Fabrication and testing of multielectrode matrix of disordered Si nanowires for brain tissue sensing*

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***Abstract*—In the field of neuroscience there is interest on manufacturing new recording devices. The relationship between individual action potentials of neurons and field potentials in multicellular records is complex. For this reason, there is a big interest in multielectrode arrays. This work describes the unconventional fabrication process of an alternative multielectrode and its use for sensing neuronal activity. It consists of a matrix of Si nanowires randomly distributed, coated with Ag nanoparticles, and with macrometric Ag back contacts. The Si nanowires are prepared by metal-assisted chemical etching of a Si wafer, which is an economical and highly reproducible technique. Recordings using the multielectrode array of randomly distributed Si nanowires look promising and comparable with recordings obtained with other multielectrode devices.**

I. Introduction

The ultimate goal of neuroscience is to understand the mechanisms underlying brain activity in order to devise different methods that help to restore normal activity in different pathological situations. Our work intends to study the activity of individual neurons and networks, investigating their electrophysiology [1, 2], focused mainly on the study of the electrical properties of cells and biological tissues. Within this field, measurements of voltage or current span a significant range from the pA current of membrane ion channels to mA in some tissues. Likewise, in the field of neurosciences, attention has been focused on the study of the electrical activity of neurons, specially, action potentials. In neuroscience the usual methods of individual electrophysiological recording in-vitro show many limitations, since they do not allow observing the interaction of complete neural networks [3]. Currently the electrophysiologists, eager to understand the collective behavior of networks of neurons, have used arrays of electrodes (multielectrode matrix), which have allowed to observe phenomena that emerge from the interaction between networks of neurons. However, the cost of these multielectrode matrices is high, because they are manufactured with highly expensive materials, such as platinum and gold.

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It is known that silicon (Si) is a biocompatible material due to its stability under physiological conditions [4-6]; therefore, it is a suitable material for the manufacture of a multielectrode matrices. However, it behaves as isolator due to the formation of a space-charge region at the analyte-Si interface. In consequence it is necessary to deposit on it some metallic material to ensure electronic transport to the array.

In order to manufacture microwire arrays of Si, it is necessary to use micromachining techniques [7-10]. Micromachining is a set of tools to design and manufacture structures and elements in the microscale. One of these techniques is lithography. Multielectrode matrices of Si manufactured using lithography are excellent candidates for the registration of electrical signals in brain tissue. However, lithography at the microscale is a process that usually requires complicated equipment such as mask aligners, which allow the definition of structures with a resolution of up to 1 μm. The proposal of this work is the development of a multielectrode matrix of Si nanowires randomly distributed, through metal assisted chemical etching (MACE). Signals are acquired on regions of nanowires defined by macrometric back contacts, and not on individual nanowires. Signal analysis may allow comparable results to those with individual point acquisition.

II. Experimental Section

The experimental procedures for the manufacture of the multielectrode array by the MACE method, the biocompatibility tests and the sensing tests of the electrical activity of neurons are described below.

A. Fabrication of multielectrode array

25 mL of 0.01 M $AgNO_3$ aqueous solution, 0.5 mL of HF (48%) and 0.5 g of polyethylenglycol (PEG) 3600 (3600 g/mol) were mixed. Subsequently, a 1 cm^2 sample of p-type Si (100) with resistivity 15-25 Ω/cm was placed in the aforementioned Ag deposition solution for 1 min, to deposit Ag particles on the Si surface. Then, it was placed in a solution with 35 mL of deionized H_2O, 4 mL of HF (48%) and 1 mL of H_2O_2 (30%), for 10 min at 35 °C to etch pores in the Si substrate. Once the chemical etching was complete, the Si sample was placed in a solution of 100 mL of deionized H_2O and 2 g of PEG 3600 at 50 °C for 30 min at constant agitation, to passivate the surface of the sample. After 30 min, the sample was placed in a solution of 100 mL deionized H_2O, 0.45 g of KOH and 2 g PEG 3600, at 50 °C for 1 min to over-etch the walls between pores producing

wires. After the etching procedure, the electrical contacts on the back side of the multielectrode matrix were defined with photoresist in an array using photolithography. The contacts are of about 1 mm in diameter.

