FACILE FABRICATION OF HIGHLY SENSITIVE PT-BLACK ELECTROCHEMICAL SENSOR FOR L-GLUTAMATE DETECTION

Sung Sik Chu[1], Paul Marsh[2], Hung A. Nguyen[2], Randall Olson[3], Carolyn E. Jones[3], Miranda M. Lim[3], and Hung Cao[1,2]

[1]Department of Biomedical Engineering, University of California Irvine, USA
[2]Department of Electrical Engineering and Computer Science. University of California Irvine, USA and [3]VA Portland Health Care System, Portland, USA

2021 IEEE 34th International Conference on Micro Electro Mechanical Systems (MEMS) | 978-1-6654-1912-3/20/$31.00 ©2021 IEEE | DOI: 10.1109/MEMS51782.2021.9375288

ABSTRACT

L-glutamate is the most abundant neurotransmitter (NT) in the human brain and is involved in essentially all activities of the central nervous system. Variations in its availability have also been linked to neuronal disorders such as secondary mechanisms in traumatic spinal cord injury. Compared to conventional assays, electrochemical sensors have been an area of interest in NT measurement due to their favorable sensitivity as well as high temporal and spatial resolution. In this work, a highly sensitive, L-glutamate specific, amperometric electrochemical sensor was developed by cost-effective electrodeposition of nanostructured platinum black (Pt-black) on platinum substrates, which increases the active surface area for sensing. A self-referencing site was integrated in order to increase the signal-to-noise ratio (SNR). After further applying m-phenylenediamine dihydrochloride as a size exclusion layer, we demonstrated good sensitivity and selectivity for the miniaturized prototypes. These devices can be further developed for implantable biosensors for in vivo dynamic measurement and evaluation.

KEYWORDS

Electrochemical; L-glutamate; electrodeposition; platinum black; biosensor

INTRODUCTION

It has been shown from multiple studies that neurological disorders are related to abnormal releases of neurotransmitters [1, 2]. For example, one hypothesized mechanism underlying autism spectrum disorder, a common developmental disorder involving impaired social behavior and cognition, is thought to be an imbalance in the ratio of excitatory and inhibitory neurotransmitters (E:I) in the brain, the two most abundant of which being L-glutamate (L-glu) and gamma-aminobutyric acid (GABA) respectively [3, 4]. Therefore, simultaneous detection of their ratios within the same brain region is of high interest and crucial for better understanding real-time changes in E:I balance in neurological disorders.

Intracranial microdialysis of brain interstitial fluid is a widely used approach in measuring neurotransmitters in vivo, in which a small catheter is inserted into the measurement site and solutes of interest passively diffuse across a semipermeable membrane as a result of the concentration difference between sides of the membrane [5]. However, microdialysis has poor temporal and spatial resolution, on the order of minutes and millimeters [5-7]. Alternatively, electrochemical sensors using microelectrode arrays (MEAs) offer second-by-second detection with submillimeter spatial resolution within the brain [8, 9]. Among various materials, platinum (Pt) has been used extensively due to its stability and biocompatibility [10-14]. Further, Pt shows better electrochemical activity during redox reactions compared to other noble metals, such as gold and palladium [15]. Despite the superior performance of Pt towards neurotransmitter sensing, Pt probes made with conventional evaporation method alone results in low output current of pA~nA range, limiting their wider use [16, 17]. This calls for a facile and cost-effective method to increase the active surface area for fabrication of Pt microelectrodes with higher sensitivity to detect the minute changes in neurotransmitters.

In this work, we develop L-glutamate sensors with electrodeposited Pt-black as the working electrode (WE) material, aiming to enhance the effective surface area. The surface of the WE was modified with a phenylenediamine dihydrochloride (mPD) layer to increase specificity for the generated H_2O_2 reaching the electrode surface [18]. In this scheme, electrooxidation of H_2O_2 occurs on the Pt electrode surface with an applied constant potential of +0.7V vs a Ag/AgCl reference electrode. The measured sensing signals were calibrated with a self-referencing sensor which was placed close to the sensing site to eliminate ambient noise.

