Team Results Document

PULSe

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SensUs

KU LEUVEN

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1. Summary (Abstract)

The Point-of-Care University of Leuven SensUs team (PULSe) is representing KU Leuven at the SensUs 2024 competition. We have successfully developed a continuous biosensor for robust and precise creatinine monitoring in interstitial skin fluid (ISF) for patients with acute kidney injury (AKI). Our aim is to enable remote patient monitoring and facilitate communication between patients and doctors through a user-friendly app, this way building a supportive community for patients, specialists, nutritionists, and psychologists.

Our sensor employs electrochemical methods with screen-printed electrodes (SPEs) for rapid response. These SPEs are integrated into a microfluidic chip with a flow chamber and pump, enabling seamless sample transitions with minimal interchange between consecutive samples. Functionalized with Nafion and polyaniline (PANI), and immobilised with creatinine deiminase via glutaraldehyde, the sensor uses open circuit potentiometry (OCP) to logarithmically measure creatinine concentrations. The compact device connects to the CreaView smartphone app via Bluetooth, communicating data with specialists.

The CreaTrack device aims to bridge the gap between patients and specialists by enabling continuous creatinine monitoring for acute kidney injury (AKI) and chronic kidney disease (CKD). Through our business model, we plan to commercialise the sensor, offering enhanced measurement quality and improving the healthcare of tomorrow.

2. Biosensor

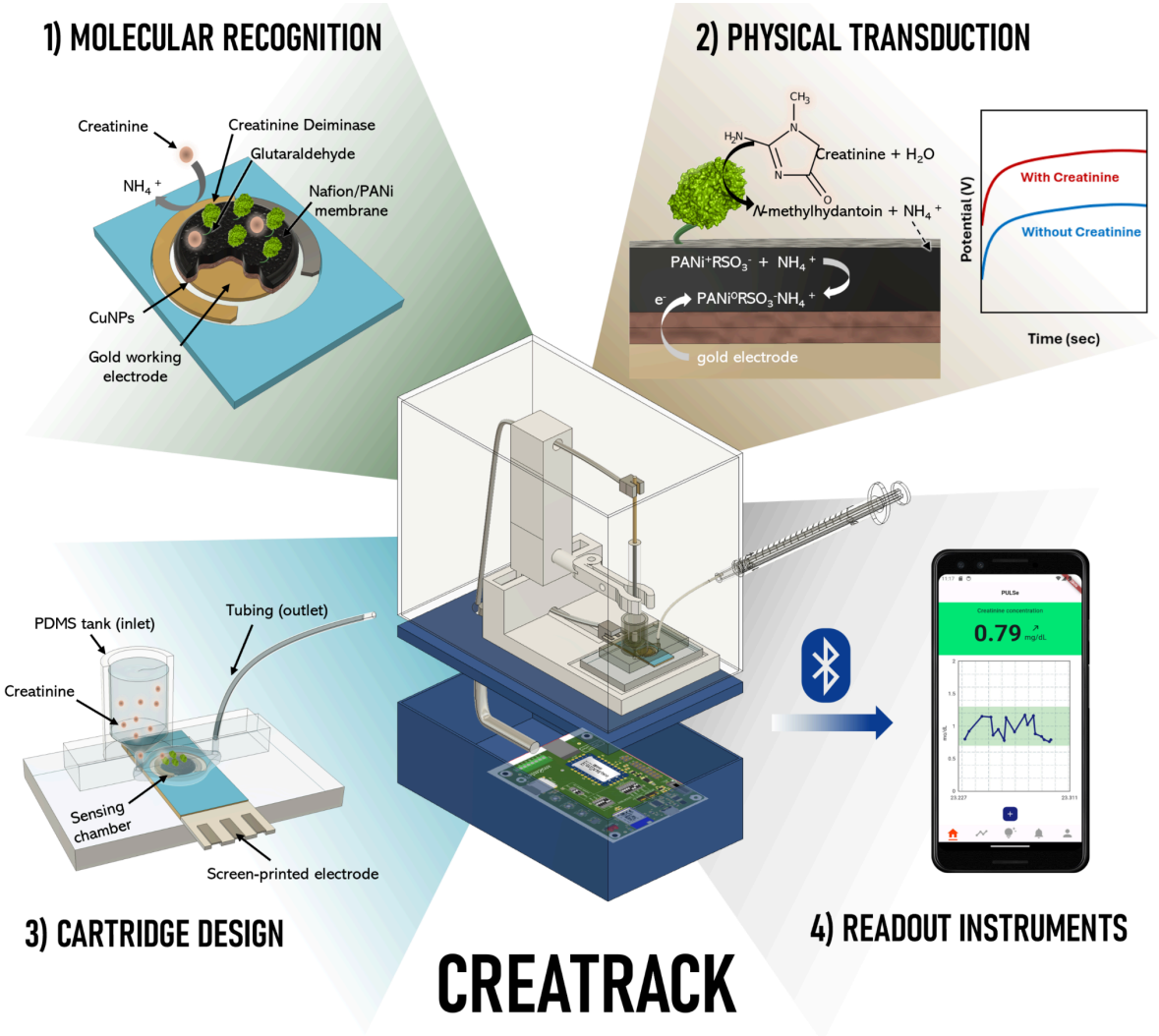

Figure 1. CreaTrack biosensor concept: (A) molecular recognition, (B) physical transduction, (C) cartridge technology, and (D) reader instrument.

2.1. Molecular recognition

Continuous creatinine detection in ISF was achieved by combining an enzymatic reaction, an ion-selective membrane, and an electrochemical readout. In CreaTrack, a single-enzyme system based on Creatinine Deiminase (CD) was chosen over other three-enzyme cascade systems to avoid complicated co-optimization of multiple enzymes with different intrinsic working condition (Pundir et al., 2019), (Killard & Smyth, 2000). In this system, ammonium is generated as a byproduct of creatinine deiminase reaction, which could be electrochemically detected via a membrane made of Nafion - an ionophore, and polyaniline (PANi) - a conductive polymer matrix (Figure 1(A)). Moreover, copper nanoparticles (CuNPs) were deposited to enhance the charge transfer rate between the gold screen-printed electrode (Au SPE) and the ammonium-selective membrane (Zhybak et al., 2016). The enzyme was covalently immobilised on the Nafion/PANi layer using glutaraldehyde as a linker molecule.

2.2. Physical transduction

In CreaTrack, potentiometry was chosen to transduce the enzyme-target interaction. This technique records the potential difference between the working and the reference electrode in thermal equilibrium condition, enabling low power consumption (Gonzalez-Gallardo et al., 2022). Furthermore, ion-selective membranes in potentiometry provide a wide detection range while being reagent-less, making them suitable for continuous sensors (Bakirhan et al., 2017). The signal is proportional to the logarithm of the target molecule concentration, following the Nernst equation (Bakker & Pretsch, 2005). The transduction mechanism is explained in Figure 1(B). If creatinine is present, the enzyme converts it into

ammonium ions. Subsequently, these ions selectively pass through the PANi-Nafion membrane, where electric charges are exchanged and accumulated to the electrode (Bobacka, 2006). As a result, an increase in electrode potential can be measured via potentiometry.

2.3. Cartridge Technology

The integrated fluidic channel is shown in Figure 2(a). The system consists of two main parts - a 50 µL PDMS sensing chamber and a 150 µL PDMS sampling reservoir where creatinine samples can be added. The Ag/AgCl reference electrode is held within the same tank by a 3D printed holder made of Liqcreate Stone Coal Black resin. A tubing is injected at the outlet, which is connected to a syringe-suction pump from CETONI Nemesys. The electrodes are connected to the EmStat Pico potentiostat.
Continuous measurement is done via sample-pipetting followed by fluid suction as indicated in Figure 2(b). In the first step, 100 µL of sample (blue) is injected to the PDMS reservoir. Then, the sample is pulled by the syringe pump to fill the chamber in the second step. All measurements are made while pausing the pump for 180 seconds. Subsequently, in the third step, the next sample (yellow) is pipetted to the tank. By pipetting, bubble formation between the two samples could be avoided. Finally, high-speed suction at 500 µL/min for 5 seconds is done to remove the old sample (blue) and introduce new sample (yellow) to the chamber. This sampling process allows minimal mixing among successive samples.

