Team Results Document
TUcanSense

SUPERVISOR
Prof. Dr.-Ing. Andreas Blaeser

COACHES
Tim Eike Weber
Leonie Maria Holderbach

TEAM MEMBERS
Jake Appelhans
Angela Arends
Moritz Birkner
Aileen Kaiser
Philipp Karnop
Svenja Keller
Felix Klecker
Janne Kühner
Susi Zhihan Li
Helena Mehler
Maximilian Mitschke
Erich Walter
Sandra Janina Weber
Elena Wiemer

TECHNISCHE
UNIVERSITÄT
DARMSTADT

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2 Abstract

Traumatic brain injury (TBI) is a serious neurological injury caused by head trauma that can lead to multiple symptoms and complications. Especially in children, there are difficulties in correctly assessing the severity of the disease and choosing the right treatment. For that reason, we (Team TUcanSense) developed a rapid and easy-to-use electrochemical biosensor, that enables a quick treatment decision by measuring the concentration of the relevant biomarker glial fibrillary acidic protein (GFAP). Therefore, we immobilised antibodies on the surface of self-made screen-printed electrodes (SPE) and use the Electrochemical Impedance Spectroscopy (EIS) to obtain a usable electrical signal, corresponding to the concentration of GFAP in a sample.

Our self-designed biosensor, the *SPEcialist,* is also cost-effective, as we manufacture a significant portion of its components in-house and have a lot of potential for reducing the total number of unnecessary stationary hospital stays and treatments through the improved diagnostic possibility. Due to its compact design and easy handling, it allows uncomplicated use in the ambulance or emergency room, as well as in hospital wards.

With our biosensor, we aim to advance the research and treatment of people injured by traumatic brain injury, staying true to our motto: "Together we can sense the future."

3 Biosensor system and assay

3.1 Molecular recognition and assay reagents

For the molecular recognition, an Immunosorbent Assay (ISA) is employed, using antibodies to ensure precise immobilization and reliable detection of the target biomarker. By modifying the SPE a robust and sensitive platform for capturing GFAP is created. The following steps outline the sequence of procedures involved in preparing the biosensor for accurate biomarker measurement.

For the immobilization of the capture antiboy, first L-Cysteine is electropolymerized onto the platinum electrode. Cyclic voltammetry with a scan rate of 50 mV/s between -0.2 V and 1.2 V for 20 cycles is used. Subsequently, incubation with 1-Ethyl-3-carbodiimide/N-Hydroxysuccinimide (EDC/NHS) (100 mM, 4:1 v/v) is performed for 30 minutes to activate the L-Cysteine, allowing the amino group of the antibody to bind. Next, the antibody 83 (30 ng/mL) is incubated for one hour as well as bovine serum albumin (BSA) (1%) to prevent non-specific binding. Throughout the process, the electrodes are washed with ultrapure water (Milli-Q) and phosphate-buffered saline (PBS, pH 7.4). The procedure is shown in Figure 1a. After the immobilization process, the GFAP sample is pretreated with Heparin (25 mg/mL) and thermal incubation. Through centrifugation the precipitated proteins are separated and removed. Next, the supernatant is incubated on the electrode, followed by an extended washing with Milli-Q. To increase the sensitivity, the secondary antibody 81 (50 ng/mL) is added, which is additionally labelled with a 40-100 nm large gold nanoparticle (AuNP). Both after sample application and secondary antibody addition, an impedance measurement is performed using a redox solution containing 0.1 M Potassium chloride, 5 mM Potassium hexacyanoferrate (III) and 5 mM Potassium hexacyanoferrate (II). During the impedance measurements, a DC potential of 150 mV as well as an AC potential of 10 mV are utilized, covering a frequency range from 1 to 100000 Hz.

The specific chemicals referenced in the text can be found in the attached appendix [10.6 App.].
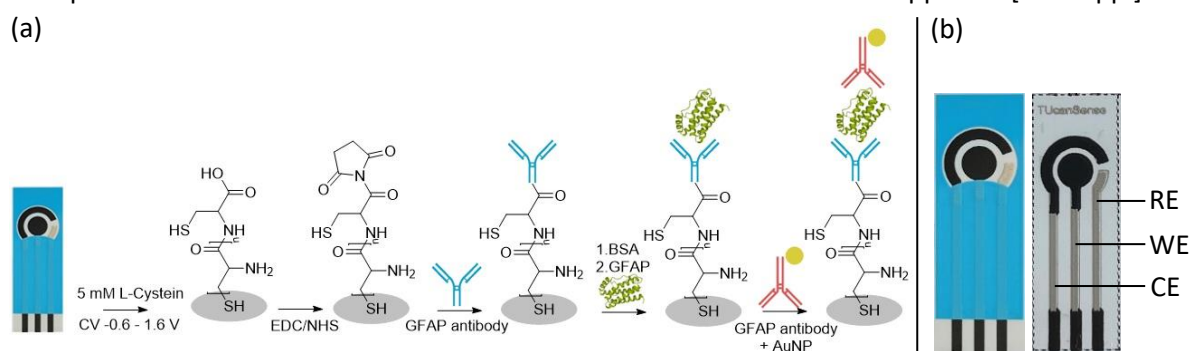

Figure 1: Schematic process of the immobilisation of the GFAP antibody on an electrode (a) [1]; SPE with protective layer (left) and without it (right) (b).

3.2 Physical transduction

The electrochemical signal is transmitted via a SPE. SPEs are measurement devices, which consist of three different electrodes. Hereby the response of the working electrode (WE) is sensitive to the analyte concentration. The reference electrode (RE) provides a constant potential to measure the WE's potential. The counter electrode (CE) completes the circuit, which should have a large surface to enable the passage of the current. To obtain a usable electrical signal, corresponding to the concentration of GFAP in a sample, Electrochemical Impedance Spectroscopy (EIS) is used. The result of this method can be represented in a Bode plot. By changing the surface of the WE with a variety of substances like GFAP, the electrochemical properties of the system change noticeably. The diffuse double layer, for example, can be interpreted as a capacitor which changes the impedance depending on the amount of bound GFAP.