MATERIALS AND METHODS

Chemicals

Phosphate buffered saline (10x PBS), glutaraldehyde, and hydrogen peroxide (H_2O_2) were purchased from Thermo Fisher Scientific (*Waltham, MA*) and L-glutamic acid monopotassium salt monohydrate, chloroplatinic acid hydrate, dopamine hydrochloride (DA), and bovine serum albumin (BSA) was purchased from Alfa Aesar (*Haverhill, MA*). L-ascorbic acid (AA), potassium ferricyanide, and m-phenylenediamine dihydrochloride (mPD) was purchased from Acros Organics (*Fair Lawn, NJ*) and glutamate oxidase (GOx) from US Biological Life Science (*Swampscott, MA*).

L-glutamate Sensor Fabrication

The overall schematic of the fabricated sensor is shown in **Fig. 1**. First, a Pt microelectrode array (MEA) was formed using photolithography on a 127-μm thick polyimide film. Then the batch of probes was laser-cut to individual micro-probes as shown in the inset of **Fig. 1** (50 μm × 100 μm for each sensing pad). Finally, the probes were connected to a printed circuit board (PCB) using copper wire and silver epoxy paste to achieve a stable platform for signal detection.

For surface modification, platinum black (Pt-black) was electrodeposited on the Pt electrodes. The probes were immersed in a 0.01 M chloroplatinic acid made by

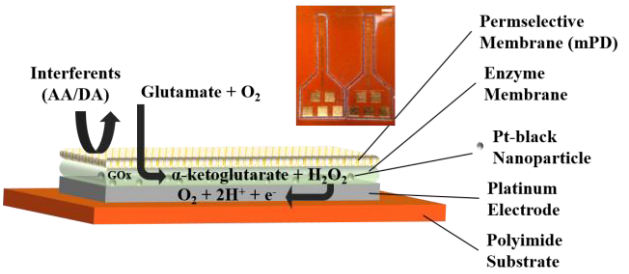

Figure 1: Schematic representation of Pt-black L-glutamate biosensor. While interferents such as AA or DA get repelled from the permselective membrane, L-glutamate diffuses to the enzyme membrane and GOx converts L-glutamate into H_2O_2. This H_2O_2 gets oxidized on the Pt-black coated platinum electrode and generates current. Inset shows mage of laser-cut probes.

dissolving chloroplatinic acid hydrate (H_2PtCl_6•xH_2O) in DI water and cycling potential between -0.4 to +0.8 V, v.s. a Ag/AgCl reference electrode in 1M NaCl, at a scan rate of 50 mV/s for 10 cycles, using a commercial potentiostat (*700E, CH Instruments, Austin, TX*). Then, the probes were rinsed with DI water and kept in air to dry before enzyme deposition.

For Glutamate oxidase (GOx) deposition, different mixtures were applied on the self-referencing and L-glu sensing sites. Initially, 10 mg of BSA was dissolved in 985µL of DI water; next, 5 µL of glutaraldehyde (25% in water) was mixed with the BSA solution by manual agitation. The mixture was set aside for 5 minutes at room temperature, then applied on the self-referencing site of the probe using a Hamilton syringe. After that, 9 µL of this enzyme-free mixture was added to 1 µL of GOx (1 U/µL) to make the final enzyme solution, which was subsequently applied to the L-glu sensing site using the same method. A total of 3 layers were applied to each site. Between each coating, 1 minute of curing time was provided. After the enzyme coating process, GOx-coated probes were subjected to 48-72 hours of curing, for full crosslinking of the protein.

Lastly, a size-based exclusion layer was formed on top of the enzyme layer to prevent block interference molecules. 5 mM of mPD solution was made by dissolving mPD into a deoxygenated 0.05 M PBS; and probes were immersed in the solution for electrodeposition. Using a commercial potentiostat, a potential of +0.5 V vs a Ag/AgCl reference electrode was applied for 15 minutes and then the probes were rinsed with DI water. After exclusion layer deposition, the probes were kept at room temperature dry for 24 hours for curing prior to calibration [19].

