Real-time Electrochemical Biosensing of Neurotransmitters

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Introduction

Electrochemical NT sensor

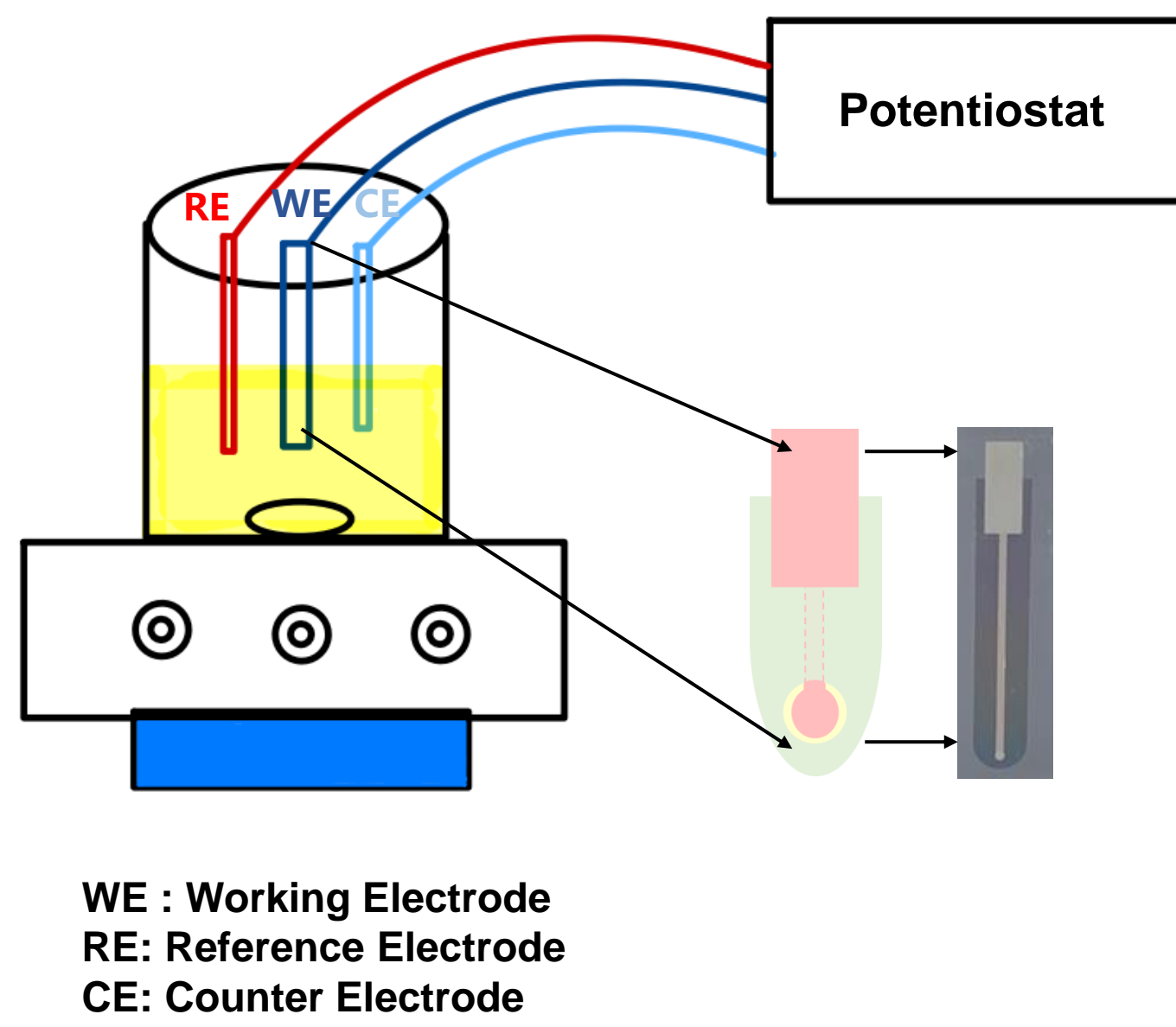**Potentiostat**

WE : Working Electrode
RE: Reference Electrode
CE: Counter Electrode

The ability to detect and measure neurotransmitter(**NT**) such as dopamine(**DA**), gamma-aminobutyric acid (**GABA**), and Glutamate(**Glu**) is essential for understanding the complex mechanisms of the brain and nervous system.

Especially, abnormal levels of DA have been linked to several neurological and psychiatric disorders, including Parkinson's disease, schizophrenia, and addiction. Plus, Abnormal levels of Glu, the most abundant excitatory NT, have been linked to Alzheimer disease, depression, and epilepsy.

Electrochemical sensors have emerged as a powerful tool for detecting and quantifying these NT. These sensors are highly sensitive, selective, and can provide real-time measurements of NT concentration, making them ideal for a variety of research and clinical applications.

In this project, we will show our electrochemical NT sensors for the detection of DA, and Glu, with a focus on the optimization of electrode designs, and coating enzyme concentration. Electrochemical sensors will open the horizon of sensors that measure various NT from brain organoids and become the core of disease treatment.

