Team Results Document

AixSense, RWTH Aachen University

SensUs 2021

Team members: Daniyar Kizatov, Anshul Prashar, Jiayi He, Shunjiro Sodei

Supervisor: Prof. Dr. Sven Ingebrandt **Coach:** Dr. Vivek Pachauri

Date of submission: 23.08.2021

0. Summary for the SensUs website

We designed a biosensor based on a miniature Ion Sensitive Field Effect Transistor (ISFET) which act as an electrical biosensor. The surface of sensors is bio-functionalized with specialized DNA-aptamer that binds specifically with the hemagglutinin molecule, thus allowing simple electrical measurement of target concentration. The sensor is cheap, reliable, provides real-time concentration measurement. Because an array of 16 sensors is located on each chip, multiplexed measurement is possible, e.g. simultaneous detection of different strains of viruses, measurements in multiple dynamic ranges. Technology allows multiple uses of each sensor cartridge, which drives costs further down, promotes sustainable and eco-friendly approaches.

1. Biosensor system and Assay

1.1 General concept

Ion Sensitive Field Effect Transistors (ISFETs) with its gate surface bio-functionalized with custom aptamers are used to detect hemagglutinin protein concentrations. Liquid sample comes in direct contact with gate surface. The exploits field effect phenomenon to detect changes in target molecule concentration in the sample. Aptamers allow very high specificity.

Aptamers bind with hemagglutinin molecules thus changing charge at the interface between the solid silicon dioxide surface and the molecules in the liquid solution. The change in the charge shifts the field effect curve (Fig. 1) thus allowing measurement of different concentration of a target molecule. In other words, different concentrations of target molecules can be registered by simply reading drain current.

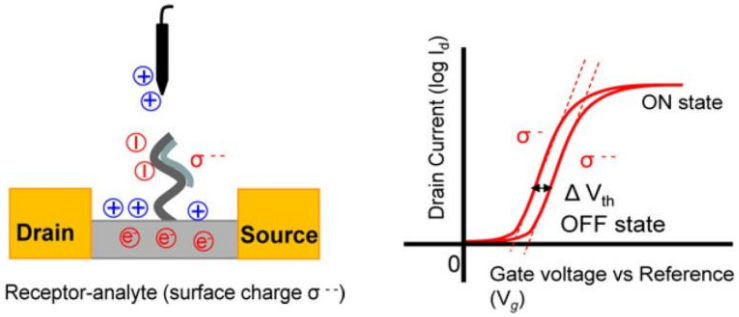

Fig. 1 General concept of the sensor system [1]

1.2 The Chip

An ISFET is a device with three terminals: source, drain and gate electrode. Transistors are insulated from liquid solution to prevent leakage currents.

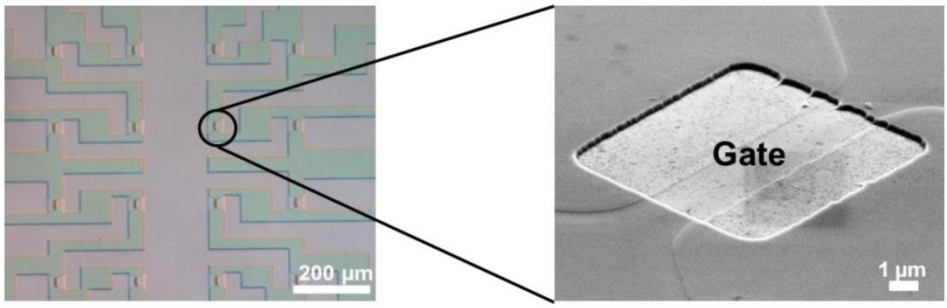

Fig.2 Microscopic image of an ISFET chip and of an individual sensor.

Each chip contains 16 individual sensors (Fig.2) in 4 x 4 layout with a distance between gates of 200 micron.

