

Microelectronic sensor system for microphysiological application on living cells

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Abstract

Living cells can be considered as complex biochemical plants. 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 in different „cellular plants“ is essential 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 have developed the so called Cell Monitoring System (CMS[®]). It allows 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. In this paper we present a measurement system with pH-sensitive ISFETs (ionsensitive fieldeffect transistors) for the measurement of extracellular pH-related signals on cells and tissues.

Keywords: ISFET, cellular acidification, semiconductor sensor array, Cell Monitoring System (CMS)

1 Introduction

Biochemical substances are sensitively recognised and processed in complex signalling and metabolic networks by living cells, either to provide life-energy or to trigger an adequate cell-type specific response [1,2]. Metazoa (multicellular organism) produce a great variety of different specialised cells with essentially universal signal processing pathways (in humans e.g. \approx 200 cell types with unique differentiation characters). In the course of biological evolution cells have adapted to many different environments as single cells or as complex organisms, consisting of different specialised cell-types.

Getting more information about the complex cellular processing of input- and output-signals (see simplified

sketch fig. 1) in different cell-types is essential for basic research as well as for various fields of mainly biomedical and biotechnological applications.

Therapeutical drugs or substances for the manipulation on cells can be improved by a better understanding of the dynamic cell-behaviour specifically for biotechnological or agricultural applications. Another important field of application is the use of cells as (bio-)sensors for toxicological monitoring of the environment [3].

Different techniques can be used to get more information about living cells. Most of them have the disadvantage to significantly disturb the cellular sub-systems for instance due to the use of more or less toxic fluorescent markers or fixation procedures for light- and TEM investigations. On-line monitoring of living cells over a longer period

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of time can provide kinetic data of the cellular reaction pattern after application of e.g. drugs to cells.

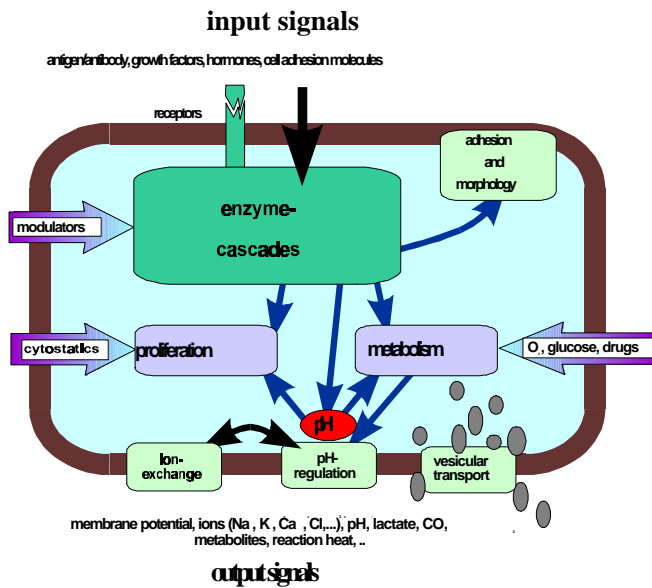


Fig. 1: A simplified sketch of signal transduction network from a living cell is shown. Input signals are connected via a complex signal transduction network with the output signals. Proliferation and metabolism of a living cell is controlled e.g. by growth factor induced enzyme cascades. Proliferation and metabolism can for instance be influenced by cytosstatic agents or other drugs. Some detectable output signals: changes in ion concentrations (mainly the extracellular pH) and other metabolite concentrations (lactate, HCO_3^- , ..), membrane potential, adhesion, morphology of the cell.

On-line monitoring is mainly performed in-vitro, where the cellular environmental conditions have to be controlled and maintained as physiological as possible. Since 1992 we are working on the development of different Cell Monitoring Systems (CMS[®]) as an advancement of our PhysioControl Microsystem (PCM[®]) (fig. 2) [4-7]. Apart of basic research applications, it is used for sensor assisted drug effects testing.

The physiological conditions of the cellular environment of the in-vitro measurements on living cells must be controlled and maintained as exactly as possible. Therefore and for the measurement of the cellular reactions the need of on-line, parallel and non-invasive measurement of different cellular parameters is crucial. Microelectronic sensors are the adequate choice for the measurement of environmental- as well as in- and output-parameters of „cellular plants“. On the one hand they are used to control the physico-chemical parameters in the measurement chamber containing cells. On the other hand they detect changes of the cellular behaviour in response to an experimental treatment.

The semiconductor microsensor fabrication process allows the combination of for example ionsensitive

fieldeffect transistors (ISFETs) [8-10] with other semiconductor sensors like temperature-, light-sensors or interdigitated electrode structures (IDES) [11, 12]. It also allows the integration of high quality standard electronics on the sensor chip and offers high cost reduction potential by mass production.

Apart from development and test of the fluid handling system, cell culture chamber unit, sensor electronic and data acquisition software, the single sensor devices (fieldeffect transistor based potentiometric sensors, IDES, oxygen- and temperature sensors, ..) are developed separately. In collaboration with other institutes they were integrated step by step into the CMS[®] [6,12].

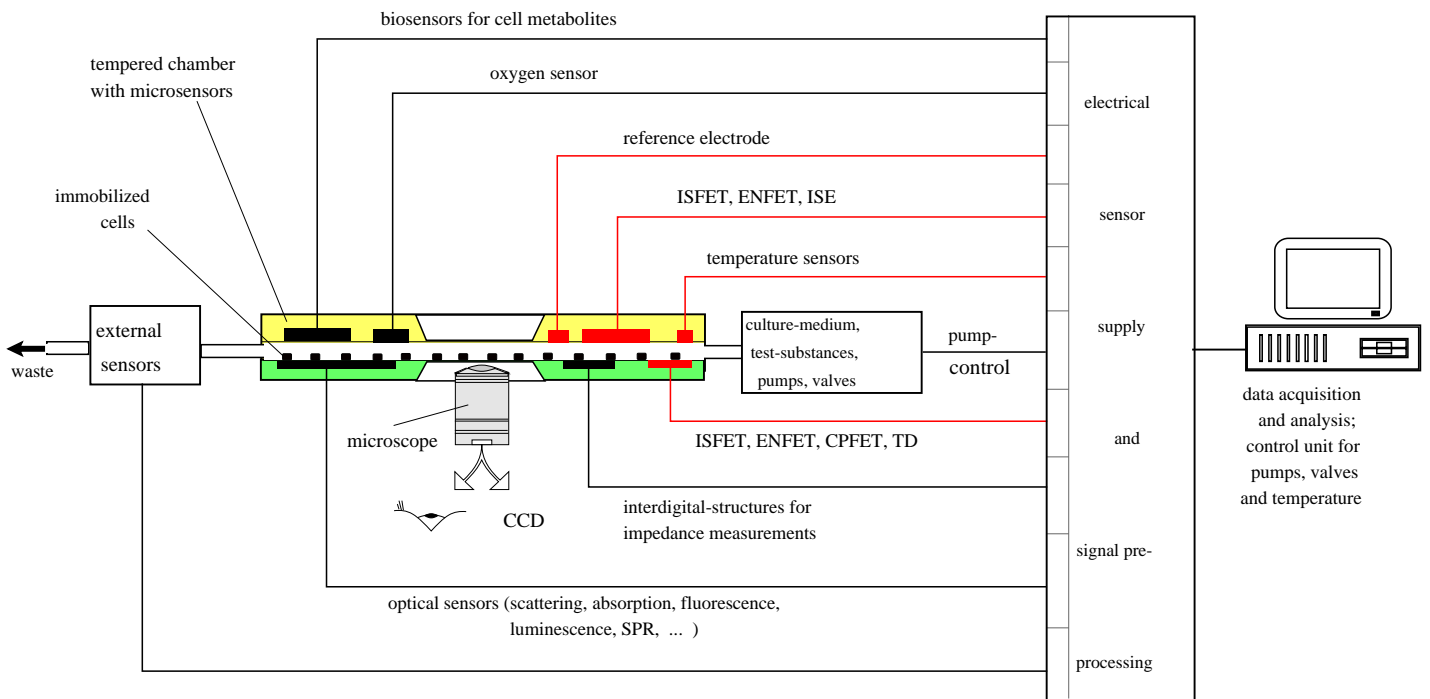
The FET-based potentiometric sensors can be used for different measurement tasks. Using additional special chemical membranes on the gateinsulator of a „basic“-FET the realisation of ISFETs for different ions (Ca^{2+} , Na^+ , K^+ , ..) or ENFETs (enzyme sensitive FETs) for other metabolites (glucose, lactate, ..) becomes feasible [13-17]. CPFETs (cell potential FETs) can be realised in order to detect membrane potential changes of nerve- or muscle-cells directly immobilised on the gate of the CPFET [8, 16-22].