Characterization and Calibration

For calculating the active surface area of the electrodes, cyclic voltammograms of electrodes were obtained using $K_3Fe(CN)_6$ as a redox couple. A test solution of 5 mM $K_3Fe(CN)_6$ in 0.1 M KCl was prepared and -0.2 to +0.6 V (vs a AgCl RE) was cyclically applied to the WE, with a scan rate of 50 mV/s. Extracted peak currents were applied to the Randles-Sevcik equation [20]:

$$i_p = (2.69 \times 10^5) n^{\frac{3}{2}} A D^{\frac{1}{2}} v^{\frac{1}{2}} C \qquad (1)$$

where i_p is the peak current, n is the number of electrons transferred in the redox reaction in question, which is 1 in this case, A is the electrode area (m^2), D is the diffusion coefficient (m^2/s), v is the scan rate (V/s) and C is concentration of analytes (mol/L).

Amperometry was used to test the performance of the fabricated probes; the data were gathered using a multichannel potentiostat from Pinnacle Technology Inc. (*8102-N, Lawrence, KS*). This enabled simultaneous recording of both self-referencing and the L-glu sensing sites. For sensitivity testing, the probe tip was soaked in 0.01 M PBS, at 37°C, under constant stirring; concentrations of L-glu (1 µM to 70 µM) and H_2O_2 (0.5 µM to 500 µM) were added sequentially while a potential of +0.7V was applied against an Ag/AgCl reference electrode. From the measurements, the limit of detection (LOD) was calculated by dividing 3 times the standard deviation of the baseline by the least squares slope.

To test the selectivity towards L-glu, the same setup was implemented but here, concentrations of interferents well known to be present in the body, such as AA and DA were introduced along with L-glu and H_2O_2. For all amperometric recordings, 20~30 minutes of stabilization time in solution was provided prior to actual calibration.

RESULTS AND DISCUSSION

Confirmation of Pt-black Deposition

As described above, surfaces of the Pt probes were modified by electrochemical deposition to increase the catalytic and enzyme immobilized surface area with a facile and cost-effective method. Deposition of Pt-black was confirmed with scanning electron microscopy (SEM), as shown in the inset of **Fig. 2**. From these SEM images, granular shaped Pt-black nanostructures were observed to be formed on top of the Pt electrode surface, as compared to bare Pt. This granular shape was expected to increase the surface area of the electrode and contribute to higher sensitivity. Thus, to further investigate the formation of Pt-black and its effect on surface area, the active surface area of both Pt-black coated and bare Pt electrodes were calculated using Randles-Sevcik equation; **Fig. 2** represents the voltammogram used for the calculation. The Pt-black electrode showed around 3 times higher active surface area compared to bare Pt, implying that the Pt-black electrode will have higher sensitivity to H_2O_2 oxidation.

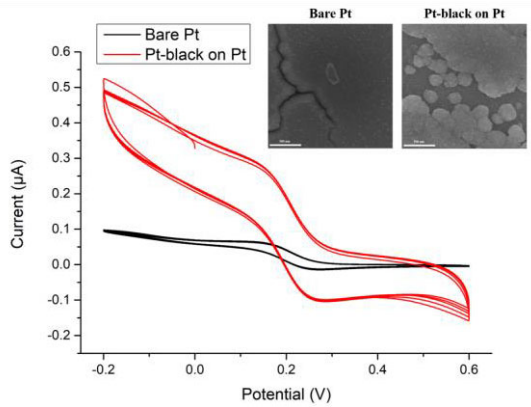

Figure 2: Cyclic voltammogram of bare Pt and Pt-black on platinum in 5 mM of $K_3Fe(CN)_6$. Inset shows scanning electron microscopic images of bare Pt and Pt-black coated Pt electrodes (bar = 500 nm)

