basic science

MEDICAL UNIVERSITY
OF VIENNA

THESIS PROPOSAL

for the Doctoral Program at the
Medical University of Vienna

Working title (without abbreviations)

Monitoring Synovial Fibroblast Responses to H_2S Exposure Using a Lab-on-a-Chip

acad. degree, first name, last name
(field of graduate study)
E-mail

[redacted]

Location: Institute and address where thesis will be performed

Main location	*Co-operating institution*
[redacted]	[redacted]

Supervisor: Name and address of supervisor

Medical University Vienna	*External Supervisor*
[redacted]	[redacted]

Supported by: Name and address of granting institution, project, etc.

[redacted]

Date: 26.04.2011

[redacted]
Signature of the applicant

Summary and Aim

The ultimate motivation for this research project is to advance personalized medicine by providing physicians with a new diagnostic tool capable of analyzing cells from individual patients' biopsies.

The intended research project aims at the development, characterization and evaluation of a bench-top sized microanalytical cell monitoring platform (Cell-on-a-Chip) capable of detecting rapid changes in cell populations. In the course of the project, a fully integrated **computer-controlled cell analysis platform will be developed** to continuously monitor *ex vivo* living human cells in a non-invasive manner. The newly developed technology will be applied **to assess cellular responses to hydrogen sulphide (H_2S) releasing molecules** using healthy and rheumatoid arthritis (RA) human synovial fibroblast cells. Although H_2S exposure has traditionally been used in sulfur containing springs (spa therapy) to treat RA patients, only recently H_2S has been described as a physiologically relevant gaseous signalling molecule [1, 2]. However, many questions surrounding therapy efficacy, safety and mechanism of action still remain. Consequently one aim of the project is to identify H_2S induced alterations of specific cell parameters such as viability, stress responses and release of inflammation mediators.

The development of the cell-chip platform calls for highly interdisciplinary expertise provided by the collaborative effort of three research partners: Ludwig Boltzmann Cluster for Rheumatology, Balneology and Rehabilitation (LBI), Siemens Aktiengesellschaft Österreich and Nano Systems at the Austrian Institute of Technology (AIT). While the effects of sulfur releasing molecules will be investigated in cooperation with LBI using ELISA, western blot and RTqPCR techniques, the benchtop-sized monitoring station will be developed by Siemens. In the final phase of the project, synovial fibroblasts will be cultivated and monitored *on-chip* to detect rapid cell responses to varying H_2S doses.

List of Abbreviations

Thesis Proposal

Abbreviation	Meaning/Definition
1BR3	Human skin fibroblast cell line
AC	Alternating current
AIT	Austrian Institute of Technology GmbH
Calcein-AM	Calcein acetoxymethylester
CO_2	Carbon dioxide
COX-2	Cyclooxygenase-2
CP	Chronic polyarthritis
CTFT	AIT Center of Thin Film Technologies
ELISA	Enzyme linked immuno-sorbent assay
ERK	Extracellular-signal regulated kinase
FBS	Fetal bovine serum
FLS	Fibroblast-like synoviocytes
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulfide
HF	Human fibroblast
HF 189 OA	Human fibroblast osteo arthritis cell line 189
HF 190 OA	Human fibroblast osteo arthritis cell line 190
HF 26 CP	Human fibroblast chronic polyarthritis cell line 26
HF 45 CP	Human fibroblast chronic polyarthritis cell line 45
HO-1	haem oxygenase - 1
HSP70	Heat-shock protein 70
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
JNK	c-Jun N-terminal kinase
LBI	Ludwig Boltzmann Institute; Ludwig Boltzmann Cluster for Rheumatology, Balneology and Rehabilitation
LoC	Lab-on-a-Chip
mRNA	Messenger RNA
µIDES	Micro interdigitated electrode structure
µTAS	Micro total analysis systems, highly integrated microdevices
NaHS	Sodium hydrogen sulphide
NFκB	Nuclear factor kappa B
OA	Osteo arthritis
p-38	p38-mitogen activated protein kinase
RA	Rheumatoid arthritis
ROS	Reactive oxygen species
RTqPCR	Real time quantitative polymerase chain reaction
TMRE	Tetramethylrhodamine
TNF-α	Tumor necrosis factor - alpha

Background

Rheumatoid arthritis (RA) or chronic polyarthritis (CP) is an autoimmune disease characterized by chronic inflammation of joint tissues, mainly the synovial membrane [3]. RA has a high prevalence (about 1% of the Austrian population) and potentially devastating and irreversible outcomes. In the absence of proper treatment RA can become a disabling, painful condition leading to substantial loss of function and mobility [3, 4].

