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Micro-sized syringes for single-cell fluidic access integrated on a micro-electrode array CMOS chip

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***Abstract*— Very-large scale integration and micro-machining have enabled the development of novel platforms for advanced and automated examination of cells and tissues *in vitro*. In this paper, we present a CMOS chip designed in a commercial 0.18 μm technology with integrated micro-syringes combined with micro-nail shaped electrodes and readout electronics. The micro-syringes could be individually addressed by a through-wafer micro-fluidic channel with an inner diameter of 1 μm. We demonstrated the functionality of the micro-fluidic access by diffusion of fluorescent species through the channels. Further, hippocampal neurons were cultured on top of an array of micro-syringes, and focused ion beam-scanning electron microscopy cross-sections revealed protrusion of the cells inside the channels, creating a strong interface between the membrane and the chip surface. This principle demonstrates a first step towards a novel type of automated *in vitro* platforms, allowing local delivery of substances to cells or advanced planar patch clamping.**

I. INTRODUCTION

For the study of electrophysiological properties of cells, the patch-clamp method [1] is today still a preferred method, since it allows recording of single ion channel currents and membrane potentials accurately.

The technique consists of bringing a glass micropipette into contact with the cell membrane; this results in excellent signal-to-noise ratio obtained by the high seal between the membrane and the glass. It is interesting for cell physiological and pharmaceutical research to investigate effects of chemical compounds released near single cells [2], [3]. For example, the effects on outgrowth, migration, adhesion and metabolism of cells can be studied by applying small amounts of active stimulating or inhibiting molecules. Local chemical delivery can be done with a micropipette, however, the disadvantage remains that the method is work-intensive and requires a skilled electrophysiologist to perform one patch-clamp recording at the time. Novel *automated patch clamp* systems have emerged the last decade, allowing a higher throughput [4], [5], [6]. Also, planar patch-clamp systems were developed [7], [8], [9], [10] with systems comprising up to 14 parallel channels have been reported [11]. Similar work towards local delivery of chemicals has been reported, involving miniaturization of micro-fluidics [12], [13], and even for chemical stimulation of the retina [14]. However, the integration of microfluidic structures with active integrated circuits is very challenging due to the very different nature of the fabrication.

Previously, we demonstrated [15] our novel CMOS-chip implemented in 0.18 μm TSMC (*Taiwan Semiconductor Manufacturing Company*) mixed-signal technology, suitable for electrophysiologcal recordings *in vitro*. On this CMOS-design, we fabricated micro-nail shaped electrodes by a custom process-flow as described in [16]. These micro-nails were developed from the hypothesis that cells have the ability to tightly engulf such structures, as demonstrated by [17], [18], [19], [20]. To further explore this effect, we developed a process flow in order to integrate *micro-syringes* with dimensions comparable to a patch-clamp pipette opening. A top-view SEM photograph of the result is illustrated in Figure 1.

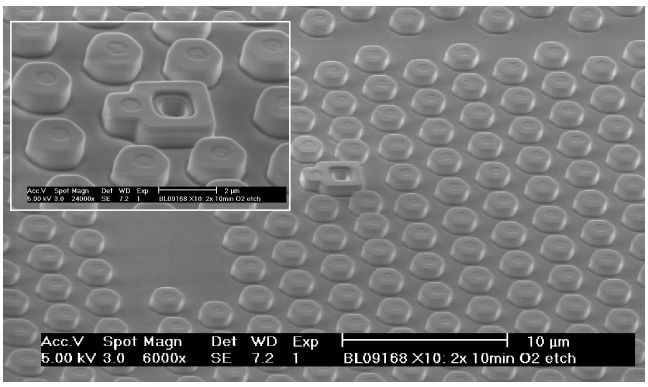

Fig. 1. SEM top-view of the micro-nail array and integrated micro-syringe on the CMOS wafer.

With this result, we demonstrate the principle of a first step towards a novel type of advanced, automated *in vitro* platforms.

