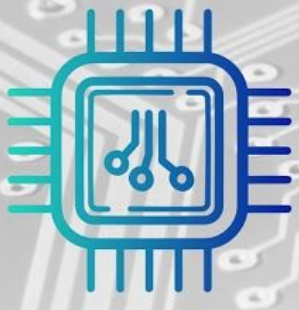
SensUs

RWTH AACHEN UNIVERSITY



AIXSENSE

Team Results Document
SensUs Competition 2018

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AixSense

RWTH Aachen University

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0. Summary for the SensUs website

We designed a biosensor to measure the concentration of the last-resort antibiotic Vancomycin in blood plasma via electrical impedance spectroscopy. The plasma is pumped through our sensor, guided by fluidic channels, and passes gold electrodes that are covered with a conductive polymer (Polypyrrole) and antibodies. The Vancomycin in the plasma bonds with these antibodies and thus “sticks” to the electrodes. The bonding causes a chemical reaction that can be translated to an electric signal. The conductive polymer provides an optimal environment for bonding and amplifies the electrical signal, helping to achieve better measurement results. When more Vancomycin bonds with the antibodies, the electrical signal changes based on changes in conductivity of the gold electrodes. This change is tracked over time with the help of a measurement tool (impedance analyzer). The data is processed computationally and translated to a value for concentration of vancomycin.

What is novel about our sensor is the use of a Polypyrrole matrix to immobilize Vancomycin antibodies on gold electrodes to amplify the signal. This approach improves the measurement results and avoids unwanted bonding of molecules. We hope to have achieved a fast and accurate way to measure Vancomycin concentration to help ensure safe and effective dosing.

1. Biosensor System and Assay

1.1. The Chip

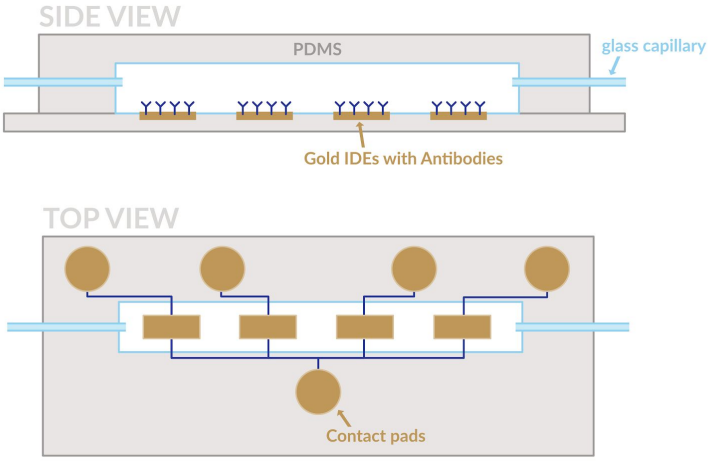

Figure 1 Microchip structure with biofunctional surfaces

The chip's microfluidic channels consist of a PDMS layer molded over the PCB with glass capillary inserts through which analyte was added. Figure 1 offers a top and side view of the microfluidic design. To create the PDMS layer, a negative was first milled out and then submerged in PDMS. The PDMS was then cured and removed from the mold before attaching it to the chip, where the negative left by the mold now created the empty volume for the microfluidic channel. The inlets and outlets, as well as access to the contact pads, were created by a biopsy punch.

Inside the microfluidic channel are four arrays of gold inter-digitated electrodes (IDEs), and each IDE has its own separate contact pad which allows for simultaneous measurements and analyses. On the whole, 20 fingers form a unique IDE with a spacing of 10 um, illustrated in Figure 2.

