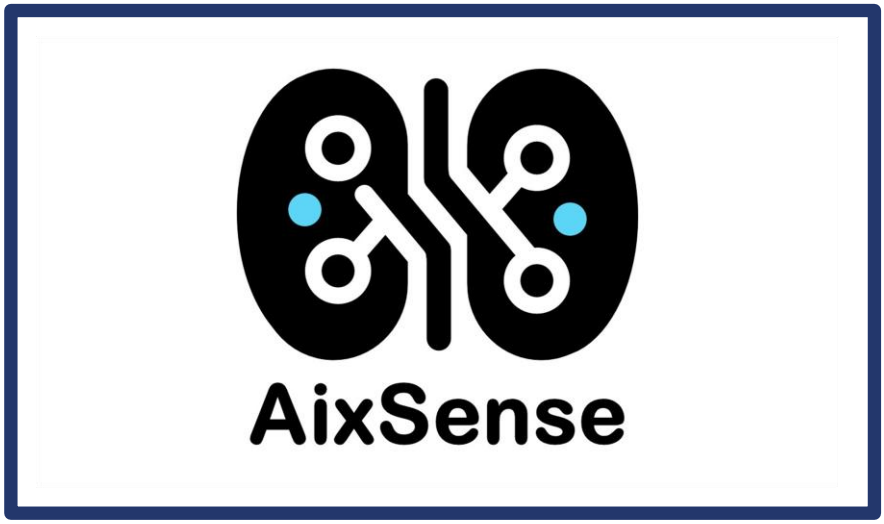
Team Results Document

AixSense



University: RWTH Aachen

Team members:

Juliette T'Kint
Stefan Kreher
Amine Baadaka
Chenyan Feng
Erdenebat Battsere
Erkai Wang
Kostadin Eftimov
Muxi Zhang
Reyyan Tağman
Seungyeon Kim
Sven Gedamke
Yijun Yan
Lin Yiyu
Yumie Nishiyama
Yusuke Tsutsumi

Supervisor:

Dr. Vivek Pachauri

Coaches:

Dibyendu Khan
Aidin Nikookhesal

09/08/2024

SensUs

AixSense

Content

1. Abstract 2

2. Biosensor 3

2.1. Molecular recognition 3

2.2. Physical Transduction 3

2.3. Cartridge Technology 4

2.4. Readout Instrument and User Interaction 4

3. Technological feasibility 5

3.1. Characterization of electrodes 5

3.2. Electrochemical measurement 5

3.3. Cartridge technology 6

3.4. Readout instrument 6

4. Originality 7

4.1 Team Captain 7

4.2 Supervisor 7

5. Translation potential 8

5.2 Stakeholder Desirability 8

5.3 Business Feasibility 10

6. Team and support 13

6.1. Contribution of Team Members 13

6.2. Individuals who have provided support 13

7. Final Remarks 14

8. References 15

9. Appendix ..... A

9.1 Biosensor System ..... A

9.2 Stakeholder Relationships ..... D

9.3 Business Model & Start-up Roadmap ..... G

9.4 Financial Statements ..... J

1. Abstract

Chronic kidney disease (CKD) affects over 10% of the global population (Hill NR, 2016; Kellum, 2021) and remains one of the leading causes of mortality worldwide (CP., 2011). For example, in low- to middle-income countries, CKD patients[1] suffering from acute kidney injury (AKI) have a mortality rate of 42% (Kellum, 2021). These conditions highlight the urgent need for effective monitoring tools that detect kidney function deterioration early.

Creatinine, a key biomarker for kidney function, plays a crucial role in monitoring these patients. Additionally, for kidney transplant recipients, a rise in creatinine levels often indicates acute rejection or viral infections, underscoring the need for efficient monitoring. Given these challenges, we designed our biosensor, "AixCrea" to continuously monitor creatinine levels, enabling early detection of AKI and more personalized care for patients.

By providing accurate, real-time data, "AixCrea" helps healthcare providers make timely decisions, like adjusting medication dosages and identifying transplant candidates earlier. Developed in collaboration with nephrologists, it addresses practical clinical needs and is grounded in evidence-based practices. Our commitment to ethical, social, and scientific values ensures that " AixCrea" is both an effective monitoring tool and a patient-centered solution. Based on feedback from healthcare professionals and solid research, we are confident that "AixCrea" will improve kidney care.

[1] G3 and aboveq

2. Biosensor

AixSense developed a biosensor for continuous creatinine detection based on Electrochemical Impedance Spectroscopy (EIS) using metal-organic framework (MOF) to trap the creatinine on the surface of the working electrode (WE). The EIS signals were recorded by a self-programmed potentiostat and afterwards displayed. Additionally, an enzymatic functionalization with amperometric measurement was explored which can be found in the Appendix 9.1.1.

2.1. Molecular recognition

We explored an approach by incorporating a MOF structure on the surface of the WE for molecular sensing (Figure 1). Creatinine and copper (II) ions are known to exhibit a high affinity for complex formation (Ngamchuea, 2022). Consequent to creatinine reaching the electrode surface, which is rich in Cu (II) ions in the form of a MOF, the complex is selectively formed. In addition to functioning as a molecular recognition site, the Cu-HHTP film also directly transduces electrical signals to the gold electrode due to its inherent conductivity (Nam, 2019). Impedance changes on forming the complex of Cu-creatinine were detected by EIS. The WE were prepared using the following processable method after some optimizations: 1. metalizing a glass wafer with titanium and gold layers, 2. dip-coating the wafer with Cu-HHTP (2,3,6,7,10,11-hexahydroxytriphenylene) in ethanol, and 3. annealing the coated wafer at 85°C for 30 minutes in an N2 gas environment.

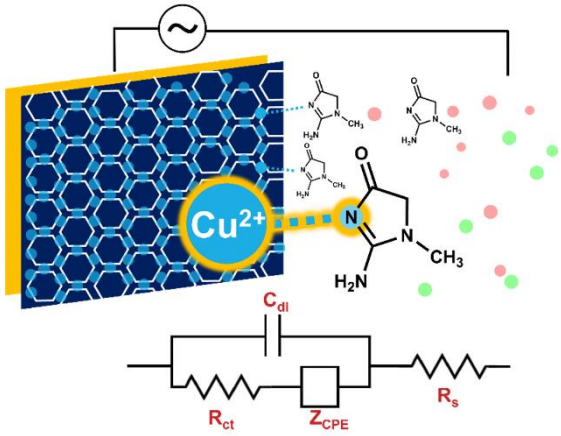


Figure 1: Schematic illustration of creatinine sensor with MOF film (upper) and its equivalent circuit.

2.2. Physical Transduction

EIS is a powerful method of measuring changes at the surface of electrodes, such as antibody–antigen recognition, substrate–enzyme interaction, or whole cell capturing (Magar, 2021). It applies a small AC voltage with varying frequencies across an electrochemical cell and measures the resulting AC current. In this two-wire setup, the response of the WE is sensitive to the analyte concentration. Meanwhile, the counter electrode (CE) and reference electrode (RE) are shorted and complete the circuit, possessing a large surface area to facilitate the passage of current while.

As seen in Figure 1, the interface of our WE can be modelled as Randles circuit (Randles, 1947), consisting of the electrolyte resistance (R_s) in series with the double-layer capacitance (C_{dl}) which is parallel connected to charge transfer resistance (R_{ct}) and constant phase element (CPE). A more detailed description can be found in Appendix 9.1.2.

2.3. Cartridge Technology

Microfluidics offers portability, low cost, and precise fluid control. Polydimethylsiloxane (PDMS) is ideal due to its optical transparency and adhesion properties (Battat, 2022). Our microfluidic chip was made by injecting PDMS into 3D-printed molds using the Digital Light Processing (DLP) printing process. The chip has four parts: two outer protective parts and two inner parts with channels, including a staggered herringbone micromixer (SHM) to disrupt fluid streamlines and enhance creatinine-copper binding in the measurement chamber (Forbes, T. P., 2012), avoiding sole reliance on diffusion. A 10:1 base-to-agent ratio was used for the gasket and inner layers, heated at 65°C for one hour, while a 4:1 ratio was used for the outer layers, cured at 85°C for three hours afterward. The layers were sealed with PDMS. The gasket was designed to ensure a 70 µL sample volume for better detection accuracy. The chip also has holes for two electrodes: WE and the combined CE and RE.

