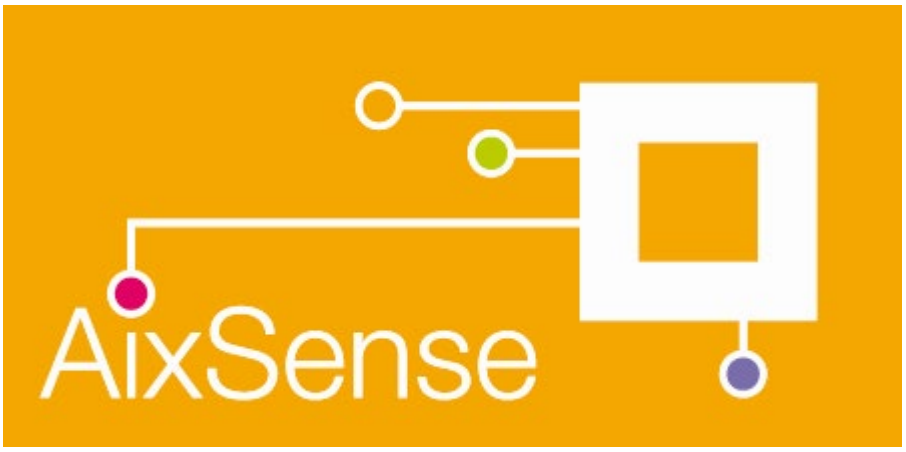
Team Results Document
AixSense



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SensUs 2023
Traumatic Brain Injury

Contents

1. Abstract

AixSense is the representative team for RWTH Aachen University in the SensUs competition. For the 2023 competition, the team developed a biosensor, AixSense TBI to detect Traumatic brain injuries (TBI).

TBI is also referred to as a silent epidemic, it is estimated that around 50-69 million people are affected by it, and many of them do not get properly diagnosed, suffering long-term symptoms without knowing the cause. As of today, the standard procedure consists of a first evaluation made by the doctor through the Glasgow Coma Scale (GCS), which assesses the need for further investigations through magnetic resonance imaging or computed tomography for example. The development of a biosensor to detect TBI could aid the doctor with a more precise and quantitative instrument to evaluate the severity of the patient's injury, reducing the amount of undiagnosed people, and avoiding the costs and risks of MRI or CT when not needed.

Aixsense therefore presents an innovative, cheap, easy-to-use electrochemical biosensor for the detection of TBI in blood. Once the sample is deposited, the chip can be easily inserted in the readout platform, which will provide the user with Glial Fibrillary Acidic Protein (GFAP) antigen concentration within a couple of minutes.

2. Biosensor system and assay

The working principle of the biosensor is based on antigen and antibody binding. The biorecognition elements are in direct spatial contact with the gate of an ion sensitive field effect transistor (ISFET). The formation of an antigen-antibody complex influences the inversion channel between the drain and source of the ISFET in such a way that the characteristic curve of the ISFET is dependent on the antigen concentration.

2.1 Molecular Recognition and Assay Reagents

The antibody immobilization strategies based on the covalent binding of antibodies are most preferred in many applications, due to its stable and rapid antibody binding properties with high immobilization density. The widely used method is crosslinking using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS), which connects amino or carboxyl groups on antibodies to free carboxyl or amino groups on the surface. Crosslinking of antibodies by their amino groups, which are located close to the antigen binding site of the antibody, may impact antibody-antigen detection. Therefore, crosslinking of antibodies by their carboxyl groups is employed for our protocol, to orient the binding of antibodies to surface away from the antigen binding site [1]. By this step, a solution containing EDC and NHS in an activation buffer (MES buffer, pH 6) is initially prepared along with anti-GFAP, and this solution is then incubated on the surface overnight [2]. This step is employed due to EDC's nature as a crosslinker reactive to both carboxyl and amino groups, which reacts first with the carboxyl group. (NHS is used to stabilize the intermediate during crosslinking)

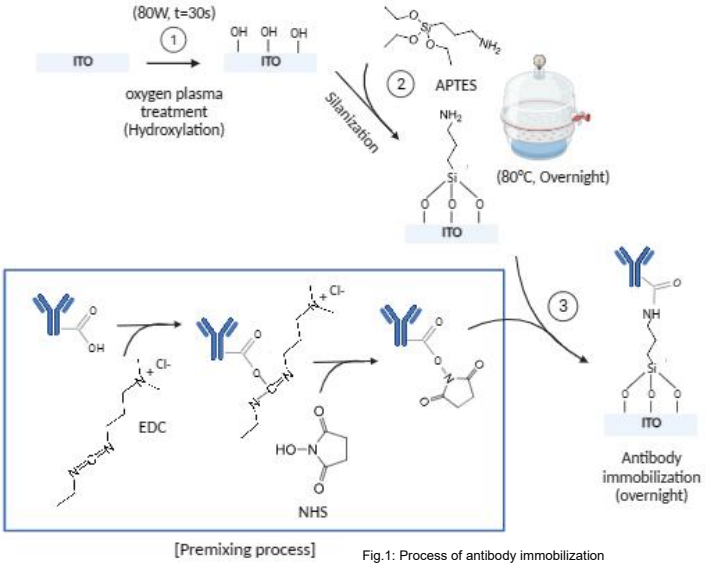


Fig.1: Process of antibody immobilization

To generate the amino groups on the surface for EDC/NHS crosslinking, silane deposition via Gas-Phase Evaporation (at 80°C, t = overnight) using (3-Aminopropyl) tiethoxysilane (APTES) is employed, which enables homogeneous deposition and the use of a small amount of silane per process [3]. Before the silanization process, the surface is activated through an oxygen plasma treatment (80W, t=30s), making it reactive to APTES.

Furthermore, to minimize the non-specific binding, Bovine serum albumin (BSA) blocking is performed to block the free primary amine on the surface.

2.2 Physical Transduction

The ISFET possesses an indium tin oxide (ITO) channel, which exhibits semiconducting properties due to the oxygen vacancies in the crystal lattice. The number of vacancies is dependent on the oxygen flow rate during the deposition step.

The biosensor transduces a biochemical signal to an electrical signal. The antigen concentration is measured by examining the change in the ISFET conductance, caused by a formation of immune complexes at the gate. The negatively charged GFAP antigens attach to the antibodies on the gate, altering the charge distribution at the interface, which causes a shift in the effective threshold voltage. This results in a change in the conductance of the ISFET. By correlating a specific conductance to an allocated range, it allows for a quantitative analysis of the antigen concentration.

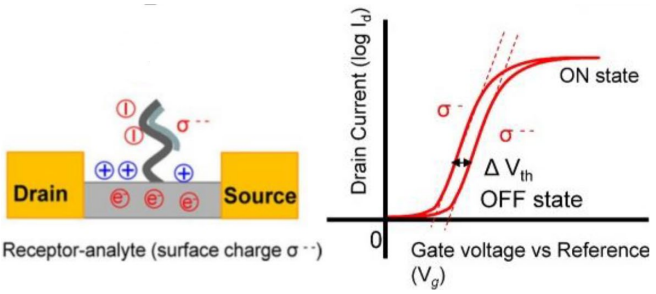
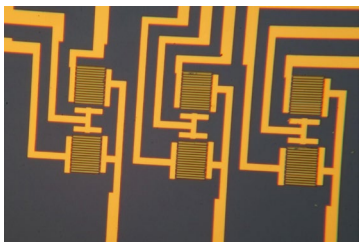


Fig.2: Illustration of the working principle of ISFET and the measured drain current-gate voltage curve

2.3 Cartridge Technology

The ISFETs are fabricated on 4-inch silicon dioxide wafers. On the top layer, a 30 nm thin film of indium tin oxide (ITO) is sputtered onto the substrate as the channel. The contact lines consist of 100 nm gold deposited onto a 30 nm adhesive titanium layer. Surface passivation is done on one half with silicon oxide and a thick photoresist (SU-8).



Each biosensor chip consists of 24 individual ISFETs comb structures. Afterwards, functionalized chips with antibodies were used to confirm the sensitivity to solutions spiked with GFAP. Thus, making it possible to correlate the drain current (channel resistance) to the concentration of GFAP in a given solution.

Fig.3: Image of 6 ISFET comb structures

Improved performance is achieved by creating a continuous flow across the sensing area [4]. The fabrication of a microfluidic component improved flow and sample handling. The components are made by injecting polydimethylsiloxane (PDMS) into a 3D printed mould. They are covalently bonded to the chip surface via a thin layer of liquid PDMS, after being cured at 60 °C in an oven to form a tight seal.

By running two parallel channels over the FETs from a common inlet, all sensing areas are reached by a single dispersion.

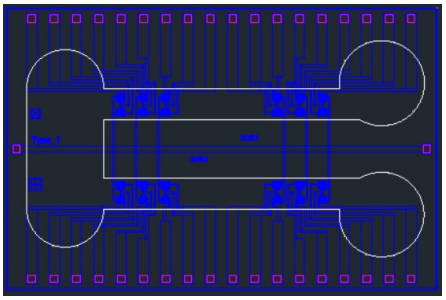


Fig 4: Layout of the chip with cartridge design

2.4 Readout Instrument and User Interaction

In this work, the potentiostat Sciospec is used as a read-out system, which is not manufactured or optimized by AixSense. The functionalized chip with the cartridge is inserted into a custom-made PCB board with a socket and switches that connects the chip to the potentiostat. The readout device is connected to a computer running a software provided by the potentiostat company, which displays the measurement values.