Spa therapy (sulfur, mud, mineral and pearl baths) of rheumatic disorders is the oldest form of treatment and is still commonly practised today [5]. In most spas mineral water with high sulfur content, originating from geothermal wells or springs is utilized. To date there is still a lack in detailed scientific investigations of the benefits of H_2S treatment. However, there is some scientific evidence that points towards the induction of a protective stress response by H_2S [6-8]. According to these studies, H_2S might act as a negative regulator of inflammation processes by activation of the stress response genes HO-1 and HSP-70. While it is well known that H_2S at high concentrations is clearly toxic [9, 10], it is still not clear whether and to what degree the concentration of sulfur applied in a typical spa treatment (>1mg/kg water [5]) is adequate to induce a protective stress response without harming organisms/cells.

Consequently, there is still a need to develop new diagnostic tools capable of rapidly assessing cell responses. Here highly integrated microdevices (µTAS) including Cell-on-a-Chip systems can provide the necessary technology to allow for fast, portable and accurate measurements of complex biological systems. The ability of microfluidic systems to conduct measurements from small volumes of complex samples (such as tissue biopsies) with high efficiency and speed has been regarded as the potentially most powerful application of Lab-on-a-Chip technologies [11]. However, to date only a small number of Lab-on-a-Chip is successfully used in real-world applications. For instance one prominent example of a µTAS capable of delivering results from complex biological samples are DNA analyzers [12-14].

The presented project envisions the development of a Cell-on-a-Chip analytical platform for the study of rheumatoid arthritis (RA) and spa therapy. While most commonly used cell based assays have a poor time resolution, one objective of the project is to continuously monitor cellular phenotype dynamics by non-invasively assessing cell viability, reproduction, and metabolic activity over long periods of time. The technology applied in our Cell-on-a-Chip system will be dielectric spectroscopy. This methodology is well established and widely used for non-destructive analysis in industry and biotechnology e.g. for analysis of biopolymers or biomass [15]. Briefly, the application of alternating current (AC) to micro interdigitated electrode structures (µIDES) creates electric fields between adjacent fingers that span perpendicular to the sensor plane into the analyte (e.g. a cell population growing on top of an µIDES) (Figure 1). Any changes of the cell status such as increase in biomass or intra-cellular variations also change the cells' electric properties resulting in a detectable signal [16] (Figure 2). We hypothesize that sulfur induced alterations of the cells' signalling pathways may lead to detectable alterations of the electrical cell properties. For our study we chose RA synovial fibroblasts as a disease model since this unique cell type is a key player in RA [17].

Operational objectives:

1st year

Aims:

Firstly, the three research partners will assess in detail the technical requirements of the entire cell monitoring platform. Based on the requirements a prototype of the cell monitoring platform will jointly be developed. Within the first year, Siemens will work on the production of a functional measurement station prototype including hard- and software as well as peripheral components such as heating or pumping devices. At the AIT we will design and fabricate a cell chip to be used with the monitoring station. The cell-chip will be capable of continuously and non-invasively monitoring cell populations under physiologically relevant culture conditions using impedance spectroscopy. LBI expertise and facilities will help to investigate H_2S induced cell reactions using standard cell biological methods.

Description:

Scientists from different fields - ranging from (software) engineering to cell biology – are collaborating in this interdisciplinary research project. To create a common understanding of the research efforts, initially any different perceptions of the project will be discussed and evaluated. Based on the initial discussion, technical requirements of the cell-on-a-chip platform will be specified in detail.

Within the first year, Siemens will design and fabricate a prototype of the measurement station including electronics as well as control and data logging software. A drawing of the preliminary hardware concept is depicted in Figure 3.

A main task for the first year of the proposed thesis project will be the design, fabrication and characterization of the microfluidic cell chip. The existing expertise of AIT (see Figure 4 for an example of a microfluidic cell monitoring chip) will be used to define a set of requirements and design constraints for the newly developed cell-chip. To allow for parallel measurements, four microfluidic proliferation chambers will be included on-chip, each comprising an integrated dielectric sensor. For physical separation of sensor and analyte a thin, non-conducting passivation layer will be deposited. Layer thickness, material, and composition will be optimized. First experiments to assess technical performance characteristics of the cell chip will be carried out using a potentiostat.