This article presents a device with pH-sensitive ISFETs adapted to the measurement of the extracellular acidification of cultures with few cells. Sensitive gate areas with dimensions of a few μm^2 for the measurement in the immediate vicinity of single cells have been realised. With gate areas from $400 \mu\text{m}^2$ up to $6000 \mu\text{m}^2$ we record the integral pH of a cell population directly on, or very close to the pH-sensitive gate of the ISFET. Small distances between sensor and cells and the use of ISFET sensors with fast response times allow high signal resolution in space and time.

In contrast to the stand alone Cytophysiometer sensor from Molecular Devices for the measurement of the cellular acidification [23, 24] the ISFETs are suited for the integration in the CMS[®] in combination with other (semiconductor) sensors and the light microscope access to the cell culture unit. Furthermore, they also allow the on-line and parallel measurement of the output signals of all sensors integrated on the chip. Due to the measurement conditions (constant temperature, relative measurements, stop and flow mode, total duration less than 2 days, ..) used in our system the well known drift-, temperature- and stability-difficulties of the ISFETs are only minor problems. An advantage of the ISFETs for the measurement on living cells is the further miniaturisation potential of the sensor device due to the integration of the ISFETs with other microsensors (together with sensor electronic) on one sensor chip. The motivation for miniaturisation is founded on the limited availability and size of cellular specimen, e.g. biopsy probes in clinical diagnostics. Currently we are working on the integration of a silicon chip with ISFETs and

Cell Monitoring System (CMS[®])

top sensors for detection of chemical parameters in culture medium



bottom sensors directly contacting cells

Fig. 2: For precise measurements of cellular signals we developed the concept of the Cell Monitoring System (CMS[®]). It consists of a thermostated cell culture chamber with a fluid handling system and different micro-sensors. Top sensors are for the detection of chemical parameters in the culture medium and the bottom sensors are directly contacting the cells. (Abbreviations: ISFET: ion selective field-effect transistor, ENFET: enzyme FET, ISE: ion selective electrode, CPFET: cell potential FET, TD: temperature diode, CCD: charge coupled device, SPR: surface plasmon resonance).

temperature sensors in a sensor carrier with IDES- and oxygen-sensors as a CMS[®]-version with optical access to perform (fluorescent-) optical and electrochemical measurements in parallel (fig. 3). So, different measurement methods can be directly compared and evaluated [25].

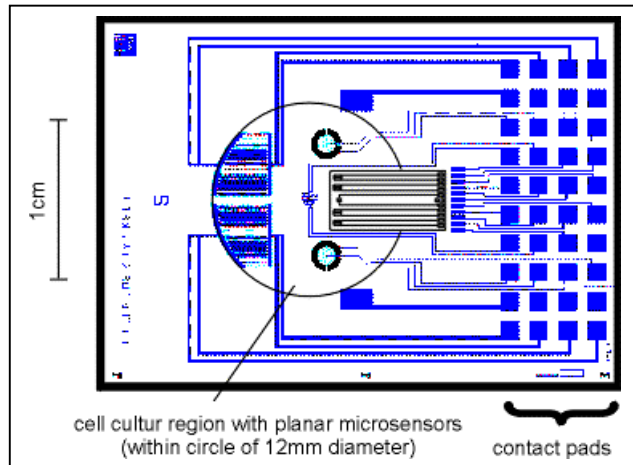


Fig 3: Layout of glass chip with two IDES, two oxygen and one Pt100 sensor and an integrated silicon sensor chip with four ISFETs and one temperature sensor. The cells are cultured directly on the chip inside a circle with a diameter of 12mm.

For further miniaturisation we already have integrated IDES and photodiodes on a silicon sensor chip with ISFETs and temperature diodes (fig. 4). Such a miniaturised system needs only very small amounts of cells to get sufficient information from cellular „biochemical plants“. First measurements with this sensor chip will also be presented in this paper.

The various CMS[®] developed in our group allow parallel, on-line and non invasive measurement of different parameters of cellular signalling. This scientific instrument can be used for basic biological and medical research as well as for applications in pharmaceutical drug development and screening in combination with cellular systems for instance.

2 Experimental

2.1 Sensor chip

2.1.1 Fabrication

Various sensor chips for the measurement on living cells have been developed and produced in cooperation with

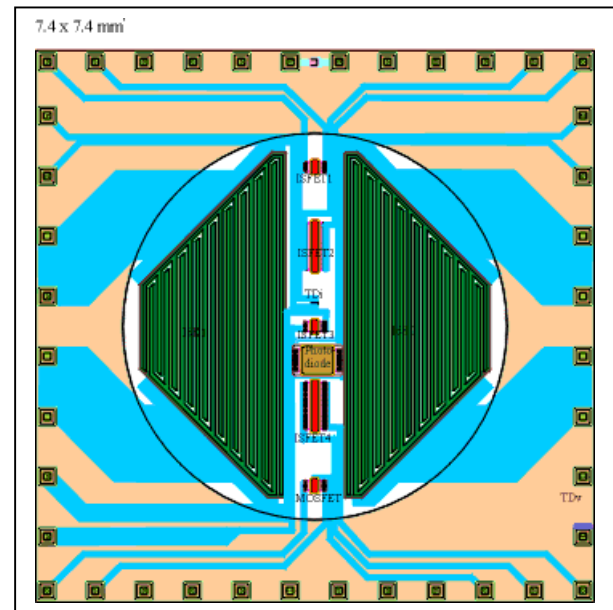


Fig 4: Layout of the silicon sensor chip with integrated ISFETs, IDESs, temperature and light sensors.

Miconas Intermetall GmbH. The first sensor chips have been realised in 5th-NMOS-technology. After the test of these NMOS sensor chips all further sensor chips have been manufactured in standard CMOS-technology (fig. 5). Due to the employment of the CMOS-technology, each n(or) p-channel FET had his own p(or) n-well and the influence of the source- substrate potential to the FET's output signal could be avoided when operating the four sensoric FETs in parallel. This work mainly presents sensor chips with four sensoric FETs (ISFETs or CPFETs), four MOSFETs and two temperature sensors integrated on one chip (fig. 6).

