# PHYSICAL IMMOBILIZATION AND POLYMERIC MICROCHANNEL NETWORKS TO ACHIEVE DEFINED NEURONAL NETWORK STRUCTURES

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**Abstract**
A CMOS-compatible chip for physical cell immobilization on an array of 16 electrodes is presented. Neurons are pulled onto the electrodes by negative pressure applied from the backside of the chip through small holes in each electrode. Neurite outgrowth is guided through SU-8 channels to ensure predictable neuronal interconnects. The chip serves as a platform to systematically grow neuronal networks with defined node positions and synaptic connections.
The fabrication of such devices, the electrical characterization of the electrodes, and the growth of neuronal networks on the chips are described. Particle immobilization was studied using fluorescent beads in a first approach.

**Keywords:**
Cell immobilization, neuronal networks, CMOS

## 1. Introduction

Measuring electrical activities of neuronal networks with extracellular micro-electrode arrays poses a challenge, as the signals through the cell membranes are minute. Owing to the low signal levels, neurons should be placed precisely on top of the metal electrodes to ensure good electrical coupling between cell and electrode. The fact that neurons tend to move on the surface during growth and may leave the active electrode area renders the signal recording task even more difficult [1].
Here, we present a physical approach to cell immobilization on a micro-chip as an alternative to patterned protein layers [2]. Negative pressure is applied from the backside to an array of 16 electrodes, each of which is perforated with a small hole, to pull the neurons onto the electrodes and make them stay on the electrode by suction. This approach is similar to micro-chip-based patch-clamp techniques [3].
The new aspect of the chip presented here is the possibility of achieving predictable, directed growth of neuronal networks with defined node positions and synaptic connections. The features leading to such controlled network architectures include (a) placing a single neuron on each electrode, (b) forcing the neuron to remain there during the measurements, and (c) guiding the neurite growth through channels to the neighboring cells.

7th International Conference on Miniaturized Chemical and Biochemical Analysis Systems
October 5–9, 2003, Squaw Valley, California USA

The chip combines silicon and SU-8 microfluidic structures with a microelectrode array while retaining full CMOS compatibility.

## 2. Device Fabrication

The 6 × 6 $mm^2$ silicon chip with an active area of 320 × 320 $\mu m^2$ is fabricated in a 5-mask process (Fig.1). A silicon membrane is formed by back-side anisotropic wet etching in KOH using 1000 nm $Si_3N_4$ as etch mask. 5-µm-diameter orifices are then etched through the membrane by deep reactive-ion etching. The array of 16 platinum electrodes (200 nm) is fabricated in a lift-off process with titanium/tungsten (50 nm) as adhesion layer. After deposition of 1000 nm $Si_3N_4$ for insulation, the layer is opened at the electrodes and at the bondpads by reactive-ion etching. The last step is the patterning of a 50-µm-thick SU-8 resist layer to form the micro wells and the channel system that connects the electrodes.

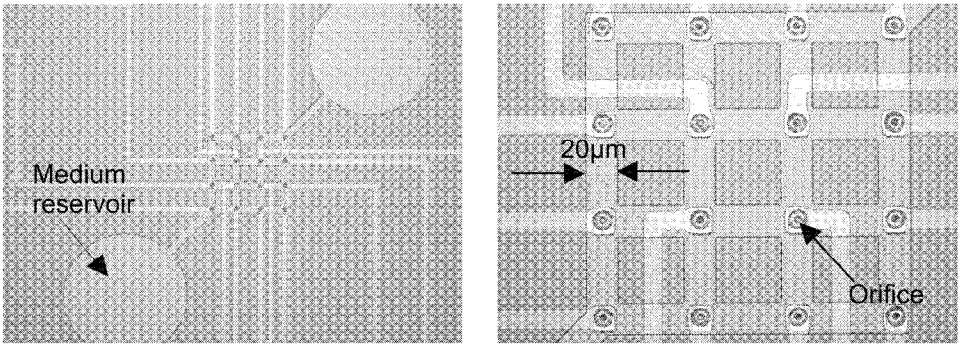


**Figure 1**: Left: micrograph of the chip with SU-8 channels and medium reservoirs; right: close-up of the SU-8 channels for guided neurite growth.

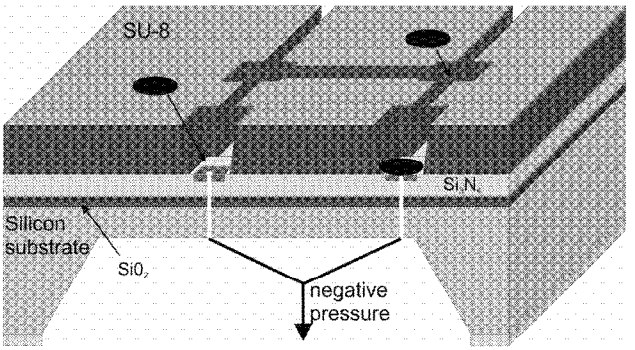


**Figure 2**: Cross-sectional view of the chip: Negative pressure is applied from the backside of the membrane, silicon dioxide and nitride are deposited for insulation of the bulk, and SU-8 micro wells are formed around the platinum electrodes. Neurons are sucked onto the electrodes and the neurites can grow along the SU-8 channels.