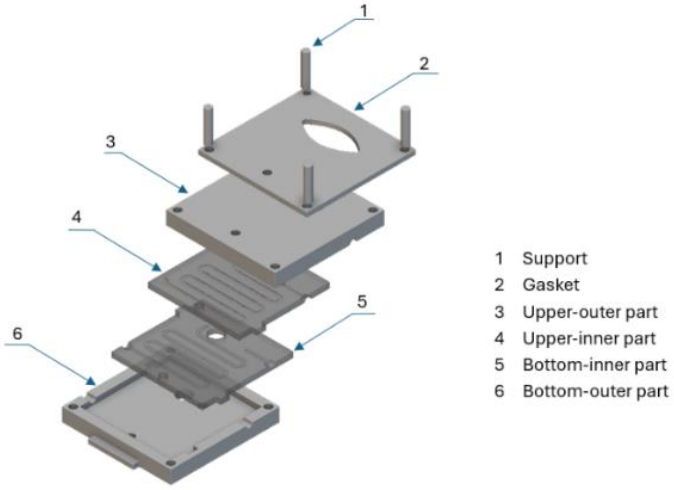


Figure 2: Layered Composition of the PDMS-Based Multi-Layer Microfluidic Chip Assembly with Highlighting Core Components.

2.4. Readout Instrument and User Interaction

The readout system utilized an impedance network analyzer and potentiostat with an integrated, programmable microcontroller (ADUCM350, Analog Devices Inc., USA), which is well-suited for point-of-care diagnostics and body-worn devices. We employed an evaluation board configuration with the ADUCM350 chip and an attached daughter board for easy access to the analog front end. This setup connects to a PC via an interface/emulator board for data communication, firmware updates, and power supply. A custom graphical user interface (GUI), which can be seen in Appendix 9.1.3, controls the measurement process, displays real-time results, and performs trend analysis for interpretable data outputs. When measuring the user can define parameters corresponding to the applied technique (Amperometry, CV, EIS) E.g. to change the applied voltage, or frequency range. Furthermore, an external pump (Spritzenpumpe LA-110, Landgraf Laborsysteme HLL GmbH, Germany) is utilized to regulate the flow from the inlet to the measuring chamber while ensuring an airtight environment. At present, the user must manually pipette the sample into the inlet chamber for subsequent processing.

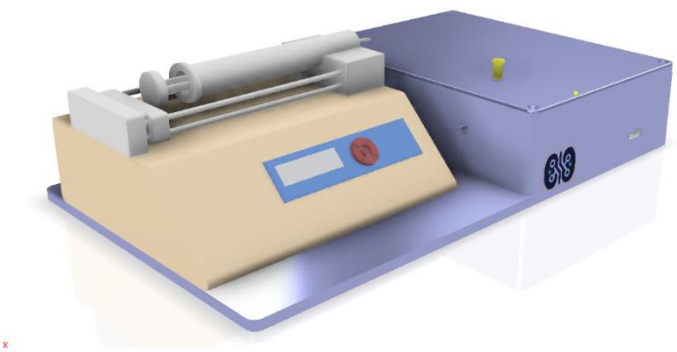


Figure 3: Graphical representation of our sensor which consists of a syringe pump on the left and the fluidic cell and µC in the right box.

3. Technological feasibility

3.1. Characterization of electrodes

The Atomic Force Microscope (AFM) was used to analyze the surface structure of our functionalized chips. Cu-HHTP flakes were around 10-20 nm height and diameter (Figure 4 (c)), which were distinctive to the deposited gold particles on glass wafer (Figure 4 (b)). As indicated by the blue color of the Cu-HHTP which corresponds to the Cu (II) complex formation (Figure 4 (a)).

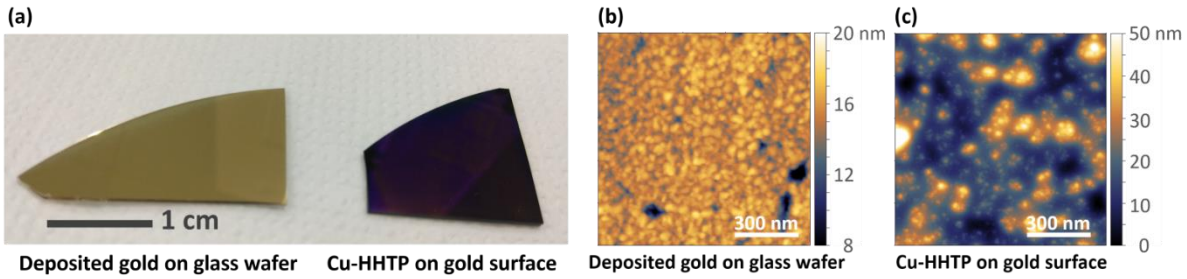


Figure 4: (a) Optical and (b, c) AFM images of gold electrodes before and after dip-coating of Cu-HHTP.

3.2. Electrochemical measurement

Differences in EIS signals were recorded as shown in Figure 5. The EIS curves shifted from left to right in the real part without remarkable changes in its shape, corresponding to the increasing concentration of creatinine. From the theoretical calculation and approximation (see appendix 9.1.2), the real part of impedance at a higher frequency corresponds to the R_{ct}. This result suggests that creatinine adsorption on the MOF surface in the solution suppresses the charge transfer. Furthermore, a correlation between impedance and creatinine levels could be more accurately established by conducting an additional test with smaller incremental concentrations.

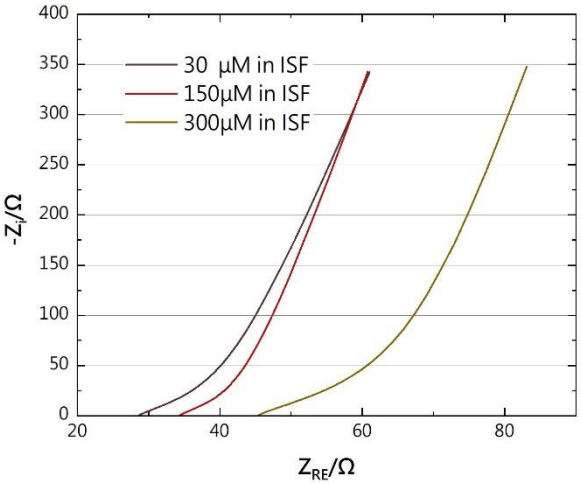


Figure 5: Nyquist plots with different concentrations of creatinine in simulated ISF.

Additionally, we observed small variations between multiple chips while measuring. This could be a consequence of slight differences in the measurement setup or due to the inhomogeneity of the MOF surface from the production of the WE which should be further addressed. Normalization can be achieved through reference measurements to account for chip variability, enabling the construction of a calibration curve. Additionally, periodic reference measurements might be necessary to maintain accuracy and account for any changes due to potential surface degradation.

3.3. Cartridge technology

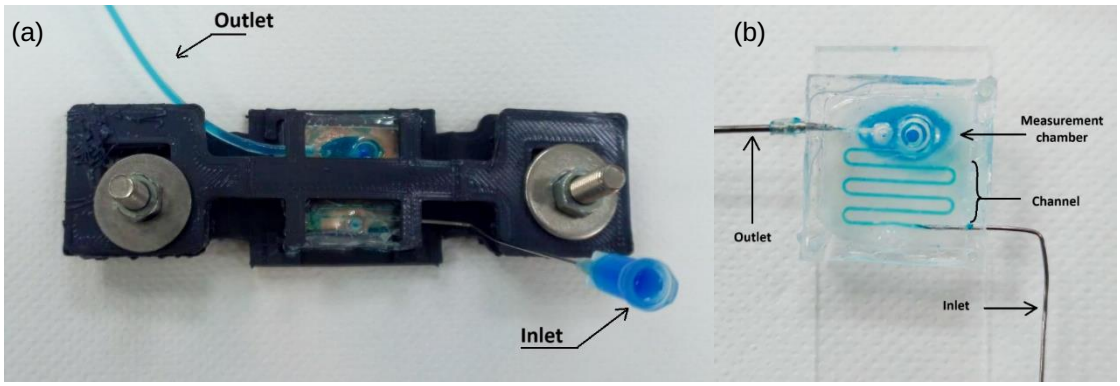


Figure 6: (a) Assembled PDMS-Based Microfluidic Chip Demonstrating Fluid Flow Through Channel and Measurement Chamber. (b) PDMS-Based Microfluidic Chip Secured in a Custom Clamp to Ensure Proper Sealing of the Components.

Experiments were performed to qualitatively evaluate flow behavior by visualizing solutions with distinct colors (Figure 6). Enhancing efficiency and accuracy can be achieved by automating the measurement process. In this setup, a syringe pump connected to the outlet creates negative pressure to draw the sample from the inlet. The sample is injected into a 45-degree curved needle at the inlet using a pipette. A custom system was designed to securely hold all components (Figure 6), including the microfluidic chip, gasket, and functionalized electrode, fitting the required geometry. To ensure proper sealing and prevent leakage during the experiments, the microfluidic chip was secured in a custom clamp, which tightly holds the assembly in place throughout the testing process.

