論文80－17－6－10

A Study on Integrated Multimicroelectrode Array
(集積回路型 多重 超微小 電極 配列에 關하여)

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要 約

最近 生体의 神經系統에서 發生하는 微小한 電氣現象의 觀察을 通하여 生体의 神經作用을 研究하는 活動이 增大 되어가고 있다.

本論文에서는 個個의 神經細胞에서 發生하는 微小電壓을 測定하기 爲하여 半導体集積回路 製造技術을 利用하여 製作한 集積回路型 超微小電極配列의 製造方法과 電氣的 特性에 關하여 記述하였다. 被測定 神經細胞의 크기와 種類에 따라 集積電極의 크기를 수μ~수10μ 範圍內에서 正確한 치수에 맞혀 製作할 수 있음을 實驗的으로 確認하였다. 光學的 photolithography方法을 使用하여 電極의 形狀을 決定하기 때문에 어떠한 形態의 電極도 만들 수 있다. 이 方法으로 만든 7素子電極위에 두께 3000Å의 유리 絶緣層을 덮었을 때에 Ringer 溶液中에서의 電極의 임피던스는 周波數 範圍 10Hz~1 KHz 範圍에서 約 1 MΩ ~ 100KΩ 程度로 比較的 낮았지만 Si_3N_4 絶緣層을 使用하면 Na+이온의 擴散도 防止되고 임피이던스 特性도 보다 좋게 된다. 이 型式의 電極은 各各의 前置增幅器와 함께 單一Si칩 위에 monolithic形態로 集積하여 製造할 수 있기 때문에 S/N 比와 임피이던스 特性을 훨씬 더 改善할 수 있다. 그리고 電極의 出力信號의 導出에 있어서는 multiplex 方式을 使用함으로써 引出導線의 數를 減少시킬 多重信號 測定도 可能하게 된다.

本方法으로 製造한 電極配列을 利用하여 實際로 生体의 神經電位를 測定한 結果를 提示하였다.

Abstract

A new type of multimicroelectrode array has been developed using integrated circuit technology in order to record action potentials from nervous systems.

The size and pattern of the electrode array are determined accurately in a few μm range by optical photolithography.

The electrical characteristics of the integrated microelectrode array were investigated.

The practical applications of this electrode array in recording action potentials from a ventral nerve of a cockroach are shown.

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1. Introduction

In neurophysiological research, recording of the bioelectrical activity of cells in the nervous system is very important. Several papers have been published on the extracellular recording of bioelectrical information from the electrodes.[1,2,3,6,7] The application of existing microelectrodes is limited to single unit

recordings. Recently several papers have been published on the techniques for fabricating multiplicroelectrodes.[4] To date, these multimicroelectrodes arrays suffer from a lack of exact reproducibility and so the electrodes characteristics vary widely from device to device.

characteristics vary widely from device to device.

A two-dimensional fixed-array prototype multimicroelectrode which is fabricated utilizing integrated circuit technology is described. The physical dimensions of this type of microelectrode can be accurately controlled within the range of 0.1μm ~ 100μm by using photolithography techniques. This technique enables the production of microelectrodes on a silicon substrate that can be used for simultaneous recording from several nerve cells. This technique has the advantage of allowing for integrating of preamplifiers on-chip with the microelectrode array, thus improving the singnal to noise characteristics of the recording system. As a specific example, the design and fabrication of a seven point multimicroelectrode is described. Its applications to recording the action potentials from the nerve cells of a cockroach are described.

2. Microelectrode Fabrication

Fig. 1 is a top view of the seven-element microelectrode array which is fabricated on a silicon wafer. Each electrode has an active recording diameter of 10μm and is 100μm apart from adjacent electrode. The passive electrodes are fabricated on a silicon-dioxide surface by adding metallization and passivation layers as shown in Fig. 2.