The layout of the SPE (Figure 1b) was further developed by us, building on the work of Team TUcanSense 2022. While our team is proficient in self-producing SPEs, we have opted not to employ them for the competition and instead use industrially manufactured ones, thus minimising the influence of manufacturing uncertainties for the prototype. Subsequently, the design was forwarded to our Sponsor Hoffmann + Krippner GmbH (Germany), who undertook the manufacturing of the SPEs on our behalf. These SPEs are fabricated on PET film with the underlying conductive pathways coated

using silver ink to ensure optimal conductivity. Both WE and CE, along with the contact ends, were layered with platinum conductive ink. A composition of silver chloride was applied to coat the RE, thus guaranteeing a stable electrochemical potential throughout the measurement process. To ensure safeguarding, the SPEs are enveloped with an encapsulating paste.

Specific Information on the used materials can be found in the attached appendix [10.6 App.].

3.3 Cartridge technology

The cartridge (Figure 2) was produced using Stereolithography (SLA) printing technology [10.6 App.]. It houses a self-designed microfluidic chip (Figure 3). A measurement is carried out as follows: The SPE is placed inside the measuring chamber, which is part of a microfluidic chip system. The microfluidic chip is compact and was manufactured by using the Digital Light Processing (DLP) printing process [10.6 App.] in an air-conditioned room with low dust exposure. Above the WE of the SPE, there is a central input channel through which the sample is applied and subsequently sealed airtight. Three reservoir chambers, each tightly sealed, are filled with the necessary solutions (PBS, Milli-Q, and the redox solution), with each chamber connected to one of the three input channels. This enables a precise introduction of the specific solutions, which is achieved through a negative pressure created by a syringe. The syringe is brought through the exhaust channel into the measuring chamber and pulled from the outside, allowing only the desired solution to enter the measuring chamber while the other chambers remain airtight. The process of washing can be accomplished accordingly in the future.

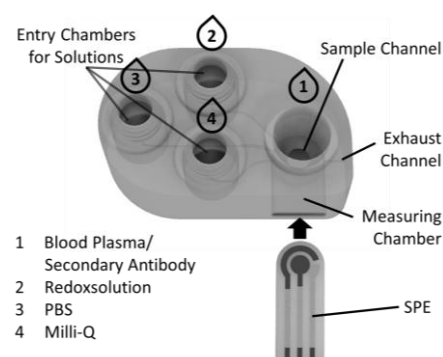Figure 2: Cartridge with microfluidic.

Figure 3: Microfluidic chip system.

3.4 Reader instrument and user interaction

The setup of the Reader Instrument is depicted in Figure 4. The Sensit-Smart Potentiate [10.6 App.] connects to a Raspberry Pi via the USB port to process the measurement results, which are then conveniently displayed on a screen. The display on the Reader Instrument provides clear visual feedback, guiding users with easy-to-understand and step-by-step instructions. To ensure a user-friendly experience, the display incorporates four buttons for simplified operation, including an easy shutdown function for the Raspberry Pi. The power supply is provided by a power bank, enabling both mains and cordless operation, making mobile application possible. Additionally, a power button on the cable allows for convenient power control. To guarantee result comparability and prevent overheating, a temperature sensor is integrated into the setup, with its readings also displayed on the screen. In addition to visualisation on the display, the setup enables the display of measurement results and their corresponding classifications on a smartphone via Bluetooth, utilizing a custom-designed app. The app could also provide additional functions for everyday clinical use, such as directly inputting measurement results into a digital patient record as well as simultaneously and remotely notifying relevant medical personnel. For enhanced convenience and functionality, a dedicated circuit board was developed that serves as a link between the Raspberry Pi, display, and temperature sensor. In the data analysis process, a Python program was developed which utilizes classification analysis to establish a robust correlation between the measured values and the concentration of GFAP. The program considers the absolute impedance and conducts a comparative analysis by subtracting the reference measurement from the sample measurement, thus allowing for a comprehensive evaluation of various GFAP concentrations.

Figure 4: Reader instrument Setup.

4 Technological feasibility

4.1 Molecular recognition and assay reagents

To identify a highly specific molecular recognition unit, antibody and aptamer combinations were compared via indirect sandwich Enzyme-linked Immunosorbent Assays (sw-ELISA), as shown in Figure 5. For this, the aptamer 5'-CCACTTCTCCTTGCAGCT-3'-Biotin and different antibody-combinations from HyTest Ltd (GFAP15cc, GFAP81cc, GFAP83cc, GFAP95cc and GFAP98cc) were tested [2].
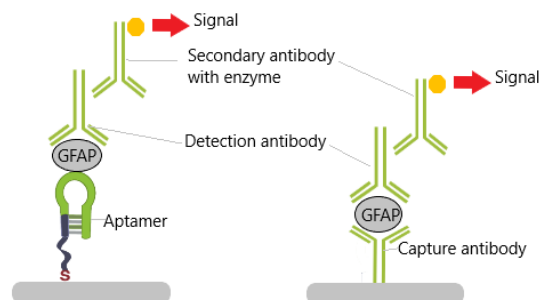

Figure 5: Schematic principle of a sw-ELISA with aptamer and detection antibody (left) and a sw-ELISA with two antibodies (right).

For the aptamer-antibody pair, antibody 81 worked sufficiently.

For the antibody-antibody sandwich, the antibody 83/81 combination showed promising results as shown in Figure 6.
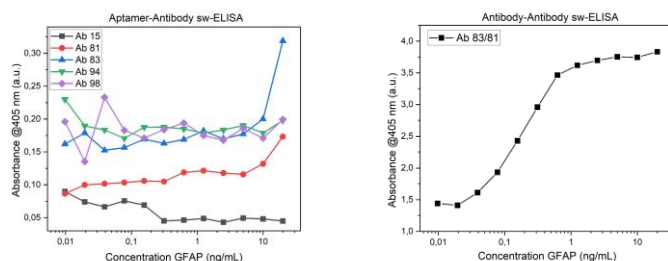
Figure 6: Different aptamer-antibody sw-ELISA (left) and antibody81-antibody83 sw-ELISA (right).

