Electroenzymatic Glutamate Microbiosensor in the Study of Parkinson's Disease

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ABSTRACT

The accurate monitoring of L-glutamate release and uptake in the mammalian central nervous system (CNS) would provide neuroscientists significant insight into the dynamics of glutamate action in a number of neurological disorders, including Parkinson's disease. A novel method is presented for microfabricating a glutamate microbiosensor. Key features of the fabrication process include: plasma etching to shape the probe, deposition of low stress silicon dioxide films as the insulating layers throughout, deposition of Pt layers for electrodes and interconnections and electropolymerization of polypyrrole on electrodes.

Such microsensors provide high spatial and temporal resolution. Flow cell measurements show that the microsensors have a response time of 3~4 seconds. *In vivo* measurements have been conducted in living mice brain tissue. The results indicate that the microsensors are able to measure glutamate uptake and release with high sensitivity and superior selectivity.

KEY WORDS: Biosensor, Parkinson's Disease, Glutamate

INTRODUCTION

The accurate monitoring of L-glutamate release and uptake in the mammalian central nervous system (CNS) would provide significant insight for neuroscientists studying a number of neurological disorders[1-4], including Parkinson's disease. Since enzyme-based amperometric sensors combine the high specificity of enzyme biorecognition and the fast response of an amperometric method, amperometric glutamate detection is among the most favorable glutamate detection approaches. There are a large number of publications describing the amperometric detection of glutamate based on redox enzymes[5-7]. An important prerequisite for an implantable sensor is that the design of the device should be amenable to mass production, resulting in inexpensive, reproducible and reliable devices. However, the microsensors mentioned above all rely on handcraft for construction, thus mass production and sensor reproducibility are hard to achieve.

Micro-Electro-Mechanical-System (MEMS) technologies have in recent years provided a feasible approach for the fabrication of high-quality, miniaturized sensors[8]. In this research, we constructed a glutamate biosensor probe on Si. A layer of overoxidized polypyrrole deposited on the working electrode gives the system, based on immobilized glutamate oxidase, superior selectivity.

FABRICATION

The microbiosensor fabrication process is shown in Figure 1. (a) First, we deposited silicon dioxide on a thin Si wafer by LPCVD (low-pressure chemical vapor deposition). A thin Pt film was deposited by e-beam evaporation followed by a lift-off process to pattern the Pt film. (b) After patterning the metal layer, another insulating layer of silicon dioxide was grown on the front side of the wafer. (c) The electrode and contact pad sites were opened by a plasma etching process. (d) The probe shape was defined by a DRIE process and the probe was released from the wafer. (e) We deposited a polypyrrole film on the working electrode to give a permselective barrier against the electroactive interferents, ascorbic acid and dopamine.

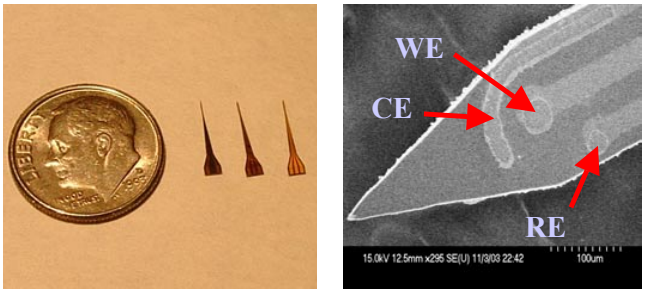

Figure 1. Micromachined glutamate sensor. The width and thickness of the sensor are 120 μm.

Figure 1 shows the micromachined sensor probe. The tip region is 120 μm in width and 120 μm thick. There are three electrodes at the tip, a working electrode (WE), a reference electrode (RE) and a counter electrode (CE).

IN VIVO MEASUREMENT

In vivo glutamate uptake and release measurements were conducted in anesthetized mouse striatum (AP +0.8 mm, ML +2.0 mm, DV – 4.5 mm).

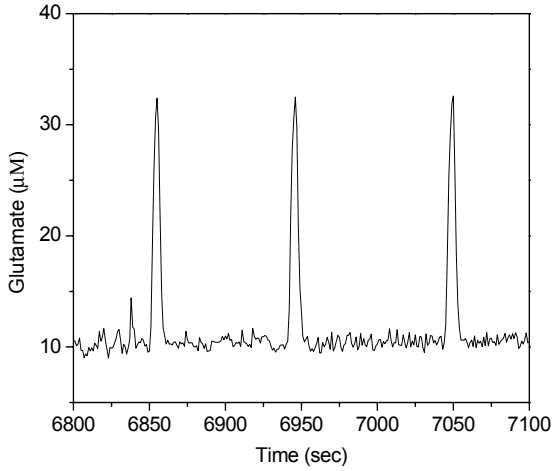

Figure 3. Glutamate uptake kinetics: 20 nL of 200 μM glutamate were injected every 100 second from a pipette fixed at the sensor probe tip.

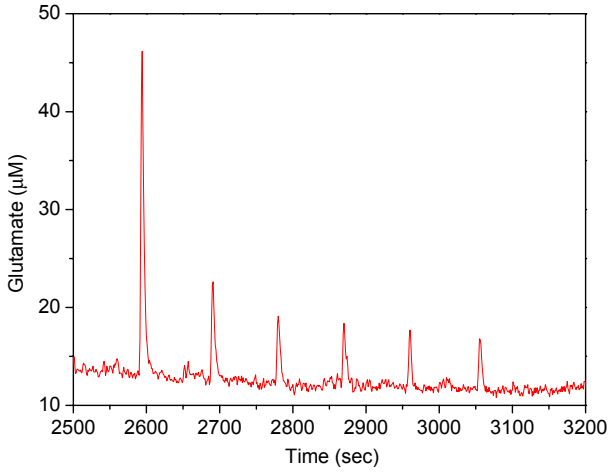

Figure 4. Glutamate release dynamics: responses to repeated 20 nL, 100 mM KCl stimulations in mouse striatum

The glutamate uptake was carried out by injecting 20 nL of 200 μM glutamate every 100 second from a pipette fixed at the sensor probe tip. The glutamate uptake curve is shown in Figure 3. Figure 4 is the glutamate release curve responding to repeated 20 nL, 100 mM KCl stimulations in mouse striatum.

SUMMARY

A new design of amperometric glutamate microbiosensor was successfully developed using MEMS technologies. The sensor system was constructed on a 120 μm-wide Si probe. In vivo glutamate uptake and release measurements demonstrated that the microsensor has excellent sensitivity, selectivity and response time.

ACKNOWLEDGMENTS

This work has been financially supported by the UCLA NIH Udall Center for Excellence in Parkinson's Disease Research.

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