Team Results Document
Aix-Sense

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1.1 Abstract

Our team designed an electrical biosensor for the detection of Interleukin 6 (IL-6). IL-6 is an enzyme in the human blood which can be used for the early detection of fatal diseases, e.g. sepsis. The goal of our sensor is to facilitate a faster and easier read-out of the IL-6 concentration in the human blood.

The measurement is done with an integrated Field-Effect Transistor (FET): The gate is formed by a liquid. The liquid is deposited on a semiconducting layer that is built up of ITO which is functionalized with aptamers. Aptamers are biomolecules, to which the IL-6 molecules bind. If IL-6 molecules attach to the surface, the current through the transistor changes proportional to the concentration of the attached molecules.

The use of the transparent Indium Tin Oxide (ITO) could facilitate an additional optical read-out, which could give a qualitative impression of the presence of IL-6.

One chip consists of 24 transistors, which enables parallel read-out to improve the signal read-out and could later be used to measure the concentration of other target molecules and thus minimize the effect of cross-sensitivities.

The sensor can read the concentrations in real time. It is a low-cost device and could be used for point-of-care applications.

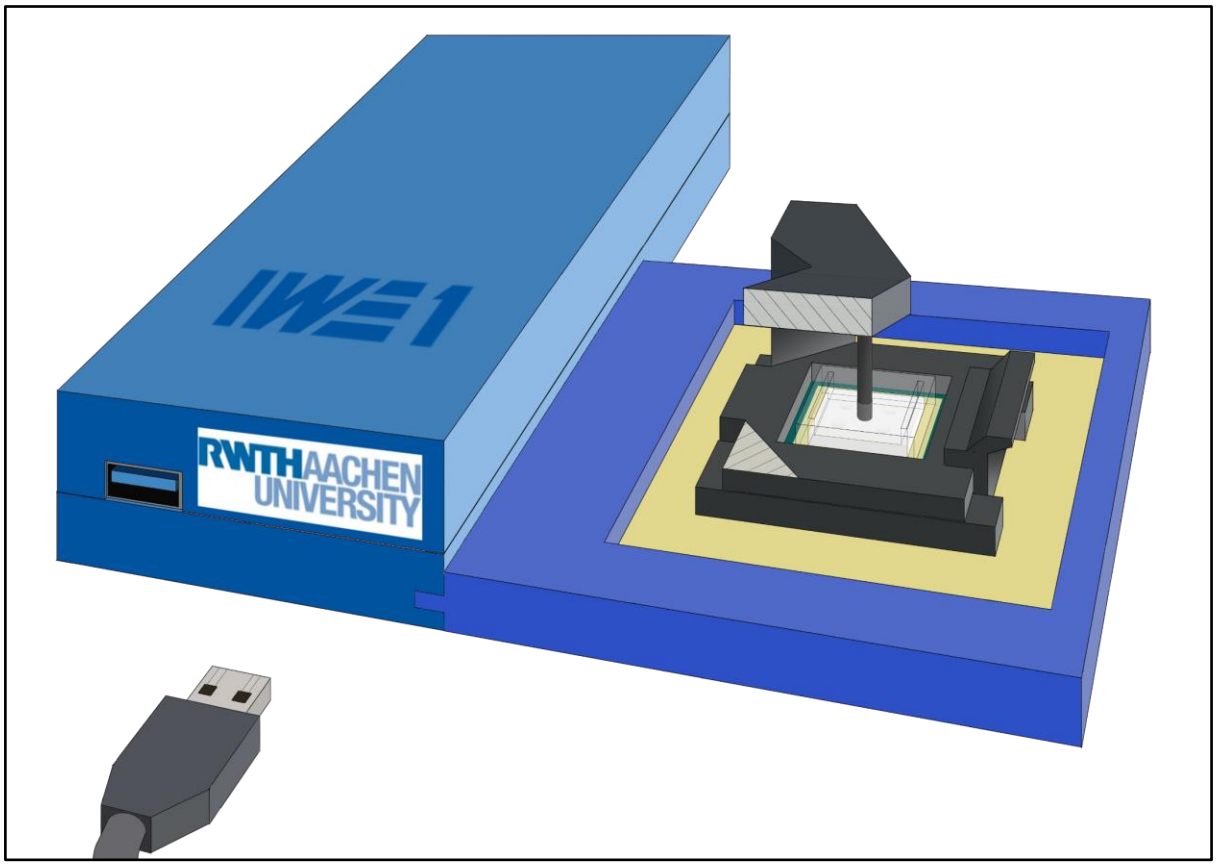


Figure 1: The sensor platform is made up of a commercial Ag/AgCl reference electrode and a disposable cartridge connected electrically via an electrical socket to a read-out set-up. That setup is powered with batteries and has a USB port to connect to a laptop for analysis.