3.4. Readout instrument

The current GUI for controlling the ADUCM350 is effective in its functionality but has room for optimization in terms of size, connectivity and mobility. Future improvements should include the integration of a more compact layout by using a custom PCB and switching to a battery-powered system. Transitioning to control the sensor with a mobile app and connecting it via NFC (Near Field Communication) would enhance the convenience and flexibility of the interface. Moreover, better workflows and analytical protocols can be implemented to simplify the use for patients and enable long-term analysis of creatine levels. In summary, our objective, as further development progresses, is to achieve a portable, on-body solution for our sensor, as shown in Figure 7.

Figure 7: Prototype of the final "AixCrea" Iteration in development. Similar to the continuous creatinine monitoring devices, the sensor will be placed onto the body

4. Originality

4.1 Team Captain

We started by reviewing several sensing principles for continuous creatinine detection such as electrochemistry and optical measurement, or such as enzymatic or non-enzymatic. We started with an amperometric measurement which utilizes an enzymatic cascade to generate H2O2. We tried both covalently and non-covalently bounded enzymes on the gold surface, but these methods needed more delicate handling and therefore more time. Consequently, we switched to another sensing principle based on EIS using Cu-HHTP as a sensor platform, utilizing well-established protocols of the IWE1 group at RWTH. We finally settled on this approach due to its stability, feasibility, cost-effectiveness, and robustness in comparison with enzymatic methods.

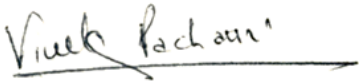
Furthermore, we created a custom flow cell for the competition to allow new samples to reach the sample platform. The software for the ADUCM350, which includes EIS, amperometric measurement, and a GUI, was developed in-house by our team. In the end as a team, we designed and built the final biosensor. The supervisor helped us in the early stage of the biosensor development in terms of feasibility and with feedback throughout the months.

4.2 Supervisor

Biologically sensitive electrical devices using AC and DC readout principles have been widely reported for detecting creatinine. Many platforms rely on enzymatic reactions and nanomaterials to enhance sensitivity. However, achieving high technological readiness (TRL) and commercial exploitation for creatinine biosensors remains challenging due to issues with sensor interface reliability and reproducibility for clinical validation.

To address real-time creatinine monitoring, the AixSense 2024 team focused on high-throughput fabrication, integrating a hybrid electrochemical surface plasmon resonance (EC-SPR) sensor platform, and evaluating enzymatic and non-enzymatic approaches. They consulted stakeholders and adjusted their development plans accordingly. Considering technological readiness and market-readiness, the team chose plasmon-active gold electrodes for a dual-mode biosensor platform. Gold's availability and use in cleanroom technology make it suitable for optical and optoelectronic applications. The team synthesized a Cu-HTTP-based metal-organic framework (MOF) thin film over gold substrates, scaling up to a 4-inch wafer for high-throughput measurements.

After the successful fabrication of the sensor platform, the team also developed original plans for the cartridge mechanism and dedicated electrical and optical readout solution (involving simulations work, additive manufacturing, micromachining, microfluidics, packaging, data-acquisition, and programming tasks) to finally demonstrate a working biosensor platform for creatinine monitoring. In addition, the platform opens up further innovation opportunities integrating array-based imaging SPR and EIS monitoring by micro-patterning, and upgrades the platform for multiplexed monitoring of a panel of biomarkers in near future. In view of all these aspects, I consider the biosensor development plan well thought out and highly original, earning my full recommendation.

Dr. Vivek Pachauri	Juliette T'Kint	Stefan Krehe
		

5. Translation potential

5.1 Business Model Canvas

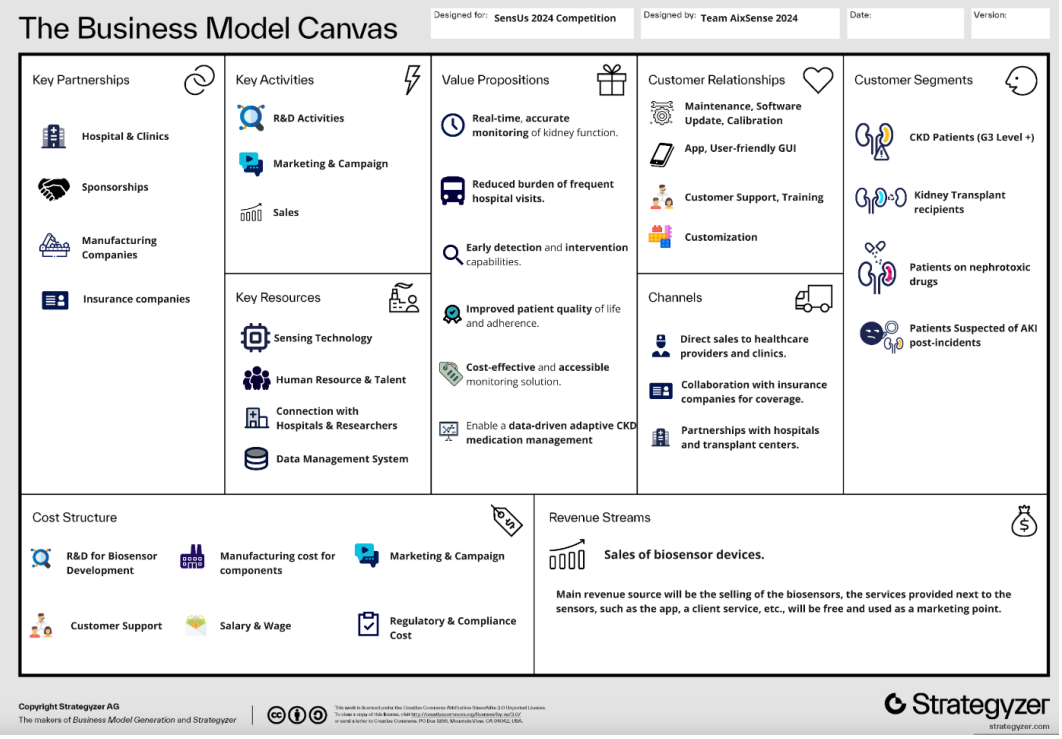


Figure 8: The Business Model Canvas of AixSense

5.2 Stakeholder Desirability

Definition of stakeholders and customers:

Our sensor is primarily designed for CKD patients (G3+ levels), kidney transplant recipients, high-risk groups including nephrotoxic drug patients, and individuals suspected of having AKI. Additionally, we will partner with hospitals and clinics for collaborative research, manufacturing companies for component supplies, and insurance companies to reduce the cost for patients.

Needs and Benefits: According to the guidelines [6], [7], [8], and through numerous interviews with medical professionals, patients with AKI should have their renal function (serum creatinine, GFR) monitored frequently few times a day depending on the severity level while hospitalized, and then at regular intervals (e.g., weekly, monthly) after discharge until stable. CKD patients should be monitored based on the stage of their condition, with G3 patients monitored on average 3 times a year and advanced-stage patients more than 4 times a year. Kidney transplant patients require intensive initial monitoring (daily blood tests for the first few weeks), which becomes less frequent over time depending on their stability. The continuous biosensor adds value through early detection, progression analysis, and prevention. Additionally, monitoring and hospitalization durations are often tailored to individual patient needs, considering factors such as the severity of kidney failure, underlying health conditions, and response to treatment. Personalized treatment can be possible due to its continuous monitoring feature.

Value Proposition:

Feature/Benefit	Non-Enzymatic Biosensor	Conventional Lab Methods
Convenience	Portable, home-based, easy-to-use	Requires frequent lab visits and commute
Real-time Monitoring	Continuous, immediate feedback	Periodic, delayed result
Integration with Technology	Smartphone, data logging, remote access	Manual-record keeping
Early-Detection	Time-series analysis, early prediction for kidney failures	Delayed result, no prediction
Cost-Effectiveness	Competitive price compared to other POC devices	Higher recurring costs (lab visits, tests)
Patient Engagement	Self-monitoring, higher awareness	Less frequent monitoring, lower engagement
Data-driven medication management	Remote data collection to reduce hospital visits and to manage CKD medication adaptively	Limited snapshot results

Table 1: The Value Proposition of AixCrea

Relationship with stakeholders:

At the core, a manufacturer supplies components to AixSense, which in turn partners with healthcare providers and pharmacies. Pharmacies distribute products to patients, whose costs are often covered by insurance companies. Additionally, AixSense directly sells to retailers, and healthcare providers collaborate with insurance companies for patient coverage. These partnerships, distribution channels, and information exchange form a crucial foundation for delivering healthcare products and services to patients. (Appendix 9.3.1)

Rules and Regulations:

AixCrea adheres to key regulations and standards to ensure safety, performance, and market compliance. In the European Union, it complies with the EU MDR 2017/745 [9], requiring CE marking for safety and performance, involving clinical evaluation and post-market surveillance. AixCrea is an in *vivo* sensor, it will be classified as II-B, meaning it will need an annual update for both its Clinical Evaluation Report and Periodic Safety Update Report. (Appendix 9.2.2)

Monetary valuation:

Our monetary valuation is based on several key factors: the anticipated number of patients in the target region (initially Europe), research and development costs, manufacturing expenses, regulatory costs, potential revenue streams, and projected investment. Compared to other point-of-care devices (Appendix 9.4.3), the final creatinine biosensor offers a lower price point, making it a compelling option for our target users. The product's core advantages lie in its convenience and ability to provide continuous monitoring.

