitro* electrochemical recording system. The electrochemical signals are measured at 5 Hz and varying gain. It is found that a selectivity of over 500:1 is achieved, and the signal to noise ratio of the recorded signal is particularly suitable for *in vivo* recordings.

Introduction

One of the central challenges in neuroscience is associated with the development of improved instrumentation for studying the central nervous system (CNS). Such instrumentation is needed both to better understand the information processing techniques used in neural structures and to aid in the development of a variety of closed-loop neural prostheses (Hoogerwerf and Wise, 1991). Multiple recordings, simultaneously performed at different, closely spaced, well defined locations throughout a three-dimensional tissue volume in the brain are of interest in neuroscience to derive useful prosthetic control signals (Prohaska et al., 1986). For over 30 years, the principle technique for studying the neural activity has been insertion of fine-tipped microelectrodes into the brain to record the extracellular action potentials generated by individual neurons (Rose and Mountcastle, 1954).

Developments in microelectronic techniques enable the fabrication of extremely small and precise structures that can be laid out in any desired way to match the recording site pattern for simultaneous neural recordings. Some of the valuable advantages offered by the thin film microprobes include a high degree of reproducibility, a precise knowledge of spatial distribution of electrode area, a high packaging density for a given implanted volume, and distribution of electrodes in a specific geometry pattern. Also, such multi-site semiconducting microprobes reduce the number of experiments necessary to collect the required data.

The John Hopkins' microprobes (Blum et al., 1991), consisting of a molybdenum-polyimide structure, exhibits poor mechanical strength, and the fabrication process yield is about 10% (all four good sites). Another major shortcoming of these probes is the polyimide dielectric material which is not suitable for chronic implants. The Michigan probe (BeMent et al., 1986), with a typical process yield of over 80%, has been fabricated on a silicon substrate with the thickness of the probes determined by the depth of a deep level boron diffusion.

In this paper, a new microfabrication technique for the fabrication of four-site semiconducting microprobes is presented. Figure 1 shows a three-dimensional view of one such four-site microprobe. The sensing electrodes of area 5580 μm^2 (155 μm by 36 μm) are placed on the tip of the microprobe and are spaced 200 μm from the center of the adjacent site. Bond pads are situated on the rear end of the microprobe and allow for external electronic connections. The microprobes are characterized *in vitro* using a high-speed computer-based electrochemical recording system. Also, the impedance and the integrity (lifetime) of the microprobe are investigated.

Materials and Methods

Microfabrication of the microprobes (electrodes) begins with a 3 milli-inches (mil) p-type <100> oriented sil-

icon wafer that acts as the host substrate. Silicon nitride (Si_3N_4) depositions are performed in a parallel-plate, capacitively-coupled, 13.56 Mhz Reinberg-type reactor (Texas Instruments Model A24C) by plasma decomposition of monosilane (SiH_4), ammonia (NH_3), and nitrogen (N_2). A modified Perkin Elmer Model 2400 sputtering machine is used to sputter successive layers of titanium-tungsten and gold on the patterned substrates. Patterns are transferred onto the substrates using a Quintel mask aligner/exposure system by a standard UV photolithography process. A Plasma Therm Model 520, parallel-plate reactive ion etcher (RIE) is used for selective dry etching of Si_3N_4, gold, and Hunt HPR-204 positive photoresist. Flow rates during both deposition and etching processes are electronically controlled by mass flow controllers. The microprobes are then separated from the host substrate by orientation dependent etching. The microprobes are then mounted on a custom made printed circuit board (PCB) carrier and either ultrasonically wire bonded using 3 mm aluminum wire with an Orthodyne Model 20 wire bonder or epoxy bonded, using ABLEBOND*967-1, to the printed circuit board carrier which allows for external electronic connections and acute recordings. A high-speed computer-based *in vivo* electrochemistry recording system is used for characterizing and qualifying the microprobes for *in vivo* recordings. Furthermore, the frequency response and impedance characteristics of the microprobes are also measured.

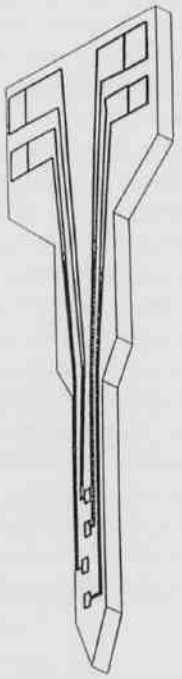


Fig. 1. Three-dimensional view of the four-site silicon microprobe.