On the ISFET sensor chip the MOSFETs are, with the exception of the gate contact, equal to the sensoric FETs. Several sensor chips with different sensoric gate areas of the p- or n-channel FETs have been produced. pH-sensitive ISFETs with small gate areas ($6 \times 1 \mu\text{m}^2$, $10 \times 2 \mu\text{m}^2$, $20 \times 1 \mu\text{m}^2$, $20 \times 2 \mu\text{m}^2$) are used to measure the pH in the proximity of single cells. ISFETs with large gate areas $100 \times 4 \mu\text{m}^2$ up to $600 \times 10 \mu\text{m}^2$ are used to measure the integral pH of the medium near to a cell population.

As gate insulator materials we chose 20-60nm thick thermic SiO_2 or a 20nm/60nm $\text{SiO}_2/\text{Si}_3\text{N}_4$ sandwich for the pH-sensitive ISFETs. The Al_2O_3 -coating for pH-ISFETs with 80nm Al_2O_3 on 20nm SiO_2 as gate insulator material was sputtered by the IMIT [26] on the sensor chips.

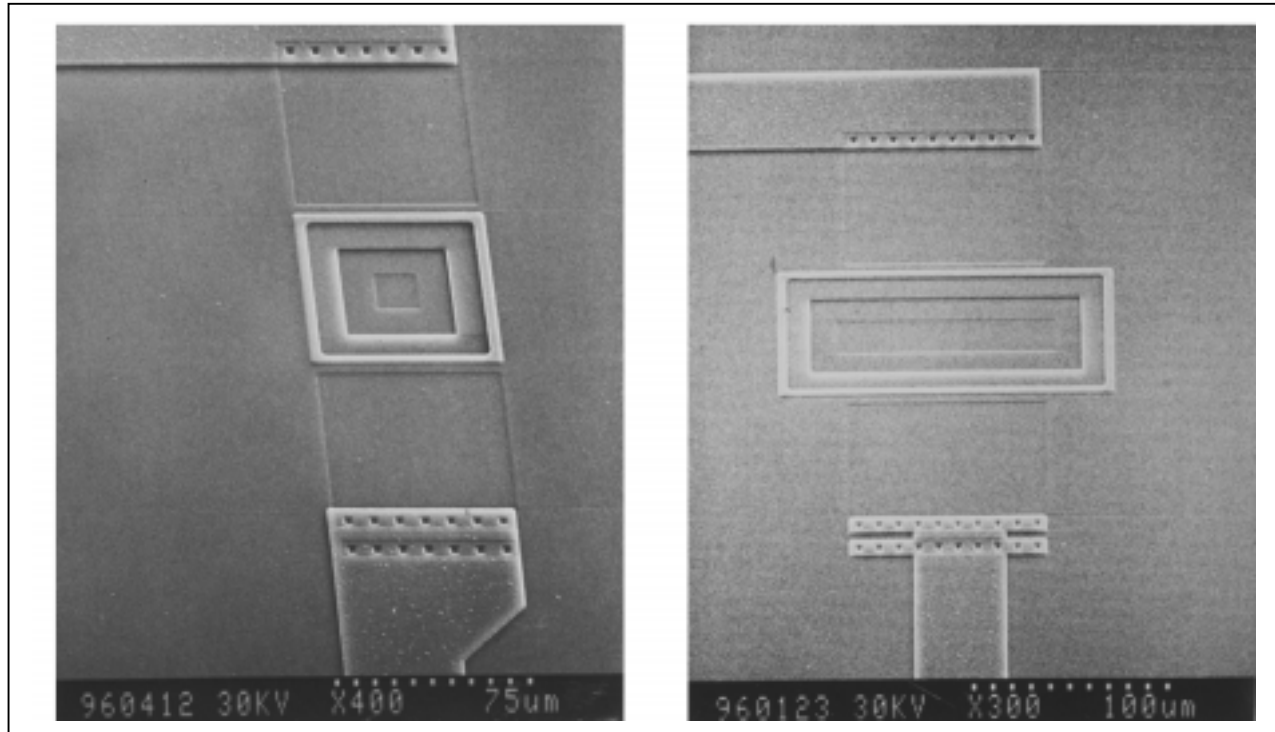


Fig. 5: SEM photos from ISFET chips with aluminium conductive strips: Left CMOS 6/1 and right CMOS 100/4.

The pH-sensitivity of the ISFETs was 15-30mV/pH for ISFETs with SiO₂ as gateinsulator material, 54-58mV/pH for Si₃N₄ and 55-58mV/pH for Al₂O₃ in the pH-range of 4-9. The pNa- and pK-sensitivity was 14-37mV/pNa and 6-30mV/pK for ISFETs with SiO₂ as gateinsulator material and less than 1mV/(pNa & pK) for Si₃N₄ and Al₂O₃. The drift of the ISFETs with SiO₂ as gateinsulator was 1-5mV/h, less than 1mV/h for Si₃N₄

and 1-10mV/h for Al₂O₃. The high drift of the Al₂O₃-ISFETs is due to the fabrication process of this insulator type. For the next Al₂O₃-ISFETs a CVD-process with a post tempering of the Al₂O₃-insulator is necessary for improved stability. Due to the operation of the ISFETs at a constant temperature of 37°C in the incubator, used for the measurements, the temperature dependence was a minor problem. The temperature diodes had an average temperature coefficient from $-2,20 \pm 0,12 \text{ mV}/^\circ\text{C}$ to $-2,32 \pm 0,01 \text{ mV}/^\circ\text{C}$ between 20°C and 60°C for all CMOS-ISFET chips from different wafers. The sensor's lifetime was at least 1 week.

The fabrication process of the new sensorchip (at present under test) with additional IDEs and light sensors on the silicon sensor chip (fig. 4) is a combination of the fabrication process of the CMOS-ISFET sensor chip presented in this work and the Pd electrode fabrication presented in [27].

2.1.2 Operating conditions

For measurement the pH-sensitive ISFETs were operated in constant charge mode with adjustable constant source drain voltage U_{DS} and source drain current I_{DS} . On sensor chips fabricated in NMOS technology the gate substrate voltage U_{GB} and on CMOS technology chips the source substrate voltage U_{BS} was constant. The ISFETs could be operated with or without MOSFET compensa-

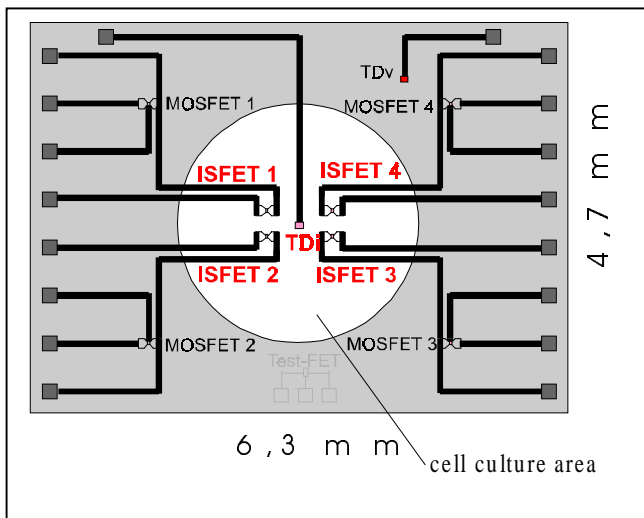


Fig. 6: ISFET-chip layout.

tion due to switching on the electronic board. Due to the significant difference in temperature- and drift-behaviour between the MOSFETs and the ISFET/reference-electrode-system the compensation was not used for the measurements. The source voltage relative to the reference electrode potential (normally a separate conventional Ag/AgCl electrode was used) in contact to the solution above the ISFET's gate insulator (U_{GS}) was used as output signal (fig. 7).