2. Biosensor system and assay

Fundamental Principle

The principle of Ion Sensitive Field-Effect Transistors (ISFETs) is shown in Figure 2. The gate of the transistor is the solution. The solid-liquid-interface at the drain-source channel is functionalized with aptamers that bind specifically to IL-6. In the presence of the aforementioned interleukin, the binding mechanism generates a change in the gate voltage. This leads to a shift of the characteristic curve, which can be related back to the IL-6-concentration in the solution.

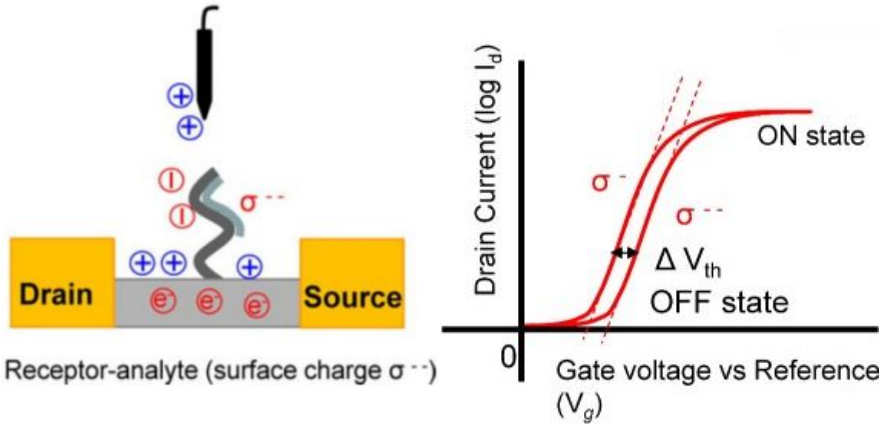


Figure 2: Fundamental principle of an functionalized ISFET [1].

Molecular Recognition and Assay Reagents

To detect IL-6, some 37 bases amino-modified aptamers specially made for that purpose and ordered from IDT, Inc. were incubated at standard temperature and pressure for a few hours. The aptamers were immobilized onto the ITO-layer through the binding to the epoxy rings of a layer of (3-Glycidyloxypropyl)trimethoxysilane (GPTMS) from Sigma-Aldrich that was deposited in a vapor phase. The aptamer sequence was proven in several different papers to have even better binding affinities compared to the standard IL-6 antibodies. So we expect to see better test results for the IL-6 detection in lower concentration ranges. Allowing us to detect ongoing sepsis already in early stages, which allows a faster medical treatment and a better prospect of recovery for the patient. For the analysis, the blood sample only needs to be centrifuged and pipetted onto the chip. Only a small sample volume (20µl) is required.

Physical Transduction

As explained in [1], the sensor is using an electrochemical transduction principle that is based on a changing potential of the ITO surface when IL-6 molecules bind to the aptamers. The potential changes are proportional to the IL-6 concentration in the analyte and can be detected due to changing characteristics of the FET. Four groups of six transistors facilitate higher reliability and can also be utilized for compensation of intrinsic measurement errors. A higher specificity is facilitated since the bio-layers of the transistors can be functionalized differently. Thus, it is possible to measure the concentration of different molecules and reduce cross-sensitivities. The structure of one chip is presented in Figure 3. The schematics of an individual transistor is presented in Figure 4.

The semiconducting layer at the solid-liquid interface is made of a thin layer of ITO with a thickness of 25 nm and dimensions of: 215 µm x 200 µm. All transistors are contacted to one main source line and have separate drain contact lines. The contact lines are made from 15 nm Au. Between Two transistors another contact is facilitated which could be modified to be used as a pseudo reference electrode which would then replace the Ag/AgCl reference electrode depicted in Figure 1.

