A system to measure, control and regulate customized physiological environment parameters on small animal models

F. Lamm¹, M. Scholtes², L. Mursina²,³, A. Weissflog¹, V. Gross², K. Sohrabi²,³

¹ThoraTech GmbH, Giessen, Germany, lamm@thoratech.de
²Technische Hochschule Mittelhessen – University of Applied Sciences, Giessen, Germany
³Competence Centre for Information Technology, Giessen, Germany

Abstract

Pulmonary diseases like COPD (Chronic Obstructive Pulmonary Disease), asthma bronchiale or pulmonary fibrosis need to be further explored, but there is a lack of technical options to collect and compare empirical data in long-term studies. In order to examine different issues in this context, various physiological environment parameters must be measured, controlled and simulated. Since such studies in humans are difficult to perform, a system called “LivingLab” was developed which allows long-term studies on small animal models under defined physiological environment parameters. The LivingLab was designed and built as a special cage system to handle up to 28 mice at once in four cages, while those are separated in a control and an experimental area. The cages are connected to a closed ventilation circuit, which ensures a controlled air and gas supply. The gas compositions may be arbitrarily chosen. Also aerosols may be administered. Based on a conducted animal research project, it could be proven, that the LivingLab offers the possibility to set, adjust and measure custom gas concentrations and to keep them constant over long periods of time.

1 Introduction

To further explore various respiratory diseases, often there is a lack of technical options to collect and compare empirical data in long-term studies. In order to examine different issues in this context, various physiological environment parameters must be controlled and simulated. Since such studies in humans are difficult to perform, a system called “LivingLab” was developed which allows long-term studies on small animal models.

The human respiration is mainly driven by arterial carbon dioxide partial pressure (pₐCO₂) and regulated by peripheral and central chemoreceptors. Usually, an increase of pₐCO₂ results in an increased breathing rate and breathing volume. Due to pulmonary diseases like COPD (Chronic Obstructive Pulmonary Disease), asthma bronchiale, pulmonary emphysema or pulmonary fibrosis, patients suffer from carbon dioxide (CO₂) retention which leads to an increased pₐCO₂ [1,2]. These hypercapnic conditions could cause an adaptation of the carbon dioxide chemoreceptors [3] leading to a decreased CO₂ chemosensitivity with a reduced ventilatory response [4].

To investigate this physiological context, a research project with the objective to establish a hypercapnic mouse model was performed. The „LivingLab“ system was used to simulate long-term hypercapnic environmental conditions.

2 Methods

According to the common principle of the “Marburger-Atemantwort-Messung” (MATAM) [3] certain gas concentrations are measured, regulated and controlled in a closed ventilation loop. Various gas, pressure, temperature and humidity sensors provide information about the current system state. Different inlet, outlet and gas valves are controlled and managed by the regulating unit which is software driven by a regulation algorithm.

The system software of ”LivingLab“ was developed with the Laboratory Virtual Instrument Engineering Workbench (LabVIEW - Version 2013 - 13.0f2 - 32-bit) from National Instruments (National Instruments Germany GmbH, Ganghoferstrasse 70b, 80339 München, Germany).

LabVIEW is a graphical programming language and a development environment at the same time. With the help of so-called Virtual Instruments (VIs), complex program sequences can be represented in block diagrams and implemented without external compilation. Since the data acquisition card was also obtained from National Instruments, necessary interface programming could be realized without any compatibility problems.

The required gases were obtained by the Linde Gas Division (Linde Gas Deutschland, Kostheimer Landstrasse 25, 55246 Mainz, Germany).

3 Results

The developed “LivingLab” was built as a special cage system which provides the opportunity to expose up to 14 test mice in two identical cages (seven animals per cage) to a defined gas concentration regarding oxygen and carbon dioxide. Up to 14 control animals can be kept to the same ambient conditions but exposed to normal ambient air at the same time (approx. 79% N₂, approx. 20.9% O₂, approx. 0.05% CO₂). Thus, the system provides the opportunity to
study the long-term behavior under controlled and specified conditions.

The four cages were made of transparent polycarbonate and are hermetically sealed by a lid. For feeding and cleaning purposes the lid can be opened by the user. The control cages were embedded in a closed ventilation circuit providing carbon dioxide concentrations up to 20% and oxygen concentrations up to 100%. The gases are conveyed at approximately 5 l/min by a fan through the gas system. In the cages the gases are distributed over different diffusers in order to ensure a homogeneous mixing. Image 1 shows the schematic structure of the entire system.

4 Conclusion

Based on the conducted small animal research project, it could be proven that the LivingLab offers the possibility to set, adjust and measure custom gas concentrations and to keep them constant over long periods of time. Furthermore, the combination of the built-in diffusers and the dual exhaust on the cage floor ensures a homogeneous gas mixture in all cages which contributes to guarantee identical life conditions while reducing stress because of the lower flow. With an area of 40 * 30 cm, one cage offers enough space for up to eight mice or other small animals. Due to the easy and intuitive interface, the handling of the LivingLab can be learned quickly.

In this case, the LivingLab was serving as a model of hypercapnia. It is also possible to generate other defined gas concentrations or to apply aerosols in a long-term altered small animal experiment if needed.

In future, a modular cage system for further applications will be implemented. Likewise, an isolated working cage with integrated gloves for examination of the animals under experimental conditions is planned. Thus, cages could be exchanged and cleaned without exposing the animals to a change of the gas concentration.

5 Acknowledgments

Image 1 Schematic diagram of the LivingLab

A software-driven intelligent measurement, control and regulation unit provides a constantly adjustable gas composition in this cage. It enables measurement and logging of temperature, air humidity, flow, oxygen and carbon dioxide concentrations in both, control and experimental area. The software regulates and stabilizes the gas composition automatically by controlling the corresponding valves in dependence of the measured values. A graphical user interface is used to set all control parameters and to represent all relevant data. If necessary, a manual control of the whole system is ensured at all times. Collected data is automatically backed up at user specified intervals.

To keep an equal noise and pressure-induced stress factor of all animals, a compressed-air valve (control area) was operated simultaneously with the CO₂ or O₂ application (experimental area). For safety, the excess CO₂ is vacuumed off the circulation by an CO₂ absorber in case of too high CO₂ concentrations. In addition, it is possible to add fresh air to the complete system using the inlet and outlet valve.

The complete system was successfully verified by several tests regarding the potential settings, the stabilization and regulation of defined gas concentrations, and the impermeability of the total system.

6 References


Spatially resolved pH measurement using iridium oxide coated micro electrode arrays
S. Lück, T. Schröder, W. Mokwa, Institute of Materials in Electrical Engineering 1, RWTH-Aachen University, Aachen, Germany

Introduction
Retinal implants restore vision for people suffering from retinitis pigmentosa. To avoid damage to the retinal neurons the influence of continuous electrode stimulation on these cells has to be studied. In this work a method for the measurement of local pH-values was developed using the IrO\textsubscript{x} coated electrode arrays, which are used for the stimulation of retinal neurons. By this the changes of pH-values due to the application of stimulation pulses over a longer period of time can be recorded in immediate vicinity and information about changes in the metabolism of retinal cells can be gained. Using the same electrodes for stimulation and pH measurement keeps the size of the device as well as the technical effort at a minimum.

Methods
Penetrating multi electrode arrays and planar electrode arrays were used during the studies (compare figures 1 and 2). Electrode diameters varied from 10 to 100 µm. The thickness of the IrO\textsubscript{x} coating was 500 nm. Electrode potentials were measured against an Ag/AgCl reference electrode in buffer solutions with known pH values between 6 and 9. To demonstrate the possibility of spatially resolved measurement, a pH gradient was generated by adding HCl solution at a defined point to the electrolyte with the electrode arrays. The time between the insertion of HCl and the reaction of the electrode potentials was measured and compared to the distances of the electrodes to the point of insertion (figure 3).