5.3 Business Feasibility

Key Resources:

Our team comprises 15 highly motivated members from diverse academic backgrounds, including electrical engineering, material science, and biology. Supported by RWTH Collective Incubator [11] and IWE1 (Institute of Materials in Electrical Engineering 1), we have direct access to state-of-the-art laboratories and cleanroom facilities. To enhance our development efficiency and productivity, we have identified several areas for improvement based on our SWOT analysis (Appendix 9.4.3). To scale our business and accelerate biosensor research and development, we recognize the need for a robust company structure with departments for accounting, business operations, ICT, and human resources. Our strategic aim is to optimize internal processes and organizational structure to support accelerated growth and innovation in biosensor development.

Key Activities:

We are ambitious to introduce AixCrea given the absence of continuous monitoring of creatinine in the market. Our core focus will be on the research and development of AixCrea.

Our market entry strategy involves an initial focus on R&D, with the goal of preparing for clinical trials by 2028 (Fig 1). Beyond in-lab research, we plan to proactively participate in pilot programs both within and outside our university, seeking technical partnerships and fundings. After the clinical trials, we will aim for regulatory approval from the EU's Medical Device Regulation (MDR). Upon obtaining regulatory approval, we will collaborate with our manufacturing partners to commence production and enter the EU market. Our long-term plan includes expanding into the US and Asian markets.

Beside the R&D process, we will create awareness and engagement with patients, we will advertise our product on our website and social media platforms, including Instagram and LinkedIn, with content that emphasizes safety and builds trust among users.

Figure 9: Startup Roadmap of AixSense

Key Partners:

AixSense benefits from a strong partnership with RWTH Aachen University, which provides crucial support, and resources as outlined in the Key Resources section. Beyond our institute, we have established valuable partnerships with Dr. Schäfer from Uniklinikum Aachen Hospital and Dr. Susa from the department of nephrology at Tokyo Medical and Dental University. These collaborations allow us to receive ongoing, iterative advice, ensuring our biosensor development remains aligned with the latest clinical needs and advancements. (Appendix 9.3.3)

As our business expands, we plan to contract with manufacturers to outsource the production process. We will work closely with regulatory authorities to ensure our product complies with relevant standards

and guidelines, guaranteeing the safety and efficacy of our products. Additionally, we will form partnerships with retailers and insurance companies to facilitate the distribution and adoption of our products. Collaborating with retailers will help us reach a broader market, while partnerships with insurance companies will ensure that our products are accessible and covered for patients, enhancing their usability and affordability. (Appendix 9.3.1)

Sustainability:

Our business plan is centered around ESG (Environmental, Social, and Governance) management, integrating these factors into our core operations. To address environmental concerns, we would investigate implementing a recycling and material recovery cycle. Users can exchange old biosensors for new ones, with used devices, particularly microcontrollers, collected for recycling. Customers receive financial incentives to participate, reducing waste and promoting eco-friendly practices. We also explore sustainable materials for products and packaging.

On the social front, we will initiate awareness campaigns about early kidney disease diagnosis through digital platforms and community outreach. By educating the public on early detection benefits, we aim to improve health outcomes and quality of life.

For governance, we will maintain transparency by openly sharing our business practices. Regularly published reports and an independent advisory board will ensure compliance with ethical standards and regulatory requirements, holding us accountable for our commitments.

5.4 Financial Viability

Cost projection

Our product is estimated to have a production cost ranging from €17.43 to €31.58 per sensor. This cost includes material expenses, variable production costs, and labor. These estimations were calculated for two scenarios: low production and mass production (see Appendix 9.4.1).

The development costs are categorized into three main areas: wages, rent, and materials for experiments. Our initial assessment estimates these costs to average[2] €1,512,909 per year of development. To sell our product in the EU, we estimate an initial cost of €1,500,000 for certification and patenting. Additionally, we project average marketing costs to be around €61,200 per year, with other miscellaneous expenses, such as team activities, estimated at €2,400 per year (see Appendix 9.4.2).

Market analysis

In our focused market, Europe, 24 828 people received a kidney transplant in 2018 (Boenink R, 2023). Furthermore, it is estimated that 11,86% of the population has a CKD of stage 3 or higher (Hill NR, 2016). This represents an addressable market size of approximately 44,5 million people when combining both kidney transplant recipients and those with advanced CKD. The number of transplants increases annually (Boenink R, 2023), and many CKD patients remain undiagnosed (Tangri N, 2023), suggesting a continually expanding market. Although our initial target is the European Union, we plan to first establish sales in Germany. Once our product is established, the market could be broadened to include patients at risk of AKI who are not hospitalized and those on nephrotoxic drugs.

Sales Price

[2] Over the 11 years of activity

We plan to sell our biosensor at a unit price of €60. As this type of product is not currently available, we based our pricing on production costs, the price per measurement of existing point-of-care devices (see Appendix 9.4.3), and the cost of continuous glucose monitoring sensors. Since a patient would need approximately 26 sensors per year for continuous monitoring, each customer would generate €1,560 annually. We intend to engage with insurance companies to develop a reimbursement policy and may adjust our pricing to ensure that as many patients as possible can benefit from our sensor.

Revenue stream

Our goal is that after 20 years on the European market, 5% of kidney transplant recipients and 0.3% of CKD patients will be using our sensors. With this target in mind and an estimated 5 years of development, this pricing model enables us to become profitable after 8 years of operation as shown in Figure 10, with an average annual return of 14.47% after 11 years (see Appendix 9.4.4).

To maximize our customer base, the key metric for our success, we will focus on providing excellent customer service, including reliable after-sales support and a convenient smartphone application. With the consent of our customers, we also aim to study their data, specifically the relationship between medication dosage, type, and creatinine levels. This analysis could provide valuable insights for doctors to optimize treatment, potentially reducing the financial burden of CKD for the wider community.

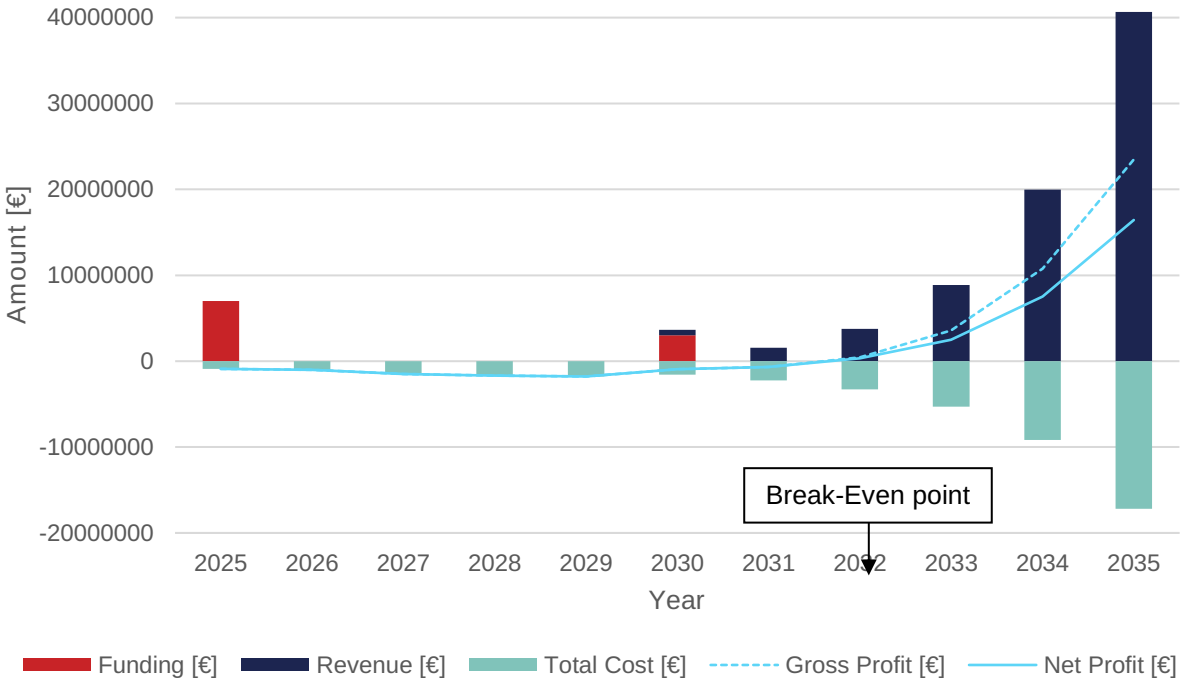


Figure 10: Financial Summary of our company over the coming 11 years

To be successful, a significant investment of around €10 million is required to gain enough market share and become stably profitable. An initial €8 million is needed to ensure a smooth, quick, and efficient development phase, with an additional €2 million required to launch our product effectively. We will initially seek grants from the European Innovation Council Accelerator, the German KMU-innovative, EIT Health Catapult, and other local funding opportunities. However, we are also open to venture capital support to fund our operations.

