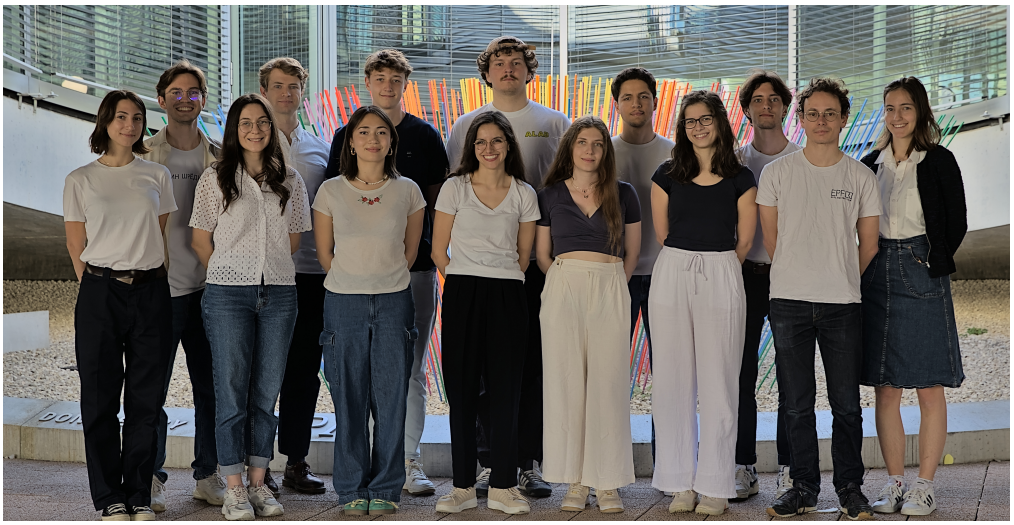
BIOSENSE EPFL

NephroPAL: an Ion-Sensitive Transistor for Creatinine Measurements



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Abstract

Chronic kidney disease (CKD) significantly impacts a large portion of the U.S. population, with about 9.2% of individuals affected. Advanced stages of CKD, such as stage 4, pose severe health risks, including heart failure, cardiovascular issues, and strokes, emphasizing the urgent need for effective management and intervention strategies. We present an innovative biosensor designed for continuous creatinine monitoring, a crucial marker of kidney function. The main principle of our sensor relies on using creatinine deiminase to break down creatinine into ammonium, which is then detected by an Ion-Sensitive Field-Effect Transistor (ISFET). This one-step process simplifies the detection and enhances accuracy. In addition, a microfluidic system has been integrated for greater accuracy. The data is post-processed and wirelessly transmitted to a smartphone app. This real-time data allows patients and healthcare providers to track kidney health and respond promptly to any changes, improving outcomes and reducing healthcare costs.

The biosensor's design emphasizes affordability, scalability, and user-friendliness. We are targeting CKM patients, namely patients affected by both cardiovascular and kidney disease. Indeed, kidney health influences cardiac health and vice versa. Our device offers a practical and user-friendly solution for managing kidney health more effectively, reducing the need for frequent hospital visits, improving and optimizing treatment management, and preventing irrecoverable outcomes. A secondary targeted group is that of working Doctors, who could access crucial information about their patients at a click of the finger.

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Summary for the SensUs website

Our project introduces an innovative biosensor designed for continuous creatinine monitoring, a crucial marker of kidney function. The main principle of our sensor relies on using creatinine deiminase to break down creatinine into ammonium, which is then detected by an Ion-Sensitive Field-Effect Transistor (ISFET). This one-step process simplifies the detection and enhances accuracy. In addition, a microfluidic system has been integrated for greater accuracy. The data is post-processed and wirelessly transmitted to a smartphone app. This real-time data allows patients and healthcare providers to track kidney health and respond promptly to any changes, improving outcomes and reducing healthcare costs.
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Chapter 1. Biosensor

1.1 Molecular recognition

Enzymes are well suited for continuous monitoring due to their selective catalyzation of biological reactions. We use creatinine deiminase (CD) (VWR, MP Biomedicals) to irreversibly hydrolyze creatinine into ammonia (NH_3) and N-methylhydantoin, as shown in Figure 1.1. CD retains most of its catalytic activity under physiological conditions, including those found in interstitial fluid (ISF) (Dasgupta et al., 2020b; Sprunger et al., 2023). Moreover, the pH of the ISF causes protonation of ammonia (NH_3) to form ammonium (NH_4^+) (Figure 1.1). In turn, ammonium can be quantified by various transducer technologies such as Ion-Sensitive Field-Effect Transistors (ISFET). Furthermore, to ensure sample-to-sample consistency, CD is immobilized by drop-casting on carbon substrates, a modified version of the method used by Dasgupta et al., 2020a. Here, the immobilization is performed on the working electrode of a carbon screen-printed electrode (SPCE) (Metrohm, Dropsens) for ease of implementation.

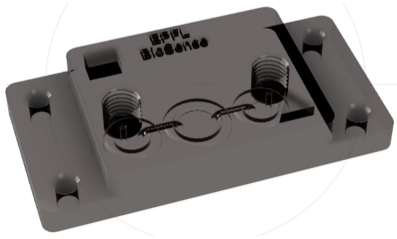
Figure 1.1: (left) Enzymatic conversion of creatinine into the measurable NH_4^+ ion by creatinine deiminase on the carbon surface of the screen-printed electrode. This reaction occurs within 1 minute at pH 7.5 and 37 °C, simulating ISF conditions. (right) Sensor prototype setup with controlled flow around the immobilized enzyme and the selective membrane of the NH_4^+ ISFETs. In non-wearable prototypes, samples are introduced via a syringe pump. The prototype uses differential NH_4^+ measurement with two ISFETs and the reference electrode of the SPCE for signal transduction.

1.2 Physical transduction

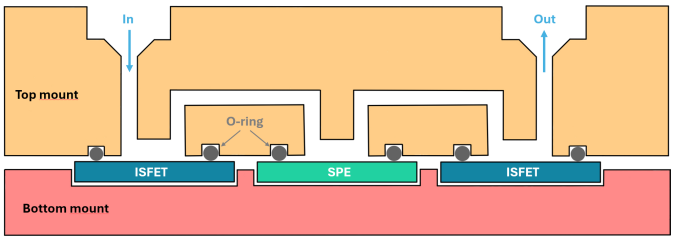
In enzymatic analyte detection, potentiometric methods (e.g ISFET) are often preferred over amperometric methods for ion sensing due to their ability to distinguish target ions in complex solutions. ISFETs are field effect transistors that makes use of an ion-sensitive membrane as a gate or on the gate. Ions change the surface potential of the gate, which affects the electric field across the insulator and modifies the current between the source and drain. The change is proportional to the ionic concentration. Furthermore, a three electrode electrochemical cell (EC) is used as a platform for the immobilization of the enzyme (on a carbon working electrode) and setting a reference potential with an Ag/AgCl reference electrode (Figure 1.1). To account for the presence of endogenous ammonia in physiological fluids like ISF, our prototype uses two ISFETs: The second one measures the enzymatically generated ions; and the other, placed upstream of the functionalized EC, measures total ion concentration. This setup allows to perform a differential measurement for isolating selectively the signal from the enzymatic conversion of creatinine.

1.3 Cartridge technology

The cartridge is built around the microfluidic system (Figure 1.2). It consists of an upper mount and a bottom mount, which, when assembled, form three sealing chambers of around $15\mu L$ arranged in series. The system consecutively delivers the solution to a reference ISFET, followed by the SPE with the immobilized enzymes, and the second ISFET for the differential measurement. O-rings are used for sealing the chambers. Inlets and outlets use 1/4-28 UNF ferrule connectors and 1/16” OD - 250 µm ID PTFE tubing.



(a) 3D render of the microfluidic cartridge



(b) Schematic of the microfluidic system

Figure 1.2: Implemented cartridge system

Samples are introduced into the flow cell using an AMF LSPone pump, a 12-valve microfluidic system, which operates at a flow rate of 5 - 7500 µL/min. The flow rate for the sensing points ranges from 25 to 100 µL/min, generating sufficient pressure to pass the fluid through the membrane and biosensor.

1.4 Reader Instrument and User Interaction

The system is powered by a LiPO battery managed by a control board that handles BMS, discharge, and charging. The ISFET and its readout board connect via UART to a microcontroller, which transmits data via BLE (Bluetooth Low Energy) to a smartphone. A custom Android/iOS app with a graphic user interface (GUI) processes and averages the values over a set interval, with "Start" and "Stop" timer functions for dynamic control.

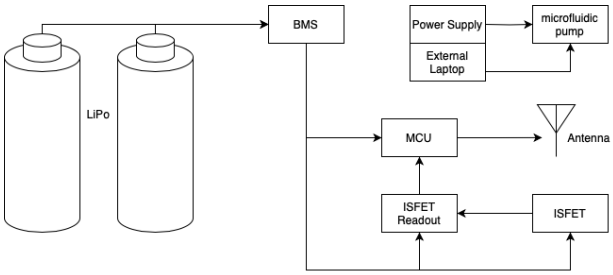


Figure 1.3: Full electronic system

The prototype electronics are compact, and the app is designed to minimize the gap to a future wearable sensor. Operation is straightforward, with automated sample flow, processing, and readout triggered by sample insertion. Users need only position the device, navigate the app, and from time to time discard the sample collector tube.

The smartphone app, developed in Dart using the Flutter SDK, communicates with the microcontroller via BLE (Bluetooth Low Energy) to read and store data sent by the sensor.
As mentioned, it processes the data to extract meaningful metrics, such as creatinine concentration and standard deviation, with a user-friendly interface tailored differently for both patients and doctors. Creatinine levels can be tracked and visualized over time, sending a warning if seeking help is necessary. The app also includes a section on best practices and rehabilitation tips to prevent further health issues (See Appendix 7.4 for GUI images).Our sensor has the potential to become a wearable device. We are developing PCBs, custom potentiostats, and BMS to reduce the footprint by a factor of 10. A redesigned microfluidic system is needed to reduce or eliminate the microfluidic pump. Possibly a microneedle could replace the pump. The ISFETs would remain unchanged and continue its normal function.

Chapter 2. Technological Feasibility

The technological development can be separated into the following aspects: ISFET measurements, enzyme immobilization, microfluidic system design, electronics system and user interface.

