SILICON MICROSTRUCTURES FOR NEURO-ELECTRONICS APPLICATIONS

HENRIQUE E. M. PERES [(1)], NATHALIA L. V. PEIXOTO[(2)], FRANCISCO J. R. FERNANDEZ [(3)] and WAGNER W. LEITE

Grupo de Sensores Integráveis e Microestruturas - SIM
Laboratório de Microeletrônica - Escola Politécnica da USP
Caixa Postal: 61548 - 05424-970 - São Paulo - SP - Brazil
Phone: **(011) 818-5310** - FAX: **(011) 818-5585**
www.lme.usp.br/sim/
[1] **hperes@lme.usp.br** [2] **nathalia@nero.uni-bonn.de** [3] **jramirez@lme.usp.br**

ABSTRACT

This work presents some results obtained within the process of fabrication of silicon microstructures for neuro-electronics applications. The microstructures are basically arrays of cavities in which culture of neuronal cells can be carried out. The cavities are connected to each other by channels intended to guide axons growth. The system includes microelectrodes in the cavities to stimulate cells or to extract biopotentials. Structures are fabricated with depths between 11 and 48 μm using KOH etch and the simple process sequence adopted showed satisfactory microstructures for this first version, from the point of view of fabrication and application. Currently, an improved version is being manufactured, in which we include resistors and diodes for temperature monitoring and control.

1. INTRODUCTION

The past few years experienced a vertiginous development in bio-engineering, especially in the use of micro-electro-mechanism systems (MEMS) designed to retrieve information from biological systems and to control biological experiments by means of transduction of biophysical–chemical in electrical quantities and vice versa [1,2]. Besides that, several important questions in developmental neurobiology can be addressed through the study of cell culture on defined micromachined substrates.

Nowadays microelectronic materials and techniques offer appropriate tools to monitor and stimulate biological systems like cells or tissue in culture [3], and special attention has been given to the study of arrays of neurons in culture with the aims of understanding growth mechanisms of axons, synapses formation and information transmission in biological systems [4], thus improving our knowledge of cellular physiology, behavior of biological neural networks and their implications on rehabilitation engineering, for example [5,6].

The objective of the work reported here is to design and to build micrometric silicon structures in the form of arrays of cavities provided with electrical access to accommodate and study neuronal cell development. The cavities are connected to each other by means of channels in order to orientate axon growth. The work involves detailed analysis of adaptation problems for conventional fabrication processes in silicon technology which includes micro machining and materials biocompatibility [7].

2. EXPERIMENTAL PROCEDURE

The basic microstructure proposed consists of an array of four cavities, as can be seen in **Figure 1**. The main dimensions are indicated as cavity size (P), channel length (C) and channel width (L). Four options for P,

L and C were designed**,** named hereupon as "L1, L2, L3 and L4", as indicated in **Table 1.** These dimensions have been determined according to the cell culture type to which they are projected. **Figure 2** shows the chip layout adopted. In addition, each cavity has an electrode that can be externally accessed by its respective contact PAD.

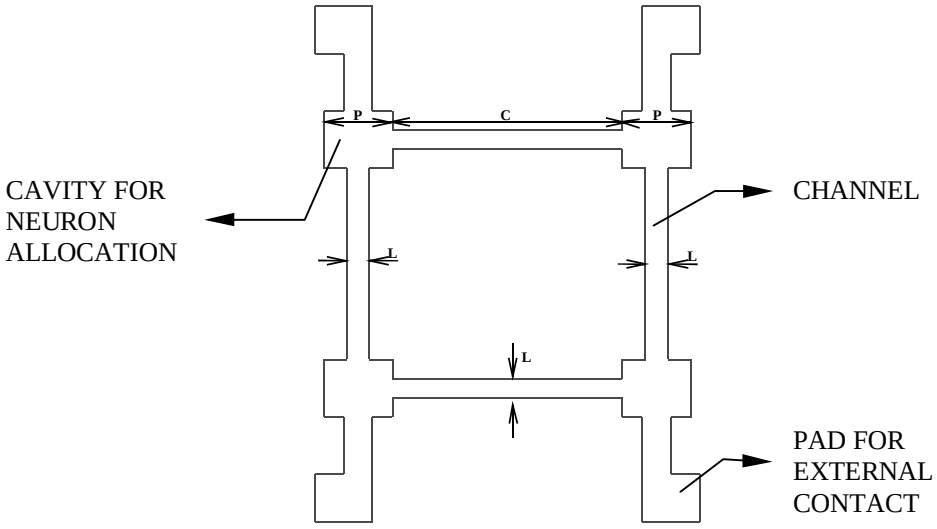


Fig. 1: Basic microstructure designed.