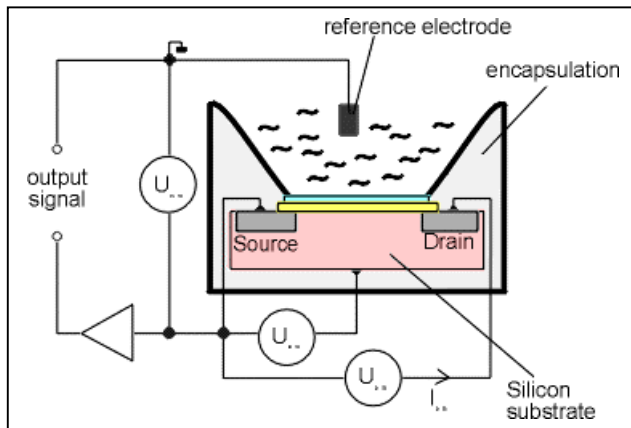


Fig. 7: Sketch of an ISFET with reference-electrode and electrical connections.

Changes in the pH of the solution above the ISFET caused a shift of the threshold voltage and the ISFET's characteristic I_{DS} - U_{DS} curve [9, 10]. This shift was compensated by the sensor electronic and could easily be measured as a shift of the same amount in the voltage U_{GS} (fig. 8). Typical operation conditions have been $U_{DS}=0.2V$, $I_{DS}=10\mu A$ and $U_{BS}=0V$. The temperature diodes have been operated with a constant current of $66\mu A$ and the temperature dependence of the diffusion voltage was used as output signal [28].

With IDES, adherent cells are cultured directly on a pair of interdigitated Pd-electrodes. The width of the electrodes and distance between the electrodes are both $50\mu m$. Impedance measurements on Interdigitated Electrode Structures (IDES) result in an integral signal which is influenced by changes in number, growth and morphological behaviour of adherently growing cells. Briefly, the cellular impedance signal results from insulation by the cell membranes. If cells are placed on

the electrodes they block the current flow in a passive way and the impedance increases [11, 12]. Complex impedance values can be specified in several equivalent ways. We have chosen an equivalent circuit with a conductance and a capacitance in parallel. The capacitance C_{par} is used for the description of the results [12].

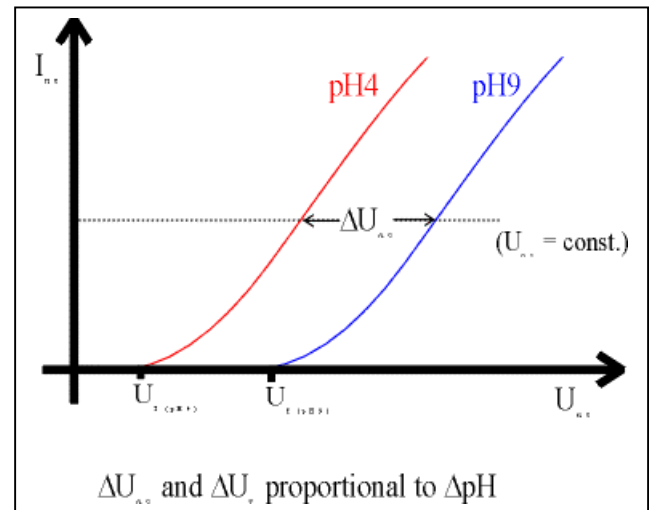


Fig. 8: Characteristic line for different pH-values. ΔU_{GS} is proportional to ΔpH .

2.1.3 Housing and encapsulation

At present the sensor chip is mounted and bonded by Micronas Intermetall in a standard 40-pin IC socket. Different encapsulation techniques and adhesives have been tested concerning stability in fluids, handling and biocompatibility. The biocompatibility was tested with different cell lines (see 2.4) [10]. The sensor chips were encapsulated with a two component epoxy from Epotec [29]. For this purpose a special encapsulation tool was developed (fig. 9). The area within the circle in figure 6 was in direct contact with fluids or cells, the outer area was encapsulated. In the opening of the encapsulation a flow injection head, connected with a reference electrode, can be inserted (fig. 10). The chamber volume of this flow injection configuration is about $10\mu l$.

2.2 Electronics and data acquisition

For the parallel operation of the FETs and temperature diodes on the chip a special electronic equipment was developed and realised. The analog output signals of the sensor electronic were recorded with a Keithley 2001 or 2000 DMM. Therefore an integrated 10-channel multiplexer for slow signals with a data acquisition rate of less than 1 Hz and a resolution of up to 24bit with the 2001 DMM was used. The instrument communication between PC and DMM is performed via a IEEE 488.2 connection. The software for the data-acquisition, -visualisation and -storage was written in Instrument Basic from Hewlett Packard. At present we use an additional data acquisition system, developed in cooperation with PTS [30] and now commercially available. In this system the analog output signals are converted with ADCs (14bit) direct on the electronic board and trans

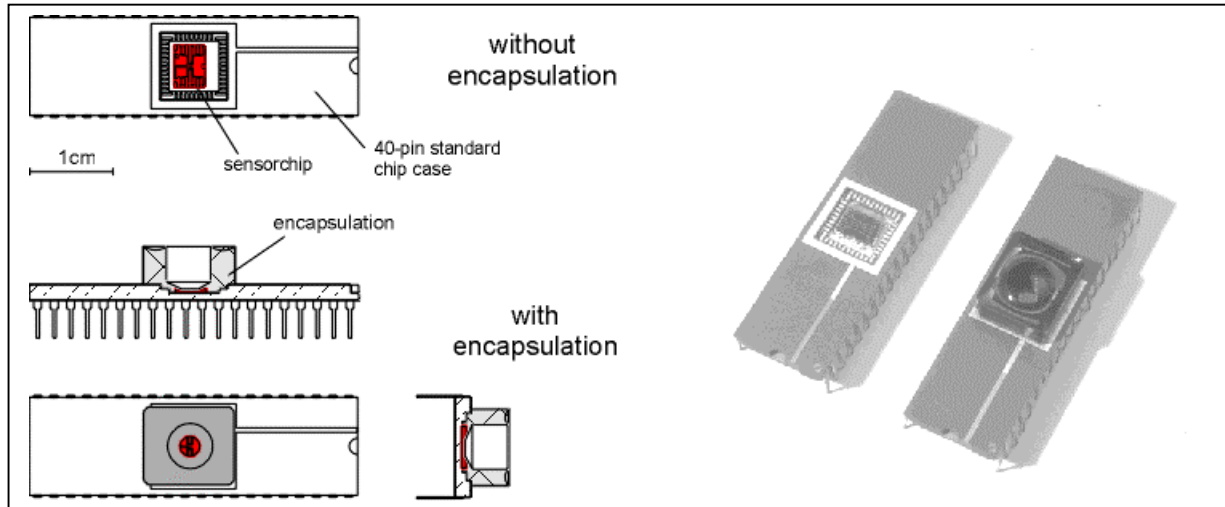


Fig. 9: Sensor chip without and with encapsulation. Left: sketch; right: photo.

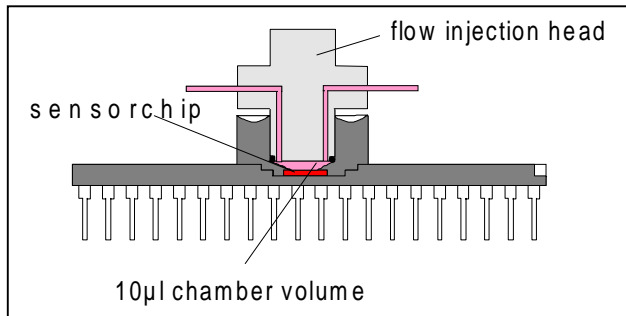


Fig. 10: Sketch of encapsulated sensor chip with flow injection system.