1.3 Bio-functional Layer

To implement the assay chip surface needs to be bio-functionalized. Transistor surface is first chemically activated with piranha solution to enable covalent attachment of functional organosilanes to the gate surface. Organosilanes can form homogeneous monolayer. 3-glycidoxypropyltri-methoxysilane (GPTMS) was used for silanization in the next stage. Amino-modified aptamers can be directly bound to epoxy rings of GPTMS. Aptamers are microspotted with high precision directly on the sensor surface (Fig.3). This allows possible application of different capture molecules.

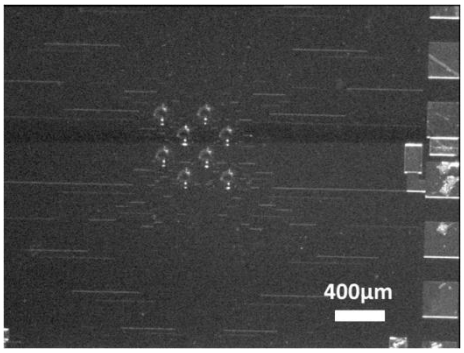

Fig. 3 Microspotting process

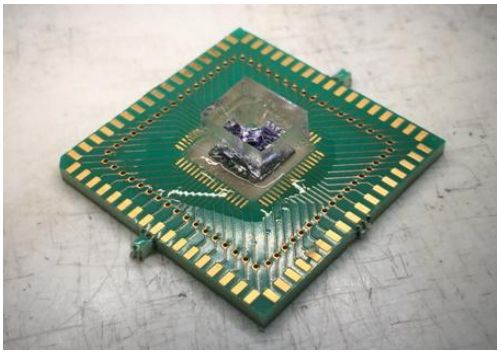

Fig.4 Encapsulated chip (Cartridge)

1.4 Cartridge technology

Each chip after being removed from wafer is placed onto chip carrier (Fig 4). It is then wire-bonded with this chip carrier. All further manipulations (surface activation, silanization, microspotting) are done with the chip attached to the carrier. After bio-functional layer is prepared chip is encapsulated, which means that a special reservoir is placed at the surface of the chip. Reservoir is made from polydimethylsiloxane (PDMS) and it allows stable measurements and easy handling of liquid samples.

The resulting cartridge is very robust, can be stored (in fridge) for at least couple of months before being used for the measurements. Moreover, the chip can be re-used or refurbished after being used, allowing cost reduction, effective recycling of chips, promoting of sustainability and ecologically friendly approaches.

1.5 Reader instrument and user interaction

Custom read-out system is used to perform electrical measurements. Ag/AgCl reference electrode is required to perform three point FET measurements. Cartridge is placed in the special adaptor which is connected to the amplifier system (Fig. 6). Voltages are applied to transistor to allow either characteristics measurement or work-point current read-outs. The sample can placed in reservoir by a manual pipette, which means that no active fluidics is required. Read-out system is connected to laptop. Signals are processed by specialized software. Results of the measurement are displayed by software real-time.

2. Technological Feasibility

Our biosensor (Fig. 4) is based on the field-effect transistor (FET) technology. When a probe molecule modified on the insulating layer binds to the target protein, the surface potential of the insulating layer changes, and the change is detected as an electrical signal. FETs are micro-scale devices advantageous for integration, which makes our sensor small and scalable. Furthermore, since the change in surface potential is detected instantaneously, it is possible to measure the concentration of the target protein rapidly.

We use DNA aptamers as molecules that bind specifically to the target protein. DNA aptamers are cheaper to synthesize than other binding molecules such as antibodies and are more stable over a long period, making our sensors useful for point-of-care analysis. In addition, the reversible structural change of DNA through 'melting' is expected to make it possible to reuse the biosensor once it has been used, thus realizing a sustainable and environmentally friendly biosensor.