Based on these results, tests with graphite and platinum SPEs were performed. Graphite electrodes are widely used but require both CV activation and gold nanoparticles for immobilization [1]. Compared to graphite, platinum electrodes possess a natural affinity to sulphur, enabling direct binding to L-Cysteine. Therefore, to minimize resources and preparation time, the focus was set on platinum electrodes. A GFAP dilution series (in Milli-Q) was measured to validate the method. The clustering (Figure 7a) of the individual concentrations shows the reproducibility of the measurements. However, it is unclear why the concentrations do not occur in orderly sequence. Despite this, the method was adapted to the more complicated sample matrix, blood plasma.

Figure 7: Dilution series of GFAP in (a) Milli-Q and (b) blood plasma on platinum electrodes. Depicted is the difference Bode plot between the GFAP and reference measurement.

First measurements showed an extremely different behavior to the Milli-Q measurement. This could be due to plasma components, *e.g.* fibrinogen, which may interfere with the surface of the electrode, leading to unconsistent results.
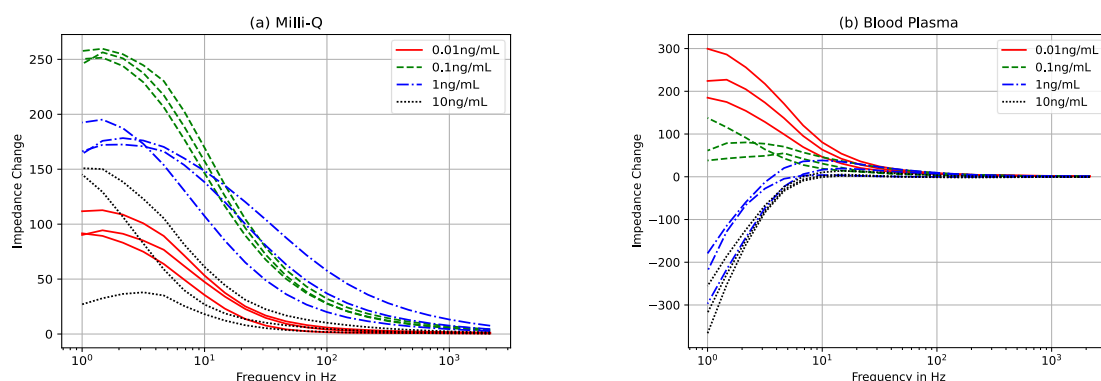
By systematically testing various pretreatments of the sample, the final method was able to eliminate the interfering factors to such an extent that concentration clustering is again obtained (Figure 7b). Further clarification is needed as the results differ in the sign of impedance change. It would be anticipated that the bound GFAP and secondary antibody always influences the impedance change in the same direction. Once this has been clarified, the application as a biosensor may be possible. The specific chemicals referenced in the text can be found in the attached appendix [10.6 App.].

4.2 Physical transduction

SPEs with different materials (gold, carbon, platinum) for the WE and CE were tested. In the beginning we printed variations of SPEs ourselves with gold and different carbon composition coatings, however self-printing is prone to error. Due to manual aligning the screen-printing device for each layer and impurities like dust inserted during the printing process, each electrode was slightly different. To avoid these irregularities, we sent our SPE design to our sponsor Hoffmann + Krippner GmbH. The SPEs they provided were printed with carbon and platinum ink on the WE and CE. Originally, we wanted to use the more cost-efficient carbon electrodes. The main reason we decided to use the platinum SPEs for our biosensor over the carbon and our self-printed SPEs is the fact that the accuracy of the differentiation between GFAP concentrations was the highest.

4.3 Cartridge technology

Laboratory experiments were conducted to qualitatively assess the flow behaviour, achieved through the visualization of solutions using distinct colours (Figure 8). A promising way to increase efficiency and precision is to automate the measurement process. This includes the relocation of the reservoirs out of the microfluidic

Figure 8: Microfluidic test with syringe.

chip. Prefabricated pump connections could be attached to the inlet and outlet duct connections. In this way, pump-driven tubing could be used to control the flow of solutions through the microfluidic system in a fully automated way, while still allowing for the necessary incubation time. These changes could not only help minimise potential air problems, but also increase the accuracy of our measurements. In addition, automation improves the workflow for the user, eliminating the need for manual application of the sample to the microfluidic and biosensor. This minimizes potential external variables that could otherwise be influenced by the examiner.

By utilizing DLP 3D printing for the fabrication of our microfluidics, intricate structures can be effectively realized. The cartridge itself is 3D printed using SLA, but it is equally feasible to produce it with recyclable material using FFF printing.

4.4 Reader instrument and user interaction

With the Raspberry Pi as a single-board-computer, the setup and evaluation are straightforward because we do not use nearly as much computing power as a Raspberry Pi offers. Using it is therefore a safe way to implement our biosensor but not necessarily the most efficient way. We are working on the possibility to use a more cost-efficient microcontroller instead of a Raspberry Pi. So far, this has failed because the microcontrollers that we tried cannot handle full-fledged python software but work with stripped-down versions like circuitpython. The appropriate scripts for the Sensit-Smart and the analysis of our results exist only as python scripts and circuitpython does not have the suitable libraries to enable an implementation. These libraries would have to be written by hand, which we decided against for the time being. However, this could be a possibility for improvement in the future.