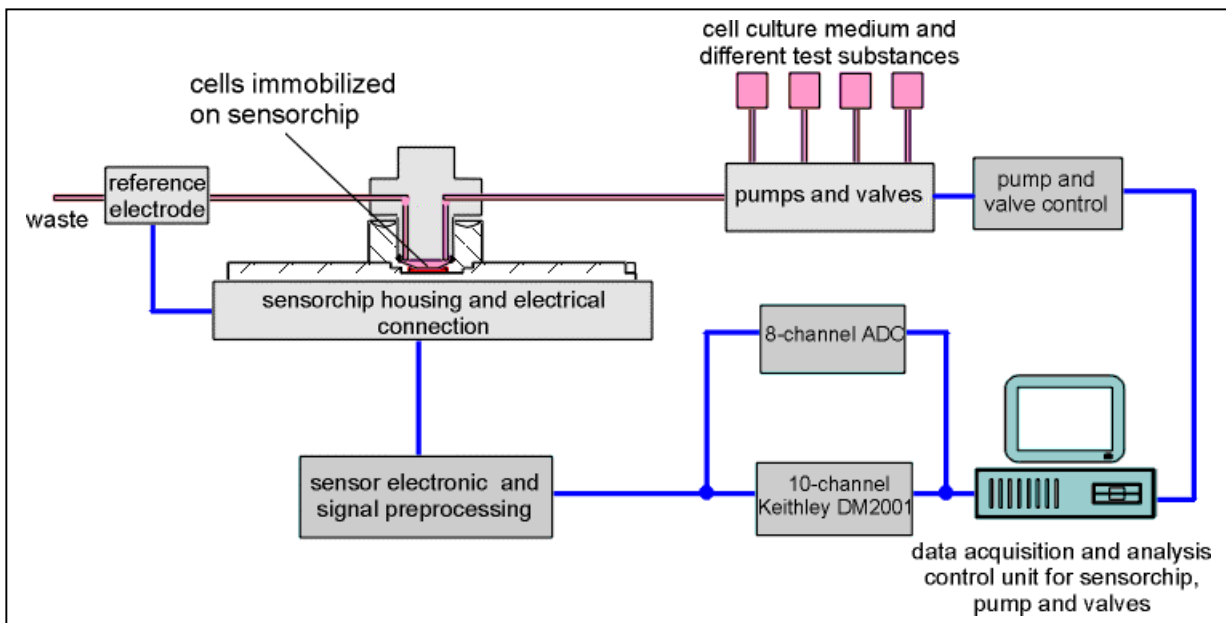


Fig. 11: Sketch of complete experimental setup of ISFET-sensor system.

ferred to the PC via a RS 232 connection. The software for this version is written in LabWindows from National Instruments

2.3 Experimental setup

Measurements with cells on the sensor chip have been performed with a flow injection system. In figure 11 the experimental setup for the system is shown.

The 40-pin IC case with the encapsulated sensor chip is mounted in a Textool socket and connected with the sensor electronic and the data acquisition setup via shielded cables. The pumps (Spetec Perimax 12 or LKB Variopex II) in the fluid handling system had a typical pump rate of $1,2\mu\text{l}/\text{sec}$. Different fluids could be switched manually with 3-way fluid switch (Novodirect) or with electrical valves (NRResearch) to the fluid system. We used Pharmed[®] tubings with an inner diameter of 0.5mm or 0.8mm. The whole flow injection setup was set in a dry incubator (Forma Scientific Model 3156) at 37°C . The measurements were performed in a stop and flow mode with 5-10min pump on and 3-15min pump off periods.

The cells were precultured under standard conditions (see 2.4) in the trough above the sensor chip. For the measurement they were transferred in the dry incubator and the chip was connected with the electronic and fluid system.

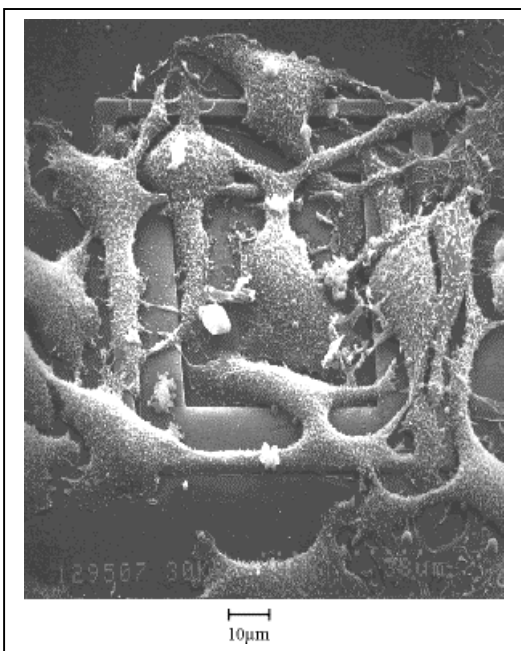


Fig. 12: The SEM-photo shows RT112 cells on a ISFET. In the middle one cell covers the sensitive gate area ($20 \times 2\mu\text{m}^2$) of the ISFET completely. The steps around the ISFET are from different insulator layers.

2.4 Cell culture

We used LS 174T (ATTC CL 187), RT 112 (German Cancer Research Center DKFZ/GCRC) HeLa (zervix carcinoma), L929 fibroblasts and algae [10, 12, 31] for measurements and biocompatibility tests (according to ISO 10993-5). The measurements presented in this paper were performed with the human colon adenocarcinoma cell line LS 174T, which grow adherently in an epithelial-like manner and have been well characterised in our laboratory [32].

All materials with direct or indirect contact to the cells in the experimental setup have been tested concerning biocompatibility. The growth behaviour of the cells on the sensor chips has been tested with different insulator, passivation and adhesive materials. Figure 12 and 13 show RT112 cells growing on a sensor chip.

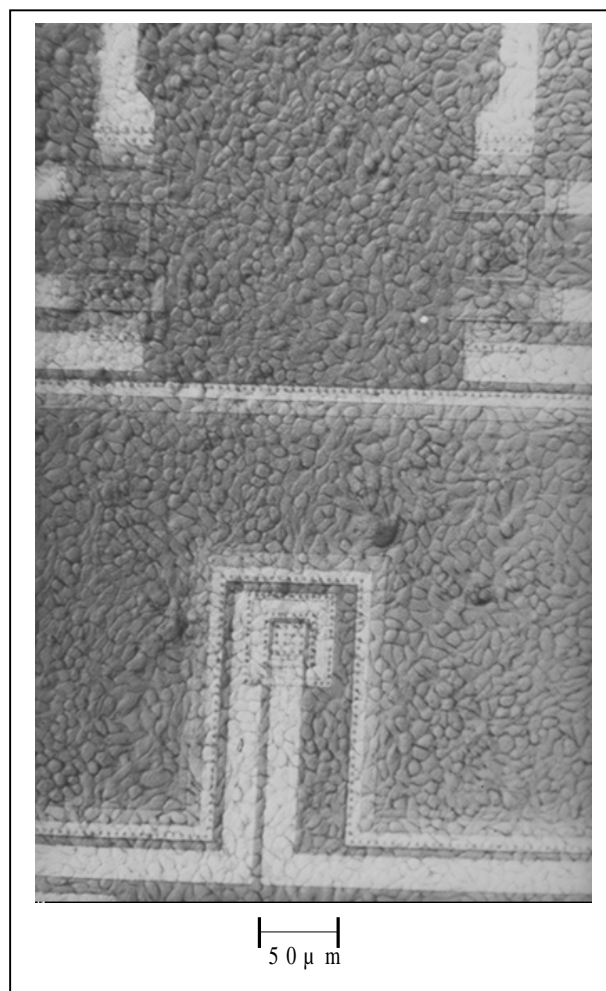


Fig. 13: The light-microscope photo shows a complete monolayer of RT112-cells on an ISFET-sensor chip.

