Team Results Document

Munich Bioneers



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SensUs

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1. Abstract: Summary for the SensUs website

We developed a parylene-based electrochemical sensor for L-Dopa monitoring and tested both single- and dual-working-electrode designs. Using a 3-axis UV laser marker, we converted selected areas of the parylene surface into conductive laser-induced graphene (LIG), forming the electrodes and feedlines.

With the single-working-electrode design, we tested non-enzymatic detection at 0.2 V using a Nafion-coated electrode. Although L-Dopa could be detected, the signal was also affected by serum and other electroactive compounds. We therefore tested enzymatic detection by electrodepositing Prussian Blue onto the LIG electrode, followed by an APTES–glutaraldehyde anchoring layer and a tyrosinase, BSA, and glutaraldehyde mixture.

We also designed a dual-working-electrode design at −0.05 V versus Ag/AgCl. Both electrodes were coated with Prussian Blue, while the enzyme layer was applied to only one. The enzyme-free electrode served as a background control, helping distinguish shared drift from the L-Dopa -related signal. Based on these results, we used the same sensing strategy for our wearable dual-working-electrode microneedle design.

2. AP award: Biosensor developed for the Eindhoven Testing Event

2.1. Molecular recognition

We investigated both non-enzymatic and enzymatic approaches for L-Dopa detection. The non-enzymatic approach does not contain a molecule-specific recognition element. Instead, a Nafion coating is used as a permselective membrane to reduce the contribution of some interfering compounds. However, other electroactive molecules and components of the serum matrix can still affect the response.

In the enzymatic approach, tyrosinase is used as the molecular recognition element. Tyrosinase oxidizes L-Dopa to dopaquinone in the presence of oxygen (Cembalo et al., 2023). To improve enzyme attachment, the electrode surface is first functionalized with APTES to introduce amine groups then treated with glutaraldehyde (Sypabekova et al., 2023; Rusu et al., 2022). A mixture of tyrosinase, BSA, and glutaraldehyde is subsequently deposited onto the surface. This creates a crosslinked protein layer anchored to the electrode via glutaraldehyde-mediated coupling, including Schiff-base formation between aldehyde and amine groups.

$$L - Dopa + \frac{1}{2}\, O_2 \xrightarrow{tyrosinase} dopaquinone + H_2O$$

In the dual-working-electrode design, tyrosinase is immobilized on only one of the two Prussian-Blue-modified working electrodes. The second electrode has the same underlying structure but no enzyme, allowing it to measure the non-enzymatic background response.

2.2. Physical transduction

2.2.1 Electrode fabrication and configuration

We fabricated the electrodes directly on a parylene-based substrate using a 3-axis UV laser marker. We used the laser to create conductive regions for the working, reference, and counter electrodes, as well as their feedlines. The untreated parylene remained electrically insulating and separated the individual electrodes. We passivated the feedlines with nail polish to prevent contact with the sample and to restrict the electrochemically active area to the exposed electrode regions. The complete laser operating parameters are provided in **Appendix A, Table A1**.

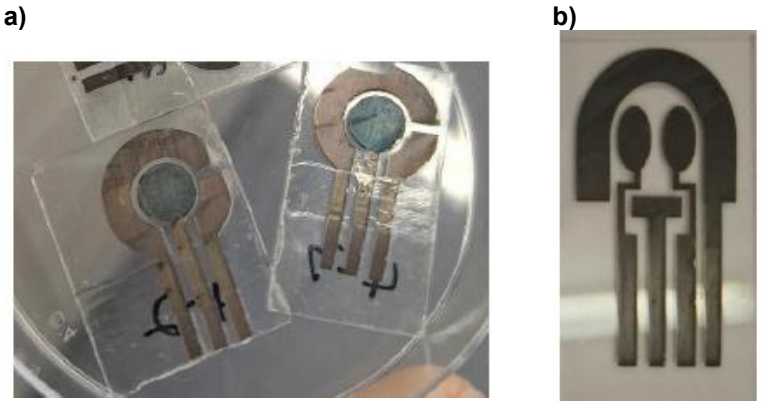


Figure 1-1 LIG-patterned parylene-based electrodes. *(a) Single-working-electrode design with Prussian Blue coating. (b) Dual-working-electrode design prior to Prussian Blue electrodeposition.*

2.2.2 Electrode fabrication and configuration

We electrochemically cleaned the laser-patterned electrodes by cycling the potential between −1.0 and +1.0 V for 250 cycles. We then applied Ag/AgCl paste to the reference electrode and baked it in an oven at 100 °C for 1 hour. For enzymatic sensing, Prussian Blue was electrodeposited from a 5 mM deposition solution by performing 40 cyclic-voltammetry cycles between −0.2 and +0.8 V. Cyclic voltammograms and electrochemical impedance spectra recorded before and after Prussian Blue deposition are presented in **Appendix A, Figures A1–A2.**

2.2.3 Non-Enzymatic Detection

When a positive voltage is applied, the electrode can oxidize levodopa. Levodopa loses two electrons while converting into dopaquinone. We can detect these electrons and use the signal to determine the amount of levodopa in the sample. A chronoamperometric measurement was performed at 0.2 V *(Figure 1-2)*. By comparing electrodes manufactured from parylene-based and polyimide, we found that polyimide is more sensitive in the oxidation region. By comparing a parylene-based sensor with and without a Nafion layer, we determined that the Nafion layer cannot perfectly prevent other molecules in serum from reacting with the sensor surface. However, it is possible to adjust the signal by accounting for the difference between the serum and PBS signals. This is non-specific detection; therefore, it is only possible when the concentration of other oxidizable molecules in human blood, such as uric acid, is maintained. It makes this method unlikely to be usable in real life

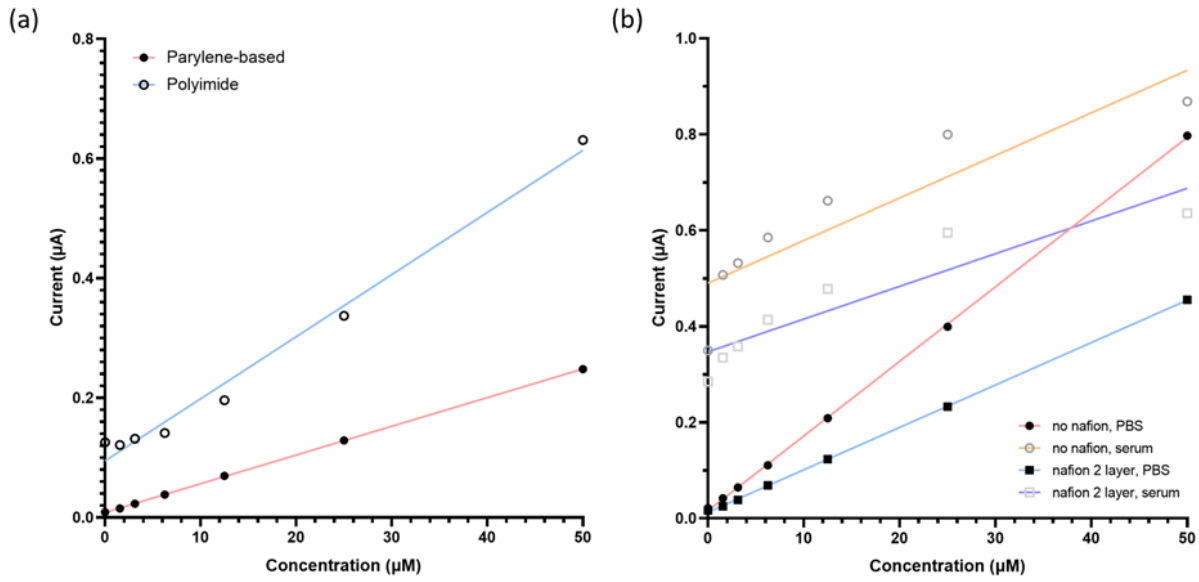


Figure 1-2: Calibration measurements performed at an applied potential of 0.2 V: *(a) comparison of the responses obtained using parylene-based and polyimide-based electrodes and (b) comparison of working electrodes with and without a Nafion coating.*

2.2.4 Enzymatic Detection

Levodopa can be oxidized by tyrosinase on the working electrode, converting it to dopaquinone. The dopaquinone is then reduced back to levodopa by applying a negative voltage, and these electrons can be detected. A chronoamperometric measurement was performed at -0.05 V. (Figures 1-3, 1-4) We found that the enzyme works properly on the

surface, with sensitivities of 0.0003792 µA/µM and 0.0003476 µA/µM. We are currently working on optimizing the recipe to achieve higher sensitivity.

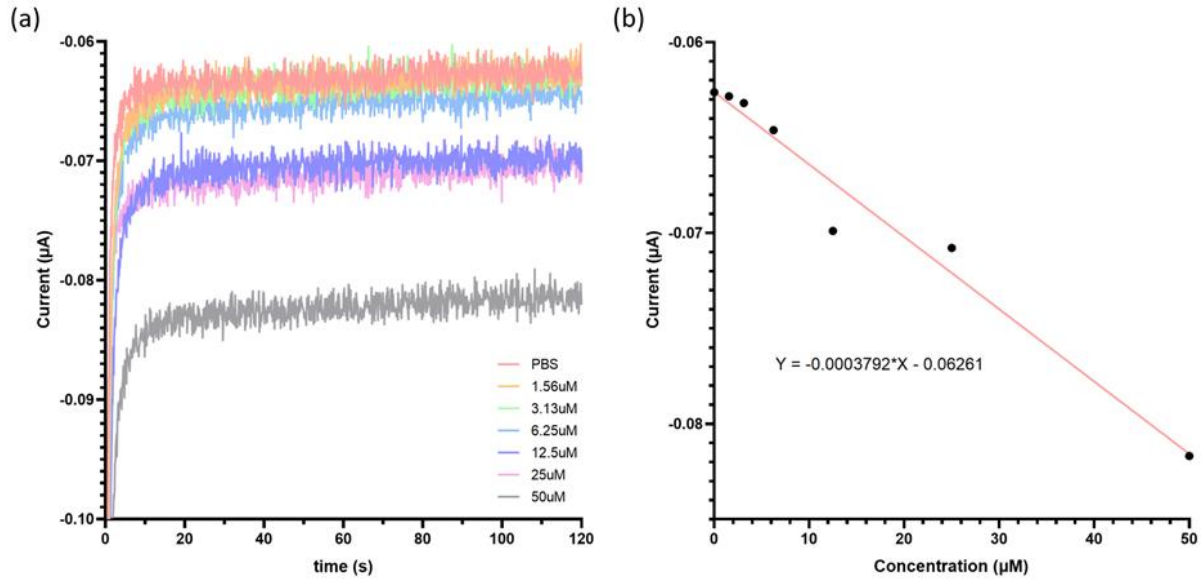


Figure 1-3: Amperometric calibration of the electrode at an applied potential of −0.05 V*: (a) current–time responses recorded for increasing analyte concentrations and (b) the corresponding calibration plot of steady-state current versus analyte concentration with the fitted regression line.*