Results
The measured potentials are stable but dependent on the electrode sizes and the electrochemical state of the IrO\textsubscript{x}. This makes a calibration necessary. The results are reproducible. A mean sensitivity of about 55 mV/pH was measured. A spatial resolution of better than 400 µm can be achieved (see figure 3).
Figure 3:

a) Time curve of the potential of three electrodes placed behind one another as a reaction on a short pulse with HCl solution, applied in the direction of the electrodes with a syringe (figure b). The diameter of the electrodes is 100 µm (area 7850 µm², offset and sensitivity were normalized)

b) Measurement setup

Conclusion

IrOx electrodes can be used for spatially resolved measurement of pH values. Technical efforts are low. A fully integratable material for the reference electrode has to be found.
MAGNETO-HYDRODYNAMIC FOCUSING – A NOVEL METHOD FOR HIGHLY SENSITIVE BIOMOLECULE DETECTION

Reis C., Siegert C., Dimitriadis N.
Fraunhofer Project Group for Automation in Medicine and Biotechnology, Mannheim, Germany

Abstract: Magnetic bead handling is a common tool in purification of biomolecules and subsequent analysis of molecule concentrations. The knowledge of forces acting on magnetizable beads in magnetic fields enables to use them not only as a purification instrument but also for analytical purposes. We wanted to use this to design and build up a novel bioanalytical instrument, which is able to analyze proteins in unpurified samples. Therefore magnetic beads are forced to describe certain trajectories via oscillating magnetic fields. This allows us to move magnetic beads periodically in a hydrodynamic system. This movement creates a dynamic fluorescence signal, which gives an insight about how many molecules are bound to the particle surface. This MHF technology can be combined with several assays, which are already on the market, maximizing signal to noise ratio.

Keywords: magnetic beads, biomolecule detection, bioanalytics

Introduction
Magnetic bead handling is a common tool in on-chip biodetection systems [1] and research in this field is improving fast. Mostly whole cells or even biomolecules are analyzed by magnetophoresis, because binding of magnetic particles change their magnetophoretic behavior [2].

To analyze biomolecules like proteins or nucleic acids, magnetic particles can be equipped with binding partners on their polymeric surface. Currently, there are many distributors on the market, producing magnetizable particles from nm to µm ranges and often pre-functionalized with streptavidin to couple biotinylated capture molecules on their surface. Thus, high specificity can be reached for bioanalysis.

Magnetic bead technologies are used to purify the molecules of interest in an upstream process. After several washing steps, an analytical process like ELISA can be performed. The purification step via magnetic beads enhances the concentration of the molecule of interest in relation to the concentration of other proteins. Therefore the signal portion of molecule of interest in the sample increases and enables higher signal to noise ratios (SNR).

We wanted to use the magnetic beads not only to purify the protein of interest but also to do an analysis in parallel. This allows us to combine purification and analysis in a one step process without washing steps. Therefore we designed an oscillating magnetic field with certain geometry. This oscillating magnetic field concentrates magnetic beads in a liquid sample in a cuvette and therefore enables local purification. The movement of the magnetic beads in certain trajectories can be used to create a dynamic signal of protein of interest. The signal is mostly dependent on the magnetic force, hydrodynamic force and the portion of molecule of interest in the sample. Signal detection can be performed via fluorescence analysis by moving the magnetic beads in known frequency through the focus point of a fluorescence light source. This focusing of magnetic beads carrying molecules of interests in a measurement spot of a light source was named magneto-hydrodynamic focusing (MHF).

An unspecific fluorescence labelling, e.g. with FITC, results in labelling of all proteins. Only in presence of molecule of interest in the sample liquid, a binding event to the functionalized magnetic beads occur. By this binding event, the fluorescence portion of bound molecules of interest is oscillating together with the magnetic beads. Combined with specific labelling e.g. by...
Methods

The MHF system consists of two optimized elec-
tromagnets and a cuvette with 160 µl total vol-
ume (figure 1).

The optimization of magnetic fields and magnet
gometry was performed using ANSYS MAX-
WELL.

The motion equation was derived from magne-
tic forces and hydrodynamic forces acting on the
magnetic beads in a periodic way (figure 2).

\[
F_{\text{magn.}} - F_{\text{hydr.}} = m_{\text{Part.}} a_{\text{Part.}} + 6\pi \eta r v_{\text{Part.}} = V_{\text{Part.}} M_{\text{Part.(x,y)}} \nabla B(t)
\]

\[
m_{\text{Part.}} \frac{d^2x(t)}{dt^2} - 6\pi \eta r \frac{dx(t)}{dt} = V_{\text{Part.}} M_{\text{Part.(x,y)}} \nabla B(t)
\]

Figure 2: Motion equation for particle movement in the
MHF system.

The system is equipped with a fluorescence de-
tection unit to enable fluorescence analysis (fig-
ure 3).

The excitation light was emitted form a metal halide lamp (Leica EL6000) which delivers the
light through a liquid light guide. The light is
collimated by a condenser lens and filtered with
an excitation filter (Semrock FF01-475/35) to
obtain excitation light in a confined spectral
band around 475nm. The light is combined with
the emission path by a dichroic mirror (Semrock
FF499-Di01).

A 10x microscope objective (Olympus RMS10X) with an NA of 0.25 is used to focus
the excitation light to a confined spot in the focal plane of the objective. Fluorescence signal
emitted from the dye is collected by the same
objective lens. The emission is transmitted by
the dichroic mirror and filtered by the emission
filter (Semrock FF01-530/43). The emitted sig-
nal is focused with the tube lens (Thorlabs
ITL200) on the photosensitive cathode of the
photomultiplier tube (Thorlabs PMM01).

The electronic voltage signal of the PMT is op-
tionally filtered with an electronic 1st order
lowpass filter with a cutoff frequency of 10 Hz
to reject high frequency noise. The voltage sig-
nal is recorded with the digital oscilloscope Ag-
ilent MSOX 2024A.

For evaluation of the system a lyophilized pro-
tein of interest in human serum was dissolved in
PBS. All proteins were dyed using FITC. 1 µg
of magnetic beads (Chemicell, 1 µm in diam-
eter, streptavidin labeled) was coated with 0.1
pmol anti-protein of interest monoclonal anti-
body. A total of 6.5 ng protein of interest with
80 µg serum protein (both FITC labelled) was
analyzed in 160 µl total sample volume. The signal was
processed using MatLab.

Results

The simulation of the magnetic field and pole
shoe geometry was performed using ANSYS MAX-
WELL (figure 4). The optimal pole shoe
geometry for generating high B-Field gradients in
the chosen distance between electromagnets and
cuvette is a tapered iron core electromagnet with
130 mm in length and a tip diameter of 8 mm. For
our experiments, we used a winding of 1100 and a
current of 3 A. In case of all magnetic beads being
on the blue reference point and the left magnet is
switching on, high B-Fields exist between both
points (figure 5).
Figure 4: Simulation of the magnetic field of different electromagnet geometries (reference points in red and blue).

For generating an oscillating magnetic field, the magnets have to be operated at a frequency of 0.1 Hz and with a 30 mm offset. The resulting B-Field at the reference point is oscillating with the same frequency (figure 6).

Figure 5: B-Fields along the distance between left and right reference point after switching on the left magnet.

Figure 6: Oscillating B-Field for moving magnetic beads periodically.

The fluorescence labelling of protein of interest and serum protein resulted in high background noise. The portion of 6.5 ng protein of interest in 80 µg labelled serum protein as background corresponds to a signal ratio of 0.008%. The sample contained a total volume of 160 µl. Total protein of interest concentration was ca. 40 ng/ml.

After processing the data with MatLab, a signal to noise ratio of 2.3% could be obtained (figure 7). Therefore the signal of 10 oscillations was added on top of each other.

Figure 7: Signal processing with MatLab gives a clear signal for oscillating protein of interest bound to the magnetic beads.

Compared with a signal portion of 0.008% this resulted in an enhancement of about 300. Further optimization can be performed by adding more oscillation signals and by increasing the sensitivity of the used photomultiplier tube.
Discussion

With the MHF-system, we could show that a one-step process for detection of biomolecules is possible. The local concentration of magnetic beads and the oscillating signal give high signal to noise ratios even if the signal portion of protein of interest is very low. Currently 40 ng/ml of protein can be detected in high background sample liquids. Usage of a secondary detection antibody instead of an unspecific labelling of all proteins will increase SNR tremendously. Due to modular setup of the biotechnological system components, a wide range of assays can be performed on the system as long as a capture molecule is available which can be coated to a streptavidin tag. The system has the capability to be further miniaturized to use it as a point of care testing device.

