Team Results Document
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SensUs

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1 Abstract: Summary for the SensUs website

Through interviews with both patients and doctors, we found out that patients treated with levodopa are seeking a biosensor that would give them similar experience that patients with diabetes have with continuous glucose monitoring (CGM) devices. Therefore, we tried to match the design of CGM as much as we could.

Our device is a small (5 cm diameter, 1 cm height) disk shaped object with a single microneedle sticking out from its bottom. The ISF sample, extracted through the microneedle, is brought to a sensing chamber containing 2 working electrodes. The first electrode is used to measure chronoamperometry of the direct oxidation of Levodopa, a robust and simple way of measuring levodopa concentrations. The second electrode contains an immobilized direct electron transfer (DET) enzyme. This DET enzyme oxidizes Levodopa and is then reduced back directly by the electrode, speeding up electron transfer drastically and producing signal directlz from the enzyme regeneration. The combination of 2 sensing methods, known as orthogonal sensing provides both selectivity and sensitivity. The rest of the device is filled with a custom made potentiostat, a battery, and a waste compartment.

The device aims provide a measured value every 10 minutes for a duration of 14 days.

2 AP award: Biosensor developed for the Eindhoven Testing Event

2.1 Molecular recognition

Our main molecular recognition element is a **direct electron transfer (DET) enzyme** that oxidizes levodopa to dopaquinone. DET enzymes do not have the ability to be regenerated by reactions with other substrates (e.g. oxygen) and are instead regenerated by an electron transfer with an electrode (see Figure 2.1). This brings many advantages for continuous biosensing, the main one being that **the regeneration of the enzyme is the source of the measured signal**.

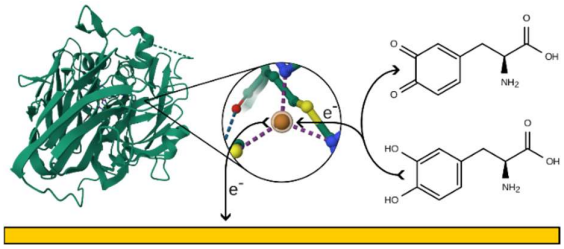


Figure 2.1 Detection using the DET enzyme.

To make such an enzyme, our team utilized the work reported in Batchu *et al*., 2025. In this work, mutations are introduced to a multicopper oxidase from Pyrobaculum aerophilum, leading to a deactivation of reaction centers responsible for enzyme regeneration. Our team modified the production and immobilization procedures to improve on the original article (see Appendix A).

A second working electrode (WE) was used for **direct oxidation** of levodopa to dopaquinone. This approach of combining two different recognition methods is known as orthogonal sensing.

2.2 Physical transduction

We utilize an electrochemical system consisting of two gold WEs, a gold counter electrode (CE), and a Ag/AgCl reference electrode (RE). The WEs and CE are arranged in a **stacked plate design** shown in Figure 2.2. **The reference electrode is positioned inside a side channel** filled with 3M KCl. An PVB gel is used to make a porous plug. The WEs and CE are treated with mercaptohexanol to have a simple antifouling layer.

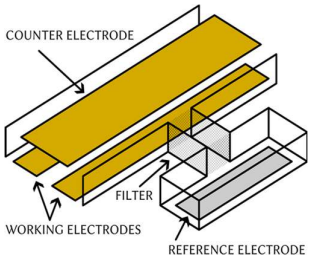


Figure 2.2 Electrode configuration. Not to scale.

Chronoamperometry was used with both working electrodes. The used potentials were +0.2 V vs RE for the DET enzyme and +0.4 V vs RE for direct oxidation. The calibration curvers in the mock ISF are available in Figure 2.3.

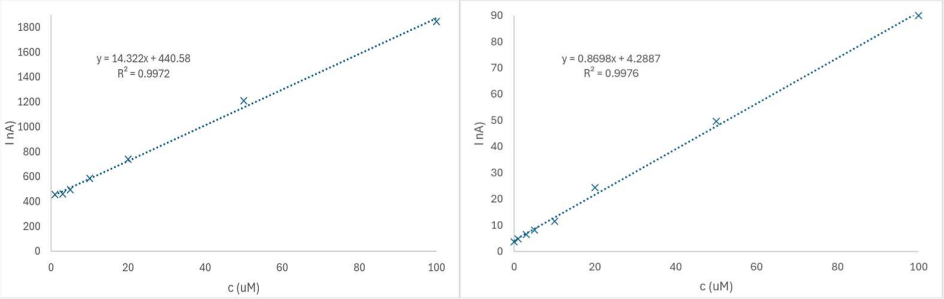


Figure 2.3: Calibration curves for direct oxidation (left) and DET enzyme (right).

2.3 Cartridge technology

The cartridge design centres around the stacked plate electrode geometry shown in Figure 2. We build it using **layers of 170µm polycarbonate foil (PCF) and 130 µm double sides adhesive (DSA)**. The microchannel geometry was cut from these layers by a **cutter plotter**. A microchannel cross section is shown in Figure 2.4 and the whole assembly in Figure 2.5. This design leads to an **internal volume of ~15 µL** for the whole cartridge with a **single WE surface area of 0.17 cm²**.

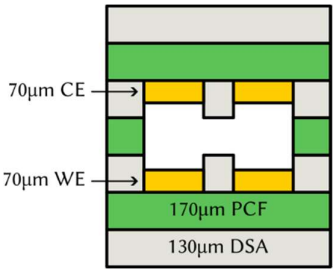


Figure 2.4 Microchannel cross section.

To handle the sample during the competition, capillary tubing is inserted into the inlet and outlet. The tubing from the outlet is connected to a syringe pump and the tubing from the inlet is immersed into the sample provided in a microtube. A flow rate of 20 µL/min will be used. The measuring cycle is: 1. Put the inlet tubing into a microtube with a sample. 2. Start the pump. 3. Let the sample flow for 2 min (exchanging the internal volume >2 times). 4. Record the measured values. 5. Stop the pump, 6. After 15s remove the microtube with the sample. 7. Repeat.

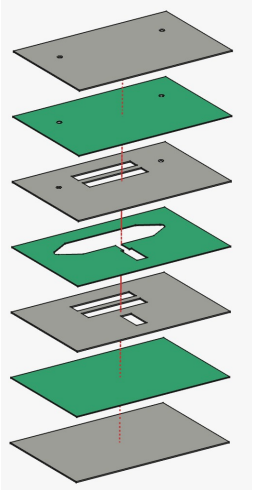


Figure 2.5 Exploded view of the cartridge layers.

2.4 Reader instrument and user interaction

We use a custom made potentiostat that has an option for Bluetooth or wired connectivity and can be powered by a small included battery. The potentiostat is capable of chronoamperometry or cyclic voltammetry either on one electrode or on both working electrodes by rapidly switching between them. The parameters of this switch and also the parameters of the electroanalytical methods can be adjusted in a GUI software developed for it by the team. Screenshot of this interface is available in Appendix B.

3 IN award: Biosensor innovation

3.1 Wearable sensor

The design of our proposed biosensor iterates on the design of the prototype used for testing during the Eindhoven Testing Event. To make a complete biosensor, the cartridge's inlet is connected to a micro needle, and the outlet is connected to a waste compartment that also serves as the source of negative pressure driving the fluid extraction and flow. These are then, together with the potentiostat and a battery, enclosed in a small (5 cm diameter, 1 cm height) circular case as shown in Figure 3.1. To increase the manufacturability of the cartridge, it was replaced by 2 thin film electrode plates connected by a single layer of double-sided adhesive (Figure 3.2).

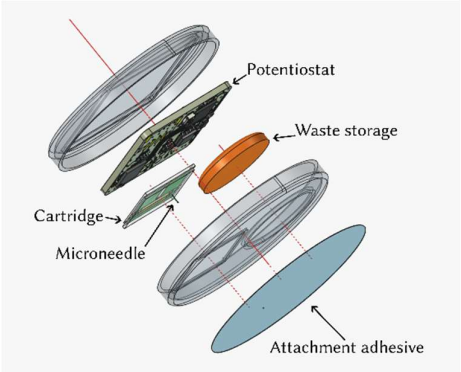


Figure 3.1 Exploded view of our biosensor.

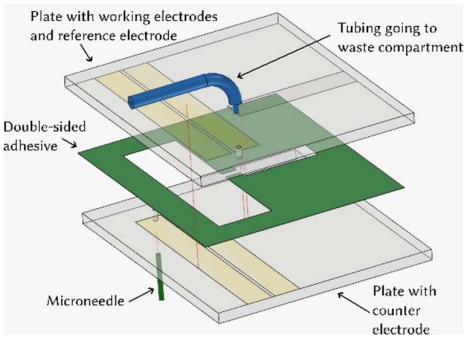


Figure 3.2 Cartridge diagram.

3.1.1 Technological novelty of wearable sensor

We consider 2 building blocks - **DET enzyme** and **cartridge design** - to be novel when it comes to wearability of our sensor.

DET enzymes (introduced in Section 2.1) are a **perfect fit for continuous wearable biosensors**. They make up the **third generation** of enzymes for biosensors. This generation introduces many improvements over the first (regeneration using oxygen) and second (regeneration using redox mediators) generations, such as **no reliance on oxygen or mediators, reduced measurement potentials, decrease in the number of reactions near the electrode surface, and decrease in fabrication steps.** However, the number of enzymes enabling DET is very low. They have been an object of study mainly for glucose detection, but none have yet found commercial success and most CGM devices on the market still use first- and second-generation enzymes. **Only one DET enzyme for levodopa detection has been reported and its discovery was reported very recently** (Batchu *et al.*, 2025). Our team utilized this exact enzyme as our main recognition element.

