Hochschule Kaiserslautern
University of Applied Sciences

SensUs

Team results document

SensAble

University of Applied Sciences Kaiserslautern (UASK), Zweibrücken, Germany

Team name: SensAble

Team members: Benjamin Heidt, Janna Papenburg, Tobias Teucke, Isabelle Schneider, Stefanie Wiedemann, Yannick David Lang, Michele Schroeter, Madeleine Born, Carolina Baus

Former members (reasons to quit the team):

(Matthias Koczy – out because of health issues; Philip Mallow – out because of change of university)

Responsible professor: Prof. Dr. Sven Ingebrandt

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Contents

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SensUs

1. Summary for the SensUs website

We are the SensUs-Team “SensAble” of the University of Applied Sciences Kaiserslautern. The team includes nine students from different fields of study such as Nano Systems and Micro Technologies and Applied Life Sciences, which will allow us to merge the professional skills of engineering and science. The profound knowledge of fabricating nano- and microsystem devices empowers the group to create and build sensors of microscale structures.

Furthermore, the interdisciplinary field of Applied Life sciences contributes the insight of biochemical processes as well as the knowledge about the interaction between antibodies and antigens. Thus, this combination of engineering and science provides the opportunity to accomplish the development of a new sensor which approaches the detection of the biomarker BNP in a new way. Thereby, the motivation and the interest arose to work interdisciplinary and to think beyond boundaries to develop a solution for this new sensor.

Furthermore, to work interdisciplinary allows students from different fields of study to obtain insight in diverse areas for instance from the developing and fabricating a sensor to the detection of the biomarker.

Hence, this idea of a biosensor has driven us to join SensUs to present our approach of a sensor as well as to learn from other groups participating in SensUs.

The goal for our team is to develop a fully functional and innovative sensor that is able to detect not only the BNP biomarker but also diverse biomarkers in human blood.

2. Description of the biosensor system and the assay:

A biosensor in general is a device which combines a biological system with a transducer and a detector. For the chip itself, the group decided to use a combination of reduced graphene oxide as a transducer nanomaterial and electrical chip made of glass and gold..

We adapted a process using interdigitated gold metal-microelectrodes (IDE) which are covered by ultra-thin films of graphene oxide (GO). The GO film, originally an insulator is further converted to semiconductor reduced graphene oxide (rGO) by a thermal reduction process. The resulting ultra-thin film of rGO of only 3 nm thickness serves as transducer layer for our electronic device concept. These sensors have shown very low limits of detection in other analytical assays. Since for our system, the goal was to detect one biomarker out of a blood serum sample, we designed a micro-fluidic housing with a sensing channel and a reference channel.

The sensors can work either in a potentiometric (DC-mode) or in an impedimentric readout (AC-mode) method. We tried and utilized both techniques in our analytical tests.

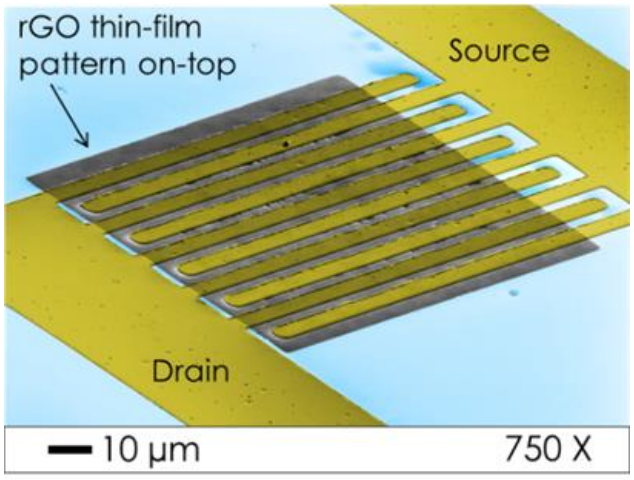


Figure 1: Scanning electron microscopy image of an IDE electrode covered by an rGO thin-film

3. Technological concept and implementation:

Sensor devices:

The manufacturing of the chips was subdivided into three big parts.