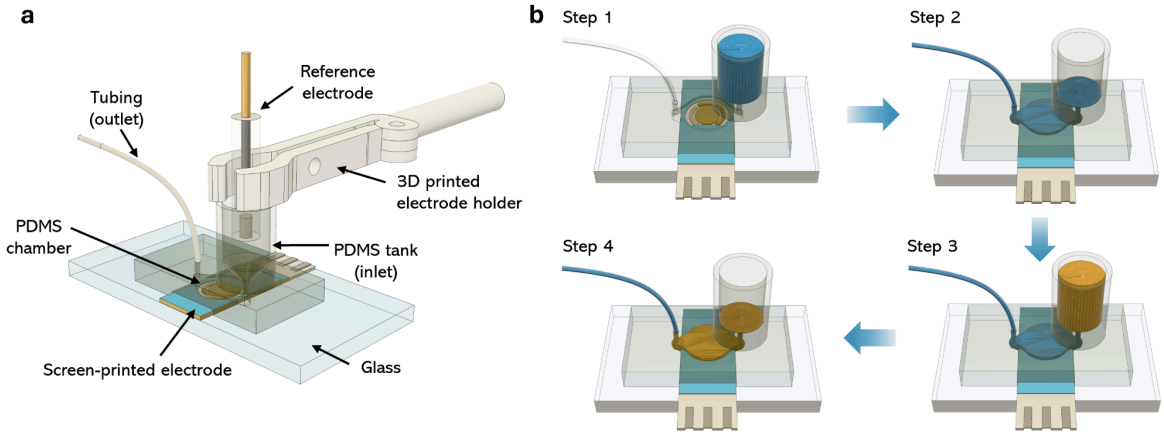

Figure 2: (a) Fluidic biosensor chip. (b) Continuous sampling process.

2.4. Reader Instrument and User Interaction

CreaTrack is 42 cm x 20 cm x 10 cm in size, including the CETONI Nemesys syringe-pump (Figure 1(D)). Potentiometric measurements are carried out by the PalmSens EmStat Pico. The data is transmitted via Bluetooth to an Android app, which converts the potential difference to creatinine concentration and presents it to the user in a graphical format.

Upon launching the app, users are presented with a login page. Once logged in, they can connect to the sensor via Bluetooth. During the initial cartridge loading, the user scans a QR code that will load the calibration curve of the cartridge into the app. The user can then view their creatinine variations. The app also creates a notification when the user's creatinine level spikes. Additionally, users can access their old readings and a lifestyle page that offers diet and exercise recommendations. Detailed screenshots of the app are provided in Figure 5.

CreaTrack has an outstanding potential to become a wearable sensor. In the future, it is planned to miniaturise the device by implementing hollow microneedles to extract the ISF passively, which eliminates the need for a pump. Additionally, a customised SPE will be developed with good reference electrode characteristics which will allow the omission of the bulky reference electrode. Additionally, CreaView can be easily integrable as a third party application into popular health trackers like the Fitbit.

3. Technological Feasibility

3.1. Bioassay

Cu/Nafion/PANi and Cu/Nafion/PANi/GA/CD-functionalized gold SPEs were tested for ammonium and creatinine detection, respectively. The equivalent ammonium concentration is 0.1 µM for 0.3 µM of creatinine, according to Michaelis-Menten kinetics. The Nafion/PANi membrane showed a Nernstian response, and a sensitivity of 34 mV/dec ammonium was observed (Figure 3(a)). After CD immobilisation, a sensitivity of 86 mV/dec creatinine is observed (Figure 3(b)), which is higher compared to the obtained sensitivity of 54.1 ± 0.6 mV/dec from a similar creatinine sensor in urine (Guinovart et al., 2017). Moreover, the sensor exhibited a decent response in the physiological range.

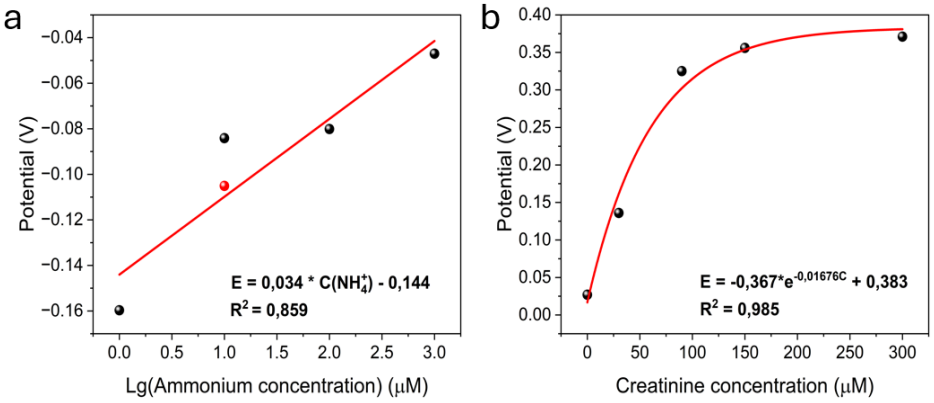

Figure 3: Calibration curves of (a) ammonium detection with Cu/Nafion/PANi modified SPE and (b) creatinine detection with Cu/Nafion/PANi/GA/CD modified SPE.

3.2. Cartridge Technology

CV measurement from -0.3 V to 0.7 V at the scan rate of 50 mV/s in ferrocyanide 10 mM and NaCl 0.1 M inside and outside the chamber was conducted outside and inside the chamber (Figure 4(a) and (b)). The characteristic curves from two set-ups match nicely as shown in Figure 4(c), indicating a successful integration. Furthermore, a continuous sampling experiment was done with blue and yellow dyes (Figure 4(d - f)) following the aforementioned protocol in Section 2.3. Insignificant mixing of the two samples and no air bubbles were observed.

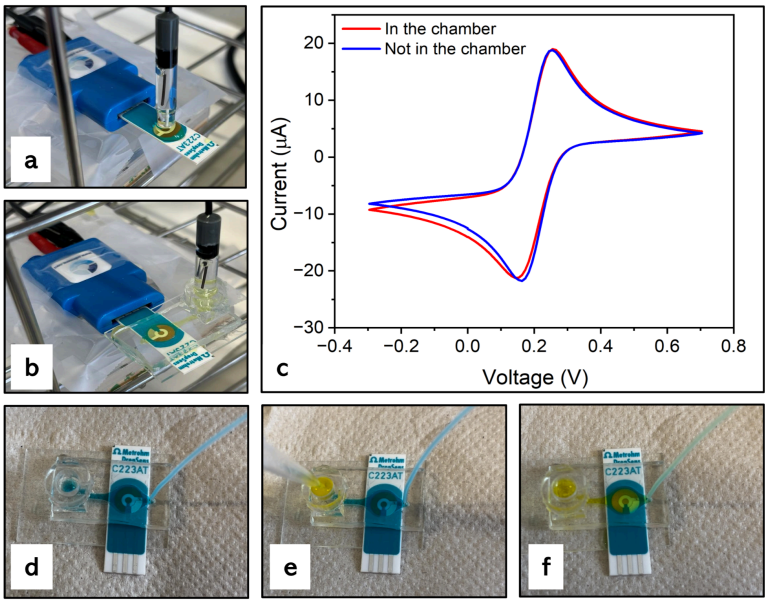

Figure 4: Electrochemical set-up of the SPE in (a) outside the chamber and (b) inside the chamber. (c) Characteristic CV curves of ferrocyanide in the two set-ups. (d - f) Continuous sampling following the process described in Figure 2(b) shows minimal mixing and no air bubbles.

3.3. Possible Improvements

Though CreaTrack provides adequate response to creatinine at physiological range, its reproducibility and stability over time could be improved. The current CreaTrack exhibits a baseline drift of 1 mV/min, which is expected to be due to a water layer that hampers electron transport between the Nafion/PANi membrane and the Au SPE. A possible solution could be introducing a hydrophobic functionalization, such as carbon nanotubes (CNTs), to block water penetration into this interface. Moreover, CreaTrack's signal could be improved by replacing the current one-junction reference electrode with a double-junction electrode. The latter reference electrode could provide higher potential stability, hence, providing a more accurate signal in potentiometry and hence, resulting in a more reproducible signal among electrodes. In addition, CreaTrack lifetime is limited mainly due to its enzyme stability. A better storage condition would be required to maintain the enzyme functionality for a longer span. Finally, the app CreaView can be improved with a number of features such as a community interaction page, where patients who use the app can anonymously share their healing journeys, as well as interaction with third party health tracker applications, and sensors for other biomarkers. These features will be vital for the financial success of CreaTrack, and will ensure a positive interaction between the sensor and the user. Screenshots of the app are shown in Figure 5.