Initially an oxide layer was thermally grown approximately 0.5μm thick in a steam oxidation furance at 950°C for about two hours. Photoresist (Shipley AZ1350J) was spin-applied to the thermally grown silicon-dioxide surface at a rotating speed of 4000rpm for 30 seconds. The coated wafer was then exposed to the ultra-violet light source for 20 seconds through the first photographic mask shown in Fig. 3 on the contact mask aligner. The exposed photoresist was developed in the 10:1 developer solution (Shipley AZ606) for 30 seconds, washed in deionized water and dried sufficiently. In order to make the metallization layer chromium was first evaporated, approxi-

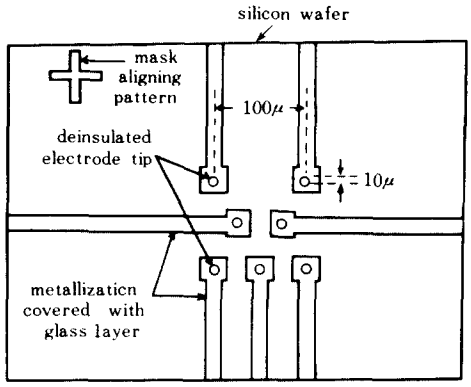

Fig. 1. 7-element microelectrodes array (top view).

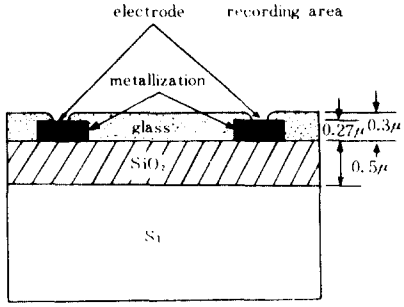

Fig. 2. Cross section of an microelectrode array.

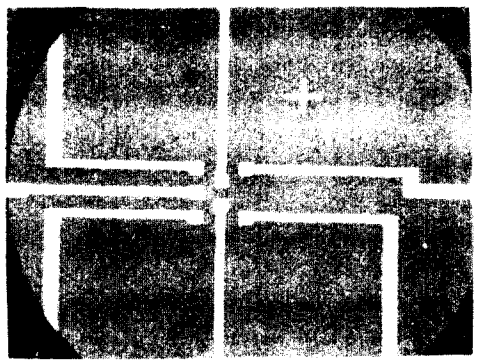

Fig. 3. The first mask pattern (for metallization).

mately 200Å thick, onto the photoresist-patterned wafer surface to assure better bonding between the silicon-dioxide layer and following surface gold layer. Gold was then evaporated, approximately 2500Å thick, on top of the chromium layer in a vacuum of 10^{-6} torr. The unnecessary metal film was removed by a process known as "lift-off". This technique

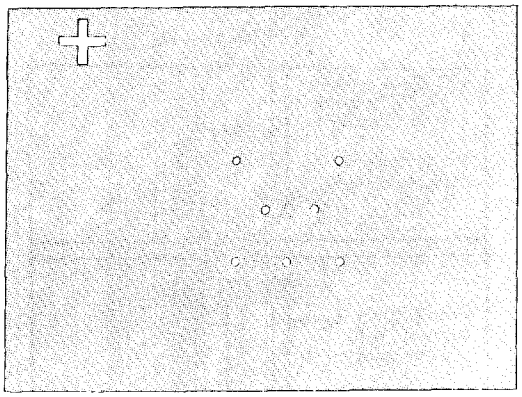

Fig. 4. The second mask pattern (for deinsulation of metal electrode active recording area).

involves soaking the wafer in acetone. The acetone dissolves the remaining photoresist under the metal, and the unwanted gold flakes off along with the unwanted photoresist not removed during the development step. Now, the gold metallization has the pattern shown in Fig. 3.

A glass solution (Emulsitone Co. glass forming solution #122) was spun onto the metallized wafer surface with a spinner apparatus at 3000rpm for 30 seconds, producing a glass layer of approximately 1500Å thickness. The glass coating was dried at room temperature for 15 minutes and then baked at 170°C for 30 minutes. To obtain approximately a 3000Å thickness, the above process was repeated twice. After the second glass layer was formed, the wafer was baked at 170°C for 5-6 hours to harden the film.

Photoresist was again applied to the glass surface on the spinner at 4000rpm for 30 seconds and then dried at 80°C for 15 minutes. Using the second mask shown in Fig. 4 aligned to the pattern defined by mask 1 the photoresist was exposed to ultra-violet light for 30 seconds on the contact mask aligner. The exposed photoresist was developed for 30 seconds in the 10 percent developer solution, rinsed and dried. The photoresist was postbaked at 170°C for 5 minutes. The wafer was then etched with a 1% hydrofluoric acid solution for 15 seconds. Microscopic investigation showed definite cutthrough of the glass layer down to the gold layer. Photoresist was stripped in photoresist remover (Shipley #1112A) at 75°C for 10 minutes followed by a complete rinse with TCE, acetone, methanol, DI water, blow dry, and baking at 80°C for 10 minutes. The wafer was then scribed into individual microelectrode chips. The chips were further hardened at 170°C for 24 hrs. Each chip was bonded to a 10-lead TO-5 header at 600°F using a conductive silver epoxy (EPO-TEX H20E, Epoxy Technology).