Figure 2 Gold IDE surface with polypyrrole

The gold IDEs are modified with a layer of vancomycin antibodies in a polypyrrole matrix. As the analyte is added to the microfluidic channel, the antibodies in the polymer layer binds any vancomycin present in the solution. The binding of the vancomycin molecule near the IDEs disrupts the baseline electric field between the fingers of the gold IDEs. Depending on how much vancomycin is

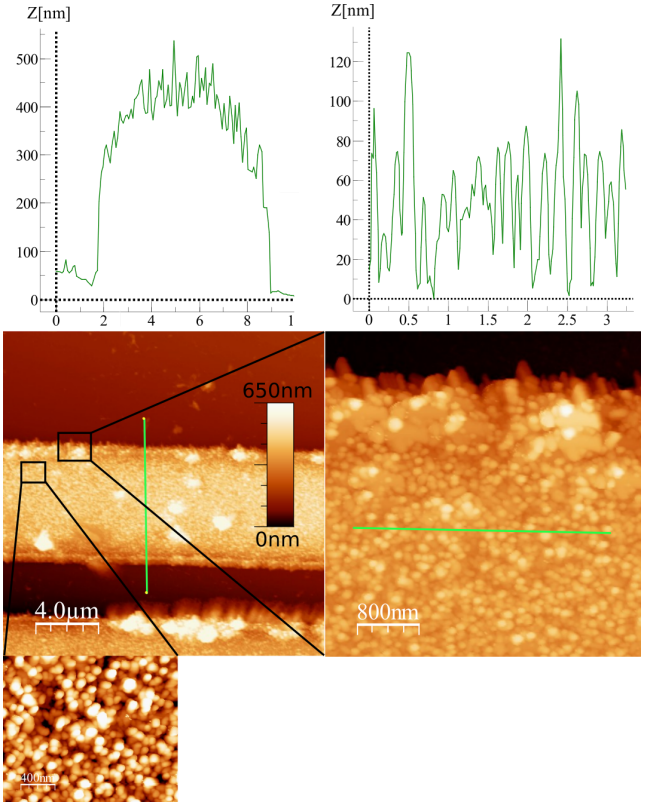

Figure 3 Picture of the AFM from the PolyPyrrole -vancomycin IDE surface

bound and the height of the MIPs layer, the electric field will be disrupted more.