### 3. Cell Growth on silicon chips

Cell growth on silicon requires thorough cleaning of the surface and the deposition of an adhesion layer. The chips are pre-treated with 30% $H_2O_2$ for 30 min. followed by drying under UV-light for 45 min. The chips are then coated with laminin (20 µg/ml) and stored approx. 2 h in an incubator at 37 °C, 5% $CO_2$. Before the cells are placed on the chips, the surface has to be cleaned 3 times with TBS buffer of pH 7.4.

The neuronal cells are harvested from 10-day-old chicken embryos. Dorsal root ganglias (DRGs) from the peripheral nervous system are dissected under a microscope and put into medium (MEM). After centrifugation at 800 rpm for 5 min., the pellet is dissolved in dissociation medium (0.03% collgenase, 0.25% trypsin in MEM) and stored at 37°C for 30 min. After another centrifugation step, the pellet is re-suspended in 1 ml medium and the DRGs are dissociated with a 200-µl pipette. The cells are placed on the chip in 0.5 ml culture medium (10% FCS, 5% chicken embryo extract, 1% pen/strep, 0.1% glutamine in MEM) with 1 µl/ml nerve growth factor (NGF) and stored in an incubator. To avoid the division and reproduction of glia cells, 4 µl Ara C (1:10 in TBS) can be added after the second day of culturing. The medium has to be replaced every 2-3 days.

After 2 days of culturing, neurite growth can be seen already. Figure 3 shows a micrograph of a neuronal network on a silicon chip, which does not exhibit holes for physical cell immobilization. It is clearly visible that most electrodes are not occupied by cells and, therefore, are not available for measurements.

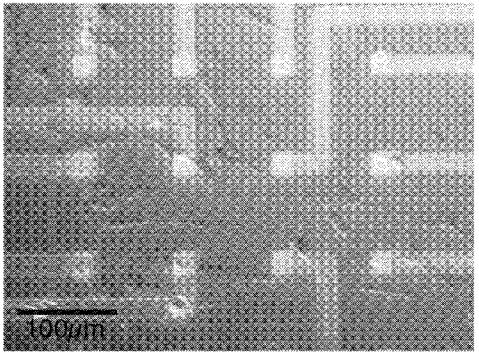


**Figure 3**: Neuronal network without immobilization. Most neurons reside outside the active electrode areas and cannot be probed.

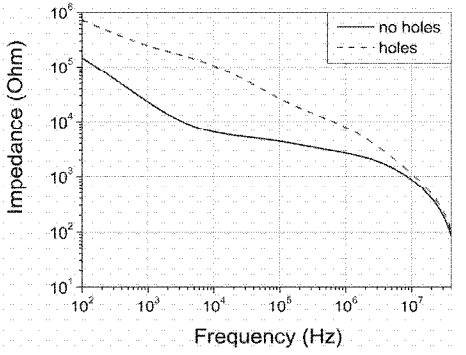


**Figure 4**: Impedance measurements with a gain-phase analyzer in physiological saline solution (frequency sweep from 100 Hz to 40 MHz, 0.1 $V_{p-p}$).

### 4. Physical Characterization

Impedance measurements were carried out to study the impact of the holes in 20 × 20 $\mu m^2$ electrodes (Fig. 4). A gain-phase analyzer (HP 4194A) has been used to carry out

measurements in physiological saline solution. As expected, the impedance of the perforated electrode is considerably higher (e.g. factor of 10 at 1 kHz) than that of a normal electrode as a consequence of the reduced surface area.
To prove that it is possible to attract particles or cells from a solution onto the holes, a negative pressure (200-400 mbar) has been applied to the membrane. First studies have been carried out using fluorescent microbeads (∅ 20 µm, polystyrene, Polyscience Inc.) (Fig. 5) and living cells. Capturing of suspended beads works very well, but if they reach the device surface before capturing, the pressure-induced flow through the orifice is not sufficient to move them onto the hole. Preliminary work indicates that a laminar flow in parallel to the chip surface will solve this problem.
Fibroblasts and PC12 cells could also be placed on the electrodes, but with less reliability since some orifices were larger than designed due to fabrication issues. Fabrication process optimization will lead to smaller holes and, therefore, to more reliable cell placement and better electrical coupling.

## 5. Conclusion

The results show that a physical approach for placing cells on electrodes is a good alternative to methods such as protein patterning.
Growth and neurite expression behavior of chicken neurons under applied pressure is currently under investigation with the aim to arrive at long-term activity measurements on the chips. The next generation of chips will additionally exhibit integrated microelectronic circuitry for on-chip recording of the neuronal electrical signals.

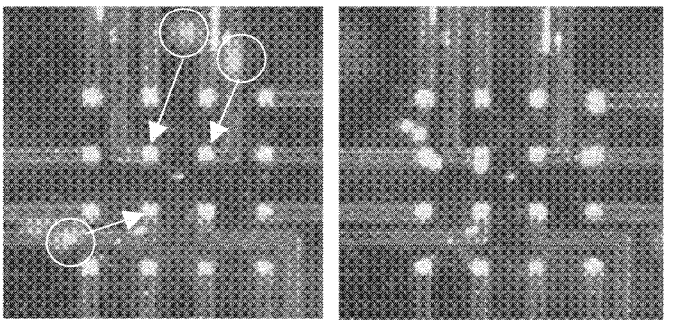

**Figure 5**: Micrograph sequence showing microbeads attracted to open holes (left). On the right side most electrode sites are occupied by beads.

## References

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