Since cell morphology, function and behaviour are strongly influenced by external conditions, it is important to create a physiologically relevant microenvironment for *in vitro* cell analysis. It is also well known that many properties of microfluidic systems are significantly different from those found under standard cell cultivation conditions [18]. As a consequence, another focus in the first PhD year will be to characterize, define and optimize important cell-chip parameters, such as shear stress or biocompatibility of chip materials.

Concurrently *off-chip* cell characterization will be performed. First, adequate fibroblast cell lines will be selected. Then time and dose-dependency of H_2S exposure of the fibroblast cells will be determined. Sodium hydrogen sulphide (NaHS) will serve as H_2S source. LBI will provide expertise in *in vitro* analysis of cellular H_2S effects. In the first year we will mainly focus on the investigation of fundamental cell characteristics such as viability, mitochondrial membrane potential and proliferation. Methods will involve alamarBlue® assays, fluorescent probes (TMRE, Calcein-AM) and standard trypan blue staining.

Operational objectives:
2nd year

Aims:
In the 2nd year all main system components of the cell monitoring platform will be integrated. The fully functional prototype will then be used for monitoring of synovial fibroblasts. Initially, adequate cell handling procedures for *on-chip* experiments will be developed. One important aim is to establish standard procedures for *on-chip* experiments.
Known stressors such as oxidative stress, shear stress and well characterized toxins will be applied to detect and interpret resulting impedance spectra.
Additionally, protocols for detailed investigation of *on-chip* cultivated cell populations will be developed. These will involve fluorescence methods as well as chip-based ELISA (IL-6).
At LBI laboratory further *off-chip* cell characterization with focus on H_2S induced signaling pathways will be performed.
Concurrently, Siemens will refine software for data acquisition, processing and interpretation. They will also assist with the implementation of any necessary adjustments and changes in soft- or hardware.

Description:
First, the cell monitoring system including all supporting components, such as syringe pumps and fluidic valves, will be set up. After initial testing of the system, cell experiments will be performed on the fully functional monitoring station prototype. *On-chip* cell handling and experimental procedures will be established, optimized and standardized.

Then well defined stresses will be applied to *on-chip* cultivated fibroblast cells and impedance spectra will be recorded. Experiments will involve the application of oxidative stress, shear stress as well as toxins with known mechanism of action. Controls performed simultaneously to each experiment in different chambers of the same chip will help to extract the relevant data from the entire spectra.

Additionally to the signals obtained from sensors integrated *on-chip*, time-lapse microscopy as well as bioanalytical measurements (ELISA) of the effluent culture supernatant will be used to gain additional knowledge on cell responses. This multiparameter analytical approach will also help to accurately relate *on-chip* data with results of state-of-the-art standard molecular biological cell assays. In order to facilitate analysis of the complex biological data sets, computational methods to identify key interaction features of sulfur containing compounds need to be developed. Since the continuous scanning of a broad frequency range will generate huge amounts of data that cannot be analysed manually, Siemens will focus on the improvement of data analysis software. Principle component analysis and partial least square analysis will be implemented.

In the 2nd year we will also continue cell characterization under standard cultivation conditions. State of the art assays (ELISA, RT-qPCR, Western blot) will be used to evaluate effects of NaHS on important signalling pathways. A 2009 study by Stuhlmeier et al. [19] on fibroblast-like synoviocytes from RA patients showed that the stress proteins HSP-70 and HO-1 can be induced by H_2S application leading to non-NFκB dependent activation of MAPK. In this paper H_2S has been reported as an inducer of ERK, p-38 and JNK phosphorylation as well as an activator of TNF-α, COX-2, IL-1α, IL-1β and IL-8 expression. However, other studies performed at LBI [7, 8, 20] report H_2S as a potential inducer of a protective stress response resulting in reduced inflammation signaling. One common aspect of the cited studies is the high time dependency of detected signal changes suggesting relatively fast, transient cell responses. Consequently identifying H_2S mechanisms of action and time dependency for the cells used in our study will be of utmost importance.