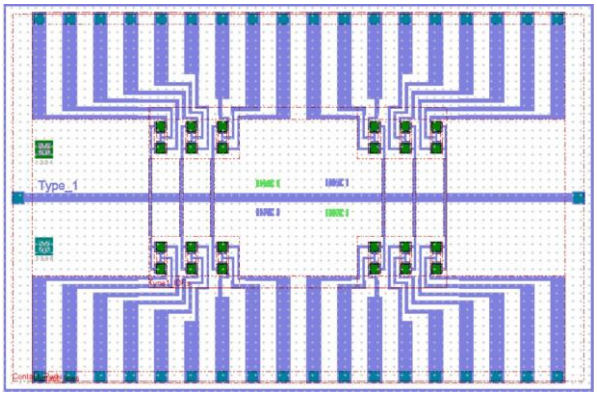


Figure 3: Structure of one chip with 24 FETs

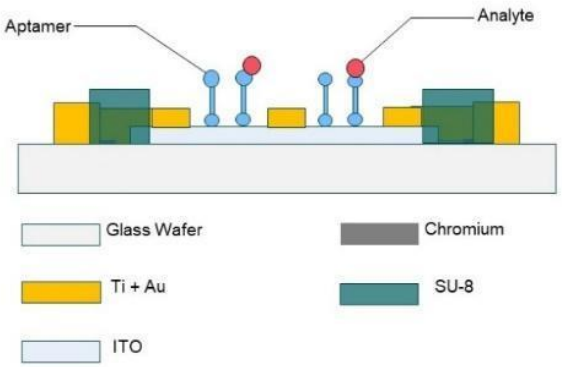


Figure 4: Schematics of the sensing chip and the materials used

Cartridge Technology

The chips are produced on a wafer, and after removal, they are wire-bonded on a chip-carrier. The bio-functionalized chip is then covered by a channel made from polydimethylsiloxane (PDMS) which guides the liquid to the sensing areas. A cellulose-strip in between the PDMS and the sensing areas causes the liquid to accumulate at the sensing areas and acts as a filter for large molecules.

Reader Instrument

The read-out is done by a custom read-out system made with a Teensy board. The transistors are supplied with voltage via the gold contact lines. A Ag/AgCl reference electrode in the liquid facilitates three-point FET measurement which could later be replaced by the pseudo reference electrode contact line. Either, the characteristic curve, or the current at the work-point can be measured to evaluate the concentration of the analyte. The cartridge is connected to an amplifier system by an adapter. Finally, the read-out system is connected to a PC, which runs a software to process the measurements and provide real-time results.

The system is itself quite small and powered with batteries, therefore easily transportable, which could be advantageous in emergency situations or for usage in remote locations.

User Interaction

To perform a measurement, not much action is required from the user. The sample needs to be centrifuged and around 20 µl of the supernatant needs to be injected into the inlet port with a pipette. Subsequently, the results need to be analyzed by the reading software installed on the PC.

3. Technological feasibility

Molecular recognition

The aptamers immobilization on an ITO layer is proven through wettability measurements. Because they are made of nucleic acids, they are known to be hydrophilic [9]. Hence, the drop of contact angle in Figure 5 could be correlated to a successful immobilization of aptamers on a substrate. The sequence used is sourced from literature, wherein the IL-6 is reported to bind specifically to the aptamer.