Afterwards, Ag was deposited in the form of nanoparticles on the Si nanowires, and in the form of a film on the back side of the sample. For the Ag deposition on the frontal face of the multielectrode matrix, that is, the area where the Si nanowires are located, a solution of 25 mL of deionized H_2O, 0.15 mL of HF (48%), 0.5 g of PEG 3600 and 5 mL of 0.001 M solution of $AgNO_3$ was used. The sample was exposed to the solution for 6 min. For the Ag deposition to produce the back contacts, 25 mL of a 0.01 M solution of $AgNO_3$, 0.5 mL of HF (48%) and 0.5 g of PEG 3600 were mixed. The sample was exposed to the solution for 3 min. To improve the electrical contact between Ag and Si, a thermal treatment was carried out at 200 °C for 5 h in N_2 atmosphere. Such an annealing procedure is typical for ohmic contacts on Si.

Fig. 1 shows a schematic of the methodology used for the fabrication of the multielectrode array of randomly distributed Si nanowires, electrically contacted on the backside.

B. *Biocompatibility tests*

Once the multi-electrode matrix was manufactured, biocompatibility tests were carried out to study cell survival on the matrix. The multi-electrode matrix was disinfected by exposure to UV radiation for 2 h. To determine the biocompatibility, Chinese hamster ovary epithelial cells CHO K1 (ATCC No. CCL61) were seeded on the electrode arrays, inside culture boxes with 2 mL of DMEM culture medium (Dulbelcco's modified Eagle's medium) enriched with 10 mL HEPES, 2 mL of glutamine, 10% fetal bovine serum, 2 mL of pyruvate and antifungal antibiotic. They were kept in an incubator at 37 °C in air atmosphere with 5% CO_2 and 95% relative humidity for 24 h. After that samples were incubated for 4 h with serum-free medium added with 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).

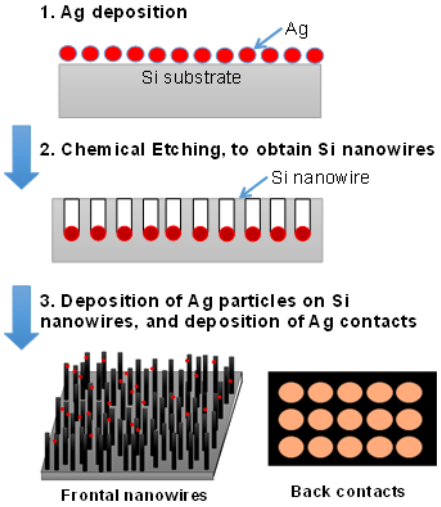


Figure 1. Schematic of the procedure to fabricate a matrix of randomly distributed nanowires electrically contacted on the backside through macrometric contacts.

Finally, the medium was removed and replaced with 85% dimethyl sulfoxide (DMSO) and the formazan crystals contained in the living cells were dissolved. The 6-well boxes with the DMSO were placed on a horizontal shaker for 10 min. Two transmittance measurements were made with a spectrophotometer (EPOC Instruments, Biotec, USA) at the wavelengths of 540 nm and 570 nm. The first measurement was made directly from the boxes (2 mL) that contained the cells plated in the electrode microarrays or in poly-D-lysine coated glasses, and the second measurement was made in 96-well boxes, with 100 µL of the formazan solution. The measurements were compared with control without materials. A negative control treated with triton-100X was also performed.

The experimental procedures involving animal models described in this paper were approved by the Institutional Animal Care and Ethics Committee. They were performed according to Official Mexican Standard 062-ZOO-1999.

C. *Electrical activity*

Primary cell culture of dorsal root ganglion neurons obtained from postnatal days (7-10) Long-Evans rats (previously anesthetized with sevofluorane) were used. The rats were provided by the animal house of the Benemérita Universidad Autónoma de Puebla. The dorsal root ganglia were dissected and incubated (30 min at 37°C) in Leibovitz L15 medium (L15: (Invitrogen, Carlsbad, CA) containing 1.25 mg/ml trypsin and 1.25 mg/ml collagenase (both from Sigma-Aldrich, St. Louis, MO).