2.1 ISFET measurements

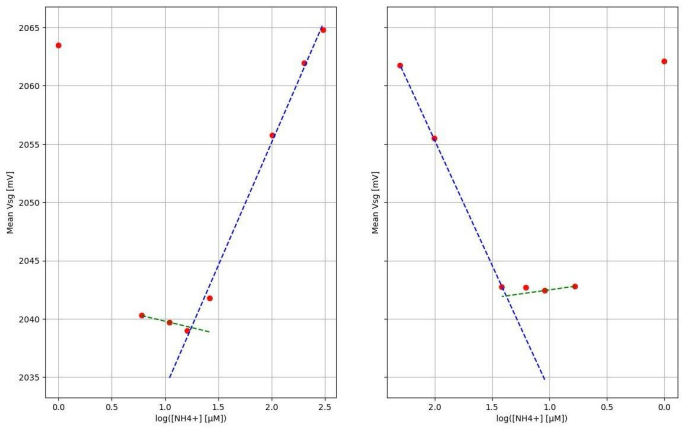


Figure 2.1: Calibration curves to obtain the lower limit of detection (LoD) in 0.1M PBS made from Na2HPO4 (Sigma Aldrich) and NaH2PO4 (Sigma Aldrich). The LoD of 17μM was found at the intersection of the two linear responses.

The Limit of Detection (LoD) of the ammonia ISFET was investigated to ensure the sensitivity in the desired final product, with the aim to obtain a linear Nernstian response, as found in theory. Microsens' ISFETs are prone to interference with K+ (Sigma Aldrich), we therefore used PBS created from Na2HPO4 (Sigma Aldrich) and NaH2PO4 (Sigma Aldrich). The measured LoD of 17μM shows no plateauing for high concentrations. A correlation between potential and ammonium concentrations in the [30 -300 μM] range can be assumed. Furthermore, through data processing and the usage of the Nikolsky-Eisenman equation, one can extract the voltages corresponding to NH4+ only Criscuolo et al., 2021.

Due to our custom boards not arriving on time, we chose to use Microsens' interface to do the analog to digital conversion.

Concerning the graph seen in Figure 2.1, the following values were obtained: during ascending concentrations of ammonium, the sensitivity slope (in blue) is: 21.09 and the R^2 value is: 0.82, indicating a promising model. During the descreasing concentration of ammonium, the sensitivity slope is: -21.42 and the R^2 value is: 0.82, also indicating a promising model. The determination of upper and lower LOD was done according to the IUPAC definition as described in the paper Criscuolo et al., 2021.

2.2 Enzyme immobilisation

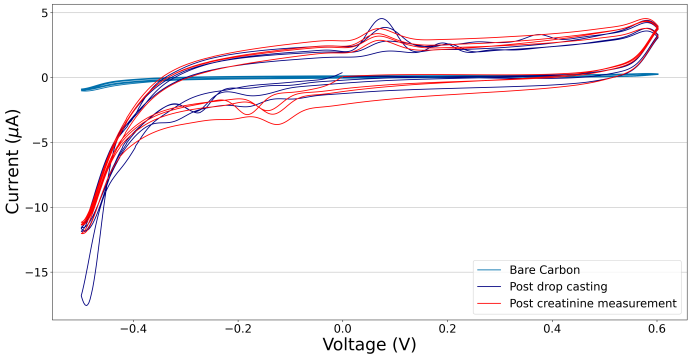


Figure 2.2: Cyclic Voltammetry performed on carbon SPE before drop casting (Bare carbon SPE), after drop casting and before measurement, and after drop casting and measurement. No significant difference between pre- and post-creatinine measurements can be seen in the response, indicating the persistence of the enzyme on the Carbon surface.

Enzyme immobilisation was performed through drop casting with two different incubation variations; (1) Overnight incubation at 4°C and (2) Drying in a vacuum oven at 45°C for 20 minutes (Dasgupta et al., 2020a). Proper deposition of the enzyme onto the carbon surface was verified using Cyclic Voltammetry (CV) with a scan range of [-0.6V, 0.5V] as shown in Figure 2.2. To minimize the effects of leaching enzymes, an excess amounts of enzymes (0.5 Units) are used. Carbon electrodes (Dropsense, Spain) are preferred over glassy carbon electrodes, contrary to methods used by (Dasgupta et al., 2020a), to lower device cost.

Clear differences can be seen between CV measurements of bare SPCEs and functionalized electrodes. Cyclic Voltammetry was also performed after each ISFET creatinine measurement on the SPCE with both drop-casting techniques.

2.3 Microfluidic system design

For our prototype, we use a resin cartridge made with stereolithography. Initial testing was done manually with a syringe and DI water to check for leaks, refining our design and processes (washing baths, curing time, and temperature) until we achieved a fully sealed cartridge. Post-processing involved 2 x 20 minutes in isopropanol and 35 minutes UV curing, yielding a precision of 0.1mm, suitable for our 1mm wide channels. Curing at room temperature provided a clearer finish and improved surface quality.
Achieving a sealed system required multiple iterations and testing with various connectors. The 16" ID 1/4-28 UNF ferrule connectors proved most reliable, ensuring a complete seal with no air gaps. Future tests will include checking for bubbles, using PBS and blood serum, and verifying compatibility with the AMF pump, which has been tuned to reduce bubble formation.
An in real life realisation of the microfluidic system can be seen in Appendix 7.11.

2.4 Electronic and software feasibility

In order to have a robust and powerful device, the Lyra24S development kit from Silicon Lab has been implemented. It is a recent cutting-edge board, that offers the possibility to communicate with the Bluetooth Low Energy (BLE) and the Universal Asynchronous Receiver Transmitter (UART) protocols, while being very tiny in size. Both protocols are widely used, because they ensure a secured and high quality transmission. It is also offering good computations resources, which are more than enough for the data processing used for this application. A small LiPO battery is used for the power supply, along with a battery management system, ensuring a long product lifetime. The overall cost is also pretty low, as seen in the business part 4.5.
Concerning the software part, the application has shown to work on both iPhone and Android. In addition, it does not require a very powerful phone to be run on, nor a large amount of storage, making it accessible to all. In the next iteration, it could be easily accessible from a cloud for download.

2.5 User interface

The User directly interacts with the sensor through the application. Therefore, it needs to be simple and user-friendly. For more details and pictures, please refer to Section 1.4 and Appendix 7.4. Here are some photos of the GUI.

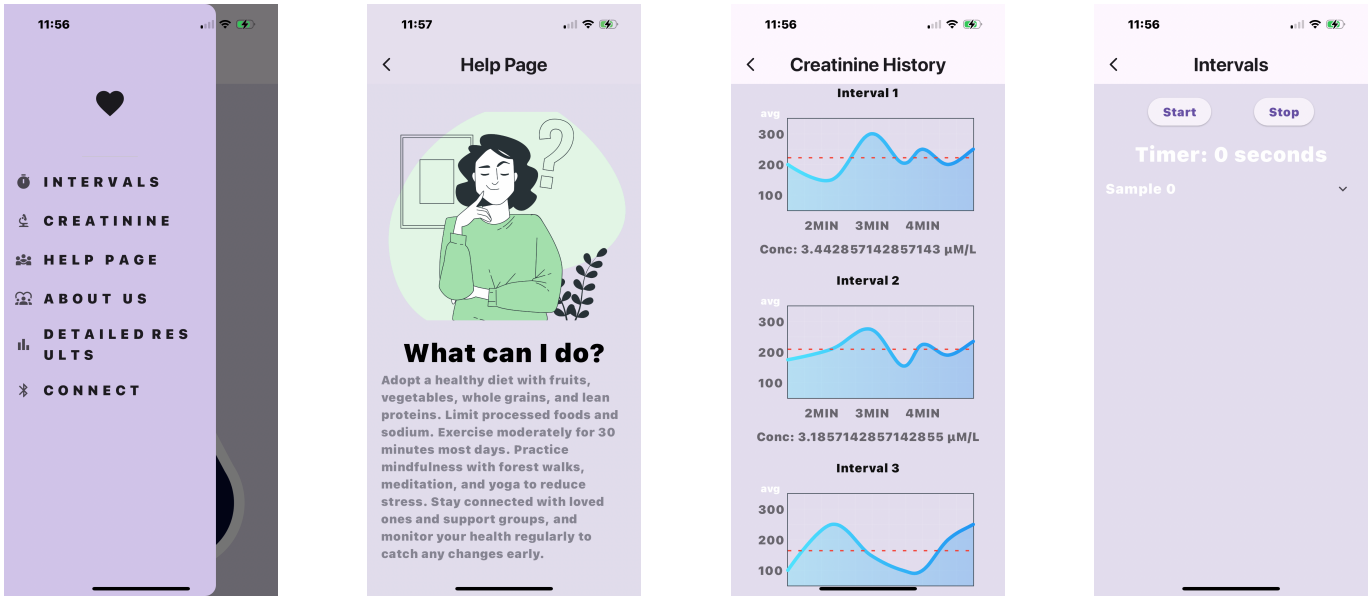


Figure 2.3: Integrated within the GUI is a menu where the user can access a help page, and view different summaries and analyses of creatinine measurements.

Chapter 3. Originality

3.1 From the BioSense EPFL team:

Current enzymatic creatinine sensing techniques predominantly measure creatinine indirectly after a three-step reaction involving creatinine amidohydrolase. In contrast, our sensor leverages the enzyme creatinine deiminase that facilitates a one-step, irreversible, catabolic, ammonia-producing reaction. This not only simplifies the detection process but also enhances the efficiency of the measurement.

Our sensor employs a potentiometric approach instead of the widely used amperometric or optical methods in the literature. Potentiometric sensing using ISFETs (Ion-Sensitive Field-Effect Transistor) provides several advantages, including better scalability and cost-effectiveness. ISFETs are smaller and are more precise than traditional electrodes (Cao et al., 2023). By using an ammonium-sensitive ISFET instead of a unspecific pH ISFET, a more accurate creatinine sensing is possible.

Our sensor design emphasizes simplicity, scalability and cost efficiency. The modular nature of ISFET technology allows for easy integration of additional measurements within the same device, such as potassium levels, by incorporating other specific ISFETs into the system. This flexibility ensures that our sensor can be adapted and expanded to meet various diagnostic needs with minimal modifications in microfluidics and data processing. Our team designed the microfluidic system compatible with the electrodes and the pump system to allow continuous and more reliable measurements. To improve the user experience, our team has developed an application that allows the user to have the readout of the creatinine levels directly on their phone. It uses the Bluetooth low energy(BLE) protocol for the transmission.

In conclusion our systems innovation lies in the never-before attempted combination of cutting-edge selective ion filtering technology, with an efficient and reliable bio-reaction. Also, the modular design makes the system very versatile, and all in a highly integrated, plug and play wireless package.

3.2 From our supervisor:

This year, the Biosense EPFL team was tasked with developing a continuous creatinine sensor, a technology currently lacking in the market for this vital kidney failure biomarker. The team evaluated several advanced detection methods but ultimately chose the most feasible approach given the competition's timeframe.

Despite initial exploration of more complex techniques such as an on-chip Mach-Zehnder interferometry and a reversible aptamer-based assay, these were set aside due to practical constraints. Instead, the final design was selected for its practicality and performance: it integrates an ISFET with an enzyme-based assay to ensure reliable and continuous measurements.

A biocompatible resin chip with multiple measurement chambers was implemented, providing a reference measurement with additional potential for multiplexing multiple analytes. Finally, a dedicated readout and wireless communication PCB is used to efficiently convert and transmit the signal to a mobile application through BLE. This solution was chosen for its power efficiency and robustness, making it ideal for wearable continuous monitoring.