The user is required to insert the chip into the PCB board prior to inserting the sample into the cartridge with a pipette. Once the measurement is done, an interpretation of the results must be done, ideally this will be done by a custom software in the future.

[Fig 5: Image our potentiostat device (Left) and 3D-render of the completed device (Right)]

3. Technological Feasibility

3.1 ITO Characterization

3.1.1 Semiconductive properties

To ensure a high sensitivity of the device, the semiconducting properties of the channel material ITO were optimized. For this, the effects of the oxygen flow rate during deposition via sputtering were examined. One sweep was performed to determine the transition from insulating to conductive behavior, with an oxygen flow rate ranging from 0 to 3 sccm. The other was performed to identify the ideal electrical properties, with an oxygen flow rate ranging from 0.6 to 1.2 sccm. The argon flow was fixed at 50 sccm. For each different oxygen flow rate, hall measurements were performed. At 0.8 sccm the following parameters were measured: sheet resistance = 983 [Ohm/sq], carrier mobility = 12.70 [cm^2/Vs], carrier density = -50 [10^12/cm^2], hall coef. = -1.25 [m^2/C], confirming the production of semiconductive ITO (n-type). Compared, these parameters were preferable because of the overall combination of parameters.

3.1.2 Hall Measurements and Raman Spectroscopy

For each of the different oxygen flow rates, Hall measurements were made to confirm the doping, conductivity and resistance of our microchips. This was an important step to choose the optimal oxygen flow rate for the desired semiconductor properties. The proper deposition of the ITO was then confirmed through Raman Spectroscopy, where measured Raman shifts were compared to values listed in literature [5].

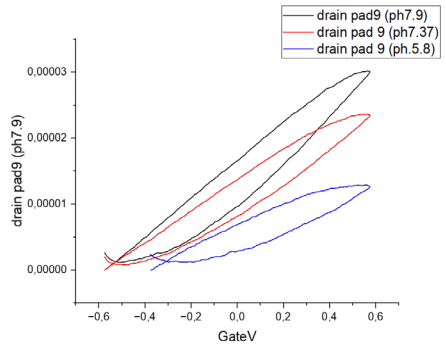


Fig 6.: resulting drain current for different pH-values

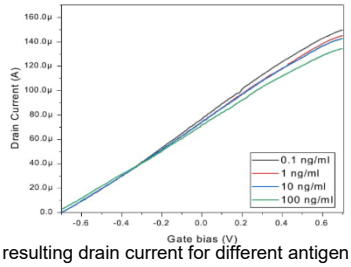


Fig 7: resulting drain current for different antigen concentration

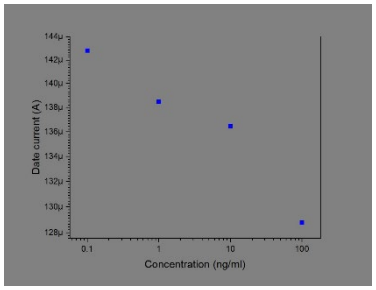


Fig 8: response curve

3.2 Device Characterization

A crucial property of our biosensor is the pH-sensitivity of the ISFETs. To test for this functionality, measurements were performed on unfunctionalized chips with PBS of the pH-values 5.8, 7.37 and 7.9. An increase in the pH resulted in an increase in the slope of the transfer curve, clearly indicating a high pH sensitivity.

Once this was established, further measurements were made with GFAP antigen samples of concentrations 100 ng/ml, 10 ng/ml, 1 ng/ml and 0.1 ng/ml. These values with large differences were initially chosen to test if the biosensor functions. The biosensor was able to successfully differentiate between the aforementioned concentrations. Further measurements would be performed using the concentration range limits defined in the competition. This is necessary to build a correspondence table of concentrations to biosensor output values. ISFET based biosensors have been proven to have an extremely low Limit of Detection [6]. Measurements performed with the samples of 0.1 ng/ml concentration produced viable results, allowing the categorization of the severity of TBI into the classes from “low” to “very high”. Research exists to support the fact that ISFET based biosensors can detect the desired protein in not only a buffer solution, but also human serum and blood [7]. Furthermore, research has shown that modifying the surface of the gate by introducing nanostructures can improve the sensitivity of the biosensor [7].

3.3 Functionalization

3.3.1 Molecular recognition

Contact angle measurements were used to determine the hydrophobic/hydrophilic character of the surface and verify that each functionalization step was successful by observing the contact angle changes at each step. Initial bare ITO had a water contact angle of 60,8° (±2,25) °. After the hydroxylation process, the surface is extremely hydrophilic, with the water contact angle (WCA) reaching an unmeasurably low degree. Hydrophobicity increased after silane deposition with a WCA of 45,92 (±2,25) °. The WCA on the surface with antibody immobilization using EDC/NHS crosslinking decreased to 23,17 (±1,71) °.

3.3.2 Fluorescein isothiocyanate (FITC) and Immunofluorescence

FITC is a fluorescein derivative that reacts with amino-terminal and primary amine groups to form covalent bonds. Its distinctive properties make it a suitable choice for verifying the presence of primary amine coverage on ITO after the silanization process. This is demonstrated by the strong signal emitted by FITC on silanized ITO, as depicted in Fig.8 (left).

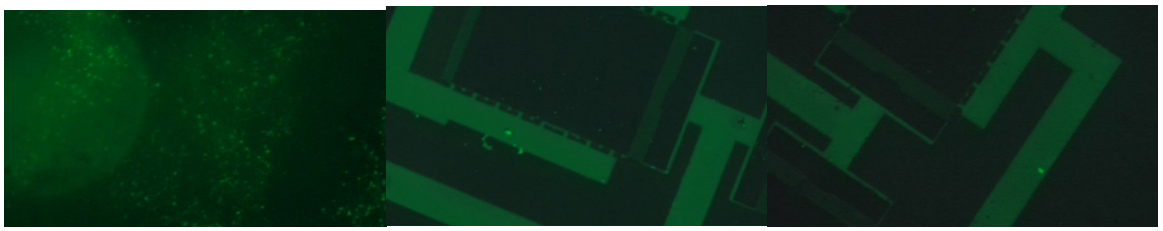


Fig.9 FITC image of silanized ITO (left). Fluorescent measurement images of anti-GFAP immobilized sample (middle) and anti-GFAP – GFAP immobilized sample(right), x5 Magnification.

An immunofluorescence measurement is performed using secondary antibody-CF 488A conjugates to check for successful immobilization and binding. A relatively strong fluorescence signal appearing as green dots is observed Fig.8 (middle). This signal arises from the attachment of the secondary antibody with dyes to the surface. A decrease in signal intensity can be observed in Fig. 8 (right) which is attributed to the binding of GFAP to anti-GFAP on the surface. This is supported by the fact that the secondary antibody, which is non-specific to GFAP, solely binds to anti-GFAP on the surface that has not already bound with GFAP.

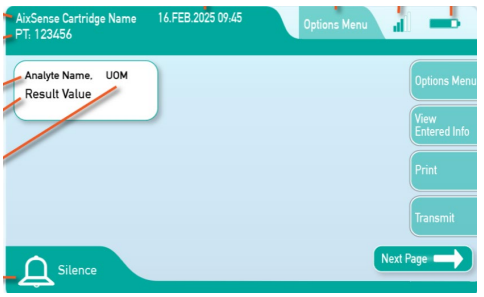
For the FITC measurements, different buffer solutions such as MES, PBS and carbonate-bicarbonate were experimented with to get the best signal. The antibody immobilization initially proved difficult, as the protocols provided in the literature did not create ideal results [2]. An adjustment of the incubation time, concentration of primary and secondary antibodies was necessary to obtain ideal results.

3.4 Cartridge Technology

The chip plays an overwhelming role in the biosensor, as it is the main transducing interface and was also fabricated internally by our team. Steps were taken during the manufacturing of the chips to provide the best results. A passivation layer was created on chips to prevent the formation of an electrical double layer at the interface between the contact line and the sample solution, resulting in lower leakage current and better performance. The microfluidic component is designed to create a continuous flow across the sensing area, improving the overall performance [4]. Due to the size of the chip and channels, very small sample sizes are required to make measurements.

3.5 Reader Instrument

The readout of the chip can be done with any adequate potentiostat device. Ideally, the information from the readout would be shown directly on an integrated display. A 3D-rendering of such a prototype has been designed, where the user would interface with the device through a graphical interface as seen in Fig.5 and Fig. 10. The user is not required to complete complicated procedures to perform measurements. (Please see Appendix 9.1)



4. Originality

Team captain: After reviewing literature on TBI detection biosensors and consulting their supervisor, the team chose to create an electrochemical biosensor using FET technology for its sensitivity, compactness, and speed. They selected ITO as the channel material.This semiconductive material is well known for its very good conductivity and transparency [9]. Despite this, it is not very used in the FET based biosensors in its natural state due to its semiconductor properties. Through literature, the team found out that by changing the sputtering parameters, one could tailor the ITO band gap, effectively tailoring its semiconductive properties, transforming it into a good candidate as a channel material for FET biosensors [8]. Compared to pricier, complex materials like graphene oxide for FET channels, ITO is cost-effective and scalable for industrial production. Its transparency also permits compatibility with optical techniques like ELISA. The team optimized ITO's conductivity via oxygen flow and concentration adjustments during sputtering and simultaneously developed an ITO functionalization protocol.Then the team designed the chip together with its integration with a microfluidic channel, which would let the sample flow slowly and continuously over the sensing area, improving the signal-to-noise ratio and reducing the volume needed for testing [10]. All the planning and experiments were carried out by the team, besides for a few manufacturing steps that could only be carried out by clean room technicians, such as the use of the sputtering machine. The supervisor helped us in the early stage of the biosensor development in terms of feasibility, and with feedback throughout the months.