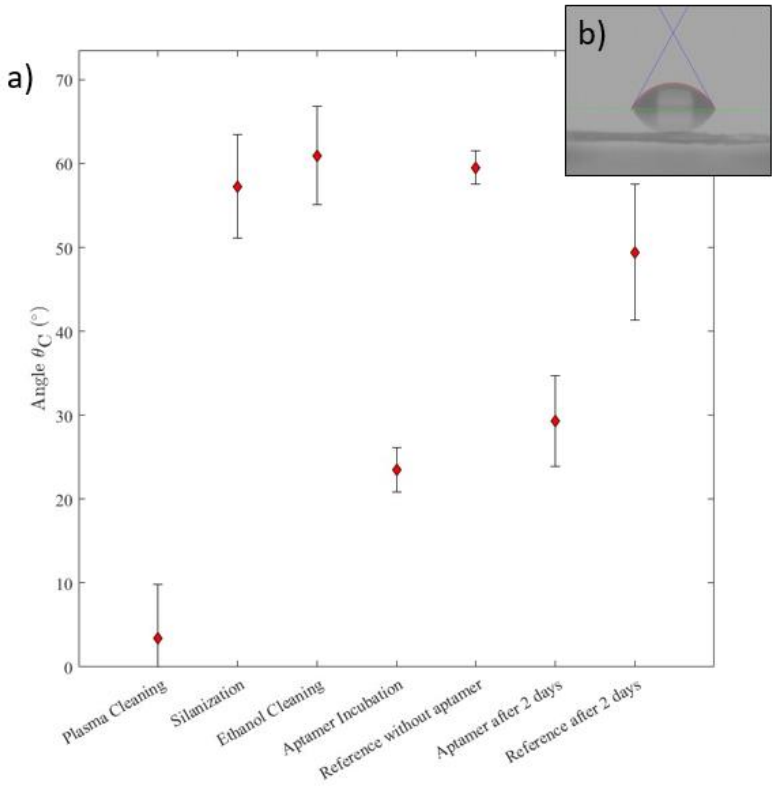


Figure 5: Change in wettability and surface energy due to surface modifications during the biofunctionalization processes. (a) Values of contact angle before and after GPTMS silanization, before and after aptamer incubation and after two days. (b) Measurement after silanization on a glass substrate covered with 25 nm ITO.

Physical transduction

ITO being an n-type semiconductor material with a large bandgap [10], it has the potential of acting as a channel material for a field-effect transistor. ITO and also the Ti/Au electrical contacts can be sputtered with standard fabrication processes. Figure 6 displays the result of fabrication for one of the sensors of a cartridge. The field-effect can be observed by varying the gate voltage and monitoring the drain current as can be seen Figure 7.

The current remaining challenge is to increase this effect by, for instance, thinning even further the layer of ITO and finding the optimal geometry for the channel. If the effect is too small, it will be overshadowed by the ambient noise and that would therefore dictate the lower limit of detection of the sensing platform.

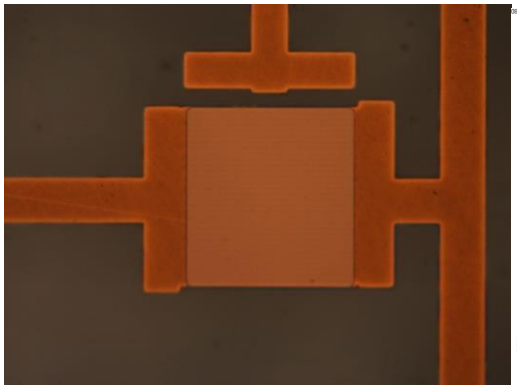


Figure 6: One of the 24 sensors of a cartridge .

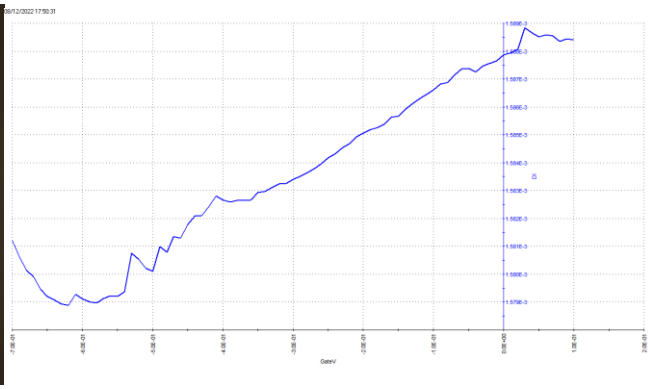
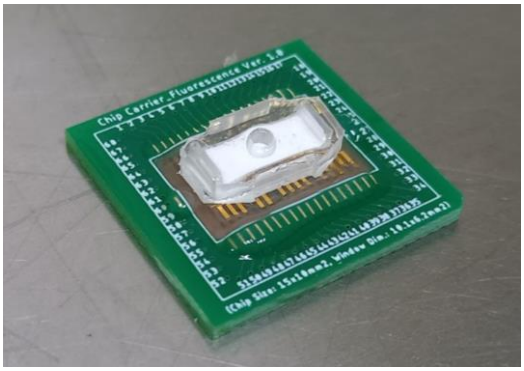


Figure 7: Transfer characteristics of an ISFET ITO chips in standard conditions.

Fluidic cartridge



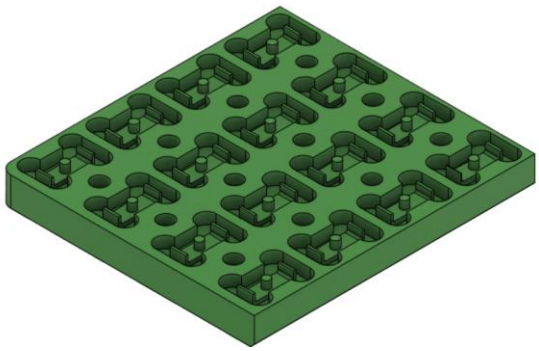


Figure 8: Fluidic cartridge made up of an encapsulated chip, a cellulose strip and a PDMS channel.