3.3 Signatures

Jiayi Tan	Grobbelaar.	
Jiayi Tan	**Maximilian Grobbelaar**	**Aleksei Kudrinskii**
on behalf of Prof. Hatice Altug	Team Captain	Team Captain

Chapter 4. Translation Potential

4.1 Introduction

Discussions with nephrology and cardiology experts highlighted that ACE inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) impact kidney health differently across chronic kidney disease (CKD) stages. While essential for heart failure management, these medications can worsen renal function, particularly in advanced CKD (stages 4-5), necessitating dosage adjustments and careful monitoring, especially in late-stage CKD patients where renal impairment risk is higher [Xanthopoulos et al., 2023]. This unmet need led us to develop a device for managing kidney health in vulnerable populations, particularly those with Cardiovascular-Kidney-Metabolic (CKM) diseases, where kidney issues are linked to heart conditions.

We identified two additional segments for close kidney monitoring: CKD patients over 65 and kidney transplant recipients. However, these segments were deprioritized since only about 10% of CKD patients progress to kidney failure [Ndumele et al., 2023], and geriatric patients often align more with the CKM category due to their heart-related treatments. Kidney transplant patients represent a smaller market than heart disease patients. Thus, our business model focuses on the first segment.

Targeting this segment offers the potential to reduce costly procedures by detecting nephrotoxicity early, enabling treatment adaptations, and lowering public health costs [Farrimond et al., 2023]. The Italian Society of Nephrology has shown interest in our device, even at the prototype stage, due to its potential to improve kidney and heart disease management based on direct feedback from nephrologists.

4.2 Business model canvas

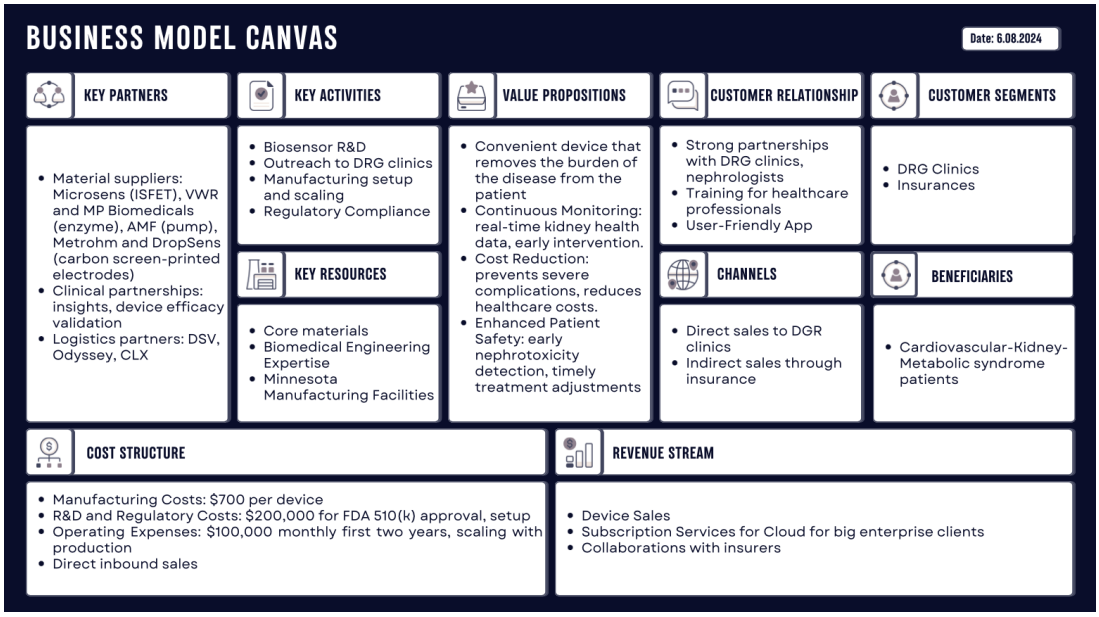


Figure 4.1: Business Model Canvas

4.3 Stakeholder desirability

Customer and stakeholders

To define our customer segment, we used the BCU (Beneficiaries, Customers, Users) framework. Figure 4.2 illustrates the benefits for each group, with the main advantage being a reduction in emergency interventions and associated costs. Prevention is key, as it is more cost-effective than treating irreversible events.

Due to the fragmented European healthcare system, we chose the more homogeneous US market, where telemedicine is advanced and widely supported. The larger US customer base, driven by factors like diet and lifestyle, further justifies this focus (Michaud et al., 2011).

We identified Diagnosis-Related Group (DRG) clinics as ideal partners, as they make independent financing decisions and handle real-world cases, making them more likely to recognize our device's benefits. DRG hospitals are incentivized to adopt cost-saving technologies, aligning with our strategy. We plan to begin manufacturing in Minnesota, targeting Mayo Clinic, Abbott Northwestern Hospital, and the University of Minnesota Medical Center—leaders in healthcare innovation and major centers for CKD and cardiac care—to ensure a strong market entry.

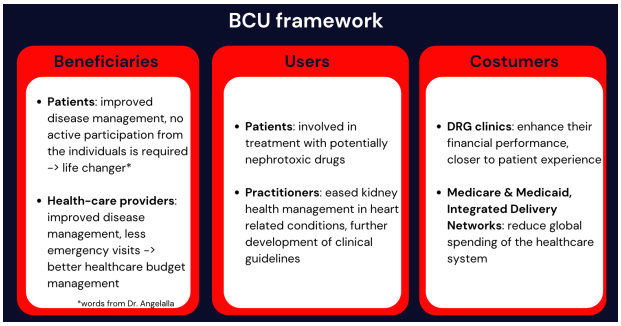


Figure 4.2: BCU framework created based on references (Farrimond et al., 2023), (Xanthopoulos et al., 2023), (Standford, n.d.).

Added value

According to the Kidney Research UK institute, over 10% of the entire population in the country will have CKD (all stages combined)(Farrimond et al., 2023). As the American Heart Association (AHA) outlined, heart and kidney complications are, in the majority of the instances, and are closely related (Ndumele et al., 2023). Therefore, from a public health point of view, it is useful to consider that being able to reduce costs related to cardiovascular diseases (through better kidney health management) is of utmost benefit (Farrimond et al., 2023). Indeed, according to the AHA: in 2016, cardiovascular diseases cost the US $555 billion. By 2035, these costs will skyrocket to $1.1 trillion. It is therefore crucial to act now, to prevent an economic bubble from forming in the healthcare sector (Association and Association, 2017).

Stakeholders needs

After multiple exchanges with field professionals, our team understood the importance that potassium has in determining kidney health. Moreover, monitoring potassium is crucial in ensuring that kidney health doesn't degrade beyond the accepted threshold once nephrotoxic drugs are prescribed to patients already exhibiting weakened kidneys (Xanthopoulos et al., 2023). To that effect, in all three technologies that have been explored for creatinine testing, the addition of potassium has also been investigated. For the chosen technology (ISFET), an ISFET selective to potassium can also be integrated. It would be the first sensor to be reached in our microfluidics, additionally allowing for the removal of some of the uncertainty concerning the ammonia membrane. Indeed, interference between the latter and potassium is a source of noise, which would be reduced by prior knowledge of potassium levels.

Competitive advantage

PROVIDER	DURATION	POINT OF CARE	COST
LetsGetChecked At Home Creatinine Test	Results in 2-5 days	At home blood sample collection, then mailed to lab for analysis	$99/per test
Nova biomedical StatSensor	Results in 30 seconds	Point-of-care testing using fingerstick capillary blood sample	$700-$800
Regular Lab Creatinine Blood Test	Results in 1-2 days from when sample arrives at lab	Blood sample collected at a lab or doctor's office, then analyzed at the lab	$17.95 - $59/per test
BioSense NephroPAL	Results in 30 seconds	Wearable at home continuous monitoring, with additional potassium measurements	$1300

Figure 4.3: While current devices on the market are promising, they still implement one-time-use measurement strategies. Our NephroPAL offers on-par time delay for measurements, with the added benefit of continuous monitoring. It is projected that within a mass production infrastructure, the cost of production for NephroPAL will significantly decrease.

4.4 Business feasibility

Timeline of the Business Strategy

This part of the report outlines the business strategy for our biosensor. The target market is the United States, and the primary considerations include manufacturing location, cost estimation, labour, equipment, business logistics, and scalability. To summarize, our timeline looks the following way 4.4.

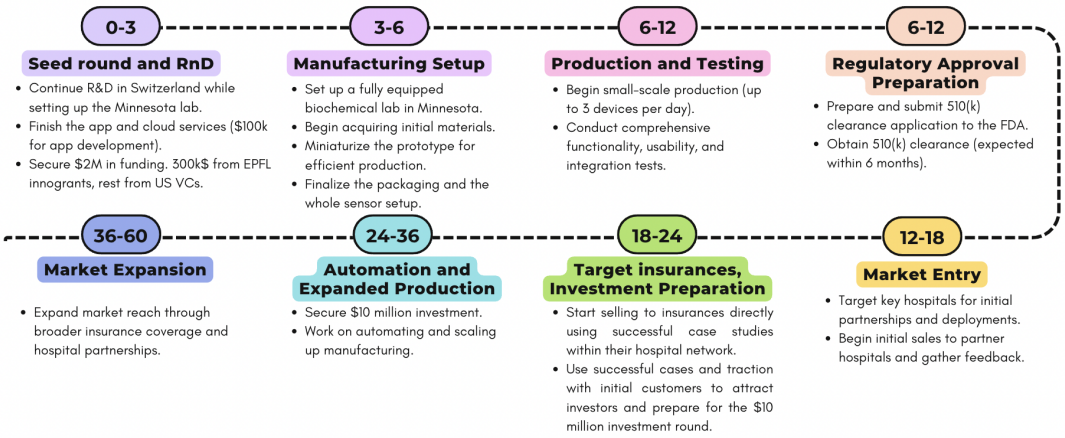


Figure 4.4: Timeline

Location Selection

After evaluating potential locations, Minnesota was chosen for several compelling reasons. Minnesota is home to major medical device companies such as Medtronic, Smiths Medical, and Nordson MEDICAL [Inven AI, 2024], which are leaders in both medical technology and healthcare innovation. The state has an established reliable supply chain, supported by a robust biotech sector working on a wide range of technologies including medical devices, pharmaceuticals, diagnostics, and therapeutics. Additionally, Minnesota boasts a skilled workforce experienced in the medical device industry, and the salaries are lower than in other major US biotech hubs: California and Massachusetts.

The decision to keep manufacturing in the US was made to avoid complex logistics and increased costs associated with overseas production. The proximity to key manufacturers and the availability of talent within the state make Minnesota an ideal location.