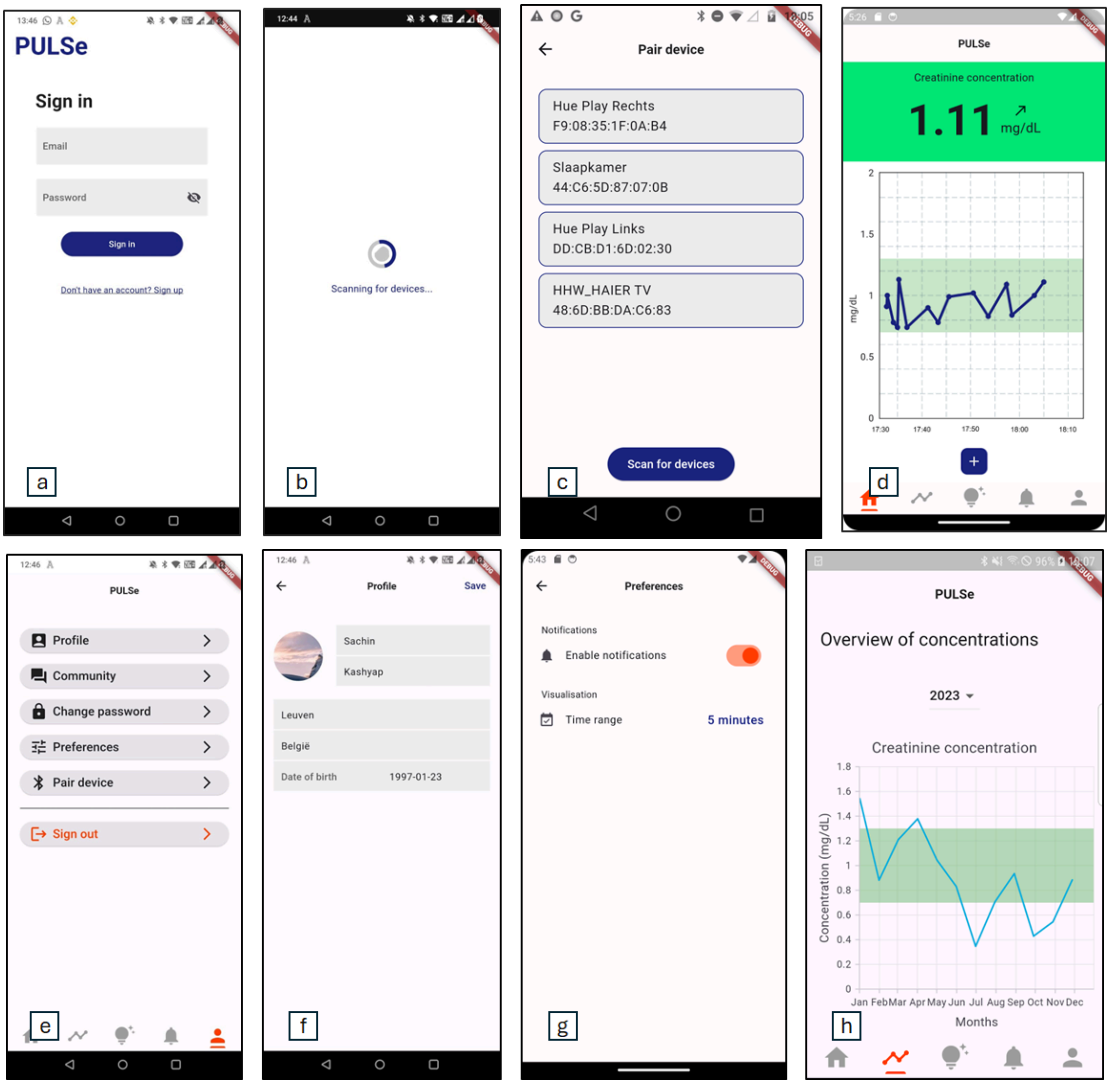

Figure 5: Screenshots of the app. (a) Login screen (b) Bluetooth scan (c) List of available devices (d) Creatinine concentration graph (e) Settings (f) Profile (g) Preferences (h) History.

4. Originality

Written by team captains

After an extensive literature review, the most promising biosensor technologies for creatinine detection are: an enzymatic solution, molecularly imprinted polymers (MIPs) and surface plasmon resonance (SPR) (Saddique et al., 2023). We ended up using the enzymatic solution due to the higher familiarity with the option and available expertise to consult with.

The decision to use a single enzyme, creatinine deiminase, was made in order to allow for a much more clear path to commercialisation and presented the least number of possible problems, which was important due to the short available time of development. The adoption of the PANi-Nafion-Cu membrane was made because they have complementary characteristics (Do et al., 2018; Zhybak et al., 2016). For the competition we have developed a first of its kind setup in which an SPE electrode is functionalized to make the detection of creatinine automatic with no need for human input outside of the refilling of the reservoir. The integration of the SPE into a water-tight PDMS chamber enables continuous fluidic measurement, which resembles a previous idea (Chen et al., 2019). A PDMS reservoir as an inlet solves the problem of air bubble formation between successive samples. A companion app, CreaView, was made to set up the calibration curve of each sensor as well as providing a great visualisation tool available both for the patient and the medical doctor showing the relevant information for either party.

The development of the sensor has been troubled by technological challenges, such as an early split in the attention by working on 2 methods: FO SPR and potentiometry. Now, although the variation between electrodes is still a problem, the experimental results gave us a proof of concept. Further optimization is needed to improve performance, miniaturise and implement microneedles.

Written by supervisors

Our PULSe team has presented here a proof-of-concept for a, to our knowledge, first-of-its-kind continuous creatinine biosensor. Inspired by the well-established biosensing concept of the glucose biosensor, the team chose a functionalized SPE with single-enzyme based reaction offering the advantage of simplicity with high quality results, combined with an electrochemical readout. The developed creatinine biosensor eliminates the need for washing steps or sample dilutions, as its minimal sample volume can be partially utilised for self-cleaning. The chosen components enable significant downscaling, including smaller pumps and readout equipment, making the device suitable for wearable applications. The novelty of the device lies in its potential to create significant socio-economic value through remote and continuous creatinine monitoring. This way enables accurate monitoring and consequently correct treatment, while easing the burden on hospitals and doctors, and ultimately improving patient's lives. In addition to the CreaTrack hardware, the sensor comes with a highly patient-oriented app: CreaView, which can be accessed on their phone via a Bluetooth connection. Next to offering the patient an easy-to-use interface to access their data, it provides them with health advice, notifies the specialist nephrologist in case of emergency, and creates a supportive community for patients to connect with each other, and share their experiences, and soften the path to healing. Throughout the development of the presented biosensor, the students resided in a strong support system (i.e. 5 motivated PhD students and myself as the supervising professor) to provide them with valuable knowledge and guidance. Nevertheless, the students were continuously encouraged to think outside the box and independently came up with innovative concepts to make their biosensor unique. Herein, the students have presented themselves as a strong, dynamic, hard-working, and creative team; showing a great work ethic and an extraordinary level of independence. This eventually led them to design this innovative biosensor that shows great potential to become both: (1) an accurate and continuous way of monitoring creatinine levels, and (2) a product with great market value for multiple target audiences. Although further optimization will be required, the presented preliminary results show great potential for the future.

