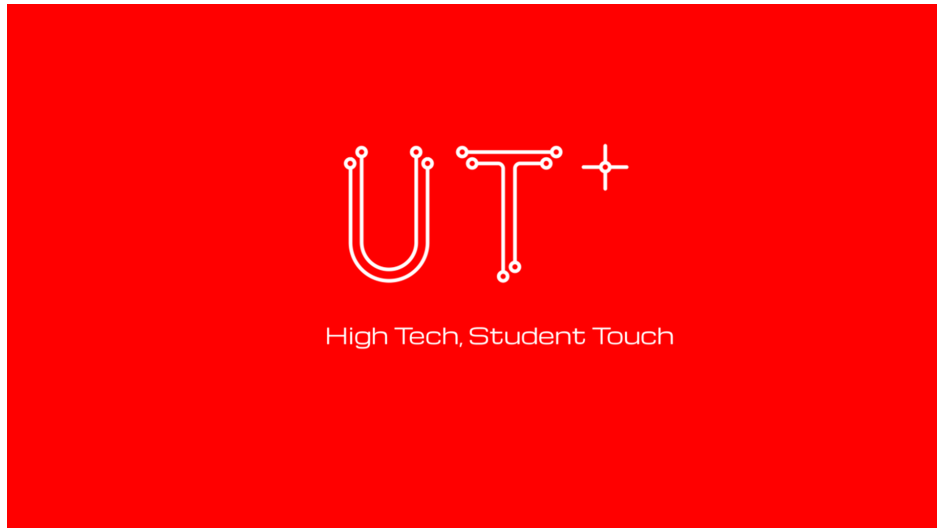


SENSUs 2020

TEAM RESULT DOCUMENT 13/08/2020



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Abstract

Epilepsy accounts for a significant proportion of the world's disease burden. Valproic acid (VPA), is one of the main antiepileptic drugs used. VPA binds to proteins contained in blood (mainly to albumin, 80- 90%). However, its remaining free fraction (fVPA) can cause overdose, leading to the need to be monitored. Point-of-care (POC) devices provide personalized diagnostic tests. However they are not available for VPA yet. Since biosensors fail mainly due to nonspecific adsorption (NSA) of other proteins in blood, their surfaces need to avoid NSA of foulants and bind specifically the biomolecule of interest. Polymer chemistry allows tuning properties of the biosensor's surface. Zwitterionic polymer brushes are so far the best antifouling materials. They can immobilized biomolecules in an oriented manner.

Here is presented the *ZwitterPlus* biosensor. A microelectromechanical system (MEMS) chip with an effective zwitterionic polymer brush antifouling coating with oriented immobilized anti-VPA antibodies to specifically capture VPA in blood plasma. This is in collaboration with Bronkhorst-High-Tech, a company leader in industrial flow sensing with an increasing interest in biomedical application. Furthermore, we included a business model proposal to bring the biosensor concept to the market.

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Biosensor system and assay and Technical feasibility

1.1 Molecular recognition and assay reagents

The *Zwitter Plus* immunosensor uses antibodies as biorecognition elements. For the capturing of the fVPA an antifouling coating loaded with antibodies can be created. The surface of a chip is modified with a covalently attached hydrophobic polymer as anchor layer, for the growth of zwitterionic polymer brushes. These polymer chemistry enables a long-term antifouling coating [1] resisting fouling from undiluted media [2–4], due to its super hydrophilicity properties [5]. It tightly binds water molecules by their charged moieties [6], generating a high resistance water layer and preventing proteins contained in blood to be adsorbed [6]. As an advantage no dilution is needed. Their polymerization technique (atom transfer radical polymerization) leaves a "living" radical (halide) at the chain end of the brush. This halide [7] allows the binding of anti-VPA antibodies in an oriented manner. Thus, the active binding sites (the red parts of the antibody from image 1) of the antibody remain available for higher specificity [8]. Anti-VPA antibodies [9] are immobilized on top. Since zwitterionic polymer brushes are biocompatible materials [10] they do not interfere either with the structure [11] nor the biological functionalities of antibodies [9]. These brushes can be unaltered in Phosphate Buffer Saline (PBS) for at least 4 weeks [7] Figure 1 is a schematic representation for visual effects, and it shows the principle of the functionalized surface with the antifouling coating. PBS is needed to wash out the samples.