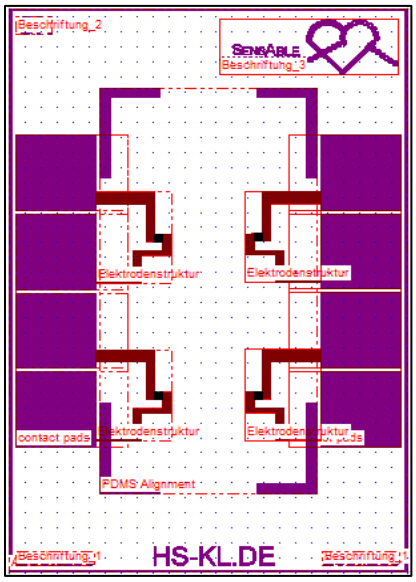


Figure 2: Chip design with the conductor tracks, IDEs and contact pads.

First of all, the lithography of the gold structures was made by using a light-sensitive photoresist. In the next step, the gold structures except for the contact pads and the inter-digital electrodes (IDE), were covered with a glass layer for mechanical protection and to prevent leakage currents. These covered structures are called conducting paths and are labeled red in figure 2. The black labeled area in figure 2 represents the measuring fields consisting of eight static IDE pairs. The advantage of using the IDEs is that they are space-saving compared to two opposite electrodes because the region of the interaction has the same length but in a more compact way (high surface-volume ratio). In the last step, the IDEs were coated with graphene oxide and thermally processed. Thereby, in the developed biosensor chip, the rGO thin-film functions as the transducer. In general, rGO shows less unspecific bindings of biomolecules than other materials therefore pre-preparation steps of the samples aren't necessary. Furthermore the electrical properties of the rGO can be tuned by different reduction protocols. The chip used for the measurements consisted of four 'sensor-fields' as shown in the figure 2, whereby one side (with two sensor-fields) is used as the reference for sensor measurements.

Surface chemistry:

To immobilize the receptor antibodies on the sensor surface, some pre-preparation steps are essential which were carried out as follows. The rGO surface was treated with EDC/NHS (1-Ethyl-3-(3-dimethylaminoprpyl)carbodiimide/N-hdydroxysuccinimide). Commonly called as the carbodiimide chemistry, EDC-NHS complex reacts with the –COOH groups present on the graphitic lattice and render it with functional Sulfo-NHS esters. Chemically modified graphitic lattice (rGO) can then readily be used for antibody binding using established surface functionalization protocols such as used for amino-modified nucleic acids. The reference side of the biosensor does not get coated with the antibodies. Free activated surface groups were blocked with ethanolamine. For the detection of the antigen binding, field effect measuring was used. Antigen (analyte) binding to the receptor antibodies leads to a change in the surface charge which can be determined. The outcome of this is that the charge-carrier density in the channels (rGO transducer) changes too. The varying conductance depends on the increasing antigen binding, with the aid of a standard curve allows determining the antigen concentration in the sample.

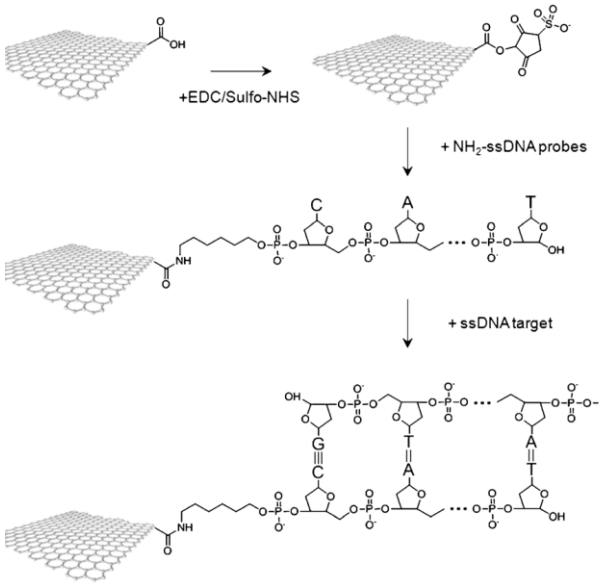


Figure 3: The surface modification of rGO sensors to immobilize receptor antibodies (image taken from Chem. Eur. J. 2012, 18, 1668 – 1673)