The use of double-sided adhesive for fabrication of microfluidics, while being quite niche, is not novel by itself. However, we think that the **integration with thin film electrodes** into

a **stacked plate design** (see Figure 2.2) makes a clever novel approach. This approach leads to a cartridge with **great manufacturability, small internal volumes, and big electrode surface areas**. Small internal volumes are very important for biosensors that extract the sample fluid (such as our biosensor), not only because the possible sample extraction rates are low, but also because the extracted fluid needs to be stored somewhere (if it is not returned back to the body) and space in a device that is supposed to be wearable is a limited resource. When used with a 12.8µm thick double-sided adhesive, the internal volume of our biosensor is less than 1µL.The separation of electrodes onto 2 plates should also lead to easier electrode functionalization steps.

3.1.2 Technical feasibility of wearable sensor

We did not have an access to make custom thin film electrodes, so we built the cartridge using thin plastic sheets, double sided adhesives, and flattened wire electrodes as described in Section 2.3. This cartridge has a bigger volume, but keeps the main features of the proposed designed. A detailed manufacturing plan together with part pricing for the proposed biosensor is available in Appendix C.

Safety is a very important aspect of any wearable device. Our device takes a sample using a single hollow microneedle and after the measurement it is stored in a waste compartment. Therefore, the main safety concerns of our device are: **the microneedle, the adhesive patch and the potential of a backflow**. The microneedle is a common 30G (311 µm outer and 159 µm inner diameter) steel needle with a length so that it penetrates the skin only 1 mm. The proposed adhesive patch is certified for long term wear, but during patient interviews some patients reported allergic reactions to the adhesive patches on CGM devices. So, while being safe, it may not provide a good experience to all patients. The potential backflow is an issue that is hard to reason about, because the flow generation is not well developed. In our proposed biosensor, the waste compartment is made out of a flexible material. Before the biosensor is put to use, this compartment is collapsed and as it expands it creates a negative pressure that drives the flow of ISF from the patient's body to the biosensor. In this design, there is always a flow from the body to the biosensor, so no backflow should be possible.

The potentiostat was shrunk down to 35 mm x 35 mm x 2 mm. It can be powered by a small battery for 2 weeks, which is the planned lifetime of the device. Measurements are targeted to be reported every 10 minutes. At this measurement rate, the device extract ~ 2 mL of ISF, that can be stored in the waste compartment of much larger volume.

3.2 Reliability of sensor output

3.2.1 Technological novelty of reliability concept

Orthogonal sensing for both selectivity and specificity

We integrated 2 working electrodes to our biosensor. One is used for direct oxidation the other is functionalized with a DET enzyme (see Sections 2.1 and 3.1.1, and Appendix A for more detail on the DET enzyme). Direct oxidation is a robust method with great sensitivity, but because of the lack of any recognition element, its selectivity is minimal. The DET enzymes (and enzymes in general) offer better selectivity, but the obtained signal values are usually much smaller. The approach of combining direct oxidation with an enzyme-based detection has been already reported for levodopa biosensing in Goud, *et al*, 2019 and has received much attention. Here, we iterate on this method by using a DET enzyme instead of tyrosinase, which brings many advantages listed in Section 3.1.1.

Electrode configuration for increased specificity and decreased drift.

Nothing in our electrode configuration is novel by itself, but the integration of thin film electrodes with double-sided adhesive brings many features that are not common in wearable biosensors. The main features are relatively large surface area and a proper reference electrode.

Since the measured sample is sandwiched in a thin layer between the WEs and CE, we can get very high working electrode surface area to sample volume ratio, that can be also easily tuned by changing the electrodes dimensions or the double-sided adhesive thickness, Greater working electrode surface area leads to higher sensitivity, which is more important for biosensors that measures levodopa than those that measure for example glucose as the concertation of levodopa in ISF is 3 orders of magnitude lower. This stacked plate design also leads to very low distances between the electrodes, decreasing the resistance through the solution.

The fabrication method also enables easy addition of a proper reference electrodes. Usually, wearable biosensors work in a 2-electrode configuration or contain a pseudo-reference electrode. Proper reference electrode is very uncommon, but its addition removes the drift from unstable potentials that makes a large contribution to the overall drift. As the device needs to function for prolonged periods of time (e.g. 2 weeks), eliminating sources of drifts is very important, so that the device reports correct values throughout its whole lifetime.

3.2.2 Technical feasibility of reliability concept

As mentioned in Section 3.1.2, we have not constructed the proposed cartridge, but instead used the one described in Section 2.3. Using our potentiostat, we can measure using either one electrode at a time or measure using both electrodes at "the same time" by rapidly switching between them, thus we can utilize the orthogonal sensing. However, a algorithm that would take data from both measurements a produce a single final reported concentration was not developed.

To assess the potential use of our biosensor for Parkinson's treatment, selectivity tests were performed against compounds with similar structures or significant electrochemical activity commonly present in the ISF of Parkinson's patients. Electrochemical responses of individual 100 µM interferents were compared with that of 100 µM levodopa, with relative differences shown in Figure 3.3. The DET enzyme showed overall higher selectivity, although dopamine produced a strong response in both cases. These results highlight the need to test additional compounds and perform interference studies to properly validate the sensor (Appendix E). It is also worth to mention that physiological level of dopamine is very low at a around 653 pM.

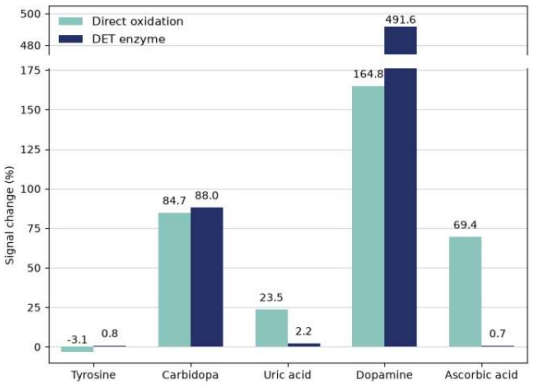


Figure 3.3 Results of selectivity tests. 100% means the same signal was obtained as from levodopa of the same concentration.

The proper reference electrode is included in the prototype used for the testing event. However, the long-term stability of the reference electrode was not testes due to time constraints of the competition.

Fouling on surface is a great source of drift in biosensors that work with biological fluids such as ISF. Fouling on electrodes decreases the effective electrode surface area and fouling on other internal surfaces can alter the flow properties, thus also causing a drift in the measured signal. In the prototype, we used and simple antifouling layer on the WEs and CE. Since most of the internal surface is made from these electrodes, we expect it to have an sizable effect. A more detailed discussion on the surface properties together with possible antifouling compounds is available in Appendix D.

Additionally, the flow rate can also change during the lifetime of the device. This would need to be determined during testing with extraction from a skin-like substance, but it is possible that some drift will be always present, so some software compensation would be needed.

3.3 Original contributions

Team Captains:

The team researched potential detection methods and identified the DET enzyme, tyrosinase, and direct oxidation as viable options. After finding screen printed electrodes (SPEs) with 2 working electrodes, the team decided that they would like to try 2 different detection options on a single device. The team consulted their intentions of producing the DET enzyme with coach Lipovová. The team came up with the changes to the production protocol and identified a possible improvement in the linker used for immobilization in the original article. The team was using the SPEs for electroanalytical experiments, but after months of no progress the mentor Slouka suggested to switch to wire electrodes. The team then came up with the idea to flatten them, so they can be integrated to a similar cartridge design as they had for the SPEs. The potentiostat was developed during the last 2 years of the competition. The team continued working on the software operating it and paid a member of last year's team to integrate the working electrode multiplexing to a newer version of the potentiostat that he continued developing for a different project. All other lab work (enzyme production, electrode fabrication, cartridge fabrication, calibration curve measurements, selectivity testing…) was planned and done by the team.

Supervisor:

The team exhibited extraordinary independence, persistence, and creativity. Numerous experimental approaches did not initially work as expected, forcing the team to repeatedly redesign several parts. Instead of being discouraged by these setbacks, the students consistently transformed them into new technical ideas. Rather than relying on a single established sensing principle, the students developed a concept combining two independent electrochemical detection approaches. A recently reported direct-electron-transfer enzyme and direct oxidation. Resulting in an orthogonal sensing strategy intended to improve the reliability of the measured signal. An equally important part of the students' work was the transformation of the sensing principle into a realistic wearable-device concept. The team designed and fabricated a low-volume microfluidic cartridge based on stacked, incorporated a proper reference electrode, and addressed practical issues including antifouling and long-term operation. Importantly, they did not approach these elements as isolated components but continuously considered how they could ultimately be integrated into a single compact device. The overwhelming majority of the concepts, experimental solutions, design decisions, and practical implementations described in this document originated directly from the team members, with the supervisors serving primarily as consultants.

Captains: 

Supervisor: 

3.4 Bonus criteria Innovation

We have conducted several studies into next steps development of our biosensor. These are included in Appendixes C, D, and E.

Appendix C contains a manufacturing plan and estimated tooling and per device costs. The total tooling price was estimated to be X and the total device price was estimated to be X. This was estimated for a small quantities when most of the actual fabrication is outsorced. It then disscuses next steps in moving some of the production inhouse during a scale up.

Appendix D disscuses the material properties are necessary for a good biosensor. It starts with how each of the parts interacts with the user and the biocompatibility certifications it will need to satisfy. It then continues the discussion on the expected compound that are expected to foul from the ISF onto the internal surfaces of the biosensor and suggests possible antifouling treatment for relevant parts.

We have conducted a limited selectivity study that was disscused in 3.2.2. Next steps should include a full interference study. Appendix E includes a list of suggested compounds with their physiological concentrations and the reason for their inclusion.

Appendix N includes SWOT analysis for sensing device.