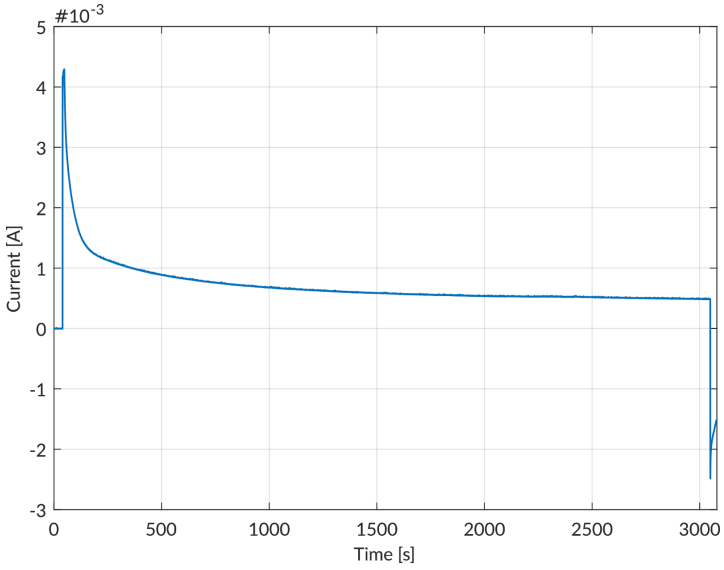

Figure 4: chronoamperometry response

2.2 The polypyrrole-antibody layer

The measurement scheme is based on the ability of the vancomycin antibodies to bind vancomycin as it flows into the microfluidic channel and over the gold electrodes. The polypyrrole-antibody layer was created by first creating a solution of 100 mM pyrrole monomer and vancomycin antibodies in 150 mM PBS. All reagents were purchased from Thermo Scientific. The chip was placed inside an enclosure having a circular cavity which was filled up with 2.5 mL pyrrole-antibody solution. The counter and reference electrodes of the CompactStat electrochemical measurement station from IVIUM Technologies were placed within the cavity. A single contact pad was hooked up to the working electrode and chronoamperometry was performed with 0.9 V for 30 seconds, during which the pyrrole polymerized onto the IDEs, entrapping the vancomycin antibodies within. The current response is shown in Figure 4. Each contact pad was then iterated through, and a layer of polymer-antibody matrix was deposited on each IDE. The presence of the polymer matrix on the gold electrodes was confirmed via light microscopy which is also shown in Figure 2. The darker areas represent the presence of polypyrrole. Furthermore, atomic force microscopy (AFM) was used to analyze the height of the polypyrrole-antibody layer, illustrated in Figure 3. In order for the bound vancomycin to disrupt the electric field between the IDE fingers, the polypyrrole layer could not be too high.

2. Analytical Performance

In order to determine which AC frequency needed to be used for our chip, impedance spectroscopy was performed on chips with the presence of PBS and PBS+Vancomycin Antibodies. In the following step, a real time measurement was performed with multiple frequencies between 1 kHz and 10 MHz at a voltage of 200 mV to avoid any kind of unwanted electrochemical reactions. For that, the impedance analyzer ISX-3mini from Sciospec Scientific Instruments GmbH was used.

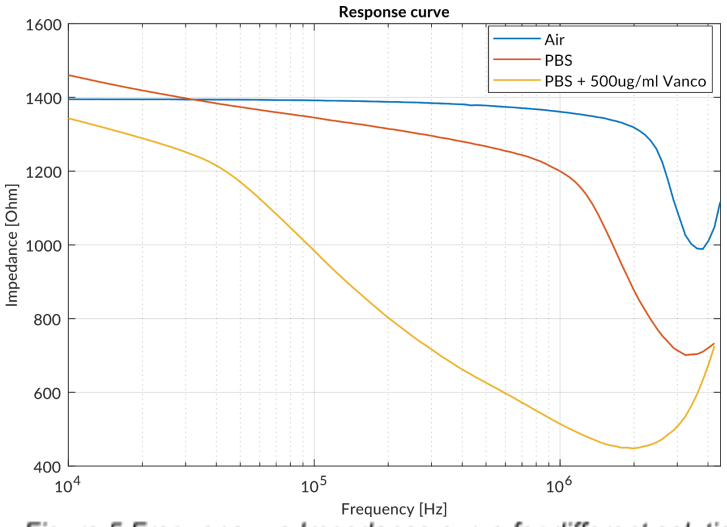

Figure 5 Frequency vs Impedance curve for different solutions

The measurement apparatus was set up and the related readings were taken in the following manner: Firstly PBS was injected. The channel was filled with a PBS solution with a certain concentration of Vancomycin Antibodies after the measured results from the previous step showed a stable response. The outcomes are illustrated in Figure 6 with concentrations from 2 ug/ml to 500 ug/ml for different frequencies. A syringe pump was used to generate a constant flow of 9 uL/min.