Figure 9: PDMS channel mold for mass-production.

The electronics part of the fluidic cartridge is fabricated with well-established scalable standard chip fabrication processes and thus proven to be feasible. The PDMS channel is made with a mold and therefore can be mass produced. The same goes for the cellulose strip. The assembly is done manually for now, but can be automated in the future.

Reader instrument

The only requirements for the read-out setup is to apply a chosen voltage, which can easily be realized with batteries and evaluate a current, which is typically done in DC mode via a transimpedance amplifier. The current is evaluated from the ratio between the output voltage and the feedback resistance. This setup has already been proven to be working in other smaller projects.

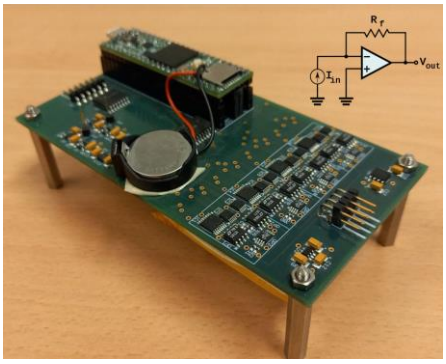


Figure 10: External read-out system used to apply voltages and measure a current.

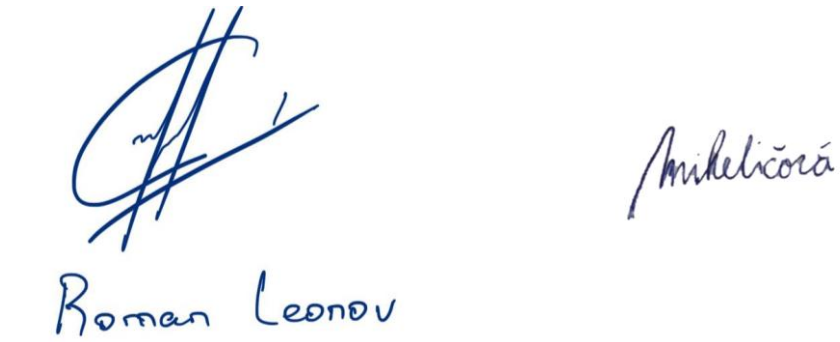
4. Originality

Part written by the team:

The use of an ISFET for the detection of IL-6 is described in [2]. ISFETs are advantageous as they provide fast and label-free real-time detection and provide the potential for high-scale production and thus low costs. Our sensor facilitates multiplexing due to many sensors on one chip. We chose the novel approach of using nanometers-thick ITO as a semiconducting layer which is hardly found in the literature. A characterization of ITO is given in [3]. This could facilitate additional optical read-out.

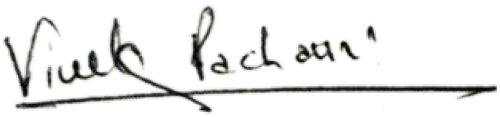
Our team developed the chip with 24 ISFETs on it, that are biofunctionalized with aptamers.

Aptamers are a relatively novel biomolecule. They stand out by their high stability and affinity [4]. Sequences for aptamers binding to IL-6 can be found in [5-6].



Part written by the supervisor:

Nanoscale biologically sensitive FETs (BioFETs) have been widely reported for biosensor applications in literature, however, high TRL development and commercial exploitation of BioFETs remains elusive till date. Typical challenges related to the high TRL development of biosensor platforms is signal fluctuations due to parasitic influences, biofouling and lack of scalability in the fabrication of biosnesors. Here, semiconducting ITO based ISFET open up a unique opportunity to realize two-dimensional (2D) BioFETs, with material and electrical characteristics representative of an ideal platform. Widely available top-down lithography techniques that can be applied for the fabrication of such biosensors at wafer scale are expected to offer an affordable platform. In comparison to otherwise expensive and elaborate processes for the fabrication of 2D FETs, the option to define ITO based BioFETs is a uniquely interesting idea that the Aixsense team came up with. Another distinct advantage of realization of 2D BioFETs based semiconding ITO is the optical transparency of the sensor devices, which can in parallel enable an optical approach to carry out an in-parallel optical detection either for alternative hybrid sensor principles, or for validation of molecular interaction at the sensor interface, and confirmation of transduction. Readily available surface fucnitonalization approaches via silanization, can also aid in fast development of such a platform for realization of high quality biofunctional layers and achieve high technological readiness.