Because the microelectrode array itself must be placed in direct contact with nerve cells to obtain recordings of the cells electrical behavior, the IC chip cannot be hermetically sealed. A header provides the mechanical base to support the chip and also provides a means of connecting the delicate chip to larger wires suitable for interconnecting the microelectrode array to other equipment, in our case, amplifiers and audio monitors. Fine gold wire is thermal-compression bonded between the bonding pads on the chip and bonding posts on the header. The bonding posts are fed through the header base to much larger diameter wires which are used to make external connections. The bonding wires are extremely fragile and require protection from any external abrasion to prevent their failure. Since a sealed top cannot be placed on our header an alternative manner of protecting the array was devised. The bonding wires as well as the bonding pads on the chip and the bonding posts on the header are coated with a silicone adhesive (Dow Corning Type A medical grade adhesive). This provides both physical protection for the delicate wires and electrical insulation from the conducting ionic solution always found externally around living cells. The complete insulated microelectrode array assembly is shown in Fig. 5.

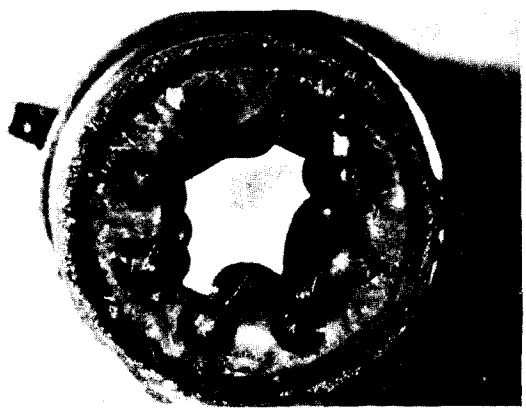

Fig. 5. 7-element microelectrode array assembly.

3. Electrical Characteristics

In this electrode system the seven integrated microprobes are spaced 100 microns apart and are formed by photolithographically deinsulating the active recording regions of the glasspassivated Cr-Au metallization which connects the electrodes to the bonding pads. The electrical characteristics of the microelectrodes are very complex, and have nonlinear and frequency dependent properties. The small-signal electrical characteristics can be analyzed by using half-cell overvoltage concepts. A spacecharge region is formed at the interface between the metal and the electrolyte. The charge and potential distributions are governed by the solution to Poisson's equation.

For an electrode with a small exchange current density, the charge-transfer overvoltage mechanism is the dominant cause of electrode overvoltage, and the current and electrode voltage are related by[5]

$$J/J_0 = \exp(\alpha Z v_t F/RT) - \exp[-(1-\alpha)Z v_t F/RT) \quad (1)$$

where J = steady-state electrode current density
J_0 = exchange current density
Z = transfer valence
α = transfer coefficient
v_t = charge transfer overvoltage
R = gas constant
F = Faraday's constant
T = absolute temperature

The facter RT/F is equal to 0.0256 volts at 25°C. For small-signal conditions, when the steady-state current through the electrode is such that $v_t \ll RT/\alpha ZF$ the incremental resistance R_t is given by $R_t = \partial I/\partial v_t = RT/ZFI_0$, where I_0 = area·J_0. For larger currents, such that $v_t \gg RT/\alpha ZF$, $R_t = RT/\alpha ZFI$. The small-signal model of an electrode consists of R_t and C_t in parallel. The incremental capacitance of the space-charge region is fairly independent of frequency, and is given by $C_t = dQ/dV$.

In addition to the charge transfer overvoltages, the diffusion overvoltage which is controlled by the diffusion of ions to or from the electrical double layer exists in the metal-electrolyte interface. The diffusion overvoltage V_d is given by[5]

$$J/J_s = 1 - \exp(-|V_d|ZF/RT) \quad (2)$$

where V_d = diffusion overvoltage
J_s = electrode saturation current density.

Hence the incremental diffusion resistance can be obtained by $R_d = RT/ZF(I_s - I) = R_d(\omega)$, where I_s is the electrode saturation current corresponding to the maximum current that the electrode will pass for a given reaction.

