# Localized temperature control in silicon microstructures for neural culture

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## Abstract

*This paper reports results on the fabrication and electrical characterization of silicon micro-structures for use in biological neuronal culturing studies. Structures are cavities for neuron allocation and contain gold microelectrodes, as well as resistors for local heating and diodes for temperature monitoring. In this way, one can control culture temperature and at the same time access the culture electrically. Cavities were obtained by KOH etching with depths ranging from 13 to 39 μm, allowing the fabrication of devices and microelectrodes by conventional microelectronic processes. Satisfactory morphology of structures was verified, as well as good adherence of poly-L-lysine (necessary to promote cell adherence). Electrical characterization was made from 25 to 55°C. Diodes show a sensibility of 2.63 mV/°C and resistors operating up to 200mW were proven adequate for local heating up to 55°C.*

## 1. Introduction

Silicon technology has shown its big potential for other applications than electronics [1]. In cellular biology, for example, there is a growing interaction with microelectronics materials and techniques in order to obtain biochemical sensors, devices and micro-electro-mechanical systems (MEMS) to monitor or to act on biological systems. In the neurobiology field, there is big interest on neuronal development in culture in order to understand growth mechanisms of axons, synapses formation and signal transmission.

The proposal of this work is to obtain silicon tridimensional microstructures for neural development investigation. Structures are provided with micro-electrodes for electrical access to neurons and devices to allow control of culture temperature. Moreover, we present preliminary results applying lysine treatment on the structures.

## 2. Design and Fabrication

The structures designed are based on a previous work [2] that utilizes a simple array of four cavities for neuron allocation. Cavities are interlinked by channels with the aim of orientating axonal growth.

As shown in **Figure 1**, there are independent microelectrodes for each cavity. For local heating and temperature measuring we designed an arrangement of two heating resistors on each side of the microstructures and a diode in the center region, as proposed by Baratto [3].

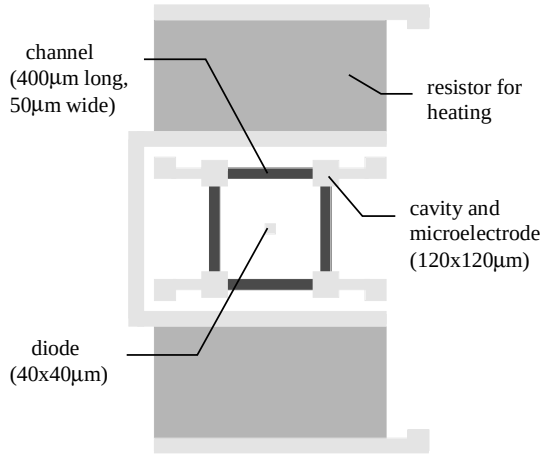


**Fig. 1: Microstructure with resistors and diode for temperature control.**

Starting material was P type silicon, <100> and resistivity 14 Ωcm. Cavities and channels were obtained by anisotropic silicon etch using KOH solution (KOH + isopropyl alcohol + water at 75°C) for different time periods, so we obtained depths ranging from 13 to 39μm. After that, isotropic etch (nitric + acetic + fluoridric acids) was made for 1 minute with the aim of smoothing superior borders of the cavities. This is desirable to avoid the rupture of metal tracks that access microelectrodes into the cavities.

Resistors and diode were obtained by

phosphorus ion implantation (energy = 50 KeV, dose = 5E15 $cm^{-2}$) and subsequent thermal annealing with thermal oxide growth. Finally, Ni-Cr alloy and gold were deposited by "sputtering" and metal tracks were defined using the "lift-off" technique.

## 3. Morphological aspects

As it can be seen in **Figure 2**, satisfactory definition of microstructures was obtained, despite of the loss of the square pattern of cavities due to the "convex corner effect" from isotropic etch, what doesn't represent a problem for the desired application. Furthermore, for 36μm depth, we obtained the "V" profile for channels.

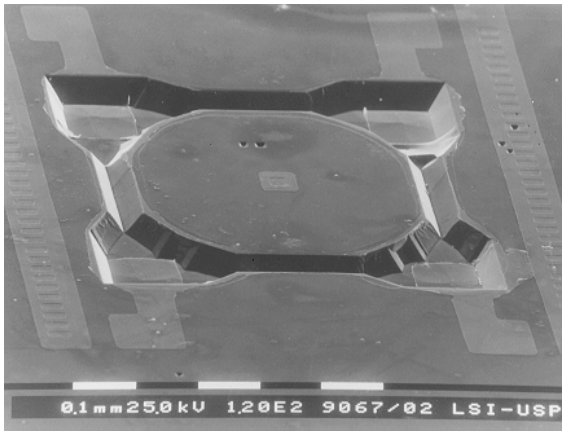


**Fig. 2: SEM picture of the 36μm depth structure**

Due to inhomogeneities of photoresist distribution when applied over non planar structures, there are some irregularities on the metal tracks definition, mainly next to the borders.

## 4. Adherence promotion

As for our application, namely, dissociated cells, it is mandatory to treat substrates with poly-lysine or laminin [4], and subsequently with growth factors (as for example bovine serum albumin or aplysia hemolymph).