Experimental Principle

Electrochemical Sensing Methods

Voltammetry
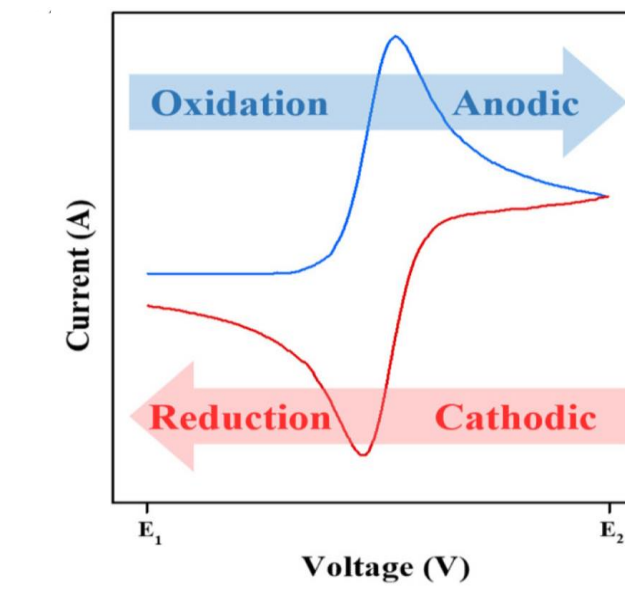
Amperometry
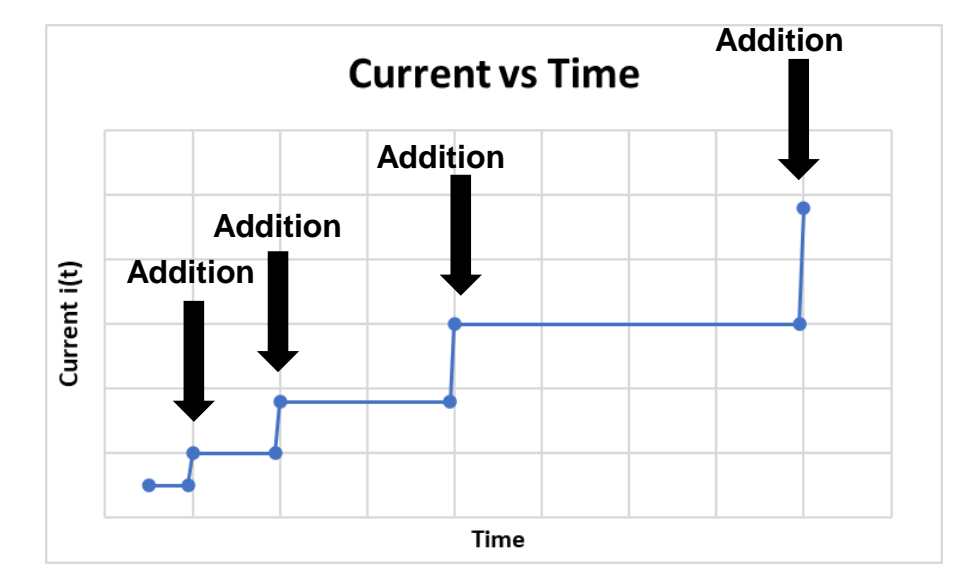
Dopamine Sensing Mechanism

$$Dopamine \longleftrightarrow Dopamine\ quinone + 2e^- + 2H^+$$
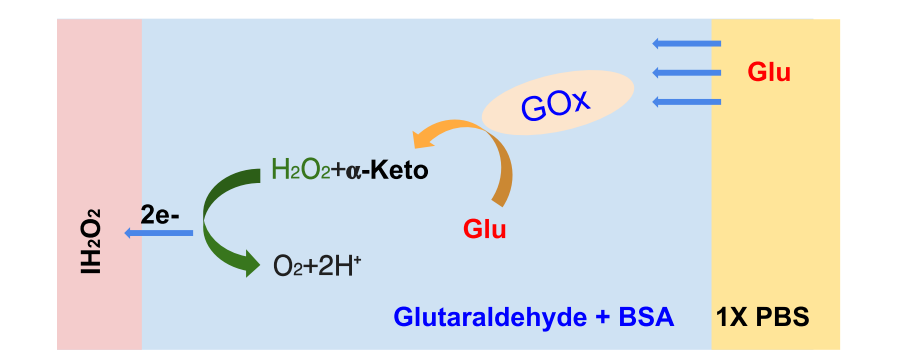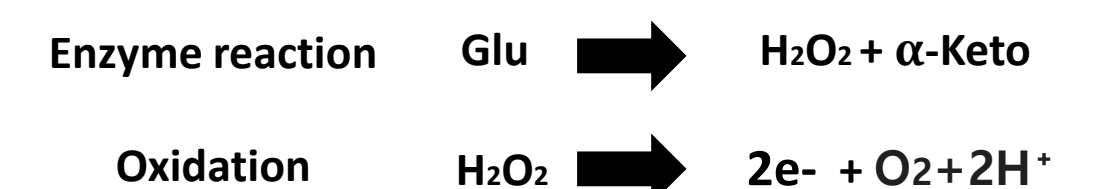
DA is a material that is self-oxidized, so it can be detected by showing an electrochemical reaction immediately when a voltage is applied.