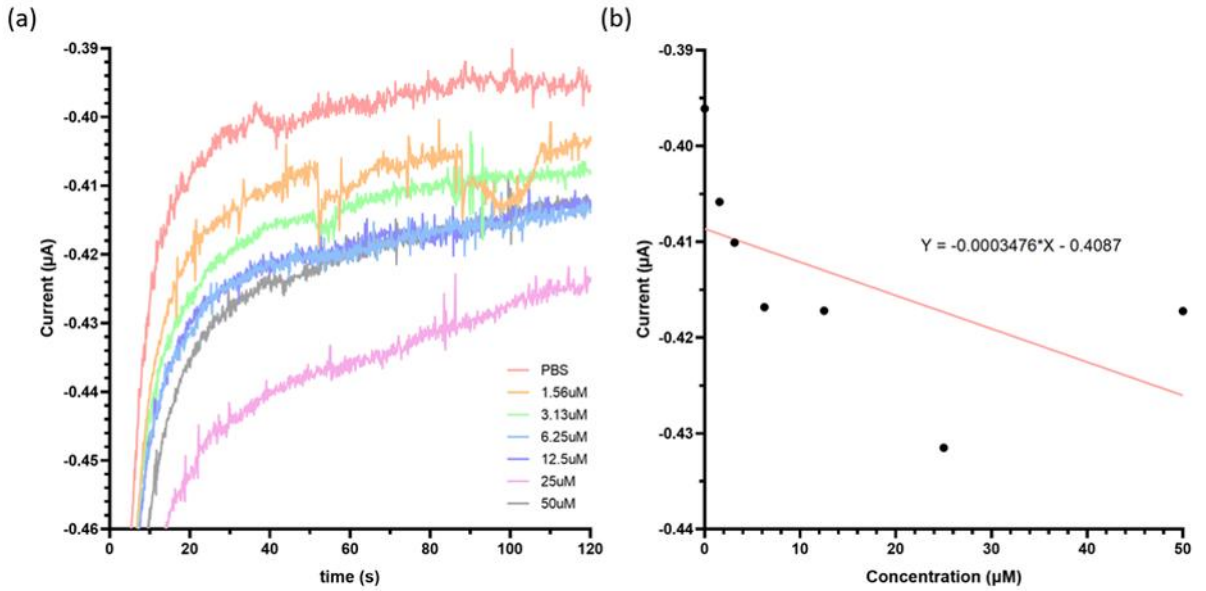


Figure 1-4: Amperometric calibration of the electrode at an applied potential of −0.05 V: *(a) current–time responses recorded in PBS and at analyte concentrations ranging from 1.56 to 50 µM and (b) the corresponding current–concentration calibration plot, with the experimental data shown as black points and the linear regression represented by the red line.*

2.3. Cartridge technology

We built a fluidic platform by stacking layers of laser-cut, double-sided adhesive tape (MCS-Foil 008) to form a 100 µL sensing chamber to match the size of the sensor. This method results in a fluidic platform that is easy to assemble, durable, and mechanically flexible.

Each sensing cycle relies on two micropumps: Pump 1 pulls 100 µL of interstitial fluid (ISF) from the reservoir into the flow cell inlet to fill the sensing chamber; Pump 2 withdraws the same volume through the outlet once the sensing interval is over. Both pumps run at a flow rate of 180 µL/min.

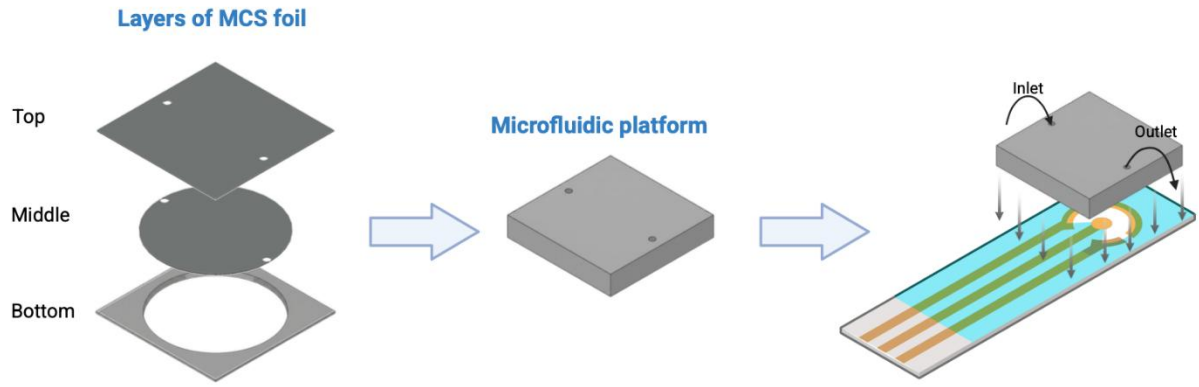


Figure 1-5: Design of the flow cell using double-sided adhesive tapes. *Three types of laser-cut adhesive layers were stacked: four top layers for the pump inlet/outlet, one middle layer sticking on one side of the top layer to shield the adhesive from the fluid, and seven bottom layers to form the sensing chamber. After being assembled, the platform was sticked onto the electrode area, creating a sealed fluidic channel.*

To confirm that the design is free of backflow, we calculated the total system backpressure as the sum of the port resistance (inlet and outlet) and the chamber resistance, as detailed below.

Port resistance (straight cylindrical channel)

Hagen–Poiseuille equation:

$$\Delta P_{port} = \frac{8\mu L Q}{\pi r^4}$$

- ΔP = pressure drop across the channel (Pa)
- $\mu = 1 \times 10^{-3}$ Pa·s (water)
- L = 2 mm
- Q = 180 µL/min
- r = 0.3 mm

This assumes fully developed laminar flow (Reynolds number in the 1 mm ports at 180 µL/min ≪ 1).

$$\Delta P_{port} \approx 1.89 \text{ Pa}$$

Chamber resistance (radial flow between parallel plates)

Hele-Shaw (radial parallel-plate) flow:

$$\Delta P_{chamber} = \frac{6\mu Q \cdot \ln(r_o/r_i)}{\pi h^3}$$

- h = 0.7 mm

- r_i = 0.3 mm
- r_o = 6.9 mm

$$\Delta P_{chamber} \approx 3.14 \text{ Pa}$$

Total system backpressure

$$\Delta P_{total} = \Delta P_{inlet\ port} + \Delta P_{chamber} + \Delta P_{outlet\ port} \approx 6.92 \text{ Pa} \ll 1 \text{ bar (pump)}$$

The total backpressure is negligible relative to the pump's driving pressure, confirming that the design prevents backflow.

After assembling, we characterized the sensor by impedance spectroscopy before and after installation of the flow cell. The Bode plot shows that both the impedance-magnitude and phase curves are similar in the two conditions, indicating that the flow cell was integrated successfully without introducing significant additional resistance *(Figure 1-6)*.

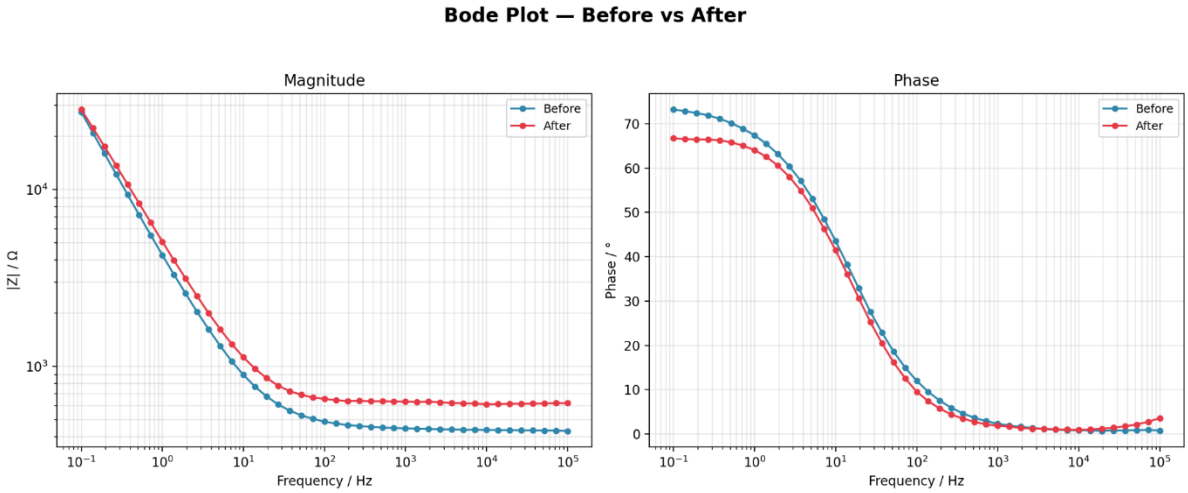


Figure 1-6: *Bode plot for impedance spectroscopy before and after flow-cell installation***.**

2.4. Reader instrument and user interaction

The electronic system was independently designed around the ADuCM355, which serves as both the main microcontroller and the electrochemical measurement platform. Its integrated analog front end enables direct interfacing via pogo pins with our electrodes and acquisition of the small currents generated at the electrodes.

The ADuCM355 also coordinates with the peripheral components of the system. The developed architecture integrates an ADXL343 motion sensor via SPI, a power-management system, Bluetooth Module BGM220P and USB communication via UART.

Custom firmware was developed to configure and control these components, perform sensor acquisition, process the measured data, and manage communication with the external interface.

The embedded software was developed separately for the two main processing units of the system. The ADuCM355 firmware was developed using Keil µVision, where the electrochemical measurement process, sensor acquisition, peripheral control, and communication functions are implemented. The BGM220P Bluetooth module was programmed using Silicon Labs Simplicity Studio, handling wireless communication and data transfer between the sensing electronics and the external user interface. Together, both firmware components enable coordinated sensor acquisition, processing, and wireless transmission of the measurement data.

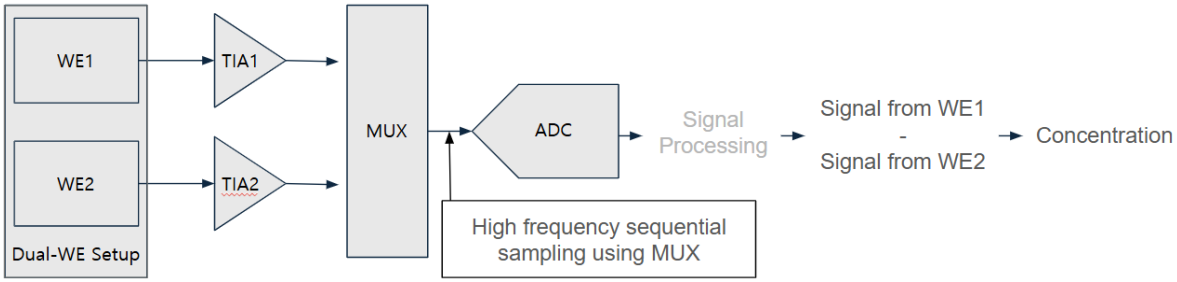


Figure 1-7: *Diagram for the signal measurement using 2 WEs setup*

The dual-working-electrode (WE) configuration enables two electrochemical signals to be measured within the same sensing system. Rather than measuring both working electrodes at exactly the same instant, the ADuCM355 rapidly multiplexes between the two measurement channels. Each WE is selected and measured sequentially within a very short time interval. Therefore, the two channels can be treated as quasi-simultaneous measurements.

The prototype PCB was designed to support both 3-electrode and 4-electrode electrochemical sensor configurations. By using configurable jumpers integrated into the PCB, the electrode connections can be adapted according to the selected sensor architecture, providing greater flexibility during prototyping and testing.

To improve the reliability and robustness of the prototype, two additional LDO regulators (XC6206) were introduced as backup power-supply options. In case of unexpected behavior or failure in the main power-management circuitry, these LDOs provide an alternative power supply.

For the testing event, only the components required for measurement, system control, and wired data communication will be used. The system will be connected to a computer via USB cable, which provides the interface for USB-based serial data communication.

2.4.1 Size and Design

A custom PCB with a size of 78mm*70mm was designed to integrate the complete sensing and control electronics into a compact platform suitable for the wearable sensor

concept. Particular attention was given to the separation and routing of sensitive analog signals, digital communication lines, power distribution, and ground connections, as the electrochemical measurements involve very small sensor currents and are therefore susceptible to electrical noise.

2.4.2 User Interface

SensusCare is the patient-facing mobile app developed alongside the biosensor, available for both Android and iOS. After a measurement, the user scans the QR code on the cartridge and the app displays the levodopa concentration in interstitial fluid. Rather than showing a bare number, it interprets the reading against the therapeutic window across the sensor's 3–50 µmol/L range, colour-coding whether the level is sub-therapeutic, well controlled, or high enough to risk dyskinesia. A history view charts readings over time so that patients and clinicians can see how levels fluctuate between doses, while short sections explain Parkinson's disease and levodopa in plain language. In future updates the sensor will transmit its results directly over Bluetooth, removing the scanning step. The app thus turns a raw sensor signal into an actionable clinical picture for the person taking the medication.