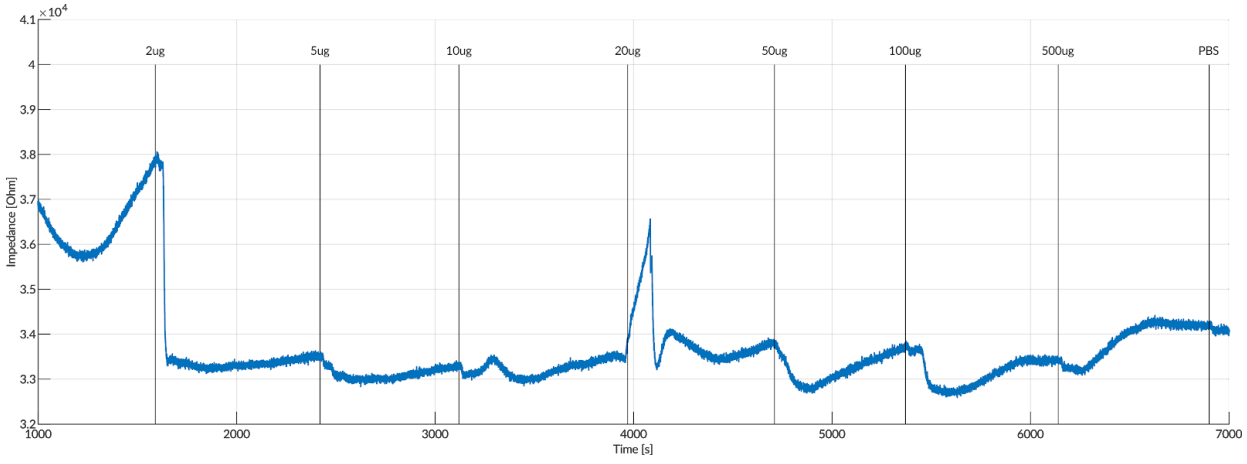

Figure 6 Impedance response for various vancomycin concentrations

It can be seen, as depicted in Figure 6 above, that the slope of the curve depends on the concentration of vancomycin. Same applies for the local minima. From that, a calibration curve can be extracted.

The advantage of measuring the rate of change in the slope in real time is that we get the resulting concentration of vancomycin within seconds.

3. Novelty and Creativity

3.1. Already available

The idea of the project is a combination of two main concepts:

The first one, related to the field of Electronics, is the use of electrodes for the measurement of change in impedance when in contact with a certain target molecule (vancomycin in this case). This is technically denoted as 'Electrochemical Impedance Spectroscopy (EIS)'. It has been established in the previous sections that at a certain frequency the impedance variation of the electrodes is maximum and this variation directly depends on the concentration of the target of interest. This influenced the selection of the type of electrode to be employed and the determination of the optimal frequency. The former was chosen to be interdigitated electrodes (IDEs). The proven versatility and accuracy of IDEs were the main reasons for their selection. The combination of different parameters such as the number of fingers, the distance between them and their width makes it easier to change and adapt the total area of the electrode and height of the electric field, which provides flexibility in terms of designing. Gold was chosen as the electrode material due to its good electrical properties and performance and its resistance to oxidation and corrosion. The frequency was experimentally determined as previously specified. This approach by EIS delivers a versatile sensor, at low cost.

The second concept, related to the field of Biology, is the use of antibodies for capturing vancomycin molecules. These act as custom size claws for the substance under control. This technique is widely used in protein detection. Our main aim was to achieve the combination of both.

3.2. New developments

Although both of the principles described above have been used before, they were not used in the measurement of vancomycin. Based on the experimental results attained by us, it is evident that vancomycin molecules in combination with the antibodies have sufficient charge to vary the impedance of the electrodes which is detectable by means of EIS. This constitutes our biggest achievement.

Moreover, as there was a need of gluing the vancomycin antibodies with the gold electrodes, a polypyrrole matrix was binded to them by means of a new technique known as 'chronoamperometry'. Polypyrrole was chosen as it is a hydrophobic matrix, thus, minimising unspecific binding. Additionally, it is a non-insulating medium, is biocompatible and reduces the insulation between the antibody and gold surfaces.

The main strength of this sensor relies in its combination of already widely used procedures, but modified for the current purpose, consequently, reaching a successfully fast, accurate and low cost result.

4. Translation Potential

4.1. Stakeholder Desirability

The biosensor is aimed at the healthcare service providers, specifically hospitals with already established protocols in collecting and monitoring vancomycin concentration in patients. These protocols and guidelines in vancomycin therapy are thoroughly identified and placed to monitor patients' conditions. Some defining factors are based on severity of patient's condition, pre-existing conditions and how patient's condition develops during treatment [1]. This ultimately means that this therapy could last several days, which translates to countless hours spent in laboratories for analysis and measurements. [1]

Thus, it places a heavy financial burden on both hospitals and patients. Costs including but not limited to analysis costs, costs associated with time and effort spent by doctors, nurses and laboratory personnel. [1]

For Doctors, and nurses alike, the process of collecting samples, transferring them to the laboratories is a time-consuming process. For laboratories, not only storing these samples for many patients is a challenge, but also analyzing all these samples is time consuming. For hospital management, storage is a huge challenge, especially small hospitals with not so adequate storage facilities. Also costs of treatment can severely limit the potential services that hospitals may be willing to offer to patients and insurance companies alike may be less willing to cover patients for such treatments.