Calibration of Pt-black Electrode

On the surface of the sensor, L-glu diffuses into mPD along with oxygen. This permselective membrane repels larger interferents such as AA and DA and only lets L-glu and oxygen to diffuse. When L-glu reaches the enzyme immobilized membrane, it reacts with GOx and is converted into H_2O_2. This H_2O_2 is oxidized by the polarized Pt, generating current (see **Fig. 1**). The sensor's sensitivity toward H_2O_2 was tested to compare the Pt-black and bare Pt electrode. The two sensors were subjected to simultaneous amperometry in 0.01 M PBS with sequential addition of H_2O_2; the *i-t* repsonse is shown in **Fig. 3(a)**. The calibration plot in **Fig. 3(b)** shows that both electrodes featured linear response to H_2O_2, with an R^2 value of 0.99. However, the sensitivity for Pt-back coated Pt was 5.93 $nA•mm^{-2}\mu M^{-1}$, which is roughly 50% higher than bare Pt (3.73 $nA•mm^{-2}\mu M^{-1}$). Thus, superior sensitivty of the Pt-black electrode, due to increase in active surface area, has been validated.

Next, the Pt-black electrode's response to L-glu was tested. As in **Fig. 4(a)**, our sensor showed a linear response to sequential addition of L-glu with a sensitivity of 5.72 $nA•mm^{-2}\mu M^{-1}$ (R^2=0.98) and limit of detection (LOD) of 1.92 µM.

Lastly, the selectivity of the Pt-black coated L-glu sensor was tested by adding AA, DA, L-glu, and H_2O_2. As seen in Fig. 4(b), addition of interferents (250 µM AA and 2 µM DA) did not result in significant increases in current in either the L-glu sensing or self-referencing sites. This indicates that the mPD layer successfully repels these molecules from diffusing into the enzyme membrane. On the other hand, addition of L-glu showed response only at the sensing site, since GOx is only present at the sensing. Lastly, H_2O_2 generated current on both sites, which can be later subtracted to only obtain signals from L-glu for obtaining higher signal to noise ratio (SNR).

CONCLUSIONS

A facile, cost-effective method of Pt-black formation on Pt electrodes, as well as Pt-black's high sensitivity towards L-glu, has been demonstrated. Electrodeposition of Pt-black, by increasing the catalytic surface area, enables the fast and highly sensitive measure of L-glu. Also, self-referencing techniques further aid in promoting SNR. This process is suitable for batch production of neurotransmitter sensors. In addition, further reduction in cost for L-glu sensing can be achieved through Pt-black deposition on other materials such as Cu or Ti. Especially, Cu electrodes can be fabricated outside of the cleanroom environment, which not only reduces cost in the material itself but also the price required for the fabrication process.

In summary, monitoring neurotransmitter release in brain with ultrahigh temporal and high spatial resolution is crucial to the investigation of the cellular and electrophysiological mechanisms underlying various neurological disorders. Although we have not yet performed in vivo validation studies, our highly sensitive biosensor potentially addresses this need. While other electrochemical sensors for L-glu have been described, none used the Pt-deposition method we are describing here, which increases surface area for L-glu detection with higher output current. This platform can be advanced with

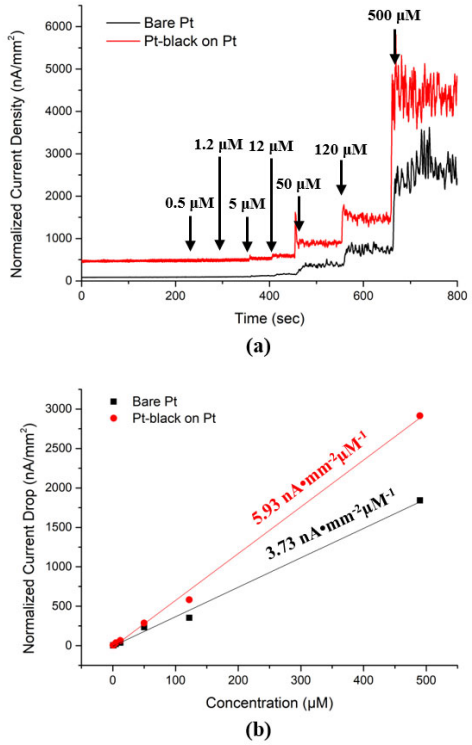

Figure 3: (a) Amperometric curve for bare platinum and Pt-black on platinum to sequential addition of H_2O_2. (b) Corresponding calibration curve with sensitivity for each material.