Cell lines were cultured under standard conditions of 37°C and 10% CO_2 in air with medium consisting of

Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

The medium was supplemented with antibiotics for LS 174T. The encapsulated sensor chip was disinfected with 70% ethanol for at least 20 minutes and rinsed afterwards several times with sterile aqua bidest. Then the sensor chip was preincubated with complete cell culture medium for at least 2 hours. The adherently growing cells (approx. 10^5 /chip) were inoculated in 100 μ l DMEM into the trough of the encapsulation (volume approx. 150 μ l). After 1-2 days they have formed a monolayer on the sensor chip. For the analysis of extracellular acidification rates it is advantageous to work without strongly buffered medium like $\text{HCO}_3^-/\text{CO}_2$ used for preculturing of the cells. Therefore RPMI (Rosswell Park Memorial Institute) medium with low buffer capacity (1mM HEPES) was used for the measurements. Two hours before starting the experiment the DMEM was replaced by RPMI medium with 10% FBS. None of the cell types used showed significant adaption effects when the medium was exchanged [10].

3 Results

For the measurement of the cellular acidification the experimental setup shown in fig. 11 was used. Adherently growing LS 174T tumour cells in direct contact to the sensor chip (fig. 6) with ISFETs (600x10 μm^2 gate area and SiO_2 as gate insulator) have been employed for the measurement in fig. 14. The pump cycle was 5min pump on and 10min pump off. During the pump off period the pH of the medium in the 10 μ l chamber above the cells decreased significantly due to the acidification of the approximately 2×10^5 cells in the weakly buffered (1,93mM) medium. In the pump on period fresh medium was pumped through the chamber and after this flow interval a new cycle started. In fig. 14 the output signals of four ISFETs on the sensor chip are shown. ISFET 1&2 were operated with $U_{\text{DS}}=0.2\text{V}$ and ISFET 3&4 with $U_{\text{DS}}=0.4\text{V}$ and $I_{\text{DS}}=10\mu\text{A}$. All ISFETs on the sensor chip overgrown with cells show the same output signal behaviour concerning the cellular induced pH-change due to the LS 174T cells.

In fig 15 the output signal of one ISFET detected in a measurement over 13 hours with pump cycles of 5min pump on and 10min off is shown. The acidification was stopped by adding of 0.1% Triton X-100 (Sigma) to the medium. This detergent kills the cells by destruction of the cell membranes and therefore stops the acidification (illustrated in fig. 15). The temporary, but significant decrease of the pH immediately after addition of Triton X100 may be caused by digestive enzymes liberated from cellular lysosomes or other cellular compartments of the cells. In figure 15 (beside the drift of the sensor) an

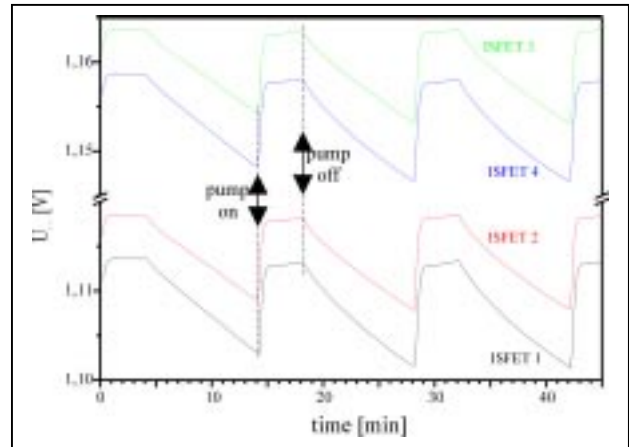


Fig. 14: Output signal of all four ISFETs on one sensor chip (ISFET 1&2 with $U_{\text{DS}}=0.2\text{V}$ and ISFET 3&4 with $U_{\text{DS}}=0.4\text{V}$ and $I_{\text{DS}}=10\mu\text{A}$). The pump cycle was 5min pump on and 10min pump off. During the pump off period the pH of the medium decreased significantly due to the acidification of the cells. In the pump on period fresh medium is pumped through the chamber and after this pump period a new cycle starts.

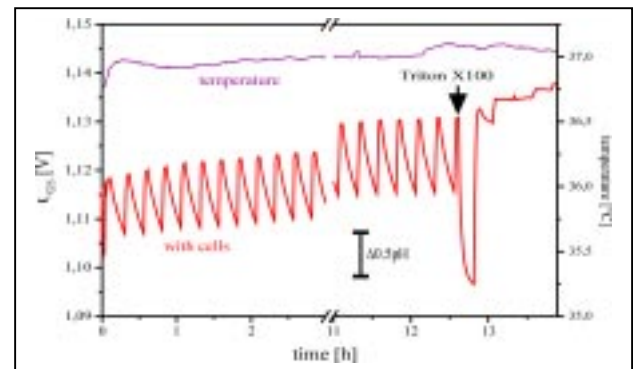


Fig. 15: The output signal of one from the four ISFETs on the sensor chip is shown in a measurement over 13 hours with pump cycles of 5min on and 10min pump off. The acidification is stopped due to the addition of 0.1% Triton X100 to the medium. So, cells were killed by destruction of the cell membranes and acidification was stopped. The impact on the detected sensor-signal is quite evident. The temperature was constantly hold at $37 \pm 0.2^\circ\text{C}$.

increase of the measured acidification from 0,065pH/min at the beginning to 0,08pH/min before the addition of Triton can be seen. This is due to the proliferation of the cells during the measurement. The temperature in the incubator was kept constant during the measurement at 37°C .

As a rough estimate ΔpH was calculated with [23]:

$$\Delta pH = \frac{1}{V_0 \beta} \sum_N \Delta H_{\text{cell}}^+$$

(chamber volume $V_0 \approx 10 \mu\text{l}$, buffer capacity $\beta \approx 1,93 \text{ mM}$, number of cells $N \approx 2 \times 10^5$, acidification rate of one cell in 1 sec $\Delta H_{\text{cell}}^+ \approx 10^8 \text{ H}^+ / (\text{cell} \cdot \text{sec})$)

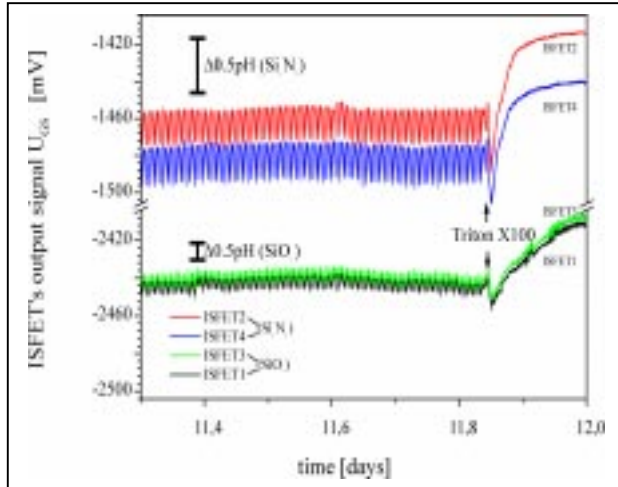


Fig. 16: The sensor chip with two SiO_2 -ISFETs and two Si_3N_4 -ISFETs was covered with a complete monolayer of LS174T tumour cells. The acidification was the same on both types of ISFETs. Due to the higher pH-sensitivity of the Si_3N_4 -ISFETs the output signal was nearly threefold higher compared with the SiO_2 -ISFETs. The output signal remained stable until the end of the measurements after 12 days.