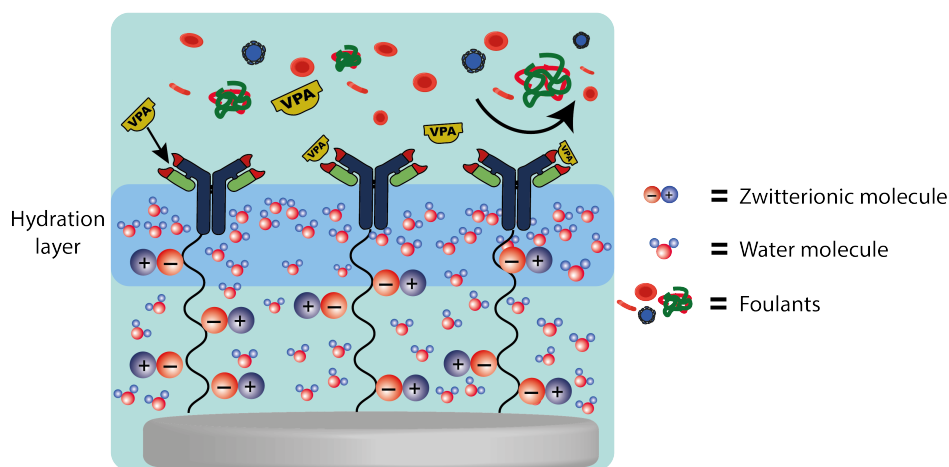


Figure 1: Schematic representation of the principle of the molecular recognition. Zwitterionic polymer brushes grown on the surface of the sensor. Water molecules are strongly bound to the anion and cation moieties of the brush forming a hydration layer, acting as a barrier. Fouling from complex media is prevented, allowing a more specific antigen-antibody interaction.

1.2 Physical transduction

Precise controlling and transmission of the sample and reaction reagents is very critical when working with small concentrations. The physical transducer comprises flow sensing technology from a collaboration with Bronkhorst High-Tech company.

A MINI CORI-FLOWTM series is chosen to be integrated with our project. This mass liquid flow sensor can cover our accuracy requirements, and its wide detection ranges from

0.05 to 200 g/hr. The micro-electromechanical systems (MEMS) chip of this flow sensor integrates a suspended micro-pipe with an inner diameter of 40 microns, and a comb-type capacitor detection unit [12], as shown in the figure 2. When an alternating current and a stable external magnetic field are applied to a silicon-based pipeline, the resulting Lorentz force will drive the pipeline to rotate [13]. At this time, the mass flow through the pipe will be subjected to Coriolis force perpendicular to the velocity direction, thereby driving the pipe to generate displacement. The comb-type capacitor at the displacement terminal of the pipeline can detect the magnitude of the displacement by detecting the change in capacitance, thereby deriving the velocity of the mass flow. The peripheral circuit can accurately control the transmission rate of the micro-pump through the feedback speed data.

This composite detection system can be used to develop fVPA concentration sensors. It can achieve high sensitivity for detecting small changes in low-speed fluid velocity. The antifouling polymer layer described above are deposited on the inner wall of the micro-pipe. Due to the capturing force between the fVPA molecule and antibody protein, the speed of the microfluid will be reduced, and the effect is related to the concentration. By detecting changes in velocity by the mass flow sensor, the concentration of the target molecule fVPA can be inferred. It is worth noting that this detection method has not been proven by published articles, however, it can be used as a reference for our project for development and integration.

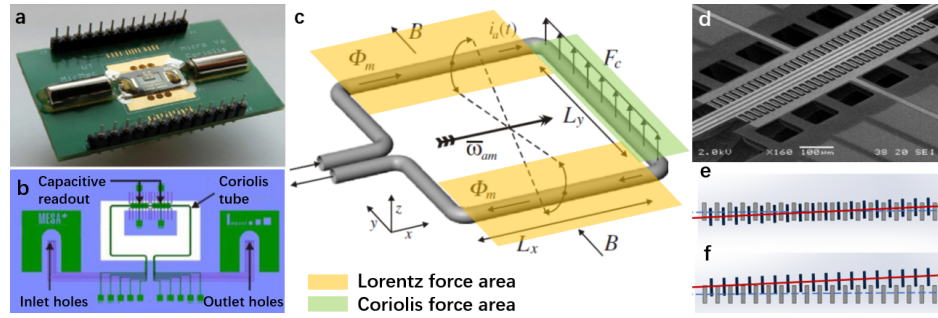


Figure 2: Schematic of the Bronkhorst mass flow sensor [12] (a) MEMS chip and integrated peripheral circuit. (b) Chip layout of the physical interfaces and electrical interfaces. (c) The micro channel is subjected to two forces in different areas marked as yellow and green, resulting in two kinds of displacement of the comb-type capacitor structure, whose scanning electron microscope (SEM) figure is shown in (d); (e) Lorentz force alone drives the displacement result when the axis rotates, (f) Coriolis force causes radial displacement to increase.