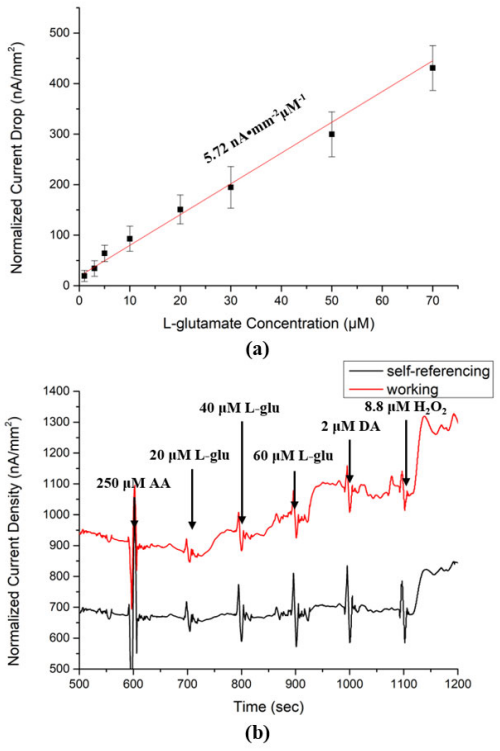

Figure 4: (a) Calibration curve and the sensitivity for Pt-black on platinum to sequential addition of L-glu. (b) Selectivity test of Pt-black coated platinum electrode.

integration of a sensing site for GABA, for simultaneous detection and real-time measurement of E:I balance in the brain. Moreover, this platform of self-referencing with facile fabrication holds the potential of being used for detecting a variety of other neurotransmitters, greatly expanding its utility in neuroscience.

ACKNOWLEDGEMENTS

This material is the result of work supported with resources and the use of facilities at the VA Portland Health Care System, VA Career Development Award, #1K2 BX002712 to M.M.L. and National Science Foundation #1926818 to M.M.L. and H.C. Interpretations and conclusions are those of the authors and do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

REFERENCES

[1] B. Engelsen, "Neurotransmitter glutamate: its clinical importance," *Acta Neurol Scand*, 1986. **74**(5): p. 337-55.

[2] M.A. Kurian, P. Gissen, M. Smith, S. Heales, Jr., and P.T. Clayton, "The monoamine neurotransmitter disorders: an expanding range of neurological syndromes," *Lancet Neurol*, 2011. **10**(8): p. 721-33.

[3] A. El-Ansary, and L. Al-Ayadhi, "GABAergic/glutamatergic imbalance relative to excessive neuroinflammation in autism spectrum disorders," *Journal of Neuroinflammation*, 2014. **11**.

[4] R. Gao, and P. Penzes, "Common mechanisms of excitatory and inhibitory imbalance in schizophrenia and autism spectrum disorders," *Current Molecular Medicine*, 2015. **15**(2): p. 146-167.

[5] S. Parrot, M. Touret, and L. Denoroy, "In vivo determination of glutamate uptake by brain microdialysis," *Biochemical Approaches for Glutamatergic Neurotransmission*, 2018. **130**: p. 431-467.

[6] S. Parrot, and P.J. Magistretti, "Neuroenergetics: Calling upon astrocytes to satisfy hungry neurons," *Neuroscientist*, 2004. **10**(1): p. 53-62.