Bibliography

A new technique for standardized assessment of the hypercapnic ventilatory response in mice

M. Scholtes1, F. Lamm2, L. Mursina1, D. Librizzi2, B. Müller3, U. Koehler4, V. Gross1, K.A. Sohrabi1, 3

1Technische Hochschule Mittelhessen – University of Applied Sciences, Giessen, Germany, michael.scholtes@kmub.thm.de
2ThoraTech GmbH, Giessen, Germany
3Competence Centre for Information Technology, Giessen, Germany
4Department of Nuclear Medicine, Philipps University Marburg, Marburg, Germany
5Laboratory of Respiratory Cell Biology, Department of Internal Medicine, Division of Pneumology, Philipps University Marburg, Marburg, Germany
6Division of Respiratory and Critical Care Medicine, Philipps University Marburg, Marburg, Germany

Abstract

Several pulmonary diseases and sleep disorders lead to hypercapnia, due to an increased airway resistance. The retention of carbon dioxide may influence the appropriate chemoreceptors and thereby cause a reduction of the hypercapnic ventilatory response. Many questions according to hypercapnia and its respiratory responsiveness, especially in the field of basic research, cannot be investigated in human beings. Hence, we developed a method for a standardized and reliable measurement of the hypercapnic ventilatory response in mice, based on our experience in human medicine and previous studies. The measurement system consists of a whole body plethysmograph, a gas mix chamber, a bias flow generator and a control and analysis unit. The system allows a continuous measurement of the ventilatory parameters and the corresponding and consistent increasing CO2 concentration. We achieved a reliable increase of the carbon dioxide concentration which allows a better comparability between several measurements. Parameters like maximum CO2 and rate of increase can be set individually, depending on the particular study design. We conclude that the measurement system named 'Marburger Mäuse Atemantwort Messung' produces reliable results and can be established as a standard method for the determination of the hypercapnic ventilatory response in mice. We have already planned further optimizations and validation studies are intended.

1 Introduction

1.1 Hypercapnic ventilatory response

Hypercapnia is a manifestation primarily caused by numerous pulmonary diseases, such as asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis [1] and obstructive sleep apnoea syndrome [2, 3]. An increased airway resistance leads to a retention of carbon dioxide (CO2) and consequently to an enhanced CO2 concentration in arterial blood (PaCO2) [4, 5]. These hypercapnic conditions presumably influence the CO2 chemosensitivity [6] and thereby the hypercapnic ventilatory response (HCVR) [7]. However, a better comprehension of the receptor's physiological characteristics would be necessary for an appropriate research of the named pulmonary diseases [8]. The investigation of HCVR has always been complicated because both CO2 and oxygen (O2) cause ventilatory drive [7]. In 1967 a rebreathing technique was evolved by Read [9]. In 2009 Sohrabi [6] developed a standardized HCVR measurement and analysis system based on these preliminary studies. In basic research in particular there are many questions according to the HCVR which cannot be studied in human beings. Therefore, it is necessary to transfer existing techniques to a system for laboratory animals.

1.2 Pulmonary function in mice

Barometric whole body plethysmography (WBP) for unstrained animals is a widely used method to evaluate the ventilatory function with several advantages [10, 11, 12, 13]. It avoids handling-induced animal stress and allows continuous monitoring of the ventilation for relatively long periods (up to 24 hours) [14]. Compared to anesthetized animal models, it avoids any pharmacological interference with anaesthetic agents and enables using the same animals for repeated measurements. This technique is often used for the evaluation of disorders such as asthma or COPD [15, 16, 17]. Nevertheless, these experiments require that the conscious animals have to be adequately acclimated to the WBP to warrant stabilized respiration [18]. An additional stimulation with defined CO2 gas concentrations enables the HCVR measurement in mice. Recent studies measured the HCVR with the help of a WBP to investigate miscellaneous questions [19, 20, 21]. They all used different methods to assess the HCVR, and comparability is not guaranteed.

Based on the experience of the 'Marburger Atemantwort Messung' [6] and the cited previous studies, we intended to develop a standardized and affordable HCVR measurement system for mice.
2 Methods

The development of the HCVR measurement system, the so-called MMATAM ('Marburger Mäuse Atemantwort Messung') has been developed in cooperation with ThoraTech GmbH (Gutleischstrasse 3-5 (D12), 35390 Giessen). The system is composed of numerous components. A whole body plethysmograph (EMMS, Unit 32, Woolmer Way, Bordon, Hants, UK, GU35 9QF) enables the acquisition of spirometric data such as respiratory frequency (RF), tidal volume (TV) and minute volume (MV). The attendant bias flow generator, model air 220 (EMMS), ensures the fresh air supply and eliminates humidity to stabilize the measurement conditions. The CO$_2$ sensor (K33 ICB SA, of Firma Driesen + Kern GmbH, Am Hasselt 25, 24576 Bad Bramstedt, Germany) facilitates the continuous analysis of carbon dioxide.

The required gases were obtained by the Linde Gas Division (Linde Gas Deutschland, Kostheimer Landstrasse 25, 55246 Mainz, Germany). The CO$_2$ mix chamber (ThoraTech GmbH) is fed by the CO$_2$ gas bottle and the ambient air to ensure an isooxic and hypercapnic gas concentration. Optionally, an O$_2$ gas bottle and an O$_2$ sensor can be connected to the system, for simplification we renounced these features in our trials. A blower inside the chamber generates the required flow to transport the current gas concentration to the WBP. The HCVR control unit detects the current ventilation data, the corresponding CO$_2$ concentration and controls the gas valve in the gas mix chamber. For the analysis of the HCVR, the ventilation parameters are matched with the appropriate CO$_2$ values and displayed in dependency. The gradient of the curve (ventilation parameter depending on CO$_2$) represents the HCVR.

3 Results

We developed the HCVR measurement system as illustrated in image 1. Mice are unrestrained and conscious during the measurement.

Image 2 shows the results of the verification tests. We achieved a very good reliability regarding the CO$_2$ behavior in the WBP plotted over time. Therefore we established a basis for exposing mice to these conditions later on. In addition, ventilation parameters (MV, AF, TV) can be detected.

![Image 2 measured CO$_2$ concentrations in the whole body plethysmograph.](image)

Image 3 shows the calculated linear regression of the matched data pairs of MV (respectively AF or TV) and corresponding CO$_2$ concentration exemplary.

![Image 3 HCVR analysis using linear regression of matched parameters (MV and CO$_2$ concentration).](image)

The HCVR is calculated using the slope triangle:

$$HCVR = \frac{\Delta MV}{\Delta CO_2}, [HCVR] = \frac{ml}{vol \% \cdot min}$$

The maximum CO$_2$ (up to 30 vol.%) concentrations and the rate of increase can be adjusted depending on the particular study design. However, we recommend a maximum CO$_2$ concentration of 8 vol.% because values above 9 vol.% can lead to respiratory depression and finally cause death [22, 23].

![Image 1 Schematic diagram of the HCVR measurement system.](image)
4 Conclusion

We could show that the HCVR system 'Marburger Mäuse Atemantwort Messung' produces reliable results and facilitates the standardized measurement of the HCVR in mice. The new technique combines Read's method of analysis [9] and the reproducibility and stability of Sohrabi's HCVR measurement system [6]. Furthermore, the user can set the required parameters for the individual study design. The system was already validated successfully in animal experiments, the collected data is in preparation for publication. Nevertheless, we are still in the progress of optimization and enhancements. We currently work on a recovering method which allows a more efficient utilization of the consumed gases. We are aiming at establishing a new standard method for the assessment of the hypercapnic ventilatory response in mice. Further studies are planned to confirm these results.

5 Acknowledgments

6 References


Microfluidic invivo model of the blood-brainbarrier: a biomimetic platform for drug development with active cell assembly

H. Kiessling1, D.Raible1, V. Rack1, B. Hagemeyer1, M.Stelzle1 and J. Schütte1

1 NMI – Natural and Medical Sciences Institute at the University of Tübingen, Germany, heiko.kiessling@nmi.de

Introduction

The development of drugs for the treatment of diseases of the central nervous system (CNS), such as Alzheimer’s disease or multiple sclerosis, is still a major challenge. The main obstacle is the blood-brainbarrier (BBB), which prevents the passage of large molecules and many small molecules into the CNS. Currently, there are no sufficiently predictive models of the BBB. Several concepts to improve cell function by mimicking their natural environment have been established in the last years1, but there is still no satisfying solution. Hence, we are establishing a microfluidic chip to actively arrange vital endothelial cells by dielectrophoresis to form a vertical tight BBB.

Chip structure and function

The microfluidic chip comprises two parallel flow channels, separated by an assembly of insulating micro pillars, and a pair of electrodes at the channel sides. Using two phase polyecondensation, a thin polyamide membrane is established between the micro pillars. Subsequently the membrane is coated to encourage cell adhesion. The electrodes are used to assemble endothelial cells between the pillars on the polyamide membrane by generating dielectrophoretic field. Furthermore they can be used for transendothelial electrical resistance measurements to validate the established cell structures. Nutrient gradients as well as shear forces on the cells were simulated during continuous flow through the microfluidic channels. Once the blood-brainbarrier is established between the channels, it can be used to test the potency and permeability of drugs.