6. Team and support

6.1. Contribution of Team Members

Juliette T'Kint, Team Captain and the business team leader, responsible for creating the business plan and communication with SensUs as well as contacting doctors and potential investors.

Erdenebat Battseren oversaw the market analysis, built a website and prepared the Instagram takeover.

Yusuke Tsutsumi prepared the business feasibility, and our Instagram campaign *Meet our member*.

Stefan Kreher, Team captain and technical team leader, responsible for the communication with SensUs, coordinated the design of the Sensor and worked on the software coding for the sensor.

Kostadin Eftimov worked extensively on the firmware for the microcontroller and on the GUI.

Amine Baadaka modelled the microfluidic system and coordinated the work on the fluidic system. Muxi Zhang, Seungyeon Kim, Sven Gedamke and Yijun Yan worked on the manufacturing of the microfluidic system and characterized the response of the biosensor.

Yumie Nishiyama developed the functionalization protocol, coordinated the work to manufacture our working electrode and evaluated the performance of the sensor.

Chenyan Feng, Erkai Wang, Reyyan Tağman and Yiyu Lin manufactured the working electrode, handeled the electrochemical aspect of our sensor and characterized the response of the biosensor.

6.2. Individuals who have provided support

Dr. Vivek Pachauri, our supervisor, provided steadfast support throughout the competition, offering continuous feedback and being available when needed.

Dibyendu Khan played a crucial role in overcoming technical challenges, being open to our input and significantly supporting us with his knowledge in various technical aspects. Aidin Nikookhesal managed the background organization, facilitating communication between the Institute and our competition requirements. He also provided valuable advice on business strategies. Both Dibyendu and Aidin assisted with organizational tasks and offered guidance on our team structure.

We also want to thank Huijie Jiang, who supported us in the MOF production and provided us with knowledge for electrochemical measurement. As well as Dr. Divagar Murugan, who gave us advice on biochemistry and on the technical aspects of the SPR. Additionally, Dr. Sota Hirokawa, who measured AFM images. Dr. med. Gideon Schäfer from Uniklinikum Aachen hospital and Dr. Susa & Dr. Sohara from the department of nephrology at Tokyo Medical and Dental University for providing expert insights on renal diseases, the potential use-cases of the biosensor, and various feedback regarding the design of the sensor.

6.3. Sponsors and Partners

We want to thank the IWE1 Institute at RWTH University which supported us with their facility, funded the project and provided mentorship. The Collective Incubator provided us with rooms for meetings on the weekend and gave us insight into pitching. TMDU (Tokyo Medical and Dental University) supported by providing insights on renal diseases, and the potential use-cases of the biosensor.

7. Final Remarks

As we conclude this document and already partially the competition, we want to thank once again our mentors, sponsors, and everyone who has supported us. Their guidance and encouragement made this journey possible. We learned a lot and are extremely grateful to have been part of this. Another big thanks to the organization team of SensUs who made this competition possible and the judges who took their time to listen to our ideas.

It's been an incredible journey, and we're excited to see where our next steps will take us.

8. References

1. Ngamchuea, K., Wannapaiboon, S., Nongkhunsan, P., Hirunsit, P., & Fongkaew, I. (2022). Structural and electrochemical analysis of copper-creatinine complexes: application in creatinine detection. Journal of The Electrochemical Society, 169(2), 020567.
2. Tsuchida, T., & Yoda, K. (1983). Multi-enzyme membrane electrodes for determination of creatinine and creatine in serum. Clinical Chemistry, 29(1), 51-55.
3. Magar, H. S., Hassan, R. Y., & Mulchandani, A. (2021). Electrochemical impedance spectroscopy (EIS): Principles, construction, and biosensing applications. Sensors, 21(19), 6578.
4. Nam, K. W., Park, S. S., Dos Reis, R., Dravid, V. P., Kim, H., Mirkin, C. A., & Stoddart, J. F. (2019). Conductive 2D metal-organic framework for high-performance cathodes in aqueous rechargeable zinc batteries. Nature communications, 10(1), 4948.
5. Randles, J. E. B. (1947). Kinetics of rapid electrode reactions. Discussions of the faraday society, 1, 11-19.
6. Chakraborty, T., Das, M., Lin, C. Y., Su, Y., Yuan, B., & Kao, C. H. (2022). ZIF-8 Nanoparticles based electrochemical sensor for non-enzymatic creatinine detection. Membranes, 12(2), 159.
7. Analog Devices (n.d.) ADUCM350 https://www.analog.com/en/products/aducm350.html
8. Kidney Disease: Improving Global Outcomes (KDIGO). (2012). KDIGO clinical practice guideline for acute kidney injury. Kidney International Supplements, 2(1), 1-138. Retrieved from https://kdigo.org/guidelines/acute-kidney-injury/
9. International Society of Nephrology. (2019). Global Kidney Health Atlas. Retrieved from https://www.theisn.org/focus/education/guidelines/
10. KDIGO 2024 CLINICAL PRACTICE GUIDELINE FOR THE EVALUATION AND MANAGEMENT OF CHRONIC KIDNEY DISEASE https://www.kidney-international.org/action/showPdf?pii=S0085-2538%2823%2900766-4
11. European Union (2021, October). MDCG 2021-24 rev. 0. [Medical Devices Coordination Group]. Retrieved from https://health.ec.europa.eu/system/files/2021-10/mdcg_2021-24_en_0.pdf
12. Boenink R, K. A.-O. (2023). Trends in kidney transplantation rate across Europe: study from the ERA Registry. Nephrol Dial Transplant, 1528-1539. doi:10.1093/ndt/gfac333
13. CP., K. (2011). Epidemiology of chronic kidney disease: an update 2022. Kidney Int Suppl, 7-11. doi:10.1016/j.kisu.2021.11.003
14. Hill NR, F. S. (2016). Global Prevalence of Chronic Kidney Disease – A Systematic Review and Meta-Analysis. PLoS ONE. doi:10.1371/journal.pone.0158765
15. Kellum, J. R. (2021). Acute kidney injury. Nat Rev Dis Primers(7). doi:10.1038/s41572-021-00284-z
16. Tangri N, M. T. (2023). Prevalence of undiagnosed stage 3 chronic kidney disease in France, Germany, Italy, Japan and the USA: results from the multinational observational REVEAL-CKD study. BMJ Open. doi:0.1136/bmjopen-2022-067386. PMID: 37217263
17. Collective Incubator. (n.d.). Home. https://www.collective-incubator.de/
18. Market Research Future. (2022). Renal Disease Market by Type, Size, Growth and Forecast – 2032 [Report]. Retrieved from https://www.marketresearchfuture.com/reports/renal-disease-market-3167
19. Forbes, T. P., & Kralj, J. G. (2012). Engineering and analysis of surface interactions in a microfluidic herringbone micromixer. Biochemical Science Division, National Institute of Standards and Technology, Gaithersburg, MD, USA.

9. Appendix

9.1 Biosensor System

9.1.1 Enzymatic functionalization

We first tried a well-known reaction of creatinine, an enzymatic cascade reaction (Tsuchida, 1983, Figure 11), which was employed as the molecular sensing part. Creatininase, creatinase, and sarcosine oxidase were covalently bound to the surface of the gold wafer by polyethylene glycol with a functional group of thiol on one end and aldehyde on the other end (SH-PEG). We expected that these reactions would result in producing hydrogen peroxide, which would allow us to detect hydrogen peroxide concentrations by using amperometric measurement.

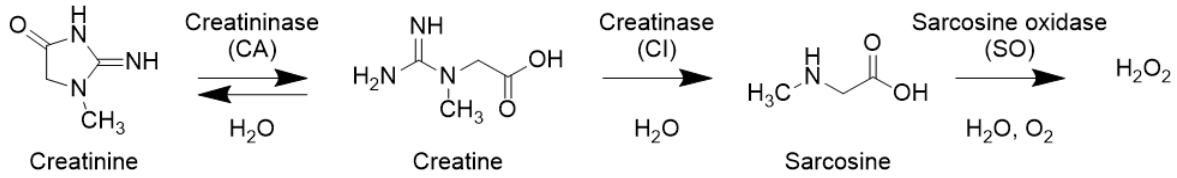


Figure 11: Creatinine enzymatic cascade reaction

Amperometric measurement of the enzymatic reactions detects the current from the oxidation or reduction of an electroactive species. In creatinine detection, the enzymatic reaction produces hydrogen peroxide. When a specific voltage is applied, hydrogen peroxide is known to be reduced to water at the electrode surface, generating a proportional current to the creatinine concentration. Various methods, including covalent binding on PEDOT:PSS and SH-PEG were employed to immobilize the enzymes onto the electrode surface. Despite various optimization of these functionalization techniques, our efforts to achieve stable and efficient enzyme immobilization were ultimately unsuccessful.