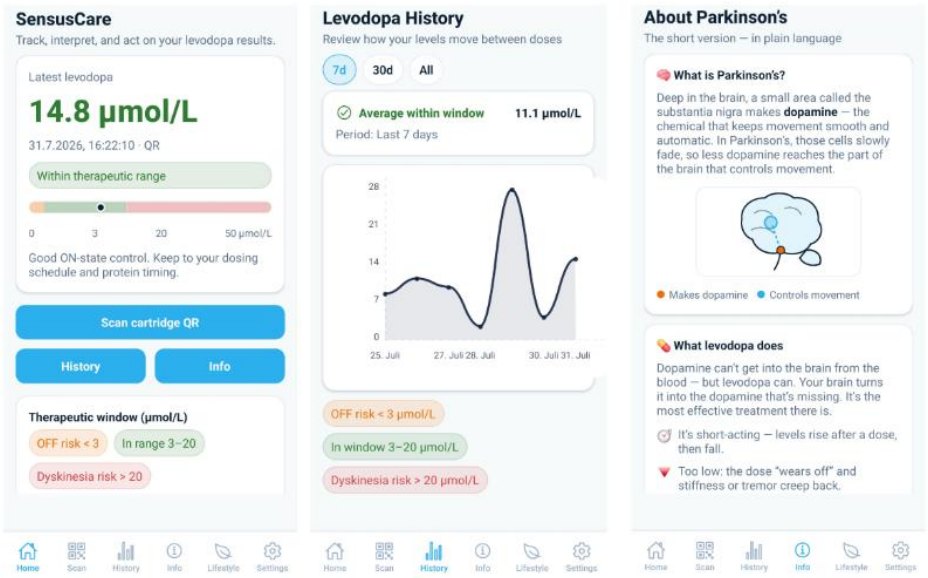


Figure 1-8: *SensUs Care App*

3. IN award: Biosensor innovation

3.1. Wearable sensor

The challenge is to develop a wearable sensor for L-Dopa monitoring in interstitial fluid that combines a mechanically suitable microneedle platform with stable electrochemical sensing and a strategy for improving signal reliability under wearable conditions. We have designed a flexible microneedle array with a four-electrode arrangement consisting of WE1, WE2, CE, and RE *(Figure 2-1)*. The array is encapsulated with parylene, while the microneedle tips are intended to be patterned with laser-induced graphene (LIG), electrodeposited with Prussian Blue, and functionalized with tyrosinase for amperometric L-Dopa detection at −0.05 V versus Ag/AgCl.

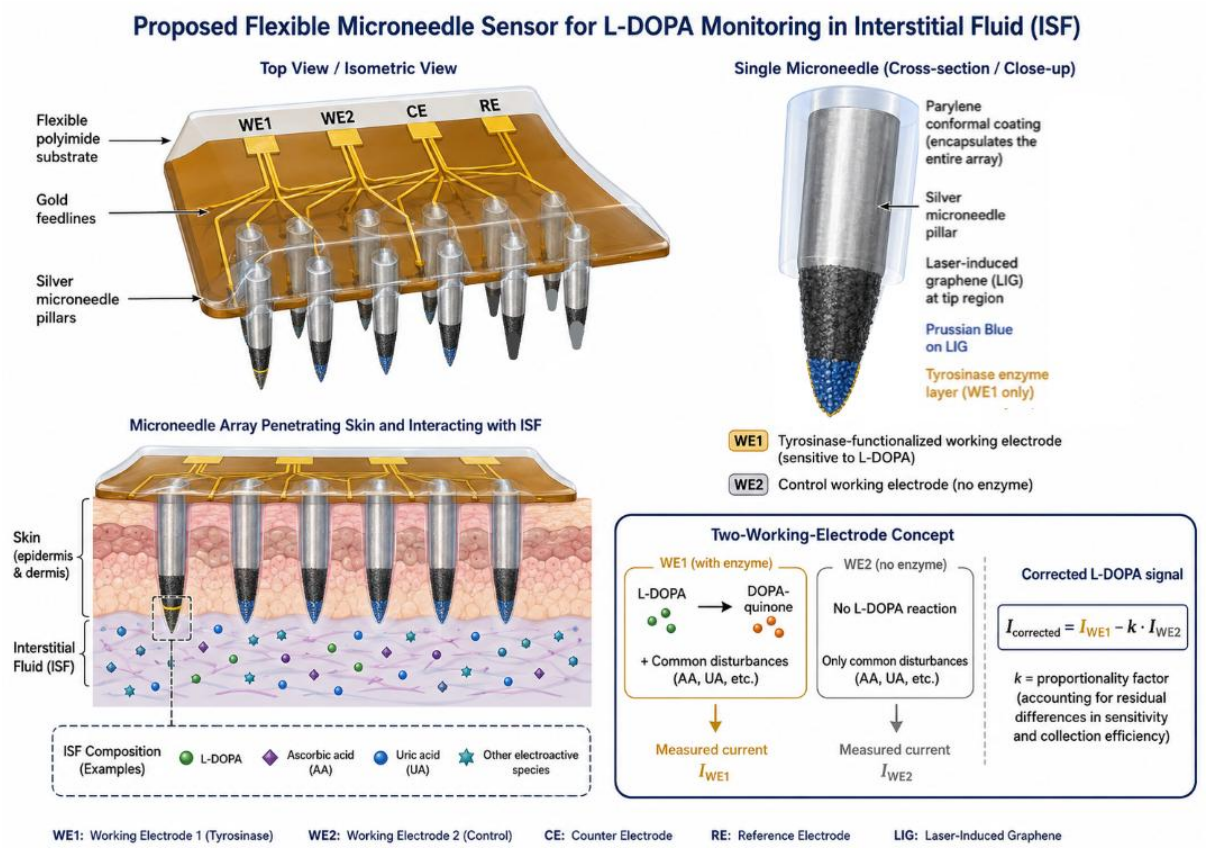


Figure 2-1. Schematic overview of the proposed flexible microneedle sensor for L-Dopa monitoring in ISF. *The sensor consists of a flexible polyimide substrate with gold feedlines and inkjet-printed silver microneedle pillars encapsulated with parylene. The working-electrode tips are intended to contain parylene-derived LIG and Prussian Blue, with WE1 additionally functionalized with tyrosinase and WE2 remaining enzyme-free as a control electrode. The array also contains a counter electrode (CE) and an Ag/AgCl paste-coated reference electrode (RE). The microneedles are intended to penetrate the skin and interact with ISF. A scaled WE2 signal is subtracted from WE1 to reduce disturbances common to both working electrodes. Schematic generated using ChatGPT (OpenAI, 2026) based on the authors' sensor design.* ***Schematic not to scale.***

The three main innovative contributions of our concept are the flexible microneedle platform with LIG-based sensing tips, the enzyme-immobilization strategy, and the dual-working-electrode design for compensation of common signal disturbances. For the wearable sensor, the technological novelty (Section 3.1.1) is discussed under two aspects: the manufacturing process and the L-Dopa sensing strategy.

So far, we have performed mechanical tests on the microneedle arrays to assess their mechanical feasibility. The proposed LIG/Prussian Blue/tyrosinase biosensing approach was tested only on flat parylene-based electrodes as a proof of principle. We also reviewed the biocompatibility of the individual materials used in the sensor design. The complete integration and electrochemical functionalization of the microneedle tips, device-specific biocompatibility testing, and experimental validation of the dual-working-electrode correction remain work in progress.

3.1.1. Technological novelty of wearable sensor

The technological novelty of our wearable sensor lies in the manufacturing process and our design strategy for L-Dopa sensing.

3.1.1.1 Manufacturing Process

Epidermis and dermis thickness show high variation according to factors such as age, BMI, and gender among individuals (Van Mulder et al., 2017). When designing a microneedle array for L-Dopa sensing, one height does not fit all. Conventional microneedle fabrication often relies on prefabricated moulds, photomasks, or etching conditions that determine the microneedle geometry and size.

In our microneedle design, we wanted to prevent this limitation. We researched the microneedle manufacturing techniques available at our university and found that Hu Peng, a previous PhD candidate in the Neuroelectronics team, had developed flexible three-dimensional electrode arrays for neural interfacing. We found that this manufacturing approach could be adapted to our wearable sensor.

The microneedle array uses a thin flexible polyimide film as the base of the device, which allows the final array to remain flexible when placed on the body. During manufacturing, the polyimide film is fixed to a glass wafer using double-sided tape for mechanical support. The planar metal feedlines, contact pads, and pillar electrode pads can be defined by either

lasering or depositing a thin metal layer using PVD, patterning the photoresist using maskless photolithography, and etching the unprotected metal areas. The feedlines are made of gold.

After the patterns are defined, silver ink is printed onto the electrode pads using a piezoelectric inkjet printer. A printing velocity of 55 mm/s was selected because it provided improved pillar uniformity under the tested printing conditions. The printing process was also tested using two nozzles instead of one. Although this reduced the printing time by half, the outer pillars became higher than the inner pillars. Therefore, one nozzle is recommended when more precise and uniform pillar sizes are required.

An important advantage of this manufacturing approach for our wearable design is that the microneedle size, height, and shape can be adjusted through the digital printing file and printing conditions. Therefore, different microneedle geometries can be produced without preparing a new mould. This flexibility is particularly relevant for a wearable sensor because epidermis and dermis thickness vary between individuals. Therefore, one microneedle size may not be suitable for everyone, and the microneedle dimensions may need to be adapted accordingly for L-Dopa sensing.

After printing and sintering, the device outlines are defined using a laser. The complete array, including the top, bottom, feedlines, and pillar sidewalls, is encapsulated with parylene using chemical vapour deposition. During this process, parylene dimers are vaporized above 100 °C and converted into reactive monomers in a pyrolysis furnace above 600 °C. The monomer vapour then enters a low-pressure deposition chamber, where it polymerizes at approximately room temperature to form a thin, uniform, and conformal parylene coating over the complete array (Peng, 2025).

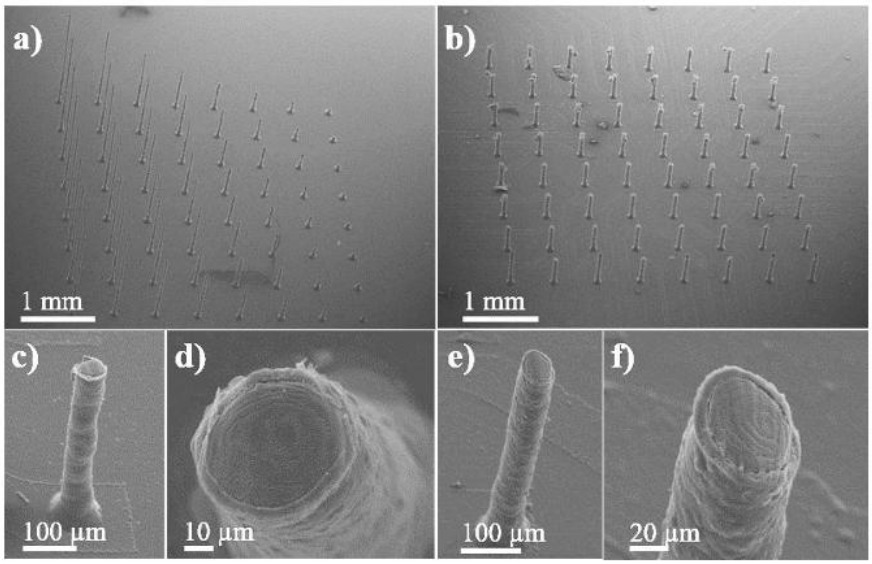


Figure 2-2: SEM characterization of inkjet-printed pillars and laser-processed tips. *(a) Pillars were fabricated using increasing numbers of deposited ink droplets, from 100 to 800 droplets per pillar, showing that pillar height can be adjusted through the printing parameters. (b) A pillar printed using 400 droplets is shown after laser ablation of the tip. (c–f) Close-up SEM images compare tip geometries produced by perpendicular and angled laser ablation.* ***(Peng, 2025)***

In the original design, the parylene at the pillar tips was removed using UV-laser ablation to expose the underlying conductive silver (Peng, 2025). Our design does not require this same tip-opening approach, because selected areas of the parylene-based surface are instead used for the formation of LIG, as described in Section 3.1.1.2.