Supervisor:	**Team captain:**
Prof. Jeroen Lammertyn	Arina Breeva
	
Jeroen Lammertyn 9/8/2024	A. Breeva 09/08/24

5. Translation Potential

5.1. Business model

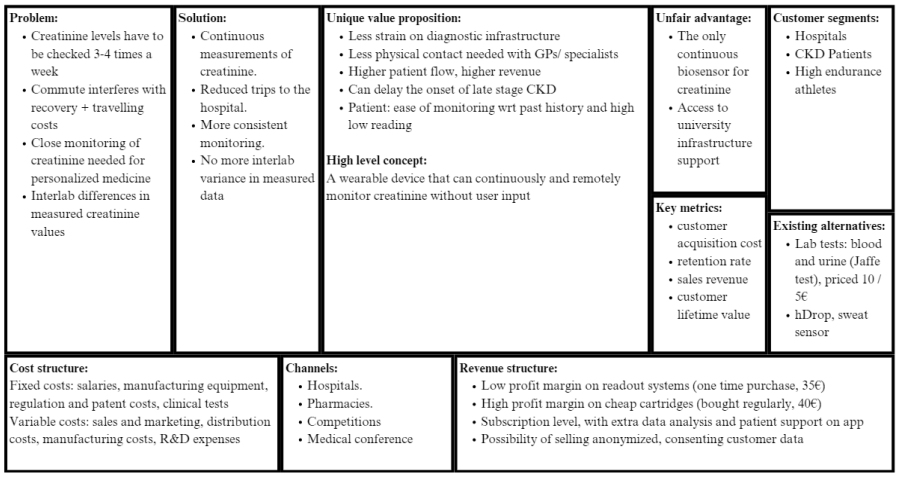

Figure 6: A lean canvas representation of CreaTrack's business model

5.2. Market description

Acute Kidney Injury (AKI), the theme for SensUs 2024, denotes a sudden reduction in kidney function. Healthy kidneys filter creatinine out of the bloodstream, thus kidney failure can be characterised by an increase in creatinine levels. AKI is frequent and prevalent, affecting 10% of the world's population and occurring in 21% of hospital admissions worldwide (Bouchard & Mehta, 2016). Currently, the gold standard test to detect and monitor AKI is the **lab-based colorimetric blood test.** There are three major drawbacks with this method. Firstly, creatinine levels fluctuate with circadian rhythms, hence significant variations in creatinine levels during the day may be ignored, leading to incorrect diagnosis (Silveiro & Zelmanovitz, 2018). Secondly, patients are required to travel to a hospital or diagnostic lab 3-4 times a week, which interferes with the patient's recovery and adds to their travelling costs which are borne by the state, while also pacing an extra burden on the infrastructure of the diagnostic lab and hospital (Theofilou, 2013). Lastly, creatinine levels vary between different diagnostic labs by almost 60%, leading to wildly inaccurate diagnoses (Lee et al., 2017), which lead to incorrect treatments. An alternative method to monitor creatinine levels, involves collecting a 24 hour urine sample to measure the average daily creatinine excreted and account for circadian variations. However, these tests have been reported to be inaccurate, with a maximum observed variation of 54% (Côté et al., 2008).

Thus, there is an opening in the creatinine sensor market for a sensor which can monitor creatinine levels: (i) **continuously**, allowing to obtain the full kinetics of the blood creatinine levels over time and therefore draw better conclusions, (ii) **remotely and autonomously**, which reduces the burden on both medical professionals and patients, (iii) **accurately**, so that variations between measurements are minimised (iv) in a **wearable package**, so that patients can use it comfortably. Our biosensor, CreaTrack, is able to meet these demands of the market. It will not only help hospitals save costs and lab resources on creatinine tests, but also offer AKI patients a more comfortable journey towards recovery.

Two specific markets that are in need of remote continuous creatinine monitoring have been identified for the above reasons: (i)**Patients suffering from Chronic Kidney Disease (CKD)**, which refers to a long term loss of kidney function. Approximately 100 million people suffer from CKD and 300 million more are estimated to be at risk of getting the disease. This imposes a significant economic and infrastructural burden on the public health system (Vanholder et al., 2021). In Belgium, 685,000 people suffer from the disease, resulting in a total patient expenditure of €2.11 billion in 2024 (Locatelli et al., 2002). Creatinine monitoring is helpful for long term management of CKD. (ii)**Patients who have just undergone an organ transplant**. These patients need to take immunosuppressants so that their bodies accept the foreign organ. However, these immunosuppressants place additional strain on the kidneys, causing kidney failure. Thus, continuous creatinine monitoring helps accurately control the dosage of immunosuppressants, while preventing kidney damage. In the EU in 2022, there were 39,000 transplantations performed, while 48,000 people were added to the waiting lists ('European Donation Day (EDD)', 2023).

Additionally, high-endurance athletes, such as ultrarunners and triathletes have been identified as an exploratory market that can be entered into at a later stage. A study conducted by RunRepeat (Dawson, 2020) suggested that 600,000 people ran an ultramarathon (race over 26.2 miles) in 2020. Additionally, around 85000 athletes entered into an ironman (140.6 mile triathlon) in 2019 ('Ironman Demographics: Overall Competitors', n.d.). CreaTrack will be useful to these athletes in monitoring the Acute Kidney Injury (AKI) arising from dehydration, since this data can help them optimise their training without affecting their kidney health. Figure 6 provides an overview of our business plan in the form of a business model canvas.

5.3. Stakeholder desirability

CreaTrack is a wearable Point Of Care (POC) device which continuously measures creatinine levels in the Interstitial Skin Fluid (ISF). The sensor consists of a fixed readout device and a replaceable cartridge which is functionalized and pre-loaded with the creatinine-sensing enzyme. CreaTrack will have a size and form factor similar to continuous glucose sensors on the market, with a diameter of 3 cm and a height of 4 mm, which will allow for easy and comfortable wearability. In addition to the sensor, we plan to develop an user-friendly app, CreaView, which will allow patients to visualise results and provide alerts when creatinine levels cross a threshold. The prototype that we present at the SensUs event will not be wearable, however, it will be a continuous creatinine sensor integrated with CreaView.

To gain insight into the needs of hospitals, our primary customer for CKD, we met Prof. Dr. Dirk Kuypers, the chair of the Nephrology department and the director of the Renal Transplantations program at UZ Leuven in Gasthuisberg. With the help of his insight and our research, we found a number of ways that CreaTrack is more beneficial than the current lab-based technique. We learned that the cost of a traditional lab test ranges from 10-25 euros per test and also takes a longer time for reporting the results (24 hours for lab tests compared to 5 minutes for CreaTrack). This results in an expense of **€300 per month** for 3 tests per week. Our biosensor, CreaTrack, will be able to bring this cost down to **€105 per month** while collecting more data in the same timeframe through continuous measurement(Appendix A1.1). This lowered cost also incentivizes insurance companies and government programmes to cover our product over the current gold-standard.

CreaTrack has a number of benefits for hospitals: **(i) Less strain on the diagnostic infrastructure**: The lab units in a hospital need not be tasked with routine creatinine measurements and can instead focus on more pressing cases. Also, hospitals would need less staff to analyse data since our app CreaView transforms the data points into easy to read graphs. Thus, the burden of data analysis and interpretation will be reduced on the doctor's side. Furthermore, the data collected from CreaTrack will be integrated into each patient's medical database, so that doctors have access to the data from each patient. (**ii) Less visits to specialists needed**: Currently, patients need to visit a hospital to take a creatinine lab test. After the test they need to make an appointment with a nephrologist to receive a diagnosis based on the test analysis. In Belgium, the waiting time for a nephrologist is around 6 months. With CreaTrack, the creatinine levels can be monitored remotely, thus a visit to the nephrologist is not needed. Furthermore, the patient and the hospital will both get an emergency notification to schedule an appointment with a nephrologist if there is a large spike in creatinine levels.**(iii) Higher patient flow, higher revenue**: Since the number of visits to the hospital is reduced, the patient flow per specialist can be increased. This enables hospitals to service more patients and reach higher revenue.

We plan to sell the sensor to **research institutions and pharmaceutical companies** to test and monitor creatinine levels while testing drugs that could potentially affect kidney function. This could also be beneficial for clinical trials, especially in the early phases where the kidneys might be impacted by the need to metabolise and excrete the drug being tested. Research institutions can also purchase anonymized data collected by CreaTrack. Thus, CreaTrack doesn't just offer value to doctors and hospitals, but to the **entire value chain** of CKD treatment.

To understand the difficulties faced by patients who undergo organ transplants, we met with Prof. Stefan de Smet, a researcher in exercise physiology for transplant patients. Prof. de Smet emphasised the need for continuous creatinine monitoring in all transplant patients, as the introduction of a foreign organ triggers an immune response. To manage this, patients are prescribed immunosuppressants, such as calcineurin inhibitors (CNIs), which put considerable strain on the kidneys. As a result, transplant patients are at a significantly higher risk of developing CKD and AKI (Bloom & Reese, 2007). Continuous creatinine monitoring is therefore essential, allowing for the close tracking of kidney function and the careful adjustment of immunosuppressant dosages.

Our biosensor could benefit high-endurance athletes, such as ultramarathoners and ultra cyclists. To explore this market, we consulted Prof. Katrien Koppo, head of the exercise physiology research group at KU Leuven. Prof. Koppo suggested that these athletes could use the sensor during training to optimise performance and prevent kidney damage caused by dehydration by monitoring kidney function and adjusting hydration and training regimens accordingly (Lippi et al., 2004). The sensor could be utilised not only during training but also starting 24 hours before the race, throughout the event, and for 24-48 hours afterward to analyse physiological changes and potentially intervene before permanent kidney damage occurs. This highlights the significant market potential for a creatinine biosensor.

We have established partnerships and secured suppliers to ensure a competitive advantage in our operations. Our key partners include Metrohm, which provides the C223AT SPE electrodes, Qiagen, which supplies the creatinine deiminase enzyme, Microfluidic Chipshop, which supplies custom microfluidic chips, and Mouser, which delivers the electronic components necessary for our readout system. To facilitate expansion and automation, we will leverage industrial connections offered by KU Leuven's Tech Transfer Office. We will focus on creating symbiotic partnerships with our partners, based on mutual growth and clear communication. This strategy enables us to secure high-quality materials and components while offering our partners a reliable customer and the opportunity to contribute to an innovative project.

