Development of Observation Method of Living Cells and Tissue Stimulated Using Fine/Local/Micro Electrochemical Devices

微細・局所・微量電気化学デバイスを用いた生細胞・生組織の刺激観察法の開発

― Glutamate Releasing Electrochemical Device　グルタミン酸放出電気化学デバイス　―

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Introduction

Bio-imaging

➢ It reveals biological features with analyzing the distribution of chemical species.

⇒ In order to analyze stimulation behavior of cells and tissues, a combination of stimulation and detection is important.

Detection
- Fluorescence microscope
- Ion image sensor

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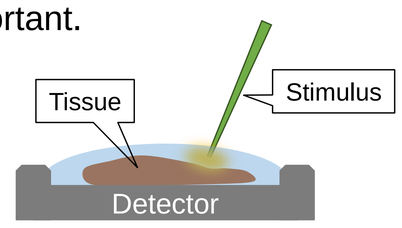
Stimulation
- Physical stimulation
- Electric stimulation
- Chemical stimulation



Chemical stimulation is excellent and effective, because it activates only specific receptors.

☐ One of chemical stimulus: Ion-releasing electrochemical devices

→ It can release chemicals at any position locally, and at any time by electrical switching.

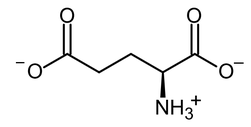
Previous work: We successfully demonstrated a calcium ion releasing electrochemical device using polymer coated electrode.[1]

We report a new glutamate releasing device. This device is unique to be able to release glutamate by the circuit switch on without external battery.

[1] Hattori, T. et al. in *Proc. 4th Asian Symp. Anal. Sci.* Sendai, Japan, Sep. 12–13, 2018.

Glutamate

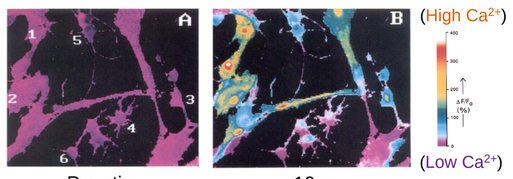
➢ a most important excitatory transmitter in central nervous system



☐ The results captured Ca^{2+} uptake by Glu^--dependent channel on the cell membrane.

Increasing of $[Ca^{2+}]_i$ of astrocytes [2]

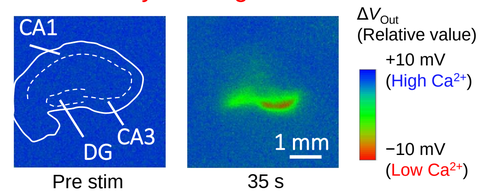
Stimulus by 500 μM glutamate



[2] Jensen, A. M. et al. *J. Neurosci.* **1991**, 11, 1674–1684.

Decreasing of hippocampal $[Ca^{2+}]_o$ [3]

Stimulus by 3 mM glutamate



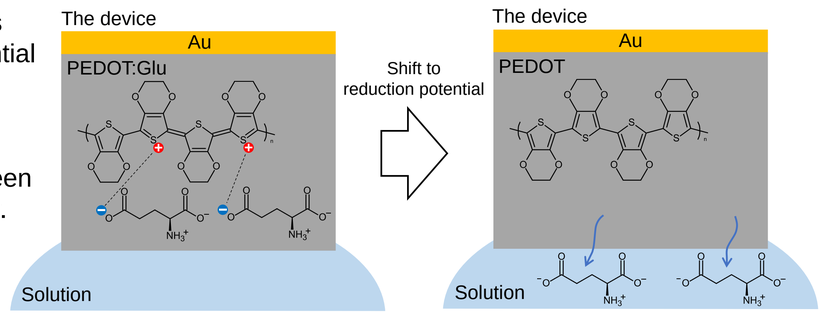
[3] Doi, H. et al. in *Proc. IEEE SENSORS 2019*, Montreal, Canada, Oct. 27–30, 2019; C3LA, 1289.

If we will have a local glutamate stimulation device, we could reveal their biological features in more details.
In this study, we report the glutamate release from Glu^--doped poly(3,4-ethylenedioxythiophene).

Glutamate ion releasing device

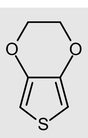
Structure & Principle

The device potential is shifted to reduction potential of PEDOT.
⇓
PEDOT is reduced, the Coulomb force between PEDOT and Glu is lost.
⇓
Glu^- are released.