Key resources and expertise

Developing a sensor that effectively monitors kidney health, particularly in CKM patients, necessitates expertise in biomedical engineering, nephrology, cardiology, and biochemistry. The integration of ISFET technology and microfluidics requires collaboration with specialists in sensor technology and fluid dynamics. We also need experienced people in the sales of medical devices.

Marketing and Sales Strategy

Our primary focus is on the US market, specifically targeting DRG clinics, due to the uniformity of the healthcare system and the advanced state of telemedicine in the region. This strategy enables us to concentrate our marketing efforts on showcasing the device's key benefit—early detection of kidney problems to prevent costly interventions.

To establish a strong sales pipeline, we will begin by directly engaging with top-tier DRG clinics and healthcare institutions in Minnesota, emphasizing the device's potential for cost savings and improved patient outcomes. Digital marketing and social media will play a crucial role in building awareness and generating leads. We'll use content marketing, such as educational webinars and white papers on kidney health in CKM patients, to position the device as an industry leader. After we have the first successful cases we will go for direct inbound sales to insurance companies.

Additionally, we plan to collaborate with key opinion leaders in nephrology and cardiology, as well as prominent healthcare institutions, to build credibility and drive the adoption of the device.

Key Partners for Manufacturing and Distribution

Microsens, based in Switzerland, will supply essential ISFETs. Collaborating with US companies like Metrohm for microfluidics and chemicals will streamline our supply chain. We will also partner with medical device manufacturers in Minnesota or Texas for infrastructure and expertise, and engage local biotech startups for innovation. Long-term relationships will be pursued through joint ventures and shared R&D initiatives, enhancing product development and mutual growth.

For distribution, partnerships with hospital networks and insurance companies will facilitate early adoption, as described above.

We will also partner with DSV, Odyssey Logistics, and CLX Logistics for managing logistics of manufactured devices and logistics of all the chemicals.

Intellectual Property Strategy

Our intellectual property strategy is designed to secure and protect our innovations, ensuring market exclusivity and competitive advantage. The strategy includes phases such as initial patent filing, portfolio updates, global protection, strategic partnerships, and long-term management. These phases address needs from securing initial patents to maintaining a strong IP portfolio. Estimated costs are approximately $153,500 - $175,500 over 5 years. A detailed description of the strategy is provided in Appendix 7.6.

Compliance and Regulatory Strategies

Our medical device, classified as a Class II device, will follow the 510(k) clearance process, which is more streamlined and cost-effective compared to the Premarket Approval process required for Class III devices Synerg BioPharma, 2024. The 510(k) process typically takes about 6 months from submission to clearance, with costs ranging from $5,000 to $50,000 and submission fees around $12,432 for 2024. In contrast, the Premarket Approval process can take 1 to 3 years or more, with total costs often exceeding $1 million Starfish Medical, 2020. Our device's qualification for the 510(k) pathway offers a more straightforward regulatory process, reducing uncertainty and speeding up approval.

We will also ensure compliance with ongoing regulatory requirements, including the FDA's Quality System Regulation (21 CFR Part 820), post-market surveillance, and Medical Device Reporting (21 CFR Part 803) [FDA, 2024] [Qualio, 2024]. Adherence to ISO standards, such as ISO 13485 for quality management systems and ISO 14971 for risk management, will further ensure our device meets all necessary regulatory and safety standards. You can find more details about our strategy in the Appendix 7.7.

Environmental Impact Consideration

Given the nature of our business, manufacturing a medical device, and minimizing environmental impact is crucial. We will prioritize sustainable, recyclable materials, especially in the microfluidics system, to reduce waste. Our energy-efficient manufacturing processes will use renewable sources to lower our carbon footprint. We will also implement protocols to minimize hazardous waste and partner with specialized companies for proper disposal. A recycling or safe disposal program for end-of-life sensors will be developed in collaboration with healthcare facilities. Additionally, we will engage in local environmental projects, provide eco-friendly training for employees, and commit to transparent environmental reporting to build trust with stakeholders. Given that the business model involves manufacturing a medical device, careful consideration of environmental impact is essential. We prioritize the use of sustainable and recyclable materials, particularly in the design of the microfluidics system, aiming to minimize waste and facilitate recycling. Energy efficiency is a key focus in our manufacturing processes, with an emphasis on using renewable energy sources to reduce carbon footprint. Additionally, we will implement protocols to minimize hazardous waste, especially from chemicals used in production, and collaborate with specialized waste management companies to ensure proper disposal. A program for recycling or safely disposing of sensors at the end of their life will be established, possibly through partnerships with healthcare facilities. As part of our corporate social responsibility efforts, we will engage in local environmental projects and offer eco-friendly training programs to employees. We will also commit to transparent environmental reporting, publishing annual metrics on waste reduction, energy use, and recycling, to build trust with consumers and stakeholders.

4.5 Financial viability

We expect our device to cost $700 to produce, which includes materials, manufacturing, and other related expenses. You can find detailed breakdown and other important estimations in Table 7.9 in Appendix.
We have decided to price each device at $1,300. This price is chosen because the device can save patients approximately $2,200 annually. We aim to keep the price low to share some of these savings with customers, considering they are early adopters and taking on some risk.
We are seeking a $300,000 grant from EPFL by the third month of our operations, which is crucial for covering initial expenses and getting the business off the ground. In the first year, we will focus on research instead of sales, refining the device, and meeting regulatory requirements.
In the second year, we plan to start selling devices, beginning with 10 units and scaling up to 600 by the end of the year, reaching a break-even point of 150 devices in seventeen months after the start of the project. This growth will be supported by adding additional hospitals to our customer base and increasing the number of devices used by existing hospitals.
In the third year, we plan to raise an additional $10 million to increase our manufacturing capacity and sales efforts. With this investment, we aim to multiply our production and sales by fourfold. Monthly expenses will rise to about $700,000, but we are confident this will lead to growth. By the end of the third year, we expect to reach $1 million in monthly profits, establishing the company as financially stable.
In the appendix 7.1, you can find detailed revenue, costs and profits projection.

Market analysis

The U.S. offers a significant market, with 9.2% of the population (about 30.6 million people) affected by CKM stage 4. Many face severe health risks, including heart failure, cardiovascular issues, and strokes. We estimate that 30% of these patients will suffer a stroke or other major heart event, creating a potential market of around 9.19 million people, highlighting the urgent need for risk mitigation in advanced CKM.
Potassium management costs primarily arise from labor and lab analysis, totaling $126 (FindLabTest, n.d.). Our device eliminates the need for routine hospital visits and lab tests, reducing costs as patients would only visit for treatment adjustments.

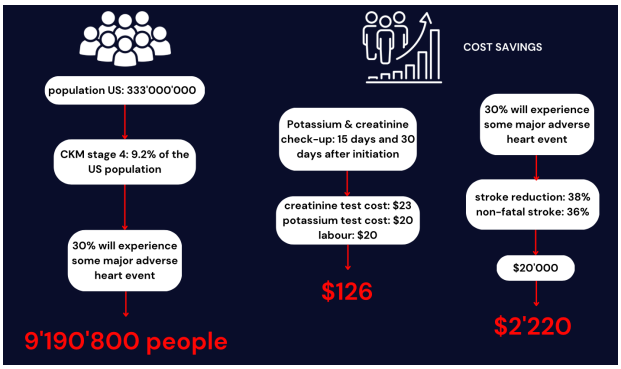


Figure 4.5: Market analysis based on data from (Papademetriou et al., 2016), (FindLabTest, n.d.), (Lorio et al., 2024).

Studies indicate that appropriate treatment can reduce stroke incidence by 37% (Papademetriou et al., 2016), saving approximately $2,200 per prevented stroke. This demonstrates that effective CKM management can significantly lower intervention costs.
We estimate the total addressable market at $20 billion, with the Serviceable Available Market at $10 billion (50% of TAM) and the Serviceable Obtainable Market at $2 billion (20% of SAM).

Chapter 5. Team and Support

5.1 Contributions of the team members

Jennifer Joey Ayer: Developed the ISFET sensing option during the semester and was the system engineer.
Ahmed Skander Ben Messaoud: Developed the optical chip needed for the photonics-based sensing system with aptamers, later helped with the microfluidics and the electrochemical measurements.
Nina Bodenstab: Developed the mobile application and worked on the Bluetooth signal transmission from the device to the smartphone.
Emile Caillol: Designed, produced and tested the microfluidics system.
Emma Genova Coimbra: Interviewed the doctors, worked on the business plan, took care of our social media outreach, and logistics, organized the team buildings and assisted in the ISFET sensing lab work.
Maximilian Grobbelaar: Team captain taking care of the administrative work, team buildings, and assisted in screen printed electrodes.
Aleksei Kudrinskii: Team captain taking care of the administrative work. Worked on the translational potential and the data processing.
Emi Myzeqari: Worked on the microfluidics system, and helped take measurements for the sensing system.
Ekin Özberk: Developed the sensing system based on screen-printed electrodes.
Cyrill Reding: Worked on the bioassay for the optical aptamer-based method, and managed the ordering of materials. Then helped with the functionalization of the ISFET system.
Pia Rosenkranz: Worked on the bioassay for the optical aptamer-based method.
Sascha Rivera: Developed the hardware of the electronics.
Laetitia Schwitter: Worked on the translational potential, managed the ordering of materials and finances and helped develop the ISFET and the aptamer-based sensing option during the summer.
Antoine Violet: Developed the app and worked on the Bluetooth signal transmission from the device to the smartphone.

5.2 People who have given support

Méline Cretegny: Helped with the team creation and gave valuable support regarding the business aspects of the project.
Julian Bär: Helped with the team creation, organizational aspects and communication with EPFL labs.
Ali Elmorsy: Gave valuable technical advice throughout the project.
Marin Bricq: Helped with the website and the organization's finances.
Ulrike Lehmann: Was our point and contact with Microsens SA and gave significant insights on the use of ISFETs.
Prof. Hatice Altug : Was our supervisor and provided critical feedback on the different systems and provided additional expertise on the biophotonics sensor.
Prof. Nako Nakatsuka: Was one of our academic advisors, gave critical opinions on all systems and provided her expertise in aptamers for the biophotonics sensor.
Prof. Sandro Carrara: Was one of our academic advisors, provided his expertise on electrochemical sensors.
PhD student Francesca Rodino: Supervised the electrochemical sensors development and provided her experience.
PhD student Jiayi Tan: Provided her help in biophotonics system as well as with ordering and labwork.
Dr. Simone Vettoretti: Helped by giving valuable insights on the market side and suggestions on our device.
Dr. Menno Pruijm: Helped with valuable insights on our device and by organizing interviews with team members and patients.

5.3 Sponsors and partners

EPFL MAKE: Offered financial aid and vital infrastructure for the sensor development by granting access to labs, machinery, and materials.