5.4. Business Feasibility

For successful deployment of CreaTrack, an estimated 2 years of R&D, clinical trials, and patenting are required. Initially, we will operate from the MeBioS lab at KU Leuven for the first year, seeking seed funding from the Gemma Frisius Investment Fund at KU Leuven, VLAIO, and the Eurostars funding programme. We will outsource legal counsel for intellectual property and regulatory compliance and establish specialised sub-teams focused on bioassay, microfluidic design, and readout for biosensor development, recruiting research scientists for each team. After one year, we plan to spin off from KU Leuven and relocate to BioIncubator facilities in Leuven to accelerate growth. During this expansion phase, we will seek additional investment from venture capital firms specialising in biotechnology. To support this transition, we will hire a business consultant and engage with the Tech Transfer Office at KU Leuven to gain expertise in building and growing our company. We are collaborating with Iene Rutten, the Valorization Manager at the MeBioS lab, who has assisted with business plan validation and IP matters.

Once we complete our clinical trials (*Assessment Time Limits for Clinical Investigations with Medical Devices*, n.d.) and legally ensure a Freedom To Operate (FTO), we plan to market CreaTrack for CKD and organ transplant patients in the Benelux region. Although we do have the unfair advantage of being the only continuous creatinine sensor on the market, we realise that in order to make CreaTrack the preferred option for creatinine sensing, we will need to grow our market share quickly. To that end, we have already begun to establish relationships with one of the most esteemed university hospitals in Flanders, UZ Leuven Gasthuisberg. We plan to contact other local hospitals in the Benelux region as well as network in major nephrology, biosensing, and organ transplant conferences in order to market CreaTrack and gain the trust of the medical community.

We are strong believers in ecological sustainability, and aim to make CreaTrack a sustainable solution with lab quality results. CreaTrack will decrease carbon emissions due to the reduction in use of hospital equipment and commute associated with the use of a single-use creatinine sensor. The small form-factor of CreaTrack will also reduce power consumption and plastic waste. Additionally, continuous creatinine sensing can help delay or in certain cases prevent dialysis, which requires a high water requirement of 75000 L per person per annum (ARUP, 2019). Thus, we believe that our product is far more ecologically sustainable than the current lab-based method for creatinine monitoring. The sustainability aspect of our product has the added benefit of making us eligible for sustainability grants from Horizon Europe, and we plan to use these funds to accelerate our growth.

To ensure the continued success of CreaTrack, we need happy and satisfied customers. Our user-friendly app, CreaView, with its multitude of features is designed to ensure user continuity. CreaView can be used by patients to monitor their creatinine levels and get answers to basic questions regarding CKD, AKI, and creatinine levels from a specialist. It can also be integrated into various popular health tracking smartwatches such as Fitbit, Apple Watch, and the Garmin fitness trackers. CreaView provides dietary and hydration recommendations to help CKD patients eat responsibly. CKD patients experience heightened levels of depression and anxiety (Duarte et al., 2009). Hence we plan to add an optional community feature to CreaView, where patients can anonymously share their experiences about their healing journeys

with other patients as a form of group therapy. We also plan to hire licensed therapists to moderate this community and provide effective Cognitive Behavioral Therapy (CBT) based suggestions in order to help patients deal with the mental health issues surrounding CKD (Duarte et al., 2009). Additionally, we plan to provide all features on the app for free to all subscribing users of CreaTrack. These initiatives will allow us to build a personalised health monitoring system, which will build closer ties with our patients and help secure our market share against incoming competition.

Furthermore, we will need a server for hosting all the recorded medical data for which AWS (Amazon Web Services) is one of the best and cheapest options in the industry. Proper adherence to GDPR guidelines will be followed and lawyers will be consulted to ensure safe practices while handling medical data (European Patient Forum, n.d.).

To achieve this vision, we have broken down the planned growth of our company into 4 phases as summarised in Figure 7.

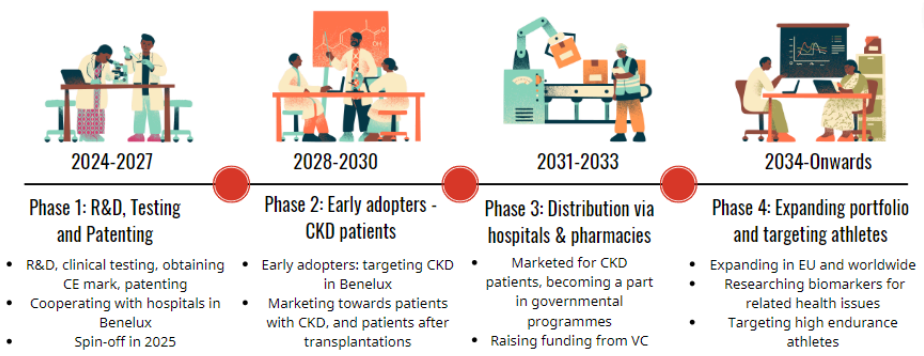

Figure 7: Schematic showing different phases of growth of our business

Phase 1 (2024-2026): Research and development will be performed in cooperation with UZ Leuven, who have already shown interest in CreaTrack, and other hospitals in Belgium. We will be performing clinical trials to obtain the CE license, as well as securing our IP and obtaining an FTO. R&D will also be performed to optimise our protocols for mass production. At the end of 2025, we plan to spin-off from KU Leuven into our own company.
Phase 2 (2027-2029): After clinical trials, patenting and obtaining the CE mark CreaTrack will be ready to enter the market. We plan to sell CreaTrack to CKD and post-organ transplant patients with a doctor's prescription. We plan to obtain 1000 customers in our first year, which will grow to 10,800 customers at the end of Phase 2.
Phase 3 (2031-2033): In Phase 3 we plan to raise further funds from venture capital in order to increase our penetration into the Benelux market. We also aim to become a part of the 'zorgtraject' government programme in Belgium for CKD patients, which compensates patients for medications, dialysis, and transplantation. Having these high-trust institutions validate CreaTrack will make it more accessible to the general public, and allow us to penetrate the market faster.
Phase 4 (2034-...): In Phase 4 we plan to expand to the EU and diversify our portfolio by selling to endurance athletes, which will not require many additional resources. We also plan to make a licensing deal with a major company like Bayer to easily market and distribute CreaTrack across the EU. CreaTrack can be added with other biomarker modules for related health issues, such as liver failure, hypertension, and autoimmune diseases to increase the functionality of our biosensor and to expand our product line. Collected patient data from the previous years can be anonymized and sold to institutions specialising in kidney research.

5.5. Financial Viability

To provide concrete support for our business plan, we made a thorough calculation of all the finances involved (Figure 8, Appendix A1, A2). We discussed this plan with our valorization manager Iene Rutten, who helped validate our assumptions. Our revenue streams comprise of selling (i) the electronic readout component, which will retail for €35, purchased once; (ii) the microfluidic cartridge, pre-loaded with the enzyme, which retails for €40 and needs to be changed every 14 days and (iii) curated data from (obliging) patients, which can be potentially interesting for research institutions and pharmaceutical companies. A key metric to measure our company's success is the continued sales of cartridges to customers who have bought the read-out devices.

We plan to price our product according to the razor-razorblade model, where we plan to have high margins on the cartridges (75%), and low margins on the electronic readout device (12%). To sustain this model, we will need to have high customer satisfaction, which we plan to achieve by developing close relationships with our customers with the help of our app, our community, and dedicated customer support. We plan to collaborate closely with customers to regularly improve the device and the app, in order to ensure that the customers receive the desired features.

Through our business model, we identified some of the major costs in the manufacturing of the sensor cartridge as labour costs, enzyme costs and distribution costs. The labour is mainly needed to assemble and package CreaTrack. Hence, we will reduce them by using simple equipment to parallelize the assembly and packaging steps. We would leverage our relationships with partners that specialise in mass production of enzymes and microfluidic chips like Qiagen and Microfluidic Chipshop. This would further help us reduce costs and help us reinvest the profits back into the product.

The distribution of the product will be helped initially by selling only through hospitals, easing the logistic requirements. For the third phase, when the sensor will hopefully extend its reach, cooperation with an established distributor of medical devices like Benetec will be needed to achieve an effective and streamlined distribution.