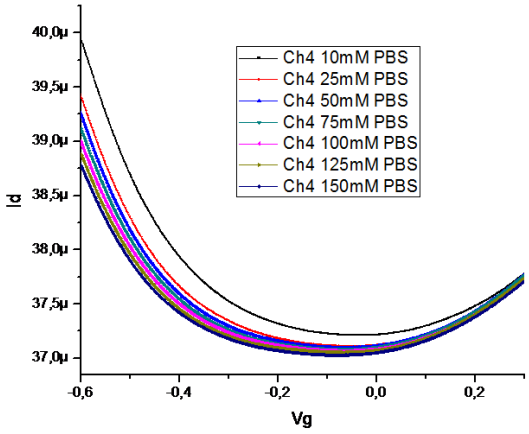
System integration:

For small sample volumes, the microfluidic structures have been established. With the aid of micropumps the whole sample volume can pass the electrodes during the measurement. The biosensor system is characterized by the diffusion of the analyte on the sensor surface. In addition to that the moving fluidic flow leads to convection. This increases the binding probability.

4. Analytical performance:

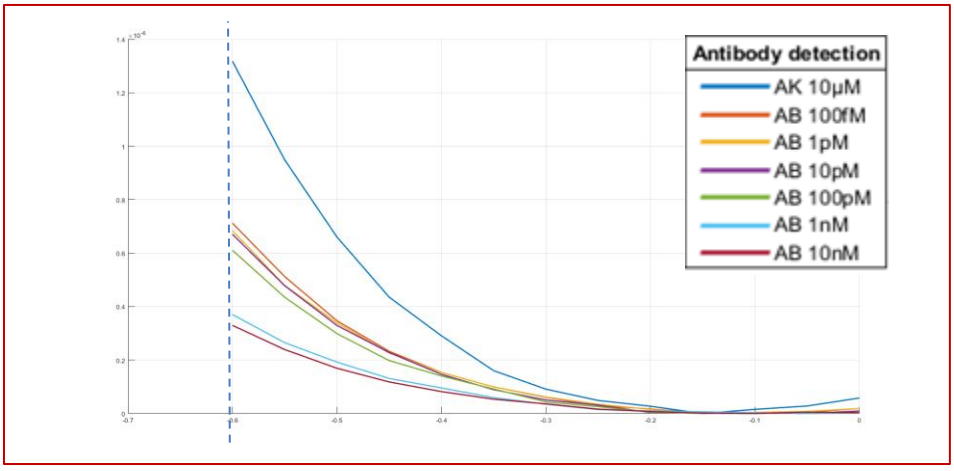
Field-effect measurement to determine various PBS ionic strength:

There is a significant change of the current flow between 10 mM and 25 mM PBS-Buffer, which decreases with higher ionic strength.