Most importantly for patients, longer stays in hospital and prolonged treatments can have negative effect on mental health of patients and increase the chances of depression and anxiety. [2] since patients requiring vancomycin treatment are in severe conditions, negative effects of depression may be more noticeable and deteriorate their chances of survival.

Based on all the above evidence, an affordable solution is necessary which not only would help with improving patients experience in hospitals, but also to allow hospitals to treat more patients while reducing the time spent on monitoring and analysis in laboratories and the costs associated with the process. [1]

4.2. Technical Feasibility

Our AixSense vancomycin measurement unit helps hospitals who want to control the amount of prescribed antibiotic to infected patients by reducing the sample analysis time and effort and enabling doctors to have on-site, real-time results to help the patients. With this value proposition, the microstructure vancomycin analyzer will enable the users to calculate the concentration of vancomycin in patients within minutes and have reliable and robust results. Real-time measurement, will provide faster and cheaper vancomycin concentration optimization and in turn will help the hospitals and doctors to improve the offered healthcare quality. The size of the device also allows treatments from patient's bedside, thus eliminating the sample transportation time.

several steps can be taken to improve this prototype and ensure its market readiness.

The prototype relies on external measurement tools that are large and come with features that are not fully utilized. Hence the measurement tool can be changed with a purpose-built unit which is integrated in the system and not only will reduce the overall size but also reduce the cost of the end-product. Moreover, the prototype lacks a robust packaging and housing which isolates the sensor from the outside world. This is of utmost importance as not only it protects the sensor from the outside hazards, but also it protects the outside world and users from the sensor.

The process for biofunctionalization can be also optimized. After the parameters for chronoamperometry were optimized, the electropolymerization process could be sped up significantly. In a manufacturing context, a template set of contacts could be designed to attach to all contact pads on the chip simultaneously, and then the polymer layers could be created by simply altering with contact was receiving power from the chronoamperometer. This would greatly reduce the amount of time and effort required for polymerization.

With these two factors in mind, the gap between the prototype and the end-product is considerable, given the effort it will take to produce a purpose-built measurement unit and a biocompatible packaging which is suitable and approved for hospital use.

To commercialize this product, several steps are needed to be followed to successfully introduce this product to the market. Once the product has been produced in small numbers, series of pre-clinical testing is needed to assess the manufacturing quality, leading to risk assessment of the device. Next step is to assess whether the device follows the economical standards in healthcare of the targeted country. Clinical trails and product validation are next logical step in initiating human trails after successful results in pre-clinical trials. In each targeted country tests must be conducted to evaluate the functionality and feasibility of the device. After successful clinical trials, device could be sold to the target markets where the device was approved. post market follow-up can be done to monitor devices' performance and report potential failures. moreover, the target stakeholders are kept in contact to address any issues that they might be facing while using the device.

4.3. Business Viability

The most expensive aspect of our current chip design is undoubtedly the antibodies present in the polypyrrole matrix. If these could be further supplemented or even replaced with other, cheaper technologies, such as Molecularly-Imprinted Polymers, the costs associated with chip production would be greatly reduced. Production of the electrically sensitive parts, such as IDEs and gold contacts have relatively small production cost and are easily producible, making large scale manufacturing of this design financially feasible and fast. The sources of revenue at first will be based on sponsoring from interested companies and other related institutes n forms of providing means to manufacture the desired parts or provide expertise and assistance in developing alternative solutions, and giving advice on market view of such products, that would benefit our sensor and boost its feasibility and market acceptance. Based on this approach size of initial market will be small and limited to the regions within the reach of the sponsors, local and international.