Glutamate Sensing Mechanism

Enzyme reaction $\quad$ Glu $\longrightarrow$ $H_2O_2 + \alpha$-Keto

Oxidation $\quad H_2O_2 \longrightarrow 2e^- + O_2 + 2H^+$
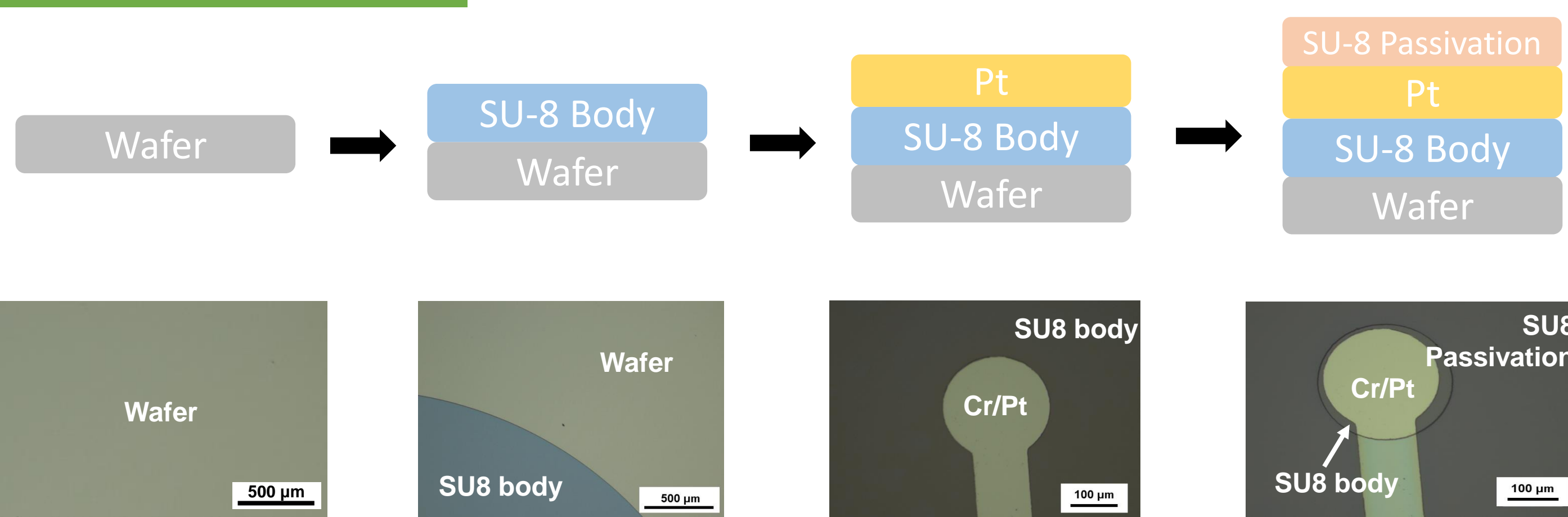

Results

Fabrication

Fig 1) Fabrication Process of electrodes & OM image

The whole process was done in the clean room.

Optimization
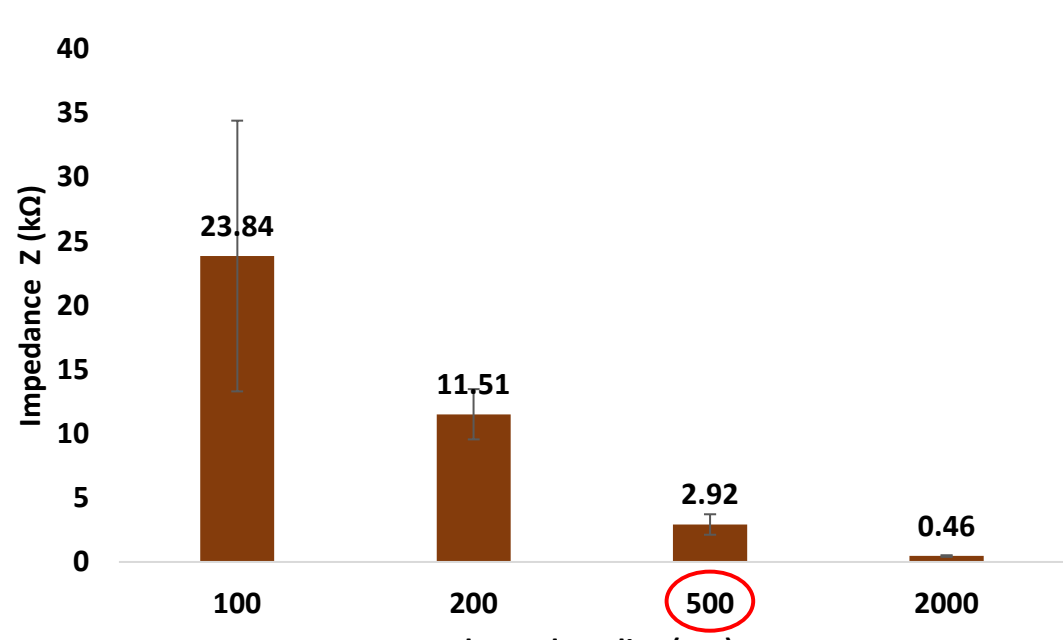
1) Size of the electrodes

Fig 2) Electrode size optimization with measuring impedance (N=20)

This experiment was conducted to optimize the area of the electrode to be made by measuring the impedance of the variety size of electrodes.