Supervisor: Biologically sensitive electrical devices based on AC and DC-based readout principles have been widely reported in literature, however, high TRL development and commercial exploitation remains elusive till date. For this year, the TBI challenge presented a unique opportunity for the AixSense team to consider an original innovation plan to address specific requirement of GFAP monitoring among patients from different demographics, age-groups, professions and even, lifestyles. The team held broad consultations from stakeholders and adjusted their biosensor development plans accordingly since the very beginning. During such consultations, it was noted that although a verity of sensor principles results in workable solutions, but do not close-in clinical diagnostics due to various technological readiness and cost-related issues determining the market-readiness of the product, securing freedom of operation towards a successful business undertaking.

Considering these factors, the team decided to use ITO based device platforms to open up a unique opportunity to realize multimodal two-dimensional (2D) devices. ITO is a widely available material and has bene adopted in cleanroom technology for a variety of optical and optoelectronic applications. ITO based substrates prepared using physical deposition techniques have also been used for development of optical biosensor principles. The ITO films, however, are available only as conductive transparent substrates so far, and not been adopted as a semiconductor material for technology applications. The AixSense team, identifying this opportunity, embarked on development of ITO as a transparent (in visible region) semiconductor and realize a wafer-scale process line for the relaization of 2D-ISFETs. In comparison to otherwise expensive and elaborate processes for the fabrication of ISFETs, the optical transparency of the sensor devices is a distinct advnatge to employ optical approaches (e.g., ELISA) in-parallel for ins-situ validation of molecular interaction at the sensor interface. In addition, the platform opens up further innovation opportunities combining alternbative optoelectrical transduction mechanism by nanoscale engineering of this unique transparent semiconductor transducer.

After successful fabrication of the sensor devices, team also developed original plans for the surface modification, cartridge mechanism and readout solutions to finally demonstrate a working biosensor platform for TBI monitoring at the competition. In view of all these aspects, I consider the biosensor development plan well thought out and highly original and give my full recommendation.

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5. Translation Potential

5.1 Business Model Canvas

<table>
<tr>
<td>Problem

accuracy & timeliness:
- head CT scans has only 10% accuracy for mTBIs [11]

- existing TBI diagnosis protocols, especially paediatric GCS scale is less reliable than the adult one leading to some limitations for a correct classification

limited resources and financial constraints for TBI diagnosis, especially in LMICs

Existing Alternatives

- ELISA, of which require lab expertise

- Abbott biosensor, of which is not intended for point-of-care-settings</td>
<td>Solution

cheap, fast, user-friendly, and point-of-care biosensor test as a companion TBI diagnosis to Glasgow Coma Scale with an accurate, quantitative result to enhance clinical decisions

Key Metrics

goal:
Number of sold chips: at least 150 chips/hospital per month.

number of customers/contracts:
599 hospitals with one-year minimum contract in 4 years

- feedback from the customers

short term goal: securing German market within 4 years after market launch.

long term goal:
entering the European market within the 5th year after launch</td>
<td>Unique Value Propositions

- a companion diagnosis for TBI that helps to avoid unnecessary medical imaging and save overall cost
- user-friendly: point-of-care with a single drop of blood and other user-centric design
- fast result within 3 minutes
- affordably priced

- provide accurate & quantitative measurement for TBI</td>
<td>Unfair Advantage

- Aachen start-up ecosystem and favourable network
- excellent university incubation system
- IWE1 institute as our key partner with facilities & mentoring

Channels

- participating in start-up pitch contests such as ATEC Aachen
- joining various exhibitions and events to broaden our network
- contacting hospitals / medical professionals directly

- strategic partnership with government and NGOs

- engagement with medical advisory board</td>
<td>Customer Segments

- hospitals/medical professionals:
neurologists, paediatric neurologists, emergency personnel
- target patient: (most value created)
children, athletes, seniors, military personnel
Early Adopters
Children Hospital Meyer
Uniklinik Aachen</td>
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<tr>
<td colspan="3">Cost Structure

- Fixed costs: production costs, personnel expenses, intellectual property, regulatory certificates

- Variable costs: cost of good sold, marketing costs, distribution costs, taxes</td>
<td colspan="2">Revenue Streams

Razor and blade model: read-out as razor (€1000 per unit) and chips as blade (€20 per unit)
1 year contract with at least 150 chips per month, for first 80 customers free trial for the first month</td>
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Market description

Traumatic Brain Injuries (TBIs) pose a significant healthcare challenge globally, affecting approximately 69 million people each year and imposing an economic burden of roughly 400 billion USD [11]. In Germany, children and adolescents account for 28% of annual TBI incidents, totalling about 250 000 cases [12]. Existing TBI diagnostic tools, such as CT and MRI scans, are either costly, not immediately accessible, or pose radiation risks. During high-demand periods, these constraints can result in diagnostic and treatment delays due to the overwhelming number of TBI patients.

Our biosensor leverages blood-based biomarkers for TBI detection and severity assessment. While it doesn't aim to replace clinical decision rules, it enhances them by offering accurate, quantitative, and timely data, enhancing the clinical procedure. In vitro diagnostic tests impact up to 70% of clinical decisions. However, in the EU-27, UK, and EFTA in 2020 and 2021, these tests represented only 1% of the total healthcare spending [13]. Our TBI biosensor reduces overall healthcare expenses by minimizing unnecessary medical imaging, proving especially valuable for LMICs with limited access to such facilities [11]. Given the challenges of limited diagnostic resources

and financial concerns, according to Arrowhead Publishers (USA), the potential market for TBI diagnosis is projected to surpass €10 billion annually.

Our biosensor is intended for healthcare professionals as a companion diagnosis of TBI in clinical settings. Nevertheless, interviews with neurosurgeons have affirmed us its relevance in emergency scenarios, telemedicine, sports, military operations, and its potential for broader application in collaborative research within the field of neurology, all while strictly complying with the Patient Data Protection Act (PDSG).

5.2 Stakeholder Desirability

Definition of stakeholders and customer: Our sensor is primarily designed for the use by medical professionals, such as neurologists, nurses and emergency room personnel as a companion diagnostic to TBI patients. We believe we create the most value for high-risk groups, of whom are children, athletes, seniors, military personnel, and people with disabilities.

Needs and Benefits: Derived from in-depth interviews with healthcare professionals and literature reviews, our biosensor caters to critical needs. It offers high diagnostic accuracy, reduces misdiagnosis risks, accommodates underreported symptoms, and efficiently addresses mild TBIs. It offers an efficient, point-of-care alternative to time-consuming, non-POC methods such as head CT scans, MRI scans, and GCS. The biosensor also helps monitor discharged mTBI patients, who risk long-term TBI development due to insufficient follow-ups.

Relationship with stakeholders: Patients suspected of having TBI receive a pre-diagnosis through biosensor before they get a CT or MRI scan. This approach is believed to be beneficial for all stakeholders in terms of both finance and time. Comparatively, a single use of a CT scan costs about €210, an MRI costs €600 (see Appendix 9.2.3), whereas the biosensor usage costs around €20. Moreover, involving insurance companies further reduces the financial burden not only for hospitals but also for end-users.

The use of the biosensor preserves patients from unnecessary radiation exposure and contributes to a smoother post-operative course, facilitating the follow-up phase, resulting in greater diagnostic accuracy and, thus, improved medical performance. A typical TBI patient journey can be seen in the Appendix 9.2.1.

A key aspect of the sensor applicability would be in the emergency department and ambulances. A diagram concerning emergency departments can be found in the Appendix 9.2.1.

For practical feedback, reference is made to actual numbers provided by the Meyer Children's Hospital in Florence:

- Case history TBI Meyer Hospital: approx. 1800/year on a volume of 45-47,000 accesses
- Main Cause: fall
- % head CT for diagnosis of intracranial lesion: at Meyer Hospital < 5%, but extremely variable on national territory.

Validated not only by Meyer's Children Hospital, but also from secondary sources, falls account for almost half of emergency department visits for TBIs[14]. People older than 65 and children under age 15 experience the most fall related TBIs. Existing TBI diagnosis protocols, especially paediatric GCS scale is less reliable than the adult one leading to some limitations for a correct classification; hence, a very strong and valid patient journey would revolve around the paediatric department. Paediatric patients present a unique case because the applied rules are particularly restrictive. Consequently, if we can develop a suitable procedure for children, it can easily be extended to all adult patients.