The measured results are in good agreement with the calculated acidification rate of approximately 0,1pH/min. The slight difference between measured and calculated values has its reason in the uncertainty of the cell number (less than 10%) and the ΔH_{cell}^+ [23]. Respecting the diffusion of the H^+ -ions in the inflow- and outflow-pipes the chamber volume has to be estimated somewhat larger.

Measurements with cells on the ISFET sensor chip (fig. 6) have normally been performed in time ranges from two hours up to 12 days. Fig. 16 shows a measurement over a period of 12 days. The sensor chip with two SiO_2 -ISFETs and two Si_3N_4 -ISFETs was covered with a complete monolayer of LS174T tumour cells. The measured acidification rate was the same on both types of ISFETs. Due to the better pH-sensitivity of the Si_3N_4 -ISFETs the variations of output signal U_{GS} were nearly threefold higher when compared with the SiO_2 -ISFETs. The output signal remained stable until the end of the measurements after 12 days.

The influence of the alkylating cytostatic agent Chloroacetaldehyde (CAA) on the cellular acidification of the LS 174T tumour cells is shown in fig. 17, where the output signal of one ISFET ($100 \times 4 \mu\text{m}^2$, Al_2O_3 gate insulator) is shown. CAA can block proteins and nucleic acids in cells. After addition of $100 \mu\text{M}$ CAA to the medium by the flow injection system a decrease of the cellular acidification can be observed due to the increased blocking of the cellular metabolism with CAA. After addition of Triton X100 the cells are killed and the acidification is stopped.

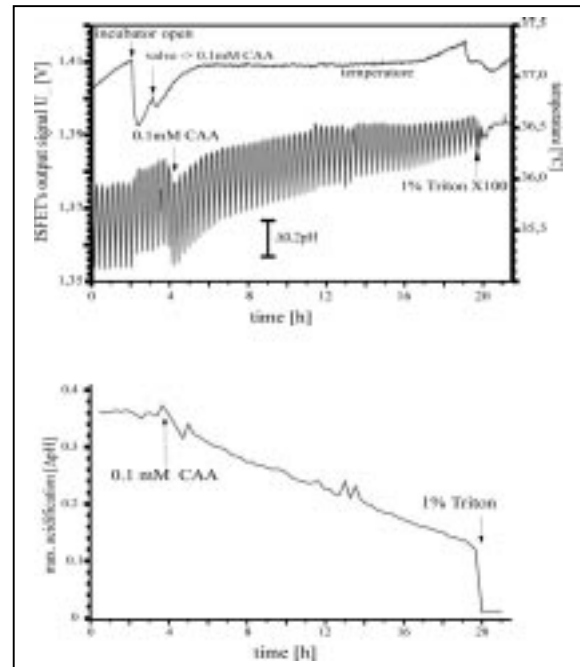


Fig. 17: Addition of $100 \mu\text{M}$ from the cytostatic agent Chloroacetaldehyde (CAA) after 4 h measurement with LS174T tumour cells on ISFET sensor chip. The raw data from the output signal of one ISFET (a) and calculated results of maximum acidification (b) are shown.

Fig. 18 shows the effect of Jodoacetate, which selectively blocks the glycolysis by inhibition of the enzyme glyceraldehyde-3-phosphat dehydrogenase. From the complete monolayer with LS174T cells from two of the four ISFETs on the sensorchip (fig. 6) cells were removed as shown in fig. 18.a. With cells growing on the ISFETs we obtained a maximum acidification rate of approx. 0.2 pH/8min in the pump off interval and without cells on the ISFET only about 0.06pH/8min (due to diffusion of the hydrogen ions from the cells to the ISFETs without cells on it). The pump cycle was 4min pump on and 8min pump off. Addition of $100 \mu\text{mol}$ Jodoacetate caused a fast decrease of the acidification rate and with the addition of Triton it was totally stopped. The increase of the pH on the cell-covered ISFET is probably due to the limited diffusion of the

hydrogen ions from the region between cell and sensor to the medium above the cells [33].

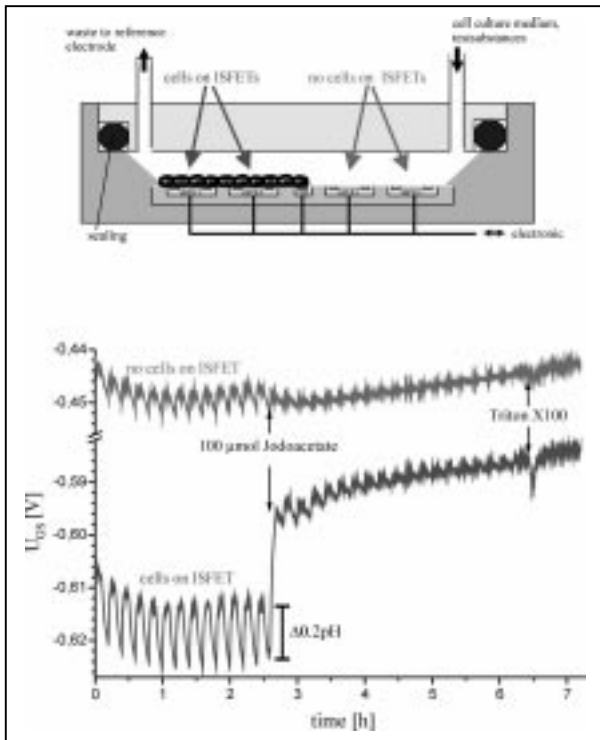


Fig. 18: LS174T cells grow only on two of the four ISFETs on the sensorchip as shown in sketch 18.a. With cells on the ISFETs we get a maximum acidification of approx. 0.2 pH in 8min and without cells on the ISFET only about 0.06pH/8min due to diffusion from the cells. The pump cycle was 4min pump on and 8min pump off. After addition of 100µmol Jodoacetate a fast decrease of the acidification can be seen and with the addition of Triton it is totally stopped.

First measurements with ISFETs and IDEs integrated on one sensor chip (fig. 4) provided extra information concerning the cellular response after addition of 100µmol Jodoacetate to the perfused medium (fig. 19). At the beginning a complete monolayer of cells is formed. In the first hours you can see the influences of the medium flow. After the addition of jodoacetate an increase in the parallel capacitance can be seen followed by a decrease to end at nearly the same values as before the addition. Nevertheless, the changes due to the medium flow are reduced. The low capacitance values show, that the cells are still close to the electrodes. This situation is changed when Triton X-100 is added at the end of the experiment and you have high capacitance values. This shows that the pH increase after addition of Jodoacetate was not caused by a permanent change of the cellular adhesion to the surface.

1 Conclusion and outlook

It could be shown that pH-ISFETs are sensor devices suited for on-line cellular acidification measurements. Changes in the extracellular acidification rates caused by the addition of drugs to the medium can be measured on-line and non-invasively. This allows a deeper insight in the kinetic of the cellular signalling. For the interpretation of drug effects on living cells the correlation of different parameters is important. On-line monitoring with microsensors seems to be particularly helpful in cellular pharmacokinetics, e.g. in analyzing drug uptake, early events of drug action or reversibility of drug effects. In this work the cointegration of ISFETs and IDEs on the same chip is shown to be useful for the detection of both cell metabolic and cell physiological responses to drugs.