Fabrication Process.--A typical process sequence is shown in Fig. 2. As shown, the fabrication process begins with a three-mm silicon wafer as the host substrate material and involves three masking steps. Silicon is strong, yet flexible when thinned, and is inert in the body. Therefore, it qualifies as a suitable substrate for chronic implants over other substrate materials.

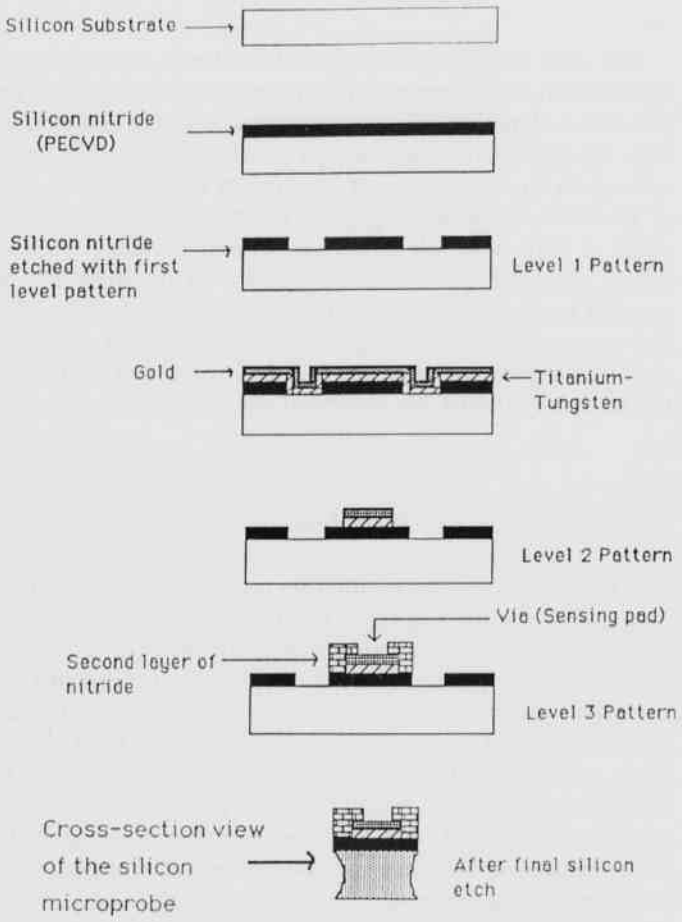


Fig. 2. A typical process sequence for the fabrication of the four-site silicon microprobe.

A sufficiently thick layer of silicon nitride, grown by plasma-enhanced chemical vapor deposition (PECVD), serves as a dielectric layer between the electrodes and the host substrate. Silicon nitride is a preferred choice since it is impervious to extracellular fluid. It is deposited by gas phase dissociation of monosilane (SiH_4), ammonia (NH_3), and nitrogen (N_2). This mixture of gases is excited by an RF plasma in which high-energy electrons dissociate reactant gases to allow deposition of solid material on the substrate at moderate temperatures (300°C). The silicon nitride deposition conditions are summarized in Table 1.

The outline of the microprobe is defined with a first-level photomask using a standard UV photolithographic process by which a pattern of photo-sensitive masking material is applied to the surface of a silicon substrate. The silicon nitride is subsequently etched from exposed areas by reactive ion etching. Table 2 provides source gases, etch rates, and other etch parameters for different materials used to fabricate the microprobes.

Table 1. The processing parameters used to deposit silicon nitride (Si_3N_4) films.

Experimental conditions:	
Deposition power (RF)	100 W (28 mW/cm^2)
Deposition frequency (RF)	13.56 MHz
Deposition temperature	300°C
Deposition rate	120 Å/min
Total gas pressure	1 Torr
Gas flow rates:	
Silane	100 sccm
Ammonia	80 sccm
Nitrogen	200 sccm

Table 2. Source gases, etch rates, and other etch parameters used to etch different materials.