II. PROCESSING

The process flow as in [16] was adapted to be compatible with a commercial Aluminum CMOS-platform. We started from full 8" wafers in a 4-metal 0.18 μm technology, processed at TSMC. First, passivation and contacts were formed by deposition of 3 μm SiO_2, i-line lithography, contact opening by deep-reactive ion etch (DRIE), tungsten chemical vapor deposition (CVD), and chemical-mechanical polishing (CMP). Then, 100 nm of Titanium Nitride (TiN) was deposited as electrode material. The electrodes were

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formed in two steps ; the first step consisted of lithography of light-field dots aligned on the Tungsten vias, and deep-reactive ion etch (DRIE) to form planar electrodes. The second step consisted of a similar pattern with a longer etch time to release the micro-nails. Experimental results using this process were published in [15].

For the fabrication of the micro-syringes, i-line lithography with 3 μm resist was patterned on top of the micro-nail matrix. The pattern consisted of holes with a diameter of 1 μm aligned on top of the micro-nail electrodes. A long, deep-reactive ion etch, forming a via with a diameter of 1 μm and a depth of 5 μm was performed, stopping on the silicon wafer.

After the top-side processing, the wafers were bonded on glass carrier wafers, and thinned until the Silicon had a remaining thickness of 100 μm. A back-side alignment and lithography with 1X mask was performed, forming openings of 20 μm aligned on the micro-syringes. Another DRIE-etch was performed, using the top-side SiO_2 as an etch-stop layer. This resulted in formation of a cavity and opening of the micro-syringe. Then, the wafers could be removed from the carrier wafer, stripped and cleaned. A schematic representation of the finished chip is shown in Figure 2. SEM-photographs of the chip after back-side processing processing are illustrated in Figure 3.

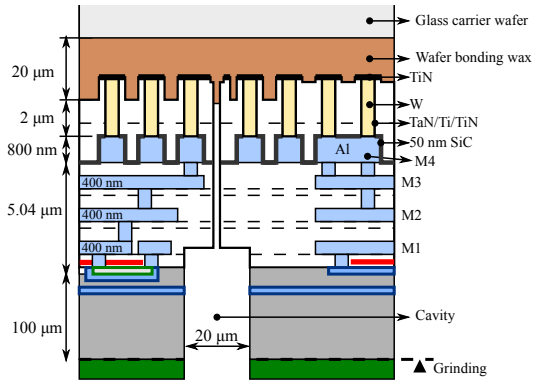

Fig. 2. Schematic cross-section illustrating the finished chip, with the glass carrier wafer on top, the thinned CMOS wafer with the micro-nails and micro-syringes on top, and the back-side fluidic cavity on the bottom.

III. METHODS

A. Packaging

The chips were packaged on a custom designed ceramic substrate of 50×65 mm with Au and Pt/Ag contacts. Packaging was performed by Gold stud bumping at 150 °C directly on the Aluminum/TiN chip bond pads. Because the flip-chip bonding technique was custom processed for our application, custom bond pads were lay-outed on top of the TSMC I/O library. The chips were flip-chipped on the substrates by ultrasonic bonding at 200 °C for 2 minutes. A bio-grade Sylgard epoxy was dispensed at the side edges of the chips and heated, resulting in under-fill and sealing of the edges around the active area.

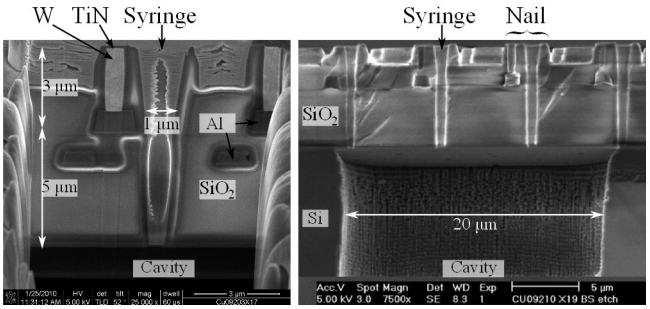

Fig. 3. **left**: FIB-SEM cross-section of the micro-syringe on top of the CMOS circuits. The Aluminum interconnects are visible. The syringe is filled with Platinum from the FIB sample preparation. **right**: Cross-section of the thinned wafer with a backside fluidic cavity and an array of micro-syringes.