By the end of Phase II, we aim to reach around 9,000 customers in the BeneLux region, which will translate to 10,800 readout devices manufactured (assuming margins for manufacturing defects), as well as 234,000 cartridges being sold per year. This will translate to a yearly revenue of €8.62 million Once we have optimised our operation processes we plan to rapidly scale up. We aim to do this by becoming a part of public health insurance programmes like the 'zorgtraject' in Belgium. This will allow us to reach a large customer base quickly. Our relationships with doctors, local hospitals, and patients, along with our cost-effective sensor should help us penetrate these programmes. To service this massive market, we plan to obtain a second round of funding through venture capital for ramping up and outsourcing our manufacturing, distribution and marketing networks. Finally, in stage 4, we aim to expand our operations throughout the EU, and service secondary markets like high-endurance athletes. We also plan to leverage our relationship with the medical system in BeneLux in order to sell anonymized data to research institutions and pharmaceutical companies, thereby ensuring another revenue stream, as well as promoting cutting-edge kidney research.

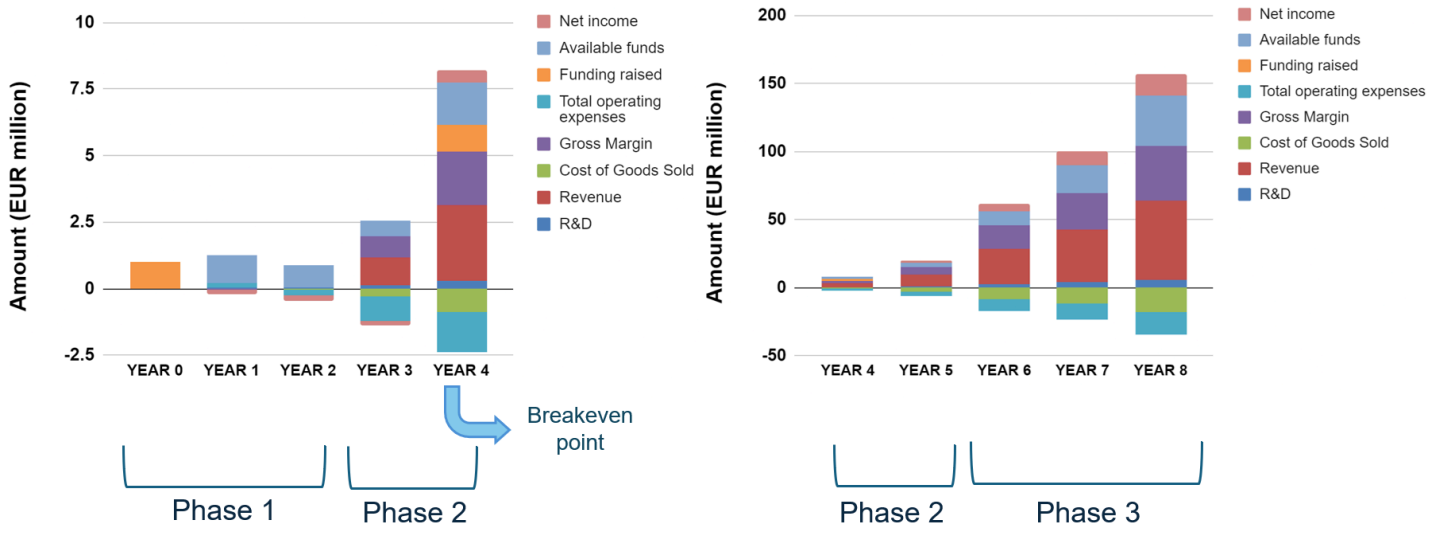

Figure 8: Financial summary of our business over the coming 8 years

Adequate and timely funding is integral to the success of our company. During Phase 1, we will look for seed funding from the Eurostars funding program, Gemma Frisius, and VLAIO,. We plan to raise €1 million for initial R&D and keep us afloat during Phase 1. Once we begin to generate revenue and spin-off from the bio-incubator, we will aim to ramp up our manufacturing and grow our market share with the help of additional funding from venture capital. We plan to raise another €1 million in the second round of funding in year 4 to accelerate our growth in the subsequent phases. According to our business model, we will reach the break-even point in year 4, as seen in Figure 8.

6. Team and support

6.1. Contribution of team members

Bioassay: Arina Breeva is our team captain. As a team member of the bioassay team made the SPE's and the dilutions, measured NH4 and creatinine, optimised the membrane. She is also a part of the business team where she organised business meetings for model validation, worked on the TRD and entrepreneurship session assignments. **Yogen Borkar** is a member of the bioassay team where he worked on ideating, fabricating and testing the biosensor. Yogen is also part of the business team where worked on developing a model for financial viability. He also contributed to the TRD. **Julia Boerjan** is a member of the bioassay team. She is a regular feature in the day-to-day synthesis and testing of the biosensor, and interpretation of the results. Julia is also our social media manager. **Matteo Fiori** is a member of the bioassay team. Alongside synthesising and testing the biosensor, he was creative in optimising testing and data analysis protocols.

Integration: Edoardo Trinca is a member of the integration team. He helped manufacturing cartridges and electrochemical testing with the bioassay team. He contributed to the TRD. He is also a member of the business team where he creatively explored new markets, helped incorporate understanding about EU regulations. **Tu Anh Le** is a member of the integration team. She was in charge of designing and manufacturing the PDMS chamber and biosensor cartridge. She optimised the pressure of the pump, experimented with the fluidic cell. She was also responsible for the illustration images and helped finalise the TRD. She also took part in some of the bioassay team's experiments, assisting with data analysis and interpretation of results. **Beatrice Marconi** is a member of the integration team. She focused on manufacturing the sensing chamber and she was the main contributor to the cartridge box design. She also handled the pump in the fluidic system. Furthermore, she assisted the bioassay team with testing the biosensor.

Readout: Sachin Kashyap is a part of the readout team. He supported the team in creating the app and assisted with the calibration of the sensor. He is also a member of the business team, where he worked on the market description and business feasibility, writing the translation potential draft and will be delivering the pitch in Eindhoven. **Khang Nguyen** is a member of the readout team. He contributed to the development of the mobile app, focusing on communication, storage and visualisation of data. He also assisted with automatization of the pump. **Jitske Van Peer** is a member of the readout team. She assisted in developing parts of the mobile app, the testing of the EmStat Pico and the Bluetooth connection.

6.2. Additional support

Firstly, we would like to express our gratitude to **Prof. Lammertyn,** founder of the Biosensor group at MeBioS, KU Leuven for providing us with all the necessary resources and support to develop our biosensor.

We would also like to acknowledge our coaches. During our weekly meetings, they provided invaluable advice for our experiments, drawing on their extensive expertise with biosensors. Their support strengthened our team and enabled the successful realisation of our creatinine sensor. **Francesca Pollet** helped out the readout team on the structure of the mobile app and possible sensor calibration methods. **Roselien Verboven**, together with **Francesca**, directed the integration team and helped them navigate possible designs and implementable features while supporting the team developing solutions to adhesion of the different elements. **Vedika Choudhari and Lieze Dankers** directed the bio-assay team, assisting with sensor functionalization and immobilisation of the biorecognition element by sharing different protocols and exploring multiple immobilisation strategies. **Lieze** also played a crucial role in developing a robust business case and validating the model with the appropriate experts. **Jalu Pradana** supported the readout team, was instrumental with the set-up of electrochemical measurements and development of calibration algorithms.

We would like to thank **Prof Dirk Kuypers, Prof Stefan de Smet,** and **Prof Katrien Koppo** for taking out time to meet with our business teams. They helped us understand prevalent issues with AKI and CKD sensing infrastructure and validated our ideas about expanding to other markets. Lastly, we would like to thank **Dr. Ir. Iene Rutten** for helping with fine tuning the translation potential and providing us with valuable insights and feedback. Finally, we extend a special acknowledgement to the following people for their assistance with the practical aspects of the project, valuable insights, and friendly advice: Claudia Scarperlini, Maarten Van der Auweraer, Ruben Cops, Dr. Ir. Lorenz Van Hileghem, Prof. Arantzazu Navarez, Prof. Irene Taurino.

