

Comparison Study between Dialysis Electrode and CNT Probe for Neurotransmitter Monitoring

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Abstract

The performance of CNT network junction probe and conventional dialysis electrode was compared for the efficiency of real time glutamate monitoring in 11 vessel occlusion (11 VO) rat model. The dialysis electrode (Sycopel Scientific Ltd., NE 38 9BZ, U.K.) was perfused with fresh phosphate buffer saline (PBS) solution containing glutamate oxidase at 0.5 μ L/min during the measurement. The glutamate oxidase immobilized CNT probe was implanted in the brain of the rat model. From the results of real time glutamate monitoring and cerebral blood flow (CBF) in 11 VO rat model, it seemed that the dynamics of glutamate release in extracellular fluid were similar both by dialysis electrode or CNT probe. However, the response of CNT probe based biosensor with L-glutamate oxidase (GLOD) to glutamate was faster (<10 sec) than that of dialysis electrode (>70 sec). The sensitivity of CNT probe to glutamate was higher than that of dialysis electrode in the rat model (approx. 20 times). Therefore, we suggest that the GLOD-immobilized CNT probe provides a fast response time and a high sensitivity for glutamate which are essential for the real-time glutamate monitoring.

Keywords: CNT probe, Dialysis electrode, Neurotransmitter, Real-time monitoring

Introduction

It is generally accepted that glutamate performed as principal excitatory neurotransmitter in the brain, and its excessive release might play a key role in neuronal death associated with a wide range of neural disorders¹. A better understanding of the excitotoxic process required accurate assessment of the time course of extracellular glutamate changes, during or after various medical incidents such as neurosurgical operation.

In vivo monitoring of the glutamate concentration in the extracellular fluid of the brain became possible through the microdialysis measurement due to the small size of electrode (for instance, Clark electrode etc.). In previous studies, the extracellular concentrations of glutamate and K⁺ have been measured by high-performance liquid chromatography coupled with the microdialysis method and a K⁺-sensitive electrode^{2,3}. However, since it takes a long time to collect a sufficient volume of sample for the test, the real-time detection of the analytes in the early period of ischemia is not possible.

Albery *et al.* implanted a dialysis electrode system which consisted of electrode and a standard microdialysis probe containing glutamate oxidase⁴. In this method, the glutamate is oxidized by L-glutamate oxidase in perfusate and produced hydrogen peroxide which can be amperometrically detected in real time on a platinum working electrode in the tip of the probe. This dialysis electrode has been used in a study of the rat cerebral ischemia model.

The field effect transistor (FET) made of carbon nanotube (CNT) has been used as a sensor due to its extreme sensitivity to local chemical environments⁵. The high sensitivity of CNT originates from its atoms located at the surface. Proteins such as enzymes and antibodies which are specific to their targets have been used as recognition elements. It is essential to immobilize biological molecules on CNTs by a reliable method to preserve their electronic characteristics. In 2003, Besteman *et al.* found that immobilization of glucose oxidase (GOD) on the sidewall of a semiconducting single-walled carbon nanotube (SWNT) decreased the conductance of the tube. As a result, they suggested that biosensor maintaining enzyme activity could be constructed at the single molecule level of an individual SWNT⁶.

In this study, we compared the performance of CNT

network junction probe (CNT probe) with conventional dialysis electrode in 11 vessel occlusion rat model.

Results and Discussion

Recently an ischemic stroke, a kind of cerebrovascular disease, is one of the main causes of the death in the world (specially in developed countries). Glutamate is the principal excitatory neurotransmitter in the brain, and its excessive release plays a key role in neuronal death associated with a wide range of neural disorders. Real time monitoring of extracellular glutamate levels in the brain would be very helpful in order to understand the excitotoxic process of neurotransmitters, as well as brain injury during or after surgery.

Figure 1 showed the ischemia-evoked response of glutamate with dialysis electrode and the change of cerebral blood flow (CBF) in 11 VO rat model. As apparent from Figure 1, during the ischemic period in which CBF declined rapidly to near zero levels, the elevation in glutamate release began approximately 70 sec after the onset of ischemia and continued to rise throughout the ischemic period. In the end of the ischemic episode, the release of glutamate showed maximum change of 31 nA ($\approx 110 \mu\text{M}$). During reperfusion, a transient increase of glutamate levels was detected at first, but then steadily declined to pre-ischemic levels. We utilized CNT probe as glutamate sensor for 11 VO rat model and compared the results with those of dialysis electrode about the change of glutamate release during the intra- and post-ischemic periods following brief (10 min) global ischemia. In Figure 2, it was shown that the increase in the current (response signal) of the CNT probe immobilized with GLOD