After the enzyme treatment, ganglia were washed three times with L15 medium, and mechanically dissociated. The cells were seeded on the multielectrode matrix and kept in culture for 24 h h at 37 °C in an atmosphere of 5% CO_2 and 95% air in L-15 medium supplemented with 15.7 mM NaHCO3 (Merck, Naucalpan, Mexico), 10% fetal bovine serum, 2.5 µg/ml fungizone (both from Invitrogen), 100 U/ml penicillin (Lakeside, Toluca, Mexico), and 15.8 mM HEPES (Sigma-Aldrich).. For the electrical recording the medium was replaced by physiological solution (in mM: NaCl 145, KCl 2.5, HEPES 10, glucose 10, $CaCl_2$ 2, and $MgCl_2$ 1, pH 7.4). For stimulation 20 mM KCl was added to the solution (to maintain the osmolarity an equimolar quantity of NaCl was eliminated). The experiments were performed at 22-25 °C.

III. Results and Discussion

A. *Resulting structures with the proposed fabrication method*

A cross-sectional SEM micrograph of the multi-electrode matrix can be seen in Fig. 2a. The structure consists of wires of approximately 3.55 µm height and approximately 100 nm in diameter. Fig. 2b shows a top view of the multi-electrode matrix, in which the roughness of the surface due to the random arrangement of the Si microwires is clearly observed. In the brain of higher vertebrates neuronal cells

are in close relationship with neuroglia [11]. Neurons and neuroglia attach themselves to maintain their mechanical stability [12-13]. Si nanowires, due to their high roughness, can serve as an anchoring site for neurons, favoring their survival.

B. Biocompatibility properties

Studies of biocompatibility of CHO cells in the Si nanowires (n = 5) shown that CHOK-1 cells have a similar survival rate than on common glass with a coating of Poly-D-lysine, which is a material commonly used for cell culture. Taking the survival percent on this glass as a reference (100%), the survival percentage on a non-coated multi-electrode matrix of Si nanowires, and on the multi-electrode matrix coated with Ag nanoparticles was calculated. The statistics are plotted in Fig. 3. The survival percentage in the three conditions was statistically similar ($P > 0.05$) indicating that Si nanowires biocompatibility is similar to that of standard culture.

The analysis of cell survival on Si nanowires coated with Ag at longer times (48 and 72 h) will be performed in the near future planning some applications. These materials could be used as implants preventing the growth of bacteria without interrupting the development and reproduction of vertebrate cells.

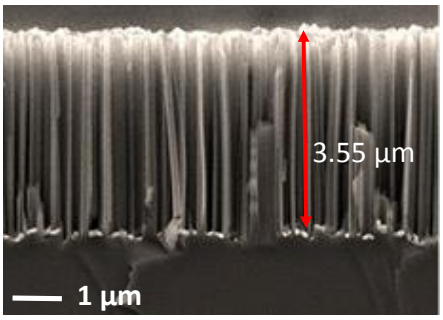
C. Electrical activity of neurons

Once the tissue was cultured on the matrix, the electrical activity of the neurons was recorded. For this purpose, a solution of KCl at a concentration of 20 mM, was used to excite the neurons grown on the multielectrode matrix. The registration protocol was as follows:

1) Wash of the recording chamber with physiological fluid for 5 min. This allowed to assure that the neuronal tissue is in a basal state, that is, without any previous excitation. 2) Stimulation of the primary neuronal tissue with 20 µL of 20 mM KCl solution. The stimulus with KCl allowed recording the electrical response of the neurons. 3) After the stimulation, the recording chamber was washed with physiological solution for 5 min.