3.1.1.2 Design Strategy for L-Dopa Sensing

In our design, a UV laser is used to convert selected areas of the parylene-based surface into laser-induced graphene (LIG). The LIG tips are then electrodeposited with Prussian Blue using a 5 mM deposition solution and functionalized with tyrosinase, as described in *Section 3.2.1.1*, to enable L-Dopa detection at −0.05 V versus Ag/AgCl. Due to time limitations, LIG patterning and electrochemical functionalization were not tested on the microneedle tips. However, the same laser-patterning and surface-functionalization approach was successfully demonstrated on flat parylene-based electrodes using the marking parameters listed in *Appendix Table A1*. These results provide proof of principle for the sensing chemistry and LIG-based electrode fabrication, while integration on the microneedle array remains a work in progress.

To the best of our knowledge, parylene-derived LIG has not previously been reported on microneedle arrays for electrochemical sensing. A recent study demonstrated LIG on

carbon-coated 3D-printed microneedles for olanzapine detection, but the LIG was produced from a carbon coating rather than parylene (Kadian et al., 2026)

3.1.2. Technical feasibility of wearable sensor

To assess the technical feasibility of our microneedle arrays for wearable use, mechanical tests were performed and the biocompatibility of the materials used in the sensor was reviewed.

3.1.2.1. Mechanical Tests performed on the Microneedle Array

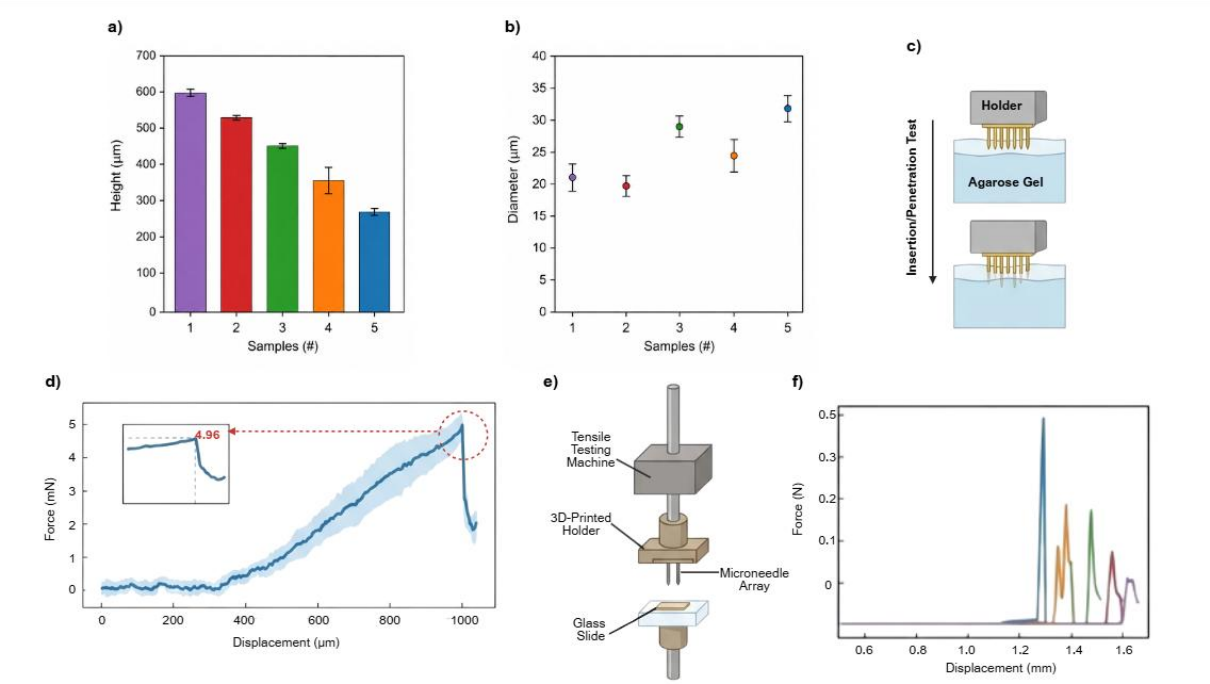


Figure 2-3. Mechanical characterization of the 4x4 microneedle arrays. *(a) Average pillar height and (b) average pillar diameter of five array samples with different geometries; error bars represent SD, n = 16 pillar measurements per array (c) Schematic of the insertion test into agarose gel. (d) Force–displacement curve for insertion of the approximately 600 µm-height array into agarose, showing a peak force of approximately 4.96 mN for the complete 4x4 array. (e) Schematic of the compression-test setup against a flat glass slide. (f) Compression force–displacement curves for the five arrays for compression test.*

To find suitable parameters for our wearable design, we performed compression and insertion/penetration tests on five 4x4 microneedle arrays with different pillar heights and diameters using a tensile testing machine. The pillar heights ranged from approximately 265 to 600 µm, while the pillar diameters ranged from approximately 20 to 35 µm. *Figures 2-3(a)* and *2-3(b)* show the measured heights and pillar diameters of the five different array samples.

The compression-test results are shown as force–displacement curves in *Figure 2-3(f).* For the compression test, the microneedles were fixed onto a 3D-printed holder and pressed against a flat glass slide, as illustrated in Figure *2-3(e).* The maximum compression force was lowest for the array with the longest pillars, ~ 600 µm. As the pillar height decreased, the required maximum compression force increased. For ~ 600 µm array, a maximum force of 0.11 N was measured, corresponding to approximately 6.88 mN per pillar. For the shortest array, with a pillar height of ~ 268 µm, the maximum force was ~ 0.50 N, corresponding to 31.25 mN per pillar. As can be seen in Figure *2-3(f),* the ~ 300 µm height arrays showed two force peaks, at ~ 0.19 and 0.29 N, because the pillars fractured in two sequential stages.

A microneedle array should not fracture or bend during insertion through the *stratum corneum*. The geometry, height, diameter, and material composition of the pillars affect microneedle insertion and penetration (Makvandi et al., 2021). An agarose gel phantom was used to measure the force required to insert the microneedles into a soft material. For the insertion test, an array with a pillar height of approximately 600 µm was selected. As shown in the force–displacement graph in Figure *2-3(d),* a peak force of approximately 4.96 mN was required to insert the complete 4×4 array. Assuming that the force was distributed equally across all 16 pillars, this corresponds to approximately 0.31 mN per pillar. The insertion force per pillar was therefore approximately 22 times lower than the maximum compression force of the approximately 600 µm pillars.

Although our L-Dopa microneedle array may not need to penetrate the skin to a depth of 600 µm, the longest pillars were selected as a worst-case scenario because taller pillars showed the lowest resistance to compression. To conclude, successful insertion of these pillars supports the mechanical feasibility of using shorter pillars made by the same manufacturing method. However, agarose gel does not mimic the layered mechanical properties of human skin. In our future work, we plan to repeat the insertion tests using the shorter arrays and a more skin-relevant model, such as a PDMS layer with an agarose gel at the bottom.

3.1.2.2. Biocompatibility of the Microneedle

ISO 10993-1 requires the biological safety of the final medical device to be evaluated based on its intended use and contact with the body (ISO 10993-1, 2025). Since our wearable sensor is still in the design stage and the complete device has not yet been finalized, we first reviewed the biocompatibility of the individual materials used in our design.

Since the microneedle tips are intended to penetrate the skin and interact with interstitial fluid, the biocompatibility of the exposed materials is important. Parylene-based materials are widely used as a coating and encapsulation material in biomedical devices, while LIG and Nafion have also shown good biocompatibility in previous studies (Chang et al., 2007; d'Amora et al., 2020; Turner et al., 1991). Prussian Blue has also been investigated for wearable biosensors and other biomedical applications (Jiang et al., 2022). However, these studies mainly consider the individual materials and therefore do not demonstrate the biocompatibility of our complete microneedle sensor.

Our final aim is to evaluate the biocompatibility of the complete integrated sensor according to ISO 10993-1 and the final classification of the device. Since the wearable remains outside the body while the microneedles penetrate the skin and contact tissue/interstitial fluid, the device would most likely be classified as an externally communicating device with tissue contact. If the intended cumulative contact time is between 24 hours and 30 days, it would fall under the prolonged-contact category (ISO 10993-1, 2025). The relevant biological endpoints for this device category and contact duration would then need to be evaluated for the final sensor.

3.2. Reliability of sensor output

3.2.1. Technological novelty of reliability concept

Our reliability concepts are centred around our two-working electrode design and enzyme immobilization strategy.

3.2.1.1. Enzyme Immobilization Strategy

APTES is commonly used for surface modification and enzyme immobilization in biosensors. It reacts with hydroxyl groups on oxide surfaces and introduces amine groups. A stable APTES monolayer is preferred because multilayers can detach during washing and processing steps; therefore, APTES concentration is important for biosensor performance (Sypabekova et al., 2022). Following APTES functionalization, glutaraldehyde (GA) reacts with the surface amine groups and supports enzyme immobilization (Rusu et al., 2022).

The amine groups of tyrosinase can react with the aldehyde groups of GA. However, excessive GA-mediated cross-linking can lead to enzyme aggregation and reduced activity.

For this reason, BSA is used as an auxiliary protective protein. BSA provides additional amino groups that react with GA, which can reduce excessive cross-linking of tyrosinase and help maintain enzyme stability and activity (Mafra et al., 2016; Ifko et al., 2023).

Initially, our enzyme functionalization strategy on Prussian Blue electrodeposited LIG surfaces consisted of three steps: (i) surface functionalization with APTES, (ii) glutaraldehyde (GA) activation, and (iii) immobilization of a tyrosinase–BSA mixture. *Table 2-1* summarizes the different immobilization strategies evaluated in our project.

For Group 1, no distinguishable current response was observed for the L-Dopa samples compared with the PBS blank. We therefore increased the tyrosinase concentration from 2 to 20 mg/mL in Group 2. This 10-fold increase enabled the detection of the two to three lowest L-Dopa concentrations tested. However, the enzyme layer was not stably immobilized and was gradually washed away during subsequent measurements *(Figure 2-5)*. Based on these results, we modified the immobilization strategy to improve enzyme retention while maintaining the L-Dopa response.

Table 2-1: Tyrosinase Functionalization Strategies on Prussian-Blue Electrodeposited LIG surface

Group	APTES surface functionalization (% v/v)	GA addition (% v/v)	BSA concentration (mg/mL)	Tyrosinase concentration (mg/mL)	GA in enzyme mixture, one-pot (% v/v)	Nafion coating (% w/v)
G1	2	2	10	2	—	—
G2	2	2	10	20	—	—
G3	2	1	10	2.5	1	—
G4	2	1	10	2.5	1	1

Cross-linked enzyme aggregates (CLEAs), referred to as the one-pot method in this report, are a carrier-free enzyme immobilization method in which enzyme molecules are aggregated and chemically cross-linked without being attached to a solid support (Blanco-Llamero et al., 2021). Accordingly, a mixture containing BSA, tyrosinase, and glutaraldehyde (GA), prepared without an oxide surface or APTES functionalization, can be considered a one-pot formulation.

Last year, a BSA–GA–tyrosinase mixture (one pot by itself) was tested by our team on commercial electrodes, but the enzyme layer did not remain on the electrode surface for more than a few hours. After the unsatisfactory results obtained with G1 and G2, we decided to integrate this approach with our current APTES–GA anchoring strategy.