Material	Gases/flow rate	Power	Pressure	Etch rate
Silicon nitride	CF_4 (25 sccm)	200 W	500 mT	200 Å/min
Gold/Ti-W	CF_4 (17 sccm) CCl_4 (25 sccm)	450 W	150 mT	914 Å/min
Photo resist	Oxygen (89 sccm)	450 W	700 mT	650 Å/min

The next step in the process is the sequential sputtering of ≈250Å of titanium-tungsten (Ti-W) and 2500 to 3000Å of gold (Au) onto the patterned microprobe. Gold, being highly electropositive and non-corrosive, is an excellent metal for chronic implants. Unfortunately, the adhesion of gold to silicon nitride is a major metallization problem. However, the thin layer of Ti-W acts as a barrier layer and promotes adhesion. In the sputtering process, the required coating material is dislodged and ejected from a target by momentum transfer due to energetic particles (argon ions and radicals in this case). A second-level mask is used to photolithographically define the Au and Ti-W to form the sensing electrodes, conducting lines, and bonding pads. As shown in Table 2, the etch rate for Au/Ti-W is ≈914 Å/min with an etch selectivity of 1.5 over positive photoresist (Ranade et al., 1993).

Figure 3 shows a scanning electron micrograph (SEM) of several 3 μm gold conductors etched using reactive ion etching. Good edge definition is achieved without significant undercutting implying that gold etching is primarily anisotropic.

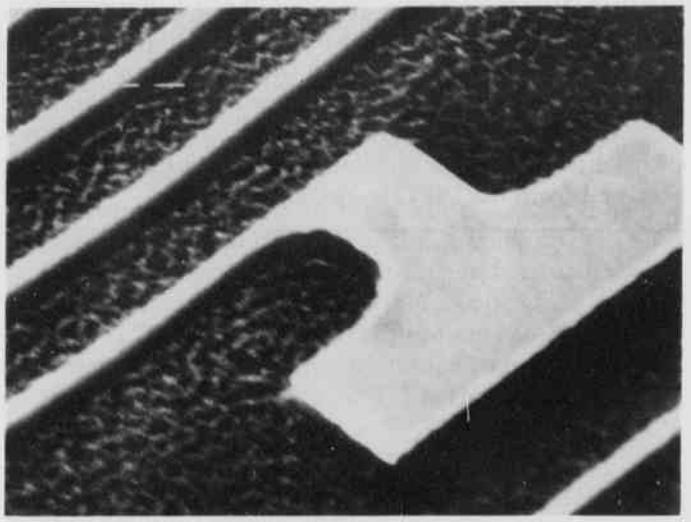


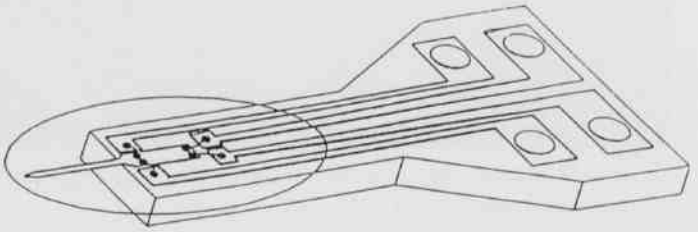
Fig. 3. Scanning electron micrograph (5000X) of several 3 μm lines etched using RIE.

A second layer of silicon nitride is then deposited, photolithographically defined, and etched to provide openings for sensing electrodes and bonding pads. Finally, the silicon microprobes are separated by an orientation-dependent etchant, ethylene-diamine pyrocatechol (EDP) in water. The thickness of the probes can be tailored to meet any requirement. The final thickness of these four-site silicon microprobes is about 50 μm. The fabrication processes are controlled to obtain a typical yield in the 70 to 80% range.

After separation, the good microprobes are mounted onto a custom made printed circuit board (PCB) carrier and wires are either ultrasonically or epoxy bonded from the microprobe to the PCB as shown in Fig. 4.

