

## Microfabricated platforms for the study of neuronal and cellular networks

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**Abstract.** In this contribution we present the development of three microfabricated devices for the study of neuronal and cellular networks. Together, these devices form an attractive toolbox, which is useful to stimulate and record signals of both electrical and chemical nature. One approach consist of microelectrode arrays for the study of neuronal networks, and allow for the electrical stimulation of individual cells in the network, while the other electrodes of the array record the electrical activity of the remaining cells of the network. We also present the use of micropipettes that can measure the extra- and intracellular concentrations of ions in cells cultures. A third approach exploits the laminar flows in a microfluidic device, to deliver minute amounts of drug to some cells in a cellular network. These three illustrations show that microfabricated platforms are appealing analytical tools in the context of cell biology.

### 1. Introduction

Microfabricated devices have the potential to solve important problems related to the study of individual cells in a cell cultures. By definition, microfabricated sensors and microsystems have active components of the sizes ranging from sub-micrometer to hundreds of micrometers –a range of sizes that overlap with the dimensions of individual cells or sub-cellular components. As a result, microfabricated sensors and microsystems represent well-suited tools for the elucidation of fundamental questions in cell biology. In this contribution, we focus on recent developments in the fields of microelectrode arrays (MEA), ion-selective electrodes (ISE) and microfluidics. Together, these techniques have the capability to perform a wide range of experiments with cellular and neuronal cultures: they are useful to collect electrical and chemical information from the cells, and to stimulate cells with electrical and chemical signal.

1.1 *Microelectrode arrays.* Among different techniques for cell biological research, extracellular potential recording of excitable cell cultures using MEA is now a well accepted technique in both fundamental neuroscience and applied electrophysiology. The functional characteristics of the current MEAs allow mid- to long-term recordings of both spontaneous and evoked neuronal activity patterns and of their spatio-temporal evolution. The mapping of the spatio-temporal evolution of the network activity i.e. of the network dynamics, offers a highly effective approach for investigating the information processing in neuronal networks and hence of the learning and memory processes. However, due to the network complexity and to the fact that both spontaneous and evoked activities tend to exhibit complex patterns synchronized over the whole network, the detailed investigation of spatio-temporal changes remains rather difficult. One of the possible approaches to alleviate this complexity is based on network patterning using adhesion promoters/inhibitors, arrays of neurocages or microchambers. In this case, however, the random nature of the network becomes relatively limited.

Our approach is based on integrating the MEAs with physical barriers for clustering the neuronal network into a number of interconnected, random sub-networks. To this end, we have realized, on a Pyrex substrate, a thin-film Pt microelectrode array consisting of 60 microelectrodes of 30  $\mu\text{m}$  diameter with integrated SU-8 clustering structures [1]. The five clustering chambers of 3 mm

in diameter are interconnected by channels of 500  $\mu\text{m}$  or 800  $\mu\text{m}$  in length and 300  $\mu\text{m}$  in width. Additionally, for a future integration within a micro-incubation chamber, two Pt resistive temperature sensors (Pt-RTD) are integrated on-chip outside the clustering structure. The biological functional evaluation was performed with cultures of rat's embryo cortical neurons in order to ascertain the device biocompatibility, the stimulation/recording capabilities and the functionality of the clusters.

1.2 *Ion-selective electrodes.* To further characterize the cell biology, we are developing micropipette-based arrays of ion-selective electrodes (ISEs) for intra- and extra-cellular measurement of  $\text{Ca}^{2+}$  and  $\text{K}^{+}$  activities in cell cultures. Ionic species play an important role in intra- and extra-cellular signaling and are known to regulate many different cellular processes. Currently, next to fluorescence measurements, glass capillary ISEs are used for ion activity measurements in cell cultures. Although these electrodes can be made very small, this technology is not adapted for parallel measurements of a number of cells in the culture. A generic technology platform for the microfabrication of silicon nitride micropipettes with diameters ranging from 6  $\mu\text{m}$  down to 250 nm has been developed. The micropipettes are realized by a combination of KOH and DRIE etching as well as a directional  $\text{SF}_6$  reactive ion etching. It allows to modulate the micropipette diameter by choosing the thickness of the oxide sacrificial layer and that of the silicon nitride walls. The silicon part in which the micropipettes are structured is completed with a Pyrex part that contains an array of microchannels with Pt microelectrodes [2,3]. Membrane cocktails based on respectively the ionophores ETH 129 ionophore for calcium and valinomycin for potassium were used for evaluating the functional characteristics of the 1.5  $\mu\text{m}$  in diameter micropipettes.

1.3 *Microfluidics.* Most of the devices for cell-based experiments require some level of control on the fluid contacting the cells. In that regard, microfluidics offers a number of possibilities with various levels of complexities. In two of the approaches presented in this contribution, we exploited features, which are unique to microfluidics. The MEA chip took advantage of microfluidics, by fabricating a network of chambers and connecting channels to define the size, the shape and the level of connectivity of the neuronal culture. The high level of freedom in design allows the users to select arbitrary the layout of the culture (such as interconnected chambers/channels in our experiments).