Operational objectives:

3rd year

Aims:

In the 3rd project year we will focus on the *on-chip* investigation of H_2S effects on synovial fibroblast cells. Experimental design will be based on the results of years 1 and 2. Data gained from previous experiments will be used for interpretation of cell signals.

One important aim will be to assess rapid cell responses that are difficult to evaluate using traditional cell based assays. The data analysis software developed within this project will be used to evaluate continuously recorded impedance spectra of NaHS exposed cells. The additional *chip-based* assays developed in year 2 will be performed to support impedance results.

Description:

In the final project year further *on-chip* experiments will be performed. A range of non-toxic to toxic NaHS concentrations will be applied to synovial fibroblasts for different time periods. Cell responses will be continuously monitored and impedance spectra will be analysed with the goal to match sensor signals to specific cell parameters. For each experiment adequate controls will be included on the same cell-chip. Supplementary cell tests will be performed as described for year 2. Additionally data acquired from *on-* and *off-chip* experiments throughout the project will help to accurately analyse impedance spectra.

The continuous and non-invasive monitoring will help to investigate time dependency of cell responses with high time resolution. Since data of existing studies [7, 8, 19, 20] indicates relatively fast, transient cell responses to H_2S, continuous monitoring might help to answer some of the open questions concerning sulphur bath therapy.

Working plan

1st year

Months 1-6

1. Cell characterization
1.a Selection of cell line

2. Design, fabrication and characterization of the Cell-Chip
2.a Design of microfluidics and dielectric sensor configuration
2.b Fabrication and characterization microfluidic biochip
2.c Surface modification, activation and functionalization strategies

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Initially meetings of all project participants will be arranged in order to reveal any contradictory assumptions of the project and get a detailed common understanding of the joint effort. The result of these meetings will be a list of (technical) requirements of the cell monitoring station.

Approximately 2 days per week are scheduled for *off-chip* cell cultivation and analysis that will be performed in the LBI facilities at Therme WienMed, Oberlaa. First experiments will be carried out to decide which cells should be used throughout the project. Main criteria will be a sufficient proliferation rate and high viability. For healthy fibroblasts we will additionally check for a (physiologically) low expression of inflammation markers.
Available cells will include various human fibroblast (HF) cell strains from donors with chronic polyarthritis (CP), osteo arthritis (OA) or healthy individuals. Cells will be obtained from AKH Wien through members of the Ludwig Boltzmann Cluster for Rheumatology, Balneology and Rehabilitation. Provided cell lines and strains include "HF 26 CP", "HF 45 CP", "VH skin fibroblasts" and "1BR3". Osteo arthritis cells ("HF 189 OA", "HF 190 OA") will be included into some experiments as controls. The cells will be cultivated in humidified atmosphere containing 5% CO_2 and constant temperature of 37°C. DMEM supplemented with 10% fetal bovine serum (FBS) and 50U Penicillin/ Streptomycin will be used as full culture medium. In some assays a reduced medium containing 5% serum will be used to keep background signals low.

The microfluidic biochip will be designed and fabricated. The chip will comprise three layers, namely the sensor layer, the fluidic layer and the connection layer. The sensor layer will consist of a 3x3cm glass substrate featuring a micro patterned metallization layer (50nm high gold film). Contact pads for connection with the system existing at AIT (potentiostat) as well as the newly developed measurement station, conductor paths and microscale sensors will be included. Requirements and design constraints for the electrode layout will be assessed in the first months of the project. In order to reduce ohmic contributions to the IDES sensor signals the electrode structures need to be insulted from the liquid sensing environment [16]. The equipment and technology provided by AIT Center of Thin Film Technologies (CTFT) will be available for the project to create an optimized passivation layer. Materials (e.g. tantalum oxide, titanium oxide, silicon oxide or silicon nitrate) and layer thickness (50-600 nm) will be optimized for mechanical strength, electrical inertness, sensor sensitivity and biocompatibility.
The second layer will feature the microfluidic channels connected to cell proliferation and measurement chambers. General design constraints for microfluidics such as avoiding sharp edges will be considered. Additionally 2D and 3D computer simulations using the COMSOL Multiphysics ® software environment will be performed to estimate flow characteristics and shear forces. The fluidic layer will be fabricated of the hard polymer SU-8. The PhoeniX Software CleWin, a hierarchical layout editor, will be used to define photomasks that can be applied for substrate patterning in lithographic production processes. The connection layer will comprise fluidic in- and outlet ports for connection with external tubes.