5 Originality

5.1 Team captains

Our team developed a biosensor utilizing an immunosorbent assay for the quantification of GFAP, combining and optimizing screen-printed electrodes, a microfluidic system, and a reader instrument. After an extensive literature review and several tests, we decided on an antibody sandwich approach on a screen-printed electrode. While a similar method has already been evaluated in recent work, for example by Ozcelikay (2022) using carbon-based electrodes, we opted for platinum electrodes. This eliminates the need for surface activation, as the thiol group of L-Cysteine exhibits a strong affinity to the platinum surface, facilitating direct binding. This decision simplifies the fabrication process tremendously and therefore saves valuable time and resources.

In addition to the electrode modification, our team undertook the complete design and production of our cartridge, ensuring durability and longevity by using epoxy encasing. The cartridge houses both a microfluidic chip and our reader instrument. Our self-designed microfluidic chip plays a critical role in minimizing errors and automating measurements, receiving validation and feedback from experts. Our reader instrument is composed of a Raspberry Pi, a custom-made printed circuit board, and a display that provides clear instructions for the operation of the *SPEcialist*. Furthermore, our team developed an app for the *SPEcialist* that connects seamlessly to our reader instrument and enables a result output directly to a mobile device. In a hospital environment, where staff is often on the move, this feature ensures easy access to results and allows for convenient notifications. With its user-friendly interface and advanced microfluidic system, our *SPEcialist* ensures minimal manipulation and effortless usability for untrained personnel.
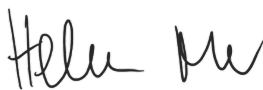
5.2 Supervisor

The TUcanSense team has successfully engineered an electrochemical antibody-sandwich-based biosensor. The journey began with extensive literature research to explore various possibilities, leading to the emergence of two promising concepts: an electrochemical approach and an optical measurement methodology.

While the optical method received substantial attention and underwent thorough refinement, the team encountered challenges related to the suspected inherent autofluorescence of the plasma, ultimately leading them to make the difficult decision to discontinue this approach.

The team then focused their efforts on the electrochemical approach, using screen-printed electrodes. They evaluated aptamer and antibody assays, and after extensive testing and careful consideration, they chose the antibody assay due to its better results. The team decided on a sandwich assay, therefore using an established method and ensuring high sensitivity. Furthermore, the team selected Electrochemical Impedance Spectroscopy, deciding on a powerful and precise technique for the analysis. The combination of the sandwich assay and Electrochemical Impedance Spectroscopy represents an approach that has not been previously documented in the existing literature.

While the use of screen-printed electrodes is not entirely novel, the team's unique approach sets the biosensor apart. They combined it with microfluidics and incorporated a sophisticated, sustainable design and user-friendly handling. Both the microfluidic system and the cartridge were self-designed and printed by the team. Additionally, they independently produced screen-printed electrodes and incorporated custom-designed electrodes from their sponsor, Hoffmann + Krippner GmbH. The unique composition of the biosensor distinguishes it from other biosensors and enables versatile use in the clinical field.

_____   _____   _____
Helena Mehler   Angela Arends   Prof. A. Blaeser
(Team Captain)   (Team Captain)   (Supervisor)

6 Translation potential
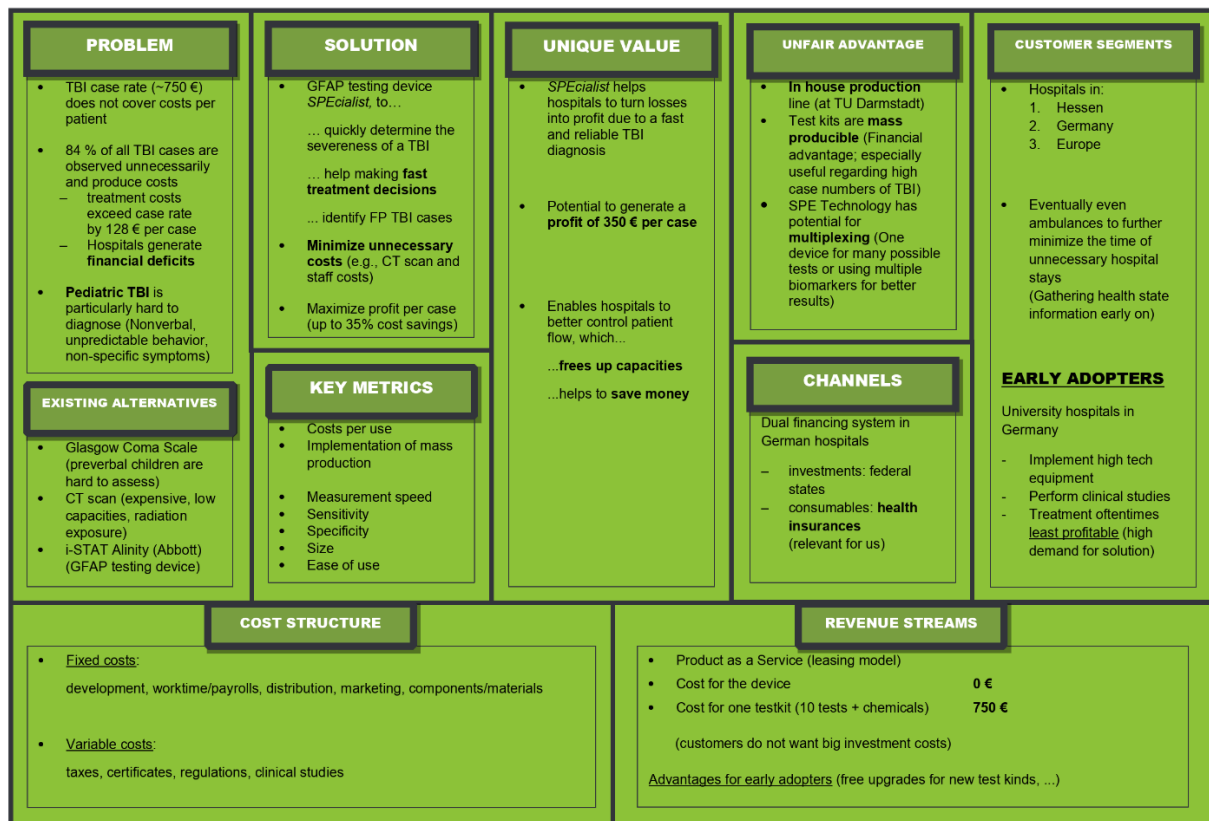
6.1 Business model canvas

Figure 9: Business model.