B. Flow-cell

In order to allow evaluation of the micro-syringe perfusion, a custom flow-cell set-up, compatible with a *Zeiss Examiner* upright microscope, was fabricated. The flow-cell consisted two Plexiglas holders tightening a stack of PDMS sheets and the packaged chip. An opening at the top of the stack was made to allow access of the $100\times$ microscope objective. In the bottom PDMS sheet, a fluidic chamber sealed around the fluidic cavity of the chip, was made; two tubes accessed this fluidic chamber. This set-up is illustrated in Figure 4. In the beginning of the experiment, the tubes were flushed with Phosphate Buffered Saline (PBS) for 5 minutes. Then, 100mM of fluorescent Dextran FITC (*Sigma*, excitation $\lambda = 485$ nm, emission $\lambda = 525$ nm was perfused through the tubes for 20 seconds. Then, the tubes were flushed again with PBS. During the procedure, the opening of the micro-fluidic channel was observed and recorded with a upright microscope (type Examiner A1, *Carl Zeiss*).

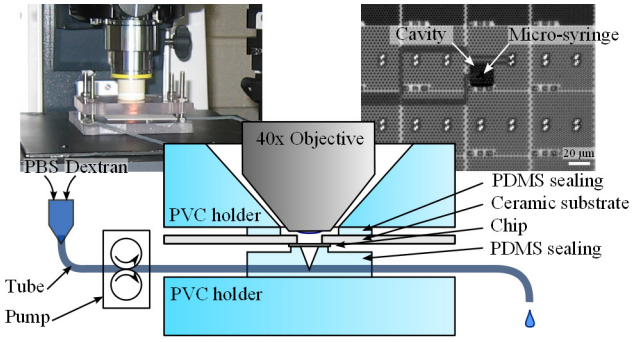

Fig. 4. **left**: photograph of the measurement set-up. **right**: bright-field image of the micro-nail array. The dark square is caused by the removed Silicon of the backside cavity ; the micro-syringe is visible in the middle. **bottom**: Schematic overview of the flow-cell for the perfusion test.

C. Cell cultures

Hippocampal neurons were isolated from 17-day-old mouse embryos as described in (Brewer, 1995). Hippocampi were dissected from the cerebral hemispheres after brain removal, incubated with 0.05% trypsin-EDTA for 15 minutes

and washed 3 times with Hanks Balanced Salt Solution (HBSS, w/o Ca^{2+} and Mg^{2+}, *Invitrogen*, Merelbeke, Belgium). Individual cells were mechanically isolated in HBSS by re polished Pasteur pipettes with decreasing tip sizes. Approximately 7000 cells/cm^2 were plated on chips coated with poly-L-lysine (500 $\mu g/ml$; 70 kDa, *Sigma*, Bornem, Belgium) in Neurobasal medium containing 12.5 mM glutamate and B27 supplement (*Invitrogen*). The medium was changed to Neurobasal medium w/o glutamate after 3 days of plating, and 5 μM cytosine arabinoside (Ara C) as added to inhibit glial cell proliferation. Neurons were maintained at 37 °C in an atmosphere containing 5% CO_2 and 95% O_2.

D. Fixation and FIB-SEM

In order to evaluate the cell adhesion on the syringes, substrates with hollow micro-nails were fabricated with a simplified process flow ; no circuits were required for this test, and the syringes were not etched through the wafer. The micro-nails had a diameter of 3 μm and a height of 2 μm, with a hole in the center, simulating the micro-syringe as described above.

After cell culturing, the neurons were fixed in a 4% sucrose, 4% paraformaldehyde solution at room temperature for 30 min followed by 3 rinsing steps in PBS (*Invitrogen*). Cells were treated with 1% osmiumtetroxide (OsO_4) in PBS for at least 2 hours. After 3 times rinsing with PBS cells were dehydrated through serial dilutions of ethanol (30, 50, 70, 90 %) and placed in absolute ethanol (100%) for critical point to avoid cell collapsing.

IV. RESULTS AND DISCUSSION

A. Flow-cell tests

After processing, the packaged chips where tested electrically. Correct functioning of the CMOS-circuits could be observed, from this, we concluded that the wafer thinning and DRIE-etch had no significant impact on our circuits.

The first flow-cell tests were performed with passive chips without electrical measurements. Future work will incorporate back-side integration of a more compact and advanced micro-fluidic system. During the flow-cell test, the micro-syringe was imaged from the top with fluorescent microcope using fluorescent light to excite the wavelength of the dye (490 nm) and an emission filter (510 nm). A region-of-interest was located on top of the opening, allowing measurement of the outflux of fluorescent Dextran molecule. This result is presented in Figure 5.