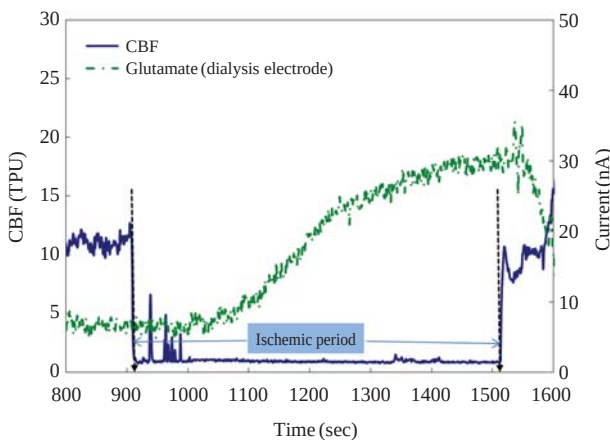


Figure 1. Real time changes in cerebral glutamate level with dialysis electrode and cerebral blood flow level in the 11 VO rat model during ischemic episode.

was detected shortly after the occlusion was initiated (< 10 sec), and rose to a peak of 580 nA during the ischemic period. Then a sharp peak (760 nA) was observed immediately at the start of reperfusion and returned to its initial value during reperfusion. In our previous study, no significant changes of current signal in the CNT probe without GLOD were observed before and after ischemia, including 10 min occlusion⁷.

From this result, the dynamics of glutamate release in extracellular fluid seemed to be similar both dialysis electrode and CNT probe, as shown in Figure 3. However, we found that the response of CNT probe with GLOD to glutamate (< 10 sec) was faster than that of dialysis electrode (> 150 sec). And the sensitivity of CNT probe to glutamate was higher than that of dialysis electrode in 11 VO rat model (approximately 20 times).

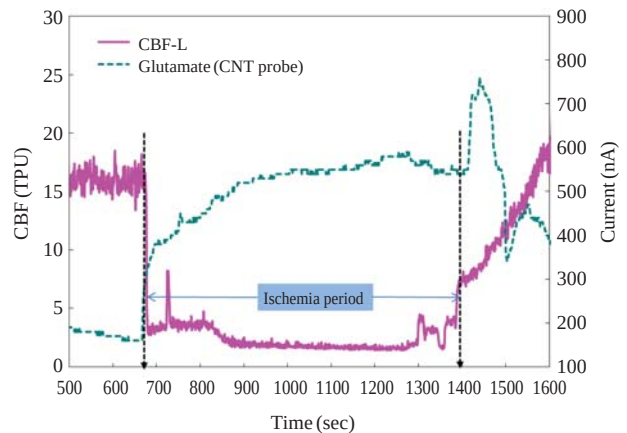


Figure 2. Real time changes in cerebral glutamate level with CNT probe and cerebral blood flow level in the 11 VO rat model during ischemic episode.

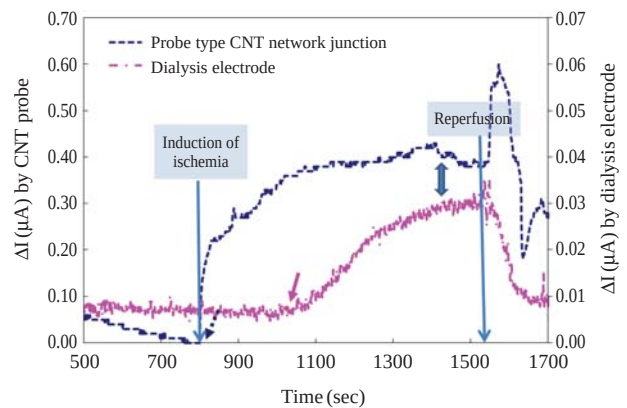


Figure 3. The comparison of extracellular glutamate release between CNT probe and dialysis electrode in the 11 VO ischemia model.

According to the previous studies, it was suggested that the initial increase of glutamate level throughout the 10 min ischemic period was attributed to the reversed uptake of glutamate transporter which was due to an ionic imbalance⁸. On the other hand, it was thought that the second and third sharp peaks were caused by a reperfusion-evoked release of glutamate. However, it is not clear why the first and second sharp peak appeared clearly only in the probe-type CNT sensor. (possibly due to the better performance of the probe-type sensor and/or the different detection mechanism between the two sensors) Therefore, our result suggested that the GLOD-immobilized CNT probe has a fast response time and a high level of sensitivity which are essential for the real-time glutamate monitoring.

Conclusions

In this paper, we demonstrated newly designed glutamate biosensor based on the probe-type CNT immobilized with GLOD and compared its performance with conventional dialysis electrode. In order to compare, we utilized 11-VO rat brain ischemia model and real-time data acquisition during the experiment.