Results

Using multiphysics simulations (CFD-ACE+) we designed and optimized the micro pillar structures for assembly of endothelial cells. Experiments confirmed the accumulation of endothelial cells between the micro pillars. Furthermore we developed a method to integrate a polyamide membrane into injection-moulded chips consisting of cyclic olefin copolymer. CaCo2-cells were successfully assembled on this membrane. Currently we are improving the processes to cultivate the cells to form a closed membrane.

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Biocompatible and actuated Carbon Nanotube-based electrodes for advanced neural interfacing

K. Tegtmeier1,*, J. Stieghorst1, P. Aliuos1, O. Majdani1, T. Lenarz1, T. Doll1

1 BioMaterial Engineering, Cluster of Excellence Hearing4All, ORL, Hannover Medical School, Hannover, Germany
* corresponding author: katharina.tegtmeier@mh-hannover.de

Introduction

Improving hearing sensations in patients using cochlear implants, whilst keeping insertion trauma at a minimum, is a major goal in auditory research. Reduction of the distance between nerve cells and the electrode shaft is important to realise stimulation with smaller electrical fields and more electrode contacts. To additionally reduce insertion trauma an electrode shaft with reduced stiffness that changes from a lateral position during implantation to a perimodiolar position after implantation is necessary. Since this is not possible with state of the art materials, a much more flexible electrode array is necessary.

Methods

To realise the self-bending of the shaft, a hydrogel swelling element was integrated, using a novel coextrusion and overmolding device. To evaluate the actuation mechanism, which is quantified using light microscopy, the shaft is stored in pure water. As a very flexible conductive material carbon nanotubes were immersed in liquid RTV-2 silicone rubber to replace the platinum wires. Conductivity was improved by varying means including alignment processes. To assure good interaction with the neurons, silicone was etched of the CNTs on the electrode contacts. To integrate connecting wires, metallic particles were included into the CNT material. Conductivities of variations of this material were measured after different treatments to improve conductivity further. Biocompatibility of all materials was shown in cell tests with fibroblasts and neuronal cells as well as the WST-1-Assay.

Results

More than anatomically needed rotations were observed, showing the proof of principle. It has been found, that the CNT-Silicone has a good conductivity which can be further improved by adding metallic particles. Etching processes lead to better conductivity and biocompatibility at the cell-interface.

Conclusion

Comparing the novel design with conventional electrodes it should allow an increased number of electrode contacts and smaller areas of stimulation with good neuron-electrode interaction, due to CNTs on the interfaces.
BioMEMS for analysis and synthesis in life sciences

M. Stubenrauch, S. Hanitsch, R. Fischer, H. Bartsch, A. Straube, M. Hoffmann, H. Witte,
TU Ilmenau, Institute of Micro- and Nanotechnologies MacroNano®, Ilmenau, Germany, hartmut.witte@tu-ilmenau.de

Introduction

BioMEMS based on silicon are valuable handling systems for cell cultures and bio-analytics. Even though other materials like polymers, ceramics, glass recently have been technologically improved and gain increasing relevance for miniaturized systems.
BioMEMS allow modular configurations for various setups. They provide reduced dead volumes as well as precise ‘cell housings’ with controlled environmental conditions and loads (stress) for cells, to initiate or modulate cell proliferation and differentiation.
The assembly and interconnection technologies presented allow the combination of different materials to enable efficient exploitation of their advantages tuned to the desired system or biological experiment.

Methods

Micromachining of standard materials (silicon, glass, ceramics) and nanostructures for the interconnection of such heterogeneous compounds enable multiple arrangements for cell culture experiments. Hydrogel based 3D-scaffolds serve as substitute and initial growth pattern for cells inside the tissue structure. They can also be coated by bio-functional molecules, to induce the desired behaviour of the cells during the experiment.
Sensors for physico-chemical parameters and standard imaging allow online observation of such experiments. In case of mechano-sensitive cells standard procedures are inverted (homeostasis takes place at the point of experiment).

Results

Osteoblasts and Chondrocytes (cell lines and primary cells) have been successfully cultivated in BioMEMS and on 3D-scaffolds made of hydrogel materials. During these experiments it turned out that integrated sensors and a higher degree of automation is desirable.

Outlook

Current work is focussed on sensor integration and innovative, mainly optical sensor systems for enhancing measurement quality and quantity. Automation of cell culture processes in a “System for Automated Cell Cultivation and Analysis” (SACCA) is on the way. Based on the systems existing, functional extension will be done in the field of synthesis of drugs or biomedical substances in cell culture BioMEMS. Drug on demand or personalized drug composition will be producible without large investments into laboratories or machinery.
Thermal stress in thin-film polyimide-based electrode arrays

Juan Ordonez, Thomas Stieglitz, Department of Microsystems Engineering, Laboratory for Biomedical Microtechnolo- 
gy, BrainLinks-BrainTools Cluster of Excellence (ExC1086), University of Freiburg, Freiburg, Germany, o-
donez@imtek.de

Introduction

Implemenation of thin-film electrode arrays in neuroprosthetics is limited by their structural long-term stability. Aware-
ness of the inherent stresses left over from the fabrication procedure is of primordial importance not only to understand 
failure, but also to counteract it to a certain extent. Even though many material specific parameters play an important 
role in the induced stress, the most important fact ends up being the interplay between the material combinations, which 

Methods

Mathematical calculations as well as finite-element methods were used to calculate the layers’ thermally induced resid-
ual stress induced from the fabrication procedure for polyimide-based thin film electrode arrays. The stress in different 
layer stacks containing symmetrical and asymmetrical layers of platinum and polyimide as well as platinum, silicon 
carbide (SiC), diamond-like carbon, gold and polyimide was calculated. The layer thicknesses were parametrized to ob-
tain an optimal material’s thicknesses for reduced stress.

Results

Both, mathematical calculations and finite element modelling conclude that platinum deposited on polyimide experi-
ences a thermally induced residual stress much higher than its fracture strength (which would lead to delamination). 
These stresses are compressive within the symmetrical layer stacking (wiring) and tensile at the electrode’s opening. 
The use of thin (~50nm) silicon carbide as adhesion layer between polyimide and platinum allows a cancelation of the 
thermal stress at the critical interface between the polyimide and the silicon carbide, shifting the stress towards the 
much more stable SiC-Pt interface.

Conclusion

Mathematical predictions are of great aid for visualizing critical structural situations on electrode arrays. Even if a 
strong chemical bond is present between the layers, knowing the optimal layer thickness is crucial for the stability of the 
system, which allows us fabricating electrode arrays with longer stability than so far achieved with this technology.
Toward thin-film based high density hermetic feedthroughs for direct on die implementation

C. Bentler, J.S. Ordonez, M. Schuettler, T. Stieglitz, Department of Microsystems Engineering, Laboratory for Biomedical Microtechnology, BrainLinks-BrainTools Cluster of Excellence (ExC1086), University of Freiburg, Freiburg, Germany, christian_bentler@web.de

Introduction
Active implantable medical devices aspire to more sophisticated applications which demand for higher integration but state of the art IC demand for protection. To take full advantage high density feedthroughs at manageable price are needed. Conventional feedthrough concepts do not provide sufficient density and simple down scaling leads to insufficient hermeticity and poor crack resistance. Thus high density feedthroughs for hermetic packaging of ICs were developed. They consist of only a few additional layers and can be realized without expensive mounting steps using only standard thin film technologies hence allowing implementation during IC production.

Methods
To investigate the influences of different geometry parameters and local defects within layers FEM simulations were conducted. To identify an appropriate conductor, metal membranes were fabricated using photolithographic methods on silicon and tested for their hermeticity using a helium leak test. SiO, SiC and TiO were considered as insulators. Thus different layers were deposit and characterized using impedance spectroscopy and CV to complete the search for an appropriate insulator.

Results
Simulations show that diffusion rates for geometries with ~100 µm diameter are sufficiently low for hermetic packaging and suggest superior crack resistance compared to conventional feedthroughs. Tests proved fabrication of stable metal membranes despite high stress and suggest leak rates below detection limit (< 10⁻¹² mbar · l/s) for them. Measurements revealed specific resistivities of 11.7 ± 0.3 Ωm and k values of 52 ± 16 for TiO made by anodization. Preliminary results for SiO and SiC encourage their use as insulators.