Months 7-12

1. Cell characterization
1.b Toxic effects of H_2S

2. Design, fabrication and characterization of the Cell-Chip
2.d Surface biocompatibility study
2.e Cell chip design revision

In the second half of year 1, cell characterization under standard cultivation conditions will be carried out. Investigations will focus on dose-dependent toxic effects of H_2S using commercially available test kits (e.g. Calcein-AM viability test, alamarBlue® proliferation assay). The sulfur containing compound sodium hydrosulfide (NaHS) will be used as exogenous H_2S donor. Interleukin 1-beta (IL1β, 10ng/ml) will be applied to induce an inflammation response in healthy fibroblasts. Data of previous studies carried out at LBI [8, 19, 20] will be used as starting points for NaHS incubation time and dose range. Different concentrations of the H_2S donor (0.1 – 10mM) and varying incubation times (15min to 24h) will be applied to assess dose dependent effects. Further experiments will be carried out to assess recovery time after exposure to sulfur containing compounds. This is of interest, because H_2S released by NaHS will rapidly evaporate from the aqueous cell culture medium, consequently limiting its time of action. This mechanism is profoundly different to *on-chip* experiments, where continuous medium supply in a gas tight system limits evaporation of H_2S. This difference has to be considered for detailed planning of *on-chip* experiments as well as for interpretation of experimental results.

Additionally, the biocompatibility of the cell-chip will be optimized. Surface modification techniques will be applied to create favorable *on-chip* culture conditions. Various surface chemistries will be evaluated in standard 6-well plates. Round cover glasses (32mm) with different surface modifications will be inserted into the wells and serve as substrates for cell adhesion. Modifications will include magnetron sputtering of defined passivation layers (e.g. tantalum or titanium oxide) alone and in combination with biomolecules frequently used in cell cultivation applications to enhance cell adhesion (e.g. gelatin or fibronectin). Main parameters of interest will be morphological changes, cell proliferation and viability.

In the last months of year 1, the electric properties of the cell chip will be characterized. For initial experiments the chip will be mounted onto the existing measurement system (potentiostat) to perform sensitivity and stability tests using various solvents (water, buffer, cell culture medium).

By the end of the first year an iteration step for cell chip design revision can be performed: In case results of the first tests should reveal any inadequacies in the cell-chip design, modifications and optimizations of the microfluidic- or sensor layout will be considered. Any necessary adoptions can easily be implemented at this stage of the project.

Working plan

2nd year

Months 13-18

1 Cell characterization

1.c H_2S induced alteration of signaling pathways

3. Cell-on-a-Chip configuration

3.a Integration of main system components for cell-on-a-chip experiments

3.b Fibroblast cultivation on chip

3.c On-chip assay standardization

4. Adaption of cell assays

4.a *On-chip* live cell imaging (fluorescent dyes) and time lapse microscopy

4.b Integration of *off-chip* ELISA analysis of inflammation markers

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In the first half of the 2nd year standard cell tests will be continued and *on-chip* cell cultivation protocols will be established.

Changes in various signaling molecules will be quantified under standard cultivation conditions. Extra cellular concentrations of the inflammation markers IL-6 and IL-8 will be quantified by performing ELISA tests of the culture supernatants. RTq-PCR will be used to determine changes in mRNA concentrations. Furthermore changes in other relevant signaling pathways will be assessed mainly by Western blots. The proteins under investigation will include the stress proteins HSP70 and HO-1, phosphorylated and unphosphorylated forms of mitogen activated protein kinases such as ERK, p38 and MEK as well as caspases 3, 7 and 9. Results of these experiments will then be used for detailed planning of *on-chip* experiments, where we will additionally aim at investigating time courses of cell responses.

A fully functional prototype of the computer controlled cell monitoring station will be set up at AIT laboratory. The station will comprise all necessary components for cell cultivation and monitoring including hard- and software as well as peripheral devices such as syringe pumps, tubes, valves and a heater.