However, subsequent experiments demonstrated that the enzymatic reaction of interest was reproducible and worked if not functionalized. This suggests that further refinement of the immobilization process is necessary to achieve effective integration with the electrode for practical applications.

9.1.2 Randles model

The Randles model was proposed by Randles in 1947, which describes the behavior of an electrical double layer. The total impedance of the model is described as follows.

$$Z_{\text{total}} = R_{\text{s}} + \frac{1}{j\omega C_{\text{dl}} + \frac{1}{\left(R_{\text{ct}} + \frac{A}{(j\omega)^{\alpha}}\right)}}$$

Where ω is frequency, R_s is the electrolyte resistance, C_{dl} is capacitance, R_{ct} is charge transfer resistance, and A and α are constant. When the frequency approximates infinity, the real part of Z_{total} is equal to (R_s + R_{ct}). If we assume that R_s is constant in any concentration of creatinine, Z_{total} equals R_{ct}, therefore, creatinine concentrations are calculated by the intersections of the EIS signal and X-axis.

9.1.3 Graphical User Interface

The GUI features three tabs for streamlined user interaction. The first tab, *Measurement*, allows users to select a measurement principle from a menu and display the real-time data in a graph. The second tab, *Config*, provides fields and sliders for adjusting specific parameters like applied voltage, measurement time, and frequency, tailored to the selected principle. The final tab, *Help*, contains general guidance and tips, providing context and help for an optimal use of the GUI in combination with the µC. While the GUI is designed to be intuitive, we provide a step-by-step example below for performing an electrochemical impedance spectroscopy (EIS) measurement.

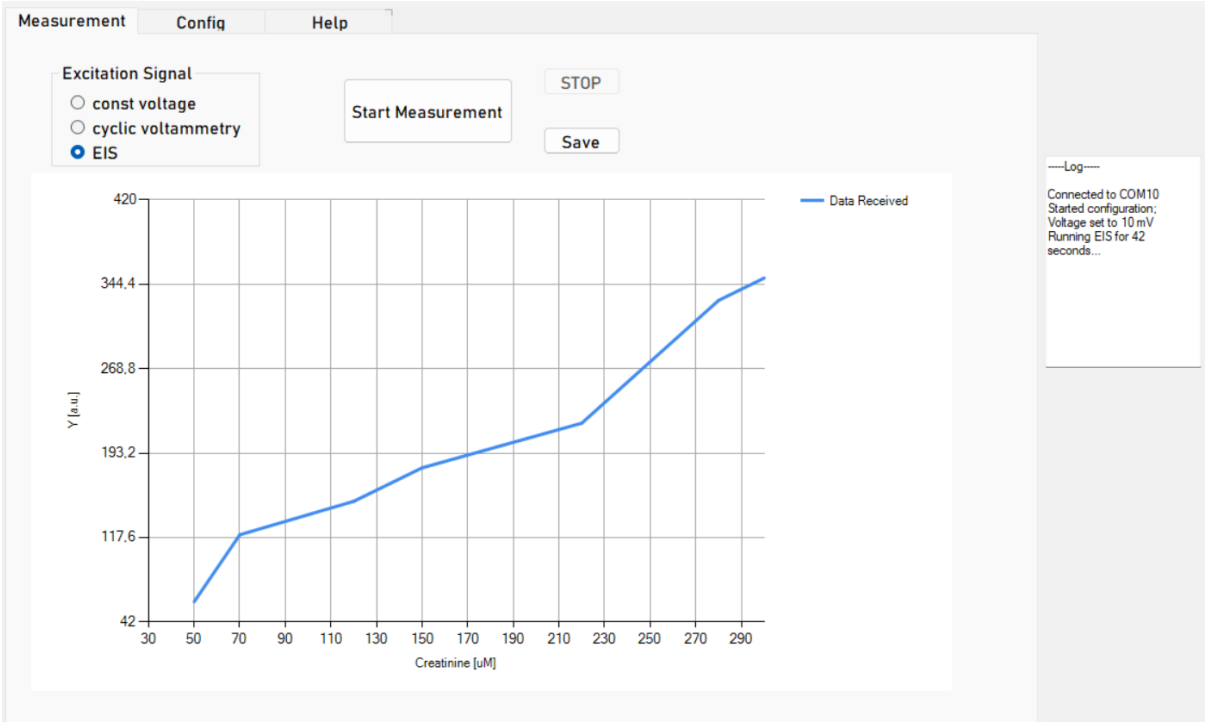


Figure 12: The Measurement Tab of the GUI displays the different measurement methods and the measuring graph

Starting an EIS measurement:

Step 1: switch to configuration tab

Step 2: choose the right port for connecting with the microcontroller and set the desired path for the measurement results

Step 3: set the desired parameters in the corresponding input fields and click ‘Change parameters’

Step 4: switch to measurement tab

Final step: select EIS and click ‘Start measurement’ !

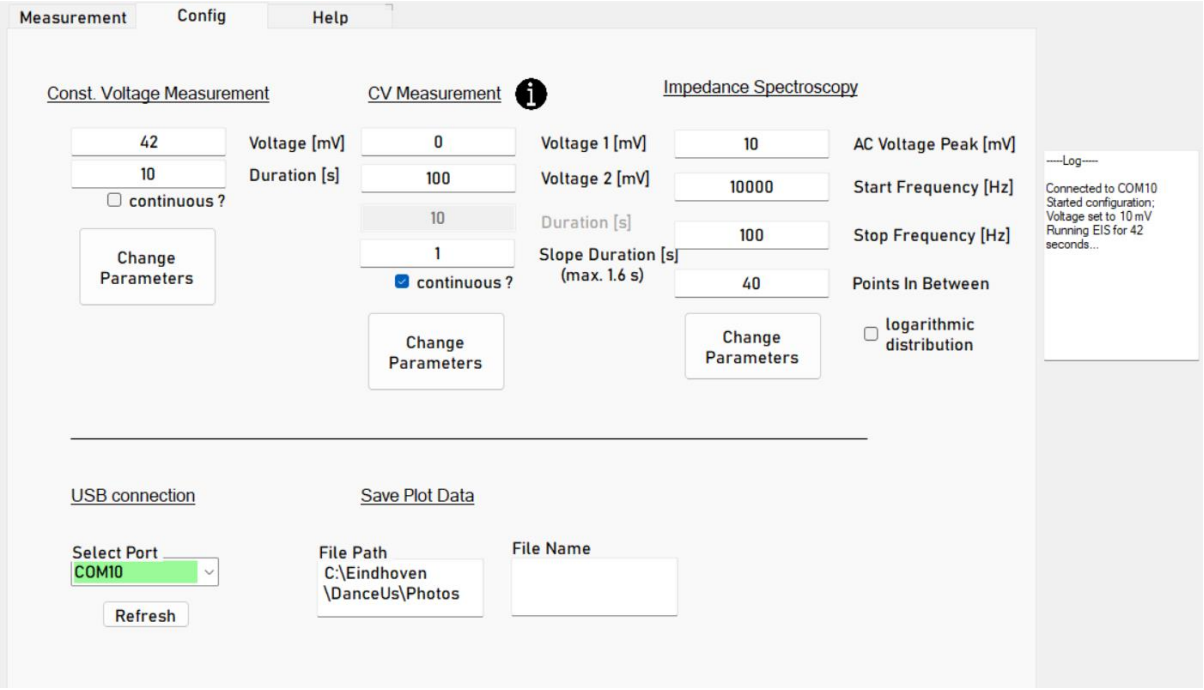


Figure 13: Configuration Tab, used for setting the measurement parameters, communication port with the µC, and the save location of measurement data