In a higher level of complexity, we used a three-dimensional microfluidic system to deliver drugs to cardiac myocytes immobilized on a sensor [4]. In this approach, the mammalian cells were cultivated onto an 8 x 8  $\text{mm}^2$  chip fabricated with CMOS technology (complementary metal oxide semiconductor). The CMOS chip combined a high density array of microelectrode (very much like a MEA) with on-chip data treatment to enhance data acquisition rate and reduce noise during signal recording. We designed the microfluidic system to fulfill two main tasks in the experiments: (i) the delivery of nutrient to the cell culture over extended periods of time, and (ii) the delivery minute quantities of pharmacology-relevant drug to a sub-set of cells in the culture, to monitor the effect of the drug on the properties of the cellular network. These two tasks took full advantage of inherent capabilities of microfluidics to automate fluid delivery, and accurately dispense small quantities of fluid into microsystems.

## 2. Results and discussion

2.1 *Microelectrode arrays.* A photograph of the clustered MEA is shown in Figure 1. The biological tests were performed at two project partners' institutes - INSERM E358 (Bordeaux) and DIBE, University of Genova. The effect of the clustering structure on the spontaneous and evoked network activities were evaluated by comparing the recordings on clustered and conventional MEAs. The results clearly demonstrated the cluster functionality for both spontaneous and evoked network activities and confirmed thus the network organization in interconnected sub-populations [1]. This proof of concept of the clustering structure functionality opens up new prospects for investigating in-

vitro interactions among large assemblies of neurons or co-cultured populations as well as the basic learning mechanisms and network plasticity.

2.2 *Ion-selective electrodes.* The array of ion-selective electrodes consists of 24 micropipettes arranged in a 2 x 8 array with 150  $\mu\text{m}$  spacing and four electrodes located on both sides of the array at 300  $\mu\text{m}$ . A photograph of part of the array is shown in Figure 2. The micropipettes have a total length of 50  $\mu\text{m}$  of which 5  $\mu\text{m}$  protrude from the silicon surface. Before filling the micropipettes with the membrane, the device is dehydrated and silanized to improve the adhesion of the membrane to the silicon nitride inner walls. The membrane cocktails of different compositions were studied with respect to the micropipette' filling by capillary forces and ion-selective electrode functional characteristics i.e. sensitivity, selectivity and detection limit. Measurements performed in buffered solutions have shown a good sensitivity and detection limits of respectively  $10^{-7}$  M  $\text{Ca}^{2+}$  and  $10^{-5}$  M  $\text{K}^{+}$ .

2.3 *Microfluidics.* The main difficulty in coupling a microfluidic flow cell to a CMOS chip is the size mismatch between the two components: the CMOS chip has an area of 64  $\text{mm}^2$  (the cost efficiency of CMOS chips is heavily dependent on the ability to reduce the size of the chips), whereas the microfluidic flow cells typically cover areas of several square centimeters (mostly because of bulky fluidic connection to the outside world). To overcome this difficulty, we proposed a three-layer sandwich (see Figure 3, left) composed of a central silicon chip with a CMOS chip bonded on its bottom surface, and a microfluidic Pyrex chip bonded on its top surface. In this arrangement, the size of the microfluidic flow cell had no impact on the size of the CMOS chips, maximizing the attractive attributes of the CMOS technology.

In the central silicon layer, we microfabricated square-shaped holes (6 x 6  $\text{mm}^2$ ) with a KOH etch. For the characterization of the flow properties of the flow cell, we mimicked the presence of a CMOS chip by bonding a Pyrex wafer on the bottom surface of the silicon wafer, and obtain square cavities. The upper part of the system was composed of a Pyrex wafer with laser-drilled holes (diameter 40-65  $\mu\text{m}$ ): 2 holes for nutrient inlet and outlet, and 6-8 holes for drug delivery purposes. The nutrient inlet was connected to a syringe pump with a supply of fresh nutrient for the cells, and the inlets for drug delivery were connected to a syringe pump with a supply of drug, through a 6-way or 8-way valve. In this arrangement, we controlled the flow of nutrient through the flow cell (between 20 and 100  $\mu\text{L}\cdot\text{min}^{-1}$ ), and injected the drug through one of the drug inlet holes. Because all fluids in the flow cell are in a laminar flow regime, the drug injected followed a streak having a width defined by diffusion only. We could adjust the width of the streak of drug by varying the flow of nutrient (see Figure 3, right): at slow volumetric rates, the residence time in the flow cell is longer, and the effects of diffusion will be more pronounced. The biological tests were performed in our partners' institute (ETHZ, Switzerland), and showed that cardiac myocytes grown on the bottom of the cavity could be treated with a biochemical reagent using the drug delivery system.