Fibroblast cells will be cultivated repeatedly in the microfluidic system and reproducibility of cell handling, seeding and *on-chip* growth will be assessed. Optimized processes will be used as standard operating procedures in the following experiments. In general a cell-chip experiment will involve the following steps: sensor testing, preparation of the fluidic, recording of background signals, cell seeding and adhesion, continuous supply of medium or test substance, monitoring of cell responses, chip cleaning.

In order to get further insight into cell responses and verify correlation with *off chip* results, additional assays will be performed. In project months 13-24 *on chip* procedures for time-lapse microscopy, live-cell imaging as well as bioanalytical measurements (e.g. ELISA) of the effluent culture supernatant will be established. Non-toxic fluorescent dyes such as calcein AM (viability) or TMRE (tetramethyl rhodamine ethyl ester, for mitochondrial membrane potential) will be used to assess basic cell parameters. The culture supernatant will be collected and cytokine content (IL-6) will be quantified using adapted ELISA strategies for small sample volumes.

Months 19-24

1 Cell characterization
1.c H_2S induced alteration of signaling pathways

4. Adaption of cell assays
4.b Integration of *off-chip* ELISA analysis of inflammation markers

5. On-chip fibroblast monitoring
5.a Monitoring stress responses

In the 2nd half of year 2 cell testing under standard cultivation conditions will be continued. *On-chip* cell tests involving know stressors will be performed and protocols for chip-based ELISA will be optimized. Siemens will focus on the development of software for (statistical) analysis of impedance spectra.

Cell tests to assess effects of H_2S on synovial fibroblast signaling pathways will be continued as described for months 13-18.

Additional adaption and optimization of ELISA strategies for chip samples will be performed. Critical aspects to consider will be sample collection, maximal time resolution and assay sensitivity. Optimized ELISA procedures will be used to assess dose dependency of inflammation marker (IL-6) expression to sulfur.

On-chip cell behavior will be monitored in presence of various well defined stress conditions. Signals of cell populations exposed to oxidative stress (H_2O_2), varying shear stress and known toxins such as cycloheximide (inhibitor of protein biosynthesis) or mitomycin C (DNA crosslinker) will be assessed. Time curves of impedance spectra will be recorded and analysed to identify sensitivity and stability of *on-chip* measurements.

The data management and analysis software will be applied to interpret impedance spectra. Since capacitive changes occurring along membranes and at cell surfaces are frequency dependent [21], recording of frequency spectra rather than capacitance changes at a single frequency can provide additional information that allows discrimination of various cell responses. However, this method produces large amounts of data requiring adequate data collection and analysis software. Siemens will therefore work on the implementation and evaluation of statistical methods for multivariate data analysis.

Working plan

3rd year

Months 25-30

5. On-chip fibroblast monitoring
5.b Cytotoxic NaHS effects *on-chip*
5.c *On-chip* detection of rapid changes in NaHS induced cell responses

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In the 1st half of year 3 fibroblasts will be challenged *on chip* with H_2S releasing compounds.

The cell-chip station will be applied to monitor healthy and RA synovial fibroblasts exposed to H_2S. Experimental parameters such as incubation time, sulfur concentration and allowed recovery time will be based on *off chip* results. First NaHS concentrations ranging from non-toxic to toxic will be applied. Recorded impedance spectra will be analysed using the software implemented by Siemens. Data will be compared to results of *cell-chip* tests performed in year 2 and interpreted accordingly. This will reveal the sensitivity of the newly developed technology towards H_2S induced cell responses. Further experiments will then be carried out to refine cell-chip results and correlate them with results of standard assays performed in year 2. Finally we will focus on the monitoring of dynamic cell responses, such as transient suppression of IL-6 expression.

Supplementary chip-based assays (ELISA and fluorescence assays) will be included into the experiments. This will help to gain additional information on cell status and support impedance results.

Months 31-36

5. On-chip fibroblast monitoring
5.b Cytotoxic NaHS effects *on-chip*
5.c *On-chip* detection of rapid changes of NaHS induced cell responses

6. PhD thesis
6.a Writing of PhD thesis

In the 2nd half of project year 3, final *on-chip* H_2S experiments will be carried out. There will be also some time allowed to repeat any experiments if necessary.

Writing of the PhD thesis is scheduled as the main task of months 31-36.