4 TP award: Translation potential

4.1 Customer interviews

4.1.1 Identification of potential stakeholders

We identified 3 key stakeholder groups: **patients, neurologists, and investors and insurance companies**. The topics, their inclusion aims to answer are listed in Table 4.1. Other parties that we had considered were caregivers, but we decided to instead focus on including patients at all stages of progression, since they would be the primary users of a potential biosensor.

Table 4.1 Main topics disscued with stakeholder groups.

	Patients	Neurologists	Investors & Insurance comp.
1	Current state of living with Parkinson's disease	Current state of levodopa monitoring	Patient costs and a market potential for a levodopa biosensor
2	Willingness to use and pay for a wearable biosensor	Possibility of using a wearable biosensor for levodopa monitoring	Qualities of a potential wearable biosensor to get investment or reimbursement
3	Qualities of a potential wearable biosensor	Possibility of integration with subcutaneous pumps	

4.1.2 Interview approach and execution

We conducted **semi-structured interviews** with the identified stakeholder groups, striking a balance between maintaining a consistent thematic focus and allowing for in-depth personal insights. Institutions were contacted directly by email and phone; patients were reached via the organization Parkinson z.s.. A specific **structured discussion guide** was developed for each target group (Appendix M) to guide the discussion towards answering the objectives listed in Table 4.1.

All participants (7 patients, 3 neurologists, 3 insurance companies, and 1 investor) provided informed consent for their data to be processed anonymously within the SensUs 2026 competition. To prevent bias, we **refrained from promoting our own technological solution** during the sessions, but instead discussed on a hypothetical wearable biosensor similar to a CGM. To eliminate human error and ensure reproducibility, all interviews were recorded and transcribed for systematic analysis.

4.1.3 Interview results and conclusions

As previously noted, the full interview questions are provided in Appendix M, while the transcribed interviews and relevant supporting documentation are accessible via the Google drive (available here). The following section synthesizes four key findings that

emerged from our primary stakeholder research and highlights how each respondent group addressed these core issues. Full analysis of the interviews is available in Appendix F.

Dosing is performed blindly: Neurologists currently adjust doses empirically ("blindly") because levodopa is only measured in research, making continuous biosensing essential to unlock real-time pharmacokinetics. This matches patient needs, as they want real-time tracking to end the reactive dosing after symptoms like tremors or stiffness appear. Investors see an unaddressed "blank spot" in a $6–7B market and suggest positioning the device as a *companion diagnostic* with pharma partners (e.g., Stalevo, Madopar). However, payers stress that reimbursement requires hard clinical proof that eliminating blind dosing yields measurable health improvements or cost savings.

Over-Reliance on Subjective Patient Diaries: Paper diaries remain neurologists' sole tool, yet they fail to capture non-motor symptoms like anxiety that correlate with dopamine drops. This rigid tracking paralyzes daily life for 29% of patients, who would gain immense psychological relief from real-time data. Because hardware commoditizes over time, investors stress that long-term value lies in replacing these subjective diaries with continuous data and algorithms (SaMD). However, insurance companies note that subjective diaries lack billing validity, meaning objective sensor data must first demonstrate clinical proof to secure catalogue reimbursement (SÚKL/VZP).

Unpredictable Condition Fluctuations: Erratic "ON/OFF" shifts paralyze daily life for 29% of patients, while another 29% suffer hallucinations from temporary over-dosing, forcing neurologists to manage these severe fluctuations in specialized centers via shorter dosing intervals, infusion pumps, or deep brain stimulation (DBS). To improve this, insurance companies challenge the myth that tech innovations save money and demand hard evidence of reduced hospitalizations (such as fewer fall injuries) before granting reimbursement. Consequently, investors highlight that proving a measurable reduction in hospital admissions is the single most critical threshold for securing reimbursement codes and achieving commercial viability.

4.2 Design of validation study

4.2.1 Selection of the problem

The overarching problem discussed in the interviews was the lack of any method for determination of levodopa levels that is currently in a routine medical use. This leads to levodopa dosing being based on the currently best available source of relevant data - patients' diaries. The data provided by patient's diaries is however insufficient for managing such complex diseases as Parkinson's is and keeping a diary is mentally taxing on patients. The lack of quality data further leads to a plethora of problems such as late or wrong adjustments in dosing that can introduce states of dyskinesia, decreasing patients' quality of life (QoL). A wearable continuous biosensor could be a solution to this problem. Both patients and neurologists claimed in interviews that there is a strong demand for such a solution. However, the last stakeholder group brought up some disadvantages that might make this type of product not the ideal solution. These include classification of wearable biosensors as medical devices and not in vivo diagnostic tools, fragmented market of levodopa formulations, and a large market share of non-levodopa medications.

4.2.2 Conceptual design and use case



Figure 4.1 App interface design.

Two different potential biosensor solutions: a standalone device and a closed-loop system with a subcutaneous pump came from the interviews as valid options. We decide to go forward with a standalone device rather than the closed-loop system because the total addressable market is much bigger, patients with subcutaneous pump reported low to no struggle with dosing, and a potential software integration is possible in the future. Therefore, the main use case of our biosensor is guiding patients using oral levodopa on dosing.

The proposed conceptual design (shown in Figure 3.1) follows the design discussed in Sections 2 and 3. Together with the biosensor design, we also showcase the design of the interface of a companion up (Figure 4.1). The data reported will be computed by an algorithm that together with the mobile app and the biosensor itself provide a complete hardware-software solution.

4.2.3 Validation study design

To validate the critical aspects of the proposed biosensor solution, we propose a 3 stage validation study (see Figure 4.2). The first part (most relevant to this competition) studies the design of the biosensor hardware and software. The next step is the clinical study.

The end goal of this study is to not validate our product only as a biosensor that measures levodopa levels, but as a complete software-hardware solution that provides patients enough useful information so that they can take informed decisions about their dosing.

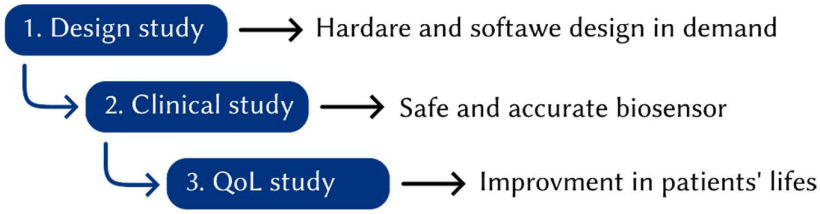


Figure 4.2 Diagram of validation study stages and their outcomes.

Design stage - In the first stage, the patients' preferences on the product's hardware and software will be studied. Patients will rate the design of the hardware based on a datasheet. The product's software (a mobile app) will be provided to the patients in the form of an image of the interface. Datasheets and app interface images of commercial CGM solutions will be used as a control. Examples are available in Appendix H. Quantitatively, usability and cognitive load will be assessed using the standardized System Usability Scale (SUS) questionnaire, while user anxiety variance regarding real-time data exposure will be evaluated on a 5-point Likert scale.

Clinical stage - Our biosensor is classified as a medical device IIb. Therefore it has to undergo a clinical evaluation assessing both its performance and safety (discussed more in Appendix I). This part will answer some potential issues that came up during the interviews. These include for example the use of interstitial fluid as the matrix, safety of the attachment adhesive, and specificity of detection for different drug formulations. The data gathered in this stage will be then used to build up a prediction algorithm that will be validated in the next part.

Quality of life – The previous stage determined that the biosensor is safe and can accurately measure levodopa levels. However, the knowledge of raw levodopa levels might not be enough to provide a dosing that would meaningfully improve patients' QoL. Effectiveness will be quantified as the shift in Time-in-Range (TIR) over and patients will fill up a PDQ-8 questionnaire (included in Appendix L).

4.3 Bonus criteria Translation Potential

The team has conducted few studies that we believe could be considered for the bonus part.

In Appendix G we have summarized key finding from drug dispensing logs provided by an insurance company.

Appendix I contains discussion on clinical trials and Appendix J contains a plan for a market entry strategy.

Appendix N includes SWOT fand MOSCOW analyses.

5 Team and support

5.1 Contributions of the team members

Member	Contribution
Ivan Bezrukov	Reader instrument software and hardware
Anastasia Bredichina	DET enzyme recombinant production
Eliška Hojdová	Translation potential
Nikol Hromádková	Translation potential
Richard Janeček	Cocaptain, recognition element, administrative things
Natália Kazimierová	Reference electrode, biocompatibility
Veronika Mejtská	Electrochemistry, outreach
Adam Benjamin Plšek	Captain, biosensor design and integration
Miriam Saganová	DET enzyme recombinant production, outreach
Lucie Slonková	Cartridge fabrication
Lucia Sučáková	Recognition element immobilization
Vojtěch Sýs	Translation potential
Hana Zikánová	DET enzyme recombinant production

5.2 People who have given support

We have received support in the form of one or more consultations with the following people:
Zdeněk Slouka, Petra Lipovová, Kateřina Kovaříčková, Jan Šťovíček, Jan Vyčítal

5.3 Sponsors and partners

We have received financial support from our school (University of Chemistry and Technology, Prague) and from the Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences.

6 Final remarks

7 References

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Appendix A - DET enzyme

Enzymes capable of direct electron transfer (DET) are recognized as a really promising strategy for developing enzyme-based biosensors in many biomedical applications, especially in continuous monitoring systems (Sowa et al., 2024). Their advantage is that they do not rely on oxygen or other external electron acceptors. This independence is especially favourable because the concentration of oxygen can change over time in response to different processes in our body (Batchu et al., 2025).

One group of DET enzymes is the group of multicopper oxidases (MCOs). These cathodic enzymes accept electrons directly from enzymatic anodic reactions and then catalyse the reduction of oxygen into water. They consist of three centres containing copper – type 1 (T1), type 2 (T2) and type 3 (T3). MCOs catalyse the oxidation of different substrates (for example, phenols) by reducing the copper in the T1 centre. Electrons can also be accepted from the electrode. These electrons are then received by the T2/T3 centre, where reduction of oxygen into water occurs. In this study MCO from hyperthermophilic Pyrobaculum aerophilum, McoP, was used (Batchu et al., 2025).

