

Multiparametric neurosensor microchip

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SUMMARY

Biochemical substances are sensitively recognized and processed by living cells, either to provide life-energy or to trigger an adequate cell-type specific response. For on-line monitoring of cellular reactions we develop(ed) different Cell Monitoring Systems (CMS[®]). In the last years we measured mainly metabolic parameters like e.g. acidification and respiration on tumor cell lines. Since nearly two years we additionally focus on the development of a CMS to measure electrical signals of neuronal networks cultured on our new neurosensor microchip. At present we optimize the sensor parameters using experimental techniques like patch-clamp in combination with PSPICE system modeling.

Keywords: multiparametric microchip, neurons, cell monitoring system

Subject category: Biosensors

INTRODUCTION

Biochemical and biophysical processes enable a cell to maintain itself, to grow, to reproduce and to communicate with the environment. Getting more information about the multifunctional cellular processing of input- and output-signals is fundamental for basic research as well as for various fields of biomedical applications. For in-vitro investigations on living cells the cellular environment differs from the native environment found in vivo. As a first approach for on-line monitoring of cellular reactions under well controlled experimental conditions we develop the so called Cell Monitoring System (CMS[®]) [1, 2]. It allows the parallel and non-invasive measurement of different parameters from cellular systems by the use of microsensors. Microelectronic sensors are the adequate choice for the non-invasive measurement of environmental as well as in- and output parameters of cells. Beside the measurement of mainly metabolic parameters like e.g. acidification and respiration [3, 4] we additionally focus on the development of a silicon sensorchip based CMS to control also electrical signals of neuronal networks cultured on our new neurochip.

The non-invasive extracellular coupling of electrically active cells with appropriate transducers permits e.g. long-term measurements of electrical signals from neural networks [5]. The quality of the

contact between the electrically active cells and the transducers is thereby of crucial importance for application of such hybrids in basic and biomedical research. Our aim is to get a sensor system where we can measure metabolic as well as electrical signals with one sensor chip for basic research and e.g. drug screening applications.

SENSOR SYSTEM

In cooperation with the semiconductor company Micronas we realised different silicon based microsensor chips for the on-line, non-invasive and parallel measurement on living cells [1, 4]. The cells are cultured on the sensorchip in a trough (see Fig. 1). The trough was formed by a polycarbonate CNC-component which we use for encapsulation. The CNC-encapsulation was glued on the sensorchip in the ceramic IC socket with a thin film of biocompatible silicon glue.

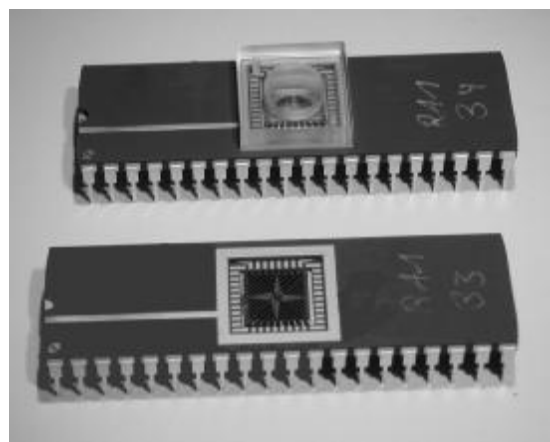


Fig. 1: Neurochip mounted in a 40-pin IC ceramic socket (rear chip with encapsulation).

The culture area is reduced to less than 15 mm² and has a chamber volume of 10 µl in the flow injection system. As transducers for the neurochip employed at present we use palladium electrodes (10 µm diam.) together with different types of cell potential field-effect transistors (CPFETs) with sensitive gate areas of 6x1 µm² integrated in combination with a temperature sensor on the chip. The palladium coating was performed in a self adjusting back end process.

METHODS AND EXPERIMENTS

Different measuring systems for the analysis of the metabolism or electrical activity of electrically active cells cultured on the silicon sensor chips using standard techniques (see Fig. 2-4) have been tested.

Dissociated tissue cultures were prepared according to the basic method established by Ransom et al. [6]. Spinal cord and cortical tissues were harvested from 14-15 day and 17 day old mouse embryos. Cells were seeded on the neurochip. Neurons were maintained for

one week in MEM, containing 10 % fetal calf serum and 10 % horse serum. Thereafter cells were fed 3-times per week with MEM containing 10 % horse serum. The cultures were maintained at 37 °C in an atmosphere of 90 % air and 10 % CO₂. The network developed spontaneous electrical activity after about 1 week and stabilized after 3 weeks.