Rules and Regulations: The TBI assessment and management follow several standards like SCAT5 [15] and Canadian CT Head Rule (CCHR)[16] to reduce unnecessary CT scans, radiation exposure, and medical costs. On the other hand, according to Dr Giordano, clinically-important TBI is relevant for paediatric patients, for whom CT might be unnecessary [17]. Our biosensor aligns with these standards and includes clinicians' insights in its development. Also, ISO 9241 and ISO 62366 guidelines ensure a safe, effective, and user-centric design in our system.

To date, our main commercial competitor is Abbott. However, we have also identified a list of emerging point-of-care market competition in Appendix 9.2.3b.

Given these premises, our main benchmark is therefore the Abbott biosensor. The main differences are analysed below:

- Abbott biosensor is not a point-of-care device because it requires laboratory procedures to obtain plasma and has an exaggerated pricing, possibly due to monopolistic market presence.

- AixSense device is cost-effective and priced reasonably, with a price of no more than €20 per test for a cartridge.

Future consideration: Indium Tin Oxide (ITO) in the chip allows the biosensor to potentially expand its application for research purpose, such as allowing optical analysis techniques like fluorescence spectroscopy due to its transparency.

Monetary valuation: The TBI biosensor offers competitive pricing compared to current diagnostic tools, making it an attractive option for healthcare providers. Furthermore, its potential to decrease the need for unnecessary CT/MRI scans has the advantage of reducing overall healthcare costs (see Appendix 9.2.3a).

Key features: Our biosensor uses blood-based biomarkers for point-of-care TBI detection and severity classification. It's not meant to replace clinical decision rules, but to enhance them by providing quantifiable, objective, and timely results, adding value to the diagnostic process.

To use our biosensor: a simple process of depositing a 20 µL blood to the cartridge, plugging in the chip, pressing the touchscreen display, and waiting for a few minutes for the results. Our current concept reader device, sized 20 cm x 30 cm x 30 cm, utilizes a Raspberry Pi 3 to serve as a microprocessor for the LCD screen. The idea is that it could serve as a data logger, wirelessly connect to the PC/laptop and interface with a compatible potentiostat. Together with a rechargeable battery, the design promotes dual-power functionality, allowing for both stationary and portable use, enhancing clinical workflows. Our housing design is currently prototyped using PLA material; however, a metalled, with grounded Faraday cage and a more resilient casing to ensure longevity and optimal performance is considered for future design. Another key aspect to consider is a thermal system for the monitoring of the cartridge temperature to (below 37 Celsius), as the denaturation of protein results in an obsolete test. To achieve truly point-of-care for the user, a single-use lanced fingertip will also be included in our biosensor packaging. For the ease of use by medical professionals, a customizable user-interface and electronic medical record (EMR) system integration will also be considered in future development. (see Appendix 9.1)

Please find Appendix 9.2 for your reference for TBI epidemiology facts and a patient's journey map, existing diagnostic methods of TBI and market comparison.

5.3 Business Feasibility

In the next two years after the SensUs competition, we are dedicated to advancing our Technology Readiness Level (TRL) by first focusing on public grants and executing a Proof-of-Concept. On the recommendation of our start-up coach, the team will officially join RWTH Incubation Program to receive professional guidance on our technological document and business plan for public grants and pre-seed investments. During this competition, the team has been active in attending entrepreneurial workshops and events from AGIT GmbH, expanding our network to no-equity stake programs, private and corporate venture capital investors. The team will take initiative on attending further start-up programs offered in the Aachen ecosystem like ATEC to further develop not only the product-market fit, shareholder's rights and employment law, but also ourselves as founders for investment pitches, networking opportunities and to learn about a company's success factors.

Technologically, our biosensor chip has shown promising results to validate Proof-of-Principle (PoP) during this competition, validating our position of approaching TRL 3. For further TRL and to establish ourselves as an individual start-up team, negotiation to the access to necessary workspaces for clean room facilities, laboratories and equipment has been ongoing, mainly with our key partner, IWE1. We plan to multiplex our biosensor with an additional TBI biomarker, specifically UCH-L1 or S100ß for the final product. Hence, a formal advisory board with knowledge of TBI and medical backgrounds will be instituted to provide insights, feedback and credibility on clinical expertise and validation of biomarkers. Thanks to our current biosensor chip design with 4 sensing areas, of which one sensing area is not functionalized to enable on-chip reference sensing, it is technologically feasible for the multiplex option. By integrating multiple biomarkers into our biosensor, we aim to achieve a high specificity and sensitivity in TBI detection. (Please refer Appendix 9.2.4)

Concurrently, from TRL3-5, in the initial phase, we plan to establish a co-development partnership with commercial partners for our readout system and control software. As we evolve, we will slowly transition to our customized biosensor reader with specific user-friendly features complementary to our biosensor chip and aligning to our intended use & purpose. Additionally, we'll collaborate with an antibody supplier essential for our in-lab tests with ELISA and Abbott's biosensor as the-state-of-the-art benchmark. We are already in close contact and collaboration with Clinical Translational Center Aachen (CTC-A) and the Ethical Committees of Hospital Meyer, understanding the complex criteria for our biosensor's clinical evaluation and regulatory frameworks. Due to the standards within

QMS and IVDR, we expect strict audits starting TRL 5 onwards; hence, we will officially consult with our contacts, consisting of IVDR expert from Vysyo GmbH, TÜV SÜD and relevant Clinical Research Organization (CRO) like Parexel to ensure smooth clinical evaluation and regulatory approval of CE marking and other sales certificates. (Please refer Appendix 9.3.2)

To develop an optimal patent strategy, we'll conduct a patent search with RWTH Patent & Standards Center (PNZ), mainly about other POC market competition (see Appendix 9.2.3c), ensuring smooth approval of our patent. We aim to acquire at least two additional patents within a six-year timeframe, specifically on our chip technology and its chip socket (sensing area), of which will be essential for our revenue model. Our main role will be the antibody functionalization and final assembly of the chips, ensuring that FQC protocol is met.

We adopt the Razor and Blade Model (see Appendix 9.3.1), with the "blade" - the chip - as our primary source of revenue. The "razor" - the reader - will be made accessible at a minimal margin by licensing our patented chip sensing platform to an established read-out production company and distribution services will be established with our contacts of Aachen's ecosystem. This strategic partnership is meant to leverage scalability and enable our team to concentrate on advancing our niche and core competency in biosensor technology, simultaneously expanding partners' market footprint. Leveraging our revenue model, we plan to deploy a compelling marketing strategy tailored for early adopters. We offer our early adopters a lower pricing structure for the reader device, complemented by a complimentary provision of the first 150 chips to be free of charge for one month, under a commitment of a one-year minimum contract. By collaborating with early adopters, we believe we could gain crucial feedback and data, enhancing our product brand and market position.

Our business is committed to not only provide economical advantages, but also offering ecological benefits. Our marketing team aims to connect healthcare infrastructure and the general communities by consistently initiating awareness campaigns, highlighting the importance of early TBI diagnosis. For networking and promotional purposes, the team will participate in IEEE Biosensors and other related events. Upholding sustainability, we will meticulously select business partners aligned with our ethos, emphasizing sustainable production and cartridge waste management. Through strategic global alliances with governments, NGOs and business partners, and while focusing primarily on our niche of a companion diagnosis of Traumatic Brain Injury (TBI), our ambition is a sustainable, healthier future for all.

Please find Appendix 9.3 as a reference to our revenue model and roadmap as per Technological Readiness Level (TRL) as a start-up in the next 6 years.

5.4 Financial Viability

Securing vital startup funding is our top priority. Our strategic approach includes applying for programs and grants during a period of 6 years, which allows us to raise an amount of €3.045.000. Additionally, we're going to actively engage with business angels and capital ventures to raise an amount of €4.500.000 during this six-year period of pre-market entry, please refer to Appendix 9.4.1 for more details.

To establish the optimal unit count and pricing strategy for our biosensors, we've undertaken meticulous market research, closely tracking trends and assessing both existing and potential patient volumes [21]. Moreover, we've thoroughly considered the potential customer base in Germany, (see Appendix 9.4.2). In consultation with medical experts, we've devised an initial pricing approach. Commencing at €20 per unit, this higher price point reflects the initial manufacturing costs (see Appendix 9.4.3), projected at approximately €5 per unit, with the initial cost of €12,44 (see Appendix 9.4.4) and the high distribution (Cold Chain Distribution) cost, due to the special environment the biosensor needs to be transported. Our launch strategy involves distributing approximately 150 biosensors per month per customer. As we advance, our commitment is to significantly reduce the biosensor price while ramping up production.

Shifting focus to the read-out device, the manufacturing cost stands at roughly €800 (see Appendix 9.4.3), while the selling price is projected at €1000. Although the profit margin is comparatively conservative, we are determined to gain traction by ensuring high-quality performance and reliability. In terms of volume, our initial projection is to supply at least one read-out device per customer.