Bio/CMOS Interfaces (BCI) Group: Hosted two students for semester projects, sponsored part of the technological development. Their advice was crucial for electrochemical sensor development.
BioNanoPhotonic Systems (BIOS) lab Hosted one student during the semester. The lab granted access to their lab, machinery, and technological advice throughout the semester and summer.
Hybrid Photonics lab (HYLAB) Hosted one student during the semester to develop the photonic circuits for the aptamer bioassay and perform measurements in the optical lab.
Laboratory of Nanoscale Biology (LBEN) lab Hosted one student during the semester to develop the aptamer bioassay.
Laboratory of Systems Biology and Genetics (Deplancke) Lab Hosted students during the semester to develop the different microfluidic systems.
Microsense Were crucial industrial advisors for the ISFET method and kindly lent us ISFETs and their interface.

Chapter 6. Final Remarks

Tasked with developing a novel continuous creatinine sensor, our team has decided to pursue an innovative solution rather than opting for established methods. The taken approach was not conventionally adopted in literature, but was developed as a result of exploring multiple research papers. The innovative character of the enzymatic ISFET based sensor meant more difficulties encountered in its development, but it was ultimately the most promising from the explored sensing options.

In fact our team has worked on two additional sensor techniques, one optical, based on an integrated photonic interferomter, and the other based on the more conventional screen printed electrodes.

The interferometric approach, though promising in terms of sensitivity and selectivity, was difficult to purse due to challenges mainly related to the lengthy fabrication process, the implementation of the setup for coupling the light to and out of the chip, and the proper coupling of the aptamers.

On the other hand, the SPE method, while straightforward, affordable, and easy to implement, is not particularily innovative, less adapted for continuous sensing, and did not perform well in complex media.

These experiences motivated us to ultimately pursue the ISFET based enzymatic sensor technology, which we believe holds the most promise.

However, we invite you to have a look at the work we have accomplished with the other two methods, which can be found in the appendix for further insights.

We would like to extend our heartfelt gratitude to all those who supported us throughout this journey. Special thanks to our supervisors, academic advisors, and coaches for their invaluable guidance and encouragement. We would like to thank the Jury and the general public for your interest in our work.

Looking ahead, we are excited about the future possibilities that our biosensor holds. Post-competition, we plan to continue refining and enhancing the ISFET technology, with the aim of bringing our innovative biosensor closer to market. Our long-term vision includes further exploring our sensor within the Biosense EPFL association through semester projects and association events.

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Chapter 7. Appendix

Our team has been developing two other sensing options, one based on interferometry and the other using screen-printed electrodes. In the following sections, we will present the work our team has done with these techniques, showcasing our results and the challenges encountered in their development.

7.1 Optical Detection

Optical detection can be harnessed to produce highly sensitive biosensors with label-free operation within small portable devices by making use of integrated photonics (Ramirez et al., 2022).

With integrated photonics, table-top setups and optical devices can be miniaturized to manipulate and process light in compact chips. The technology builds on the legacy of IC and microfabrication, enabling ultra-scaled and low-cost devices.

Our team has explored this path by designing and implementing a biosensor based on a silicon photonic Mach Zehnder interferometer.

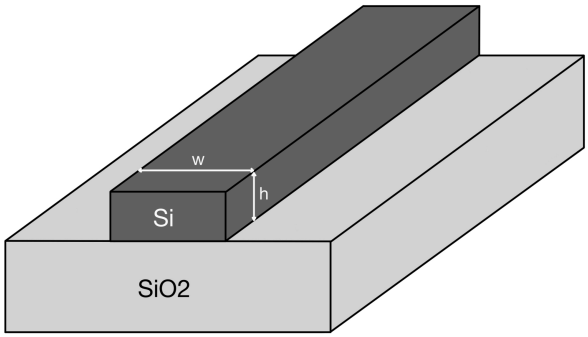


Figure 7.1: Schematic of silicon waveguide on a silicon oxide substrate

The basic building bloc of a photonic integrated circuit is the waveguide, which is a structure that confines and guides the light using total internal reflection. A schematic of a silicon waveguide with silicon oxide substrate can be seen in fig.7.1. The waveguides are patterned on an SOI wafer using e-beam lithography, with typical dimensions of $w = 450\ nm$ and $h = 220\ nm$.

The light propagating in the waveguide is not fully confined in the silicon core, a part of it propagates outside the core in what is called the evanescent field. The evanescent field can be used in biosensing to sense the presence of target molecules in the vicinity of the waveguide. This is done by functionalizing the surface of the waveguides with bioreceptors which specifically capture the target analyte, as seen in fig.7.2.

The presence of the target molecule will affect how the light propagates along the waveguide. The variation of the local mass density surrounding the waveguide will alter a property of the propagating light, the effective refractive index. This will translate into a variation of the phase accumulated by the light along the waveguide.

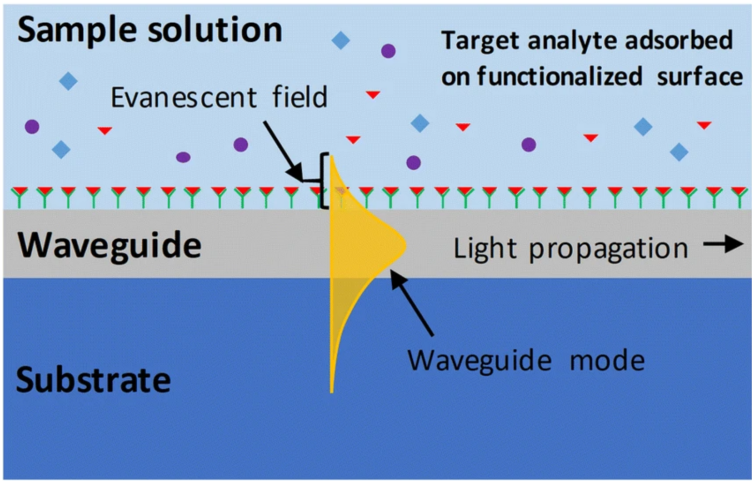


Figure 7.2: Schematic of evanescent field sensing (Ma et al., 2020)

This variation of accumulated phase should then be translated into a variation of light intensity which can be measured using a photodiode. This can be achieved by placing the functionalized waveguide in an interferometric configuration as shown in fig.7.3.

Infrared light from an input laser is coupled to the interferometer using a grating coupler. It is then split between two waveguides, one used as a reference, and one for sensing.

The reference waveguide is isolated from the sample, by cladding it with silicon oxide (fig.7.4a), such that the light propagating in it accumulates a fixed phase.

In the sensing arm, a window is opened to expose the waveguide. The waveguide is then functionalized with aptamers (fig.7.4b), and sample delivery is ensured through a microfluidic cell. Aptamers, short single-stranded DNA or RNA, can bind to a specific analyte similarly to antibodies. For the photonic sensor, a 5' thiol ending anti- creatinine aptamers were immobilized through silane chemistry on the sensing arm. This brings creatinine selectively into proximity of the waveguide, which in turn would suggest refractive index change. With a strong immobilization and aptamers relative strong stability (Thiviyanathan and Gorenstein, 2012), this system too, is ideal for a continuous sensing approach.

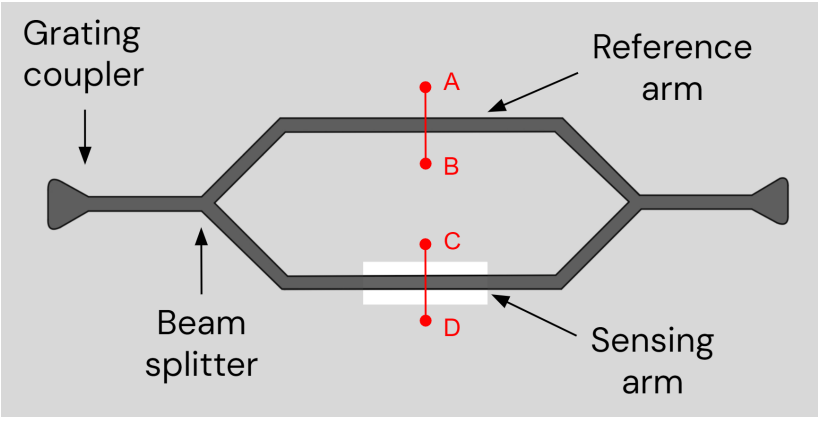
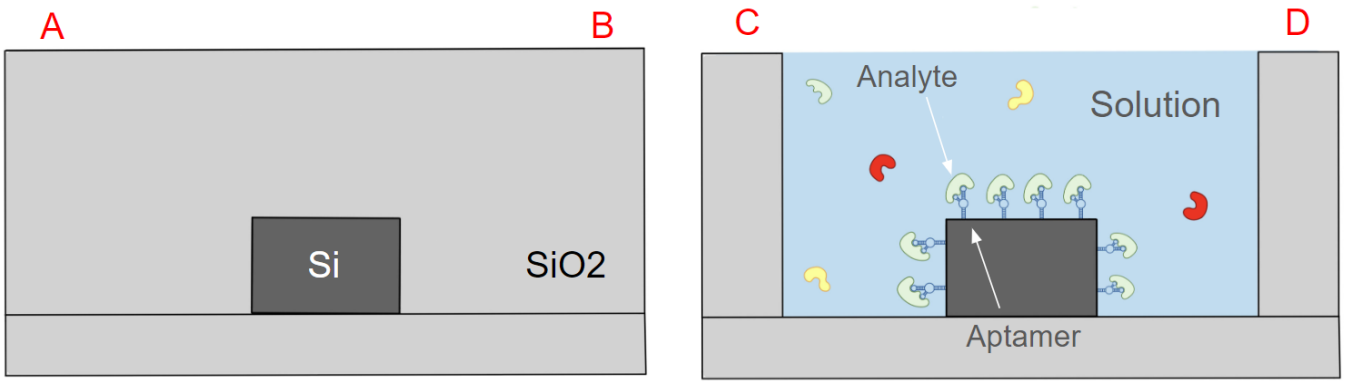


Figure 7.3: Top schematic of the Mach Zehnder interferometer



(a) Cutline of the reference arm

(b) Cutline of the sensing arm

Figure 7.4: Cross sections of the reference arm and sensing arm

As the analytes gradually bind to the receptors, the phase accumulated in the sensing arm will gradually shift. As a result, when recombining the light that has propagated in both arms, interference will occur: $I_{out}(t) \sim cos^2(\frac{\Delta\Phi(t)}{2})$ where $\Delta\Phi$ is the difference in accumulated phase between the two arms. The resulting signal is then collected from the output grating and the intensity is measured through a photodiode. From the output signal, the phase shift in the sensing arm can be retrieved and linked to the concentration of the analyte.

We conducted the fabrication of the photonic circuits at EPFL's cleanroom facilities, fig.7.5 showcases an SEM image of the implemented design following the patterning of the waveguides. The waveguide arms are implemented through compact spirals to increase the interaction length while maintaining a compact footprint. Figure 7.6 showcases the device following the deposition of the SiO2 cladding and the opening of the window in the sensing arm.