Conclusion
Promising results encourage continuation of development toward a first realization of the high density feedthroughs for hermetic packaging of ICs.
Proposal for a contribution for the BMT Focus Session: Integrated Technologies support Bio-Medicine

Methods for modelling 3D cellular environments
Schober, A et al.

Combining modern methods in microsystem technology with the latest advancements in the life sciences, namely those in tissue engineering and advanced cell culturing, is promoting the development of a promising toolbox for modeling biological systems. The core problem to solve using this toolbox is the design of 3D artificial cellular environments, both in fluidic systems and on solid substrates. The construction of 3D cell cultures on substrates involves various fabrication techniques which use different polymers and biopolymers processed by micromachining, chemical pattern guided cell cultivation, photopolymerization, and organ printing methods. These methods together have the potential to create an artificial system with the complete hierarchical, geometrical, and functional organization found in an actual biological system [1].

Starting from our work concerning 3D cultivation as a first step towards more biological modelling of cellular environments [2] we performed different studies concerning the use of such systems as pharmacological test platforms [3], the different genetic behaviour of the cells in 2D and 3D [4], different structuring techniques [5, 6] towards the integration of different organ like systems on one chip [7].

In this contribution we will explain our approach to gain complex cellular structures while using chemical and mechanical modification of thin polymer foils. Due to folding and stacking of this pre manufactured cell sheet layers it is possible to achieve complex cellular and fluidic entities which are integrated in micro bioreactor systems.

With the combined application of different methods it is possible to mimic complex tissue like structures of different organs. Preferable with the liver lobe we demonstrate the construction schema of such a multilayer techniques.

We will discuss the different methods of designing such structures. Open questions of cocultivation different cell types, structuring Disse space like areas and the methods thereof will be analyzed and conclusions are given.

The liver plays a crucial role for the metabolism of both nutrients and drugs. Understanding and modelling of organomimetic cultivation substrates is a key technology with high potential for future developments in pharmaceutical drug discovery and tissue engineering.


Biofeedback – Smart Modality Fusion for Clinical, Home and Outdoor Health Monitoring

B. Venema1, N. Blanik1, V. Perlitz2, S. Laffar2, H.G. Ortlepp3, S. Borik4, J.-P. Jansen5, M. Koeny1, V. Blazek1, S. Leonhardt1

1 Philips Chair for Medical Information technology, RWTH Aachen University, Aachen, Germany
2 Department of General, Visceral and Transplantation Surgery, Uniklinik RWTH Aachen, Aachen, Germany
3 CIS Research Institute for Microsystems and Photovoltaic GmbH, Erfurt, Germany
4 Department of Electromagnetic and Biomedical Engineering, FEE, University of Zilina, Zilina, Slovakia
5 SZ Pain Center Berlin GmbH, Berlin, Germany

Abstract

The demographic transition in many industrialized countries may also lead to problems in the health system. In the elderly population, the vulnerability for diseases of affluence may increase significantly. Therefore, an objective of increasing relevance will encourage the patient to assume a more active role into prevention, therapy and rehabilitation (training, relaxation exercises) which will not only optimize treatment but eventually economize public health expenses. Non-invasive sensor concepts and portable 24/7-biofeedback systems may take over a pivotal role responsible even for monitoring vital bodily functions supplying support in times of crises. Based on this idea, the current research project Ear-Biofeedback-System aims to develop a photoplethysmography-based biofeedback system for treatment of vegetative dysfunction.

1 Introduction

A great number of medical conditions relate to dysregulations within the autonomic nervous system (ANS) since many vital bodily functions are under ANS control, including e.g. cardiovascular, respiratory, or metabolic processes. Commonly referred to stress, such dysregulations often originate from situations exceeding the individual’s faculties to cope with internal or external challenges. Insofar, either of these challenges may be result or source of stress, eventually manifesting diseases. Any therapy is likely to be successful when applied individualized adaptations. In this respect, non-invasive diagnosically and/or treatment approaches bear potential to gain substantial advantage since they promise to abide by definition of the fundamental medical rule of *nil nocere* - avoiding harm. New approaches often aim to substantiate therapeutic success through the active involvement of the patient (“patient in the loop”). The implementation of this combination of diagnosis and treatment is the aim of this research project. For this, a highly integrated biofeedback system was developed based on photoplethysmography (PPG).

PPG is a non-invasive, optical method to obtain blood volume shifts at the point of measurement. Blood volume shifts are generally caused by blood pressure variations coming from various physiological processes. Therefore, PPG is capable to derive many different vital signs like heart and respiratory activity, blood pressure variations etc. We expect our in-ear PPG system to be capable of detecting rhythmic, vital phenomena that are directly linked to central nervous system (CNS). With a user feedback in form of a mobile phone (smartphone), a technological/biological regulation loop will be realized whereby the patient will be trained to influence (regulate) vegetative functions to reduce stress.

Besides the in-ear biofeedback system, algorithmic approaches are presented in this work that are important for CNS related biofeedback. In addition, medical application scenarios are discussed.

2 In-ear PPG Biofeedback

2.1 Treatment rationale

Physiological functions can be controlled using biofeedback if they are connected to cortical structures of the CNS [1]. Further, many vital functions are related to the two branches of the ANS, the sympathetic nervous system (SNS), and the parasympathetic nervous system (PNS) [2]. The SNS mediating stress responses and the PNS mediating the “relaxation response” [3], supplies evidence of a maze of possible applications since the ANS effects almost all vital functions, be it temperature, respiration, cardiac activity, food and liquid intake, reproduction etc. Consequently, there should be a vast number of medical conditions susceptible to biofeedback treatment. In spite of such evident considerations, corroborated soundly by common sense, it remains enigmatic why this apparently powerful approach receives poor attention among medical professionals.

Currently, biofeedback of essential arterial hypertension, asthma, constipation, stress incontinence, anxiety disorders, as well as few somatoform disorders such as functional dyspepsia, tension headaches, migraine, and back pain has received scientific attention. In these conditions, biofeedback is applied usually as part of a therapy concept spanning various treatments. It needs to be emphasized that biofeedback was demonstrated to be the most effective non-pharmacological treatment of pain patients suffering from headaches, chronic back pain, and inconti-
ence [1,4]. A more recent pilot study of biofeedback treatment of migraine patients confirmed the validity of this treatment rationale [5].

2.2 System concept
Prerequesition of a mobile, unobtrusive biofeedback system is the development of a remission PPG sensor element to be placed in the outer ear channel, which does not impede in daily life while being a physiological keyhole to the heart. Combined with a small, high-precision interface device that can be worn at the backside of the concha, pre-processed vital data is send via Bluetooth to a mobile device for visual biofeedback. For medical support in case of emergency or for therapy control, data can be broadcasted to clinical facilities. The entire biofeedback is schematically shown in image 1.

Image 1 Schematically illustration of the in-ear PPG biofeedback setup. Adapted from [6].

2.3 Sensor hardware design
The remission in-ear-PPG was previously developed in BMBF (Federal Ministry of Education and Research, Germany) funded research projects INMONIT and LAVIMO. In image 2, the progress in the construction of in-ear photoplethysmographic sensors at CiS is shown: two modifications are the main contribution in sensor further development regarding the reduction of motion artefacts.

Image 2 Sensor assembly on flex-print.

A possible source of motion artifacts are movements of the optical module against the skin. To reduce such movements both the optical module and the ear mold have been changed.

The silicon chip containing both the light emitters and the photo-receiver that is now assembled directly on a flex-print board, without the former used glass carrier (image 2). Thus the seismic mass of the optical module is reduced almost by factor two, and we expect that head movements can be better followed by this new module because of less inertial force. During chewing or speech the upper and lower parts of the ear move against each other. The former used carrier of the optical module – a stiff ear mold known from hearing devices – could not follow such deformations. Thus both the lateral position and the pressing force of the sensor changed during movement of the lower jaw. A new soft ear mold has been developed to reduce artefacts caused by de-placing of the sensor (image 3). Presently the evaluation of the soft ear mold equipped with the new sensor assembly is in progress.

Image 3 Stiff and soft (elastic) ear mold.

With respect to power consumption, one of the most difficult aspects in low power wireless sensor applications is wireless transmission of vital data. In the developed interface electronic, we implemented the Bluetooth (BT) 4.0 communication standard using a BLE112-A Bluetooth module including low energy profile. Today, this offers the most energy effective, standardized wireless transmission for Smartphone applications. Although the BLE112-A consists a programmable microcontroller, an additional MSP430F169 (Texas Instruments, ultra-low mixed signal processor) microcontroller as main processing unit is used with respect to the complexity of the digital signal processing. With the remission sensor concept used, cardiac signal component is ten times smaller than the alternating signal component measured with transmissive sensors. Therefore, a 24-bit AC/DC converter is used.