Operational expenses (see Appendix 9.4.5) include Research & Development, where we'll initially rely on grant funds and later allocate 19% of net income for innovation. During an interview with the Director of the Patent & Standards Center (PNZ) at RWTH Aachen University we concluded that the patents would cost approximately €316.000, ensuring 20 years of patent rights across 5 key EU countries. Clinical evaluation and regulatory approval

are central to our future, with an estimated investment of €4.720.000 for a 5-year period we expect to fulfill every need, so the biosensor would be ready for the market. Our dynamic marketing strategy involves active participation in industry related events such as: IEEE Biosensor, AACC Annual Scientific Meeting, SmartTech Asia etc, and having a digital presence such as a professional website in order to reach out to our potential customers. During the first year of operation, our strategy involves giving away 150 biosensors for the first month to our first customers (see Appendix 9.4.6). Employee wages are carefully budgeted, amounting to €92.160 during the startup phase (40 hours per week with min. €12 per hour) and €365.500 later (see Appendix 9.4.7). Facility usage will transition, initially utilizing IWE1 for biosensor development, followed by specialized production facilities. Additionally, we have budgeted for essential professional services, including legal, financial, and tax advisors. Particularly significant is our investment in distribution advisors, recognizing the substantial impact distribution has on our expenses. This strategic allocation aims to optimize our distribution processes and manage costs effectively.

Considering taxation in Germany, our structure involves a 30% total tax rate, comprising Income Tax (Körperschaftssteuer), Trade Tax (Gewerbesteuer), and Sales Tax (Umsatzsteuer).

A detailed financial breakdown, including Net Profit, Gross Profit, and Earnings Before Tax (EBT), is provided in Appendix 9.4.1, ensuring transparency and insight into our company's financial performance.

6. Team and support

6.1 Contributions of the team members

Luca Terenzi, and **Hojeong Lee**: our team captains handled the organizational side of the process, designing together with the team the next steps and facilitated interpersonal communications between team and external partners/supervisors.
Niklas Meyer and **Muchen Yang** designed and manufactured the cartridges and sensing elements (chips) of our biosensor.
Minju Kim, Michelle Heinsch and **Antigoni Karavelaki** developed the functionalization protocol and were responsible for handling antibodies and antigens, as well as conducting the fluorescence tests.
Stefan Noppeney and **Min Young Jeong** characterized the response of our biosensor and gave feedback on its performance.
Francesca Parrotta, our business team leader, worked on understanding hospital workflows and patients' journeys by working with our key partner, Meyer Children's hospital.
Chop Way Lee actively represented the team in networking events and concluded business feasibility with our start-up roadmap and has also designed our 3D housing of the reader.
Henki Xhepa worked extensively on market research and the financial aspects of our start-up and was a bridge between biosensor and business since he knows well about both parts.
Ryota Ishii oversaw stakeholder desirability and creative parts such as making videos and posts for Instagram takeover.
Zoe Alissa Buchholz supported measurements and helped with research.
Jan-Hendrik Meyer helped with literature reading and planned the Gantt-chart for the biosensor development process.

6.2 People who have given support

Dr. Vivek Pachauri, our supervisor, supported us throughout the competition with continuous feedback and availability in case of need. He was responsible for the organization side of the institute, putting the team in contact with helpful researchers and technicians that could help us in case of need.
Dibyendu Khan and **Aidin Nikookhesal** greatly helped us in the technical parts, whenever we faced challenges. They were always receptive to our opinions and played a significant role in addressing various technical aspects. They also supported and advised the team regarding silanization and organizational tasks. We also thank **Dr. Divagar Murugan** for helping us with the biochemistry part, especially the fluorescence measurements, and **Animesh Singh** for technical support for manufacturing and characterization.
Dylan, Dan, and **Roman** team captains from previous years supported us with valuable advice based on their own experience participating in the competition and helped us to get insightful details.

6.3 Sponsors and Partners

IWE1 institute at RWTH university supported us with facilities, funding, and mentoring.
Collective Incubator provided us a coworking place for meetings and workshops.
We would also like to thank the **Dental Materials and Biomaterials Research and Teaching area** for their prompt assistance and provision of fluorescent antibodies for the initial testing.

7. Final Remarks

We thoroughly enjoyed the SensUs competition and have learned tremendously from the plannings of the organization! Can’t wait to finally present our biosensor in Eindhoven 😊

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Interviews

[1] Interview with Univ.-Prof. Dr. med. Hans Clusmann, Director of Neurosurgery Uniklinik RWTH Aachen. 21 March 2023.

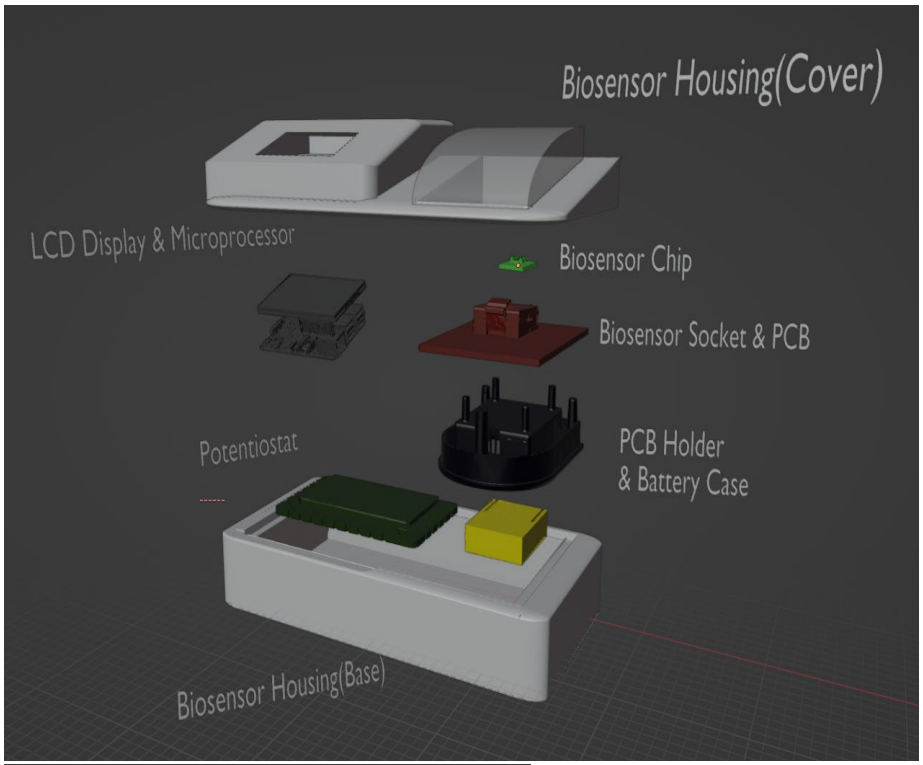
[2] Interview with Dr. Flavio Giordano, Head of Paediatric Neurosurgeon of the Meyer Children's Hospital. (Multiple interviews, including with his team)

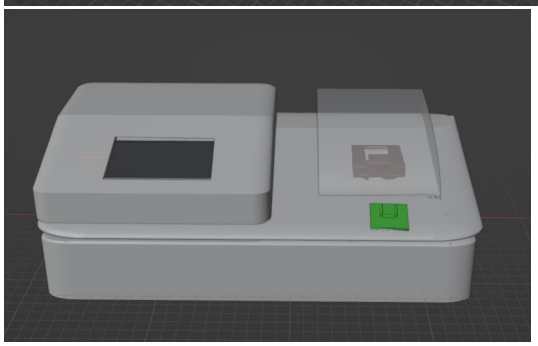
[3] Interview with Dr. rer. nat. Rainer Schuckelt, Coordinating General Manager of the Center for Translational & Clinical Research, CTC-A and Dr. Myriam Lipprandt, Head of Translation Center. 2 August 2023.

[4] Interview with Dr Susanne Ruffert, Director of the Patent & Standards Center (PNZ) and Kristin Jirka. 6 June 2023.

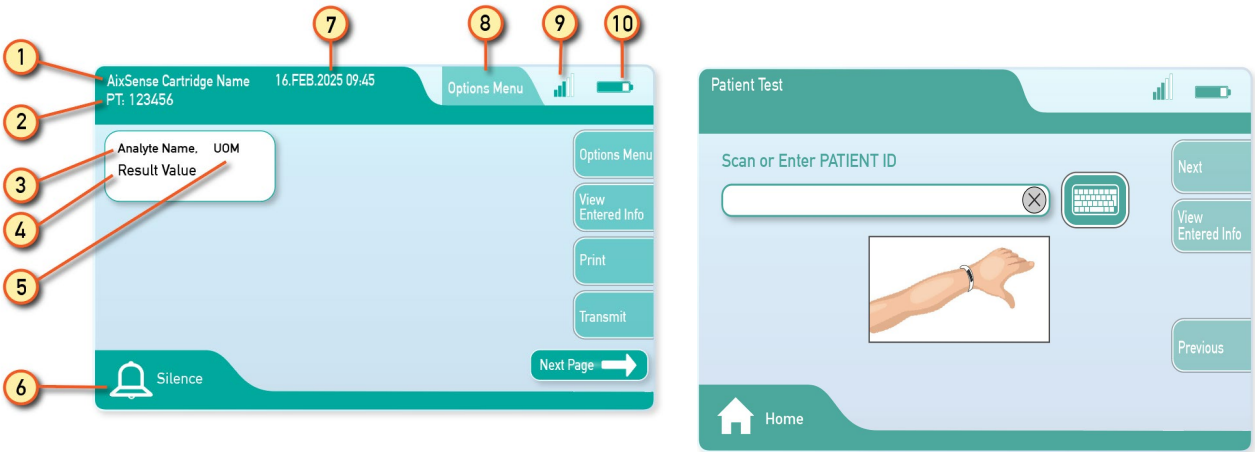
9. Appendix

9.1 Biosensor System and Assay





The figures show the **AixSense Reader's** design, which prioritizes the safeguarding of its core components. The sensitive biosensor chip is shielded from external contaminants, ensuring consistent and reliable readings. This structural integrity plays a pivotal role in maintaining the device's performance and longevity. The reader is sized approximately 20 cm x 30 cm x 30 cm.