Characterization.--In vitro tests are performed to obtain most of the information required to qualify an electrode for electrochemical and electrophysiological studies. Quantitative measurements of microamines are performed with the microelectrodes using a high-speed chronoamperometric recording technique. Fig. 5 shows typical chronoamperometric measurement data. The microelectrodes were dip-coated with a thin layer of nafion, a perfluorosulfonated derivative of Teflon, to increase the selectivity of cationic neurotransmitters such as dopamine, norepinephrine, and serotonin *in vitro*. Their selectivities versus anionic interferents such as ascorbic acid in extracellular recording were also characterized (Van Horne et al., 1990). Dopamine calibration in 2 μM increments, challenged with 250 μM ascorbate, was

performed to the study sensitivities and recording characteristics of sensing electrodes. Square-wave pulses of 0.0 to +0.55 V, with respect to a Ag/AgCl reference electrode, were applied to the working electrode. The measurements are performed at 5 Hz and varying gain settings. The resulting oxidizing and reducing currents were digitally integrated for a fixed recording period. Figure 6 shows linear regression data for the sensing electrode obtained from the IVEC recording system.



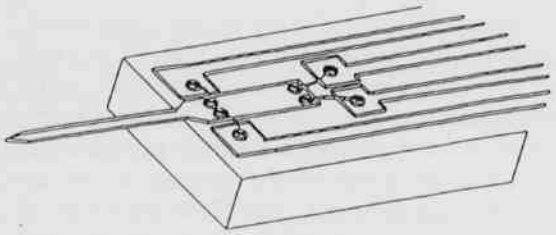


Fig. 4. A complete probe assembly consisting of microprobes bonded onto a printed circuit board carrier.

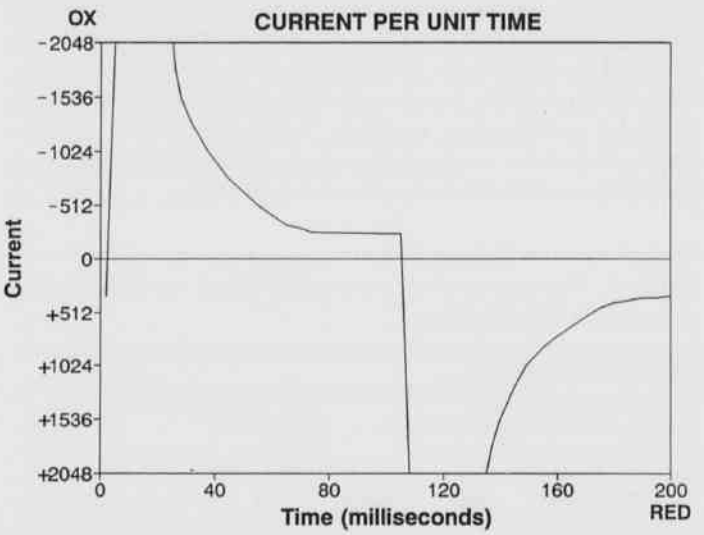


Fig. 5. Typical calibration data for the sensing electrode recorded using chronoamperometry.

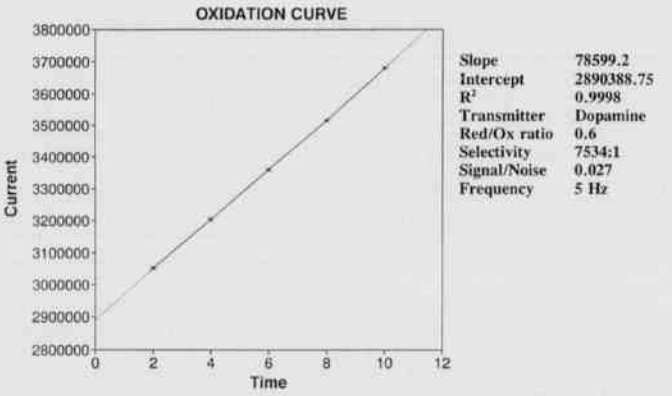


Fig. 6. Linear regression data for oxidation of the sensing electrode of Fig. 5.

Results and Discussion

As can be seen in Fig. 6, the electrode responded linearly to increasing concentrations of dopamine with the linear regression correlation coefficient of the calibration curves being greater than 0.998. Typical selectivities for dopamine detection versus ascorbic acid achieved lie in the range of 300-900:1. Furthermore, the detection limit of the microelectrodes range from 25-150 nano-molar at a signal-to-noise ratio of 3. Current state-of-the-art carbon fibre probes have less than a 50 nano-molar detection limit at a signal-to-noise ratio of 3 (Gerhardt et al., 1984). The oxidation curve is found to have a slope of better than 100,000. A typical red-ox current ratio of about 0.6 to 0.8 was recorded.