1.3 Cartridge technology

For the handling of the sample and biosensor our group has created the following device: It consists of a turntable with four different and modular components on it. The diluted, *via* pipette, sample is placed inside the device, then ultrasonic mixing, reagent addition and the physical transduction of the electro chemical signal, measured by the sensor, is preformed. After the cycle is done, the tested sample returns to the initial position. High emphasis was put on modularity and future adjustment of the system.

For the mixer there will be used a method of an ultrasound speaker generating a high frequency sound and mixing the solution of blood with its reagents and dilution fluid. The frequency of this mixing device normally lays between 1 and 10 MHz.

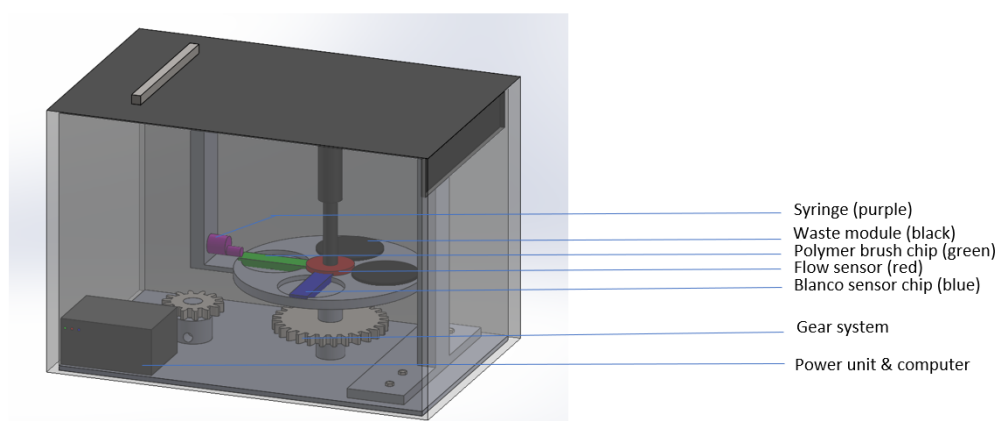


Figure 3: The 3D model of the testing system. It is composed of the MINI CORI-FLOW in red, a functionalized chip with an antifouling polymer brush-antibody layer in green, a functionalized chip with just an antifouling polymer layer in blue.

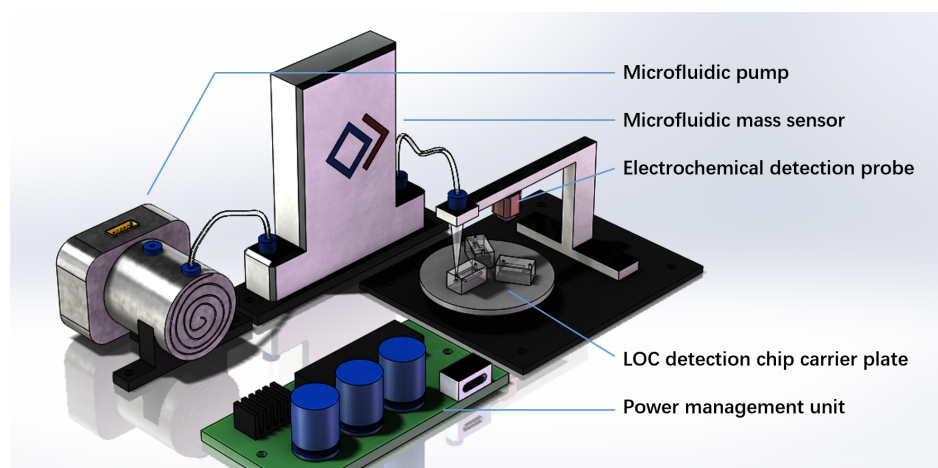


Figure 4: The 3D model of the testing system.