[7] R.A. Saylor, S.R. Thomas, and S.M. Lunte, "Separation-based methods combined with microdialysis sampling for monitoring neurotransmitters and drug delivery to the brain," *Compendium Of In Vivo Monitoring In Real-time Molecular Neuroscience-Volume 2: Microdialysis And Sensing Of Neural Tissues*, 2017: p. 1.

[8] P.A. Garris, J.R. Christensen, G.V. Rebec, and R.M. Wightman, "Real-time measurement of electrically evoked extracellular dopamine in the striatum of freely moving rats," *J Neurochem*, 1997. **68**(1): p. 152-61.

[9] G.A. Gerhardt, W.A. Cass, J. Hudson, M. Henson, Z. Zhang, A. Ovadia, et al., "In vivo electrochemical studies of dopamine overflow and clearance in the striatum of normal and MPTP-treated rhesus monkeys," *J Neurochem*, 1996. **66**(2): p. 579-88.

[10] J.J. Burmeister, and G.A. Gerhardt, "Self referencing ceramic based multisite microelectrodes for the detection and elimination of interferences from the measurement of L-glutamate and other analytes," *Analytical Chemistry*, 2001. **73**(5): p. 1037-1042.

[11] J.J. Burmeister, F. Pomerleau, M. Palmer, B.K. Day, P. Huettl, and G.A. Gerhardt, "Improved ceramic-based multisite microelectrode for rapid measurements of L-glutamate in the CNS," *Journal of Neuroscience Methods*, 2002. **119**(2): p. 163-171.

[12] H. Cao, A.L. Li, C.M. Nguyen, Y.B. Peng, and J.C. Chiao, "An integrated flexible implantable micro-probe for sensing neurotransmitters," *Ieee Sensors Journal*, 2012. **12**(5): p. 1618-1624.

[13] S.K. Hamdan and Z.M. Zain, "In vivo electrochemical biosensor for brain glutamate detection: a mini review," *Malaysian Journal of Medical Sciences*, 2014. **21**: p. 12-26.

[14] Y.B. Hu, K.M. Mitchell, F.N. Albahadily, E.K. Michaelis, and G.S. Wilson, "Direct measurement of glutamate release in the brain using a dual enzyme-based electrochemical sensor," *Brain Research*, 1994. **659**(1-2): p. 117-125.

[15] R.D. O'Neill, S.C. Chang, J.P. Lowry, and C.J. McNeil, "Comparisons of platinum, gold, palladium and glassy carbon as electrode materials in the design of biosensors for glutamate," *Biosensors & Bioelectronics*, 2004. **19**(11): p. 1521-1528.

[16] M. Inaba, M. Ando, A. Hatanaka, A. Nomoto, K. Matsuzawa, A. Tasaka, et al., "Controlled growth and shape formation of platinum nanoparticles and their electrochemical properties," *Electrochimica Acta*, 2006. **52**(4): p. 1632-1638.

[17] J. Kim, C.W. Roh, S.K. Sahoo, S. Yang, J. Bae, J.W. Han, et al., "Highly durable platinum single-atom alloy catalyst for electrochemical reactions," *Advanced Energy Materials*, 2018. **8**(1): p. 1701476.

[18] N. Wahono, S. Qin, P. Oomen, T.I.F. Cremers, M.G. de Vries, and B.H.C. Westerink, "Evaluation of permselective membranes for optimization of intracerebral amperometric glutamate biosensors," *Biosensors & Bioelectronics*, 2012. **33**(1): p. 260-266.

[19] K.N. Hascup, E.C. Rutherford, J.E. Quintero, B.K. Day, J.R. Nickell, F. Pomerleau, et al., "Second-by-second measures of L-glutamate and other neurotransmitters using enzyme-based microelectrode arrays," *Electrochemical Methods for Neuroscience*, 2007. **1**: p. 407-450.

[20] M. Zaib, and M.M. Athar, "Electrochemical characterization of a Porphyridium cruentum-modified carbon paste electrode by cyclic voltammetry," *Instrumentation Science & Technology*, 2018. **46**(4): p. 408-425.