9.2 Stakeholder Relationships

9.2.1 Patient Journey Map

Patient Group	Stage	Timeframe	Key Steps
AKI	Initial Stage	0-7 days	Diagnosis: Serum creatinine, urine output; Immediate Treatment: Fluid management, stop nephrotoxic drugs, dialysis if necessary; Monitoring: Daily renal function; Hospitalization: Likely required
	Early Recovery	1-4 weeks	Ongoing Monitoring: Every 2-3 days; Treatment Adjustment: Gradual change in medications;
	Long-term Recovery	1-3 months	Regular Follow-up: Weekly to bi-weekly; Rehabilitation: Nutritional support, physical therapy; Preventive Measures: Lifestyle modifications
	Stable Phase	3-6 months	Periodic Monitoring: Monthly; Management of Comorbidities: Control hypertension, diabetes
CKD	Early Stage	0-1 year	Diagnosis: GFR, albuminuria; Initial Treatment: Lifestyle changes, BP and glucose control; Monitoring: Every 3-6 months
	Moderate Stage	1-5 years	Ongoing Treatment: Medication adjustments, dietary restrictions; Monitoring: Every 1-3 months; Patient Education: Adherence to treatment
	Advanced Stage	5+ years	Intensive Management: Preparing for dialysis/transplant; Frequent Monitoring: Monthly or bi-monthly; Supportive Care: Psychosocial support
After Kidney Transplant	Immediate post-surgery	0-1 month	Hospitalization: 5-10 days; Intensive Monitoring: Daily blood tests
	Early Recovery	1-3 months	Frequent Follow-up: Weekly visits; Patient Education: Medication adherence, signs of complications
	Mid-term Recovery	3-12 months	Regular Monitoring: Bi-weekly to monthly; Lifestyle Management: Diet, exercise, infection prevention
	Long-term Care	1+ year	Stable Phase: Quarterly to bi-annual check-ups; Chronic Care: Management of comorbidities, continuous medication adjustment
On Medications Affecting Kidney Function	Initial Stage	0-1 month	Risk Assessment: Identify high-risk patients, baseline tests; Patient Education: Inform about side effects
	Ongoing Monitoring	1-12 months	Regular Follow-up: Bi-weekly to monthly tests; Adjustments: Modify dosages, switch medications if needed
	Long-term	1+ year	Periodic Monitoring: Every 3-6 months; Preventive

	Monitoring		Measures: Encourage hydration, avoid nephrotoxins
	End-of-Treatment Follow-up	Varies	Post-Medication Monitoring: Continued monitoring; Rehabilitation: Address residual effects, dietary, lifestyle advice

Frequency of monitoring glomerular filtration rate (GFR) and albuminuria in people with chronic kidney disease (CKD).

				Albuminuria categories Description and range		
				A1	**A2**	**A3**
CKD is classified based on: **• Cause (C)** **• GFR (G)** **• Albuminuria (A)**				Normal to mildly increased	Moderately increased	Severely increased
				<30 mg/g <3 mg/mmol	30–299 mg/g 3–29 mg/mmol	≥300 mg/g ≥30 mg/mmol
GFR categories (ml/min/1.73 m²) Description and range	**G1**	Normal or high	≥90	Screen 1	Treat 1	Treat 3
	G2	Mildly decreased	60–89	Screen 1	Treat 1	Treat 3
	G3a	Mildly to moderately decreased	45–59	Treat 1	Treat 2	Treat 3
	G3b	Moderately to severely decreased	30–44	Treat 2	Treat 3	Treat 3
	G4	Severely decreased	15–29	Treat* 3	Treat* 3	Treat 4+
	G5	Kidney failure	<15	Treat 4+	Treat 4+	Treat 4+

Low risk (if no other markers of kidney disease, no CKD)
Moderately increased risk
High risk
Very high risk

9.2.2 Rules and Regulations

MDR (Medical Device Regulation) Classification:

- Short-term Use: Normally intended for continuous use for between 60 minutes and 30 days.
- Invasive Device: Any device which, in whole or in part, penetrates inside the body
- Active device intended for monitoring: detecting, diagnosing, monitoring or treating physiological conditions

MDR Rules:

- Rule 5 Class II-A: intended for short-term use,
- Rule 9 Class II-B: All active devices intended to control or monitor the performance of active therapeutic class IIb devices, or intended directly to influence the performance of such devices are classified as class IIb.
- Rule 11 Software Class II-A: Intended to provide information used to take decisions with diagnosis or therapeutic purposes.

International Standards (First identified – Approvals will be taken based on market expansion plan)

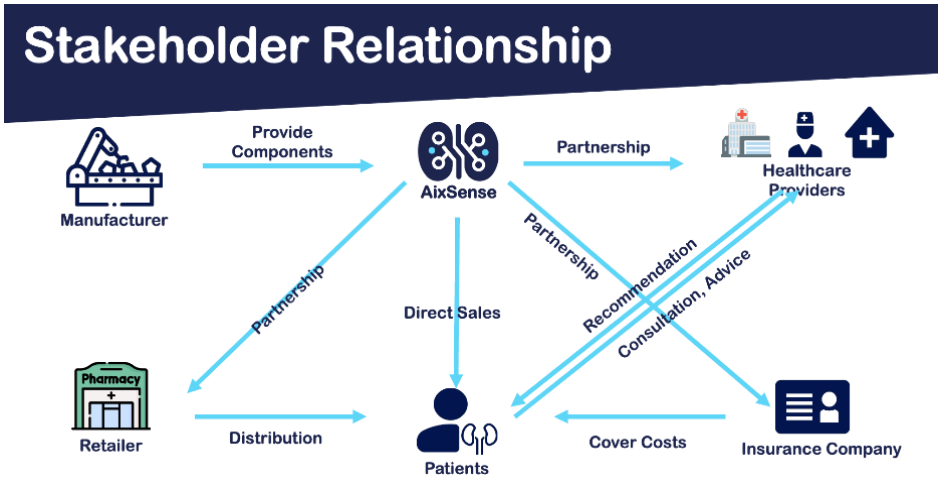
- ISO 13485: Quality management system (QMS) for medical devices.
- ISO 14971: Risk management for medical devices. Processes for identifying, evaluating, and controlling risks associated with medical devices throughout their lifecycle.
- ISO 10993: Biological evaluation of medical devices, ensuring biocompatibility.

IEC Standards

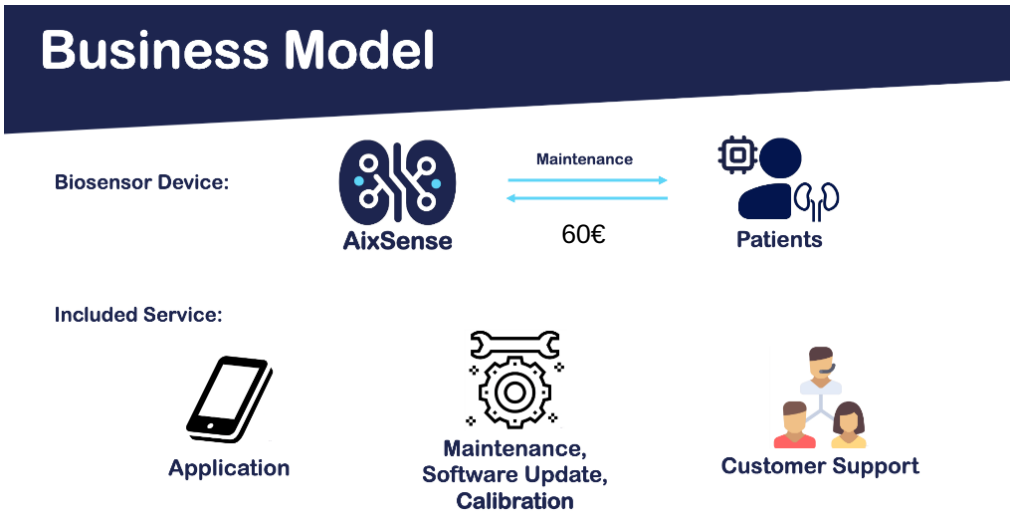
- IEC 60601: Standards for the safety and performance of electrical medical equipment. Relevant parts of this standard ensure the electrical safety of biosensors.
- IEC 62304: Establishes requirements for the life cycle processes for medical device software, including biosensor software.
- ISO 9241: ensures ergonomics and human-centered design processes.

9.3 Business Model & Start-up Roadmap

9.3.1 Stakeholder Relationship



9.3.2 Business Model



9.3.3 Partnerships

IWE 1 Institut für Werkstoffe der Elektrotechnik 1

UNIKLINIK RWTH AACHEN

collective incubator

TOKYO MEDICAL AND DENTAL UNIVERSITY TMDU

9.3.4 SWOT analysis

Strengths

- Diverse Expertise: The team comprises members with varied expertise in chemistry, electronics, optics, programming, and business, fostering comprehensive development of the

biosensor.

- International Backgrounds: The team's diverse national backgrounds enhance understanding of regional properties, aiding global market entry.
- Established Partnerships: The team has established partnerships with doctors from Germany and Japan.
- University Support: Full backing from RWTH Aachen University provides both developmental and financial support.
- Motivated Team: The team's high motivation and eagerness to improve healthcare contribute to a positive and energetic working environment, crucial for the biosensor's development.

Weaknesses

- Startup Experience: Most team members lack startup experience, which may present challenges.
- Development Stage: The biosensor is still in development and far from the ideal concept.
- Need for a robust company structure: Departments for accounting, business operations, ICT, and human resources are needed in the future
- Limited Resources: The team is relatively small and has limited resources compared to competing biotech companies or startups.
- Market Competition: Point-of-care biosensors currently dominate the market, posing strong competition.