1.4 Reader instrument and user interaction

The reader instrument will consist of a box with all components inside, such as the sensing kit, microcontroller, and 10V power adapter. The design focuses on an automated process requiring the user only to dilute the sample and manually load it into the device. By this approach, the ease of use is significantly increased and the risk of contamination of the sample greatly reduced. In the future, we plan to fully automate the system providing even greater accessibility. That would reduce all the actions taken by the user to inserting the sample, initiating the measurement sequence and removing it afterwards. Due to the current circumstances, the focus of the team has shifted towards modularity and the possibility of expansion and refinement by future teams. The flexibility of the design allows for adjustment of certain components without complete overhaul of the device resulting in time and resource savings.

The user interaction, after sample dilution and insert of the sample, with the device relies on a 7" LCD touch screen. This allows for adjustments based on received feedback and

further pursues the concept of user-centered design for reduced complexity and access by all age groups. The user would press a start box to initiate the measuring sequence, the screen would indicate that process has started and after its completion, a result would be displayed as well as an indication in form of a message that the cartridge can be removed. A simple yet informative GUI provides the user with the necessary data. In the future, we plan to add a mobile device application, which would fully utilize Raspberry Pi 3 B+ potential, that could store the results history and support the treatment process.

The prototype would be produced using additive manufacturing, preferably stereolithographic printer (e.g. Form 3L or Fuse 1 by formlabs) as it provides necessary dimensional accuracy and does not cause common, for conventional 3D printers, issues such as layer separation and warping. Further, the lack of layers improves the cleaning of the device further improving anti-cross-contamination capabilities.

1.5 Originality

The UT⁺ team has been working on integrating several concepts to address the challenge of a biosensor design posed by this year's challenge. The main issues were identified and different possible technical solutions to overcome it were brainstormed. The feasibility of different approaches led us to create a collaboration with one of the most important companies in flow-sensors: Bronkhorst High-Tech. They already have a long standing collaboration with the University of Twente and were therefore open to collaborating with us.

The UT⁺ team received guidance from a group of UT supervisors that provided a coral feedback in their varied areas of expertise. To complement the academic interaction, the supervisors facilitated some industrial contacts from their research activities (i.e. Lionix, Demcon, Qurin). This kick-started industrial collaboration and motivated the students to proactively seek for new views and interactions. A special emphasis was placed in clinicians and practitioners in order to collect valuable user feedback for platform technology development. Therefore, the team conducted stakeholder interviews with Dr. Kris Movig, pharmacist at the local MST hospital, an epilepsy patient who wishes to stay anonymous and a successful entrepreneur, founder of the start-up Medimate, Steven Staal.

Despite this year's hardship, the team has displayed creativity and originality by providing a concept of proof for the biosensor. The team organized itself in different working groups in order to cluster expertise, be more efficient and make room for closer supervision from the supervisor team. The teams addressed different challenges, and are outlined in more detail in the Appendix (5.1 'Overview/Breakdown of Working Groups).

We would like to emphasize the following: Our supervisor Dr. Sissi de Beer pioneered the polymerbrush technique for gas sensing and is currently working on combining it with optical sensing, together with supervisor Prof. Dr. Ir. Sonia García Blanco. In this project we attempted to adapt the concept to biosensing. A biosensor's main component is molecular recognition [14]. Therefore, special attention was taken. The by the team selected approach was utilizing antibodies as biorecognition element, due to their high specificity [15] towards an analyte. In order to prevent fouling from proteins contained in plasma, and to provide an oriented antibody immobilization polymer brushes are used. Super hydrophilic polymer brushes can provide resistance to fouling and have been shown to provide the best antifouling coatings [11, 16, 17]. They can also provide functional groups where specific parts of the antibodies can be attached to. Zwitterionic brushes have the advantage that they are stable, biocompatible [10] and have shown to preserve not only its antifouling character [6], but also have the ability of keeping antibodies functionalities without causing denaturation [18]. The selected approach of combining zwitterionic brushes with the mass flow meter is a key invention and has never been done before, which is why we are proudly presenting our novel concept in this document.

Translation potential

Please find the business model canvas in the Appendix (5.2.1).

2.1 Stakeholder desirability

In the European Economic Area (EEA), there are about 4.9 million cases of active epilepsy and 142 000 in the Netherlands [19]. For each of them, the seizure frequency ranges from once per year to multiple times a day [20]. To combat seizures, the most versatile and effective broad-spectrum anti-epileptic drug (AED) is VPA, as it can be used for all types of seizures with the highest success rate [21]. Thus, VPA is often used after the first seizure and for the first period of treatment of a patient. However, VPA is one of the AEDs with the most severe side effects and overdosing can have serious downsides to a patient's life.