Image 4 Miniaturized medical/technical high precision interface device with low power Bluetooth connectivity.
The miniaturized device (image 4) is built with support of Binder Electronics GmbH, Sinsheim. Since it contains a Li-Ion battery that can be charged via micro-USB connection, it represents an energy self-sufficient health care product. Several firmware-related energy saving strategies are implemented. More technical details can be found in [6-8].

3 Advanced biofeedback relevant algorithm strategies

3.1 Heart and respiratory activity related stress control

From PPG, heart and respiratory activity can be derived. In order to be able to differentiate relaxation from stress, analysis of heart rate and respiration rate are needed, for stress is likely to go along with increases in these parameters. Nevertheless, stress is a physiological phenomenon with very high inter- and intra-individual variation. Therefore, an “absolute” quantification of a stress-level is challenging. Newest research focus on variations in respiration and heart frequency since this is more promising for stress-level quantification.

One major purpose of our biofeedback system is autogenous treatment of pain. Pain is a subjective feeling that is influenced by many various factors. In general, pain assessment is stress assessment as well. Due to a narcosis, during surgical interventions external influences are reduced. Currently used algorithms for pain assessment like for example the Analgesia Nociception Index (ANI) [9] or Surgical Stress Index (SSI) [10] analyze the heart rate, amplitude and heart rate variability based on the ECG or PPG [11].

3.2 Tissue perfusion related stress control

The tissue perfusion index (TPI) as an assessment of dermal perfusion permits relevant insights into the development of stress level of the monitored patient [12]. The TPI is calculated from recorded data, which can be directly derived from the recorded PPG curve (compare image 5):

$$TPI = \frac{I_{AC}}{I_{DC}} \frac{1}{t_{PPT}}$$

(1)

Hereby, the perfusion amplitude $I_{AC}$ is normalized by the steady component ($DC$) of the signal $I_{DC}$, and the peak-to-peak distance of two neighboring maxima of two separate beats $t_{PPT}$, which is reciprocal to the momentary heart rate (HR). As result, undefined effects of constant, bloodless tissue absorption are suppressed. However, time-depended stress-correlated changes of tissue perfusion or HR will lead to relative changes of the TPI [13], which are utilized in the feedback process. Further, TPI allows an assessment of signal quality as well as pulsatility of dermal perfusion.

2.3 Pulse wave form related stress control

The Oliva-Roztocil index (ORI) gives insight into the influence of arterial elasticity on the PPG signal that is highly significant for PPG pulse wave form and therefore important for the interpretation of PPG signals [15]. Since biofeedback has also a high potential for the elderly with (usually) reduced arterial stiffness, the inclusion of this parameter is of importance. Blood pulse waves propagate through the whole arterial tree in various forms depending on its location towards periphery. The pulse wave contour (PWC) is similar to the PPG signal and influenced by physiological or pathological changes of arterial system. Arteriosclerosis is the one of the primary pathological PWC modulators and there are many indices which are used for assessment of the arterial tree state, e.g. for assessment of arterial elasticity [14].

Image 5 Description of TPI and ORI parameters from typical physiological pulse wave form.

To distinguish between different degrees of arteriosclerosis and to visualize the influence of stress and pain on vascular wall characteristics ORI can be used. Comparing to another commonly used arterial elasticity indices this index offers heart rate normalization and its use can be more powerful at differentiation of lower degrees of arteriosclerosis [15]. ORI calculation is based on the ratio of two time ranges (Eq. 2).

The first time range is measured between ascending and descending arm of PWC at two-thirds of signal amplitude $AC$ (image 5). The second time range represents heart rate period $PPT$ [15].

$$ORI = \frac{P}{PPT}$$

(2)

Simulations were performed to correlate arteriosclerotic changes with the ORI. The simulation was based on an electromechanical model of arterial system (based on the works of [16 - 18]). ORI calculation was used for determination of changes in PWC and the simulation was performed several times for different values of arterial elastic moduli, which was increased step by step using modulation coefficient $c_m$. ORI increases with the higher values of arterial elastic moduli (Table 1) suggesting of arteriosclerosis presence...
possibility in arterial tree. Using the simulation, the performed measurements were validated [15].

Table 1 Dependency of ORI on arterial elastic moduli

<table>
<thead>
<tr>
<th>Elastic moduli</th>
<th>$c_m$</th>
<th>ORI</th>
</tr>
</thead>
<tbody>
<tr>
<td>physiological</td>
<td>1</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>0.262</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
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</tr>
<tr>
<td></td>
<td>4.5</td>
<td>0.373</td>
</tr>
<tr>
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<td></td>
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</tbody>
</table>

4 Conclusion and future application scenario

In this progress report, a biofeedback concept for the treatment of dysfunctional diseases is presented that is based on a wireless, wearable and nonobtrusive in-ear PPG sensor system. This work is part of an ongoing BMWI-funded research project. The technical components and the system design was presented. The overall biofeedback concept and algorithmic strategies relevant for stress-level assessment were discussed.

In the future, biofeedback can be used to improve the treatment of autonomic dysfunctions. One important medical scenario and the main objective of this project is the treatment of patients suffering from chronic pain. Chronic pain results various pathological consequences, such as arterial hypertension or specific manifestations of depressions. Constant exposure to pain frequently restricts the patient’s attention and perception focus on “their” pain, thus distracting it from well-being of other parts of their body. Haunted by the pervasive presence of pain, biofeedback has been demonstrated to restore freedom relying mostly on the body’s own faculties. Nothing less should be expected from any well conceived remedy.

5 Acknowledgements

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6 References


CMOS integrated biosensor for the detection of biomarkers

Kim Burmester¹, Andreas Goehlich¹, Yusuf Celik¹, Radostina K. Manova², Luc Scheres³, Esther Roeven³, Anke Trilling³, Andreas Schmidt¹, Ulrike Hutten¹, Katrin Neureiter¹, Sebastien Pierrat¹, Teris A. van Beek², Holger Vogt¹
¹ Fraunhofer Institut für Mikroelektronische Schaltungen und Systeme, Finkenstraße 61, 47057 Duisburg, Germany
² Laboratory of Organic Chemistry, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, The Netherlands
³ Surfix B.V., Dreijenplein 8, 6703 HB Wageningen, The Netherlands

In this contribution we report on results of a micromechanical sensor intended for the detection of allergens and biomarkers, which has been developed within the framework of the Euregio funded project “UniHealth”. This project aims at the development of a cost effective label free biosensor system that is intended for point of care applications concerning the detection of large range of allergens e.g. the papain enzyme, gluten and peanuts and the detection of biomarkers e.g. cholera toxine B on GM1 saccharide. The typical mass of allergens and biomarkers is in the range of 20 kDa and 80 kDa. For the label free detection of allergens and biomarkers a mass sensor with micro and nanostructures is a promising detection principle. In this work we report on electrical measurements of membrane structures that have been realized with CMOS compatible pressure sensor technology.

The adopted sensor principle relies on electrostatically driven resonating micromechanical membrane structures with a functionalized surface that allows the selective binding of allergens and biomarkers, as shown in Figure 1. The analyte binding causes an increase of the effective mass of the membrane and therefore induces a decrease of the mechanical resonance frequency. The performed measurements of the electrical spectra show nonlinear behaviour, which is explained by a nonlinear oscillator model. As shown in Figure 2, the calculation of the derivation of a sharp transition has the advantage of a sharp peak, i.e. high frequency resolution, where the position of the peak can be easily determined.

The sensor element consists essentially of a free standing membrane, which is electrostatically actuated to an oscillation with a resonance frequency of about 4 MHz and a mass sensitivity in the picogram range. We report on experiments with streptavidin on biotin as it is shown in Figure 3, with cholera toxin subunit B biomarkers onto GM1 functionalized sensors with a diameter of 60 µm.

Key words: mass sensor, CMOS integrated circuits, biosensor, MEMS

Figure 1: Cross section of the sensor element.