In dialysis electrode, the glutamate is oxidized by L-glutamate oxidase in the probe perfusate and produced hydrogen peroxide which is amperometrically detected in real time on a platinum electrode in the tip of the probe. On the other hand, the detection method with CNT probe is based on the principle that the conductance between source and drain connected with CNT

network was changed by its chemical environments. In our previous study, we suggested that the conductance increased by the L-glutamate addition was attributed to the charge transfer from carbon nanotubes to hydrogen peroxide⁷. In other words, hydrogen peroxide worked more effectively as an electron transfer mediator compared to the ammonia to change the conductance signals from CNT networks treated by the ethanolamine.

The continuous, real-time changes of glutamate release in the 11 VO rat model were successfully measured with the GLOD-immobilized CNT probe. The initial release of glutamate by the glutamate re-uptake system was rapidly monitored in the intra-ischemic period. In addition, the CNT probe even detected the slight glutamate release evoked temporarily by reperfusion. From these results, the CNT network junctions built in the probe possess great potentiality as a new device for the real time monitoring of the neurotransmitter due to its fast response and high sensitivity.

Materials and Methods

The Preparation of Dialysis Electrode

The conventional dialysis electrode was purchased from Sycopel International Ltd. (Type: General 20-10-4-4, U.K.) Figure 4(a) shows the diagram of dialysis electrode. The dialysis electrode was filled with PBS to electropolymerize the O-phenylenediamine on the platinum electrode at 0.65 V for 20 min with Sycopel BD2000 potentiostat. Subsequently, the dialysis elec-

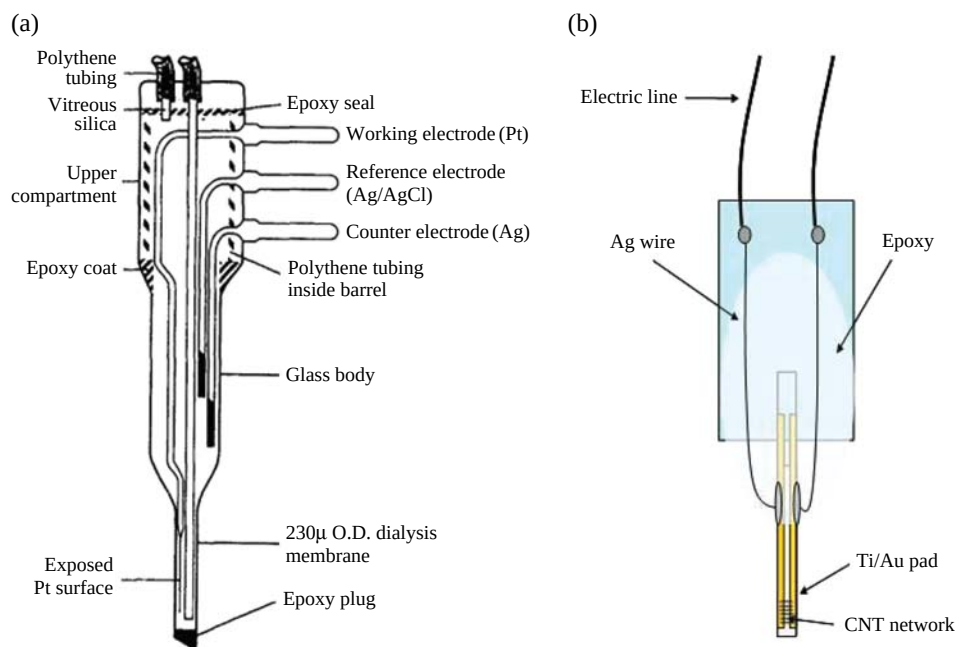


Figure 4. Schematic structure of dialysis electrode (a) and probe-type CNT network junction (b).

trode was perfused with fresh PBS containing GLOD (Yamasa Corp., Tokyo, Japan) at a flow rate of 0.5 $\mu\text{L}/\text{min}$. The dialysis electrode showed linear response in the concentration range of 50–150 μM standard glutamate solution with a sensitivity of 0.22 nA/ μM ($R^2 = 0.998$).