The personalized high accuracy device we propose would help bridge this gap, as most VPA dosage is prescribed by weight and then controlled by a blood exam a few weeks later in a hospital [22]. This exam is often inaccurate and causes patient suffering [22]. Thus, initially prescribing the right dosage and constant and reliable monitoring are important. To find the right dosing, measurements of the drug concentration in the blood are necessary. Then, it is possible to prevent seizures in 70 per cent of the patients [23]. A decrease/elimination of the amount of seizures increases the patient's quality of life [24]. Thus, our biosensor increases the patient's quality of life, as it can be used for drug levels measurements at home, leading to a more controlled dosing of the drug. Further, commuting and waiting times at the hospital will be reduced as the data measured at home is sent to the hospital. Most patients are either younger than 18 years or older than 60 years. Both may need assistance and more time to get to the hospital. Thus, our biosensor enables them a more autonomous, flexible, and free life. In the current COVID-19 pandemic, remote testing, especially for elderly and thus more vulnerable patients, increases the value of the biosensor even more. Further, it benefits patients living in extremely remote areas like the Australian outback. There, 250 000 epilepsy patients exist and mostly call a phone line for questions regarding dosing and seizures, as they only go to the doctor around twice a year [25]. With our biosensor, they can get immediate feedback on their fVPA blood levels and could then discuss it online with a nurse or a doctor, leading to more accurate treatment.

The biosensor also decreases treatment costs for hospitals and insurances compared to treatment in the hospital. While a measurement at the hospitals costs 25 EUR, it might only cost 10 EUR with our biosensor [stakeholder interview]. Patients might need this measurement multiple times per week. Assuming a patient needs the measurement two times a week, our biosensor reduces the cost per year by 1300 EUR. Additionally, it would free the hospital's testing capacities. Finally, non-compliance can be decreased by at-home testing and thus, prevent seizures and thus, save money.

2.2 Business feasibility

Key Partners: We will create partnerships with competent companies in the Enschede region, like Lionix and Demcon, to avoid added costs for manufacturing abroad. We choose Lionix as our main manufacturer as they make customized MEMS in scalable production volumes. Our supplier would be Demcon as they deliver high-end technology systems and products. We already established a partnership with Bronkhorst High-Tech and are confident

that it will continue. Bronkhorst High-Tech has an interest in working with us, as they can sell their kit to/through us, which is why they will be our mass flow meter supplier.

Further Investors will be tapped via pitching events, connections to industry and establishing partnerships. Another financing opportunity lies within seed funding and venture capital. All this can only happen once we have a working prototype and a more concrete estimation of costs and (potential) revenue streams. We would also apply for funding for RD from 'Pioneers in Health Care Innovation Fund' of the UT/MST/ZGT, TURBO (UT/Radboud) or Open Mind (TTW).

Key Resources: We first need to finish the RD phase, including clinical testing and getting a CE certification before we can start selling our device. We need to acquire funding for this as well as for IP rights. Additional necessary resources we will need in a later phase is a highly qualified sales force, to make our marketing strategy a success.

Key Activities: Once we have produced a high quality biosensor for fVPA levels, we would like to further extent our business and produce biosensors for more medication measurements with our novel polymer brush technique, for example lithium, phenprocoumon (also known as Marcumar), glucose, cholesterol (small molecules). Ideally, we will have one high end sensor in the end, that is able to measure a variety of different molecules accurately and customizable. Another key activity we would like to focus on is marketing, to reach our customer and adequately communicate our value proposition. This would be supported by partnerships with Hospitals, Health Insurances and family physicians. Further, we will continue to entertain established relationships to industry and continue to explore our market opportunities. Manufacturing will be outsourced to competent partners, and our main focus will be on customer services to build a good, long lasting relationship with them.

2.3 Financial viability

The prevalence of epilepsy in the European region is at 0,82 % for active cases and a further 2% of the population are predicted to have suffered from epilepsy at some point in their lives. [26] Assuming an even spread of the cases in Europe and also that all active patients could make use of the device at some point, brings the number of people that could make use of our device in the Netherlands to a total number of about 142 000 people as per its population. [19] Further, in the EEA, with a population close to 595 million inhabitants [27], we can assume there are about 4,9 million active cases of epilepsy, and including the USA in our market potential would add an extra 3,4 million people. [28]

To be able to scale our production and commercially sell our devices in the EEA and USA markets we will first need to obtain the CE mark and FDA approval. Those require lengthy and expensive clinical trials. They generally cost many millions [29] [30], meaning that we will need funding and support from partnerships to cover the costs. So, for now we shall estimate the costs for the first phase of development of the device which is creating a working prototype as a proof of concept before we can start looking for funding.