Arrays of ISFETs can be easily produced using standard CMOS techniques. They improve the measurement statistics and can provide resolution in space and time. Additional information can be obtained by the evaluation of parallel sensor signals from overgrown sensors and cell-free sensors [34].

Regarding accessory sensor performance we test at present ion selective membranes (ISM) for Ca^{2+} on our sensor chip. In collaboration with Micronas Intermetall we are currently developing appropriate back end processes for the sensor chip fabrication to establish membrane processes for different ISMs, ENFETs and oxygen sensors for mass production. A first measurement with a combination of FET based sensor arrays with other semiconductor sensors (IDES, temperature and light sensors) on one sensor chip has been presented (fig. 19). The additional integration of oxygen sensors is tested at present. CPFET and electrode arrays on one chip are available since end of 1998 for tests with muscle- and nerve cells.

Sensor based test systems for on-line measurements on living cells in-vitro can be used in basic research as well as for e.g. reduction of animal experiments in drug screening applications.

Consideration of scientific and market demands leads us to the development of three CMS versions with integrated sensor chips.

1. A two channel sensor device with light microscopic access to the cell culture units and a glass chip with integrated microsensors with a relative large cell culture area ($\leq 12\text{mm}\varnothing$) for mainly basic research in biology and medicine [5-7, 34]. Standard (fluorescence-) optical techniques in combination with microsensors on the glass chip allow to measure different cellular parameters in parallel.
2. A multi tester for the parallel evaluation of the effectiveness of drugs. For example a multiwellplate with

sensor chips integrated in the bottoms of the single wells for drug screening (fig. 20) or for parallel testing of different chemotherapeutic drugs on tumour biopsies from cancer patients before application of the drugs to the patients.

- Cellular biosensors for monitoring or as early warning systems concerning toxicological substances in the environment are under development.

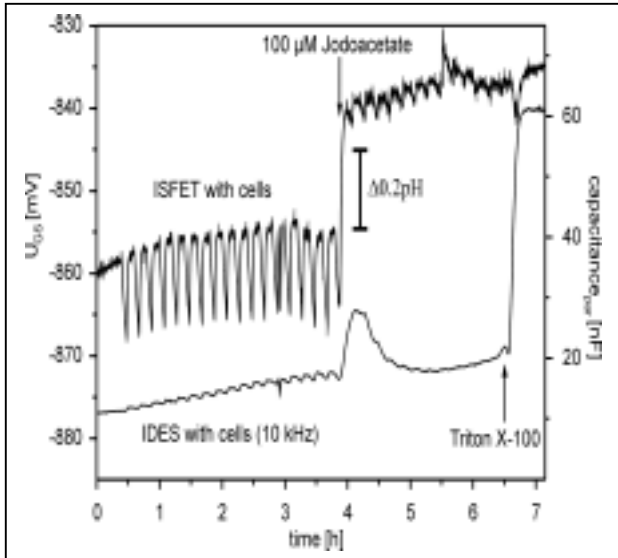


Fig. 19: LS174T cells grown on the sensor chip with four ISFETs and two IDESs. The pump cycle was 4min pump on and 8min pump off. After addition of 100 μ mol Jodoacetate a fast decrease of the acidification was obtained and totally stopped after the addition of Triton. With cells on the ISFETs we got a maximum acidification of approx. 0.2pH shown also in fig 18. As an additional signal impedance measurement with the IDES structures were performed. The (capacitance) output signal of the IDES showed only a transient change and stabilised again approx. 1h after the addition of the Jodoacetate.

At least for the last two CMS versions the preprocessing of the great quantity of sensor data is necessary. With the modelling of cellular systems, e.g. the Structured Biological Modelling (SBM) [1], and the modelling of the sensor systems it is possible to realise an automated evaluation of the sensor data and a better adaptation and data evaluation of the sensor system to different measurement requirements. Microstructure and semiconductor technologies are the basic hardware technologies for the fabrication of the sensor- and fluid handling-systems as well as for the necessary data acquisition and evaluation systems. The functional testing of cellular signalling with the CMS in combination with the modelling of cellular systems is a very important method to understand cellular reaction patterns.

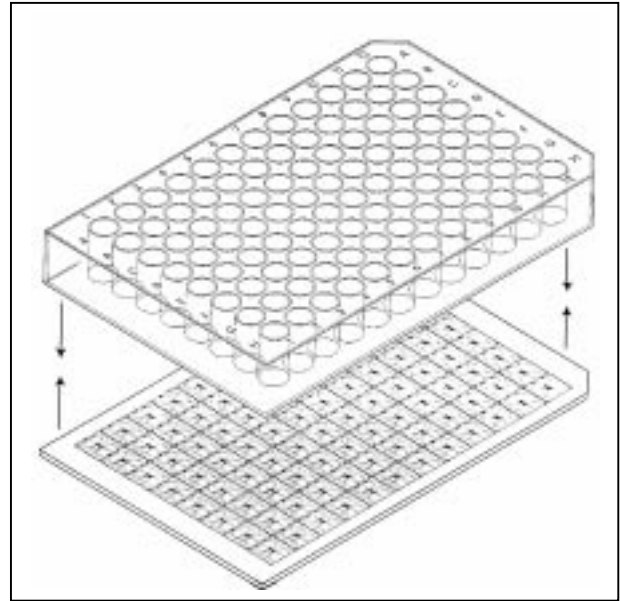


Fig. 20: Multiwellplate with integrated sensor chips in the bottom of the wells.

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4 Biographies:

Werner H. Baumann worked after a 3 year apprenticeship in electromechanics and electronics for 2 years in a research laboratory for magnetic materials and test-systems in the Robert Bosch GmbH in Buehl, Germany. Afterwards he studied Physics in Freiburg and received the Diploma in 1991 and the graduation in Biology in 1996. Since 1992 he is working in the Group of Prof. Wolf in the Institute of Immunobiology in Freiburg. His main scientific interests are the development of the silicon sensor chips, sensor electronics and fluid handling systems for measurement on living cells with micro-sensor systems (especially with ISFETs and CPFETs).

Mirko Lehmann began 1995 his physics diploma thesis in the group of Professor Wolf with the topic: „Development and testing of semiconductor biosensors“. Since then he worked also as part of his diploma thesis with Micronas Intermetall a semiconductor company in Freiburg. After his diploma thesis in the end of 1996 he continued his work as part of his PhD in biology concerning cellular biosensors both at the university and Micronas.

Anne Schwinde, Technical Assistent, worked in several departments of the University of Freiburg since 1970, e.g. in Limnology, Cell Biology, Pathology and Immunobiology. Her special interests are in cell culture and electron microscopy techniques.

Ralf Ehret received his Diploma in Physics in 1991 and the graduation in Biology in 1997. Since 1992 he is a member in the group of Prof. Wolf. His scientific interests are the detection of cellular behaviour with IDES, optical and scanning electron microscopy techniques. For this, his special interests are the adhesion of cells and related phenomena for application and exploitation in microsensor systems.

Martin Brischwein received his diploma in Biology in 1992 from the University of Freiburg. Afterwards he joined the group of Prof. Wolf at the Institut for Immunobiology and received the graduation in Biology in 1998. His main interest is the detection of cellular metabolism by various microsensor techniques under microscope optical control.

Bernhard Wolf is Professor for Biophysics at the University of Rostock. His main interests are analytical

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