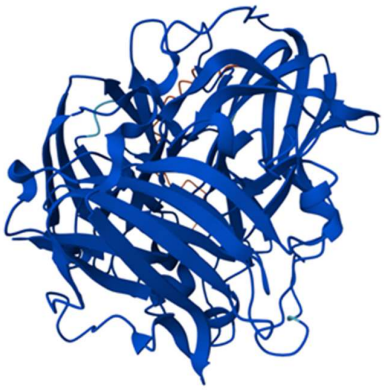


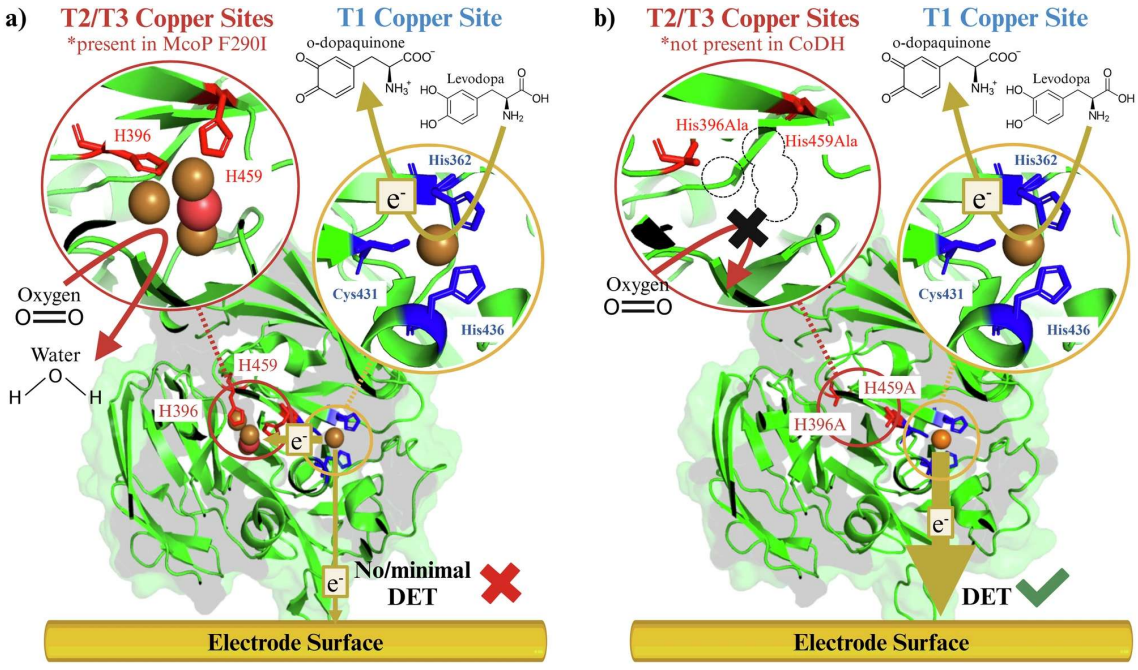
Figure 1 AlphaFold-predicted structure of McoP (Jumper, J *et al.*, 2021, Bertoni D. *et al.*, 2026).

This enzyme is advantageous due to its high specificity towards levodopa, however, the use of oxygen by this enzyme is problematic. Since continuous electrochemical sensing is limited by its dependence on oxygen. Therefore, mutations in T2/T3 copper centres were introduced (H396A, H459A - as previously described by Batchu *et al.*, 2025). This mutated McoP does not contain T2 and T3 copper centres, oxygen is not reduced during the reaction mechanism and the DET-ability remains due to T1 copper center (Figure 2). The P290I mutation was also introduced, as it showed increased catalytic activity compared to unmutated McoP, as described by Batchu *et al*., 2025. This enzyme was named copper dehydrogenase (CoDH) and can be advantageously used for developing a chronoamperometric enzymatic biosensor for continuous levodopa measurement (Batchu *et al.*, 2025).

The presence of copper in the enzyme was confirmed via Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

The recombinant production protocol used in this project was based on the method described by Batchu et al. Plasmid vector pET-16b containing the required gene was transformed to bacterial cells *Escherichia Coli* BL21 (DE3), with added ampicillin (AMP) as a selective agent. The following step was protein expression induced by isopropyl β-D-1-thiogalactopyranoside (IPTG). The cells were harvested by centrifugation after overnight cultivation. Lysis proceeded using lysozyme, sodium deoxycholate, DNase, RNase and sonication before enzyme purification.

However, we have made several modifications differing from the Batchu et al. method. The main modification was shortening the original plasmid before transformation, using the polymerase chain reaction (PCR) to remove the histidine tag sequence. Secondly, we have altered incubation temperature after adding IPTG. For lysis procedure, we have used a one shot cell disruptor as well, while running another production process. Both sonication and one-shot methods were successful. Subsequently, we have chosen Phosphate-Buffered Saline (PBS) as a buffer for storage purposes. Minor changes include slightly altering used concentrations of AMP and IPTG.



Another key aspect of usage of DET enzymes is immobilization on the electrode. In this work we tried two different linkers: N-hydroxysuccinimide ester of lipoic acid (LIP-NHS) and dithiobis(succinimidyl propionate) (PRO-NHS). These linkers firstly form a self assembling monolayer (SAM) on the gold surface of electrodes via S-Au bond. To cover the pinholes in the monolayer 6-mercaptohexanol was used (only for LIP-NHS). After the formation of the

SAM, electrodes were washed and solution containing the enzyme was incubated with the electrode. The primary amino groups of DET enzyme, mainly in lysine, reacted with N-hydroxysuccinimide ester group via amino transesterification.

LIP-NHS and PRO-NHS have both an advantage over the linkerdithiobis succinimmidyl hexanoate (HEX-NHS) used by Batchu *et al.*, 2025. LIP-NHS has as well as HEX-NHS six carbons in its chain but contains two thiol groups (at normal conditions they form intramolecular disulfide bond), which makes the bond between the linker and Au surface stronger. PRO-NHS has only single thiol group but its carbon chain is only made of three carbon atoms.

To confirm the formation of SAM and successful immobilisation and to confirm that the enzyme stays active two experiments were made. For these experiments instead of DET enzyme we used tyrosinase, so its activity is more easily tested. Two different control electrodes were used, first one was bare gold only with assay solution and the other one was electrode without linker with tyrosinase.

Firstly, the activity was successfully measured via spectrofotometric assay (Juan C. Espín *et al.*, 2000) using L-dopa.

Secondly, to double check that protein structure was successfully immobilised on the gold surface even after several washing with a buffer and incubation in a new buffer solution overnight. Electrodes were analyzed by direct on-surface proteolytic digestion followed by LC–MS/MS. This method also confirmed the presence of tyrosinase on the electrodes.

Proteolytic assay: After removal of the storage solution, the wires were washed with 200 µL of 50 mM ammonium bicarbonate and incubated for 4 h in the same buffer with 4 µL of chymotrypsin. Chymotrypsin was selected instead of trypsin because tyrosinase had been immobilized through lysine residues, which could prevent tryptic cleavage and leave large protein fragments attached to the surface. The released peptides were collected, dried in a SpeedVac, reconstituted in 30 µL of 0.1% trifluoroacetic acid, purified using C18 ZipTips, and analyzed by LC–MS/MS. Protein identification was performed by database searching against Swiss-Prot and the UniProt *Agaricus bisporus* proteome supplemented with common laboratory contaminant sequences.

Appendix B – Devices and software

We have not included photos of the actual produced devices and software in the main part, because of the limited space. You can find below a photos of the user interface, potentiostat, and the cartridge.

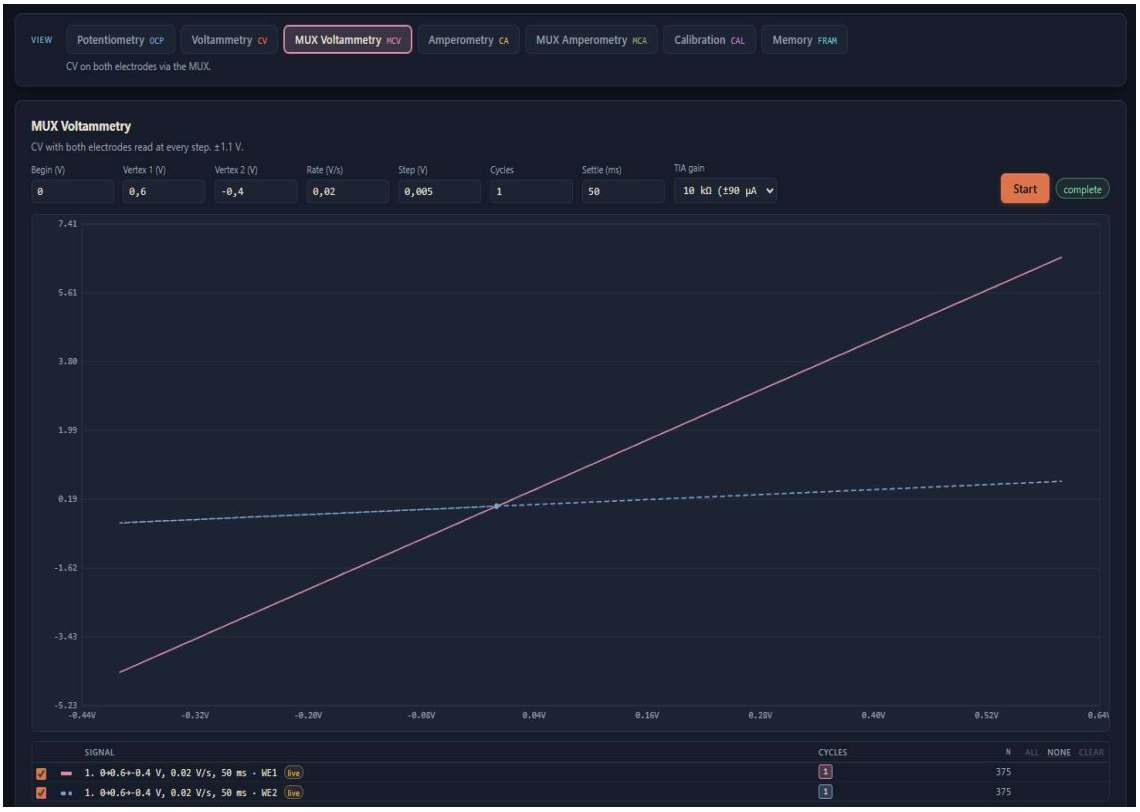


Figure 8.0.1 Screenshot of GUI application used for operating the potentiostat.
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Figure 8.0.3 Semi-assembled cartridge with visible channel geometry and counter electrode.