Cost analysis for the initial development phase:

Firstly, our team consists of a broad spectrum of specializations and of many people willing to diversify their expertise through hands-on learning, meaning that there will be no need to spend money on staff during the initial development phase. Further than that, with the support of MESA+ Institute, our team is provided with expert advice and supervision at no extra costs.

First step for our device is developing the antifouling chip in a cleanroom. MESA+ Institute is supporting us during the development phase and after consulting with them it is estimated that it would take about 6 months for one to two engineers full time to develop the chip. The estimated cost is of 700 € per engineer per day in the clean room plus the materials. The cost of materials during this phase is negligible compared to the cost of the cleanroom so we will round up the costs to an estimate between 100 000 and 200 000 € to creating a working prototype (depending if we use one or two engineers; the real cost should be somewhere in between). See appendix for overview of recurring cost estimations per sensor (5.2.2)

Once we have a working prototype we can produce a more accurate cost analysis that will take into account and estimate all of those costs and variables, as by then we will know exactly the process and obstacles to face and we will be finally able to propose a final market price. This more accurate analysis will also include the very significant cost of clinical trials and obtaining the CE and FDA certifications meaning that we will finally be able to bring forward a proposal to potential investors.

The expectation is to reduce the costs per device by scaling up production and developing long term partnerships with our suppliers to reduce cost of production. Hopefully, by buying in bulk and having an exclusive partnership we could negotiate significantly lower prices, which in turn would make our sensor much cheaper and maybe accessible even to individuals on a wide basis.

For the time being, “A typical family physician in Europe will have 10-20 persons with epilepsy among his or her patients” [26] and they could be the main person of contact with our device in the case of a first seizure or for adjusting the dosing prescribed of the initial VPA with much more regular visits due to proximity. This would both save precious time for the patients and the hospitals while also making it cheaper to test more locally and not overcrowding hospitals. This does not mean that neurologists will become redundant but results from the device could be sent online to the neurologist for evaluation and they could give a new prescription or decide whether a visitation is in order.

Thus, our selling strategy would be to convince healthcare systems, governments, insurance agencies, hospitals and physicians, using our partners’ established distribution channels, to try our device to alleviate the day to day suffering of their epileptic patients. This course of action will be beneficial as most people do not live next to hospitals and it could potentially be cheaper to operate this way. In this scenario there would be the need for about 14 200 devices to cover the need of the Netherlands and about 830 000 devices to cater for the needs of the EEA and USA markets; if every family physician needed to have one of these devices in their practice, assuming each physician has an average of 10 epileptic patients.

In short, the value proposition is a sensor that will diminish the suffering of epileptic patients thanks to often testing and adjusting their drug dosage. The aim is for this often testing to be done as close and convenient for them while being cheap enough (less than 25€ per testing over a year) so that healthcare systems and insurance companies will see a clear benefit in investing in them while providing better services to their clients and cutting down on direct and indirect cost. We believe this to be achievable and financially lucrative too, considering the number of people that could make use of this device alone and not taking into account the potential branching off our innovative process into sensing other molecules. As for the income stream we shall have two of them, with one being smaller income by selling the device at a reasonable price in to gain the interest of many people and then charging a yearly or monthly subscription for use/quality control which would guarantee us a steady and predictable income stream that will allow us to plan for our future steps.

Team and support

3.1 Contributions of the Team Members

Martijn Lam was a team captain. He organized meetings with external people and potential business partners. Katharina Kück was a team captain. She functioned as the main point of contact for team, organization and supervisors. Marlen Braun was responsible for the marketing of the team. **Entrepreneurship team:** Katharina Kück, Marlen Braun and Nicolas Georgilopoulos all participated in the entrepreneurship sessions, setting up and executing stakeholder interviews and are responsible for our business proposal. **Testing platform team:** Gijsbert Pelleboer focused on making a 3D-CAD model for the design to show how all components act together to perform certain operations of the device. Jakub Bladdek focused on designing the reader instrument as well as and user interaction platform with the developed device. **Molecular Recognition team:** Paloma Dueñas was in charge of the chemistry needed to create an antifouling coating to increase the specificity of the biosensor. Miguel Rodriguez focused on research of different molecular recognition methods apart from the antibodies approach, and supported Paloma with the chemistry. Yujia Kong was responsible for identifying the physical principles and laying the foundation for the next steps. **Flow Sensing team:** Qi Wu was responsible for analyzing the working principle of the sensor in combination with the needs of the project. At the same time, based on the existing sensors, he was responsible for designing alternative detection schemes and conducting theoretical studies on the feasibility.