Figure 2: Measured shift of the resonance frequency due to additional mass of streptavidin (red) onto a biotin functionalized sensor with a diameter of 60 µm.
Development of a microchip based cell sorting device

S. Kahnert¹, A. Goehlich¹, D. Greifendorf¹, H. Vogt¹, K. Lennartz², U. Kirstein³, B. Goellner¹, U. Michelsen³, F. Bartels³, F. Schreiber⁴, A. Rennings⁴, D. Erni⁴
¹Fraunhofer IMS, Finkenstraße 61, 47057 Duisburg, stefan.kahnert@ims.fraunhofer.de
²Universitätsklinikum Essen, Institut für Zellbiologie (Tumorforschung), 45147 Essen, Germany
³Bartels Mikrotechnik GmbH, Otto-Hahn-Str. 15, 44227 Dortmund
⁴Universität Duisburg-Essen, Allgemeine und Theoretische Elektrotechnik (ATE), and CENIDE – Center for Nano-integration Duisburg-Essen, D-47048 Duisburg, Germany

Abstract

Cell sorting is broadly utilized in the field of biological and clinical research. Scientists frequently apply the technique to investigate the biology of stem cells or to develop therapies against neurodegenerative diseases [1]. Currently flow cytometry is the state of the art procedure to cope with cell separation tasks. In comparison to these commonly used systems, which rely on a serial sorting concept, the microchip based cell sorting device promises an increasing sorting velocity due to a parallel cell throughput, a more gentle cell treatment and an improved protection against biohazardous materials by encapsulating the sorting process in a microfluidic channel. The planned sorting device should be capable to sort droplets, which contain fluorescent cells by using the electrowetting effect. We present preliminary fluorescent microscopic investigations on a microfluidic system to demonstrate first separation events of different fluorescent labelled beads. In addition a layout and first images of the intended microchip will be introduced.

1 Introduction

The aim of the project MINAPSO (Microchip Navigated Parallel Sorting-Device) is the development of a cell sorting device, which relies on the concept of digital microfluidics. It is thought to transport cell containing fluid droplets on the surface of a microchip, while intermediate droplet splitting steps are used for cell separation. To detect and distinguish between different cell species, a fluorescence microscope is utilised.

1.1 Basic concept of electrowetting

The technique to realize the motion of the droplets is commonly known as electrowetting on dielectric (EWOD). The principle is based on an electrode, which is covered by a dielectric layer. A voltage, which is applied between droplet and electrode, changes the free energy on the dielectric surface and modifies the contact angle of the droplet (Image 1).

Image 1 Principle of electrowetting on dielectric

The droplet movement can be achieved by an asymmetric change of the contact angle, which induces a pressure shift within the droplet and finally causes the motion of the droplet (Image 2).

Image 2 An asymmetric change of the contact angle induces droplet motion.

2 Fluorescence measurement on a digital microfluidic system

As described in the previous section a digital microfluidic system is intended to be used for cell sorting. In this work a proof of principle of all relevant fluid operations is presented.

2.1 Experimental setup

The experimental setup mainly consists of an controllable, digital microfluidic system (developed by Bartels Mikrotechnik GmbH) to carry out the fluid operations and a
fluorescence microscope (AxioZoomV.16, Zeiss AG) for investigating the fluorescent objects within the droplets. (Image 3).

**Image 3** Fluorescence microscopic investigation of a digital microfluidic system

In order to simulate real conditions, a solution of different labeled beads replaces a suspension of biological cells. The fluorescence of the bead species is separated by two filter setups (Table 1) and recorded by a sensitive CMOS-camera (Hamamatsu, Orca Flash).

<table>
<thead>
<tr>
<th>Filter</th>
<th>λ\text{Excitation [nm]}</th>
<th>λ\text{Emission [nm]}</th>
</tr>
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<tbody>
<tr>
<td>DAPI</td>
<td>300-400</td>
<td>410-460</td>
</tr>
<tr>
<td>Fluorescein</td>
<td>450-495</td>
<td>500-550</td>
</tr>
</tbody>
</table>

**Table 1** Filter setup for recording different bead-species

### 2.2 Investigation of a sorting step

The controllable microfluidic system were manually filled with a suspension of two different bead species. A MATLAB programm enabled the user to carry out all fluid operations on the electrode matrix.

**Image 4** Proof of principle of the relevant fluid operations

To find out if a splitting step generates droplets with a single bead species or if further steps are necessary to separate the cells, a fluorescence microscopic analysis is required before and after droplet splitting (Image 5) and the bead quantities have to be determined. During the experiment any working step has been carried out manually, while in later applications a computer aided sorting algorithm will undertake these tasks.

**Image 5** Fluorescence microscopic investigation of a droplet before and after droplet splitting

Furthermore the circumstance, that an applied AC voltage provokes a circulation of the beads within the droplet (Image 6) and therefore allows mixing of the different bead species guaranties a homogeneous particle distribution and was already shown by Mugele et al. [3].

**Image 6** Images of moving (A) and stationary beads (B) in order of an applied and an unapplied AC voltage

### 3 Development of a microchip based sorter platform

The presented experiments were based on a electrowetting system with only a few electrodes (12x12). In order to perform an effective and fast cell sorting, the number of electrodes has been increased (here 456) and the electrode placement was adapted to a sorting algorithm [4], which automates the sorting process. As dielectric layer a 100 nm thick Ta₂O₅ layer will be deposited on the surface of the chip by ALD (Atomic layer deposition) and will finally spray coated with a hydrophobic Teflon film. The microfluidic channel will consist of photoresist, which functions as spacer [5] and a lid made of ITO structured glass [6]. For applying and regulating the required voltage the contact pads of the chip will be wire bonded to a pcb, which comprises a control unit.

#### 3.1 Chip fabrication

The chip layout has been designed by the IC design software “Cadence”. It consists of electrodes (blue), which are connected by multilayer conductor paths (yellow, ma-
genta) to a frame of contact pads. The dimensions of the electrodes account for 350 x 350 µm and their contact sides are spline shaped to accelerate the droplet transport. The spacer layer (green) surrounds the matrix completely and forms reservoirs at the upper area of the chip. The prominent structures in the centre and on the lower left side are alignment structures to adjust the lithography during the manufacturing process (Image 7).

4 Conclusion

In this conference paper, we present characterization measurements on a microfluidic system and introduced a concept for a cell sorting device based on digital microfluidics. We demonstrate all sorting relevant fluid operations (generation, transporting, splitting) with the aid of a microfluidic chip (12 x 12 electrode matrix). Therefore a solution with different labeled fluorescence beads has been used and finally analysed by fluorescence microscopic investigations. Like other authors, we could observe a circulation of the beads due to an applied AC voltage. That leads to the idea to generate homogeneous particle distributions within the droplet by using this mixing effect [3]. In addition we show a layout of a microfluidic sorting chip (456 electrodes), which were fabricated in the L035 process line of the Fraunhofer IMS.

Acknowledgement

We thankfully appreciate the support of the research project MINAPSO by the Ziel-2-Programm of the Ministerium für Wirtschaft, Energie, Industrie, Mittelstand und Handwerk of the state North Rhine-Westphalia within the competition NanoMikro+Werkstoffe NRW.

5 References

Detection of thermally induced denaturation of muscle tissue by impedance spectroscopy

M. Bellof¹, M. Ulbrich¹, S. Leonhardt¹

¹Chair for Medical Information Technology, RWTH Aachen University, Aachen, Germany
marco.bellof@rwth-aachen.de

Abstract
In this work, the cooking process of skeletal muscle tissue is analyzed by measuring its complex electrical impedance. With the monitoring of the electrical conductivity at different measurement frequencies it is possible to evaluate the cooking process by an autonomous measurement. To get a better impression of the electrical impedance change, skeletal muscle tissue was examined microscopically at different degrees of doneness. The analysis of the microscopic structure shows denaturation of the connective tissue and destruction of the cell membranes. This leads to an impedance value change and a structural change in impedance in the $\beta$-dispersion frequency range. A standalone hardware was developed which is able to evaluate the doneness of muscle tissue autonomously. The device works with a low cost impedance converter network analyzer and has a user-friendly interface for managing the measurement.

1 Introduction

Food, that most people call "meat", is a part of the skeletal muscle from mammals and poultry. The right preparation of meat is a complex process, that is difficult to control for the layman in food preparation. Supporting techniques exist, like the method of monitoring the core temperature of the meat. However, this method is very inflexible and not easy to use for small parts of meat. Furthermore, it is not user-friendly to stab a measuring electrode into the tissue, and the consequent harm of the piece of meat causes an increased fluid loss. Hence, the core temperature measuring method is not suitable for the preparation of small pieces of meat, such as steaks with a thickness 1 to 5 cm. With the help of modern impedance spectroscopy, a measurement hardware will be able to detect the doneness by monitoring the electrical conductivity of meat. The electrical impedance is dependent on the microscopic cell structure of the muscle tissue. With this method, a measurement by using only an electrical contact with the meat is possible and the tissue does not have to be harmed.