Opportunities

- Increasing Demand: The need for continuous creatinine monitoring is rising.
- Market Gap: There are no existing commercial continuous creatinine monitoring biosensors in the market.
- Integration Potential: The biosensor has the potential to be integrated into wearable devices.
- Sustainability: The biosensor can be improved through sustainable materials and production processes.
- Public Awareness: The company can promote the importance of early diagnosis for kidney disease, fostering empathy and public relationships.

Threats

- Competitive Methods: Point-of-care detection methods are currently sufficient and affordable, potentially limiting demand for continuous monitoring.
- Complexity: The complex sensing method may present challenges during development and research.
- Conservative Adoption: Doctors may be conservative towards new treatment methods,

preferring proven techniques unless new methods are 100% reliable and accurate.

- Resource Dependence: The team relies on RWTH Aachen's materials and facilities, which could lead to high costs and material shortages. Future cost reduction and material availability will require finding original manufacturers.

9.4 Financial Statements

9.4.1 Details of the sensor cost

	Low production [€]	Mass-production [€]
Material		
Stainless steel needle	1,4000	0,5000
Gold	0,0016	0,0016
Cu(OAc)2	0,0011	0,0008
HHTP	0,0017	0,0014
Ethanol	0,3342	0,0334
Membrane	0,0512	0,0128
µController	18,6000	13,6400
Housing	2,0000	0,5000
NFC Chip	1,4000	0,8000
Production		
Oven process	0,030	0,003
Labor cost	7,756	1,939
Total sum	31,576	17,432

Figure 14: Material and Production Price per Sensor

9.4.2 Cost Analysis

Year	R&D Cost [€]	Employee number	Wages [€]	Rent [€]	Miscelleneous [€]	Cerification Cost [€]	Cost of Goods Sold [€]	Marketing Expenses [€]	Total Cost [€]
2025	100000	15	780000	10000	1000	0	0	2217	893217
2026	200000	15	780000	10000	1000	0	0	4397	995397
2027	200000	15	780000	10000	1000	500000	0	8599	1499599
2028	200000	18	936000	10000	1000	500000	0	16365	1663365
2029	200000	20	1040000	10000	1000	500000	0	29673	1780673
2030	10000	23	1196000	30000	1000	80000	220816	49772	1587588
2031	10000	23	1495000	30000	4000	80000	538986	75000	2232986
2032	10000	27	1755000	30000	4000	80000	1304771	100229	3284000
2033	10000	30	1950000	50000	4000	80000	3089245	120328	5303573
2034	10000	30	1950000	50000	4000	80000	6958416	133636	9186052
2035	10000	35	2730000	50000	4000	80000	14178982	141402	17194384

Figure 15: Description of our cost structure over 11 years

The R&D cost includes material needed for our experiment, external analysis, budget for buying equipment, etc.

9.4.3 Competitor Analysis

- Creatinine POC Biosensor

Criteria	(1) StatSensor & Xpress	(2) iSTAT System (CREA Cartridge)	(3) Evidence MultiSTAT
Technology	Point-of-Care Creatinine Testing	Point-of-Care Creatinine Testing	Point-of-Care Creatinine Testing

	(Electrochemistry)	(Electrochemistry)	(Electrochemistry)
Sample types	1.2 μL blood	65 μL blood	Blood, Urine, Oral fluod 200μL
Parameters Tested	Creatinine and eGFR	Depends on the cartridge	Depends on the cartridge
Results Speed	Results in 30 seconds	Results in 2 minutes	17 minutes
Automation	Fully automated	Portable, requires minimal user interaction	Simple and direct without the need for calibration or configuration
Usability	● Portability ● Rapid results	● Versatility ● Connectivity	● Broad application ● Multiplex testing
Applications	● Clinic / Hospital ● Emergency department ● ICU	● Clinic / Hospital ● Emergency department ● ICU	● Clinic / Hospital ● Emergency department ● Drug testing
Portability	266g	650g	48kg
Cost	€680 + €6.3(per strips)	US$ 4,950＋€12.4(per test)	€45,000

(1) StatSensor® & StatSensor Xpress™

Quick and Simple Testing: Uses single-use biosensor test strips for creatinine and eGFR measurement, providing results in 30 seconds from a 1.2μl blood sample.

Ease of Use: Designed for any healthcare professional at the point of care, allowing rapid and easy assessment of kidney function.

Applications: Useful in radiology and cardiac catheterization to prevent risks from contrast media, supports immediate renal function screening which aids in safe imaging procedures.

Operational Efficiency: Avoids the need for time-consuming central lab tests, facilitating immediate on-site screening.

Enhanced Patient Care: Enables quick renal function assessment before chemotherapy in oncology, improving safety and treatment efficacy.

Emergency Use: Allows quick renal function tests in emergency settings, supporting swift and accurate triage and treatment

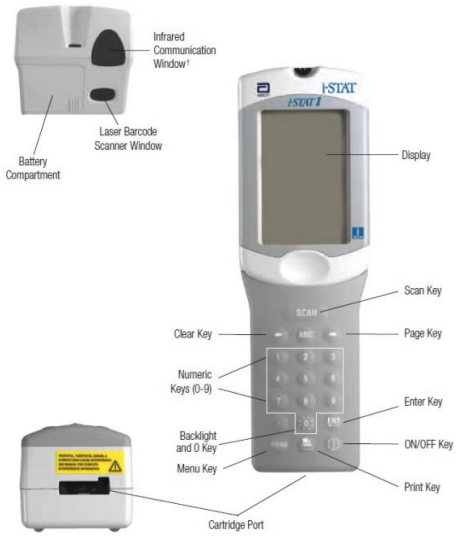
decisions.

Source:

https://nsiframe.ggwebcast.com/smlps/production/1711/collateral/StatSensor%20Creatinine%20Brochure.pdf

(2) iSTAT System

Versatile Testing Menu: Allows for a wide range of tests using disposable i-STAT test cartridges on a portable platform.



Ease of Use: Simple four-step testing process requiring only a few drops of whole blood.

Rapid Lab-Quality Results: Most tests, including blood gases and electrolytes, deliver accurate results within about 2 minutes.

High Portability: Facilitates bedside testing to streamline processes and reduce errors.

Connectivity: Links with Abbott Info HQ® data management system to integrate test results with lab systems and electronic health records.

Source:

https://www.globalpointofcare.abbott/jp/ja/product-details/apoc/i-stat-system.html

https://www.ruh.nhs.uk/pathology/documents/poct/SOP_Abbot_iSTAT_user_guide.pdf

(3) Evidence MultiSTAT

Mainly for drug tests:

Uses Biochip Array Technology (BAT) for simultaneous multi-analyte testing.

Capable of testing up to 21 different drugs from a single sample.

Testing Matrices:

Compatible with multiple sample types including blood, urine, and oral fluid.

Specific kits designed for optimal performance with each sample type.

Applications:

Broad application range including emergency rooms, workplaces, rehabilitation centers, mining industry, and more.

Used in forensic contexts, such as prisons and anti-doping for sports.

Analytical Process:

Simple loading of reagent and tip cartridges.

Sample and conjugate are dispensed into the Biochip well, which is then incubated and imaged to measure chemiluminescence.

Device Features:

Touch screen interface for easy navigation.

Connects to peripherals such as barcode scanners and printers.

Data export functionality and connectivity with Laboratory Information Management Systems (LIMS).

Source:

https://www.randox.com/wp-content/uploads/delightful-downloads/2019/06/LT650TOX-MultiSTAT-JAN19.pdf

9.4.4 Financial Statement

Year	Number of custumer	Unit Sold	Total Cost [€]	Funding [€]	Revenue [€]	Gross Profit [€]	Net Profit [€]	Cash Balance [€]	Cumulative profit [€]
2025	0	0	893217	7000000	0	-893217	-893217	6106783	-893217
2026	0	0	995397	0	0	-995397	-995397	5111386	-1888614
2027	0	0	1499599	0	0	-1499599	-1499599	3611787	-2494996
2028	0	0	1663365	0	0	-1663365	-1663365	1948422	-3162964
2029	0	0	1780673	0	0	-1780673	-1780673	167749	-3444038
2030	406	10556	1587588	3000000	633360	-954228	-954228	2213521	-2734901
2031	991	25766	2232986	0	1545960	-687026	-687026	1526495	-1641254
2032	2399	62374	3284000	0	3742440	458440	320908	1847403	-366118
2033	5680	147680	5303573	0	8860800	3557227	2490059	4337462	2810967
2034	12794	332644	9186052	0	19958640	10772588	7540812	11878274	10030871
2035	26070	677820	17194384	0	40669200	23474816	16432371	28310645	23973183

Figure 16: Assumed financial statement for the coming 11 years

For calculating the average annual return, this formula is use:

$$Average\ annual\ return = \left(1 + \frac{Cumulative\ profit}{Investement\ volume}\right)^{\frac{1}{number\ of\ years}} - 1$$

For our sensor, this value is 14,47% after 11 years.