During the stimulation, the electrical activity was recorded at a sampling frequency of 1 kHz. The recording of the electrical activity was obtained from the channel marked with dashed line and the stimulus with the KCl solution was made approximately at the point marked with a star (see the insets of Fig. 4). Fig. 4a presents a recording corresponding to the case when the excitation is done at the same point of the measurement. In this case, depolarization is clearly seen; that is due to the flow of K^+ ions into the neurons, producing an increase of the positive charges inside the neurons. Subsequently, a repolarization is observed, since the neurons restore their resting membrane potential. Fig. 4b shows the recording obtained from a channel close to stimulating region (marked with dashed line), fixing the position of the excitation (marked with a star). In this case the depolarization and repolarization are also distinguished.

a)



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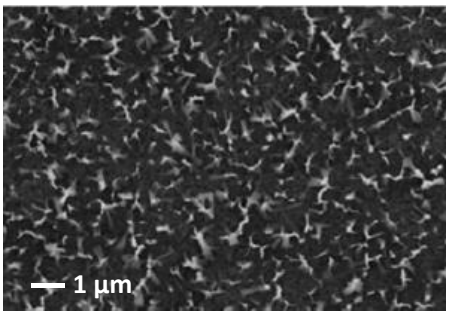


Figure 2. a) Cross-sectional SEM micrograph of the matrix of Si nanowires. The wires have an average height of 3.55 µm and a diameter of about 100 nm. b) Top view of the matrix, exhibiting the random distribution of the nanowires.

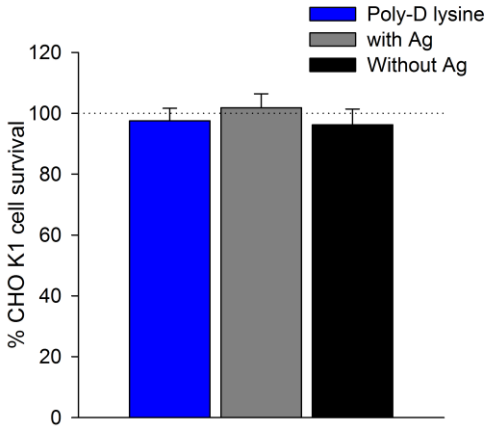
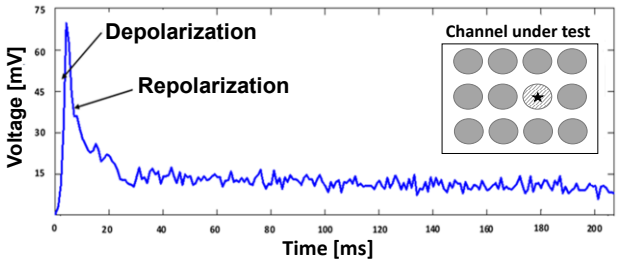


Figure 3. Percentage of survival of the CHO-K1 cell line. The highest percentage of survival was obtained with Si nanowires with Ag particles. Although differences between the three conditions and the control are not statistically significant $P < 0.5$ (n = 5 experiments for each condition).

The reduction of voltage from the first to the second recording may be due to the distance between the recording channel and the stimulation region. However, it is also possible that the response to stimulation is lower when performing the second recording. The registered potentials are field potentials, that is, potentials that emerge due to the collective activity of the neurons that are contacting the matrix of Si nanowires.

a)



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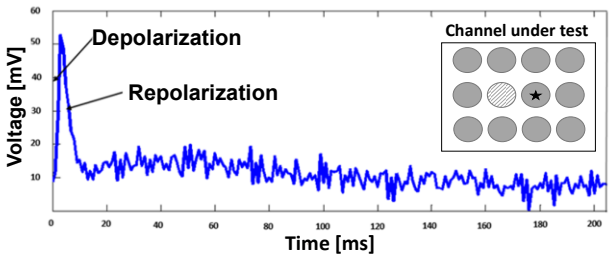

Figure 4. Recordings after excitation of neurons with a 20 µM KCl solution. a) Excitation was performed at about the point marked with a star, and measuring at the dashed spot. b) Changing the electrode position for measuring.

IV Conclusion

The matrix of randomly distributed Si nanowires shows a good biocompatibility with different types of cell strains, such as the cell line CHO-K1, and the neuronal cells of the dorsal root ganglia. The high level of biocompatibility could give a guide in future work to create implantable multielectrode devices, which could allow the recording of the electrical activity of an in-vivo neural network. The results obtained from the records of the electrical activity of the neurons grown on the multielectrode matrix, show that it is possible to obtain results similar to those obtained with devices manufactured with more expensive micromachining techniques. The development of the multielectrode matrix could allow the study of networks of different types of excitable cells.

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