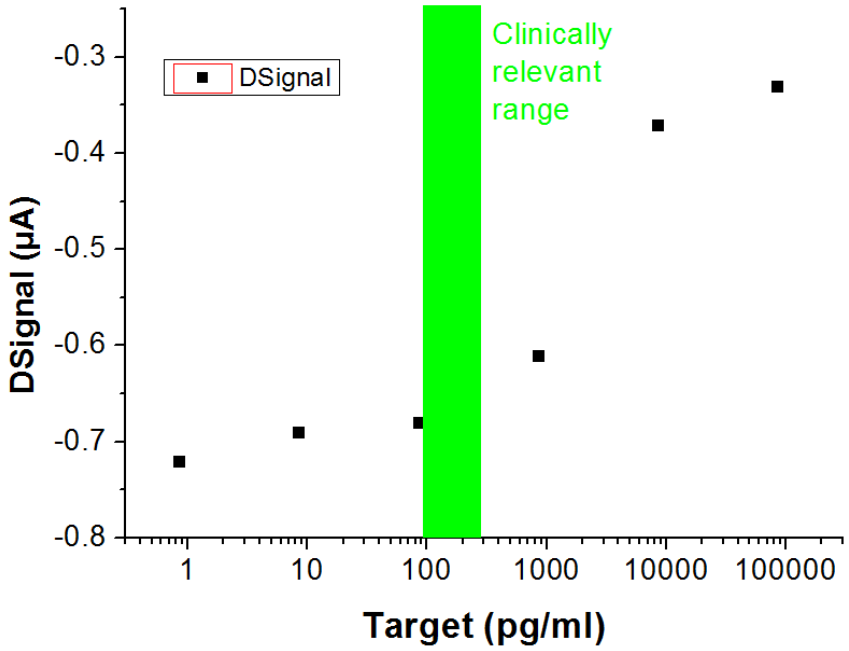


Graph1: *Changes of the current flow with different ionic strength of PBS. (Measured with Keithley SC 4200; 200x200 µm² IDE rGO ISFET). Sensor shows good stability.*

Field-effect measurement to determine the surface saturation and range of detection for the AB-target interaction:



Graph2: *Changes of the current flow with different antibody concentrations in 150 mM PBS Buffer. (Measured with Keithley SC 4200; 200x200 µm² IDE rGO ISFET). Values are taken at -0.6 V against Ag/AgCl as data points.*



Graph3: *Evaluation of binding isotherm with different antibody concentrations in 150 mM PBS Buffer. Values were taken at -0.6 V against Ag/AgCl as data points and raw data is plotted against target concentration. We evaluate a K_{on} of roughly 2000 pg/ml for this particular experiment. The sensor is reacting above clinically-relevant range.*

Note: Calculations between molar concentrations and pg/ml concentrations as used in clinical practice were done with following calculator: http://unitslab.com/de/node/163

In this particular experiment it seems that the limit of detection of the system is not sufficient and we can only detect slightly above the clinically relevant range. The sensor has a K_{on} above the clinically-relevant range. This is a problem, which has to be fixed in the near future to guaranty a detection of NT-proBNP for clinical use.

This behaviour could be seen on several different channels of the sensor.

CONCLUSION:

The system shows a good sensitivity but further tuning of the assay would be necessary to get a linear response in the clinically-relevant range. This behaviour could be seen on several different channels. We need further tests to confirm this behaviour.

5. Novelty and creativity:

The main concept for reduced graphene oxide biosensors was adapted from an existing concept of AG Ingebrandt and developed further into a system integration approach.

Available prior to the project:

- Graphene oxide
- Protocol for sensor encapsulation
- Portable readout instrument (to be used at team event)
- Protocols for microelectrode fabrication

Developed themselves:

- New sensor layout embedded into microfluidics
- Fabrication of new sensor chips
- Development and fabrication of microfluidics
- Development of a readout program with Raspberry PI
- Development of a readout circuit for AD5933 impedance device (unfortunately not finished)
- Adaptation of the assay onto rGO platform
- Installation of microfluidic system
- System integration

6. Translation potential

A. Healthcare application potential:

The graphene oxide sensors are at the moment realized on glass chips. The fabrication process could also be transferred onto flexible substrates – eventually in reel-to-reel processing offering a potential for high-density low cost manufacturing of disposable test chips. For healthcare applications, this sensor approach sets forward a generic pathway for the realization of a high-performance and versatile sensor platform offering technology transfer and implementation to other bio-assays as well. The field-effect and/or impedance-based sensing offers time-dependent readout, which is expected to enable bed-side continuous monitoring and might be useful in other bioassays dealing with critical illnesses, rapid infections, transplants, healing, etc.

B. Industrialization potential:

The graphene-oxide sensors do have a very high industrial potential. The source material was the main topic in PhD theses of Mr. Ruben Lanche, Mr. Walid-Madhat Munief and Ms. Xiaoling Lu (supervised by Dr. Vivek Pachauri). In their works they compared the source material with commercially available GO and confirmed higher quality for the GO material produced in AG Ingebrandt.