Figure 8.0.2 Fully assembled cartridge with visible wires used for connection to the potentiostat.

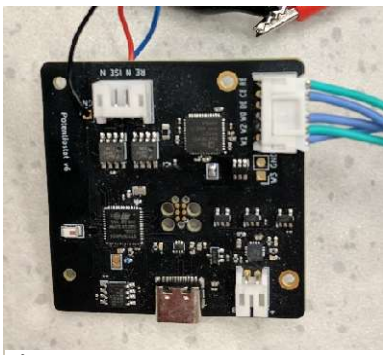


Figure 8.4 Photo of the potentiostat.

Appendix C - Manufacturing processes and costs

Part	Input materials	Manufacturing method	Cost per unit	Tooling costs
Case	Polycarbonate pellets	Injection molding	0.20€	10000€
Microneedle	Hypodermic tubing	Laser cutting	0.10€	0€
Attachment adhesive	Longwear adhesive, TPU adhesive, Double sided adhesive	-	0.50€	0€
Thin film electrodes	Glass, gold, silver, and chromium target	Thin film deposition	8€	6000€
Porous plug	Polyvinyl butyral	Liquid dispensing	0.05€	12000€
Sealing adhesive	ARcare 8890	Laser cutting	0.05€	1000€
Waste compartment	Polydimethylsiloxane	Casting	0.50€	10000€
Total			9.40€	39000€

The manufacturing plan is as follows:

1. Holes are made for inlet, outlet and for filling the reference electrode chamber into the glass plates used for electrodes.
2. Electrodes are deposited on the glass plates.
3. AgCl layer is electrodeposited onto the Ag electrode
4. Enzyme is immobilized onto one of the working electrodes. All gold electrodes are functionalized with an antifouling agent.
5. Sealing adhesive is cut and attached to one of the plates.
6. The porous plug is made by dispensing PVB solution into the microchannel.
7. The cartridge is closed by attaching the second plate.
8. Reference chamber is filled and closed.
9. The microneedle is cut from the hypodermic tubing and attached to the cartridge.
10. The cartridge is connected to the waste compartment that was cast into its shape.
11. The cartridge is connected to the potentiostat and inclosed in an injection molded case.
12. The case is sealed and the attachment adhesives are added to the bottom of the case

In the begging, most fabrication processes except for casting of the waste storage compartment and cutting the adhesive are outsourced. During the scale the fabrication of the thin film electrodes is brought inhouse, because it has large upfront costs but can reduce the price per device.

Appendix D - Materials properties

When preparing a wearable biosensor, it is important to choose such materials that are biocompatible, meaning they do not interact with the human body nor the sample in any harmful way. The standard series which take biocompatibility into consideration are the ISO 10993 standards. Proof of safety is established through targeted chemical characterization and biological testing. First table shows all of the materials used for constructing our sensor, their interactions with body and a sample, and their needed biocompatibility standard.

Part	Material	Interaction with body or sample	Necessary biocompatibility standards
Case	Polycarbonate	Occasional contact with epidermis	ISO 10993-1 ISO10993-10
Microneedle	Medical grade stainless steel	Prolonged insertion in dermis, interaction with the sample	ISO 10993-5 ISO 10993-10 ISO 10993-11
Attachment adhesive	3 layers of medical adhesives	Prolonged contact with epidermis	ISO 10993-5 ISO 10993-10 ISO 10993-11
Electrode plates	Glass	Interaction with the sample	ISO 10993-5 ISO 10993-18 ISO 10993-23
Electrodes	Gold	Interaction with the sample	ISO 10993-5 ISO 10993-18
Porous plug	PVB	Interaction with the sample	ISO 10993-5 ISO 10993-18
Sealing adhesive	Acrylate based adhesive	Interaction with the sample	ISO 10993-10 ISO 10993-18
Waste storage	PDMS	Interaction with the sample	ISO 10993-5 ISO 10993-18 ISO 10993-13

All of the materials above can cause leaching which may pose a problem mainly with parts that are interacting with the sample. Such leachables may act as an accelerators for compounds in the ISF, for they can foul the sensor faster than they normally would on an inert and a clean surface. The following table provides additional information about fouling, what compounds from the ISF can cause the leaching and possible antifouling methods which help prevent such degradation.

Part	Material	Surface charge	Expected fouling compounds	Possible antifouling treatment
Microneedle	Medical grade stainless steel	Slightly negative at physiological pH	Triglycerides, fatty acids, lipophilic proteins, collagen	Oleophobic /Zwitterionic hydrogel coatings
Electrode plates	Glass	Highly negative	Positively charged proteins	Silanization with fluorosilanes or PEG-silanes
Electrodes	Gold	Neutral to slightly negative	Adsorpted non-specific ISF proteins	Self-Assembled Monolayers of alkanethiols with oligo-ethylene glycol
Sealing adhesive	Acrylate based adhesive	Neutral to slightly negative	Sweat components, micro-fluidic lipids, unreacted monomer residues	Plasma surface oxidation followed by PEG grafting
Waste storage	PDMS	Hydrophobic, neutral to slightly negative	Lipids, hydrophobic proteins, cell debris, small lipophilic molecules	Oxygen plasma treatment

Appendix E – Interference tests

Proper validation of the biosensor would require interference tests against a wide range of compounds. Due to limited resources, these experiments were not conducted; instead, we propose a testing protocol.

A mixture of 10 µM levodopa and each interferent at a concentration representative of its physiological level would be measured, and the percentage difference in response would be calculated relative to 10 µM pure levodopa. The following table lists the selected compounds, their proposed concentrations, and the rationale for their inclusion.

Interferent	Concentration	Reason to test
Carbidopa	10 µM	Adjunct medication, structural similarity
3-O-Methyldopa	48 µM	Metabolic pathway
Homovanilic acid	450 nM	Metabolic pathway
Dopamine	653 pM	Metabolic pathway, commonly present in body
3,4-Dihydroxyphenylacetic acid	10.7 nM	Metabolic pathway, structural similarity
Ascorbic acid	114 µM	Electroactive, commonly present in body
Uric acid	50 µM	Electroactive, commonly present in body
Noradrenaline	3.7 nM	Commonly present in body, structural similarity
Acetaminophen	200 µM	Electroactive, can be oxidised by gold electrode
Tyrosine	10 µM	Structural similarity
Glucose	10 mM	Electroactive, commonly present in body
3,4-Dihydroxyphenylethanol	11.9 nM	Structural similarity
Lactate	2.5 mM	Commonly present in body
Urea	14.5 mM	Commonly present in body
Amantadine	29 µM	Common medication
Na+	150 mM	Commonly present in body
K+	5 mM	Commonly present in body
Adrenaline	327 pM	Commonly present in body, structural similarity

Appendix F - Analysis of interviews

Patients:

1. According to patients, the dosing is based on diaries (included in Appendix K) that they need to keep. A majority of patients had a problem with dosing at some point in their life. Some of those patients had difficulty with adjusting the dosing and that led them to try different drug intake methods such as nighttime levodopa or subcutaneous pumps. Patients that tried nighttime levodopa reverted back, but patients using subcutaneous pumps noted that they did not need to do dosing adjustments much anymore.

2. When asked if they would use a wearable biosensor similar to a CGM, the overwhelming portion replied that they would. Only a minority of patients responded that they would not be willing to pay for a biosensor if it was not covered by their insurance. Those that were willing, were split equally about the money they were willing to spend into ranges of up to 80€ , up to 160€, and over 160€.

3. Further questions regarding the qualities of a hypothetical biosensor revealed that the CGM like form factor is what the majority of patients would like. A non-negligible number of patients added that it was critical to them that a biosensor would not constrain their movement (e.g. not catching on things like doorframes or being waterproof so that they can swim with it). Few patients had an experience CGMs opened up on issues such as allergic reactions to adhesive patches that they had encountered while using CGMs

Neurologists

1. Neurologists confirmed that levodopa levels are not measured in medical practice, but only in research studies and they therefore have to rely on the patients' diaries. The treatment journey they described starts with dopamine antagonists and continues with oral levodopa, subcutaneous pumps and in severe cases ends with a brain surgery. They referred to oral levodopa as the gold standard, that is very effective and can be used for most patients by adjusting the dosing as the disease progresses.

2. A wearable biosensor was described as definitely useful. They mentioned that it could also provide valuable data on pharmacokinetics and non-motor symptoms such as anxiety. According to neurologists, patients seek a wearable biosensor and regularly ask about it. Neurologists however, were not familiar with ISF as the medium. They agreed that a form similar to a CGM would be a good fit, but they did not hold the view that it was very important.

3. Integration with subcutaneous pumps was welcomed by neurologists. They answered that a closed loop system that could automatically adjust the dosing would be ideal, but an independent device would also provide valuable information to adjust the dosing correctly.