We could clearly observe three phases in the fluorescence profile. 1: The fluorescent Dextran was added in the tubes for 20 seconds, and afterwards, flushed with PBS. The delay of roughly 2 minutes was caused by the pumping through the tubes. 2: After this delay, the backside cavity got filled with Dextran, allowing diffusion through the micro-syringe. This was observable by a steep increase of fluorescence. 3: The concentration of Dextran in the chamber was diluted by PBS, so the out-flux through the syringe reduced, while the Dextran diffused in the medium, resulting in an exponential decrease of observed concentration.

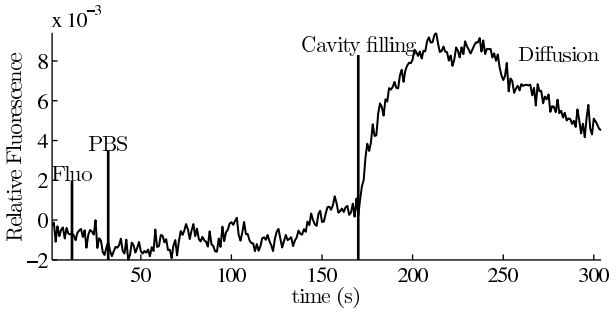

Fig. 5. Recorded relative fluorescence in the region-of-interest centered above the micro-syringe.

From this first experiment, we concluded that the principle of the process flow for micro-syringe integration was demonstrated successfully.

B. FIB-SEM of neuron cultures

Primary hippocampal neurons where seeded on the test substrates with hollow micro-nails, and put in culture for 4 days *in vitro*. Then, the cells were fixated using paraformaldehyde. The result is presented in Figure 6 (left). FIB-SEM cross-section was performed, resulting in the images Figure 6 (right). A protruding part of the cell membrane is clearly visible, which resulted in the conclusion that the cell is adapting its cell membrane to the shape of the syringe.

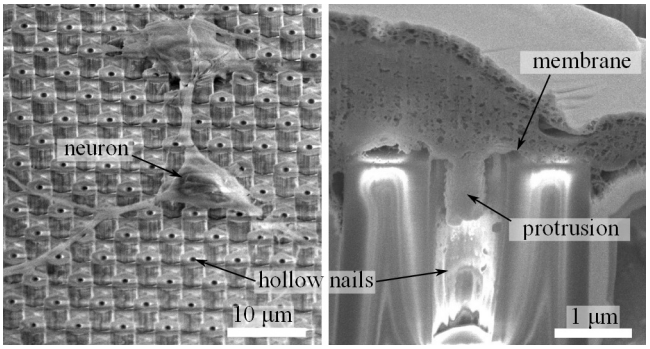

Fig. 6. **left**: SEM photograph of the fixated cells after 4 DIV on the substrates with hollow micro-nails. **right**: FIB-SEM cross-section of a neuron on the micro-syringe.

V. CONCLUSIONS AND FUTURE WORK

A. Conclusions

In this paper, we presented a CMOS chip designed in TSMC 0.18 μm technology with integrated micro-syringes suitable for local chemical delivery and integrated planar patch-clamp combined with micro-nail shaped electrodes and readout electronics. Perfusion tests were performed, demonstrating the functionality of the micro-fluidic channels. Hippocampal neurons were cultured on top of an array of micro-syringes, and FIB-SEM cross-secions reveal protrusion of the cells inside the channels. This protrusion effect could have substantial consequences. Firstly, it might result in a further increase of the seal resistance, without the need

for suction, as in the case of a patch-clamp system. This could also lead towards a novel type of electrode without the need of a micro-fluidic system. Finally, the protrusion effect itself might be caused by cell phenomena which are interesting to study.

B. Future Work

Future work will consist of developing a micro-fluidic system which makes the chip suitable for electrical tests *in-vitro*, in parallel with electrical recording or stimulation. A detailed study of the observed phenomenon has to be performed: from an electrical point-of-view, what is the impact of the protruded membrane on the on the seal formation, or from a physiological point-of-view, what are the properties of this piece of cell membrane inside the hollow structure.

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