6.2 Market description

TBI, or the so called "silent epidemic", represents a major global health issue, responsible for 419.507 annual stationary treatments (2016) in Germany alone [3].

In 2015, 71.516 patients under the age of 15 with a TBI diagnosis were treated stationary in German hospitals. 91 – 97,3 % of these cases were considered as "mild", whereas only 1 – 5 % were considered "moderate" to "severe" [3]. The mortality of a TBI is highly dependent on the case. It lies at around 0,5 % for most cases but there is a noticeable increase in those cases that are classified as severe [3]. An even more alarming trend can be noticed for patients in the age group of 1 – 3 years. Here the mortality for severe cases lies at 17,8 % and it represents the leading cause of death in this age group [4, 5]. This critical characteristic of paediatric TBI is aggravated by the difficulties that lie in the detection of the disease in children, since a lot of the established diagnostic methods are not well suited for children. For example, the number of safe CT scans for children is lower than for adults and the general TBI symptoms are harder to assess for children because they naturally behave unpredictably or could be nonverbal [6].

The by far largest portion of TBI cases is classified as mild, for which the assessment of the severity can be very challenging and expensive (e.g., CT scan). The treatment can quickly exceed fixed case rates, leaving hospitals with a financial deficit [7].

Therefore, the market for TBI in Germany has a lot of potential for reducing the total number of unnecessary stationary hospital stays and treatments, especially in regard of paediatric TBI, which holds a lot of difficulties itself. This sort of saving measures for hospital capacities is particularly appealing in Germany, considering the decreasing capacity for stationary hospital stays each year [8].

6.3 Stakeholder desirability

Through intensive research and interviews with medical professionals, Dr. Merker, senior physician in paediatric intensive medicine and Dr Isselstein, senior physician in paediatrics and adolescent

medicine, we identified the most important customers for *SPEcialist* as university clinics with specialized paediatric intensive care units and non-urban ones without a CT-scanner. The needs of hospitals with paediatric care units lie in maximizing profit from diagnosed related groups (DRG) case rates, which are budgeted at 750€ per TBI case [7]. Furthermore, it is important to detect the high false-positive (FP) TBI rate of 84% [10.4 App.] which results in relieving fully utilized hospital capacities and therefore optimizing positive outcome focused patient care. Implementing *SPEcialist* into existing diagnostic work streams provides an estimated 35% [10.2 App.] more cost-effective solution through time saving by minimizing stationary observation and precise detection of TBI severeness in non-responsive infants. This advantage can be generated due to an estimated cost per use of only 75€ leading to an overall viability [10.2 App.] At this point of time healthcare units in Germany are facing negative cash flows. Unnecessary treatment procedure in FP TBI cases costs clinics 26.044.301€ in Germany every month [10.4 App., 10.2 App.].

The use of *SPEcialist* could pose a significant advantage for university clinics with a broad treatment spectrum to generate profits from TBI cases. With an estimated 30-minute sample to result period, 84% of mild TBI cases could be identified increasing the patient turnover rate significantly [10.4 App.]. By maximizing the full diagnostic potential of our GFAP-based sensor TBI could be detected with 75.6% sensitivity and 78.3% specificity [9].

University clinics provide additional qualifications to implement novel detection methods as their medical service goes hand in hand with research opportunities which our *SPEcialist* detection-platform provides. Moreover, the cooperation in clinical trials is an important factor for our consideration process in regards of provision of our sensor [7]. While conducting Interviews, senior physicians explicitly stated high interests of university clinics in a new TBI diagnostic method if it can be accompanied in a scientific context (studies, etc.) [7].

For our entry market we deliberately decided on the German federal state Hessen to ensure personal supply of early adopters in the first two years to mitigate costs in delivery and service. After reaching market saturation, expansion into the entire German market is planned in year three [10.1 App.]. We identified the German healthcare market as conservative but ideal starting point to scale up supplies for the European market after 10 years due to uniform admission standards and high prestige of German engineering.

As the market of rapid testing for diseases gained immense significance/popularity due to the COVID-19 pandemic, investor's interest also increased exponentially [10]. Considering the expected growth (7.5% p.a.) of the TBI market in the upcoming years, the increasing market volume can be expected to attract several private investors and healthcare companies [10].

Investing in our sensor can be of particular interest as we are providing a dynamic development process with a wide scope for adaptation to the wishes of our cooperation partners and donors. In comparison to competitors such as Abbott Laboratories (*i-STAT Alinity Instrument*) we present a highly dynamic team which is able to explicitly respond to the wishes of the customers and develop the sensor for optimal workflow implementation. The steady research and development advancements go hand in hand with regular updates of software and hardware components of *SPEcialist*. Furthermore, the multiplexing ability of our platform can be adapted and implemented into a broad spectrum of diagnostic work streams in medical facilities. Since currently, we are able to produce the majority of the parts for our sensor in-house and rate this as a clear advantage to our competitors, we plan to keep it that way for the rest of the development phase and the first two years after market entry until we reach production limits. To meet European certification standards for medical equipment (ISO13485) it is considered to consult quality assurance companies such as SGS Institute Fresenius or the quality assurance department of our partner Merck KGaA. Furthermore, the production of high-turnover consumables such as SPEs can be outsourced to specialized companies such as our current partner Hoffmann + Krippner.

6.4 Business feasibility

As mentioned, we are able to produce the majority of our sensor components (cartridge, electrodes, microfluidic, testing solution cartridges) in-house at the IDD at TU Darmstadt in a cost-efficient and scalable way. We will continue to outsource production of our electrical components such as the molecular recognition unit "Sensit Smart" to our current supplier PalmSens. As the molecular

recognition unit poses the most expensive part of our sensor at the current point of time, a possible partnership with PalmSens in the near future is conceivable to steadily increase production scale and decrease procurement costs.