The Preparation of CNT Probe

The CNT probes were fabricated by surface programmed assembly method. The fabrication process of probe-type CNT transistor was as follows: purified SWNTs were dispersed in 1,2-dichlorobenzene with ultrasonic vibration around 1 hr. For SWNT assembly, the molecular patterned capillary tube (glass) was dipped in SWNT solution for 10 sec, and then rinsed thoroughly with 1,2-dichlorobenzene followed by thorough drying with N_2 gas. Thereafter, 100 Å of Ti and 300 Å of Au were deposited by thermal evaporator. The glass tube with CNT network junctions was supported by wafer connected with silver wire and epoxy. The schematic structure of probe-type CNT transistor is shown in Figure 4(b). The CNT probes were incubated in methanol containing 1 mM 1-pyrenebutanoic acid succinimidyl ester (Molecular Probes, Inc., USA) for 2 h while stirring, then washed with methanol⁹. Next, the probes were exposed to the GLOD solutions in 10 mM PBS (pH 7.4) overnight. After rinsing with clean PBS, the 100 mM ethanolamine was added onto the probes to deactivate and block the excess reactive groups remaining on the surface and followed by incubation for 30 min.

After washing with PBS, the GLOD-immobilized CNT probe was dipped in 20 mL of fresh buffer solution

for electrochemical measurements at room temperature. We measured the change of current between source and drain electrode (I_{sd}) when L-glutamic acid was added to the PBS buffer solution in which the probe with and without GLOD was dipped. A drain-source voltage (V_{sd}) of 10 mV was applied by DC power supply (Agilent E3646A) and the conductance change was measured with picoammeter (Keithley 6485).

In Vivo Measurements

For real-time in-vivo glutamate measurements, we prepared the 11 VO (vessel occlusion) rat model. 11 VO rats were prepared by division of the omohyoid muscle to allow the larynx to be retracted, and exposing the ventral surface of the clivus following retraction of longus coli muscle. 3 mm craniotomy was drilled through the clivus, centered just caudal to the basioccipital suture. After opening the dura and arachnoid, the distal basilar artery was coagulated just caudal to the superior cerebellar arteries, and divided. The pterygopalatine arteries were coagulated prior to the entrance into the tympanic bullae. Both occipital arteries and the superior thyroid arteries were identified, coagulated and transected. Snares were placed around the external carotid arteries (ECAs), between the occipital arteries proximally and the superior thyroid arteries distally, and snares were placed on the common carotid arteries (CCAs).

Animals were placed in stereotaxic head holder for real-time glutamate monitoring. Changes in CBF were monitored by laser-Doppler flowmetry with cortical glutamate levels by CNT probes and dialysis electrode.

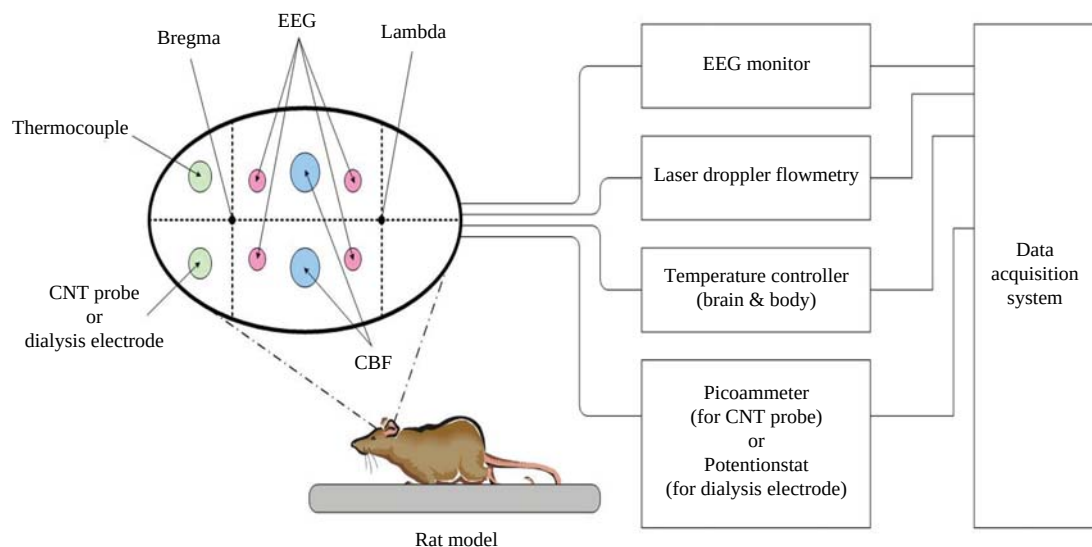


Figure 5. The diagram of experimental setup for real-time glutamate monitoring system in 11 vessel occlusion rat model. Zoomed image showed the coordinates for craniotomy (EEG : electroencephalogram).

Both CNT probes and dialysis electrode were inserted into the motor cortex at coordinates A 1; L 4; V 2 mm (from the bregma and the dura), as shown in Figure 5. The ten minute 11 VO cerebral ischemia was initiated by pulling the snares on the CCAs and ECAs. The snares were released and withdrawn after 10 min.

Acknowledgements

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