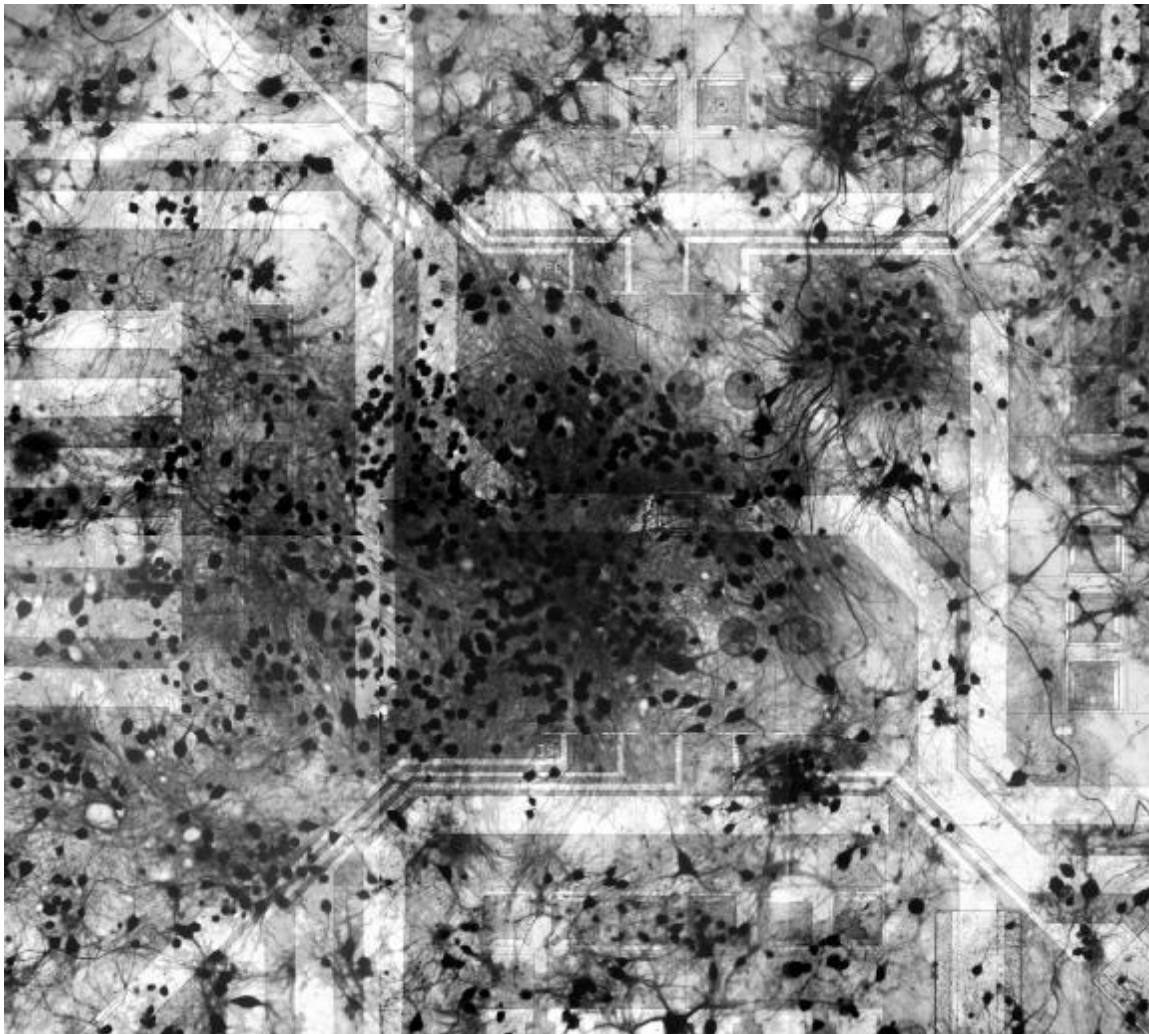


Fig. 2: Stained neuronal network grown for 30 days on the neurochip.

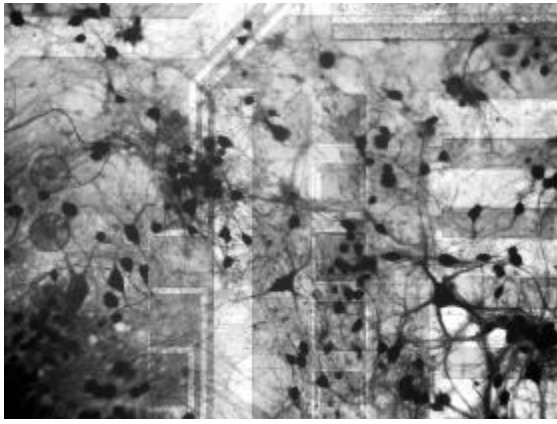


Fig. 3: Stained neuronal network on the neurochip. On the left side you can see a row of electrodes and on the right side the CPFETs under the network.