Figures show a customizable graphical user-interface and electronic medical record (EMR) system integration to be considered in future development.

Feature	Description
1. Cartridge Name 2. Patient Identification	Ensures unique cartridge and patient identification.
3. Analyte name	GFAP protein
4. Result – value 5. Unit of Measurement	Core output providing a quantitative measure of the analyte.
6. Audible Cue	Notifies completion of the test; valuable in busy settings.
7. Date & Time of Test	Tracks injury progression and test intervals.
8. Options Menu	Allows customization and access to advanced biosensor features.
9. Wireless Signal Strength	Indicates connection strength for data transmission; ensures server connectivity.
10. Battery Strength	Indicates device readiness; crucial for portability and uninterrupted testing.

Table shows feature considered for user interface.

9.2 Stakeholder Relationships

9.2.1 Patient Journey Map

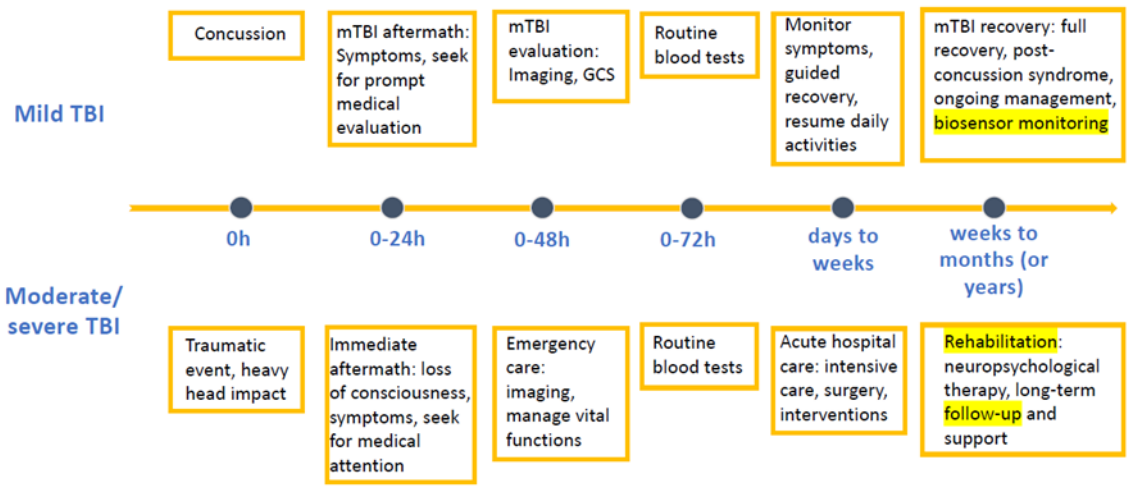


Figure shows a typical TBI patient journey map.

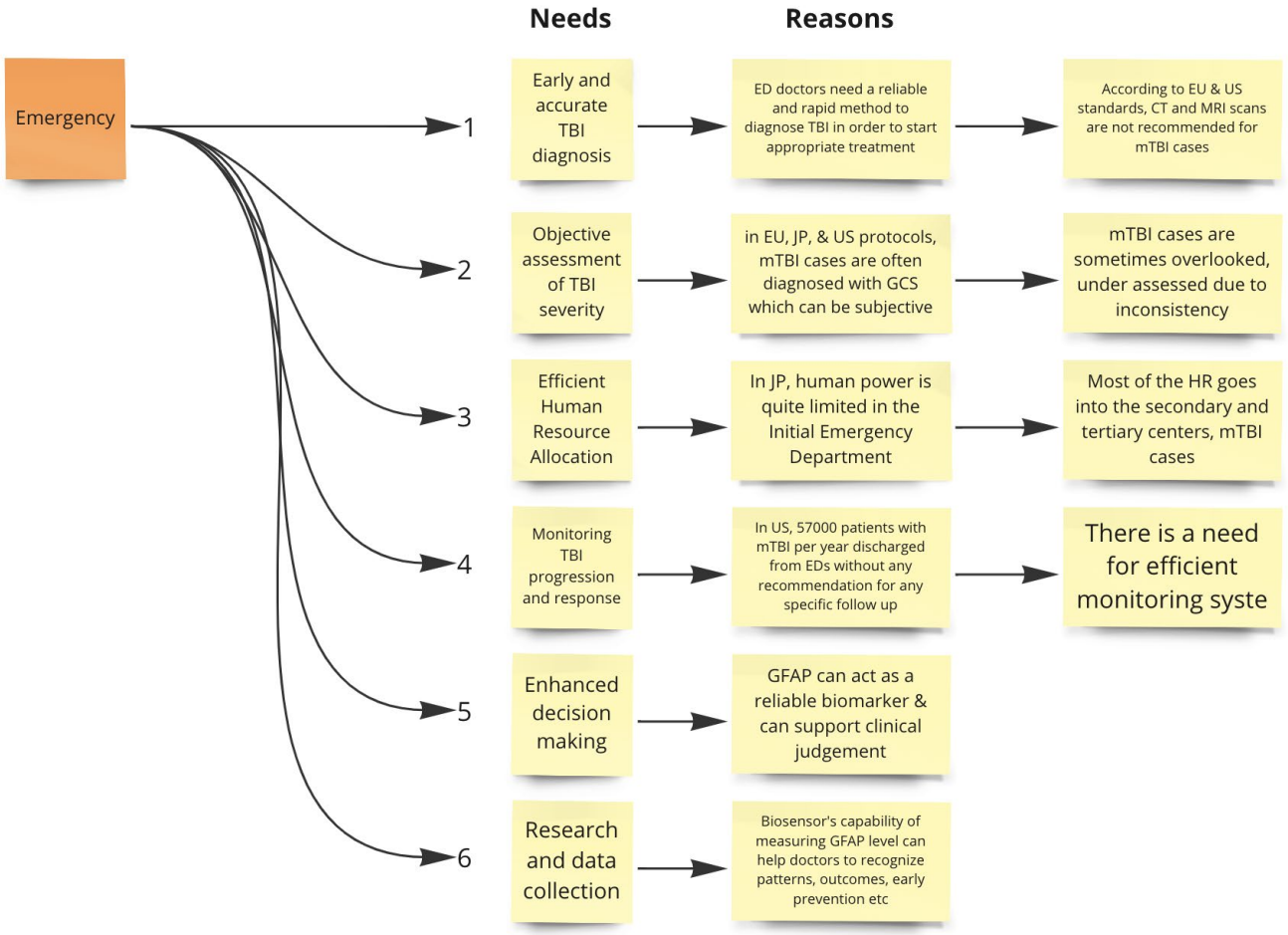


Figure shows a TBI patient's needs and reasons in an emergency scenario.

9.2.2 Epidemiology Trend of TBI and its Heatmap Representation between the Stakeholders and Their Needs [14]

Legend: Red – High impact Orange – Mid impact Yellow - Low impact	Accurately Diagnose mTBI	Mobility	Long-term monitoring	Efficient manpower allocation	Reliability	Speed
Neurologist						
Paediatric doctor						
Family doctor						
Emergency doctor						
Natural disaster workers						
Children						
Athletes						
Seniors						
People with disability						
Military people						

Figure shows heatmap representation between the stakeholders and their needs.

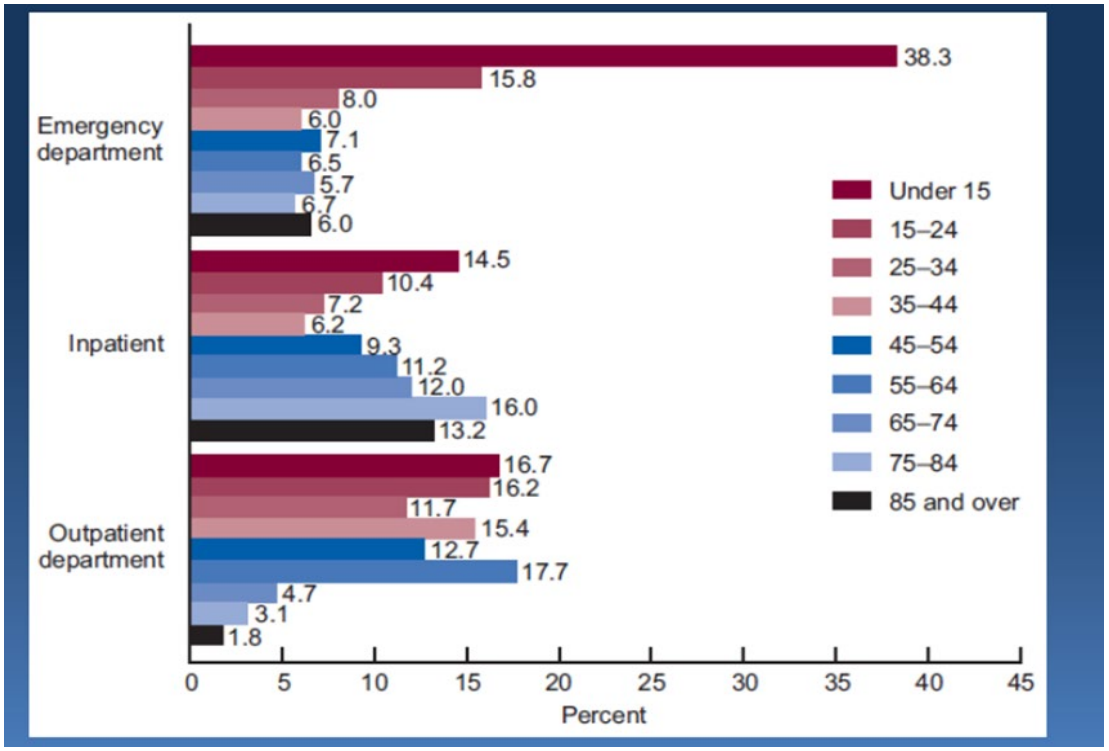


Figure shows the epidemiology of Traumatic Brain Injury (TBI).