For G3 and G4, we combined the pre-mixed BSA–GA–tyrosinase formulation with the APTES- and GA-treated electrode surface. In this way, the enzyme mixture was cross-linked within the formulation while also being anchored to the functionalized electrode surface. The mixture contained 10 mg/mL BSA, 2.5 mg/mL tyrosinase, and 1% (v/v) GA. L-Dopa measurements performed 34 hours after immobilization, shown in *Figure 2-4*, suggested that the enzymes were not washed away from the electrode surface when this combined immobilization method was used. These results indicate that combining the two immobilization approaches may improve enzyme retention; however, further experiments are required to confirm this effect and understand the underlying mechanism.

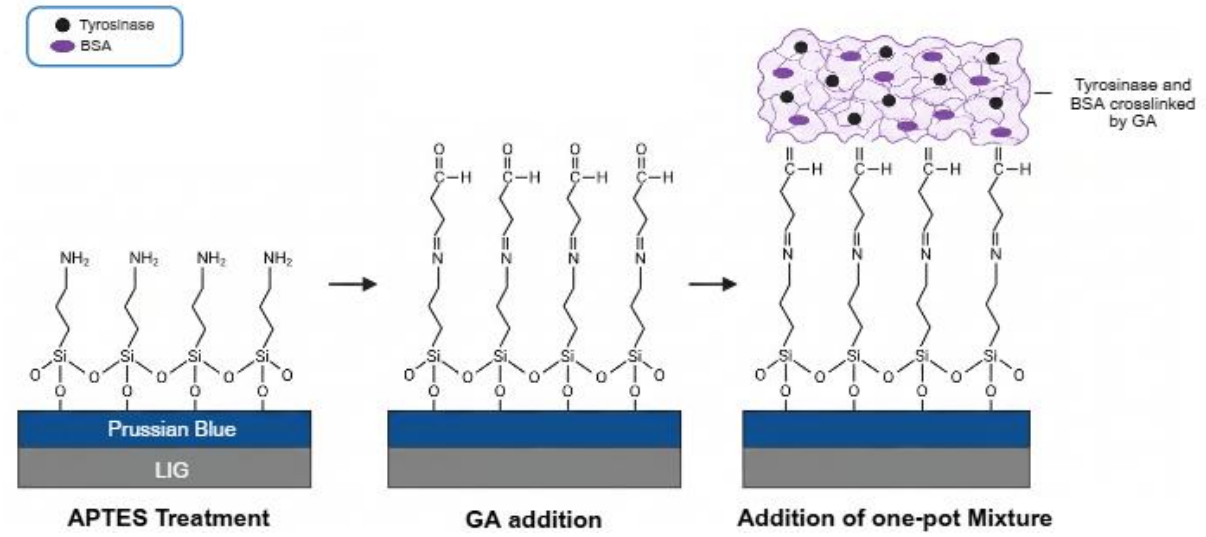


***Figure 2-4: Schematic of the combined enzyme-immobilization strategy used for G3 and G4**. The Prussian Blue-modified LIG surface is first functionalized with APTES to introduce amine groups, followed by GA activation. A pre-mixed BSA–GA–tyrosinase formulation is subsequently added, allowing the enzyme mixture to be cross-linked within the formulation while also being anchored to the functionalized electrode surface. G4 additionally contains a 1% Nafion coating on top of the enzyme layer, which is not shown in the schematic*

The main difference between G3 and G4 was the addition of a 1% Nafion coating in G4. During the 50 µM L-Dopa measurement with G3, the electrode was not washed properly, and the measured response was closer to that expected for approximately 12.5 µM. This altered experimental condition may have affected the response, although the exact mechanism cannot be determined from the current experiment.

Therefore, the calibration was evaluated both with and without the 50 µM point. Both regressions are shown to illustrate its influence on the calibration. The point should not be considered a confirmed outlier, and repeated measurements are required before establishing an exclusion criterion.

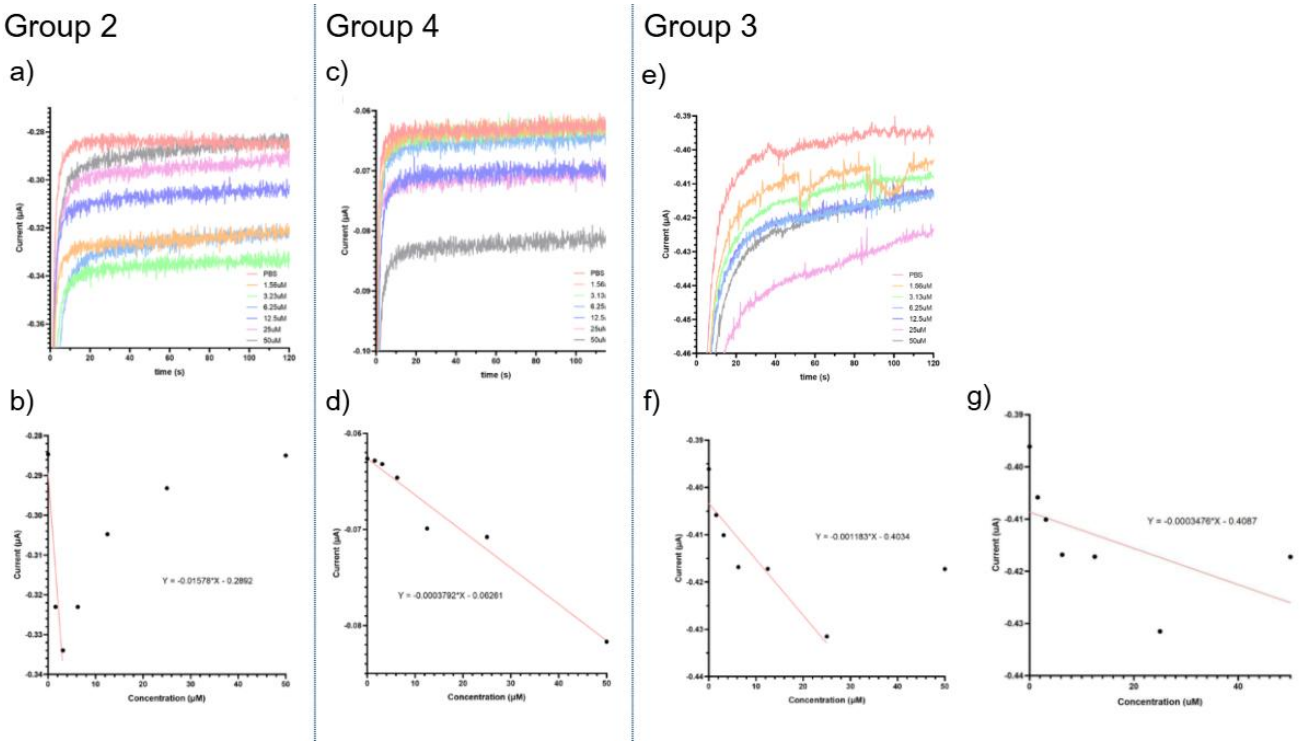


Figure 2-5: Chronoamperometric responses and calibration curves of tyrosinase-functionalized Prussian Blue/LIG electrodes for L-Dopa detection. *Chronoamperometric responses and corresponding calibration curves are shown for (a, b) Group 2, (c, d) Group 4, and (e–g) Group 3. Measurements were performed in PBS with L-Dopa concentrations of 1.56, 3.13, 6.25, 12.5, 25, and 50 µM at −0.05 V versus Ag/AgCl. Groups 3 and 4 were measured 34 h after enzyme immobilization, and Group 4 additionally contained a 1% Nafion coating. For Group 3, the 50 µM measurement deviated from the trend and was obtained after the electrode had not been washed properly. Therefore, the calibration is shown both (f) excluding and (g) including the 50 µM point in the linear regression. The 50 µM measurement is retained in both plots and is not considered a confirmed outlier. Overall, Groups 3 and 4 showed a concentration-dependent L-Dopa response, but repeated measurements are required before reliably comparing sensitivity or the effect of Nafion.*

Overall, the results obtained with both G3 and G4 were promising and suggested that the modified immobilization approach improved enzyme retention during the measurements. Differences in response were observed between the electrodes with and without the Nafion coating; however, the limited number of measurements and the deviating 50 µM measurement for G3 do not yet allow a reliable comparison of their sensitivities. Formal sensitivity, repeatability, and LOD were not calculated at this stage because sufficient repeated measurements are not yet available. The experiments therefore need to be repeated before drawing a strong conclusion regarding the effect of Nafion. Our aim is therefore to avoid Nafion if possible, provided that sufficient enzyme retention can be achieved without it, since the additional coating may limit analyte diffusion to the electrode surface.

3.2.1.2. Two-working electrode Design

The second reliability concept of our sensor is the use of two working electrodes. WE1 is functionalized with tyrosinase and is intended to measure the L-Dopa-dependent electrochemical response together with background changes affecting the electrode system. WE2 has the same underlying electrode structure and Prussian Blue coating but does not contain the biorecognition element. Therefore, WE2 is intended to act as a control electrode for disturbances that are expected to affect both working electrodes.

These common disturbances may include baseline drift, temperature-related changes, protein adsorption, and reference-electrode potential shifts. Since these effects can influence both WE1 and WE2, the signal measured at WE2 can be used to correct the response measured at WE1. The corrected signal can be expressed as:

$$I_{corrected} = I_{enzyme} - kI_{control}$$

where k is a scaling factor determined from baseline measurements without L-Dopa.

The scaling factor is included because WE1 and WE2 may not produce identical background currents even when exposed to the same conditions. Differences in electrode area, surface modification, impedance, and baseline current can lead to different signal magnitudes between nominally similar electrodes. Similar differential sensing approaches have therefore used baseline or offset correction before subtraction of the control signal (Maier et al., 2019; Spring et al., 2021). In our design, k will be determined experimentally from baseline measurements without L-Dopa.

The purpose of this design is to reduce disturbances that are common to both working electrodes while maintaining the L-Dopa-dependent contribution measured at WE1. However, the correction is not expected to compensate for effects that occur specifically at the enzyme-functionalized electrode, such as enzyme degradation, enzyme-layer loss, or unequal fouling. The dual-working-electrode correction has not yet been experimentally validated and remains part of the future work described in Section 3.2.2.

3.2.1.3. Electronics

The reusable main electronics include the major system components, such as a potentiostat/analog front-end (AFE) for electrochemical signal acquisition, a

microcontroller (MCU) for system control and signal processing, a Bluetooth module for wireless data transmission, and a battery with a power management unit to power the system.

The electronics will be designed to be as compact and lightweight as possible to improve the portability and wearability of the sensor. To minimize power consumption, the system will employ low-power operation strategies, including sleep modes and selective activation of individual system components only when required for measurement or data transmission.

The disposable substrate contains conductive traces that electrically connect the microneedle electrodes to contact pads on the substrate. The electrochemical signals from the microneedles are routed through these traces to the contact pads, which interface with pogo pins on the reusable main PCB. The pogo pin connection provides a detachable electrical interface between the disposable microneedle substrate and the reusable electronics while allowing the microneedles to remain on the skin-facing side of the device.

3.2.2. Technical feasibility of reliability concept

The dual-working-electrode correction has not yet been experimentally validated. As a next step, WE1 and WE2 will be characterized separately under baseline conditions to determine the scaling factor k and to account for differences between the two electrodes.

Future work will include long-term chronoamperometry, protein-fouling tests, temperature and reference-electrode stability measurements, and experiments with both increasing and decreasing L-Dopa concentrations. The corrected signal will then be compared with the uncorrected enzyme-electrode response and, at a later stage, with a reference method such as HPLC.

Conclusion

In conclusion, we introduced a flexible microneedle sensor concept for L-Dopa monitoring that combines an adaptable inkjet-printed microneedle platform, parylene-derived LIG sensing tips, Prussian Blue/tyrosinase-based detection, a modified enzyme-immobilization strategy, and a two-working-electrode reliability concept.