### 3. Conclusion

The various Microtechnology approaches investigated in this contribution provide essential information for studying the behavior of cells in a culture. This includes the ability to (i) generate a wide range of stimuli to cells by electric means, or with drug delivery, and (ii) record electric signals and variation in extra- and intracellular ionic concentration. These tools are generally useful for cell biologists, and especially interesting for researchers in fundamental neuroscience and applied electrophysiology. The ability to monitor cellular activity during stimulation with a drug (or a drug candidate) also has a wide range of applications in pharmacology. Because these techniques have the potential to be combined (to some extent) into a single device, the functionality of integrated system can be extended to match the requirements of many cell biology experiments.

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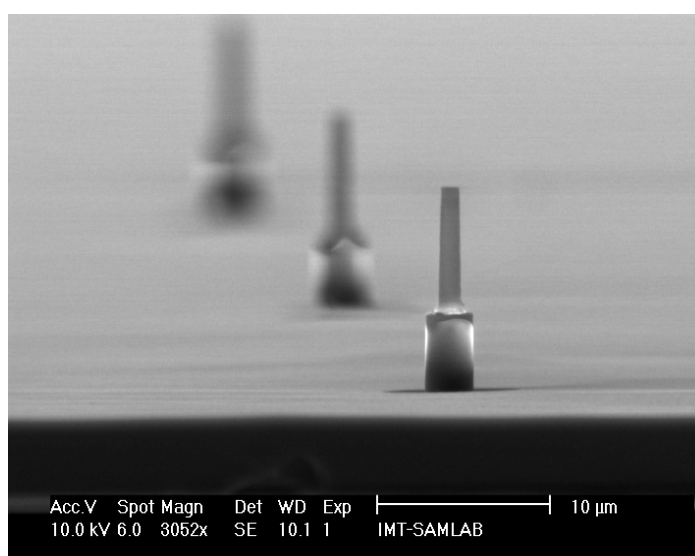


Figure 1: SEM photograph of a part of micropipette-based ISE array.

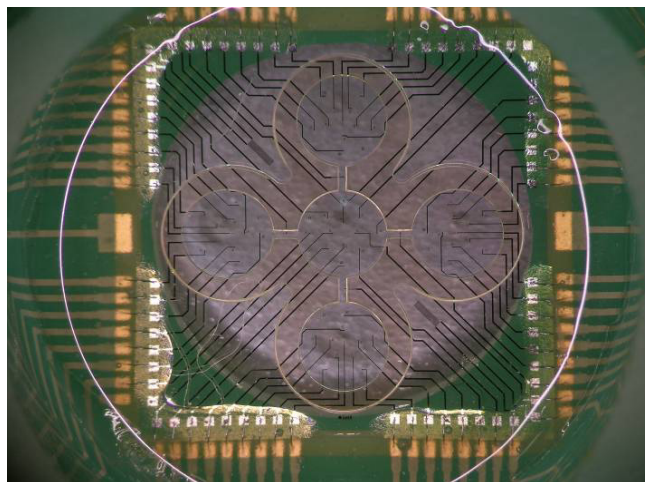


Figure 2: Photograph of a Pt MEA with five interconnected clustering chambers

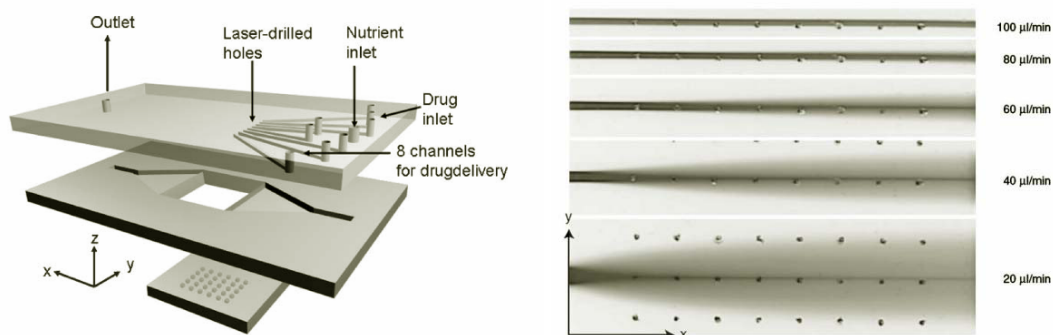


Figure 3: Microfluidic chip for drug delivery experiments. (Left) overall design of the chip: a silicon chip with a square-shaped through-hole formed the heart of the device, with a CMOS chip bound underneath and a Pyrex cover plate above, forming altogether a cavity. Fluidic connections were established from top to perfuse the system with nutrient (from right to left), and to deliver drug from the array of holes above the entrance of the cavity. (Right) the width of the drug streak in the cavity can be varied by adjusting the volumetric flow rate of nutrient (here, nutrient flows from left to right).