Impedance is a kind of resistance, and when the impedance is low, electrons are easily moved and current flows well. The lower impedance, the better electrode performance.

Impedance is the lowest at 2000 μm radius. However, the electrode size is too large for use in *in vivo* or organoid. Therefore, we selected a 500 μm radius electrode.

→ Optimization: 500 μm radius electrode

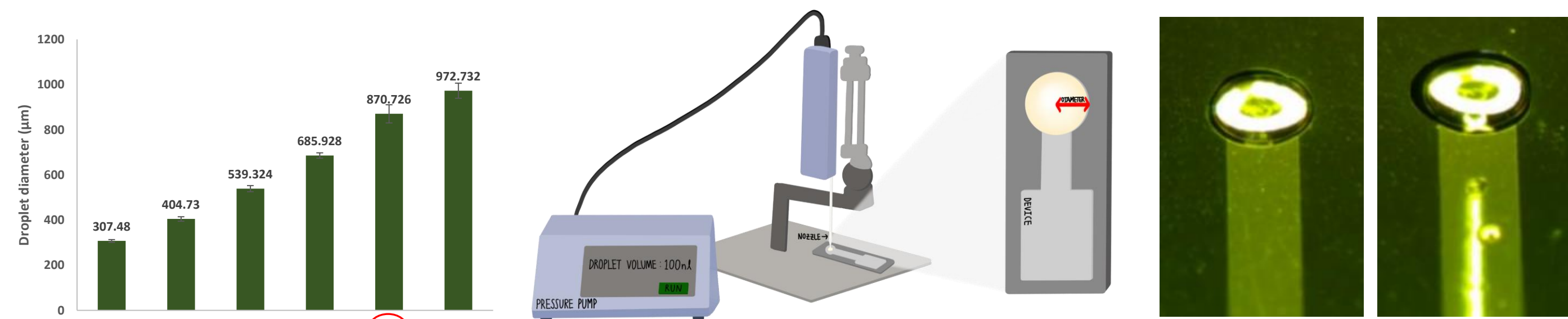#### 2) Enzyme coating volume & Coating condition

Fig 3) Droplet diameter optimization with changing droplet volume

Fig 4) Coated Electrode Before RIE & After RIE

In the case of NTs that are not self-oxidized, enzymes that convert them into oxidizable substances must be coated on the electrodes to detect and measure the NTs in an electrochemical method.
Coating conditions were necessary to coat the electrode with the same concentration of enzyme. We set the volume of a drop of the enzyme solution and experimentally looked with microscope at the diameter of the circle when this volume was dropped, and the left side is the result of this experiment.
Also, Reactive ion etching(RIE) was treated only on the electrode part to coat wider with an enzyme of the same capacity.