Figures

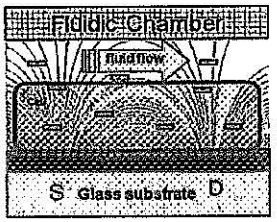

Figure 1: Schematic drawing of a dielectric microsensor integrated within a microfluidic device: The external electrical field penetrates the passivation layer as well as the fluidic chamber where it interacts with the dielectrical properties of the analyte (symbolized by yellow rectangles). (Graphic from AIT Nano Systems)

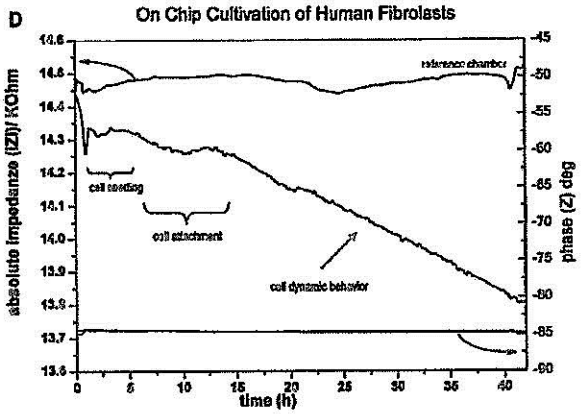

Figure 2: Bioimpedance traces obtained in the presence and absence of live fibroblast cells: A Cell-on-a-Chip system was used to continuously and non-invasively monitor human fibroblast cells. Absolute impedance of contact-less dielectric microsensors underneath the proliferation chamber correlated with the status of cells present in the electric field. (Data from AIT Nano Systems)

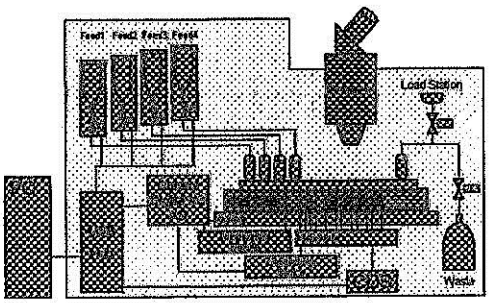

Figure 3: Hardware concept of the Cell-on-a-Chip platform (Diagram from the original FFG bridge application)

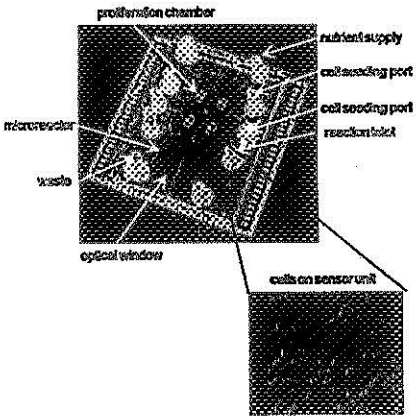

Figure 4: The picture shows a multiparamter microfluidic biochip developed at AIT Nano Systems including the sensor layer (gold structures fabricated using lithography), the fluidic layer (fabricated of a soft polymer (PDMS)) featuring channels, chambers and reaction sites as well as a glass cover holding microfluidic ports for connection to an external tubing system. The detail image shows human fibroblast cells seeded over the dielectric sensor unit of the cell-chip.

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Workplan Thesis Proposal N094

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Workpackage	1st year		2nd year		3rd year	
	months 1 - 6	7-12	13 - 18	19 - 24	25 - 30	31 - 36
1. Cell characterization						
1.a Selection of cell line						
1.b Toxic effects of H_2S						
1.c H_2S induced alteration of signaling pathways						
2. Design, fabrication and characterization of the Cell-Chip						
2.a Design of microfluidics and dielectric sensor configuration						
2.b Fabrication and characterization microfluidic biochip						
2.c Surface modification, activation and functionalization strategies						
2.d Surface biocompatibility study						
2.e Cell chip design revision						
3. Cell-on-a-Chip configuration						
3.a Integration of main system components for cell-on-a-chip experiments						
3.b Fibroblast cultivation on chip						
3.c On-chip assay standardization						
4 Adaption of cell assays						
4.a On-chip live cell imaging (fluorescent dyes) and time lapse microscopy						
4.b Integration of ELISA analysis of inflammation markers						
5. On-chip fibroblast monitoring						
5.a Monitoring stress responses						
5.b Cytotoxic NaHS effects on-chip						
5.c On-chip detection of rapid changes of NaHS induced cell responses						
6. PhD thesis						
6.a Writing of PhD thesis						