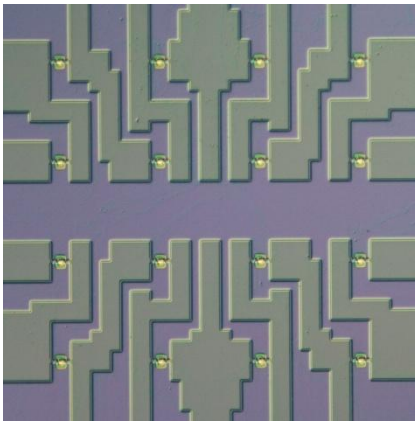

Fig. 5 Enlarged view of the sensor chip

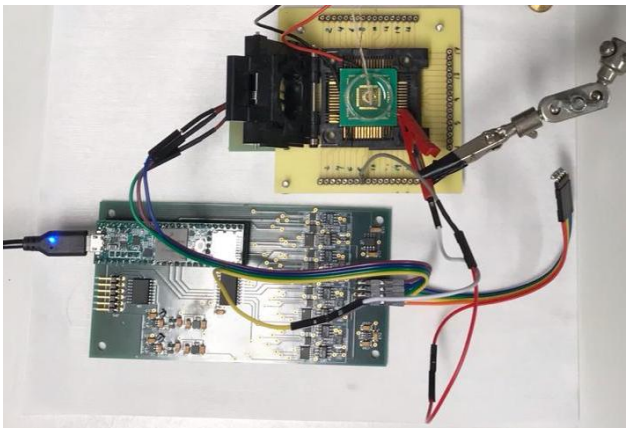

Fig.6 Cartridge connected with the external readout system

Our sensor is composed of 16 FETs (Fig. 5). This combination makes statistically reliable measurements possible. In addition, the surface of the insulator of each FET is separately modified with aptamer molecules using a microspotter. Therefore, different proteins at different measurement ranges can be measured simultaneously by varying the type and density of aptamer molecules in each FET. A sensor with more FETs can be built if needed.

A change in the surface potential is detected as a change in the drain current vs. gate voltage. Our sensor can automatically measure it with an external readout system (Fig. 6). We have performed actual measurements using a PBS buffer solution containing hemagglutinin and confirmed that the drain current becomes lower as the concentration of hemagglutinin increases (Fig. 7). The plot with the hemagglutinin concentration as the x-axis and the drain current as the y-axis gave a sigmoidal curve is drawn as expected, and a significant drain current change was observed from 100 fM to 100 pM (Fig. 8). ***Although, there was no drain current change observed at 10 fM, which is the specified concentration range for this competition, we are now conducting experiments to correct the dynamic range by adjusting the density of the DNA aptamer. Further experiments are needed to optimize the properties of the sensor.***

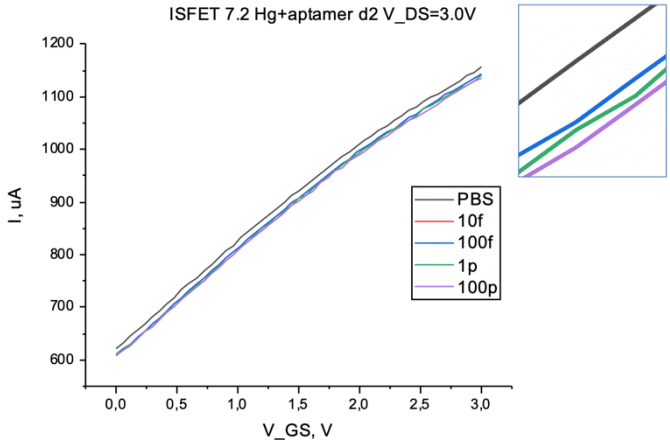

Fig. 7 Drain current vs. gate voltage

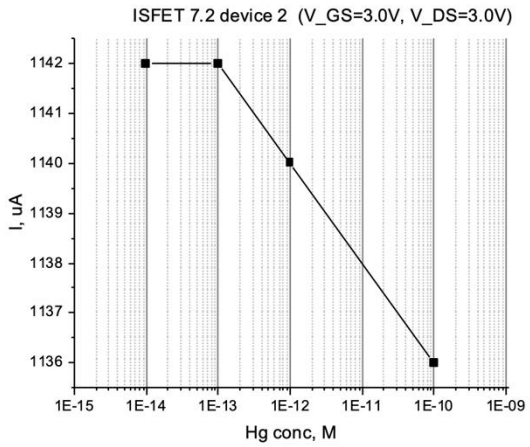

Fig. 8 Drain current vs. hemagglutinin concentration

The above results suggest that this sensor is helpful for the detection of hemagglutinin, but additional experiments are planned before the competition.