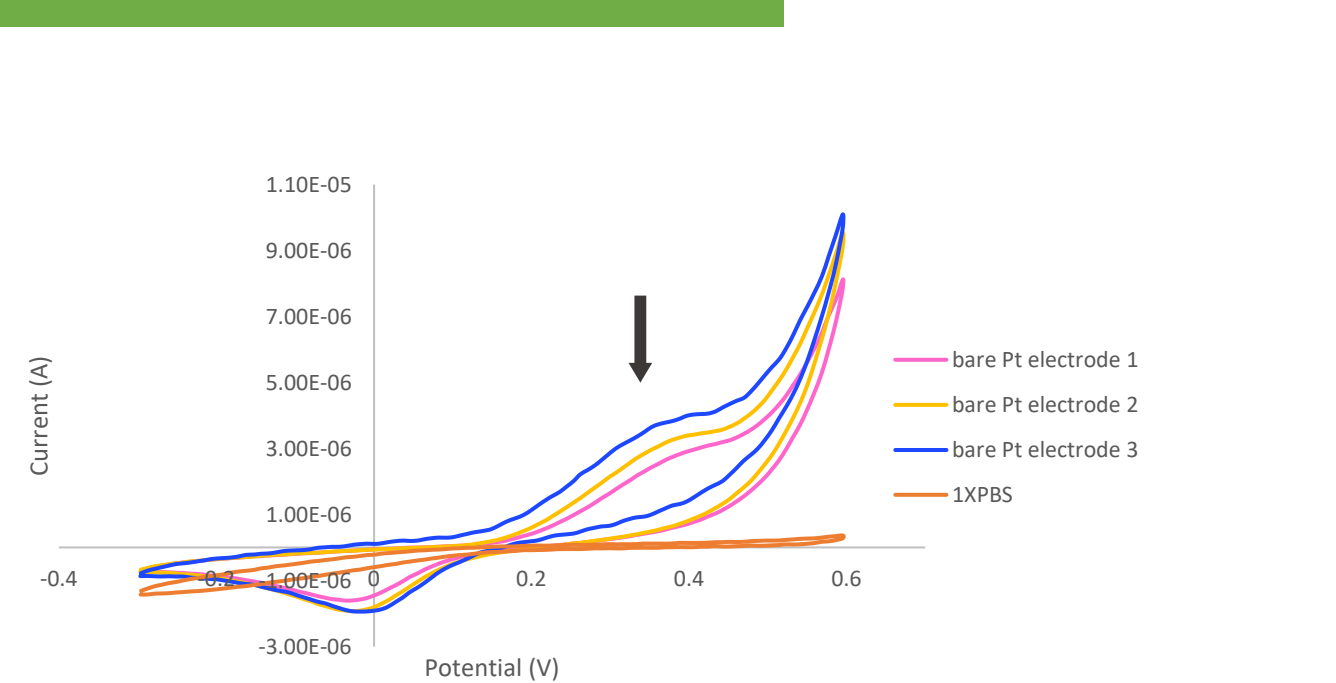**→ Optimization: 100nL Enzyme volume**

Dopamine Sensing

Fig 5) Bare electrodes' Cyclic voltammetry results in DA10mM in 1XPBS(pH7.4)

Fig 6) Amperometry results in DA 400μM in 1XPBS(pH7.4)

Cyclic Voltammetry was performed to use an electrochemical method to sense self-oxidizing NT, DA. The peak potential shown in the Cyclic Voltammetry graph in the left figure is 0.37V, so we decided to proceed with Amperometry with this potential value.
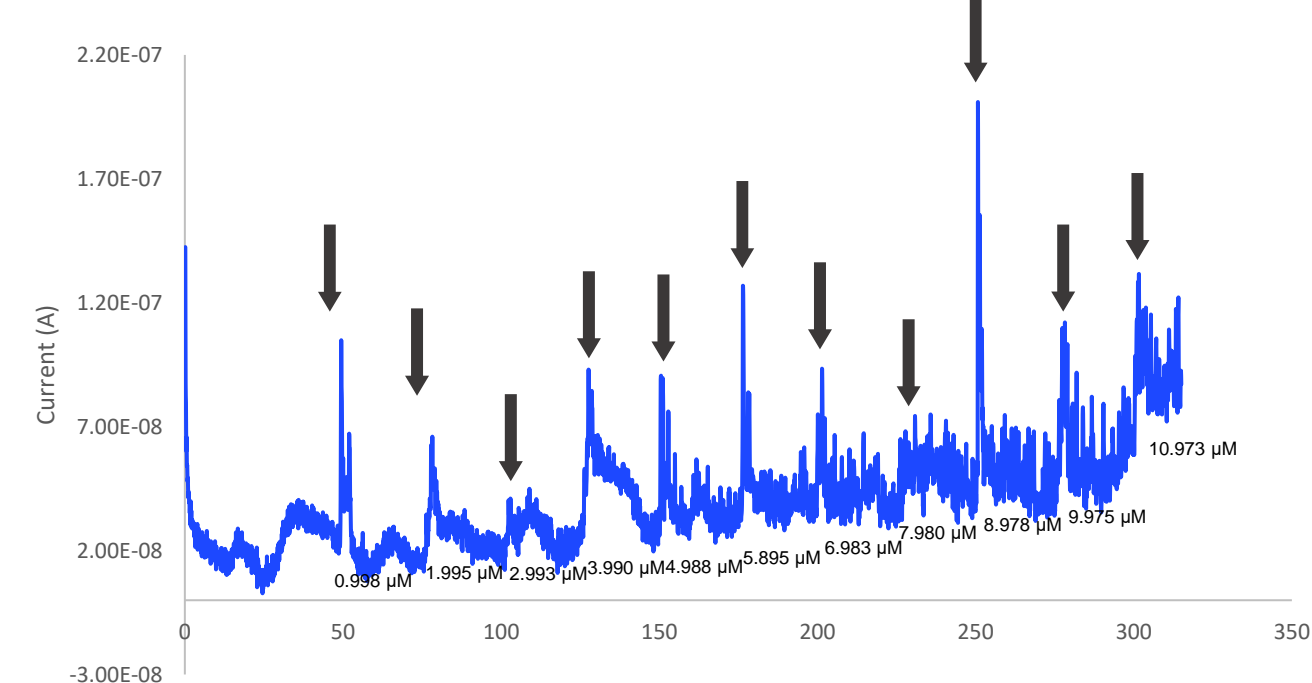
This graph shows that the current rises when the concentration of DA rises.
Also, Sensitivity and LOD can also be obtained through this graph.

The smaller the sensitivity of the sensor, the lower concentration of the material can be sensed. Accordingly, sensor is possible to detect an extremely low concentration of neurotransmitter *in vivo* or organoid.