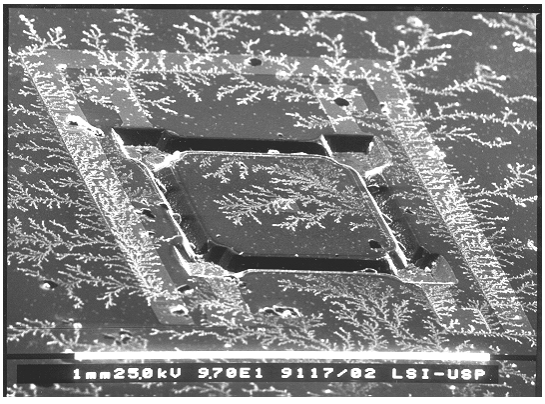


**Fig. 3: SEM picture of microstructure with poly-L-lysine.**

Because silicon covered by silicon oxide layers and gold is not a usual substrate in cell culture, it is necessary to adapt methods, as cell adhesion factors [5], for the structures fabricated. Preliminary results show good adherence of poly-lysine on silicon as well as on gold. An evidence of lysine adhesion is shown in **Figure 3**. It is a SEM photography of dry poly-L-lysine over the fabricated structure. We observe a tendency of spreading over gold tracks, although some material is also present on the cavities.

## 5. Electrical Characterization

In order to utilize a diode for temperature measuring, it's convenient to operate it under constant direct current and to relate the temperature with resulting diode voltage. By ploting the IxV curve for diode (**Figure 4**) an operation current of 7.5μA was chosen. Such current is adequately low to avoid considerable device self-heating (for a 40 x 40μm diode), although it is within the recombination current range for that diode.

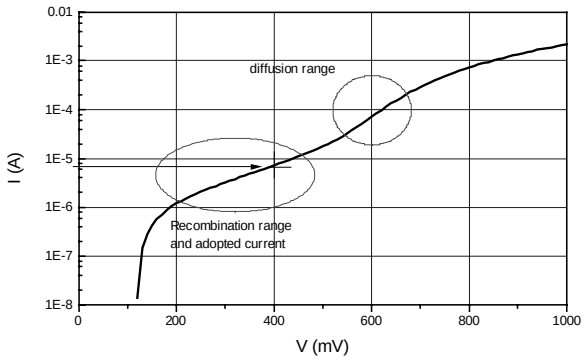


**Fig. 4: IxV curve for 40 x 40μm diode**

A functional diode characterization was made from 25 to 55ºC, that is the interesting range for any biological application. The overall structure was externally heated and temperature was measured by a calibrated termocouple, so we could plot the diode voltage vs. temperature, as shown in **Figure 5**.

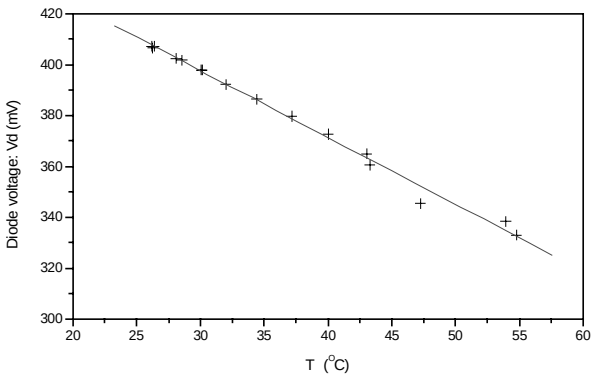

**Fig. 5: Obtained Diode Voltage (Vd) vs. Temperature and resulting linear fit.**

Linear fit over that data results on a sensibility of (2.63 ± 0.06) mV/°C and was recorded as the calibration of that temperature sensor. Moreover, the linear correlation coefficient was found to be more than 99%. These results reveal a good fit over the analyzed temperature range, what enable the diode for the desired application.

For local heating evaluation, resistors were polarized under different currents, at the same time diode measures temperature. So that, to obtain temperatures around 55°C near the structures, we need resistors power of 180 mW (40mA, 4.5 V) as it can be seen in **Figure 6,** that shows a plot of temperature (measured by diode) vs. resistors power. Furthermore, after each step increment on resistor current we observed fast temperature stabilization, within 30 seconds.

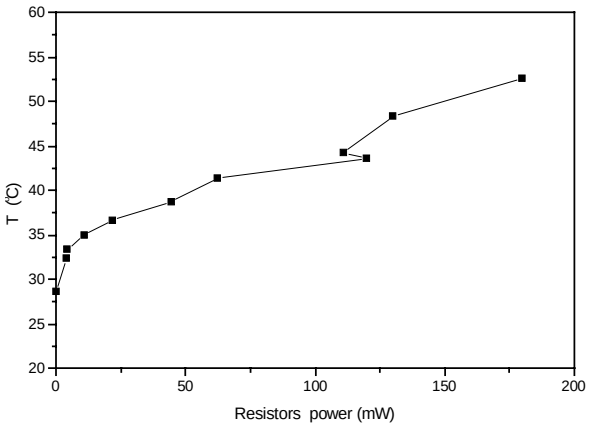


**Fig. 6: Temperature (measured by diode) vs. heating resistors power.**

In this way, from the point of view of functionality, the obtained temperature sensor and heating elements present good characteristics to work together for implementation of localized temperature control, that is: good sensibility, fast response time and low power consumption.

## 6. Conclusions

Satisfactory microstructures can be obtained for biological neuro-electronics applications using simple and conventional microelectronics techniques. In the presented structures, some parameters have been altered, maintaining the same basic geometry presented in [2], in order to investigate alternative process sequence and device integration.

With the aim of showing adequateness of microelectronics materials it is necessary to adapt cell culture procedures such as chemical treatment and sterilization methods. Here we have shown the adhesion of lysine to the structures, wich is a necessary step towards cell adhesion.

Electrical characterization of diode and resistors fabricated on non planar microstructures indicate the feasibility of integrated temperature monitoring and control with adequate sensibility, response time and power consumption. The possibility of device integration compatible with cell culture procedures opens a vast field of applications in biomedical field.

Future work will focus on microelectrode geometry, with the objective of reducing contact sizes and increasing number of contacts.

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