TABLE 1: P, L and C values (in microns) for 4 options of structures designed.

STRUCTURE →	L1	L2	L3	L4
P	50	85	120	120
L	20	30	40	50
C	50	100	400	400

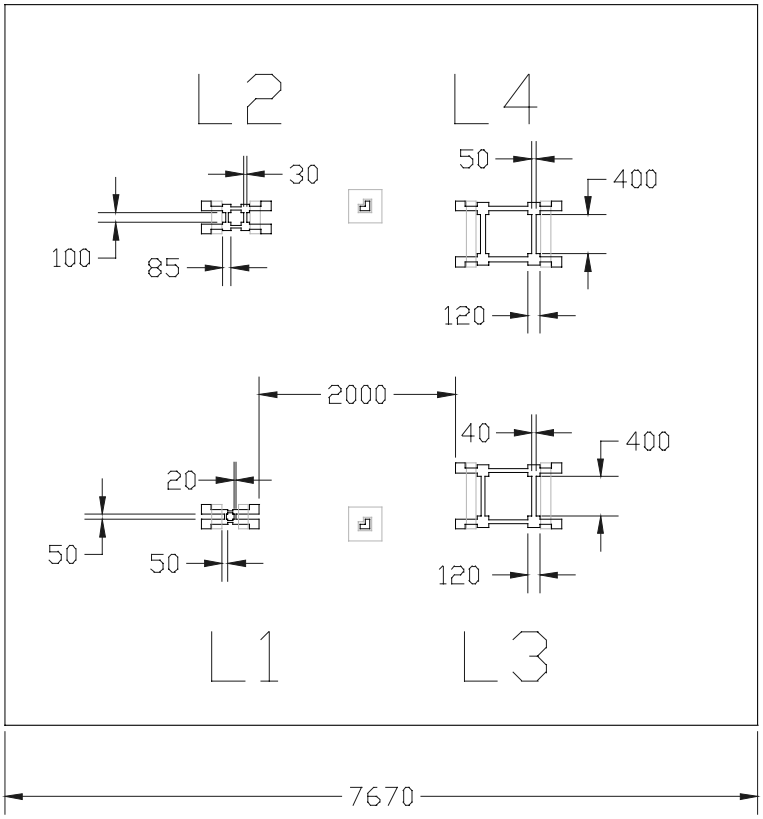


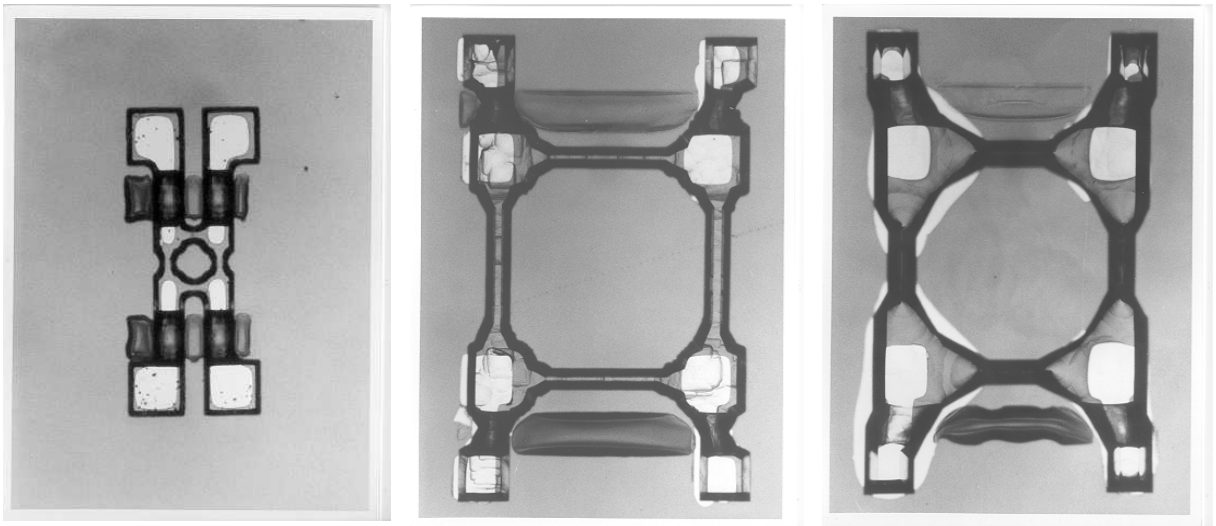
Fig. 3: Designed chip showing adopted layout and relevant dimensions (in microns)