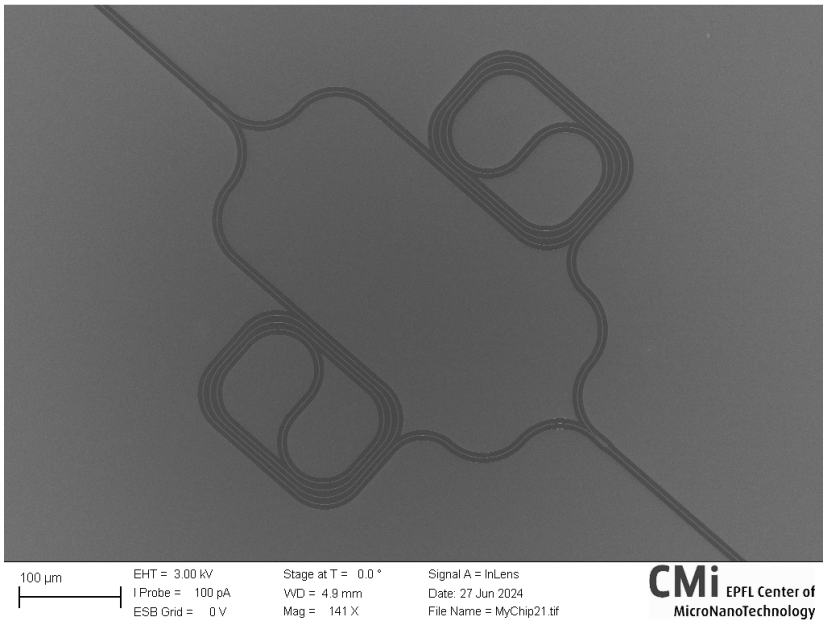


Figure 7.5: SEM image of the implemented device structure

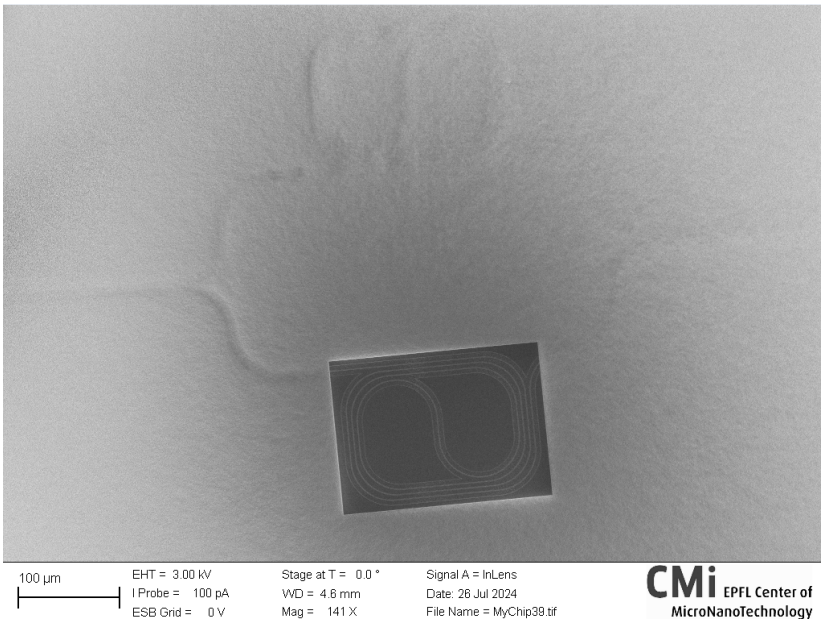


Figure 7.6: Top schematic of the Mach Zehnder interferometer

The functionality of the photonic circuits is tested by sweeping the input wavelength (Halir et al., 2013). An interferometric signal, combined with the pass-band response of the grating coupler is observed (fig. 7.7), confirming the proper functioning of the interferometer's components.

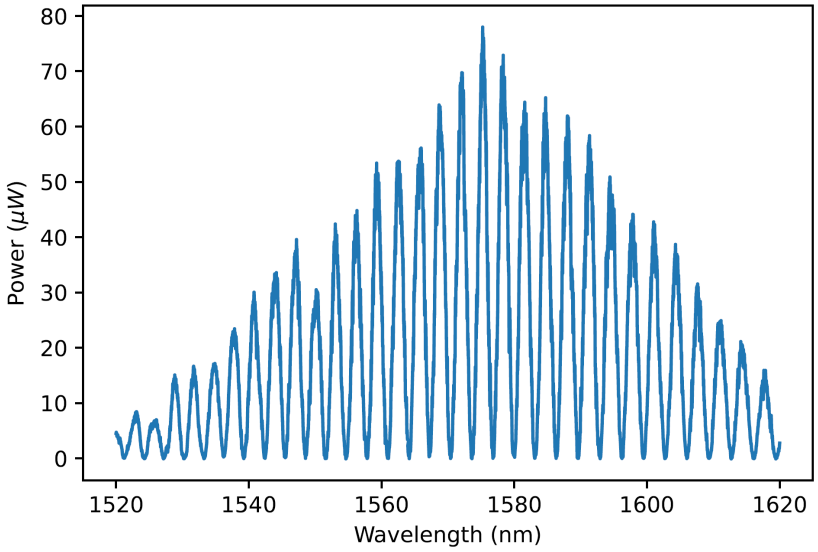


Figure 7.7: Top schematic of the Mach Zehnder interferometer

Unfortunately, important delays have been encountered in the fabrication of the devices as well as the shipment of the aptamers. Due to the testing delays and complexity associated with building a prototype out of this approach (especially in terms of coupling the light and integrating the microfluidics), pursuing this approach was no longer realistic in the available timeframe, it was therefore aborted to focus on building the electrochemical sensor's prototype.

7.2 Enzymeless Creatinine Detection using Screen Printed Electrodes

Screen-printed electrodes (SPEs) provide a cost-effective, precise, and user-friendly method for electrochemical measurements (Paimard et al., 2023). These devices are structured as a three-electrode cell, consisting

of a working electrode, a counter electrode, and a reference electrode. They are printed with conductive inks such as gold, silver, platinum, or carbon onto substrates like ceramic or plastic. SPEs are favoured for their affordability, ease of reproduction, and adaptability in modifying the working electrode to enhance sensitivity for specific analytes.

Due to their accessibility, low cost, and versatility, SPEs are highly suitable for electrochemical sensing. In our research on various sensors for creatinine detection, we chose to explore SPEs for creatinine measurements. Creatinine does not react with common conductive inks like carbon, platinum, silver, or gold. To overcome this, we modified the electrodes by adding copper. This allowed us to use a method where copper interacts with creatinine to enable redox reactions, facilitating the detection of creatinine without enzymes using copper-modified SPEs. (Domínguez-Aragón et al., 2023)

Our initial approach involved a basic creatinine sensing method, as detailed in the referenced paper Domínguez-Aragón et al., 2023. We began by pretreating the electrode in 0.1M PBS through chronoamperometry, followed by cyclic voltammetry. We then electrodeposited copper by immersing the electrode in a copper sulfate pentahydrate solution and applying a constant -0.6V for 50 seconds to deposit copper nanoparticles on the working electrode. This process is visually outlined in Figure 7.8.

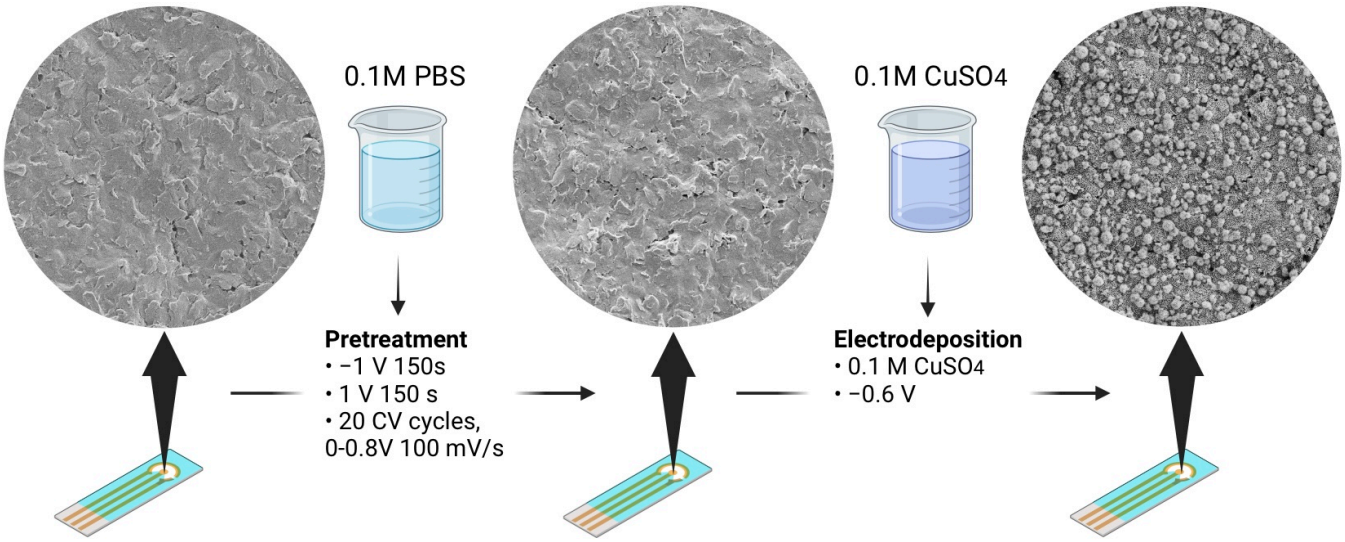


Figure 7.8: Pretreatments and Copper-Electrodeposition Process Flow for Carbon SPEs.

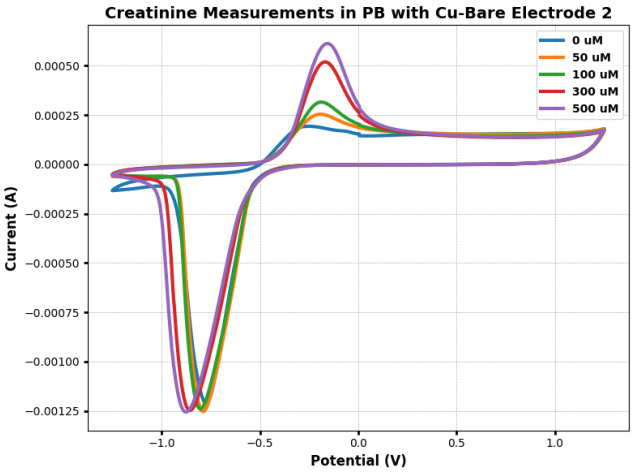


Figure 7.9: Cyclic Voltammetry Results for Varying Creatinine Concentrations in Phosphate Buffer using a Cu/Carbon SPE.