Sensitivity (μA μM⁻¹cm⁻²)	LOD (μM)
0.65	0.998

Fig 7) Sensitivity(0.65 μA μM⁻¹cm⁻²) & Limit of Detection(**LOD**)
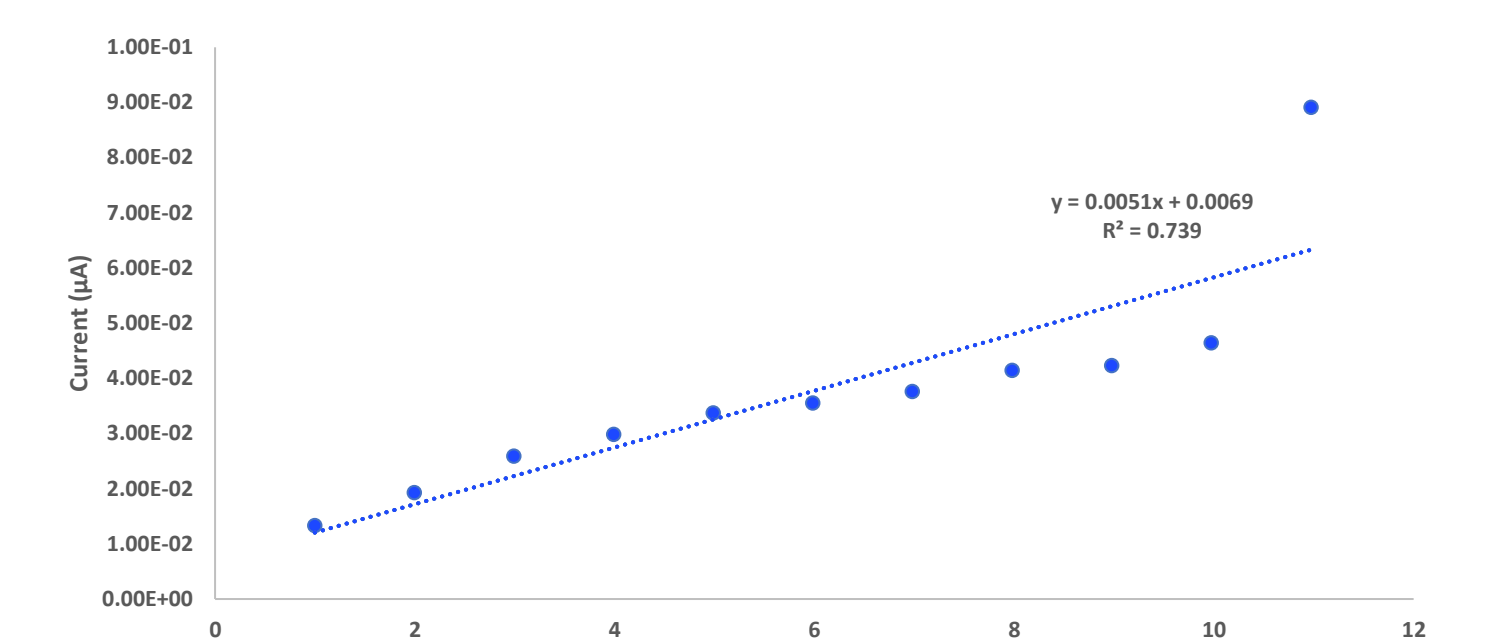

Fig 8) DA sensing electrode calibration curve
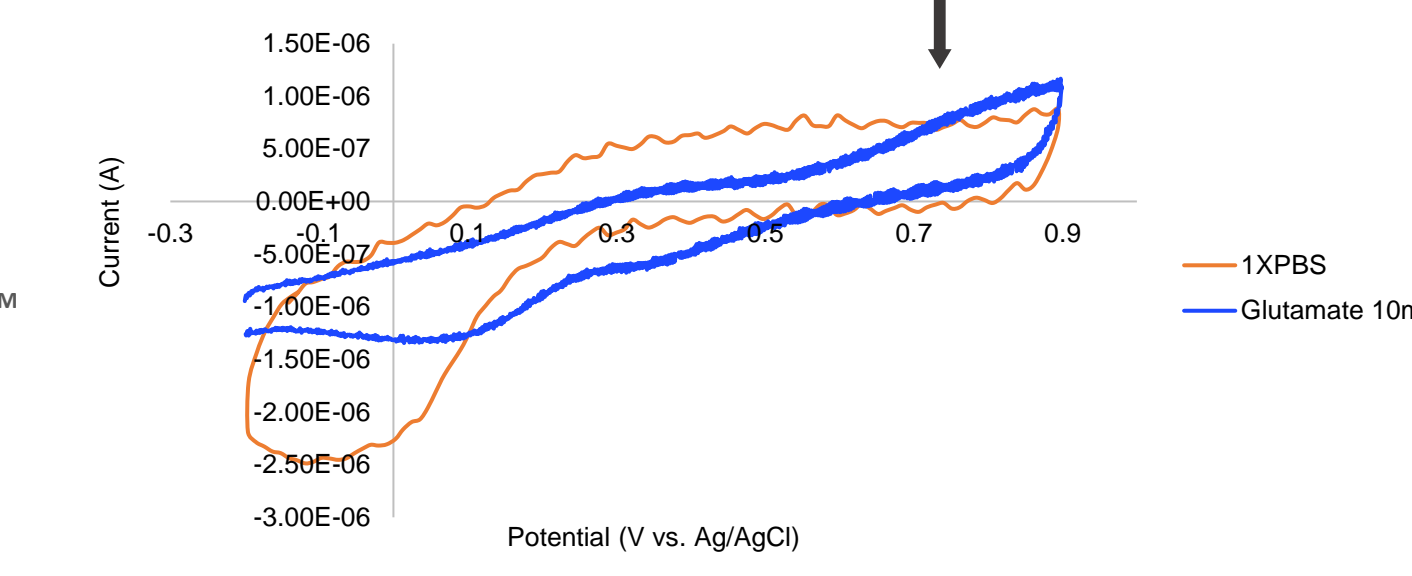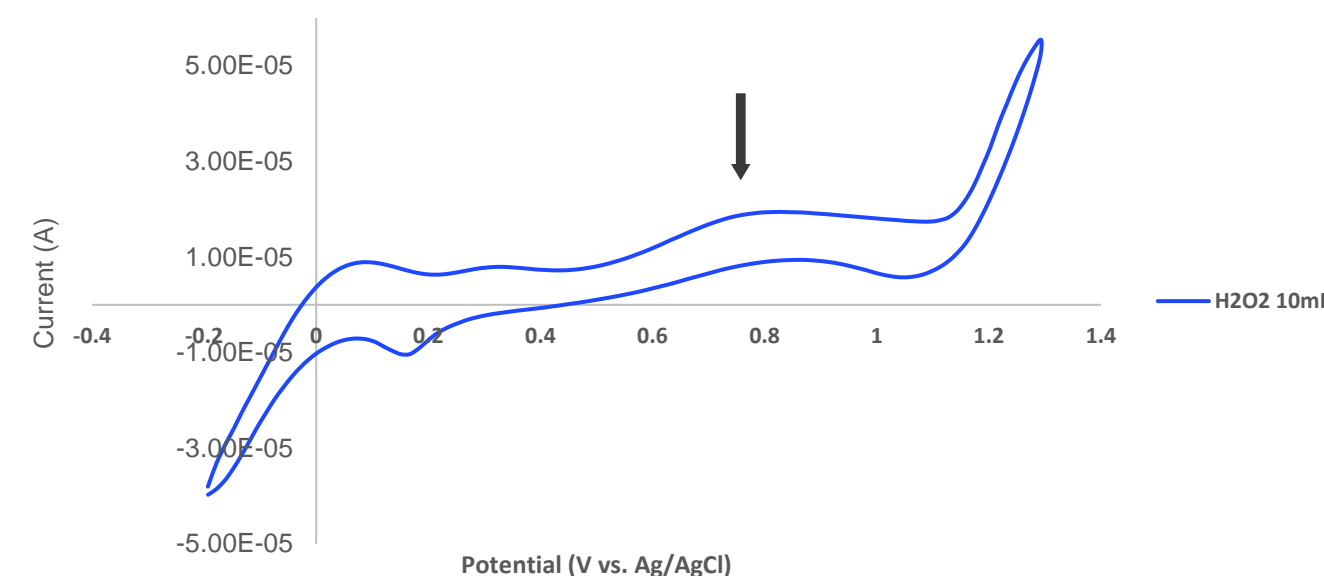
Glutamate Sensing