7. Final remarks

We hope that this document persuaded you of the potential of our sensor *CreaTrack* which integrates continuous biosensing with electrochemical approaches like potentiometry. In this device, enzymatic detection was utilised to achieve a high sensitivity of 86 mV/dec for creatinine. Future studies would need to test the device in reconstituted interstitial fluid (ISF) to simulate the sensor's performance in the human body. Furthermore, the team encountered certain problems with baseline potential drift present in Au screen printed electrodes. CNT based working electrodes and a double junction reference electrode can be used as they have been known to showcase higher signal stability. This might also enable the device to achieve higher LoD and sensitivity for creatinine in ISF. To implement the sensor into a minimum viable product, the current chamber design will be optimised and further research into a biocompatible microneedle-incorporated sensor will be done. The current readout system holds great promise for direct implementation due to its multiple innovative features like Bluetooth connectivity and the supporting app *CreaView*. This would enable the devices usage with minimal dependance on diagnostic infrastructure and especially in delicate cases like post transplantation or in remote areas. After incorporating the features outlined in the business plan, we are confident that CreaView can become a pioneering app in personalised medicine.

Over the past nine months, we have learned an entire portfolio of skills, going from technical and business aspects, but also some soft skills such as communication and interdisciplinary teamwork. We are grateful for the support we received from our coaches, supervisor and the KU Leuven support system. We would also like to sincerely thank the SensUs organisation for organising this remarkable competition which gave us an opportunity to learn a variety of skills and ideas concerning biosensor development and translation research. We are eagerly awaiting the innovation days in Eindhoven and excited to meet all the participant teams.

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Appendix:

A1: Business Plan Assumptions

In this section, we have provided a comparison between our sensor and the gold standard present in the market. In addition, the assumptions made in the business plan have been presented. Finally, the cost breakdown for each component of our sensor is provided.

Table A1.1 Comparison of other creatinine sensors in the market

Name	Frequency of testing over 1 month	Price (€)
Hospital test(*Example Creatinine Cost*, n.d.)	3 times a week	300
CreaTrack	2 sensor cartridges	80 (105 with readout device)

Assumptions in the model

a. The product was priced such that there 75% margin on the sensor cartridge and 12% margin on the electronics, so as to account for the other manufacturing costs. A quick survey of other available technologies (Appendix) tells us that the sensor is still competitively priced with ample margin for price fluctuation.
b. Manufacturing costs were estimated as 1.2x of the total labour cost needed per product, since a major component of manufacturing will be labour.
c. Material cost assumptions for making the sensor and the electronics components are listed in the Tables A1.2 and A1.3 below. Note that the current prices are for smaller, lab scale lots. As we increase our quantities the costs for these reagents (especially the enzymes and chips) will decrease further and increase our margins. Most of the reagents were sourced from Sigma Aldrich website. The equipment cost was similarly estimated using data from different ecommerce websites.
d. Year 1 and 2- some nonzero cost to make the products are needed for testing etc. No new personnel is hired since the team is supposed to work out of the lab in KU Leuven. We can still hire some project staff and marketing managers to help out in Year 2, extra funding for these kinds of expenses can be acquired from lab project funding or Eurostart. No lab space is required during this time.
e. Estimates for IP costs were taken from the KU Leuven Tech Transfer office website. Legal, business development and financial counsels are assumed to be outsourced since their need for a small team like ours, would be less than 1 full time equivalent.
f. Year 3 after opening the product to the market, is assumed to capture 1000 patients in CKD and post transplantation markets in BeNeLux. For phase 2, a modest growth rate of 3x users year on year is assumed.
g. For phase 3, the current assumed growth rate is 1.5x year-on-year so as to not necessitate excessive funding in scaling and allow us to mature in the market. However, there is room to scale this up to 10x, as even after this rate we only occupy 1% of the CKD patients market in Europe. This would lead to much higher profit margins in phase 3, than presented in Appendix A2.
h. Second round of funding amounting to 1M is assumed to be raised in year 4 to make the transition to a completely automated production process. This would enable us to scale up our production exponentially. The newly raised funding is assumed to be invested from the next year after in two batches of 400K each, to account for lag in raising and investing.
i. In year 2, the team consists of around 8 full time employees. This was calculated by assuming one lab technician can make at least 100 sensors a day. Hence we need 3 technicians to fabricate, assemble and package. We assume the production capacity per person will increase to 10x (i.e) 1000 sensors a day post investment in automation in Year 5. Beyond year 5, the personnel composition is scaled according to this production capacity.

j. All material costs and manufacturing costs are indexed at 2%pa. However our model does not account for the actual decrease in manufacturing costs that will occur as we scale up the process. This has been done to act as a buffer on any additional costs in the components or manufacturing.
k. Server, app store costs are assumed based on prices available in the market.
l. Post Year 5, we might need to expand to a larger setup. This has been accounted for by adding extra facility space costs. (The current estimates are for 250 sqm. space without equipment in Bioincubator Leuven)
m. Each year we assume 20% of income is invested in Sales and marketing and 10% of income is invested in new research for further development of the product (eg. adding new features, adapting for newer markets, etc).
n. 33% corporate tax is only applied when the operating profit is positive.

Cost breakdown

Table A1.2 Sensor component (cartridge):

All prices were taken from inventory listings on Sigma Aldrich.

Reagents	Explanation (values from protocol)	Lot cost	reagent amount/ sensor	Cost
Total per sensor cost				**5.70**
Aniline	€45/100mL	0.45	0.0194	0.00873
Acid	assumed similar price for other acids required for synthesis €92/500ml	0.184	0.015495	0.00285
Nafion	€325/25mL	12.96	0.0008	0.01037
Enzyme	€132/250U	0.528	5	2.64
Glutaraldehyde	€71/1000ml	0.071	0.00008	0.00001
MilliQ water	€14.5/10L, 25ml per sample	1.45	0.025	0.03625
PBS	€ 0.5 per liter, 10ml per sample assumed	0.5	0.01	0.00500
CuNO3	€40/100g	0.4	0.00116295	0.00047
Chip	Assumed for injection molded chip, designed inhouse			3.00

Table A1.3 Electronics component:

Modules+ chips	Explanation (values from protocol)	Costs
Total per device cost		**20.5**
Electronic components	amplifier, resistors, switches, contacts, etc	18
Battery	assumed 10% of total device cost	2
Assembly		0.5

Table A1.4 Other details for scale up post Phase 1:

Main expense	Details	Cost per item	number of items	Cost
Total expense				**14380**
	Pipettes (entire set)	1500	2	3000
	Parallelization pipettes	600	2	1200
	Fume hood	700	2	1400
	commercial potentiostat	850	2	1700
	electrochemical bath	300	1	300
	Pt counter electrodes	26	10	260
	Chemical storage cabinets	400	2	800
Instruments needed	Fridges	360	2	720
Misc				5000

A2 : Financial Projections

In this section, we have provided detailed financial projections for the next 8 years.

TABLE A2.1 : FINANCIAL SUMMARY TABLE

	YEAR 1		YEAR 2		YEAR 3		YEAR 4		YEAR 5		YEAR 6		YEAR 7		YEAR 8	
	Amount	Revenue (%)	Amount	Revenue (%)	Amount	Revenue (%)	Amount	Revenue (%)	Amount	Revenue (%)	Amount	Revenue (%)	Amount	Revenue (%)	Amount	Revenue (%)
Revenue	0	0	0	0	1,082,000	100%	2,874,000	100%	8,622,000	100%	25,866,000	100%	38,799,000	100%	58,198,500	100%
Cost of Goods Sold	15,130	0	19,301	0	286,074	26%	874,603	30%	2,675,503	31%	8,186,536	32%	11,767,811	30%	17,824,338	31%
Gross Margin	-15,130	0	-19,301	0	795,926	74%	1,999,397	70%	5,946,497	69%	17,679,464	68%	27,031,189	70%	40,374,162	69%
Personnel	0	0	55,000	0	422,800	39%	430,656	15%	539,269	6%	777,365	3%	825,386	2%	841,893	1%
General & Administration	60,000	0	60,000	0	388,421	36%	714,343	25%	1,764,116	20%	4,517,749	17%	6,514,456	17%	9,431,273	16%
R&D	50,000	0	50,000	0	108,200	10%	287,400	10%	862,200	10%	2,586,600	10%	3,879,900	10%	5,819,850	10%
Depreciations	14,380	0	11,504	0	9,203	1%	7,363	0%	405,890	5%	724,712	3%	579,770	1%	463,816	1%
Total operating expenses	154,380	0	206,504	0	940,424	87%	1,524,762	53%	3,721,475	43%	8,756,426	34%	11,949,511	31%	16,706,832	29%
operating profit (EBIT)	-169,510	0	-225,805	0	-144,498	-13%	474,635	17%	2,225,022	26%	8,923,038	34%	15,081,678	39%	23,667,331	41%
Corporate tax	0	0	0	0	0	0%	0	0%	712,749	8%	2,944,603	11%	4,976,954	13%	7,810,219	13%
Net income	-169,510	0	-225,805	0	-144,498	-13%	474,635	1%	1,512,273	3%	5,978,436	10%	10,104,725	17%	15,857,112	27%