Initial measurements were conducted in Phosphate Buffer (PB) using Cyclic Voltammetry across various creatinine concentrations. The voltage range was set from -1 V to 1 V with a scan rate of 100 mV/s. Distinct redox peaks around -0.1 V were noted for each concentration, as depicted in Figure 7.9, which shows the CV curves for creatinine in PB using Cu/Carbon-SPE. However, this method proved insufficient in phosphate-buffered saline (PBS) where added chlorine salts (NaCl and KCl) caused significant interference. The reactive chlorine hindered the detection of creatinine-copper complex redox peaks. Consequently, we substituted carbon electrodes with platinum, significantly enhancing the measurement system, as detailed in the referenced paper Chen and Lin, 2012.

In further experiments, we used copper electrodeposition on platinum screen-printed electrodes (SPEs), successfully correlating the current at the redox potential of -0.1 V with the creatinine concentrations, as depicted in Figure 7.10. These measurements were conducted using cyclic voltammetry between -0.25 V and 0.0 V, with a scan rate of 100 mV/s. Despite these advancements, the enzymeless detection method using SPEs was found to be insufficient for precise creatinine measurement in artificial interstitial fluid, due to numerous interferences in this complex medium.

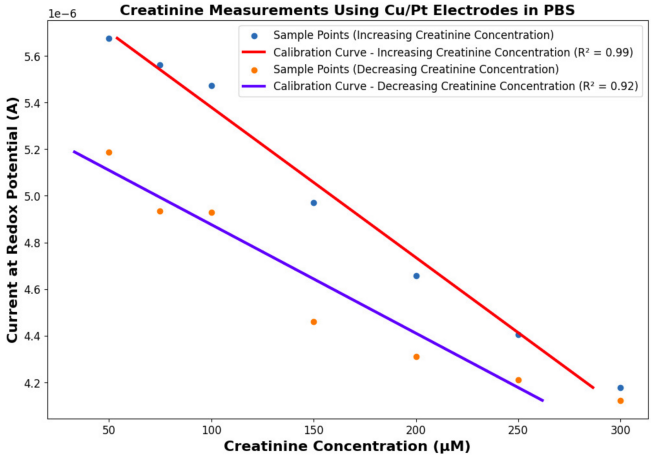


Figure 7.10: Current at Redox Peak of -0.1V for Cyclic Voltammetry Measurements for Varying Creatinine Concentration in Phosphate Buffer Saline using a Cu/Pt SPE.

7.3 Microfluidics

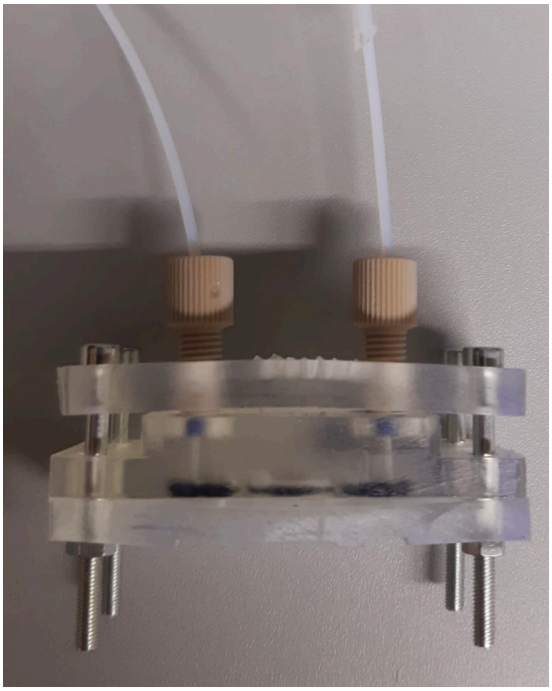
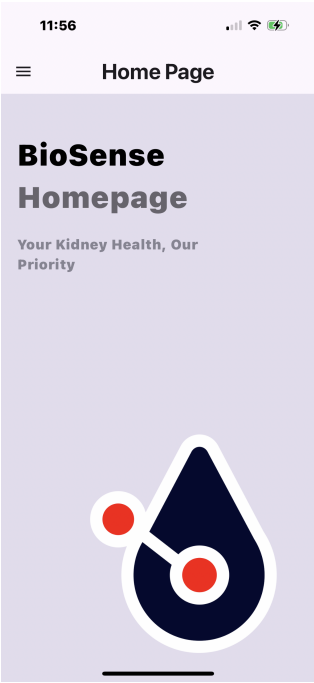
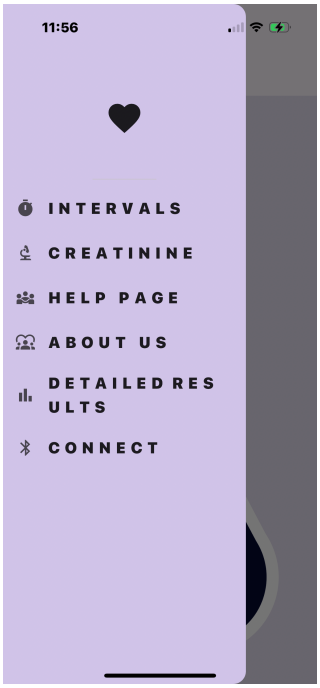
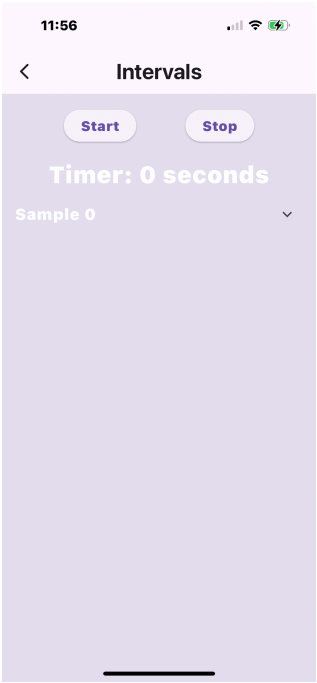


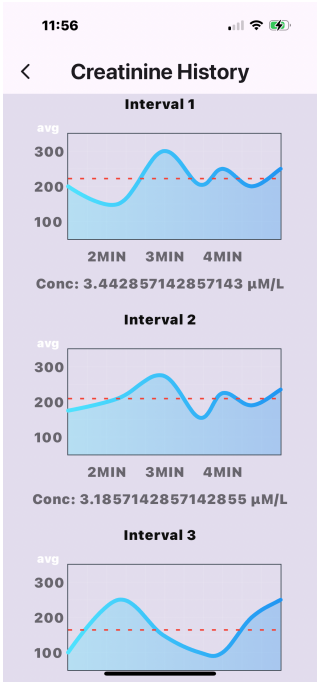
Figure 7.11: In real life realisation of the microfluidic system.

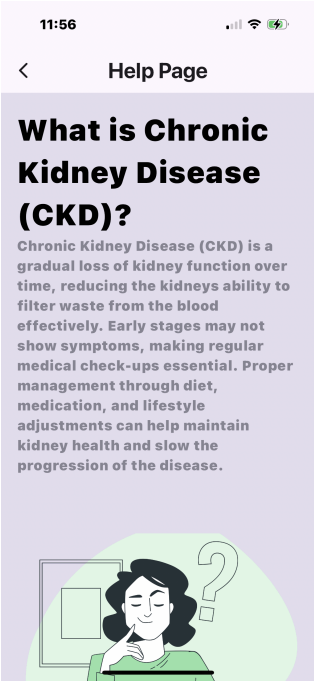
7.4 Images of smartphone app GUI

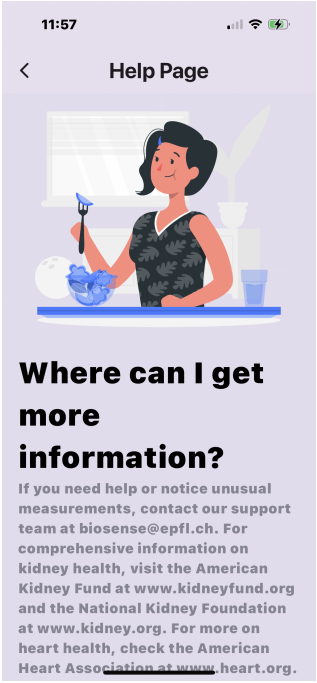


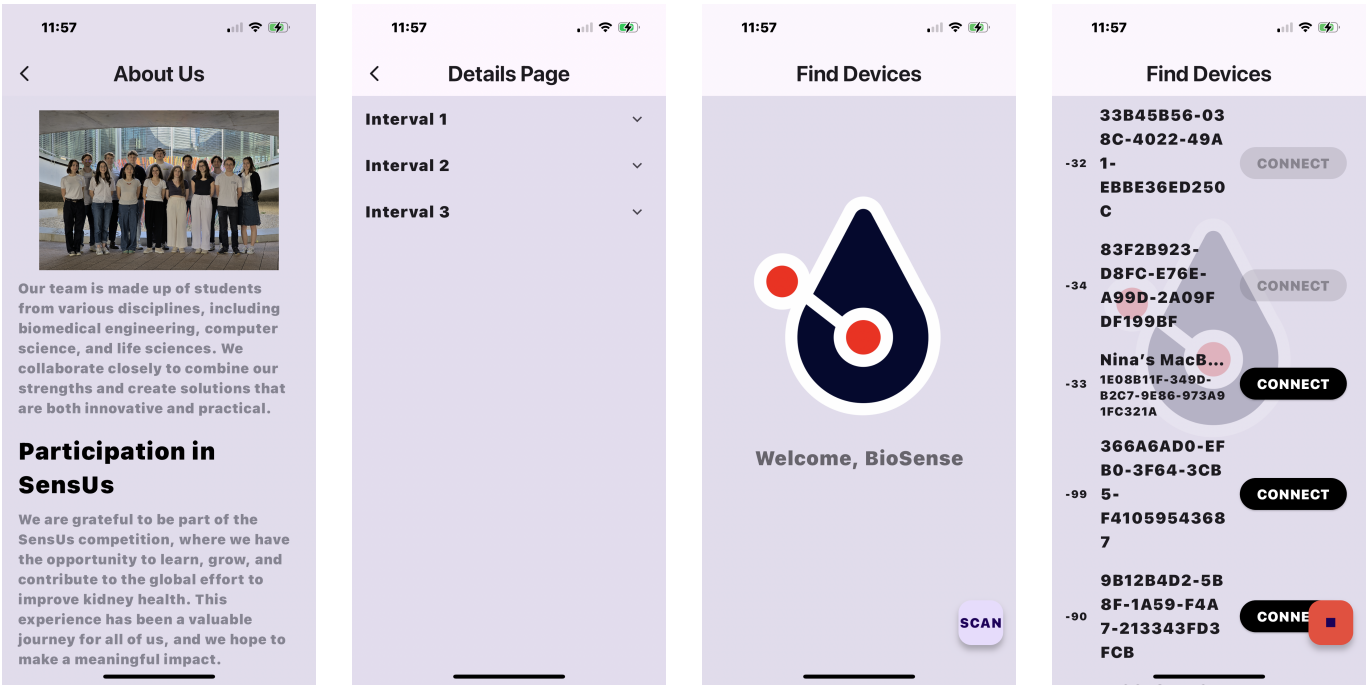












The images used in the app are open-source and copyright-free, please see references: Storyset, 2024b, Storyset, 2024a, and Storyset, 2024c. Other resources used are the extensive documentation by fl_charts: FL Chart App, n.d. as well as the official Flutter documentation: Flutter Documentation, n.d. To implement the Bluetooth, the following "flutter blue plus" GitHub was cloned KG, 2024.