5. Translation potential

When starting work on a biosensor or any other device, manufacturers always need to think about business model validation. It consists of three important aspects: user (or stakeholder) desirability, technical and business feasibility and economic viability. User desirability means how necessary and important the development is, whether analogs currently exist and which characteristics they have. Such information helps to understand if such a development is reasonable. Technical and Business feasibility means that it is technically possible to implement the idea. The plan must be realistic and achievable. Moreover, it is important to understand which companies to work with in the future and how they can benefit from developing the product. Financial viability is based on statistics and already existing inventions in the field. It is important not only to invent a sensor, but also to be able to monetize the product.

Stakeholder desirability

Sepsis affects almost 50 million people around the world every year, about 20 percent of them die. This number depends on the quality of life and level of medicine in a certain country or region. Each 3 seconds a person dies due to sepsis. It is the most common cause of death of most infectious diseases. Sepsis occurs when a person's own tissues and organs are damaged by the immune system while fighting an infection. Many surviving patients suffer from the consequences of sepsis for the rest of their lives, due to the need of severe surgeries, to save their lives. Risk groups include people with weakened immune systems, adults over 60, children under one year, people with chronic diseases, e.g. lung, liver, heart diseases, people with AIDS or diabetes, people with no spleen. Thus, there is no doubt that the earliest possible detection of sepsis is an important factor in reducing mortality from this disease and the prospect of recovery.

Technical feasibility

Unfortunately, modern sepsis detection methods (here ELISA is mostly meant, as it is the main used way to detect Interleukin-6) take about 5-16 hours. The goal of this work is to contribute to the development of research in this field and to produce a sensor that works much faster with comparable detection limit and range.

As our work leads us to only prototype development, the following development should be done with the support and supervision of companies having an experience in the same field. On one hand, the companies can provide technical expertise for further development, on another hand - they have the technical ability to produce large batches of the sensors. In addition, they have the marketing divisions, able to find the customers in the best way.

Financial viability

In comparison to previous methods for detection of interleukin-6 the costs can be reduced due to following factors:

- No need to prepare the biological substance for analysis. Doctor or nurse should be able to take a patient's blood, centrifuge it and make a test;
- It is not necessary anymore to have laboratory assistants because testing procedure is very easy to do;
- Faster analysis (≈ 15 min instead of 16 hours).
- Main readout system only needs to be purchased once and additional detection units can be purchased as needed

Calculation of a self-cost of a sensor batch

One wafer contains 42 devices (around 35-40 wafers in a batch due to production process limit). The cost of the wafer itself is 40 euros (1400-1600 euro for batch). According to the experience of RWTH laboratory, one batch takes 5-7 working days. If we consider a normal working day of 8 hours and its cost of around 25-30 euros per hour, the staff cost to produce one batch of sensors is around 1000 – 1680 euro. The approximate cost of consumables (mostly chemicals used in production) for one batch is 200 euro.

Table 2: Costs for producing one batch

	Number	Cost, euro	Sum, euro
Wafer	35-40	40	1400-1600
Working hours	5-7 days * 8 hours	25-30	1000 – 1680
Consumables	-	-	200

Thus, a rough estimate of the cost per batch is approximately 2600 – 3480 euro (2,9 euro for one single sensor if 1200 out of 1600 in one batch are produced successfully). In addition, readout system costs, clean room costs, marketing costs and patent costs should be taken into account. Their impact will depend on the number of sensors to be produced. In total, the readout unit only needs to be purchased once and then additional detection units can be reordered at a considerably low price of around 5 to 10 euro per piece which will lower the long term operating costs of the device.

Product translation potential

Each time one introduces a new product or service, one has to estimate whether it has enough potential for translation to the market. It is difficult to estimate this directly, but there are some aspects that help to do that.

Market size

The first and most important factor is the size of the market of the product. The size of the market is the market sales potential of all the companies together. In our case, the potential size of sales depends directly on the trend in sepsis statistics (Figure 11).