Detailed breakdown on calculation for revenue and COGS are provided in Table A2.3
Detailed breakdown on total operating expenses are provided in Table A2.4

TABLE A2.2 : SUMMARY OF CASH FLOW

The cashflow is based on our assumptions in Appendix A2 and financial summary in table A2.1

	year 0	*year 1*	*year 2*	*year 3*	*year 4*	*year 5*	*year 6*	*year 7*	*year 8*
net income	*0*	*-169,510*	*-225,805*	*-144,498*	*474,635*	*1,512,273*	*5,978,436*	*10,104,725*	*15,857,112*
depreciations	*0*	*14,380*	*11,504*	*9,203*	*7,363*	*405,890*	*724,712*	*579,770*	*400,000*
investments	200,000				500,000	500,000			
net cash flow	***200,000***	***-155,130***	***-214,301***	***-135,295***	***-18,002***	***1,418,163***	***6,703,148***	***10,684,494***	***16,257,112***
cumulative net cash flow	*200,000*	*44,870*	*-169,431*	*-304,726*	*-322,728*	*1,095,435*	*7,798,583*	*18,483,077*	*34,740,189*
Capital raised	1,000,000				1000000				
available funds (>0)		*1,044,870*	*830,569*	*695,274*	*1,677,272*	*3,095,435*	*9,798,583*	*20,483,077*	*36,740,189*

TABLE A2.3: PROFIT AND LOSS STATEMENT

	Phase 1		Phase 2			Phase 3		
	YEAR 1	**YEAR 2**	**YEAR 3**	**YEAR 4**	**YEAR 5**	**YEAR 6**	**YEAR 7**	**YEAR 8**
Income	***0***	***0***	***1,082,000***	***2,874,000***	***8,622,000***	***25,866,000***	***38,799,000***	***58,198,500***
Sensor + microfluidic chip revenue	0	0	1,040,000	2,730,000	8,190,000	24,570,000	36,855,000	55,282,500
Unit Price	40	40	40	35	35	35	35	35
Number of units	0	0.0	26,000.0	78,000.0	234,000.0	702,000.0	1,053,000.0	1,579,500.0
Electronic component revenue	0	0	42,000	144,000	432,000	1,296,000	1,944,000	2,916,000
Unit Price	35	35	35	40	40	40	40	40
Number of units	0.0	0.0	1,200.0	3,600.0	10,800.0	32,400.0	48,600.0	72,900.0
Cost Of Goods Sold (COGS)	***15,130***	***19,301***	***286,074***	***874,603***	***2,675,503***	***8,186,536***	***11,767,811***	***17,824,338***
Sensor + Chips Cost Of Good	950.0	4,845.0	256,978.8	786,355.1	2,406,246.7	7,363,114.9	10,508,326.3	15,897,676.2
Material unit cost	5.70	5.81	5.93	6.05	6.17	6.29	5.70	5.70
Manufacturing unit cost	3.8	3.88	3.95	4.03	4.11	4.20	4.28	4.37
Number of units	100.0	500.0	26,000.0	78,000.0	234,000.0	702,000.0	1,053,000.0	1,579,500.0
Electronic readout component	13,800.0	14,076.0	28,715.0	87,868.0	268,876.1	822,761.0	1,258,824.4	1,926,001.3
Material unit cost	21.00	21.42	21.85	22.29	22.73	23.19	23.65	24.12
Manufacturing unit cost	2.0	2.04	2.08	2.12	2.16	2.21	2.25	2.30
Number of units	600.0	600.0	1,200.0	3,600.0	10,800.0	32,400.0	48,600.0	72,900.0
Total App Costs	380	380	380	380	380	660	660	660
Server costs	280.0	280.0	280.0	280.0	280.0	560.0	560.0	560.0
App store costs	100	100	100	100	100	100	100	100
GROSS MARGIN	***-15,130***	***-19,301***	***795,926***	***1,999,397***	***5,946,497***	***17,679,464***	***27,031,189***	***40,374,162***

TABLE A2.4 : DETAILS OF BREAKDOWN OF TOTAL OPERATIONAL COSTS

		Phase 1				Phase 2						Phase 3					
		YEAR 1		YEAR 2		YEAR 3		YEAR 4		YEAR 5		YEAR 6		YEAR 7		YEAR 8	
PERSONNEL COST		**0**		**55,000**		**422,800**		**430,656**		**539,269**		**777,365**		**825,386**		**841,893**	
Total #FTE		**0.0**		**1.5**		**8.0**		**9.0**		**10**		**17**		**18**		**18**	
CEO	*Cost per year*		0	0	0	120,000	120,000	122,400	122,400	124,848	124,848	127,345	127,345	129,892	129,892	132,490	132,490
	# FTEs	0		0		1.0		1		1		1		1		1	
Engineering lead (CTO)	*Cost per year*	0	0	0	0	100,000	100,000	102,000	102,000	104,040	104,040	106,121	106,121	108,243	108,243	110,408	110,408
	# FTEs	0		0		1.0		1		1		1		1		1	
Server maintenance engineer	*Cost per year*					30,000	30,000	30,600	30,600	31,212	31,212	31,836	31,836	32,473	32,473	33,122	33,122
	# FTEs					1		1		1		1		1		1	
Marketing & sales (CMO)	*Cost per year*			50,000	25,000	51,000	51,000	52,020	52,020	53,060	53,060	54,122	54,122	55,204	55,204	56,308	56,308
	# FTEs			0.50		1.00		1		1		1		1		1	
Lab scientists	*Cost per year*		0	0	0	0	0	0	0	50,000	100,000	51,000	102,000	52,020	104,040	53,060	106,121
	# FTEs	0		0		0		1		2		2		2		2	
Lab technicians	*Cost per year*		0	30,000	30,000	30,600	91,800	31,212	93,636	31,836	95,509	32,473	324,730	33,122	331,224	33,785	337,849
	# FTEs	0		1		3		3		3		10		10		10	
Admin support	*Cost per year*		0	0	0	30,000	30,000	30,600	30,600	31,212	31,212	31,836	31,836	32,473	64,946	33,122	66,245
	# FTEs	0.0		0.0		1.0		1.0		1		1		2		2	
R&D COST			***50,000***		***50,000***		***108,200***		***287,400***		***862,200***		***2,586,600***		***3,879,900***		***5,819,850***
DEPRECIATIONS ON EQUIPMENT			***14,380***		***11,504***		***9,203***		***7,363***		***405,890***		***724,712***		***579,770***		***463,816***
GENERAL & ADMINISTRATION			***60,000***		***60,000***		***388,421***		***714,343***		***1,764,116***		***4,517,749***		***6,514,456***		***9,431,273***
Start-up cost		50,000	50,000														
		1															
Office and lab space	*Rental Cost per ye*	102,000	0	104,040	0	106,121	106,121	108,243	108,243	110,408	220,816	112,616	337,849	114,869	344,606	117,166	351,498
	# units	0		0		1		1		2		3		3		3	
General and Administrative expenses			0		0		30,000		50,000		75,000		100,000		125,000		125,000
Sales and Marketing			0		0		162,300		431,100	0	1,293,300	0	3,879,900	0	5,819,850	0	8,729,775
IP costs			0		50,000		75,000		75,000		100,000		100,000		125,000		125,000
Travel and subsistence			10,000		10,000		15,000		50,000		75,000		100,000		100,000		100,000
Accreditation			0		20,000		30,000										
OUTSOURCING			***30,000***		***30,000***		***120,000***		***85,000***		***150,000***		***150,000***		***150,000***		***150,000***
Legal Counseling			10,000		10,000		10,000		10,000		75,000		75,000		75,000		75,000
Business development counseling			10,000		10,000		60,000		75,000		75,000		75,000		75,000		75,000
Financial counseling			10,000		10,000		50,000										
TOTAL OPERATING EXPENSES			***154,380***		***206,504***		***1,048,624***		***1,524,762***		***3,721,475***		***8,756,426***		***11,949,511***		***16,706,832***
OPERATING PROFIT (EBIT)			***-169,510***		***-225,805***		***-252,698***		***474,635***		***2,225,022***		***8,923,038***		***15,081,678***		***23,667,331***
cumulated loss(profit)			-169,510		-395,315		-648,013		-173,378		2,051,644		8,923,038		15,081,678		23,667,331
taxable basis			0		0		0		0		2,051,644		8,923,038		15,081,678		23,667,331
CORPORATE TAX (%)	**33%**		*0*		*0*		*0*		*0*		*677,043*		*2,944,603*		*4,976,954*		*7,810,219*