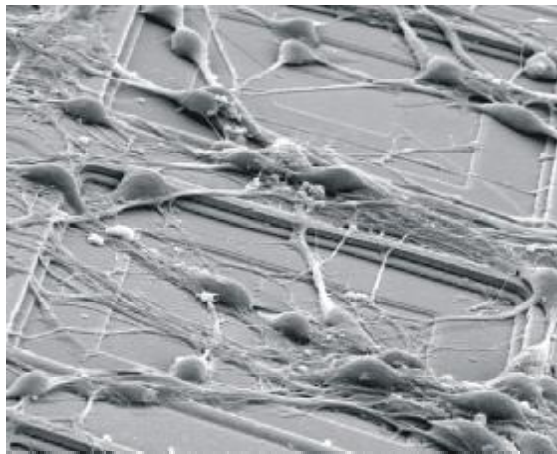


Fig. 4: SEM picture of neuronal network at the beginning of growth (after 3 days) on the sensorchip with CPFETs. After about 3 weeks the culture area of the chip is completely overgrown with cells.

The preselection of all materials in contact with the cell culture resulted from biocompatibility testing.

Basic biophysical questions concerning the electrical coupling between cells and transducers have been studied with patch-clamp techniques in combination with the electrical modelling of the cell/transducer coupling in PSPICE. The results are used to optimise the parameters of the sensorchip design and coating as well as the sensor control electronic.

Different electronic modules for the control of the sensors as well as the data preprocessing (e.g. filtering, amplification, operating point adjustment) have been developed and tested. They have been optimised with the aid of PSPICE models.

For data acquisition and analysis of the electrical signals from the neuronal network on the silicon chip we used the Multichannel Acquisition Processor System commercially available from Plexon Inc. (Dallas, TX). The experiments with the silicon neurochip (connected to the Plexon system with our electronic modules), have also been compared with the

measurements performed with the sensorchip from Gross et al. (CNNS, Denton, TX) [5].

The measurement of the electrical activity was mainly performed without flow through system. The sensorchip was kept at constant 37 °C. A humid atmosphere with 10 % CO₂ was provided over the trough with the cells. This setup was similar to the setup with the Gross MEA chip. The measured electrical activity with the electrodes on the neurochips of our first design used at present, have been comparable to the signals from the Gross chip (see Fig 5-7).

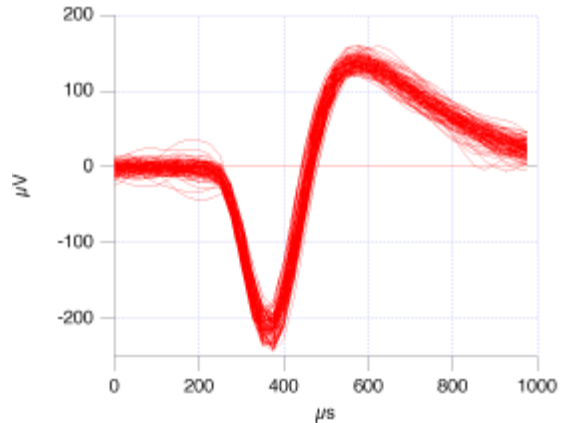


Fig. 5: Selection of extracellular recordings with good S/N from a Gross glass chip electrode; approx. 100 superposed action potential waveforms (Data from Konstantin Jügel, University Rostock).

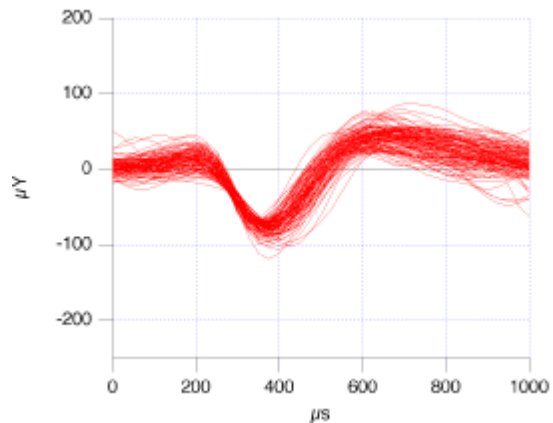


Fig. 6: Selection of extracellular recordings with ordinary S/N from a neurochip electrode; approx. 100 superposed action potential waveforms. The S/N is still not as good as in Fig. 5.

The S/N of the neurochip will be improved in the next redesign of the silicon chip. Modelling of the system with PSPICE revealed a dramatic influence of parasitic capacitances (see Fig. 8) which will be significantly reduced in the next redesign of the neurochip. Their influence can also be reduced due to a decreased electrode impedance.