Fig 9) Gox coated electrode's Cyclic Voltammogram in Glu 10mM,1XPBS(pH 7.4)

Fig 10) Bare electrode's Cyclic Voltammogram in H_2O_2 10mM,1XPBS(pH 7.4)

Glutamate is not a substance that can go over redox reactions on its own. Therefore, it can be detected by coating enzyme on the surface of the electrodes called GOx and using the redox reaction of H_2O_2 from the enzyme reaction. Therefore, the cyclic voltammogram was drawn by putting the enzyme-coated electrode into the Glutamate solution and the bare Pt electrode into the H_2O_2 solution The peak results are the same confirming that GOx enzyme produces H_2O_2 from glutamate.
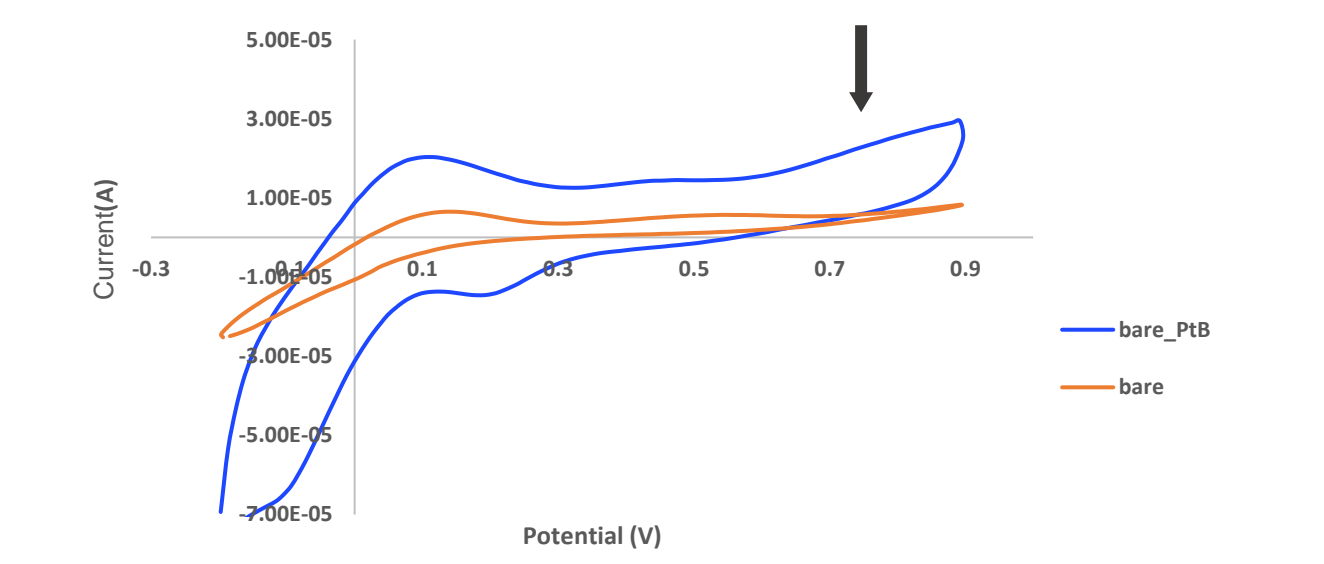**PBS: negative control, H_2O_2: positive control**