9.2.3a Analysis of Diagnostic Methods of TBI

Established Diagnostic Methods of TBI

*Inquiry obtained through email

** excluding triage and radiology analysis

Diagnostic Method	Setting Required	Device Cost (Euro)	Cost Per Test (Euro)	Time to Results	Notable Disadvantage
Head CT Scan	Radiology Department	Between 100,000 and 250,000	166 (secondary sources) 209,82 (Uniklinik Aachen*)	10 mins** whole process up to 6 hours	Only 10% sensitivity on mTBI
Head MRI Scan	Radiology Department	Between 1,250,000 and 3,500,000	256 – 461 (secondary sources) 589.85 (Uniklinik Aachen*)	30 mins **	Potential allergic reaction on contrasting agent (Gadolinium)
ELISA Method	Clinical Laboratory	680 for 96 tests	7	90 mins	Used mostly for research, not point-of-care
i-STAT TBI Plasma Test	Clinical Laboratory	8000	40	15 mins	Not intended to be used in point-of-care settings

9.2.3b Analysis of Alternative Diagnostic Methods of TBI

Device	Description	Device Cost (Euro)	Cost Per Test (Euro)	Time to Results	Notable disadvantage
i-STAT TBI Plasma Test	- TBI test using blood plasma with - multiple patents - 95% clinical sensitivity - 99.3% negative predictive value	8000	40	15 mins	Not intended to be used in point-of-care settings;
AixSense TBI Test	Goal: Point-of-Care TBI test 95% clinical sensitivity 99% negative predictive value	1000	20 (at mass production)	3 mins	Still in early stages of development with no patent

9.2.3c Analysis of Emerging Competitors of TBI Diagnosis

Company	Description (Point-of-care)	Biomarker(s)	Key Features	Regulatory Status
Medicortex	Diagnostic kit using urine and saliva samples	Carbohydrate-based glycans	• no professional interpretation needed	• A prototype diagnostic kit expected in two years • Completed pre-clinical trials
ABCDx	Biomarker panel tests	IL-10, H-FABP	• Working on a variety of brain injury conditions ranging from traumatic injury to stroke and post-stroke infections	• Secured exclusive commercial rights to a cluster of patent applications
BRAINBox Solutions	Blood biomarker and neurological test	GFAP, NSE, NRGN	• Provides a quantitative interpretation of test results & computerized neurological assessments	• Received FDA's Breakthrough Device Designation • Initiated clinical study
EzDiatech	Bight Field image coding system using Rod-Shaped Magnetic Particles (RSMPs)	GFAP, UCH-L1	• No professional operators needed • Fully automated analysis • No pre-treatment required	• Received CE certification in April 2022
BrainScope	EEG Brain Function Index, (BFI)	Brain electrical activity	• the only FDA cleared non-invasive MD that objectively assesses head injury	• FDA approved

9.2.4 Biomarker of TBI

Upon interviews with neurosurgeons Dr. Hans Clusmann and Dr. Flavio Giordano, and fortified by a CENTER-TBI study [18], we've concluded that biomarker S100B, already well recognized and integrated into national guidelines for CT scan triaging in mild TBI cases, has high sensitivity and a strong negative predictive value. The correlation between GFAP and S100B strengthen over time [19], with a clear, strong correlation beyond 36 hours, suggesting their potential combined use as an accurate TBI monitoring tool.

Meanwhile, the FDA's 2018 endorsement of biomarkers GFAP and UCH-L1 heralded a significant leap in blood biomarker development for neurological disorders. [20] Notably, the ALERT-TBI trial revealed that GFAP and UCH-L1 can discriminate against patients with CT abnormalities. The TRACK-TBI study underscored biomarkers' potential as screening tools for MRI anomalies in patients with normal CT results.

GFAP, detectable in serum hours post-trauma, peaks around 20 hours after an injury and declines over 72 hours. GFAP assists in differentiating between hemorrhagic and ischemic strokes, as its levels rise within two hours and peak between 6–12 hours for the former, while for the latter, the increase occurs later. On the other hand, UCH-L1 peaks at 8 hours post-injury and declines rapidly over 48 hours. However, their clinical utility remains unclear, warranting further exploration for an effective multi-marker strategy.

Therefore, we propose that a development of two separate cartridge system, mainly cartridge A (functionalized with UCH-L1 and GFAP) and cartridge B (GFAP and S100ß) or a combination of 3 biomarkers must be considered as they provide a comprehensive severity overview of TBI. Yet, we understand that meticulous review and approval by an Ethics Committee is crucial. Monitoring these biomarkers non-invasively helps gauge TBI severity and monitor its evolution and associated complications.

9.3 Business Models & Roadmaps

9.3.1 Razor and Blade Revenue Model

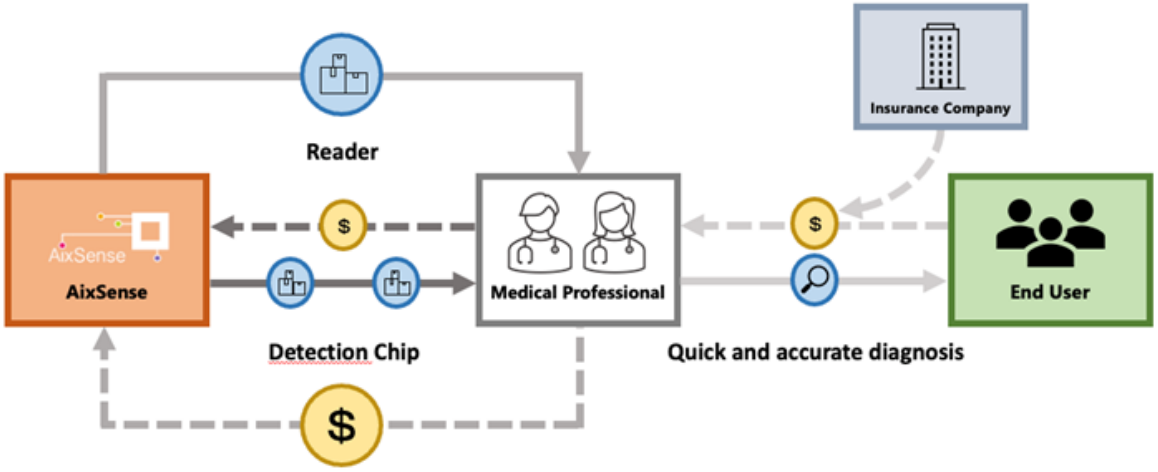


Figure shows our adopted revenue model to Razor and Blade

9.3.2 Roadmap of our Start-up

Table below shows our roadmap based on Technology Readiness Level (TRL).