To the best of our knowledge, parylene-derived LIG has not previously been reported on microneedle arrays for electrochemical sensing. Although a recent study demonstrated LIG

on carbon-coated 3D-printed microneedles for olanzapine detection (Kadian et al., 2026), the LIG in that system was produced from a carbon coating rather than parylene.

A further innovation is the combination of an APTES/GA-activated electrode surface with a separately prepared BSA–GA–tyrosinase formulation for Prussian Blue-modified LIG electrodes. In addition, our two-working-electrode concept uses an enzyme-functionalized WE1 for L-Dopa sensing and an enzyme-free WE2 as a control for common disturbances, allowing a scaled correction of the WE1 signal.

We demonstrated the mechanical feasibility of the microneedle arrays and proof of principle for the LIG/Prussian Blue/tyrosinase sensing approach on flat parylene-based electrodes. Integration of the complete sensing system on the microneedle tips and experimental validation of the two-working-electrode correction remain to be investigated.

3.3. Original contributions

By Supervisors

Munich Bioneers independently worked on the main innovative ideas of their wearable sensor, including the microneedle design, dual-working-electrode design, tyrosinase-based recognition, and adaptation of an existing microneedle architecture for L-DOPA sensing. Building on earlier work from the Neuroelectronics group on flexible microneedles for neural interfacing, the team designed a four-electrode microneedle array with laser-patterned LIG tips specifically adapted for L-DOPA sensing. Due to time constraints, the device remained at the design stage. As a proof of concept, the team independently designed and fabricated planar electrodes on a parylene material and demonstrated L-DOPA sensing using the intended enzyme immobilization strategy.

Munich Bioneers also independently developed the complete electronic readout and control platform for the wearable sensor. A custom PCB was designed and fabricated around the ADuCM355 electrochemical analog front end and microcontroller, enabling direct interfacing with the electrochemical sensor and acquisition of low-level sensor signals. The board integrates dedicated power management, USB/UART communication, Bluetooth connectivity, motion sensing, and programming/debug interfaces into a single platform. In parallel, the team independently developed the embedded firmware and software interfaces required for sensor control, data communication, and visualization. Together, the custom electronics and software establish a complete sensing chain from the electrochemical sensor to digital processing and user-level data access.

Through the team's hard work, dedication, and continued effort, Munich Bioneers made significant progress compared with the previous year.





Bernhard Wolfrum Bernhard Gleich Can Dincer

By Team Co-Captains

We, Munich Bioneers, independently developed the main features of our wearable L-DOPA sensing concept. We selected microneedles as the sensing platform, chose tyrosinase as the biorecognition element, and designed a system with two working electrodes to improve signal reliability.

Our design was based on a parylene microneedle platform originally developed by Hu Peng in the Neuroelectronics group for neural interfacing and silver nanoparticle detection. We adapted this concept by proposing a four-electrode microneedle array with laser-induced graphene (LIG) sensing surfaces.

Instead, we demonstrated the sensing concept using planar parylene-based electrodes. While the substrate material was provided by the Neuroelectronics group, we designed, laser-patterned, and fabricated the electrodes ourselves. The resulting L-Dopa measurements supported the feasibility of our planned microneedle sensing approach.

Our team independently developed the complete electronic readout and control platform for the wearable sensor. A custom PCB was designed and fabricated around the ADuCM355 electrochemical analog front end and microcontroller, enabling direct interfacing with the electrochemical sensor and acquisition of low-level sensor signals.
The board integrates dedicated power management, USB/UART communication, Bluetooth connectivity, motion sensing, and programming/debug interfaces into a single platform.

In parallel, our team independently developed the embedded firmware and software interfaces required for sensor control, data, communication, and visualization. Together, the custom electronics and software establish a complete sensing chain from the electrochemical sensor to digital processing and user level data access.



Tayis Arslan Melisa Karatas Jaehee Choi Eyed Fetni

4. TP award: Translation potential

4.1. Customer interviews

To learn insight information about design requirements for the device, we conducted various interviews with different stakeholder groups i.e. neurologists, patients, patient

organisations and reviewed literature of previous interviews with stakeholders such as nurses, caregivers, physiotherapists and wearable/pump user patients.

For the interviews we conducted, we first made contact by attending the Parkinson´s Information conference at LMU, where we met doctors and patients, and then scheduled interview dates with the people we met in person. At the same time, to gather more data, we examined existing literature and articles based on previous interview from all over the world. A full list of interview questions we used in Appendix B and C.

4.1.1. Identification of potential stakeholders

Our primary stakeholders group consisted of three patients with Parkinson´s disease (Mr. Rauscher, Mr. Karlheinz and Mr. Albien), and three neurologists experienced in treatment of Parkinson´s disease (Dr. Zwergal, Dr. Köglsperger and Dr. Höglinger). Patients were selected to gain first-hand experience into daily L-Dopa use, medication fluctuations, recognition of On/Off Periods and expectations regarding a wearable L-Dopa biosensor. Neurologists were included to learn the limitations of current clinical methods and clinical relevance of continuous L-Dopa measurements. In addition, Patient organizations we contacted (PingPongParkinson) complemented the individual patient perspectives by representing broader group of patient's needs and experiences.

The primary stakeholder interviews were complemented by secondary stakeholder evidence from published qualitative studies and documented patient experience reports. These additional stakeholders included Parkinson nurses and physiotherapists (Evers et al., 2023), patients receiving continuous L-Dopa pump therapy (Soileau et al., 2025), patients with experience regarding wearable monitoring systems (Kenny et al., 2022) (Cancela et al., 2014) and caregivers of people with Parkinson´s disease (Pigott et al., 2022)

4.1.2. Interview approach and execution

To systematically explore stakeholder needs, we conducted semi-structure interviews with Parkinson´s patients and neurologists. Separate interview guides were prepared for each stakeholder group while maintaining a set of core questions to enable comparison between participants.

Patient interviews focused on everyday experiences with L-Dopa therapy, On/Off fluctuations, medication-related challenges, unmet needs and expectations for wearables. Neurologist interviews addressed current treatment and monitoring practices, limitations in L-Dopa dose adjustment, relevant patient groups, and the potential clinical value and limitations of continuous L-Dopa monitoring. The predefined questions provided a common structure, while open-ended follow-up questions allowed participants to elaborate on individual experiences and unexpected topics.

Participation was voluntary and participants were informed about the purpose of the interviews and the intended use of their input for the project. The interviews were documented and reviewed for recurring themes, unmet needs and contrasting perspectives, which were subsequently compared across stakeholder groups and with published stakeholder evidence.

4.1.3. Interview results and conclusions

A central finding across the patient interviews was that the effect of L-Dopa is not always stable or predictable throughout the day. Stress, physical activity, food and protein intake, medication timing, and individual circumstances can influence the treatment response. Mr. Albien described stress-related OFF phases, while Mr. Karlheinz emphasized that the duration of medication effectiveness can vary from day to day. Importantly, patients may recognize decreasing medication effectiveness only after symptoms have already appeared. Mr. Rauscher described noticing insufficient medication through muscle cramps and increasing movement difficulties.

Patients generally considered continuous L-Dopa monitoring meaningful as a complement to movement-based monitoring. However, acceptance was strongly dependent on usability. The sensor should be small, comfortable, practical, hygienic and unobtrusive. The interviewed patients showed no major resistance toward a microneedle-based approach when it provides a clear benefit. Trust was another important requirement: reliable measurements, clinical validation and physician recommendation were considered important for adoption, while patients should remain involved in treatment decisions.

The neurologist interviews confirmed a major limitation in current Parkinson's disease management: L-Dopa adjustment is predominantly based on clinical examination, patient history and self-reported symptoms rather than a direct objective treatment parameter. A short clinical appointment provides only a snapshot of a condition that can fluctuate substantially throughout the day. Symptom diaries can provide additional longitudinal information but require considerable patient effort. Objective and continuous monitoring could therefore provide a more representative picture of ON/OFF states and treatment fluctuations.

The clinicians emphasized that this problem becomes increasingly relevant as Parkinson's disease progresses. Early-stage patients can often maintain relatively stable symptom control with a limited number of oral L-Dopa doses, whereas more advanced disease is associated with increasingly complex medication schedules, OFF periods, dyskinesia and sometimes unpredictable fluctuations. Continuous monitoring may therefore provide its

greatest initial value in patients with pronounced treatment fluctuations, particularly those receiving or being considered for continuous L-Dopa pump therapy.

Two particularly relevant potential applications emerged from the clinical interviews. First, continuous L-Dopa sensing could provide an early-warning signal when drug levels begin to decrease, potentially allowing intervention before the patient experiences a clinically relevant OFF state. Second, L-Dopa concentration could potentially serve as one feedback parameter for future adaptive pump therapy.

Consequently, the temporal development of L-Dopa concentration and its patient-specific relationship with clinical symptoms may be more informative than a single absolute concentration value. Objective measurements should therefore complement rather than replace the patient's subjective experience and functional state. Since clinical response is influenced by factors beyond circulating L-Dopa concentration, its temporal development and patient-specific relationship with symptoms may be more informative than a single absolute value.

Published stakeholder evidence supported these findings: Parkinson nurses and physiotherapists reported difficulties in patients recognizing symptoms such as OFF states or distinguishing tremor from dyskinesia, while symptom diaries can impose an additional burden (Evers et al., 2023).

Pump users reported more predictable symptom control, greater freedom and improved social participation, but also physical burdens including infusion-site problems, stoma discomfort, maintenance and body-image concerns (Soileau et al., 2025). These experiences indicate that therapeutic benefit alone is insufficient for long-term acceptance and that the physical burden of the device and skin interface should be minimized.

Wearable users valued objective information for medication management and communication with clinicians, particularly because some found it difficult to distinguish insufficient medication from overmedication (Kenny et al., 2022) (Cancela et al., 2014).

Caregiver studies further showed that, particularly with increasing cognitive or communication difficulties, caregivers may become important in observing changes and communicating the patient's condition to healthcare professionals (Pigott et al., 2022).

Taken together, the primary interviews and complementary stakeholder evidence refined both our problem definition and the requirements for the proposed solution. The central unmet need is not simply the absence of an L-Dopa measurement, but the lack of objective, longitudinal and patient-specific information capable of capturing treatment fluctuations between clinical visits. The findings therefore support a minimally invasive and unobtrusive system that monitors the temporal profile of L-Dopa while allowing this information to be interpreted in relation to the patient's clinical and functional state.

4.2. Design of validation study

4.2.1. Selection of the problem

Across all our interviews, the same observation returned: a patient's actual levodopa status is never measured. Clinicians infer it from a physical examination during the appointment, and patients judge it from the symptoms they happen to feel at that moment. Both described this gap independently, which gives us confidence the problem is real. Its main consequence is that dosing becomes reactive: patients wait for symptoms before taking the next dose, and as one patient put it, by the time you feel it, it is usually already too late. This defines the problem we set out to address. Put simply, there is no way to measure a patient's levodopa level. More usefully, we cannot see how a given level corresponds to a patient's clinical state, meaning how well they actually move and function, and this correspondence differs from one patient to the next. Making that individual link visible is the problem our sensor aims to solve.

4.2.2. Conceptual design and use case

Design: A small wearable patch worn on the arm, built around a microneedle array that measures the levodopa concentration in the interstitial fluid just below the skin. The patch samples continuously and sends the readings to a phone application over a wireless link, showing not only the current level but how it is changing, so that the direction of movement is visible rather than a single isolated value.

Use case: In its near-term form, the device guides the patient's own dosing at home while the same continuous data is shared with the treating neurologist for remote follow-up. In the longer term, the signal could feed a levodopa pump to adjust the dose automatically, the closed-loop approach several interviewed clinicians described as the expected direction of the field.