Moreover, we identified the biochemical preparation of our SPEs to be the most time consuming and error prone manufacturing step. We are planning to solve this bottleneck in scalability by automating the process either through an in-house approach using an industrial printing machine or by outsourcing to a medical technology service provider.

Until this point we will continue to advance and optimize our sensor to achieve constant accuracy parameters and multiplexing capabilities to detect further TBI biomarkers such as S100B. Moreover, our reader instrument team is steadily improving our software to analyse and store test results. Regarding the ongoing advancement of artificial intelligence in the healthcare sector it is thinkable to feed the accumulated test results into an AI driven database. Here, we are already in close contact with the German software as a service company lifespin GmbH which uses proprietary algorithms to store and process health data at a large scale. Also, a smartphone application which allows direct communication of the result is already in development.

To verify our sensor as a medical device, certain approval procedures are planned in the next two years. Here, we plan to obtain the CE certification to provide us a legal foundation in production and distribution. As our sensor functions as an in-vitro diagnostic instrument it is classified under "in-vitro-diagnostic-regulation" (IVDR) in risk class C. The smartphone application might have to be categorized separately. To further validate our product for commercial use, clinical studies must be conducted. Costs for comprehensive clinical studies are estimated to be 2.5 million € until full approval. Therefore, the cooperation with contacted university clinics (University Clinic Frankfurt, Clinical Center Fulda) and our commercial partners such as Merck will be beneficial for execution and cutting cost. To secure sufficient capital funding we are planning to apply for the "START-interaktiv"-program by the German ministry for education and research to secure comprehensive financial support in the upcoming years of the development phase. Furthermore, we intend to get in contact with angel investors via steadily expanding our network. Also we hope to profit from the European Investment Fund (EIF) and via venture capital investors.

Our commercialization method for the German entry market is optimized to supply our early adopters which are medical care units in Hessen with our in-house production capabilities, followed by outsourced production after expansion into the German market. The distribution and maintenance of our sensor will be outsourced to specialised logistics and biotechnology firms.

Furthermore, optimization steps in production will be implemented to cut costs in cartridge manufacturing process such as switching from 3D printing to plastic injection moulding.

To enter the conservative German market, early adopters will be able to partake in a free trial month, including a full refund guarantee if technical problems occur or promised performance parameters cannot be achieved. After that *SPEcialist* is sold as a rental service with a monthly rental fee of ~1.000 € for the basis contract (12-13 tests per month) covering all running costs. Additional test can be purchased by upgrading the basic contract to a premium package, priced at 1.500 €, including 300 tests per month. This should cover monthly cases of most German clinics [7].

6.5 Financial viability

The computed tomography scan (CT scan) after an initial categorization of the patient according to the GCS can be seen as the current golden standard for the detection of TBI. This procedure can quickly become expensive and take up hospital capacities before the treatment even begins. Considering that around 90 % of all TBI cases are considered as mild [4] and only around 7 % of these show signs of a severe intracranial injury, a lot of unnecessary CT scans accumulate to be a substantial financial burden to the hospitals [11]. Adding to this, some CT scans do not show any injuries during the most critical hours after an accident, which was noted by Dr. Merker (paediatric unit of the University Hospital Frankfurt a.M.) as part of an interview [7].

SPEcialist has the potential to prevent a large portion of these unnecessary costs, by providing the means to detect a potentially critical TBI, while using low-cost materials for each test. It also enables the users to make multiple tests in a short amount of time, which, for example, would be useful for

carrying out multiple consecutive bedside tests, which are often used in 30 minutes intervals when dealing with paediatric TBI [7].

The costs produced by caring for a patient with an endured polytrauma in a shock room in Germany (head and thorax CT scan, 24h intensive care unit, 24h regular stationary) are not covered by the proceeds of the DRG case rates. Therefore, an estimated deficit of 128 € per case is a burden to the treating hospitals [12]. Some of these costs can be avoided by using *SPEcialist* as a cost-efficient testing instance before making a head CT scan. Ideally the costs of the head CT scan and the costly stationary care can be avoided and the treatment of TBI patients even made profitable for the hospital by creating an estimated profit of 275 € [10.2 App.].

By making use of the mass producibility of our consumables, the costs of each SPE are negligible even when the more costly chemical components and printing inks are taken into account. Considering the high numbers of annual TBI cases worldwide, this cost efficiency of our consumables gives us an advantage over competing businesses using more costly consumable designs (e.g., i-STAT Alinity [13]). First steps for in-house mass production at our university were already taken care of but are yet in need of a sufficient Proof of Concept.

The production of the device itself costs around 650 € in its current state [10.3 App.]. The most expensive components are the Sensit-Smart potentiostat (PalmSens) and the pump system for the microfluidics. We expect these costs to be lowered drastically by making custom manufacturer contracts for our business. The costs of the microfluidic chip can also be lowered significantly because it is mass producible, which was confirmed by Micronit.

To generate sufficient revenue to be financially viable, our business strategy lies in selling *SPEcialist* as a Product as a Service. An annual leasing fee is preferable because this way, we can generate a constant stream of revenue while creating a dependence of our consumables that must be compatible with the device our customers are using. This way it is most attractive to potential customers because the healthcare segment prefers low investment costs and the customized solutions we plan to provide enable them to pay for precisely the testing capabilities they need [7].

We divided our available market into three segments, following the TAM-SAM-SOM method. The first market (Serviceable obtainable market) we plan to supply is our home state market of Hessen. With 151 hospitals, Hessen has a sufficient market volume to provide the first base of customers as a start for reaching the break-even-point [10.1 App.] [8]. It also enables us to start our business with the mentioned in-house production because all customers are nearby and the customer support can be done efficiently.