2 Methods

2.1 Change of muscle tissue by thermal influence

Muscle tissue consists of many muscle fibers which are kept together by connective tissue which consists mainly of a collagen network. These collagen fibers can resist forces which are tens of thousands times lagier then their own weight force. Because of this reason, muscle tissue is not chewable without destroying the collagen network in a thermal, chemical or mechanical way. The connective tissue also contains fat and water storages.

During the cooking of animal muscle tissue, many chemical and physical processes occur. The collagen structure begins to denature [3], releasing the fat and water storages in the connective tissue. Because of the high tear strength of collagen, the thermal destroying of this protein should be the first part by cooking meat. The second part is to achieve a low water loss during the cooking process. These two processes work against each other in the tissue. Hence, temperature and time are the most important factors for cooking meat. Furthermore, the thermal action should eliminate a potentially bacteria or parasite infestation of the meat.

Figure 1 Doneness of meat: well-done (left), medium (middle), rare (right)

Mainly three degrees of meat doneness with a corresponding core temperature are established in the society: Rare (ca. 50 °C), medium (ca. 60 °C) and well-done (70 °C) (Figure 1). These degrees are divided in sub-degrees, such as medium-rare (ca. 55 °C) or medium-well (ca. 65 °C).
2.2 Bioimpedance spectroscopy

Bioimpedance spectroscopy is a method for measuring the complex impedance of tissues over a wide frequency range [2]. A small alternating current is injected into the measurement object by using electrodes and the voltage drop over the sample is measured. So the impedance can be determined by the Ohm’s law.

\[ Z(j\omega) = \frac{u(j\omega)}{i(j\omega)} \]  

Although skeletal muscle tissue is very inhomogeneous, it can be described by the Cole-model [1]. This model describes fluid-filled cells in an extracellular fluid (Figure 2(a)). Larger structures, such as collagen networks, nerve fibers and vasculature, are neglected in this model. The cell membrane is a lipid bilayer, and has dielectric properties. So this layer can be approximated as a capacitance. At low frequencies, the current flows around the cells and at high frequencies, the current flows on the shortest way through the cell membranes. The current conductivities of the intra- and extracellular fluids can be simulated by the ohmic resistors \( R_i \) and \( R_e \). An electrical equivalent circuit can be derived from this behavior (Figure 2(b)). If the total impedance is calculated for the complete frequency spectrum, the result is a semicircular impedance curve grey in the frequency locus plot (Figure 2(c)).

For small frequencies, the impedance of the capacitance increases and measured values approximately equal to the resistance \( R_e \) (right). For very high frequencies, the capacitance can be represented as a short circuit and the total resistance is \( R_e || R_i \) (left).

2.3 Measurements

The measured impedance of muscle tissue during the cooking process depends on many different factors which can be divided into two groups: wanted and reducible influences. Core temperature, change in structure by denaturation of collagen, cell destruction, fluid loss, ion concentration change, change in the fat content, etc. belong to the first category. This effects are directly connected with the process of cooking and should be detected by the measurement.

The second category includes the cooking temperature, the temperature gradient inside the tissue, an increased contact resistance by crusting and electrode characteristics. These influences can be reduced by using a special cooking method. The raw meat is placed in the oven and cooked at a low temperature of 100 °C. If the final core temperature is nearly reached, the meat is shortly fried to create a crust. Thus, the crust has no influence on the electrical contact resistance, because it does not exist during the measurement. Furthermore, the low cooking temperature of 100 °C minimizes the temperature gradient in the meat, so that the cooking result becomes more homogeneous. The electrical impedance is measured at two pieces of muscle tissue with a precision LCR meter EA4980 (Agilent). In addition, the core temperature is determined on another piece of meat with a similar size. Furthermore, tissue samples for microscopy were put in the oven.

2.4 Microscopy

To explain the progressive impedance changes, four tissue samples were microscoped: a raw, a rare (51 °C), a medium (61 °C) and a well-done (70 °C) sample. The tissue pieces were cooled in sample cups to -40 °C, after they were filled up with an embedding medium (Tissue-Tek). Cryosections were cut from the frozen samples with a thickness of 2 µm.

The samples were cleaned from the Tissue-Tek with phosphate-buffered saline and fixed on the microscope slides with methanol and acetone. Afterwards, cryosections were stained with hematoxylin and eosin (H&E stain). Hematoxylin stained all acid structures such as the cell nuclei blue and acid structures are colored red by eosin. The cells and cell nuclei were software-supported counted to find leaky and destroyed cell membranes.
3 Results

3.1 Evaluation

The duration of the measurement was 68 minutes until the core temperature has risen to 66 °C. Figure 3 shows the result of the every 4 minute impedance measurements. The impedance curve at a core temperature of 22 °C was taken as reference measurement. In this measured curve the typical features of a cell structure can be seen (Cole-Model) which are primarily determined by the capacitive property of the cell’s lipid bilayer.

Two significant impedance changes can be observed with increasing core temperature. On the one hand, the reduction in impedance magnitude, and on the other hand, the structural change of the Cole curve. Both effects can be used to detect the doneness of meat. These effects were converted in one-dimensional values for checking to thresholds.

Figure 4 shows the impedance magnitude decreasing to a certain temperature after which an increase can be observed. This effect can be explained with the ion-conductor properties of biological tissue. The conductivity of the sample increases (|Z| decreases), because the mobility of the ions rises with thermal energy supply. This process is stopped by further due to the fluid loss which has a negative effect on the conductivity (|Z| increases).

The structural change of the Cole curve can be mapped to one-dimensional value (rel.abs) as follows:

$$rel.abs = \frac{Re\{Z_{middle}(t_i)\} - Re\{Z_{right}(t_i)\}}{Re\{Z_{middle}(t_0)\} - Re\{Z_{right}(t_0)\}}$$  \hspace{1cm} (2)

To apply this equation, the frequency of the impedance $Z_{middle}(t)$ with the most negative imaginary part must be determined (Figure 3). In addition, the frequency of the impedance $Z_{right}(t)$ with the greatest imaginary part and a lower frequency as $Z_{middle}(t)$ needs to be also assessed. The difference of the real parts from these two impedances is calculated at each measuring time. This difference is normalized with the first calculated difference to get a percentage result in terms of the reference measurement. Figure 5 shows the results of this calculation rule.

To be able to apply these new knowledge in practice a low cost hardware was developed (Figure 6). The impedance converter network analyzer chip AD5933 (Analog) is used.
for measuring the impedance. As measuring amplifier is used an operational amplifier (AD820). The measurement is controlled from a MSP430F2272 (Analog) microcontroller which provides a user-interface in connection with a full graphic display and switches. The measuring chip be calibrated automatically with the help of the signal relay before each measurement.

The evaluation is performed on a continuous speed determination of the absolute impedance value change. The impedance change is determined by a regression straight over five measurements with 10 seconds time difference. The regression line is determined by a minimization problem which is solved by a Given rotation. After further averaging, it is possible to determine the doneness medium rare or well done by a threshold value query.

![Figure 7](image_url) muscle tissue with 200× magnification

The results of the microscopy is shown on the Figure 7. The connective tissue between the cells was not stained and appears white under the microscope. This tissue consists of water- and fat storages which are located in a collagen network. A strong involution of the connective tissue can be seen at a higher degree of doneness. This suggests a high loss of extracellular fluid.

![Figure 8](image_url) relative number of counted cell nuclei

The results of the nuclei count in relation to the number of cells can be seen at Figure 8. This statistical analysis of the samples shows decrease of nuclei with a higher degree of doneness. The loss of cell nuclei suggests a destruction of the cell membrane. Thus, an increase of destroyed cell membranes with higher temperature is identified.

## 4 Conclusion

Within this work a method was found which can non-invasively determine the doneness of meat by an electrical impedance measurement. In addition, the change in impedance with rising temperature has been suggested microscopic structural changes in the tissue. Two methods were found with which the progressive cooking process can be determined by an impedance measurement. With this knowledge it was possible to build a low-cost hardware that can be used to cook meat with different adjustable doneness.

In the future it would be possible to improve this technology by an integration in kitchen equipment. Thus, a non-invasive control of the cooking process would be possible. It might be possible to use this measurement method for vegetables, because these also consist of biological tissue.

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## 5 References