Added value: The central problem was that a patient's levodopa status is never measured, so the sensor makes that hidden variable visible and continuous. Because it shows a trend rather than a single value, the patient can act before an OFF period arrives instead of after it, turning dosing into a proactive decision. A movement-based wearable cannot offer this, as it detects symptoms only once they are present. Since one level does not mean the same clinical state for every patient, the reading is calibrated individually against each patient's own threshold. Patients therefore gain fewer and shorter OFF periods and less uncertainty about dosing, clinicians gain an objective parameter to steer therapy by that does not exist today, and the same signal would make adaptive, closed-loop dosing possible in the future.

Feasibility and critical aspect: Feasibility is supported by the earlier sections, where the enzymatic detection of levodopa and the mechanical insertion of the microneedle array were demonstrated. The aspect on which the entire value proposition depends, and which is therefore placed at the centre of the validation study, is whether the interstitial fluid levodopa concentration follows the blood level closely and quickly enough to deliver the promised early warning.

4.2.3. Validation study design

Setting and participants

- **Where.** A two-centre study at the Departments of Neurology of LMU Klinikum Großhadern and Klinikum rechts der Isar, TUM, both specialised movement-disorder units with established Parkinson outpatient clinics and contacts made during the stakeholder interviews.
- **Who.** Approximately 24 patients in the mid-to-advanced stage with documented motor fluctuations. Early-phase patients are excluded, as the interviewed clinicians indicated this group has little need for continuous monitoring.
- **Conditions.** Conducted in accordance with the Declaration of Helsinki and Good Clinical Practice, following ethics committee approval, with written informed consent and pseudonymised data handling under the GDPR.

Aim of the study

Two claims are tested:

1. **The levodopa level falls before symptoms appear**, the condition that makes an early warning possible.
2. **Access to this information changes behaviour**, so that patients dose earlier and achieve better control across the day.

The order is deliberate: if no usable warning exists, the second claim cannot be evaluated, so Part 1 also functions as a decision point for Part 2.

What will be measured

- **Warning time.**

$$\Delta T = T_symptoms - T_sensor$$

  A positive value means the sensor signalled first; the mean ΔT quantifies the early warning.
- **Time in the individual good range.** The proportion of the day within the band where the patient is neither under- nor over-active, determined individually beforehand, following the time-in-range principle used in diabetes care.

- **Daily disease burden.** Hours spent in OFF periods and number of unplanned additional doses.

How the measurements are obtained

- **Sensor.** Continuous levodopa readings with timestamps, so the onset of a falling level is known precisely.
- **Motor diary.** A structured log, already established in neurological practice, in which patients record their state at fixed intervals.
- **Blood samples.** Drawn across one dosing cycle and analysed by HPLC or LC-MS/MS as the reference against which the sensor is verified.

How the study is carried out

- **Part 1 — does the warning exist?** Patients wear the sensor over several days with the motor diary and blood sampling across one dosing cycle, yielding the mean warning time, verification against the laboratory reference, and each individual good range.
- **Part 2 — does it help?** Each patient is followed over two weeks, one with their usual routine and one with the readout visible, serving as their own comparison given the strong variation between individuals.

4.3. Bonus criteria Translation Potential

One of the strongest translational aspects of our project is that the biosensor platform has potential applications in two different settings. The first is the intended patient-facing application, in which the microneedle-based sensor could be used for continuous and longitudinal L-Dopa monitoring in people with Parkinson's disease. This represents the long-term clinical application of the technology and would require extensive clinical validation and regulatory approval.

At the same time, we identified a potential research-use-only (RUO) application. The sensor could be provided as a research tool to laboratories, clinical research groups, or pharmaceutical researchers interested in measuring and investigating L-Dopa concentrations. This creates an alternative and potentially earlier translation pathway while the patient-facing medical device continues through further development and validation. The dual-use approach therefore provides two potential routes for value creation. Rather than depending exclusively on the longer clinical translation pathway, the technology could first establish utility in research environments and subsequently progress toward patient applications. Together, these opportunities suggest a stepwise translation strategy as, in the near term, the biosensor could be further developed and validated as a research tool for L-Dopa measurement. Following sufficient analytical and clinical validation, the technology could progress toward a wearable monitoring system for selected Parkinson's patients. In the longer term, longitudinal sensor

data could support personalized treatment decisions and potentially contribute to adaptive L-Dopa delivery systems.

This staged approach provides a pathway for generating value and practical experience at earlier development stages while progressively addressing the greater technical, clinical and regulatory requirements associated with direct patient use.

5. Team and support

5.1. Contributions of the team members

Tayis Arslan, team co-captain, was leading the wet lab and innovation teams. Her main responsibilities included coordination of the wet lab team, development of the innovation strategy, and electrode design and manufacturing. She was also part of the enzyme immobilization team and helped organize accommodation and transportation to Eindhoven.

Melisa Karatas, team co-captain, was a member of translational potential team (lead and reviewed interviews/validation study/business strategy), and a member of electronics team(Bluetooth Connection, Motion Sensor, Power Management Unit design, Layouting)

Jaehee Choi, team co-captain, was a member of innovation (researching for advanced electronics), was the lead of electronics team (AFE design, ADuCM355 Integration, Power Management Unit design, Layouting), and contacted IST Jobst for sponsorship.

Eyed Fetni, team co-captain, was a member of the translation potential team (lead customer interviews/validation study), a member of electronics team (microcontroller debugging/firmware), and the lead of software team (developed the SensusCare mobile App).

Jiun Seo was responsible for the calibration curves. She tested the electrodes in PBS and human serum with different concentrations of L-Dopa, using both enzymatic and non-enzymatic approaches.

Thi Trang Tran was responsible for the flow cell. She designed and manufactured the flow cell for testing event.

Alexander Emrich was responsible for enzyme immobilization. He worked on and tested different enzyme immobilization protocols in the lab.

Pouya Taleei was responsible for enzyme immobilization. He tested different enzyme immobilization protocols in the lab.

Caroline Wendlandt was a team member in the electronics team (Power Management Unit design) and contacted Aisler and Würth electronics for sponsorship.

Elif Neva Yıldırım was responsible for content planning, visual design, and managing the team's social media presence.

5.2. People who have given support

Julia Schiemainski: Julia was our sample preparation officer for the Distributed Testing Event for the second year in a row. She also helped us with ordering materials and guided us in the proper handling of chemicals and biomaterials.

Matthis Bünning: Matthis was our data officer for the Distributed Testing Event. He supported the team with the data-related tasks during the event.

Elisabeth Haider: Elisabeth supported us with the financial organization of the project. She also helped with ordering the equipment and materials needed for our work.

5.3. Sponsors and partners

Aisler: Aisler supported our project through PCB manufacturing sponsorship. Their contribution enabled the production of our custom-designed printed circuit boards, which were required for the electronic integration of our system.

Würth Elektronik: Würth supported our project through sponsoring electronics components. Their contribution helped us to find relevant components for our PCB.

IST Jobst Technologies: IST Jobst Technologies supported our project by sponsoring the micropump used in the system. The provided component was important for the development, integration, and testing of the fluid-handling part of our setup

6. Final remarks

We would like to thank our professors and coaches for their continuous support and guidance throughout the project. We are also very grateful to the Munich Institute of Biomedical Engineering for giving us access to the laboratories, materials, and resources, and for sharing their expertise with us.

For half of our team, this is already the second year participating in the competition. Being part of this experience has been truly life-changing for us, both scientifically and personally. It gave us the opportunity to work across different disciplines, learn from experts, take responsibility within a team, and develop an idea from an early concept into a real biosensor project.

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8. Appendix

Appendix A

Table A1: *Operating parameters used for UV laser fabrication of the parylene-based electrodes.*

Laser System	3-axis UV Laser Marker
Power	54%
Scan Speed	16 mm/s
Pulse Frequency	300 kHz
Repetition	1
Type	Fill
Pattern	Slant
Angle	90°
Fill interval	0.005 mm

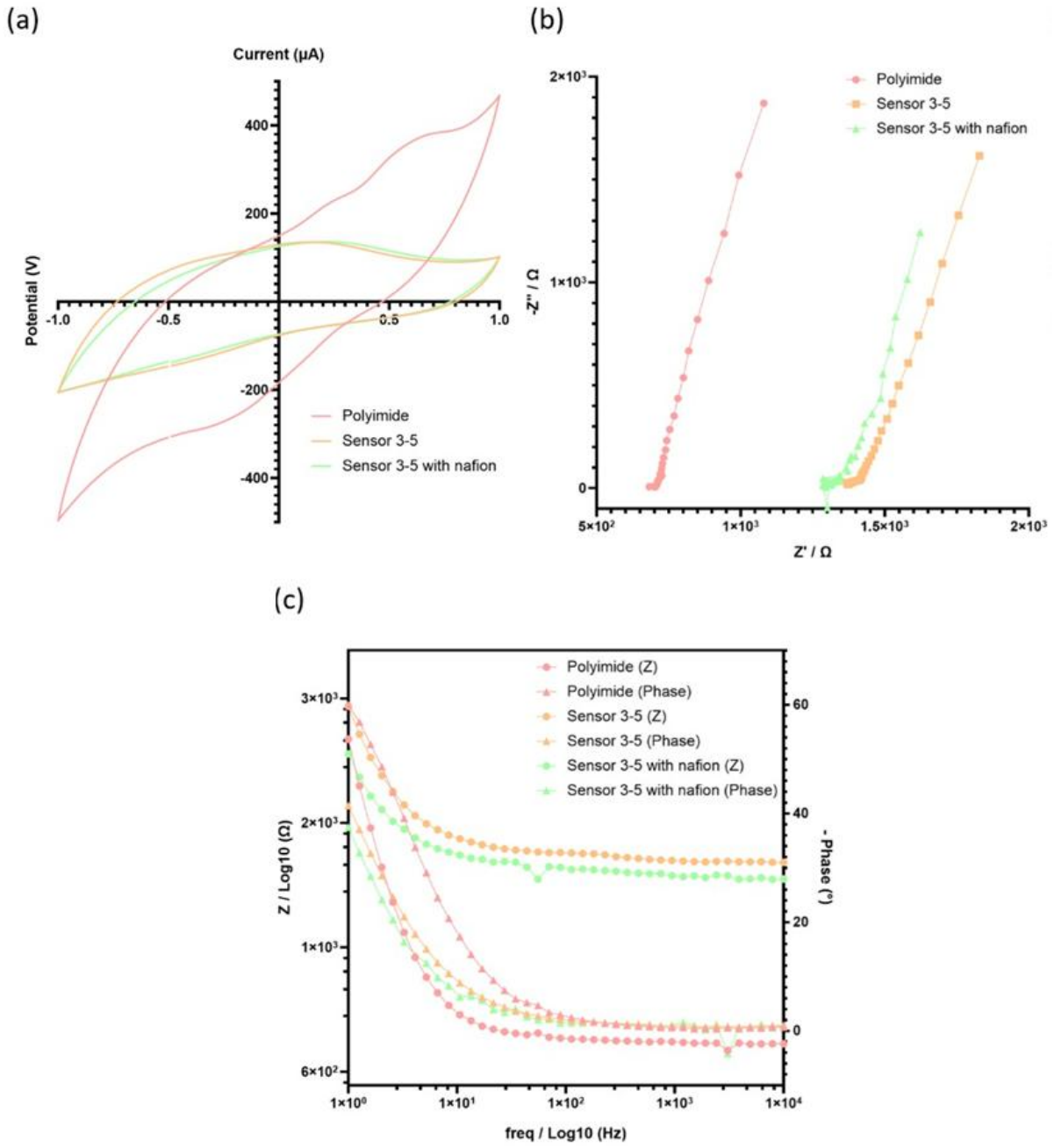


Figure A1: Electrochemical characterization of the electrode configurations used in the non-enzymatic sensing approach. *(a) Cyclic voltammograms of polyimide, Sensor 3-5, and Sensor 3-5 with Nafion. (b) Nyquist plots from electrochemical impedance spectroscopy (EIS), showing −Z'' versus Z'. (c) Bode plots from EIS, showing impedance magnitude and phase angle as a function of frequency.*