The reference electrode is certainly a limiting component of a system. It needs to be in contact with the sample to conduct measurements. It is relatively bulky and greatly limits miniaturization of the system. One of possible ways to solve this challenge is deploying sensors with integrated reference electrode.

3. Originality

3.1 Team part

This original combination of microISFETs and aptamers makes the advantages of our biosensor.

MicroFET

By sensing changes in the conductivity of the source-drain channel arising from the electric field of the environment of the channel, FET-based biosensors have many advantages.

Due to their capability of integration and scalability, novel on-chip devices have attracted more and more attention with the ability of high-density fabrication. They can integrate with different techniques and materials including microfluidics, CMOS, MEMS, electrical circuits and with different nanomaterials and assay formats. Besides their high throughput and almost instantaneous detection priority, a high number of sensors and multiplexing by micro-spotting individual sensors can also be achieved. Previous efforts have been made in different chemical modifications and immobilization strategies. For instance, a microFET-based sensor based on CMOS and MEMS technologies for the detection of HbA1c/Hb ratio was reported [2].

Our team developed the sensor with an array of 16 ISFETs modified with aptamer.

Aptamer

Due to the huge advantages of aptamers over other biomolecules (antibodies), our devices have shown great strengths.

Firstly, with a smaller size than antibodies, aptamers can reach previously blocked or intracellular targets. Secondly, re-usable sensors can be developed due to the reversible denaturation of aptamers while antibodies are not able to reform to their original conformations by restoring the optimal temperature [3]. Moreover, aptamers can be easily produced via chemical synthesis, which reduces the cost and duration of production [4]. Furthermore, aptamers can be more stable and easily attached to a detecting agent compared to other biomolecules [5].

3.2 Supervisor part

Nearly 1000 days into the pandemic, we have seen several strains of coronavirus emerge and spread in the populations worldwide. With the superfast transmission of the viral strains, while some of them with very highly contagious, identification of virus strains at the transport hubs and other social gathering nodes presents an opportunity to manage such pandemic. The AixSense team identified this challenge and aimed at designing a sensor platform that will have the potential to provide ultrafast multiplexed (demonstrated by microspotting of aptamers onto sensor chip) quantification of viral strains. With the ongoing developments in selection and development of aptamers with high selectivity towards different viral proteins, exploiting aptamer-protein binding at system-integrated electrical biosensors presents a truly feasible approach to realize a platform for real use. Interestingly, instead of employing a nanomaterial based transducer, the team decided to just use microscale ISFETs of silicon for realization of sensor platform – which push the invention to a high TRL. Such a sensor platform can be easily configured into integrated circuits and can be mass-produced at low prices. Usefulness of such electrical biosensors for high throughput analysis therefore is a key attribute towards carry out clinical approvals and push the invention in the next stages of product development cycle.

4. Translation Potential

4.1 Stakeholder Desirability

The biosensor that we have developed aims to benefit healthcare service providers and patients. Despite enormous scientific and technological advances in the area of biosensors, a gap is observed between this progress and actual products incorporating biosensors being commercially available [6]. This gap is largely attributed to difficulties and costs of manufacturing, shelf life of the biosensors and reproducibility on a large scale. Hence, this area of medicine still suffers from a lack of products satisfying the needs of point-of-care applications, despite viral diseases posing a great risk to our way of life.

Because of these limitations, whenever there is a viral outbreak no matter a local one or a global one, hospitals are usually overwhelmed and healthcare professionals are unable to provide adequate service to everyone. This also puts financial burden on hospitals and patients. Hospitals have to take on high expenses to offer these diagnosis tests since it is usually not a one-time test. Every patient would probably need to take them at regular intervals. A portable diagnosis test that a patient can do at their home alleviates these burdens greatly and also helps the hospitals and clinics financially.