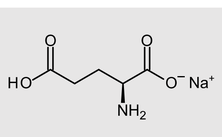


Fabrication methods

A gold electrode was applied voltage in an aqueous solution including EDOT and NaGlu.



3,4-ethylenedioxythiophene (EDOT)



mono-sodium L-glutamate (NaGlu)

	Potnetiostatic mode	Potentiodynamic mode
WE	Au disk (φ3 mm)	
CE	Platinum wire	
RE	Ag|AgCl (sat. KCl)	
Electrolyte	EDOT 0.01 M, NaGlu 0.1 M in H_2O (3 mL, stirring)	
Applied voltage	+1.2 V for 30 min.	−0.9 ~ +1.2 V at 25 mV s^{-1} for 10.5 or 20.5 cycles

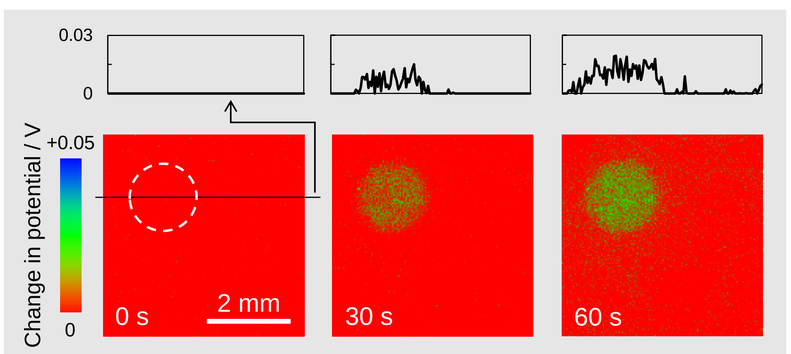
Real-time monitoring of Glu^- released from the releasing device

A Glu^- image sensor is used to evaluate the amount of Glu^- released from the device.

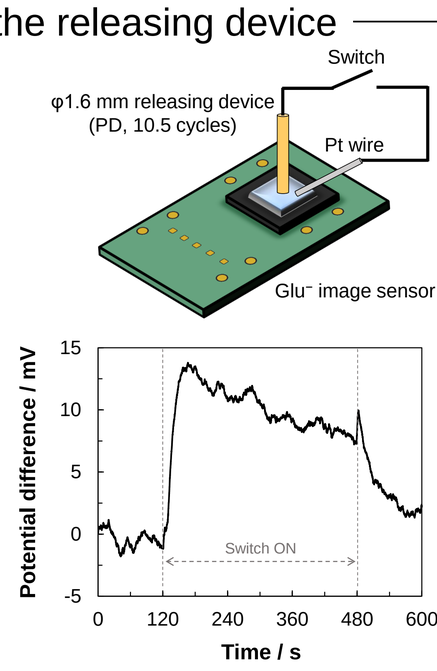
Glu^- image sensor [4,5]

It can monitor 2D distribution concentration of Glu^- in real-time.

[4] Mizutani, S. et al. *Sens. Mater.* **2017**, 29, 253–260.
[5] Okumura, Y. et al. in *Proc. IMCS 2018*, Vienna, Austria, July 15–19, 2018; pp.141–142.



Real-time monitoring by the Glu^- image sensor.



Variation just under the releasing device.
15 mV → $[Glu^-] = 10^{-6}$ ~ 10^{-5} M

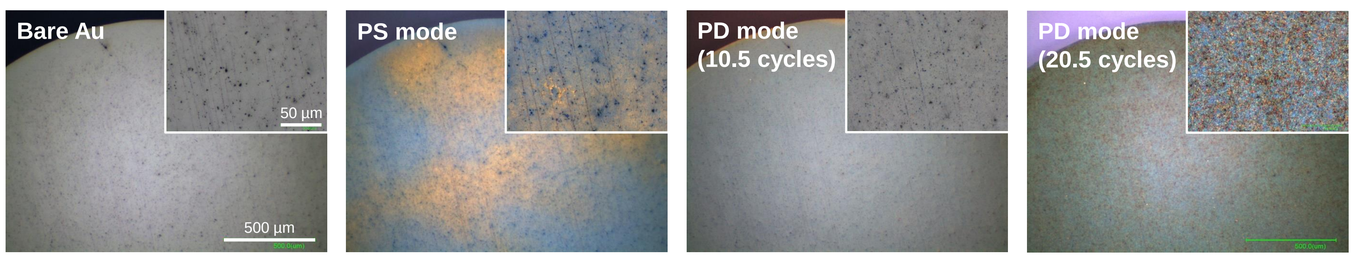
Effect of different polymerization conditions on the Electrochemical Device

The morphology and conductivity of the device electrode change depending on the polymerization conditions of PEDOT.[6] PEDOT:Glu membranes were fabricated by two different *V-t* modes; ➢Potentiostatic (PS) mode: constant potential, ➢Potentiodynamic (PD) mode: linear triangle scan of potential.