7.5 Business Model Canvas

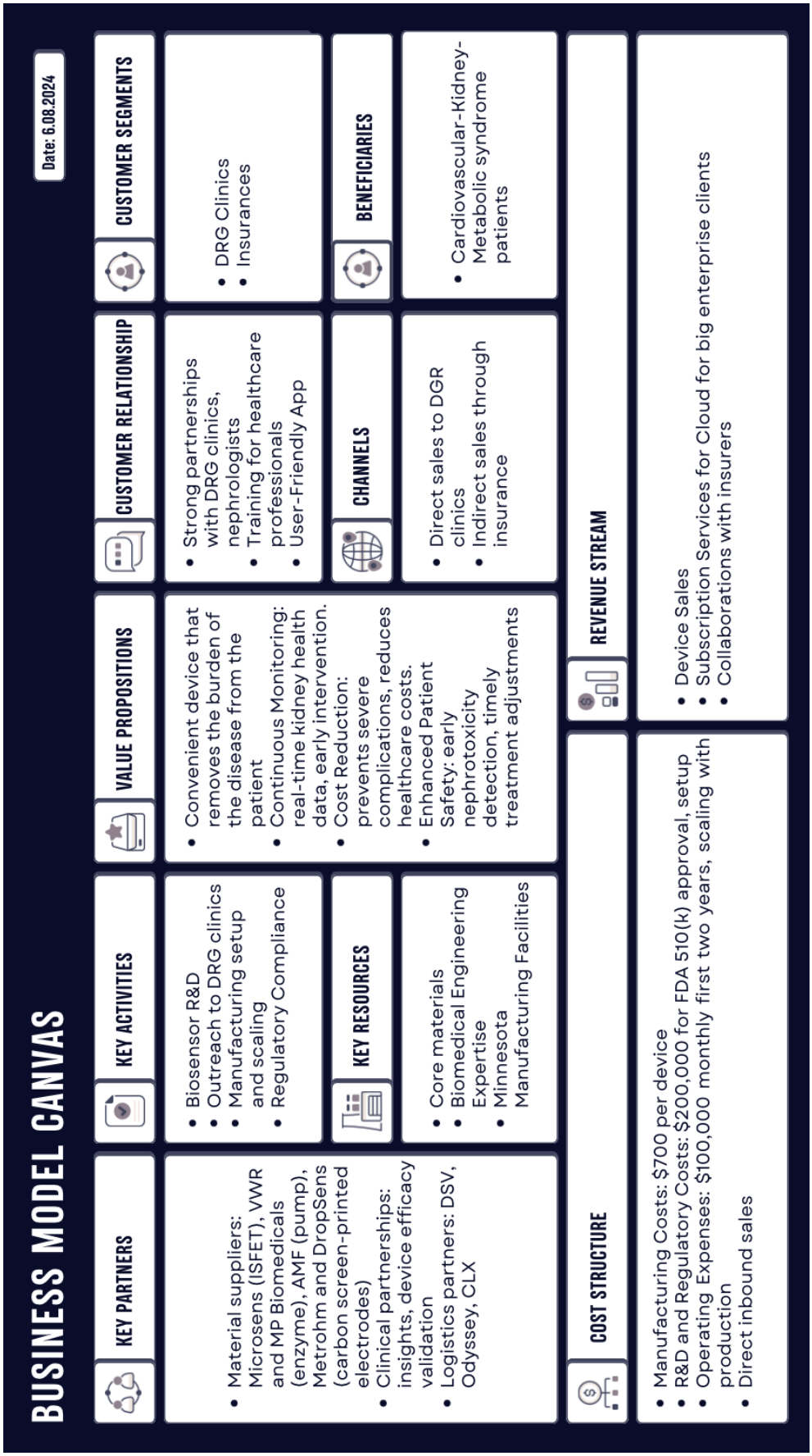


Figure 7.12: Business Model Canvas, updated to 6.06.2024.

7.6 Intellectual property strategy

1. Initial Patent Filing (Months 0-12): We begin by filing a provisional patent application to establish a priority date, costing approximately $2,500 and taking about 3 months. This is followed by a comprehensive patent search to confirm the novelty of our invention, costing around $5,000 over another 3 months. Finally, we prepare and file a non-provisional patent application based on refined invention details, with costs ranging from $10,000 to $15,000, over 6 months. This phase is crucial for securing our intellectual property early on.

2. Portfolio Updates and Global Strategy (Months 12-24): As we continue development, we file additional patents for any new features or modifications, with each modification costing around $5,000. We plan to file these every 3-6 months to keep our portfolio up-to-date. By months 18-24, we file a Patent Cooperation Treaty (PCT) application for international protection, costing $4,000 to $5,000, plus $2,000 to $3,000 per country for national phase entry, initially targeting five key markets. This phase ensures that our intellectual property is protected globally.

3. Strategic Licensing and Partnerships (Months 24-36): We identify and negotiate licensing agreements with established medical device manufacturers, incurring legal fees of around $10,000 over 6 months. We also set up monitoring services to detect potential patent infringements, costing $5,000 per year. These actions help monetize our patents and protect our market position.

4. Continuation Applications and Design Patents (Months 36-48): To refine our patent claims and address technological advancements, we file continuation applications costing $3,000 to $5,000 each. Additionally, we secure design patents for our device's unique features, with costs ranging from $1,500 to $2,000 per design. This phase ensures our intellectual property remains current and comprehensive.

5. Long-Term Patent Portfolio Management (Months 48+): We conduct regular Freedom-To-Operate analyses annually, costing $10,000 per analysis, to ensure we do not infringe on existing patents. Additionally, we maintain and renew our patents, with annual fees ranging from $2,000 to $3,000 per patent. This phase focuses on sustaining and managing our IP portfolio over the long term.

7.7 Compliance and Regulatory Strategies

510(k) Clearance [Synerg BioPharma, 2024]

- **Timeframe:** Typically 6 months from submission to clearance.
- **Costs:** $5,000 to $50,000 for the process; submission fee of $12,432 (FY 2024).
- **Advantages:** Faster market entry, lower costs, and a more predictable regulatory environment by demonstrating substantial equivalence to an already approved device.

PMA (Premarket Approval) [Starfish Medical, 2020]

- **Timeframe:** 1 to 3 years or longer.
- **Costs:** Often exceeding $1 million, with a submission fee of $368,000 (FY 2024).
- **Requirements:** Extensive clinical trials and rigorous review process.
- **Applicability:** Required for high-risk Class III devices.

FDA Regulations[FDA, 2024]

- **Quality System Regulation (QSR):** 21 CFR Part 820 requires adherence to quality management systems, including design controls and production processes.
- **Post-Market Surveillance:** Continuous monitoring of device performance in real-world settings, reporting adverse events, and conducting post-market studies.
- **Medical Device Reporting (MDR):** 21 CFR Part 803 mandates the reporting of incidents where the device may have caused or contributed to a death or serious injury.

ISO Standards [Qualio, 2024]

- **ISO 13485:** Specifies requirements for a quality management system for medical devices, recognized by the FDA as part of its Quality Management System Regulation (QMSR).
- **ISO 14971:** Provides a framework for risk management in medical devices, outlining processes for identifying hazards, estimating and evaluating risks, controlling these risks, and monitoring the effectiveness of the controls.

7.8 Costs, revenue and profits projection

Table 7.1: Costs / Revenue / Profits Projection

Month	Sold Devices	Cost	Revenue, 1k$	Profit, 1k$	Cumulative Profit, 1k$
1.0	0.0	200.0	300.0	100.0	100.0
2.0	0.0	100.0	0.0	-100.0	0.0
3.0	0.0	100.0	1700.0	1600.0	1600.0
4.0	0.0	100.0	0.0	-100.0	1500.0
5.0	0.0	100.0	0.0	-100.0	1400.0
6.0	0.0	300.0	0.0	-300.0	1100.0
7.0	0.0	100.0	0.0	-100.0	1000.0
8.0	0.0	100.0	0.0	-100.0	900.0
9.0	0.0	100.0	0.0	-100.0	800.0
10.0	0.0	100.0	0.0	-100.0	700.0
11.0	0.0	100.0	0.0	-100.0	600.0
12.0	0.0	100.0	0.0	-100.0	500.0
13.0	10.0	107.0	13.0	-94.0	406.0
14.0	10.0	107.0	13.0	-94.0	312.0
15.0	10.0	107.0	13.0	-94.0	218.0
16.0	10.0	107.0	13.0	-94.0	124.0
17.0	50.0	135.0	65.0	-70.0	54.0
18.0	100.0	170.0	130.0	-40.0	14.0
19.0	200.0	240.0	260.0	20.0	34.0
21.0	300.0	310.0	390.0	80.0	114.0
22.0	400.0	380.0	520.0	140.0	254.0
23.0	500.0	450.0	650.0	200.0	454.0
24.0	600.0	520.0	780.0	260.0	714.0
25.0	600.0	520.0	10780.0	10260.0	10974.0
26.0	600.0	1120.0	780.0	-340.0	10634.0
27.0	800.0	1260.0	1040.0	-220.0	10414.0
28.0	1200.0	1540.0	1560.0	20.0	10434.0
29.0	1600.0	1820.0	2080.0	260.0	10694.0
30.0	2000.0	2100.0	2600.0	500.0	11194.0
31.0	2200.0	2240.0	2860.0	620.0	11814.0
32.0	2400.0	2380.0	3120.0	740.0	12554.0
33.0	2600.0	2520.0	3380.0	860.0	13414.0
34.0	2800.0	2660.0	3640.0	980.0	14394.0
35.0	3000.0	2800.0	3900.0	1100.0	15494.0
36.0	3400.0	3080.0	4420.0	1340.0	16834.0

7.9 Financial estimates

Item	Cost (USD)
Production Costs	
Materials, manufacturing, and other related expenses	700
Breakdown of key components:	
ISFETs	200
Enzymes for sensing	40
Chemicals for enzyme functionalization	50
Readout circuits	20
Microfluidics system materials	30
Bluetooth transmission electronics	5
Total cost per device	**700**
Selling price per device	**1,300**
Profit per device	**600**
Additional Expenses	
Lab setup (initial equipment, R&D)	100,000
501(k) regulatory approval process	200,000
Monthly operating expenses (first 2 years)	100,000

Table 7.2: The table summarizes the production costs, selling price, and additional expenses associated with the device. The production cost per device is $700, including key components such as ISFETs, enzymes, and microfluidics system materials. The device is priced at $1,300, aiming for a profit of $600 per unit.