An impedance tester was used to measure the impedance of each of the recording sites. The recording site impedance depends on the site material, site area, surface roughness, electrolyte, signal frequency, and current density (Prohaska et al., 1986). The measured impedance of the electrode is based on the potential developed at the electrode-electrolyte interface. A typical site impedance of 2 to 5 MΩ is measured at 2 KHz, which is in the appropriate range for a good neuronal recording (Ang et al., unpublished). Furthermore, tests of probe lifetime for continuous immersion in physiological saline solution

conducted on the microelectrodes over an extended period of time (180 hours), suggest that the site impedance remains relatively stable over the soak duration (Ang et al., unpublished). Such measurements are accomplished by calibrating the system using resistors for the microelectrode and correlating the measured potentials with a resistor value.

Summary and Conclusions

A high-yield microfabrication process has been successfully developed for the fabrication of a novel semiconductor-based, four-site silicon microprobe for electrochemical and electrophysiological recordings. The electrochemistry of these silicon microprobes was characterized quantitatively by *in vitro* measurements.

The microprobes were characterized for the sensitivity and recording characteristics of dopamine calibrated against ascorbic acid. A typical selectivity of over 500:1 for dopamine versus ascorbic acid was achieved. Also, the microelectrodes responded linearly to increasing stock solution (concentrations) of dopamine. Furthermore, the detection limit of the microelectrodes was found to lie in the range of 25-150 nano-molar at a signal-to-noise ratio of 3.

A typical electrode site impedance, in the range of 2 to 5 MΩ, was measured at a frequency of 2 KHz. The integrity of the microprobe dielectrics was examined by observing the stability of the site impedance during a 180 hour soak test in a saline solution. Finally, the results of this work suggest that semiconductor microprobes will play an important role as real time biological sensors to analyze presynaptic neurotransmitter dynamics and activity of multiple neurons.

Acknowledgements

The authors would like to express their appreciation to Biographics, Inc., Winston-Salem, North Carolina and Rocky Mountain Center for Sensor Technology, Denver, Colorado for their financial support.

Literature Cited

Ang, S.S., G.A. Gerhardt, G. Sreenivas, A.S. Salian, R.M. Ranade, B.J. Hoffer and **D.J. Woodward.** Unpublished data.

BeMent, S.L., K.D. Wise, D.J. Anderson, K. Najafi and **K.L. Drake.** 1986. Solid state electrodes for multi channel multiplexed intracortical neuronal recording, IEEE Trans. Biomed. Eng. BME-33: 230-241.

Blum, N.A., B.G. Carhuff, H.K. Charles, Jr., R.L. Edwards, and **R.A. Meyer.** 1991. Multisite microprobes for neural recordings: IEEE Trans. Biomed. Eng. BME-38: 68-74.

Gerhardt, G.A. A.F. Oke, G. Nagy, B. Moghadda and **R.N. Adam.** 1984. Nafion-coated electrodes with high selectivity for CNS electrochemistry, Brian Research, 290: 390-395.

Hoogerwerf, A.C. and **K.D. Wise.** 1991. A three-dimensional neural recording array. International Conference on Solid-State Sensors and Actuators, IEEE Electron Devices Soc. 120-123 pp.

Prohaska, O.J., F. Olcaytug, P.Pfundner and **H. Dragaun.** 1986. Thin-film multiple electrode probes: possibilities and limitations, IEEE Trans. Biomed. Eng. BME-33 (2): 223-229.

Ranade, R.M., S.S. Ang and **W.D. Brown.** 1993. Reactive ion etching of thin gold films, J. Electrochem. Soc. Vol. 140 (12): 3676-3678.

Rose, J.E and **V.B. Mountcastle.** 1954. Activation of single neurons in the tactile thalamic region of the cat in response to a transient peripheral stimulus, Bull. The Johns Hopkins Hospital, 94: 238-282.

Van Horne, C.G., S. Bement, B.J. Hoffer and **G.A. Gerhardt.** 1990. Multichannel semiconductor-based electrodes for in vivo electrochemical and electrophysiological studies in rat CNS,: Neurosci. Let. 120: 249-252.