Fig 11) NT electrode Cyclic Voltammogram of H_2O_2 10mM in 1XPBS(pH 7.4) and Pt Black deposition NT electrode Cyclic Voltammogram of H_2O_2 10mM in 1XPBS(pH 7.4)

Fig 12) Bare electrode & Pt Black deposited electrode (L:OM, R:SEM)

By depositing Pt black, it was possible to achieve 100times more current amplification than the bare electrode.

Conclusion & Further Study

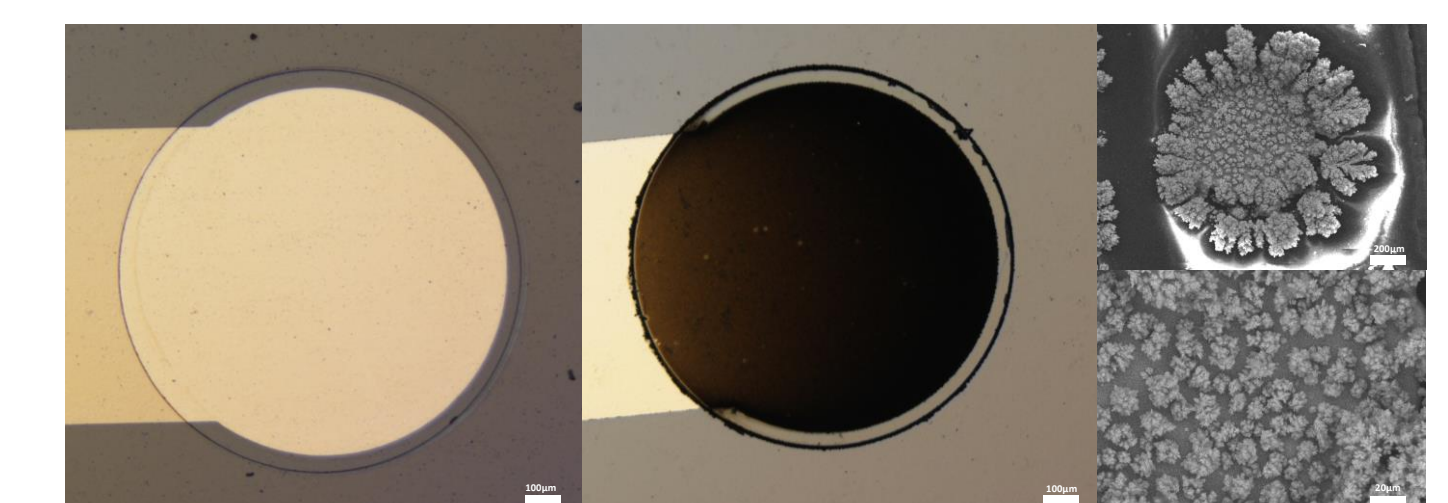### Conclusion

With this sensor fabrication and electrochemical sensing methods, we have created sensors that can sense various neurotransmitters such as dopamine and glutamate. We also optimized these sensors by varying surface areas and enzyme coating volumes so that these sensors can detect dopamine with LOD : 0.998μM and sensitivity 0.65μA μM⁻¹cm⁻² with electrode radius 500μm.

Further Study

Even though our sensor now can only detect dopamine successfully, we can further develop this idea of electrochemical sensor so that we can detect glutamate, GABA, and other neurotransmitters. Furthermore, we can apply this sensor for monitoring neurotransmitters that are produced from organoids as we have already optimized the sensor size for organoid. By doing so we hope that this sensor can work as a part of overall health-monitoring platform device.

Reference

[1] Imran Hossain, Chao Tan, Phillip T. Doughty et al., *Frontiers in Neuroscience*, 2018, A Novel Microbiosensor Microarray for Continuous ex vivo Monitoring of Gamma-Aminobutyric Acid in Real-Time
[2] Nicolaie Moldovan, Iuliu-Ioan Blaga, Sanjeev Billa et al., *ELSEVIER*, 2021,Brain-implantable multifunctional probe for simultaneous detection of Glutamate and GABA NTs
[3] Sung Sik Chu, Paul Marsh, Hung A. Nguyen et al., *IEEE Engineering in Medicine & Biology Society(EMBC)*, 2021, Fabrication of Highly Sensitive Pt-black Electrochemical Sensors for GABA Detection
[4] Taewhan Kim, Woosung Choi, Heon-Cheol Shin et al. , *Journal of Electrochemical Science and Technology*, 2020, Applications of Voltammetry in Lithium Ion Battery Research