3.2 People who have given support

First of, a huge thanks to our supervisors! Prof. Dr. Ir. Loes Segerink was responsible for establishing the SensUs competition at our University and helped to secure funding for the team. She supervised the Testing Platform team together with Dr. Ir. David Fernández Rivas. He gave great advice on the development of the cartridge technology and reader instrument. Dr. Sissi de Beer helped the molecular recognition team with the chemistry and lab work. Prof. Dr. Ir. Sonia García Blanco provided additional insights on the sensor and the support to test our antifouling coating on her devices. Dr. Ir. Pep Canyelles Pericàs was the main point of contact between the team captains and the supervisor team. He supervised the Flow Sensing team and initiated contacts with some companies himself. All supervisors were involved and enthusiastic with the team and supported us with their expertise, feedback and advice. Without them we would not have gotten nearly as far in the development as we did. Thank you!

3.3 Sponsors

Our main sponsor was the Mesa⁺ institute. They helped us out energetically, for example by funding logbooks and our lab materials (antibodies, etc.). Without them we would have had no budget and our development would have been slowed down significantly. Thank you for your support!

Final Remarks

As a final remark, we would like to thank all the members of the SensUs competition. This year will definitely be remembered. The competition has been a great opportunity to explore the medical field and understand the challenges that companies, patients, and people have to go through. We are thankful for the motivation shown by the organization, especially through these difficult moments. Even though a real time competition is not possible under current circumstances, the way everything was handled and coordinated is remarkable. This was our first year participating and we definitely learned a lot. We hope to be able to participate again next year, and this time with more experience to be able to develop our proposed sensor, with small changes for the specific future target molecule.

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Appendix

5.1 Overview/Breakdown of Working Groups

- Molecular recognition team. A biosensor’s main component is molecular recognition [14] . Therefore, special attention was taken. The by the team selected approach was utilizing antibodies as biorecognition element, due to their high specificity [15] towards an analyte. In order to prevent fouling from proteins contained in plasma, and to provide an oriented antibody immobilization polymer brushes are used. Super hydrophilic polymer brushes can provide resistance to fouling and have been shown to provide the best antifouling coatings [11, 16, 17]. They can also provide functional groups where specific parts of the antibodies can be attached to. Zwitterionic brushes have the advantage that they are stable, biocompatible [10] and have shown to preserve not only its antifouling character [6], but also have the ability of keeping antibodies functionalities without causing denaturation [18].
- Flow sensing team. This group used the contact with Bronkhorst High Tech provided by the coach Pep Canyelles Pericàs. Bronkhorst High-Tech has a long-standing collaboration with the UT. As such they kindly agreed to provide their flow sensing platform for testing fVPA concentration at no cost. Lockdown restrictions obstructed intensive testing, but flow sensing concepts using MEMS were explored.
- Testing platform team. This team developed a cartridge and an instrument concept under the supervision of David Fernández Rivas and Loes Segerink.
- Entrepreneurship team. They developed the business case by taking into account the stakeholders’ views and the advice received from the SensUs organisation, as well as what is currently known in business and research. Insights gained during the stakeholder interviews are included in the translational potential section.

5.2 Additional Information Translational Potential Award

5.2.1 Business Model Canvas

We made this business model canvas by considering the insights gained from stakeholder interviews. We furthermore considered input from the ’mini-lectures’ linked for the en-

trepreneurship sessions and in discussion with Professor Bobelyn, we revised and improved this through multiple iterations.