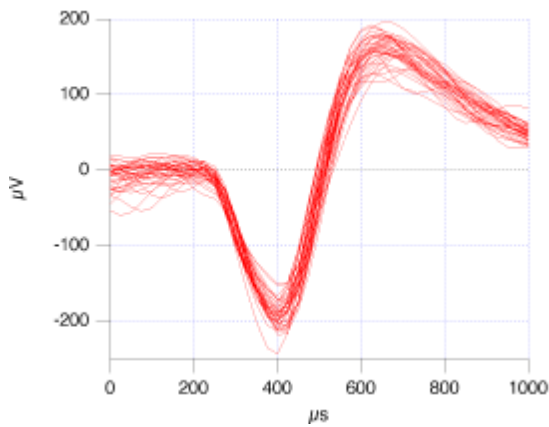


Fig. 7: Selection of extracellular recordings with quite good S/N from a neurochip electrode; approx. 40 superposed action potential waveforms. The S/N is comparable to those in Fig. 5.

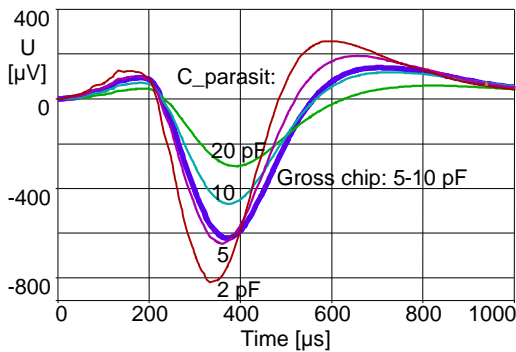


Fig. 8: PSPICE simulation of the output signal from neurochip electrodes with different parasitic capacitances at $R_{seal} = 20 \text{ MOhm}$ compared with an electrode from the Gross chip (thick line).

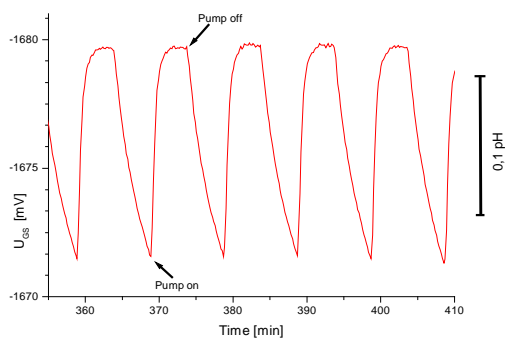


Fig. 9: Cutout from an extracellular acidification measurement of a neuronal network on a silicon sensorchip with ISFETs in a flow through system. The acidification was measured at each pump off cycle. During the pump on cycle the medium was completely exchanged with fresh medium.

Up to now with the CPFETs we couldn't measure electrical activity due to the adverse S/N of our CPFET sensors. The CPFETs will be optimised in the next

redesign including reduced noise characteristics, improved filter and sensor electronic as well as topological changes in the sensitive gate region of the CPFETs.

In addition we tested e.g. ISFET sensors for pH measurement to control the acidification as a metabolic parameter of cell cultures on a sensor chip. The measurement was performed at 37°C in a flow through system. The flow through Ag/AgCl reference electrode was placed after the sensorchip in the flow system to avoid influences from potassium. The pump cycles have been typically 5 min pump on and 5 min pump off (see Fig. 9).

CONCLUSION AND OUTLOOK

A silicon based neurochip for the measurement of the electrical activity of neuronal networks has been realised. In our next redesign S/N improvements will be implemented and the new chip will be mounted in a 68 pin PLCC type chip carrier. At present we focus on the integration of the sensors for the measurement of metabolic and bioelectrical parameters together with FET electronic, preamplifiers and multiplexers on the sensor chip.

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REFERENCES

- [1] Baumann, W., et al.: Microelectronic sensor system for microphysiological application on living cells, *Sensors and Actuators B*, B 55 (1999), 77-89.
- [2] Ehret, R., et al.: Multiparametric cellular Biosensor chips for screening applications, *Fresenius J Anal Chem*, 369 (2001), 30-35.
- [3] Henning, T., et al.: Approach to a multiparametric sensor-chip-based tumor chemosensitivity assay, *Anti-Cancer Drugs*, 12 (2001), 21-32.
- [4] Lehmann, M., et al.: Simultaneous measurement of cellular respiration and acidification with a single CMOS ISFET, *Biosensors & Bioelectronics*, 16/3 (2001), 195-203.
- [5] Gross G.W., et al.: The use of neuronal networks on multielectrode arrays as biosensors. *Biosensors & Bioelectronics*, 10 (1995), 553-567.
- [6] Ransom B.R., et al.: Mouse spinal cord in cell culture, *J. Neurophysiol.*, 40 (1977), 1132- 1150.