Considering that 90 % of all TBI cases are considered as mild and only 7 % of these show severe intracranial injuries, around 84 % of all TBI cases do not need to be taken intensive stationary care of [4, 11]. The market volume (possible profit for hospitals) of our first market lies at around 5,6 million €, assuming that in 80 % of the defined cases a precautionary head CT scan is done nevertheless after testing negative with *SPEcialist*. Therefore, each hospital in Hessen could make an annual profit of ~26.000 € by lowering unnecessary treatment costs with the use of *SPEcialist*.

Our revenue will be generated by selling the test kits via a monthly subscription model which enables us to provide our services without the need of high investment costs to establish the device in existing healthcare environments. High investment costs are a main pain factor for our customers because they come with laborious procedures and unnecessary costs, while monthly expenses for consumables are much easier to justify and realize.

The costs for the market introduction of SPEcialist are estimated at 3,1 million €, consisting of 100.000 € for the process of the CE regulation, 2,5 million € for the affiliated clinical studies and 500.000 € accumulated during the development of the first product variant. These debts, additionally to new production and operating costs of our business must be overcome by our revenue streams, to reach breakeven [10.5 App.]. We will start by providing the first university hospitals near us with 4 devices in total. By assuming a steady growth of leased products, we aim to make manufacturer contracts after 3 years to lower the overall production price of each device. To enter the second and larger market (Germany), an inclusion of our products into the catalogues of service of the major German healthcare

insurance companies is a vital key element. By achieving this, we expect our product to gain traction in the German healthcare market and being able to grow more rapidly, with the financial drawback of not being able to deliver the new market with our in-house production line and the need to outsource the production by then.

Without adjusting the annual fees, we will be able to reach breakeven after 11 years in the first two market segments (Hessen, Germany). By then, our accumulated expertise and partnerships, together with our standing in the German healthcare market will enable our business to thrive in all of Europe.

7 Team and support

7.1 Contributions of the team members

MR	Aileen Kaiser	Contact with business partners / sponsoring / lab work
	Janne Kühner	Updated sponsors and supporter / lab work / microfluidics
	Angela Arends	Team captain / lab work / planning of experiments
	Erich Walter	Project management / data analysis / app development / lab work
	Philipp Karnop	Conducted interviews with experts / business plan / lab work
	Jake Appelhans	Contact for chemical questions of all kinds / lab work / TRD
PT	Susi Zhihan Li	Master of taking spontaneous pictures / electrode printing
	Moritz Birkner	Business plan / microfluidics / design of SPEs
	Elena Wiemer	Sponsoring / electrode printing
	Svenja Keller	Social media / research in microfluidics / electrode printing
RI	Sandra Janina Weber	Hardware setup and soldering / software coding / TRD
	Helena Mehler	Team captain / software coding / data analysis
CD	Felix Klecker	Cartridge design / organizing the documentations / TRD
	Maximilian Mitschke	Project management / microfluidics / specialist for 3D printing

7.2 Support

We are thankful for the support of the following people:

Prof. Andreas Blaeser, our supervisor and head of the biomedical printing group of TU Darmstadt. He has granted us access to his laboratories, machines, and materials. Without his generosity our participation in the competition would not have been possible.

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7.3 Sponsors and partners

We extend our gratitude to our sponsors **Merck KGaA** and **iocto GmbH** for their insights and generous financial contributions, which have been instrumental in making our participation in the competition possible. Additionally, **Hoffmann + Krippner GmbH**'s advice and in-kind support with printing colours and electrodes has been incredibly helpful. Without the continuous interest and backing of our sponsors, we would not have had the means to pursue this project and showcase our innovations.

8   Final remarks

In conclusion, our journey in developing the biosensor has been marked by dedication and relentless effort. We wholeheartedly believe that our innovation has the potential to contribute significantly to the advancement of biosensing technology. None of this would have been possible without the tremendous support and guidance from the "Institute of printing, science and technology" of TU Darmstadt, Prof. A. Blaeser, and our team coaches Leonie Maria Holderbach and Tim Weber. We express our gratefulness to the TUcanSense team from last year, whose support and estimation have been instrumental in our success. With great appreciation, we acknowledge the collaborative efforts that have made our participation in this project a reality.

We additionally want to express our sincere appreciation to the SensUs team for their exceptional organization of this event, which has provided us with a unique opportunity to form an interdisciplinary team and engage in a meaningful scientific project.

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10 Appendix

10.1 Break even analysis

Year	Leased devices	Production costs (€) (adding to next year's debts)	Debts (€) (implementation process)	Revenue (€) (130 annual tests per device)	Target Market	Development steps
1	4	2.600,00	3.100.000,00	39.000,00	Hessen	contracts with component manufacturers (optimization of device manufacturing cost 670 → 400 €)
2	6	3.900,00	3.063.600,00	58.500,00		
3	10	4.000,00	3.009.000,00	97.500,00		
4	15	6.000,00	2.915.500,00	146.250,00		
5	30	87.000,00	2.775.250,00	292.500,00	Germany	Entering next market (Germany by being included into the catalogues of services of the major German health insurance companies. Outsourcing of production (increased costs)
6	45	130.500,00	2.569.750,00	438.750,00		
7	65	188.500,00	2.261.500,00	633.750,00		
8	80	232.000,00	1.816.250,00	780.000,00		
9	100	290.000,00	1.268.250,00	975.000,00		
10	105	304.500,00	583.250,00	1.023.750,00		Break even point will be achieved in the German market → investing profit into capacities to supply the European market
11	110	319.000,00	-136.000,00	1.072.500,00		
12	140	406.000,00	-889.500,00	1.365.000,00	Europe	

Figure 10: Break even analysis.

10.2 Cost per case

without *SPEcialist*		with *SPEcialist*	
compensation for TBI (DRG B80Z)	1.230,37 €	compensation for TBI (DRG B80Z)	1.230,37 €
shock room (with head CT)	-819,88 €	shock room (without head CT)	-696,44 €
ICU (24 h)	-366,01 €	ICU (12 h)	-183,01 €
stationary care (24 h)	-172,44 €	no stationary care	---
		1x *Specialist* test	-75,00 €
Revenue	-127,96 €	Revenue	275,93 €
Revenue increase per case		**403,89 €**	

Figure 11: Cost per case.