[6] Castagnola, V. et al. *Synth. Met.* **2014**, 189, 7–16.

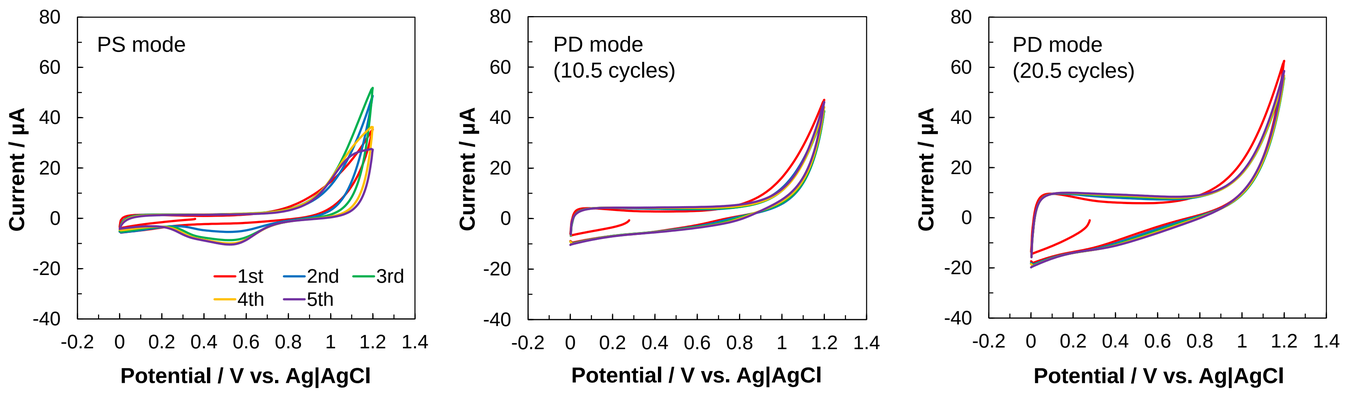
Optical microscopic images of polymer surface

The surfaces of the device electrode after polymerization were observed.



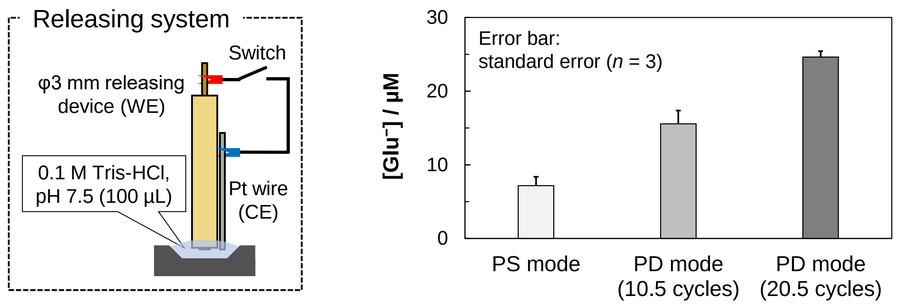
Cyclic voltammograms of the releasing devices

Cyclic voltammograms were carried out in aqueous solution including 0.1 M NaGlu. The potentials were scanned at 0.1 V s^{-1}.



The trace amount of Glu^- released

The amounts of Glu^- released from the releasing device are quantitative by L-Glutamate Assay Kit YAMASA NEO (YAMASA, Japan). Samples were obtained by closing the following release system in the solution for 5 min.



	PS mode	PD mode
Polymer surface	non-uniform	uniform
Cyclic voltammogram	irreversible	reversible
The amount of Glu^- released	lower	higher

In the PS mode, rapid polymerization is carried out since the PEDOT oxidation potential (1.2 V) is applied immediately. On the other hand, in the PD mode, slow polymerization is carried out since the device potential is gradually increased to the PEDOT oxidation potential. This affects uniformity of the membrane, Cyclic voltammogram, and the amount of Glu^- released. Moreover, in the PD mode, the larger number of cycles result in the larger amount of Glu^- released.

Currently we fabricate the Fine/Local/Micro Electrochemical Stimulation Device for cells and tissues.

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