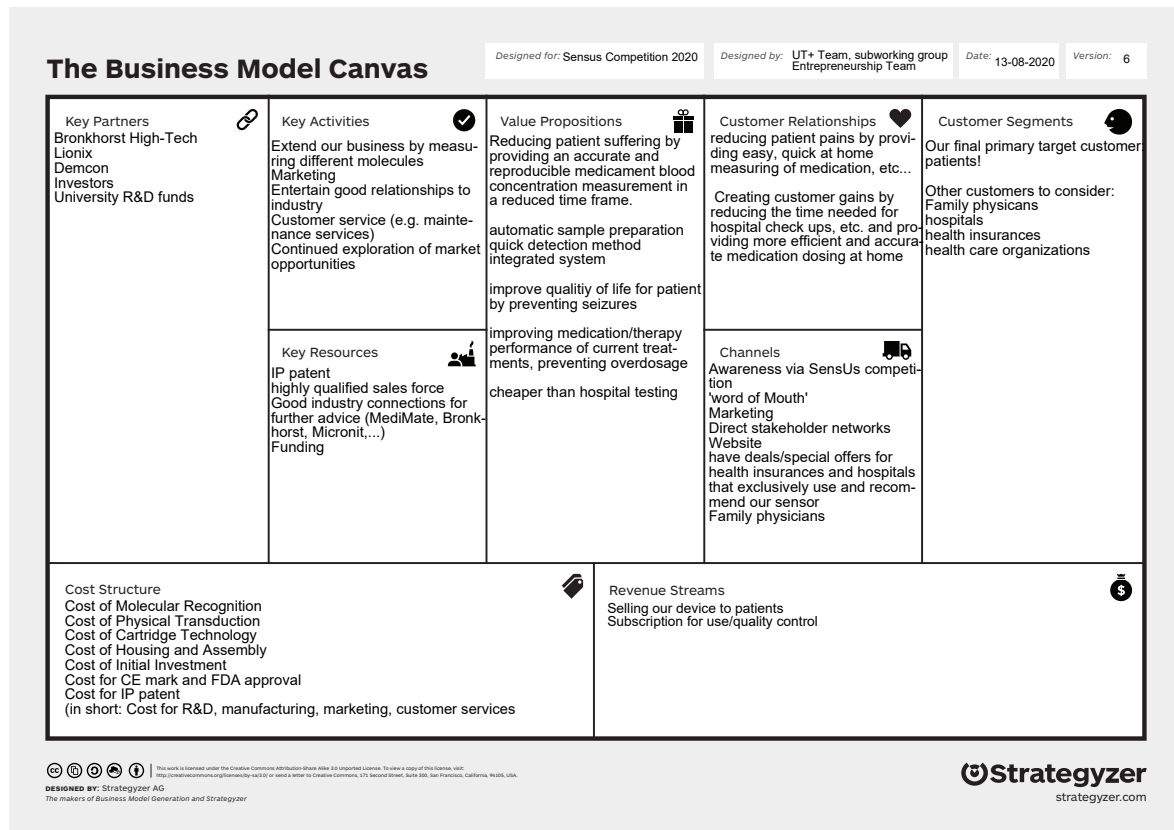


Figure 5: The Business Model Canvas

Our main conclusions from the Business Model Canvas and discussions with experts are that our market niche lies within providing fast, accurate, at home measurements and our sensor has the most value in rural areas where quick access to the hospital is scarce.

5.2.2 Recurring costs estimations per sensor

Sensing mechanism:

Table 1: Approximated costs for the biosensor. *Commercial price of the MINI CORI-FLOW™ ML120V21, through volume production and a partnership the price is likely to decrease.

Components	Material use, estimated by UT ⁺	Cost per sensor
Zwitterionic monomer [31]	2,5g per 15x15cm wafer (225; 1x1cm chips)	0,267 €
Polymeric anchor [32]	50mL per wafer (225; 1x1cm chips)	0,019 €
SiO ₂ Wafers [33]	15x15cm wafers	0,351 €
Reagent: PBS [34]	10mL per sensor	0,085 €
MINI CORI-FLOW™ [35]	Best fit for our requirements MINI CORI-FLOW™ ML120V21 -> 1 per sensor	6300 €*
CCD-based camera [36]	Generic commercially available CCD camera -> 1 per sensor	100 €
Ultrasound speaker [37]	Generic 1-10 MHz ultrasound transducer -> 1 per sensor	1 €

Cartridge technology, casing and user interaction:

Components	Cost per sensor
Micro-controller [38]	30 €
10V power adapter [39]	10 €
7" LCD touch screen [40]	75 €
3D printing up to 2kg of plastic [41]	60 €

There also need to be considered costs that are hard to predict accurately during this initial development phase such as marketing, housing and storing (about 60 euros per square meter for industrial spaces [42]), RD for optimization, provisions for further RD in order to expand the customer base for our sensor by addressing other molecules too, unpredicted costs, staff, transportation, scaling up process and finally profits.