Insurance companies

1. Requirements and reimbursement pathways depend primarily on mandatory MDR certification and the CE mark. Reimbursement follows two paths: patient self-use uses a voucher system (via a new legal category or uncategorized group 11), while healthcare professional use relies on specialized performance codes or separately billed material (ZUM). Risk-sharing agreements (e.g., 100% reimbursement tied to proven outcomes) are key for innovations.
2. Economic viability and cost impact are heavily scrutinized by payers who challenge the idea that innovations save money, noting that total healthcare costs usually rise alongside health improvements. They require cost-benefit modeling and peer-reviewed studies demonstrating direct savings (e.g., reduced hospitalizations or severe dosing complications). The choice between a "device-as-a-service" rental model and a one-time purchase depends on component lifespan and pricing structure.
3. Go-to-market strategy and prescription channels should avoid direct-to-consumer marketing, which is ineffective and unaffordable for academic startups. Instead, engaging specialist neurologists (KOLs) serves as the primary prescribing channel. Payers advise startups to build financial models and conduct informal consultations with insurance boards and medical societies before investing in costly certification.

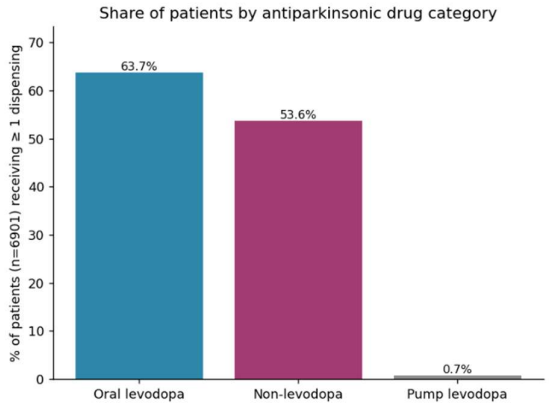
Investors

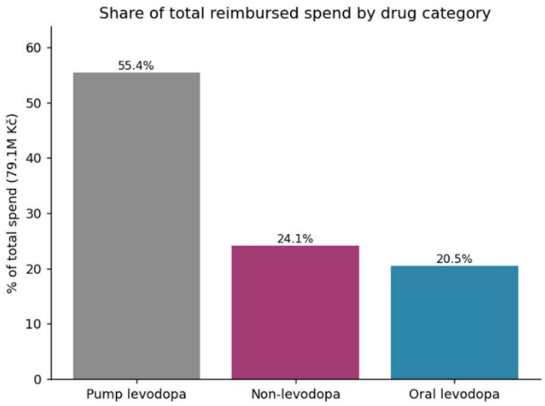
1. Investors acknowledge Parkinson's disease as an attractive market (~10M patients; $6–7B market) where continuous monitoring is a major blank spot, but emphasize that market size is strictly defined by reimbursement. Positioning the sensor as a companion diagnostic alongside specific pharmaceutical manufacturers (e.g., Stalevo, Madopar) provides upfront development funding and higher commercial viability than standalone devices.
2. Success relies primarily on clinical validation and clear regulatory pathways (favoring EU CE mark / FDA 510(k) over PMA). Technology alone is insufficient; the biggest killer of MedTech startups is underestimating MDR certification costs (2–4 years, millions of EUR) and reimbursement friction, which exhaust capital before exit.
3. Software, data, and closed-loop algorithms (SaMD/AI) are essential for long-term defensibility as hardware commoditizes. Execution must be serial—securing European CE approval before US expansion—targeting a 5–10x exit multiple over 7–10 years (25–35% IRR),

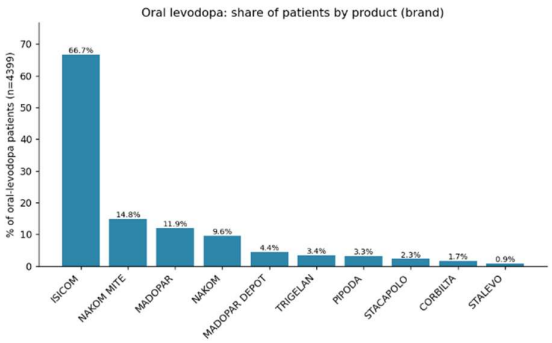
most realistically via strategic acquisition by incumbents like Medtronic, Abbott, Dexcom, or Roche.

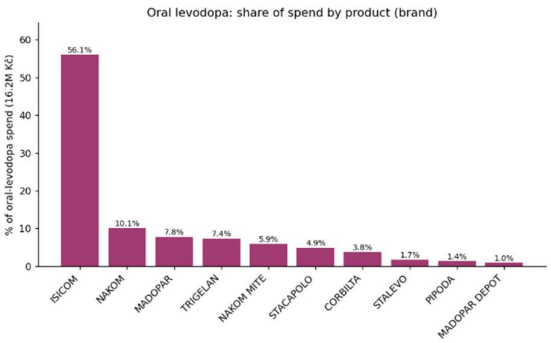
Appendix G – Analysis of drug dispensing logs

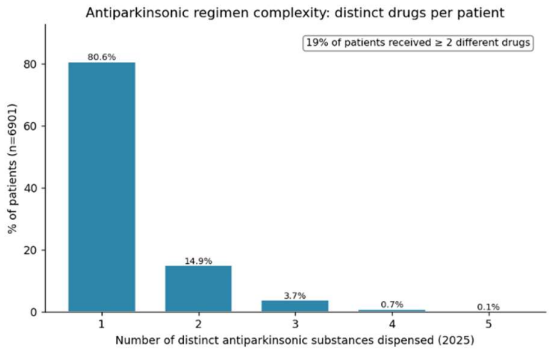
One of the insurance companies we interviewed gave as logs of antiparkinson drug dispensing for 6901 patients for the whole year of 2025. The main takeaways used during the competition are summarized in graphical form below.







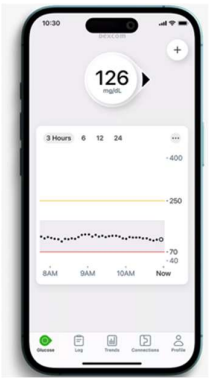




Appendix H - Design study

In the Design stage, patients rate the hardware and software of the proposed biosensor against two commercial CGM systems used as controls: Dexcom G7 and FreeStyle Libre 3. Table X summarizes the hardware specifications shown to patients on each datasheet and app interface.

Table x: Hardware datasheet and app interface comparison used in the Design stage (proposed biosensor vs. commercial CGM controls).

	Proposed biosensor	**Dexcom G7**	**FreeStyle Libre 3**
Form factor	Circular disc, 5 cm diameter, 1 cm height	Oval disc, 2.74 x 2.41 x 0.47 cm	Circular disc, 2.1 cm diameter, 0.29 cm height
Sample matrix	Interstitial fluid	Interstitial fluid	Interstitial fluid
Measuring frequency	Every 10 min	Every 5 min	Every 1 min
Wear duration	Up to 14 days	10 days	14 days
Attachment	Adhesive patch	Adhesive patch	Adhesive patch
Water resistance	Immersion for 2 hours	Withstands immersion up to 2.4 m for 24 h	Withstands immersion up to 1 m for 30 min
Connectivity	Bluetooth	Bluetooth	Bluetooth
Alerts / warnings	Predictive OFF-state warning	Customizable high/low glucose alerts	Customizable high/low glucose alerts
Interface			

Appendix I - Pre-clinical and clinical studies



Pre-clinical studies

Pre-clinical investigations are an important part of the evaluation of every new implantable device, especially those classified as Class IIb. Firstly, we propose to perform bench tests to verify that our biosensor functions properly under controlled in vitro conditions. The analytical performance of our biosensor will be evaluated by measuring the calibration curve and other analytical parameters, such as sensitivity, precision, limit of detection, selectivity, interference, and limit of quantification.

Clinical studies

Every stage of our clinical studies should be conducted with a diverse group of participants of different ages and sexes (Sampaio *et al.*, 2026). Initial clinical studies are preliminary studies involving the first small group of participants. This is the first use of our medical device in humans. At this stage, data should be collected to assess safety and identify early adverse events. If this stage is successful, it can be followed by early clinical studies involving a larger group of participants. The goal of this stage is not only to demonstrate safety but also to evaluate the performance of the biosensor and compare it with existing medical devices (Fraser *et al.*, 2025). During this stage, participants should also be asked about the wearability of the biosensor.