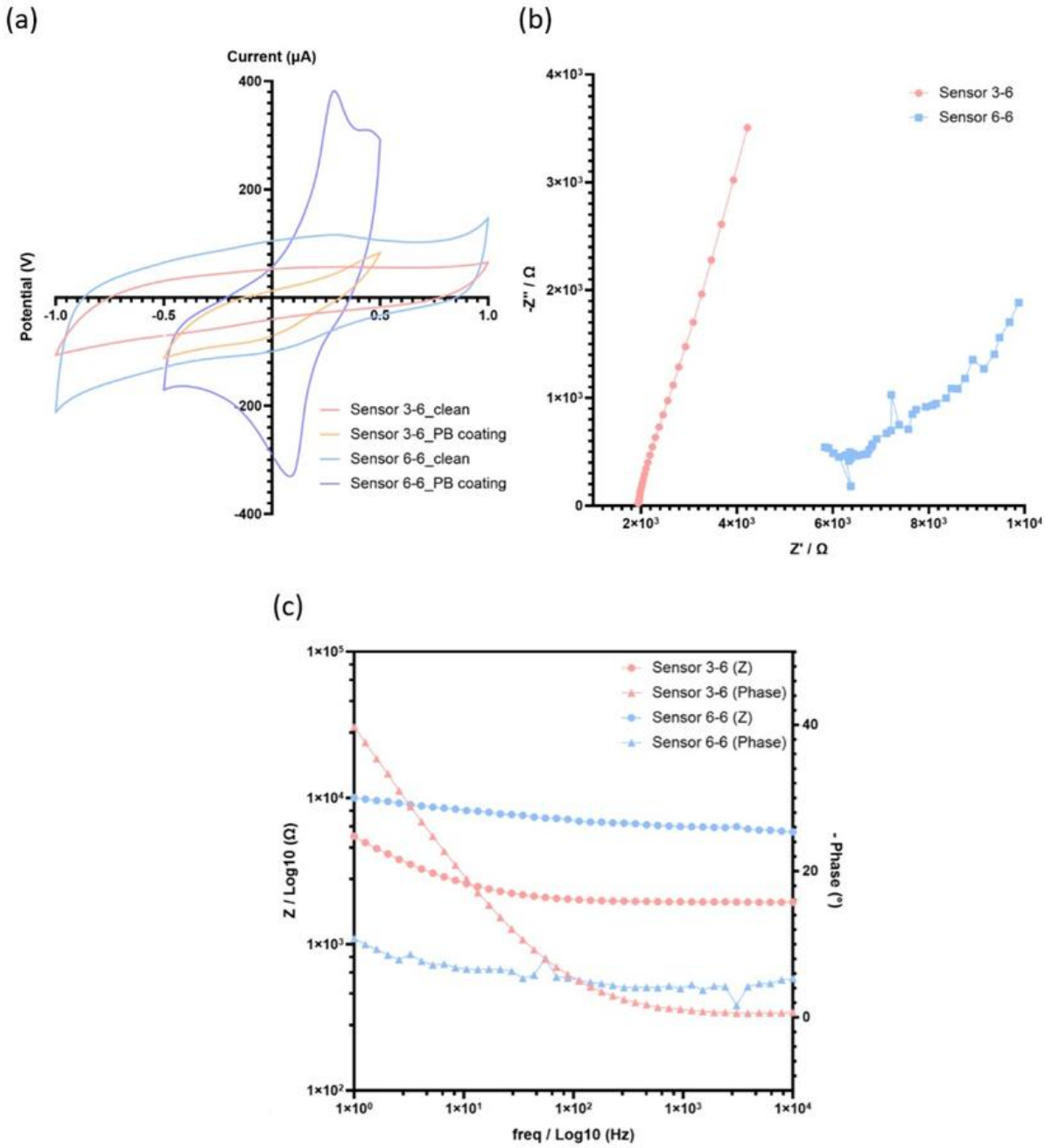


Figure A2: Electrochemical characterization of Sensor 3-6 and Sensor 6-6 used for enzymatic approach. *(a) Cyclic voltammograms of Sensor 3-6 and Sensor 6-6 before and after Prussian Blue (PB) deposition. (b) Nyquist plots obtained by electrochemical impedance spectroscopy (EIS), showing the imaginary impedance component (−Z'') as a function of the real impedance component (Z') for Sensor 3-6 and Sensor 6-6. (c) Bode plots obtained by EIS, showing the impedance magnitude and phase angle as a function of frequency for Sensor 3-6 and Sensor 6-6.*

Figure preparation: Unless otherwise stated, schematic figures in this report were created by the authors using BioRender. Figure 2-1 was created by the authors with assistance from ChatGPT (OpenAI, 2026).

Appendix B

Interview Questions for Parkinson's Patients

1. Introduction & Background

- How long have you been living with Parkinson's disease?
- How has your daily life changed since your diagnosis? Can you remember what changed in your daily life shortly before you were diagnosed?
- Which symptoms affect you the most at the moment?
- How would you describe your current stage of Parkinson's disease: early, middle, or advanced?

2. Medication & Experience with L-Dopa

- Which Parkinson's medications are you currently taking, for example L-Dopa?
- How many times per day do you take your medication?
- How would you describe your experience with L-Dopa?
- Can you feel when the medication starts working and when its effect begins to wear off?
- Do you experience so-called "On" and "Off" phases?
- What difficulties do you experience when taking your medication?
    - For example: timing, side effects, remembering to take it, or fluctuations in its effect.
- Is your condition relatively stable throughout the day, or does it change between the morning, afternoon, and evening?
- How often do your symptoms change or fluctuate during a typical day?

3. Treatment & Contact with Your Doctor

- How often do you visit your doctor for check-ups or adjustments to your treatment?
- Do you feel that your doctor provides you with enough information about your treatment?
- Would you be comfortable with us contacting your doctor, with your permission, to learn more about your treatment and experience?

4. Disease Progression & Diagnosis

- Were any other conditions or symptoms identified around the same time as your Parkinson's diagnosis?
- How have your symptoms changed over time?
- Do you have any other medical conditions in addition to Parkinson's disease?

5. Monitoring & Unmet Needs

- What aspect of Parkinson's disease has the greatest impact on your everyday life?

- Do you feel that your medication is always optimally adjusted to your needs?
- Do you think a continuous monitoring system, such as a wearable sensor, could help you?
    - For example, by helping to optimize your medication or reduce fluctuations in your symptoms.

6. Therapy & Support

- Do you use any additional therapies, such as exercise, physiotherapy, or speech therapy?
- Do you experience any symptoms or side effects that affect your mood, emotions, or psychological well-being?
- Are your Parkinson's medications, such as L-Dopa, fully covered by your health insurance, or do you have to pay part of the cost yourself?
- Have there been situations where certain medications, therapies, or treatments were not covered by your health insurance?
- Are you part of a Parkinson's patient group, support group, or community?

7. Attitudes Toward Sensors

Comfort & Design

- At which locations on your body could you imagine wearing a sensor or monitoring device?
- Which features would be most important to you?
    - For example: size, weight, material, visibility, L-Dopa monitoring, movement monitoring, or AI-based assistance.
- How long per day would you be willing to wear such a device?
    - For example: during the day, at night, while eating, during physical activities, or continuously.

Microneedle Sensor

- How would you feel about using a sensor with very small microneedles, similar to continuous glucose monitoring devices such as FreeStyle Libre?
- Would you have any concerns about using microneedles?
    - For example: pain, safety, hygiene, skin irritation, or infection.

Function & Trust

- Could you imagine using a sensor that helps you manage or optimize your medication?
- What would you need in order to trust such a system?

    - For example: a recommendation from your doctor, clinical studies, approval by medical authorities, or positive experiences from other patients.
- Do you have any general concerns about medical technologies or the possibility of technical errors or device malfunctions?

8. Future & Openness to New Technologies

- Would you be willing to participate in a study or test a new monitoring device?
- Would you be interested in understanding how the sensor works technically?
- Would you be willing to buy such a product yourself, or would you prefer to wait until it is prescribed by your doctor and/or covered by health insurance?
- Could you imagine a sensor helping to better adjust or manage your medication dosage in the long term?

9. Questions About the Parkinson's Organization

- Approximately how many Parkinson's patients are part of your organization?
- How often does the group or organization meet?
- How has the organization personally helped you?
- How does the organization support other Parkinson's patients?
- Is the organization only for patients, or are family members, caregivers, and healthcare professionals also involved?
- Does your organization collaborate with health insurance companies or medical technology companies?
- What kind of support or collaboration do you currently have with these organizations or companies?
- Would you like to see more collaboration or support in this area?

10. Extra Questions — If Time Allows

- Approximately how many people with Parkinson's disease are there in Europe?
- Approximately how many people with Parkinson's disease are there in Germany?
- Approximately how many people with Parkinson's disease are there in Bavaria?
- In your opinion, in which country are Parkinson's patients best supported, and what does that country do particularly well?

Appendix C

Interview Questions for Neurologists

Part 1 — Background (5 min)

- For how many years have you been treating patients with Parkinson's disease?
- Approximately how many Parkinson's patients do you see per week or per month?

Part 2 — Workflow (15 min)

- Could you describe how you decide whether a patient's current L-Dopa dose needs to be adjusted?
- What difficulties do patients experience in taking their medication regularly?
- What are the most common problems your patients experience during L-Dopa therapy?
- How many different formulations of L-Dopa are currently available and used in clinical practice?
- Could you describe how a typical Parkinson's patient is managed in your clinical practice?

Part 3 — Problems (20 min)

- From your perspective, what are the biggest challenges in treating patients with Parkinson's disease?
- How difficult is it to determine the optimal L-Dopa dose for an individual patient?
- What problems do patients report most frequently?
- Are there situations in which symptoms are particularly difficult to predict?
- Do patients frequently experience fluctuations in their symptoms throughout the day?
- If you could immediately improve one aspect of current Parkinson's treatment, what would it be?

Part 4 — Monitoring (10 min)

- In clinical practice, how do you determine whether a patient's condition is improving or worsening?
- How do you currently monitor disease progression?
- Are there situations in which you wish you had more information about a patient's L-Dopa levels? When is it particularly difficult to determine whether the current L-Dopa dose is optimal?
- Is there a typical L-Dopa dose, or does it vary significantly from patient to patient?

Part 5 — Technology (10 min)

- What role could digital technologies or wearables play in Parkinson's treatment?
- What concerns do you have regarding such technologies?
- How do you see the future of Parkinson's treatment?
- Do you think patients will be able to monitor and manage their condition more independently in the future?

- What type of innovation do you think could make the biggest difference for Parkinson's patients?

L-Dopa Sensor & Microneedles

- When using microneedles, where could L-Dopa ideally be measured?
- Where on the body would be the best location for measuring movement?
- How quickly would the measurement results need to be available—real time, within minutes, or within hours?
- Which body locations would patients be most likely to accept for wearing or using such a sensor?
- What level of accuracy would such a sensor need to achieve to be clinically useful for you?
- What measurement frequency would be appropriate?
- How important would the long-term stability of the sensor be?

Data & Clinical Decision-Making

- Should the collected data primarily be accessible to the physician, the patient, or both?
- How should the data ideally be presented—for example, as trends, alerts, or warnings?
- What is the typical delay between L-Dopa intake, changes in blood concentration, and the clinical effect?
- For clinical decision-making, is the absolute L-Dopa concentration more important, or is the change over time the trend more relevant?
- How quickly do L-Dopa levels typically change under real-life conditions?
- Would near-continuous monitoring be necessary, or would measurements at discrete time points be sufficient?

Interstitial Fluid & Safety

- From your perspective, would measuring L-Dopa in interstitial fluid (ISF) be sufficient, or would direct blood measurements be necessary?
- What risks or concerns do you see regarding the long-term use of microneedles?
- Are there physiological factors, such as nutrition or other medications, that could interfere with or influence the measurements?

Clinical Use Case

- Can you think of a specific patient or clinical situation in which better monitoring of L-Dopa levels would have been particularly helpful?