The established fabrication process, which was adapted and optimized towards usage in a microfluidic container, is generating very uniform device characteristics and fabrication of individual sensors is very cheap in comparison to other nanoscale sensor platforms. It has a large potential for industrial upscale, in principle also offering multiple biomarker detection after integration of multiplexing approaches.

C. Commercialization roadmap

The commercialization of the **SensAble** sensor system is so far not planned. Intermediate commercialization of the graphene oxide material and the thin-film deposition and wafers is covered by a patent application at the UASK. Commercialization as a spin-out from AG Ingebrandt has already started.

7. Team and support

A. Summary of the main contributions of the team members:

Benjamin Heidt: Group organization and planning, hard and software aspects of the readout device, contribution of the microfluidic system and part of the antibody incubation.

Janna Papenburg: Microfluidics, simulation with COMSOL of several microfluidic structures

Tobias Teucke: Assay tests and sensor usage for bioassays, chip preparation for the antibody binding, measurement of the field effect

Isabelle Schneider: Assay tests and sensor usage for bioassays, chip preparation for the antibody binding, measurement of the field effect, chemical orderings for the bioassay group

Stefanie Wiedemann: Clean room process and electrical connection of the chip, group organization and planning

Yannick David Lang: Design of the sensor and all CAD tasks (microfluidics and sensor), clean room process and the electrical connection of the chip

Michele Schroeter: Assay tests and sensor usage for bioassays

Madeleine Born: Assay tests and sensor usage for bioassays, chip preparation for the antibody binding, measurement of the field effect

Carolina Baus: Assay tests and sensor usage for bioassays, chip preparation for the antibody binding, measurement of the field effect

B. People that have given support:

Prof. Dr. Sven Ingebrandt: Supported by opening his laboratories and offered help out of the different PhD projects. He also sponsored partly from his research group budget the SensUs 2017 project. He generally supported the SensAble team during team meetings and with general scientific instruction and guidance.

Dr. Anette Britz (Assistant - AG Ingebrandt): Helped with the general organization and material orderings. She helped with group coordination in the starting phase. She also helped for the general match making with the technical workshop and with travel organization.

Dr. Vivek Pachauri (Postdoc - AG Ingebrandt): Helped with general instruction and explanation about the technical and biophysical background of the technological approach. He helped also with the microfluidic handling and installation.

Dr. Jessica Ka-Yan Law (Former postdoc - AG Ingebrandt): Helped with the concentration calculations, instruction for sensor usage and sterile handling of samples. She helped with group coordination. In the starting phase she helped with team organization.

Dr. Vania Silverio (Visiting postdoc - INESC Microsistemas eNanotecnologias, Lisbon, Portugal): Helped with the general instruction to microfluidics and simulations. Helped with the initial design of microfluidics.

Prof. Dr. Joao Conde (Visiting postdoc - INESC Microsistemas eNanotecnologias, Lisbon, Portugal):Gave a lecture for general instruction to microfluidics and simulations.

WalidMadhatMunief (PhD student - AG Ingebrandt):Helped with the clean room instruction, design and fabrication of the devices. In addition he provided graphene oxide material for the sensors and he instructed for the field-effect experiments with graphene-oxide sensors. He also instructed the Raman and AFM experiments for material analysis.

Xiaoling Lu (PhD student - AG Ingebrandt): Helped with the usage and instruction for impedance-based readout and assay development.

Felix Hempel (PhD student - AG Ingebrandt): Helped with the microfluidic mold design and with the process of curing PDMS microfluidic structures. He was instructing students in the chip encapsulation protocol.

Lukas Trumpler (PhD student - AG Ingebrandt):Helped with the construction of an impedance meter on the basis of an AD5933 with Raspberry Pi control in SensUs2017. The goal was the construction of a mobile, touchscreen-controllable and user-friendly device. Due to delivery delays, the prototype was not finished in time and cannot be presented at the team event.

C. Sponsors:

No sponsors

Zweibrücken, Germany, 05.09.2017

(Team SensAble)