The capacitance $C_d(\omega)$ related to the diffusion overvoltage is also a frequency dependent component, and appears in parallel with $R_d(\omega)$.

It can be shown that for conditions close to equillibrium and for a single ion species dominating diffusion the resistance $R_d(\omega)$ and the capacitance $C_d(\omega)$ are given by[5]

$$R_d(\omega) = \frac{RT}{Z^2F^2}\sqrt{\frac{1}{\omega}}\frac{1}{C_0\sqrt{D}}$$
$$C_d(\omega) = \frac{Z^2F^2}{RT}\frac{C_0\sqrt{D}}{\sqrt{2\omega}} \quad (3)$$

where D is the diffusion constant and $R_d(\omega)C_d(\omega) = 1/\omega$.

The electrifical model for each electrode is the combination of R_t, C_t, $R_d(\omega)$, and $C_d(\omega)$ as shown in Fig. 6, where bulk resistance R_s of electrolyte and the resistance of metal conductor R_m are usually less than several hundreds ohms and therefore can be neglected.

The complete model of an action potential measuring electrode system can be formed as shown in Fig. 7, where the shunt capacitance C_s is the capacitance between the electrode metal surface and ground with the glass passivation layer acting as the dielectric. Ecell is the action potential of a nerve cell.

The electrode impedance of the experimental

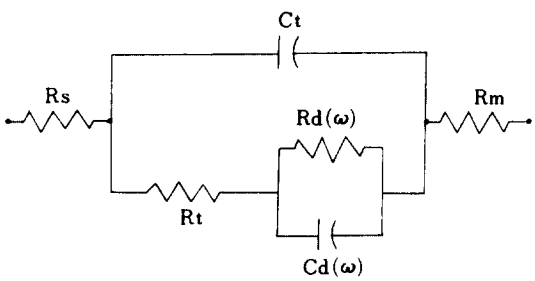

Fig. 6. Equivalent circuit of an electrode.

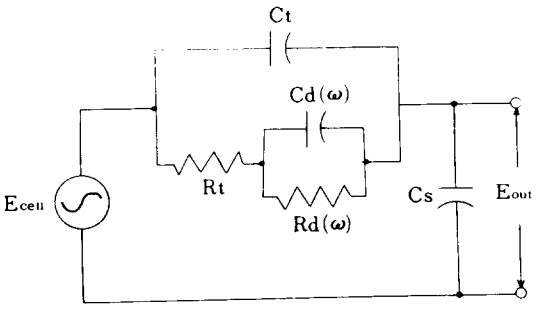

Fig. 7. Complete model of an electrode system.

microelectrode was measured with an a.c. circuit which included a signal generator, a known resistor in series with an electrode pair. The measured impedance was approximately 1 megohms at 10Hz and decreased with increasing frequency as shown in Fig. 8. This electrode impedance includes two parallel components: that of electrode tip and that of shunt component from the gold conductor through the insulation to the saline. The gold-insulation-saline layer represents a capacitor and this capacitance is the limiting factor of the shunt impedance, being less than one megohm for the case of several thousands of angstroms thick glass insulator at 1 kHz.

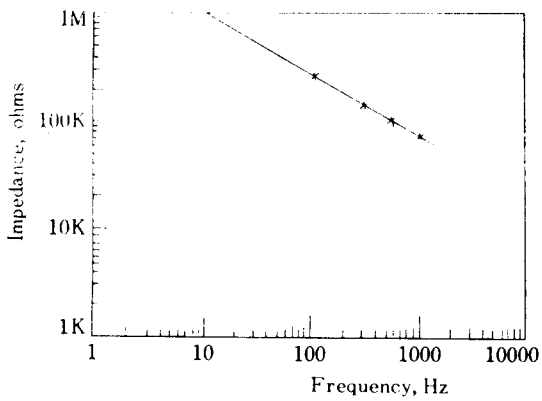

Fig. 8. Frequency characteristics of an electrode.