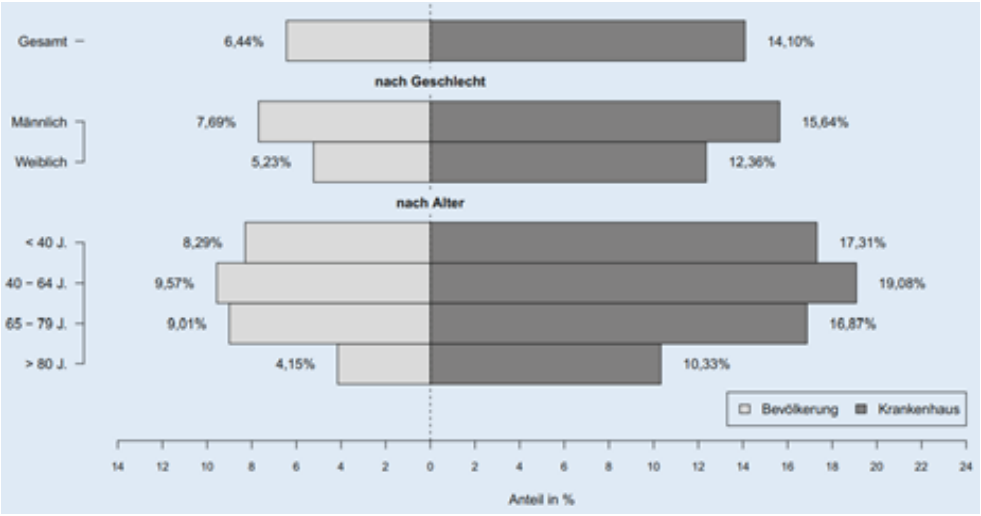


Figure 11: Sepsis-associated deaths in hospital and Germany-wide deaths in 2016 in % [8].

Statistics on sepsis according to age group are also important (Figure 12).

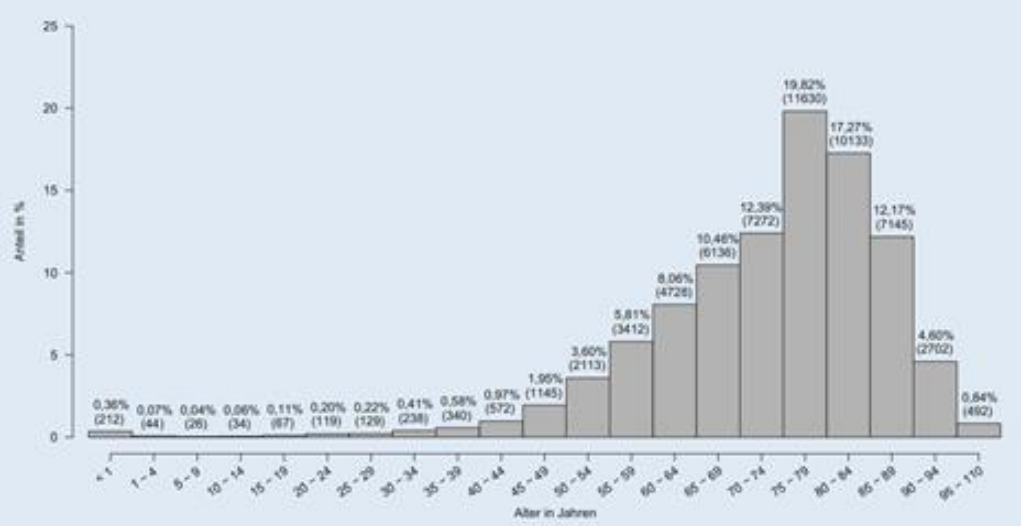


Figure 12. Age distribution of hospital deaths associated with sepsis in Germany in 2016 [8].

Market Growth Rate

Figure 13 shows the German population for 2016 and 2022, with a distribution of the population by age groups. Importantly, the number of elderly people will prevail in 2022 compared with 2016. This indicates that the potential incidence of sepsis is higher in 2022.