Our biosensor addresses these complications by requiring low cost of manufacturing, since it contains no active fluidics and the biofunctional layer is made up of aptameters, which are cheap. Aptameters exhibit greater stability than other bio-molecules which results in a much longer shelf life for our biosensor, on the scale of decades before bio-functionalization and months after bio-functionalization.

4.2 Business Feasibility

We already have a functioning prototype device that is giving us impressive results. Once the platform is optimized further for handling requirement of the real-world applications, it will provide healthcare professionals the ability to diagnose patients with H1 viruses within minutes. Real-time measurement will help hospitals to improve their service and offer it to more patients. Once the device is ready, a patent should be sought for IP protection. This would require the help of a patent firm for a smooth application and certification process.

To go from our prototype to a stage where we have a marketable device, the limitations of the biosensor need to be addressed. One is the requirement of a reference electrode, which is crucial for reliable detection of H1 molecules. One solution to this is integrating the electrode into the sensor. Special measures should also be taken to compensate for drift that might possibly develop in the reference electrode.

For commercialization of the product, a strategy needs to be defined on how to introduce the product in the market. Before introducing the product in the market, a small number needs to be manufactured and sent for clinical trials. After approval, the steps include contacting a manufacturing firm for manufacturing, assembly and shipping of the final device, and marketing of the device. Afterwards a market analysis can be done to assess the performance of the device in the market and future developments that could be done.

4.3 Financial Viability

Since the cost of manufacturing is low, the final device can easily be manufactured in large numbers at relatively low costs. And the fact that the device would be re-usable, customers would also save a lot of money as compared to other one-time tests. But before the availability in the market, sources of revenue would be companies interested in the device and other institutions which would agree to provide funding for manufacturing and marketing. The experience of these companies and institutions could also help improve the design for better market acceptance. Starting with a small, local initial market, further expansion could be looked into.

5. Team and Support

5.1 Contributions of the team members

- **Daniyar Kizatov**: Team captain, technical part of the project
- **Anshul Prashar**: Chip encapsulation
- **Jiayi He**: Read-out system
- **Shunjiro Sodei**: Report and documentation

5.2 People who have given support

- **Dr. rer.nat Sven Ingebrandt**: Supervisor, Head of the project
- **Dr. Vivek Pachauri**: Coach, technical advisor
- **Dr. Xuan Thang Vu**: Introduction to wire-bonding, microspotting
- **Aidin Nikookhesal**: silanization
- **Linda Wetzel**: Media support
- **Fabian Brings**: Read-out system
- **Stefan Leisten**: Sample preparation

Bibliography

[1] V.Pacauri, S.Ingebrandt, “Biologically Sensitive Field-Effect Transistors: From ISFETs to NanoFETs”, *Essays Biochem*, 2016 Jun 30;60(1):81-90. doi: 10.1042/EBC20150009

[2] Q. Xue, C. Bian, J. Tong, J. Sun, H. Zhang, and S. Xia, “FET immunosensor for hemoglobin A1c using a gold nanofilm grown by a seed-mediated technique and covered with mixed self-assembled monolayers,” *Microchimica Acta*, vol. 176, nos. 1–2, pp. 65–72, Jan. 2012

[3] Jayasena, Sumedha D. "Aptamers: an emerging class of molecules that rival antibodies in diagnostics." *Clinical chemistry* 45.9 (1999): 1628-1650.

[4] Cho, Eun Jeong, Joo-Woon Lee, and Andrew D. Ellington. "Applications of aptamers as sensors." *Annual review of analytical chemistry* 2 (2009): 241-264.

[5] Toh, Saw Yi, et al. "Aptamers as a replacement for antibodies in enzyme-linked immunosorbent assay." *Biosensors and bioelectronics* 64 (2015): 392-403.

[6] V. Zucolotto, “Specialty Grand Challenges in Biosensors,” *Front. Sensors*, vol. 0, p. 3, Aug. 2020, doi: 10.3389/FSENS.2020.00003.