4. Recording Nerve Signal

A large male cockroach, Periplaneta Americanus was selected for recording ventral nerve action potentials. It was first anesthetized by placing in freezer until just immobilized. After removing the legs and wings, it was pinned ventral side down on wax material. The dosal plates which cover the abdomen were cut away, and the gut and other internal organs were cleared away. The creamy white fatty tissue from around the nerve cord was carefully picked away. Under the dissecting microscope, the almost transparent nerve cord is easily identified. Using micro-scissors the tracheae (fine silvery tubes) were cut away from the nerve cord. Then, the nerve cord was elevated, and all connective tissue was cut away from the cord, freeing it so that recording electrodes can be placed underneath the nerve cord. The nerve cord was kept wet by continuously adding Ringer solution. Within the shielded Faraday cage, the nerve cord was placed on the electrode array. (See Fig. 9a)

The measuring circuit was set up as shown in Fig. 9b. The output leads of the electrode array are connected to the preamplifier (Glass Instrument Co. Model P15) which has variable gains of 10, 100, and 1000 over the frequency range of 0.1Hz to 50kHz. The output singnal of the preamplifier is applied to an active filter (Ithaco Co. Model 4302). Covering the 10Hz to 1MHz frequency range, this filter consists of a pair of identical 24dB/octave filter channels, each of which can be used as a high-pass or low-pass filter with selectable gains of 1 or 10.

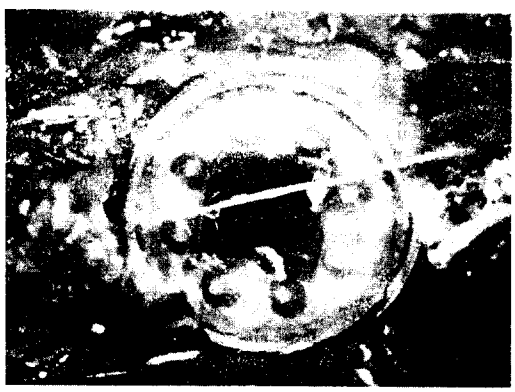

Fig. 9a. Photo of nerve cord over array.

The two filter channels can be connected in series to produce a single 24dB/octave bandpass, a 48dB/octave highpass, or a 48dB/octave low-pass filter, with selectable gain of 1, 10, or 100. Butterworth or Bessel modes can be selected by front switches. A storage oscilloscope (Tektronix Model 5111) was used in order to take photographs of the signal waveforms.

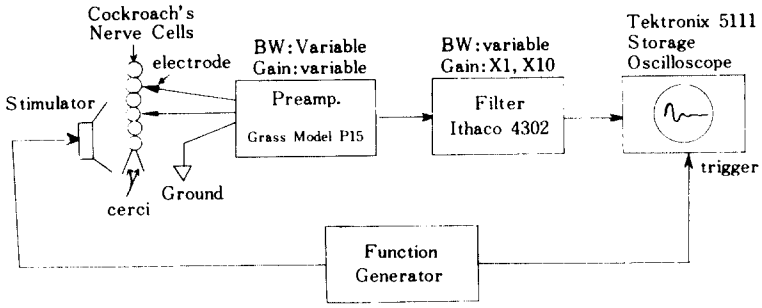

Fig. 9b. Nerve signal measuring circuit.

(a) (b) (c)

Fig. 10. Measured nerve signals (action potentials) from a ventral nerve of a cockroach: Vertical scale; 1 mV/div., Horizontal scale; 0.1ms/div.

The stimulator consists of a dynamic speaker modified to produce a puff of wind of small cross sectional area.

This is driven by a low frequency function generator. This wind puff stimulates the cerci which in turn send action potentials propagating down the nerve cord to be recorded by the electrode array.

Fig. 10 show the oscilloscope traces of action potentials taken from the cockroach ventral nerve cells using the measuring system as Fig. 9.

5. Conclusion

It has been experimentally confirmed that photolithographic method can be used in fabricating integrated multimicroelectrodes which will be used for recording bioelectric phenomena. Using these methods the diameters of the microelectrode-recording areas can be accurately controlled within the range of microns to tens of microns corresponding to the sizes of the nerve cells under investigation. The distance between each electrode can also be controlled with an accuracy of better than 1 per cent. The glass passivation layer several thousands angstroms thick produces comparatively low shunt impedance ranging from 100 kiloohms to 1 megohms for the frequency range of 10Hz to few thousands hertz.

Silicon nitride, Si_2N_4, has also been tried as an insulation passivation layer. It has the advantages of higher impedance as well as being impervious to the diffusion of Na^+ (sodium) ions present in the electrolyte solution surrounding the nerve cord.

With an anticipation that this microelectrode can be integrated with preamplifiers on the same chip, several multimicroelectrodes were fabricated experimentally. This experimental seven-element microelectrode has been successfully used in measuring action potentials from the ventral nerve cell of cockroach.

Acknowledgement

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