Appendix J - Market Entry & Stakeholder Engagement Strategy

Our strategic framework bypasses generic push-marketing in favor of a targeted multi-channel strategy designed to reduce technological uncertainty, build institutional trust, and drive adoption across three critical stakeholder groups:

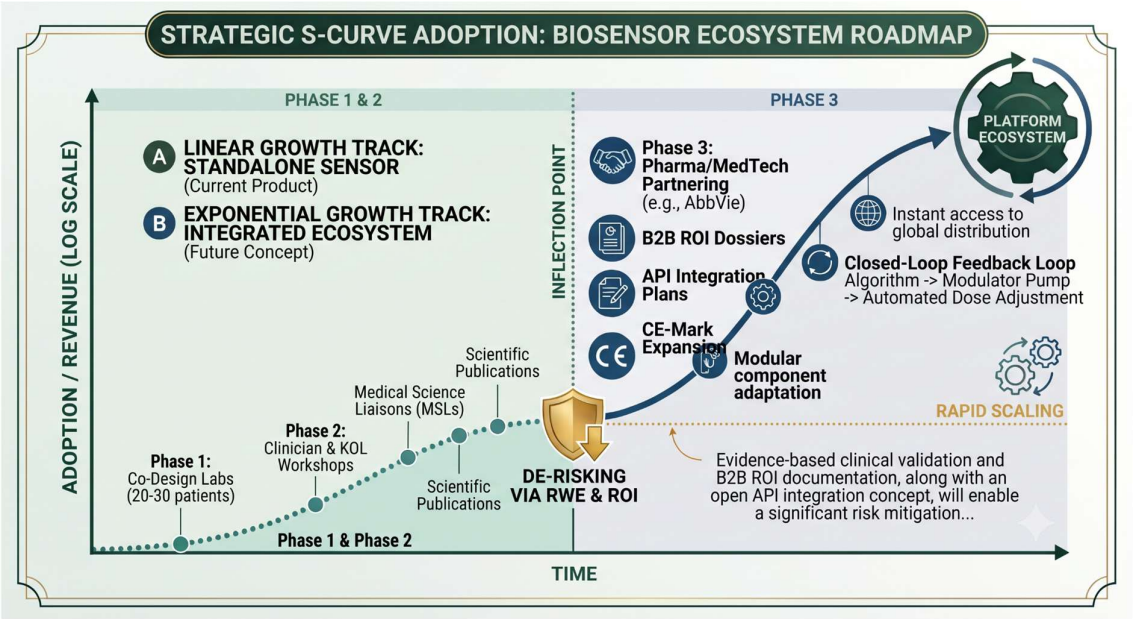
Phase 1: Patient-Centric Co-Design & Digital Outreach (User-Driven Innovation): Recognizing that device usability is critical for elderly populations with tremors, we leverage a user-driven innovation process. Partnering with advocacy leadership (*EPDA, Parkinson-help z.s., Společnost PARKINSON, z.s.*), early "Co-Design Labs" will engage 20–30 patients (Hoehn & Yahr II–IV) to refine user interfaces. Educational hubs, plain-language infographics, co-branded newsletters, video shorts, and podcasts will build awareness. Appendix F *details a comparative daily timeline (Standard Care vs. Biosensor-Guided Care) demonstrating direct clinical value.*

Phase 2: Scientific Engagement with Clinicians & KOLs (Knowledge Transfer): To overcome clinical inertia and drive adoption among key opinion leaders (KOLs), we utilize Medical Science Liaisons (MSLs) and CME workshops demonstrating how continuous diagnostic data reduces therapeutic trial-and-error (*Append*ix F). Engagement tactics include live device demos at congresses (*MDS International Congress, EAN*), peer-reviewed publications, targeted LinkedIn campaigns, and clinical sampling kits for pilot testing.

Phase 3: Strategic B2B Partnerships & Closed-Loop Integration (S-Curve Ecosystem Roadmap): *While our biosensor currently operates as a standalone diagnostic tool, Phase 3 outlines our future scaling vision* (see Figure X: Strategic S-Curve Adoption).Building on the clinical validation and market traction established in Phases 1 and 2, Phase 3 represents the prospective inflection point of our adoption S-curve—moving from linear direct adoption to potential exponential growth. To achieve this, we plan to target MedTech/Pharma leaders (e.g., AbbVie, producers of Duodopa pumps) at major partnering events (BIO Europe, MEDICA).

Once supported by real-world clinical evidence and upon developing targeted B2B ROI dossiers alongside open API integration concepts, we would be in a position to significantly de-risk future partnerships for pharma incumbents. Through this approach, our standalone sensor can be positioned not merely as an isolated device, but as a candidate module for future integration—enabling the potential to upgrade conventional drug delivery pumps

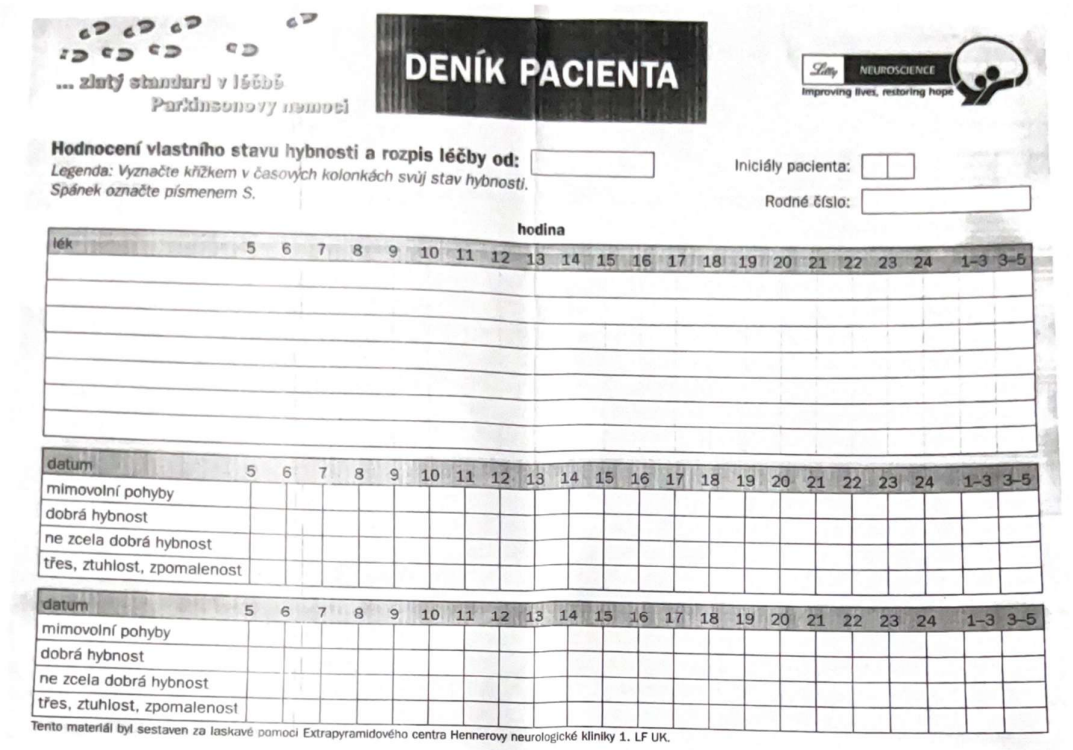
into an automated, closed-loop platform ecosystem (*sensor measures → algorithm decides → pump adjusts dose*). This long-term strategy provides a clear pathway toward global distribution networks and the steep growth phase of the adoption S-curve.



Regulatory Pathway & Socioeconomic Impact (Appropriability & Value Capture): Market exclusivity and appropriability are secured via core sensor/algorithm patents and early ISO 13485 QMS implementation. Compliance aligns with EU MDR and SaMD rules; while initial certification targets the standalone device, future joint B2B filings with pump partners will streamline CE-mark expansion for the integrated closed-loop system. Socioeconomically, levodopa stabilization extends "ON-time" and prevents expensive hospitalizations (falls/dyskinesia), securing a strong reimbursement cost-benefit ratio

To understand the clinical value of continuous monitoring data, we modeled a comparative daily timeline evaluating standard care against biosensor-guided precision medicine for advanced Parkinson's disease management.

Appendix K - Patient diary



... zlatý standard v léčbě Parkinsonovy nemoci

DENÍK PACIENTA

Lilly NEUROSCIENCE
Improving lives, restoring hope

Hodnocení vlastního stavu hybnosti a rozpis léčby od:

Legenda: Vyznačte křížkem v časových kolonkách svůj stav hybnosti.
Spánek označte písmenem S.

Iniciály pacienta:

Rodné číslo:

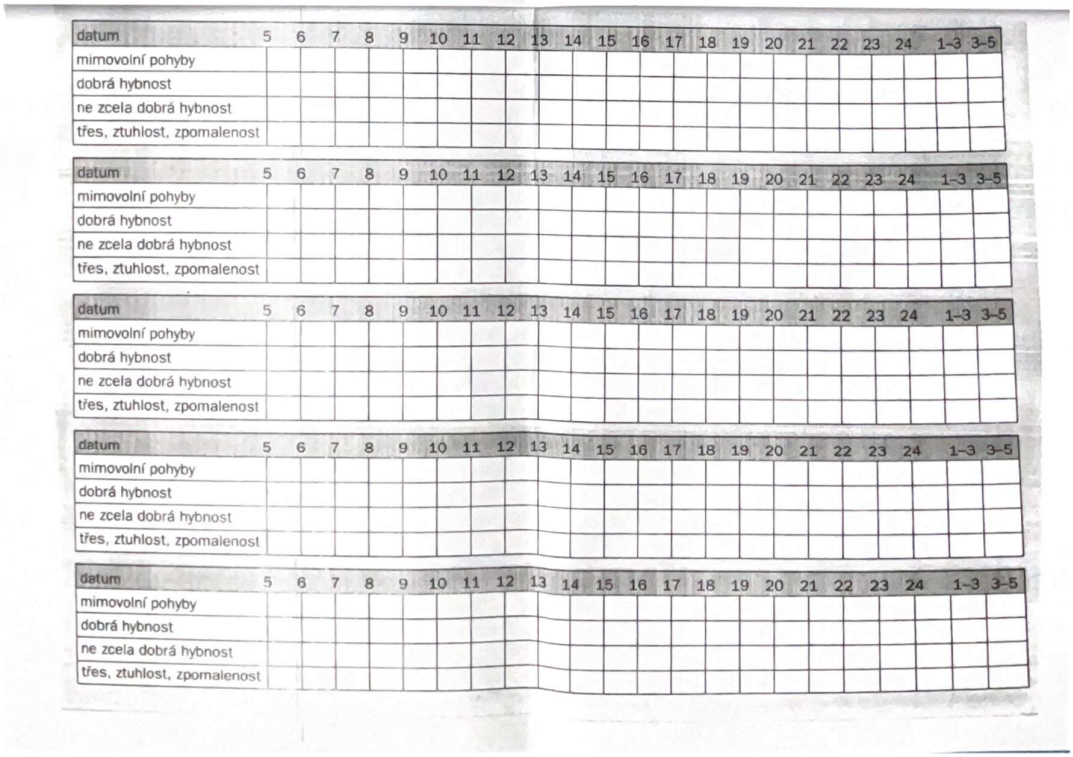
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datum	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	1–3	3–5
mimovolní pohyby																						
dobrá hybnost																						
ne zcela dobrá hybnost																						
třes, ztuhlost, zpomalenost																						

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mimovolní pohyby																						
dobrá hybnost																						
ne zcela dobrá hybnost																						
třes, ztuhlost, zpomalenost																						

Tento materiál byl sestaven za laskavé pomoci Extrapyramidového centra Hennerovy neurologické kliniky 1. LF UK.



datum	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	1–3	3–5
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mimovolní pohyby																						
dobrá hybnost																						
ne zcela dobrá hybnost																						
třes, ztuhlost, zpomalenost																						

... the gold standard in the treatment of Parkinson's disease

PATIENT DIARY

Mobility self-assessment and medication schedule from: ☐ Patient Initials: ☐☐

Legend: Mark your mobility state in the time columns with a cross (X)..
Mark sleep with the letter S.