5. Team and Support

5.1. Contributions of the team members

***Patrick Döll**:* Responsible for the antibody immobilization, data analysis and measurement and involved in the construction of the microfluidic channel.

***Kaiwen Feng**:* Participation in the chip fabrication including workflow design, cleaning room work and dicing; part of PCB solder and measurements.

Alexander Grade: Team member for organizational purposes and group leader of the PCB/Chip holder group.

Michael Halwes: Helped develop polymer-antibody layer.

***Sarah Hilker**:* Graphic, web & information design, social media takeover

***Firas Khader**:* Member of the chip fabrication team (process development, clean room work, dicing)

Stefan Meeger: Involved in measurement, calibration of the chips, data acquisition and data analysis.

***Parham Mohajerani**:* Team member for designing microchip mask with CAD software. Assisting microfluidic fabrication and measurement teams.

Paula Palacios: Participation in the mask design and in the chip fabrication. IDE's and microfluidic channel design with AutoCad and respective collaboration in the clean room.

Mohit Suranglikar: Involved in the fabrication of the microchip and the designing of the microfluidics. Designed the team logo, the 3D models of the Microchip and PCB adapters and the PDMS mould.

Tianyun Wang: Vancomycin-targeted MIPs growth on the IDEs, which including polymer layer deposition and antibody immobilization.

Qiwei Zhang: Responsible for design of PCBs regarding the type of the different chips(chip with a complete centre pad and with split centre pad); PCB soldering.

5.2. People who have given support

Dr. rer. nat. Sven Ingebrandt: Head of the entire project.

Dr. Vivek Pachauri: Technical advisor for Microfluidics, Measurement of Signals and Data Analysis.

Xiaoling Lu: Technical advisor for the project.

Stefanie Wiedemann: Supervisor for the organization and management of the project.

Tom Kremers: Technical advisor for Mask Design and Fabrication Process.

Dorothee Breuer: Microchip Fabrication supervisor and consultant.

Ewa Sekula: Microchip Fabrication supervisor.

Thomas Hoffmann: Mechanical workshop consultant.

5.3. Sponsors

Sciospec Scientific Instruments GmbH:
The impedance analyzers imperative for the measurement of the signals were provided by Sciospec Scientific Instruments GmbH. The training required for operating the same was also provided by them.

University of Maastricht:
The novel approach of using MIPS for measurement purposes was tried and studied at the University of Maastricht.

6. Final Remarks

To summarize, we have devised an effective sensor which can detect different concentrations of vancomycin, characterised by different slopes of the measurement curve.

In our biosensor, antibodies are used to recognize and bind Vancomycin with high affinity and selectivity. The disruption of the baseline electric field between the fingers of the IDEs make the concentration of the vancomycin intelligible. The most expensive component of our current sensor is the antibodies. To reduce the cost of our sensor, we had tried a novel approach which uses MIPs (Molecularly Imprinted Polymers) to bind vancomycin. But due to the scarcity of time for studying and implementing this approach in detail and also the difficulty encountered in growing MIPs on chips, this approach could not be pursued. However, we will continue to study the use and efficiency of MIPs in detail in the near future. Our appetency for improvement of our biosensor will not die down and we will be motivated to carry on with this work to make our sensor system more complete and efficacious. Our main aim in the future would be to commercialize the sensor with a focus mainly on the improving the portability, accuracy and response time, providing user-friendly display and other such features that would aid the user and most importantly reduction of the cost of the whole system. We have an amazing team composed of incredible individuals from different backgrounds, who contribute great, vibrant and creative ideas and always strive to make this a great success.

7. References

[1] James, Christopher W., and Cheryle Gurk-Turner. Advances in Pediatrics., U.S. National Library of Medicine, Apr. 2001, www.ncbi.nlm.nih.gov/pmc/articles/PMC1291340/

[2] Shoar, Saeed, et al. Advances in Pediatrics., U.S. National Library of Medicine, May 2016, www.ncbi.nlm.nih.gov/pmc/articles/PMC4852078/.