Readiness Level (TRL)		
1	Basic Principles Observed & Reported	Completed/2023 Q1
2	Technology Concept Formulated	Completed/2023 Q2
3	• Lab-based prototype test with GFAP observed • User-friendly reader conceptualized • Financed through public grants	Proof-of-Principle achieved/2023 Q3-Q4
4	• Proof-of-Concept validation • carry out rigorous benchmark tests with ELISA (with GFAP) • Identify an additional biomarker (UCH-L1 or S100ß) and work on its technological integration • Hytest official partnership • Initial consultation with Center for Translational & Clinical Research Aachen (CTC-A) of RWTH Aachen • Initial patent submission • Co-development of biosensor ready with a partner • Financed through pre-seed investment	2024 Q1-Q4
5	**Pre-clinical Phase(Technology validated in relevant environment):** • Good Clinical Practice (GCP), Quality Management System (QMS), biosensor's conformity to IVDR, audits and inspections by Notified Bodies like TÜV SÜD • Partnership with IVDR experts and Clinical Research Organization (CRO) • Benchmark analysis (specificity & sensitivity) with the state-of-the-art, for example ELISA & Abbott TBI plasma • Initial risk analysis and mitigation strategies • Financed through seed-investment	2025 Q1–Q4
6	**Pilot Program & Phase 1 Clinical Study (Technology demonstrated in relevant environment):** • Close collaboration with Clinical Research Organization (CRO) for clinical trial design and regulatory strategy • Ethics Committee positive decision highly required • Consideration on venture capital	2026 Q1-Q4
7	**Phase 2 & 3 large-scale Clinical Study** (System prototype demonstration in an operational environment) • Partnership with multiple Clinical Research Organization (CRO) to conduct observational study with up to 2000 subjects (clinical trial study of "BRAINBox Solutions Inc", an emerging market competition as a reference) • Consideration and planning of merger and other exit strategy	2027 Q1-Q4
8	**System Completion & Final Validation** • Submission of all documentations (TRL 3-7) through a Notified Body • Market authorization begins • Technology transfer completion (if present)	2028 onwards
9	**Commercial Deployment** • Post-market surveillance reports • Continuous improvement documentation	2028 onwards

AixSense Short-term Roadmap

R&D plan	2023/Q3-4	2024/Q1-2	2024/Q3-4	2025/Q1-4	2026/Q1 and onwards
Microfluidic cartridge development for TBI	GFAP	GFAP and multiplex options			
Medical prototype device development	Prototype	Functional Prototype	MVP		
Evaluation of the prototype					
Initiation of regulatory process					
Production of prototype batch				Manufacturing and Assembly	
Clinical evaluation of the final product					Final product for clinical trials (required for CE marking)
New patent application		MVP		From MVP to Final Product	

Figure shows our short-term roadmap.

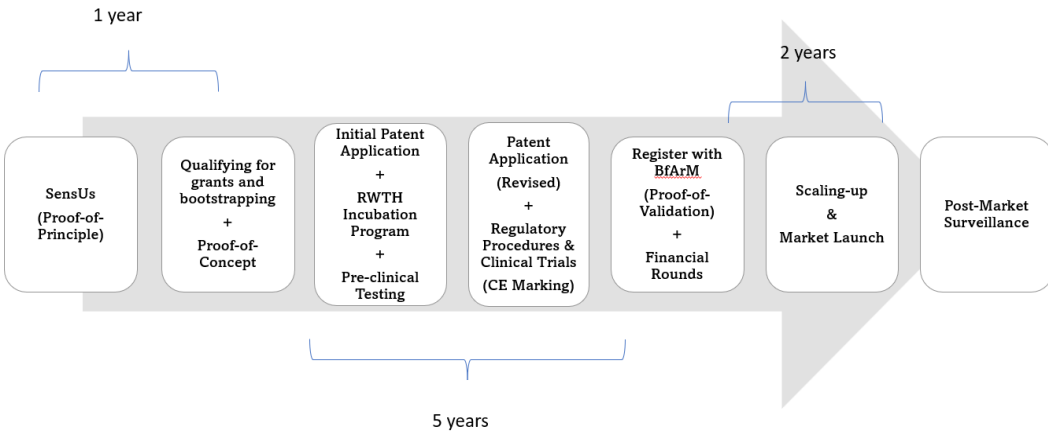


Figure shows our general roadmap.

9.4 Financial statements

9.4.1 Financial breakdown

		PRE-MARKET ENTRY						POST-MARKET ENTRY			
		2023	2024	2025	2026	2027	2028	2029	2030	2031	2032
Inflows											
Type of Investment	Capital venture	0€	0€	650.000€	1.400.000€	0€	400.000€	0€	0€	0€	0€
	Business angles	0€	330.000€	1.000.000€	500.000€	0€	200.000€	0€	0€	0€	0€
	Start-up grants	0€	35.000€	10.000€	0€	3.000.000€	0€	0€	0€	0€	0€
	TOTAL	0€	365.000€	1.660.000€	1.900.000€	3.000.000€	600.000€	0€	0€	0€	0€
Sales	Biosensors no. of units	0	0	0	0	0	0	144.000	342.000	644.400	1.078.200
	Biosensors sales	0€	0€	0€	0€	0€	0€	2.880.000€	6.840.000€	12.888.000€	21.564.000€
	Read-out no. of units	0	0	0	0	0	0	80	110	168	237
	Read-out sales	0€	0€	0€	0€	0€	0€	80.000€	110.000€	168.000€	237.000€
	TOTAL	**0€**	**0€**	**0€**	**0€**	**0€**	**0€**	**2.960.000€**	**6.950.000€**	**13.056.000€**	**21.801.000€**
Outflows											
	COGS	0€	0€	0€	0€	0€	0€	784.000€	1.798.000€	3.356.400€	5.580.600€
	OPEX	0€	359.059,69€	1.663.759,69€	1.840.758,69€	1.990.759,69€	1.781.759,69€	809.782€	896.381,69€	1.426.382,69€	2.093.381,69€
	Taxes	0€	0€	0€	0€	0€	0€	409.865,4€	1.276.685,493€	2.481.965,493€	4.254.305,493€
Profits	EBT	0€	0€	0€	0€	0€	0€	1.366.216€	4.255.618,31€	8.273.217,31€	14.181.018,31€
	Net income	0€	0€	0€	0€	0€	0€	**998.352,6€**	**2.978.932,817€**	**5.791.252,817€**	**9.926.712,817€**
	Gross profit	0€	0€	0€	0€	0€	0€	2.176.000€	5.152.000€	9.699.600€	16.220.400€

Table shows a detailed financial breakdown, including Net Profit, Gross Profit, Earnings Before Tax (EBT) etc.

9.4.2 List of customers

POST-MARKET ENTRY CUSTOMERS					
List of Customers	**Year**	**2029**	**2030**	**2031**	**2032**
Hospitals/Clinics		80	181	341	566
Military Hospital		0	3	5	5
Sport Clubs		0	6	12	28
TOTAL		**80**	**190**	**358**	**599**

Table shows potential customer base in Germany after market launch.

9.4.3 Cost of goods sold

Cost of goods sold		**POST-MARKET ENTRY**			
Year		**2029**	**2030**	**2031**	**2032**
Manufacturing	Biosensor	720.000€	1.710.000€	3.222.000€	5.391.000€
	Read-out	64.000€	88.000€	134.400€	189.600€
TOTAL		**784.000€**	**1.798.000€**	**3.356.400€**	**5.580.600€**

Table shows estimated manufacturing costs post-market entry.

9.4.4 Cost of a biosensor

Cost of a single biosensor		
Material	**Cost of production**	**Cost of mass-manufacturing**
Substrate: Si	0,83€	0,332€
ITO	1€	0,4€
GFAP antigen	10,42€	4,168€
APTES	0,034€	0,0136€
NHS	0,005€	0,002€
EDC	0,006€	0,024€
BSA	0,146€	0,0584€
PBS	0,0013€	0,00054€
MES	0,002€	0,0008€
TOTAL	**12,44€**	**4,99€**

Table shows manufacturing costs today of a single biosensor.

9.4.5 OPEX

Operating expenses (OPEX)		PRE-MARKET ENTRY							POST-MARKET ENTRY				
Category	Name	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	TOTAL
R&D	Biosensor & Read-out	0€	30.000€	45.000€	80.000€	150.000€	150.000€	50.000€	150.000€	560.000€	1.000.000€	0€
	Patents	0€	0€	8000€	150.000€	230.000€	0€	0€	0€	0€	0€	0€
	Clinical evaluation & Regulatory approval	0€	220.000€	1.500.000€	1.500.000€	1.500.000€	1.500.000€	0€	0€	0€	0€	0€
Marketing	Events / Fairs / Other	0€	1000€	2000€	2000€	2000€	2000€	62000€	3000€	3000€	3000€	0€
	Travel and Entertainment	0€	500€	1500€	1500€	1500€	1500€	1500€	2000€	2000€	2000€	0€
	Website/social media	0€	399,69€	99,69€	99,69€	99,69€	99,69€	5000€	99,69€	99,69€	99,69€	0€
Salaries & Wages		0€	92.160€	92.160€	92.160€	92.160€	92.160€	365.500€	365.500€	365.500€	365.500€	0€
Rent	Lab/Factory	0€	15.000€	15.000€	15.000€	15.000€	15.000€	67.782€	67.782€	67.782€	67.782€	0€
	Devices	0€	0€	0€	0€	0€	0€	153.000€	153.000€	153.000€	200.000€	0€
Services	Professional services	0€	0€	0€	0€	0€	21.000€	5000€	5000€	5000€	5000€	0€
Distribution	Cold chain distribution	0€	0€	0€	0€	0€	0€	100.000€	150.000€	270.000€	450.000€	0€
TOTAL		0€	359.059,69€	1.663.759,69€	1.840.758,69€	1.990.759,69€	1.781.759,69€	809.782€	896.381,69€	1.426.382,69€	2.093.381,69€	0€

Table shows operational expenses, including Research & Development etc.

9.4.6 1-Month free trial expenses

1-Year Contract for the first customers		
Customers	Units	Cost
80	1.200	60.000€

9.4.7 Salaries and wages

Salaries/Wages		
Category	Position	Salary in € / yearly
Director	CEO	100.000€
	CFO	80.000€
Engineer	Biomedical	53.300€
	Electrical	51.000€
Technicians	Lab technicians	37.700 €
Administration	Sales manager	43.500 €
	TOTAL	**365.500€**