10.3 Costs for device and consumables

Device component	Costs per unit (€)	Quantity	Costs (€)
Cartridge	30,00	1	30,00
Microfluidik	3,00	1	3,00
Pumpsystem	200,00	1	200,00
Electronic components	20,00	1	20,00
Display	12,00	1	12,00
Sensit Smart	400,00	1	400,00
Raspberry Pi Pico	5,00	1	5,00
Total Costs			670,00

Consumables (1 Test-Kit)	Costs per unit (€)	Quantity	Costs (€)
SPE	0,01	130	1,30
SPE Catridge	3,00	1	3,00
Chemicals	0,25	4	1,00
Chemical Cartridges (4x)	3,00	4	12,00
Total Costs			17,30

Figure 12: Costs for device and consumables.

10.4 Market volume

Figure 13: Market volume.

Market volume

market	volume
TAM	
TBI diagnosed annual stationary hospital stays (**Europe**)	1.500.000 patients total
-> ~90 % mild TBI	1.350.000
-> ~7 % severe intracranial injuries	94.500
number of unnecessary CT-Scans (**TAM**)	1.255.500
SAM	
TBI diagnosed annual stationary hospital stays (Germany)	275.000 patients total
-> ~90 % mild TBI	247.500
-> ~7 % severe intracranial injuries	17.325
number of unnecessary CT-Scans (**SOM**)	230.175
SOM	
TBI diagnosed annual stationary hospital stays (Hessen)	26.057 patients total
-> ~90 % mild TBI	23.451
-> ~7 % severe intracranial injuries	1.642
number of unnecessary CT-Scans (**SOM**)	21.810

Possible annual profit for our customers

TAM		
unnecessary CT-Scans	1.255.500	
hospitals in **Europe**	15000	
possible annual total profit	346.423.837,50 €	(80 % head CT scans nevertheless)
annual profit per hospital	23.094,92 €	

SAM		
unnecessary CT-Scans	230.175	
hospitals in **Germany**	1887	
possible annual total profit	41.414.236,88 €	(80 % head CT scans nevertheless)
annual profit per hospital	21.947,13 €	

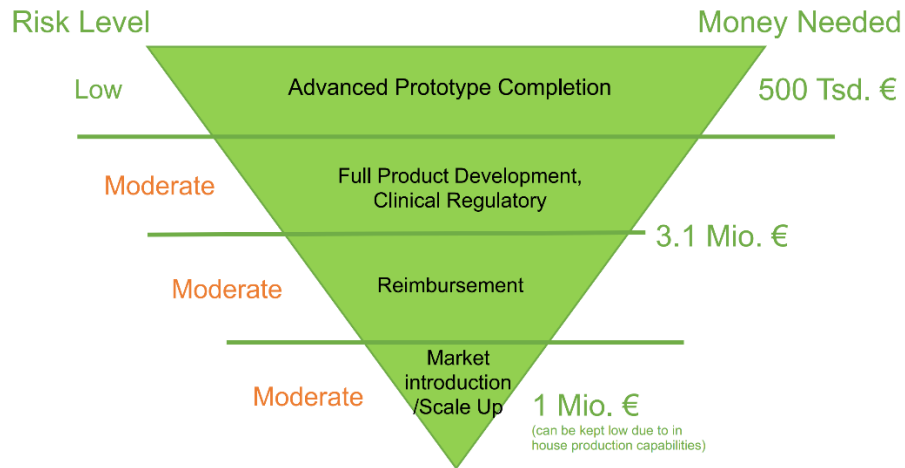| SOM		
unnecessary CT-Scans	21.810	
hospitals in **Hessen**	151	
possible annual total profit	3.924.111,89 €	(80 % head CT scans nevertheless)
annual profit per hospital	25.987,50 €	

10.5 Financing roadmap

Figure 14: Financing roadmap.

10.6 Product information

The following tables list specific information about the products we used for our biosensor. In the text the products are cross-referenced with [10.6 App.].

chemical substances:

product name	product information
Glial fibrillary acidic protein	HyTest Ltd, 8G45, Finland
L-cysteine	Sigma-Aldrich, 168149, USA
1-Ethyl-3-carbodiimide	Sigma-Aldrich, 259-53-8, USA
N-Hydroxysuccinimide	Sigma-Aldrich, 130672-100G, USA
Bovine serum albumin	Sigma-Aldrich, USA; 0948-46-8
Milli-Q	ultrapure water produced with Milli-Q® water treatment system
phosphate-buffered saline	standard recipe
Potassium chloride	Carl Roth GmbH + Co. KG, 6781.1, Germany
antibody 83	HyTest Ltd, GFAP83cc
antibody 81	HyTest Ltd, GFAP81cc,
Plasma	Sigma-Aldrich Co, P9523-ML, USA
Potassium hexacyanoferrate (III)	Carl Roth GmbH + Co. KG, P746.3, Germany
Heparin sodium salt	Carl Roth GmbH + Co. KG, 76901, Germany

technical components:

product name	product information
Sensit-Smart	PalmSens BV, Netherlands
silver ink	Loctite EDAG PF410, Henkel Group, Belgium
PET film	Melinex 339, DuPont, UK
platinum conductive ink	DuPont, BQ321, UK
Silver Chloride	Dupont, 5874, UK
encapsulating paste	DuPont, 5018, UK

3D-printing components:

product name	product information
SLA 3D-printer	Form 3L, Formlabs, USA
SLA printer resin	Formlabs RESIN Clear v4, Formlabs, USA
DLP 3D-printer	MAX X27, ASIGA, Australia
DLP printer resin	Asiga PlasCLEAR, ASIGA, Australia

10.7 Sponsors