The structures were fabricated over <100>, N type silicon wafers with resistivity around 14 Ωcm. The processing sequence was as follows:

1- Thermal silicon oxidation.
2- Application of first photo-mask: Definition of silicon areas for etching.
3- Anisotropic silicon etching using KOH solution (50g KOH + 108ml H_2O + 51ml isopropyl alcohol, T=75°C) for different times, such that depths between 11 and 48μm could be obtained.
4- Total oxide remotion.
5- Thermal silicon oxidation: electrical isolation between cavities and bulk silicon.
6- Application of second photo-mask to make use of the "lift-off" technique: definition of microelectrode areas.
7- Metal deposition: Ni-Cr alloy and gold. The Ni-Cr alloy improves adherence over silicon oxide.
8- "Lift-off": laying of metallic microelectrodes.
9- Application of third photo-mask: placement of a photoresist barrier between each cavity and its PAD for physical separation between the biological tissue and the external contact. After this, the wafers were warmed up to 100°C for 1 hour in order to "fix" the photoresist barriers.

3. RESULTS AND DISCUSSION

By controlling etching times, we obtained cavity depths of 11, 15, 19, 32 and 48 μm. **Figure 3** shows optical microscope photographs representatives for microstructures obtained with 15, 32 and 48 μm depths. The alteration of square pattern of cavities can be seen as they become deeper. This is caused by the well known "convex corner" effect for KOH etching.



3a: L1 structure, 15μm depth. *3b: L3 structure, 32μm depth* *3c: L4 structure, 48μm depth*

Fig. 3: Optical microscope photographs of L1, L3 and L4 structures with indicated depths.

Particularly for 48μm depth, one can observe that the cavity superior view looks like a triangle and that it is not possible to devise the channel depth, indicating that it reached the "V" transversal profile. The increasing on cavity area means that the embedded gold microelectrodes will touch only a fraction of the neuronal cell into that cavity. Furthermore, the distance between cavities becomes shorter. Therefore, the "P" effective dimension

increases and the “C” dimension decreases, what must be taken into account later, for the evaluation of the development of cell cultures. It is possible to minimize this effect by designing structures with compensatory geometries on convex corners.

The definition of metal tracks by means of the “lift-off” technique and the definition of photoresist barriers were satisfactory even for 48 μm depth. Subsequent electrical tests of metal tracks confirmed the continuity between cavity and its respective PAD and the open circuit between neighboring cavities.

Usually photoresist application over an irregular surface produces inhomogeneous photoresist thickness and impedes contact between photo-mask and deeper regions on structures, so difficulties could be expected in the photoresist light exposure step. In fact, the period of time needed for light exposure was longer than the usual duration, but good microelectrode definition was subsequently verified into cavities. Furthermore, for 48μm depth one can see the effect of photoresist “slip” from structure borders to cavities bottom that allows undesired metallization around structure but without affecting microelectrodes electrical functionality. Thus, simple and conventional photo-mask technique can be used to produce satisfactory microstructures.

The design of four options of structures in the chip constitutes an appropriate layout for biological application and can be easily improved by adding electronic devices. Preliminary biological tests were carried out utilizing neuronal cells extracted from the cerebral and buccal ganglia of the snail *Helix aspersa* [8]. Biocompatibility was observed between cells and the utilized materials (silicon oxide, gold and photoresist) and good growth of axons was verified on the substrate as a whole. It is still not known if the geometry proposed is adequate to the maintenance in culture and to the development of these neuronal cells.

4. CONCLUSIONS

The development of a first version of microstructures by using simple and conventional microelectronics techniques shows satisfactory results from the point of view of fabrication and application. This helps us to establish processing sequences and, thus, to obtain improved microstructures in a short time.

We would like to point out that the biocompatibility tests constitute an unavoidable step to the continuation of structure fabrication. Besides that, the size of each channel has to be determined as a function of the cell type used. In our case, depending on the age of the animal, neurons can present soma diameters from 50 to 200μm, and the axons can extend up to 500μm. This means that in order to promote synapse formation it may be necessary to enlarge the "C" dimension. We cannot yet state that the observed alteration on the square pattern for "P" impedes the development of the neurons. In order to analyse cell adhesion and culture behavior both (square and triangular) forms will be tested in culture.

Giving continuity to this work, a second version of structures is being fabricated in which there are resistors and diodes beside cavities to allow measuring and control over cell temperature. This is the first step towards a more sophisticated system to monitor cell metabolism and control its activity and development.

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