Identification Number: ☐

Medication	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	1-3	3-5

Date	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	1-3	3-5
Involuntary movements																						
Good mobility																						
Suboptimal mobility																						
Tremor, slowness																						

Date	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	1-3	3-5
Involuntary movements																						
Good mobility																						
Suboptimal mobility																						
Tremor, slowness																						

Date	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	1-3	3-5
Involuntary movements																						
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Involuntary movements																						
Good mobility																						
Suboptimal mobility																						
Tremor, slowness																						

Appendix L – PDQ-8 questionnaire for QoL study

Parkinson's Disease Quality of Life Questionnaire (PDQ-8)

Due to having Parkinson's disease,
how often during the last month have you…

*Please **tick one box** for each question*

		Never	Occasionally	Sometimes	Often	Always or cannot do at all
1.	Had difficulty getting around in public?	☐	☐	☐	☐	☐
2.	Had difficulty dressing yourself?	☐	☐	☐	☐	☐
3.	Felt depressed?	☐	☐	☐	☐	☐
4.	Had problems with your close personal relationships?	☐	☐	☐	☐	☐
5.	Had problems with your concentration, e.g. when reading or watching TV?	☐	☐	☐	☐	☐
6.	Felt unable to communicate with people properly?	☐	☐	☐	☐	☐
7.	Had painful muscle cramps or spasms?	☐	☐	☐	☐	☐
8.	Felt embarrassed in public due to having Parkinson's disease?	☐	☐	☐	☐	☐

Please check that you have ***ticked one box for each question***.

Thank you for completing the questionnaire.

U-Turn Parkinson's

1/1

Appendix M - Questions for stakeholders

Patients

1. Have you ever had a problem with dosing?
2. How was your dosing subsequently adjusted?
3. And how often are such adjustments needed?
4. Does it limit you?
5. To what extent does the unpredictability of the onset of medication effects (so-called “off” and “on” states) limit you in daily life?
6. Has it never happened that a dosing adjustment was incorrect? And did it lead to any serious problems?
7. Would you be willing to wear a biosensor similar to the ones worn by diabetics? Do you think it would limit you?
8. Is there a preferred location on your body for the most suitable sensor placement (dimensions: X:Y:Z)? If so, what would it be?
9. Would the promise of a more stable health condition convince you to try this sensor in exchange for wearing it regularly?
10. Do you believe that this sensor would give you a greater sense of security due to the constant monitoring of your health, or would there be concerns about this technology? If so, please specify what your concerns would be.
11. In the event that such a device was not covered by insurance, would you consider paying for it yourself? If so, at approximately what monthly amount?
12. In the case of partial monthly coverage by the insurance company, up to what maximum amount would you be willing to pay out of pocket for the sensor?
13. Would you be interested as a patient to know about the levodopa level in your body in real time? Can you feel it somehow?
14. What physical characteristics would be key for you in a sensor? (e.g.: size, necessity/type of charging, water resistance, design...)

Neurologists

1. Do you treat many patients with medications containing levodopa as the active ingredient?
2. Does the subcutaneous pump administer levodopa according to the patient's current needs?
3. Are the patients asking for a system similar to glucose sensors used in diabetes?
4. Can excessive or insufficient levodopa levels cause significant side effects?
5. Do patients regulate the pump themselves?

6. Apart from pumps, how is levodopa currently administered? How do you manage fluctuations in advanced Parkinson's disease?
7. Is treatment initially started with oral medication?
8. Are pumps introduced only after patients have been treated with tablets for some time?
9. How effective is levodopa treatment?
10. Do you think a biosensor would be useful?
11. Could a biosensor replace existing treatments, or would it only be an adjunct?
12. The biosensor measures levodopa in interstitial fluid. Do you think this is an appropriate medium?
13. Are there any body locations that would be preferable for measuring levodopa?
14. Do you think patients would accept wearing a biosensor similar to those used in diabetes?
15. At what stage of treatment is brain surgery performed?
16. Are there any physical design considerations specific to Parkinson's patients?
17. Are there any other biomarkers that would be valuable?
18. So overall, do you think a biosensor could become an important tool in Parkinson's disease treatment?
19. Do physicians currently have direct information about levodopa levels in patients?

Insurance companies:

1. What are the average annual costs per patient with advanced Parkinson's disease?
2. Do costs increase significantly at a levodopa dosing threshold? For example, due to hospitalization following a fall or severe dyskinesias.
3. Do you think that such a biosensor would allow a reduction in costs for patients with Parkinson's disease?
4. And how would this device exist on the market — would it be realistic for it to be reimbursed through health insurance? If so, under what conditions would your insurance company be willing to reimburse the sensor?
5. What clinical evidence would be key for you when it comes to inclusion in the reimbursement list?
6. If you decided to partially or fully reimburse this sensor, would the sensor need to demonstrate a reduction in overall treatment costs (for example, fewer hospitalizations), or would an improvement in the patient's quality of life alone be sufficient?

7. In the case of partial or full reimbursement of the sensor, would you prefer a payment model for a “service” (i.e., rental of the sensor plus consumables), or a one-time purchase of the device?

Investors:

1. How do you assess the market potential of biosensors for monitoring Parkinson's disease treatment?
2. What factors would you consider key to the success of a product in the market?
3. What risks do you see when investing in biosensor development and what do you consider to be the biggest risk for MedTech startups in the field of diagnostics (e.g. technical failure vs. certification)?
4. What are your expectations regarding the return on investment in the field of health technologies and what rate of return (ROI) do you expect for a product that requires demanding certification, such as biotechnology?
5. Several formulations are available on the market and it is very likely that such a biosensor will be accurate only for one specific formulation. Do you think this is a problem?
6. The product also includes software. Do you see it as an essential part of investing in the early development of a biosensor?
7. Do you consider global scalability, in the sense of immediate entry into markets such as the US/EU, a necessity in the early stages of development?
8. Which investment conditions are key for you? Would strong property protection (grants) be sufficient for you, or is “time-to-market” more important to you?

Appendix N – SWOT and MoSCoW analyses

SWOT analysis -Translation Potential				
	Positive		Negative	
Internal	Strenghs		Weaknesses	
	1	· Highly motivated, multidisciplinary team (hardware/software, biochemistry, chemistry, microfluidics, business)	1	· Significant R&D adjustments needed to achieve a market-ready, user-friendly wearable design
	2	· Strong institutional support from UCT for continued R&D and favorable patent licensing terms	2	· Reliance on some approaches and components covered by existing patents
	3	· Established network of research and MedTech transfer experts for a future advisory board	3	· Small team size and limited resources compared to large biotech competitors
External	Opportunities		Threats	
	1	· Addresses a major 'blank spot' for continuous monitoring in a $6–7 billion Parkinson's disease market.	1	· Class IIb medical device classification requires costly and time-consuming EU MDR certification, which is a major risk for MedTech startups.
	2	· Provides objective, real-time data to replace subjective, error-prone patient diaries.	2	· Possible discovery in future of better treatment of Parkinson's disease without levodopa
	3	· Market expansion driven by aging populations in Western countries	3	· The market is fragmented with various levodopa formulations and a large share of non-levodopa medications.
	4	· Strict European clinical trial standards offering easier subsequent entry into diverse global markets	4	· Insurance companies require hard clinical proof of cost savings (e.g., reduced hospitalizations from falls) to grant reimbursement.
	5	· Potential B2B partnerships with pharma and MedTech companies	5	· Stringent data privacy (GDPR) and cybersecurity compliance requirements for connected MedTech devices

SWOT analysis -Sensing				
	Positive		Negative	
Internal	Strenghs		Weaknesses	
	1	· Utilizes orthogonal biosensing by combining direct oxidation and a DET enzyme to achieve both selectivity and sensitivity thanks to our custom made potenciostat.	1	· The DET enzyme still experiences interference from structurally similar compounds, such as dopamine and carbidopa.
	2	· The third-generation DET enzyme (mutated McoP) does not rely on redox mediators and has much higher specifity towards levodopa compared to tyrosinase.	2	· The adhesive patch required for wearing the device may cause allergic reactions in some patients.
	3	· Inclusion of a proper Ag/AgCl reference electrode helps eliminate signal drift.	3	· Stability of linker and DET enzyme have to be tested
	4	· Stacked plate cartridge design offering a high electrode surface area-to-volume ratio	4	· Biosensor will have to contain buffer solution for the electrodes while storing to prevent enzyme denaturation.
External	Opportunities		Threats	
	1	· The biosensor can serve as a platform to test other DET enzymes, as there is not assay to prove their activity	1	· Emerging non-enzymatic sensing technologies or alternative continuous biomarker platforms
	2	· Possibility to integrate the biosensor with subcutaneous pumps to create an automated, closed-loop dosing ecosystem.	2	

MoSCoW Analysis