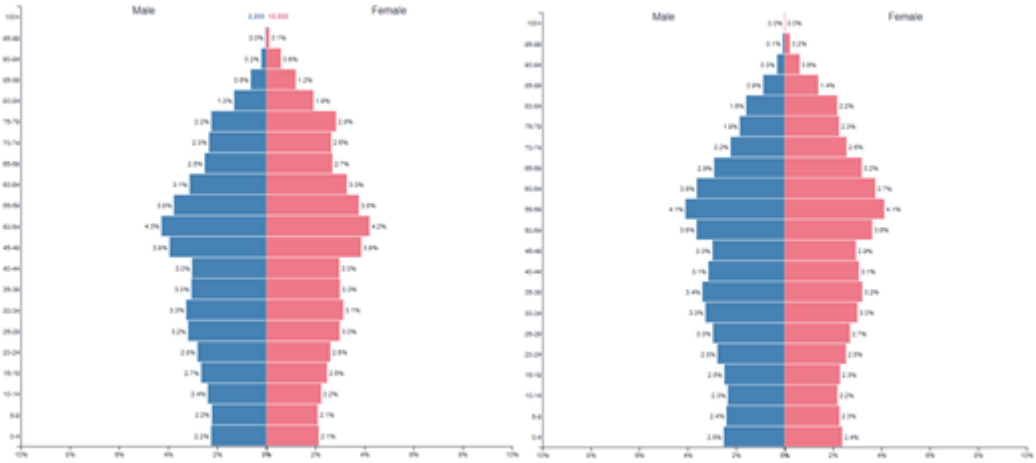


Figure 13. Population in Germany in 2016 and 2022 respectively.

Competition ability

In the case of product line extensions (which means that product does the same as before but with a little difference) entering the market may be quite expensive. The product should not cost much more than already existing ones. At the same time, manufacturing costs may be much higher. This makes the company lose money.

According to the advantages of the new sensor, changing the clinical treatment procedure and entering the market should not be very expensive. The sensor works based on technology that takes much less time, so the sensor may become a good replacement for existing ways of detecting interleukin-6.

Type of product and consumer

It is important whether the product will be bought regularly or is a one-time investment. The intention is that tests such as ours are bought systematically by hospitals, e.g. once a year or month based, so manufacturing is constant and income will be relatively stable.

The purchase of the medical devices (such as the developing sensor) is very specific, because the solution making is complicated. One side is that the patients actually take the advantage of the new method, since their prospect of recovery get better. On the other hand, the doctor makes the decision to perform the test and to start antibiotic treatment. The third involved part is the hospital, since it purchases the sensors based on the experience of doctors, its financial state, strategy and other factors. Finally, the regulation authorities and insurance companies are having a big impact on the purchase and cost covering of the particular sensor.

From our side, we are going to start to work with doctors, showing the ability of the new detection method to speed up and improve the treatment procedure. We are going to use the help of big medical technology companies (such as Siemens Healthineers) to get in contact with the hospitals, which will be the first step to distributing our sensors widely.

6. Final Remarks

The sensor platform that we developed is based on the concept of a reusable read-out unit combined with disposable detection units that can be sold separately. These detection units could be designed for further detection than just IL-6 by biofunctionalization of the ITO surface with highly specific receptors. This would enable the end user to conduct a wide variety of tests on one single readout unit. For this purpose, it is also necessary to establish a process to utilize the extra gold contact line as a pseudo reference electrode by processes such as galvanic growth.

Further developments in microfluidics, such as integrating a filter membrane prior to the cellular stripes in the fluid chamber, would erase the need for sample centrifugation in the analyte preparation step. This would allow testing with direct blood, which is even more advantageous in emergency situations or in remote locations.

7. Team and support

Contribution of Team Members

Roman Leonov: Team Captain, organization, chip fabrication

Annamària Miheličova: Team Captain, organization

Dylan Nguyen: Team Captain, organization, microfluidics, technical read-out,
chip encapsulation, biofunctionalization

Giorgi Kurkhuli: Masks design, chip fabrication

Magdalena Schone: Microfluidics, chip encapsulation

Janic Töx: Microfluidics, technical read-out, chip encapsulation, biofunctionalization, chip fabrication

Sven Töx: Biofunctionalization

Kenichi Akutagawa: Biofunctionalization

Maria Vakaeva: Translation Potential

People who have given support

Dr. rer. nat Sven Ingebrandt: Head of the Project

Vivek Pachauri: Supervisor, technical advisor

Dibyendu Khan: Introduction to the chip fabrication processes

Animesh Singh: Introduction to the chip fabrication processes

Jochen Heiss: Metallisation step

Linda Wetzel: Media support

Chunling Li: Introduction to chip encapsulation

Marcel Tintelott: Introduction to biofunctionalization processes

Dr. Divagar Muragan: Advices for the aptamers

Dr. Yogesh Singh: Insight on the clinical aspects.

Fabian Brings: Read-out system design

Institute for Biotechnology RWTH Aachen: Laboratory support

8. References

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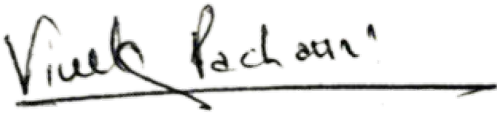

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