# Platinized Aligned Carbon Nanotube-Sheathed Carbon Fiber Microelectrodes for In Vivo Amperometric Monitoring of Oxygen

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**ABSTRACT:** The abnormal level of $O_2$ could disturb various neurochemical processes and even induce neural injury and brain dysfunction. In order to assess critical roles of $O_2$ in the neurochemical processes, it is essential to perform in vivo monitoring of the dynamic changes of $O_2$. In this study, we develop a new electrochemical method for selectively monitoring $O_2$ in vivo, using platinized vertically aligned carbon nanotube (VACNT)-sheathed carbon fibers (Pt/VACNT-CFs) as the electrodes. The VACNT-sheathed CFs (VACNT-CFs) are produced via the pyrolysis of iron phthalocyanine (FePc) on the surface of CFs, followed by electrochemical deposition of platinum nanoparticles to form Pt/VACNT-CFs. The resulting Pt/VACNT-CF microelectrodes exhibit fast overall kinetics for



the $O_2$ reduction via a four-electron reduction process without the formation of toxic $H_2O_2$ intermediate. Consequently, effective and selective electrochemical methods are developed for the measurements of $O_2$ in rat brain with the Pt/VACNT-CF microelectrodes, even in the presence of some species at their physiological levels, such as ascorbic acid, dopamine, uric acid, 5-hydroxytryptamine, and of the $O_2$ fluctuation in rat brain in the early stage of global cerebral ischemia/reperfusion, mild hyperoxia, and hypoxia induced by exposing the animal, for a short time, to $O_2$ and $N_2$, respectively, and hindfeet pinch. The use of VACNT-CF as the support for Pt effectively improves the stability of Pt, as compared with the bare CF support, while the FePc pyrolysis ensures the VACNT-CFs to be reproducibly produced. Thus, this study offers a novel and reliable strategy for preparing new microelectrodes for in vivo monitoring of $O_2$ in various physiological processes with a high sensitivity and selectivity.
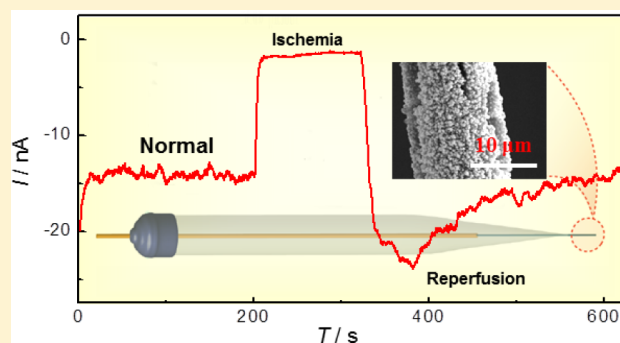
In vivo monitoring dynamic changes of $O_2$ has drawn increasing attention, because $O_2$ plays critical roles in various neurochemical processes and its abnormality disturbs these neurochemical processes and even induces neural injury and brain dysfunction.[1] For example, the deficiency of $O_2$ can be the consequence of many pathophysiological conditions, such as ischemia.[1] Nowadays, brain ischemia with various origins, such as embolism and mechanical disruption of cerebral blood flow followed by irreversible neural injury and brain dysfunction, has been recognized as one of the major causes of death and disability.[2] Brain ischemia induces selective neural injury in vulnerable brain regions, such as the hippocampus, which is highly sensitive to ischemic hypoxia, and thereby damages brain functions.[3] Since the neurochemical processes in the acute ischemic period trigger pathologic damage to the brain and eventually lead to neuron death,[4] it is of great physiological and pathological importance to understand the neurochemical processes including energy failure, anoxic depolarization, glutamate excitotoxicity, calcium overload, and oxidative stress in the early stage of cerebral ischemia. Motivated by

understanding of the neurochemical processes in acute ischemic period, we have recently developed electrochemical methods to continuously monitor the nature real-time activities of some chemical species, such as glucose, lactate, ascorbic acid and $Mg^{2+}$ that are highly involved in the neurochemical processes during ischemia.[5] In the cerebral system, the $O_2$ level varies with brain regions (striatum, hippocampus, etc.) and $O_2$ heterogeneity is involved in the different vulnerability of brain regions during ischemia.[1] As an essential element in neural injury and brain dysfunction during ischemia, $O_2$ plays critical roles in the complicated neurochemical processes.[6] Moreover, as a common electron acceptor of over 100 known enzymes, $O_2$ is involved in many biochemical reactions to generate energy in vivo, including adenosine triphosphate (ATP) metabolism.[1] On the other hand, $O_2$ is also involved in the synthesis, metabolism, release, and uptake of neurotransmitters.[6] Under ischemia

conditions, the decrease of cerebral blood flow (i.e., lower availability of $O_2$) has been reported to disturb these processes, even leading to death and disability.[4] Thus, in vivo monitoring of $O_2$ fluctuation during brain ischemia could offer a straightforward approach to better understanding of neurochemical processes involved in this process.

Although the pressing need for in vivo measurements of $O_2$ has greatly activated intensive interests in developing direct and indirect methods for $O_2$ sensing, the complexity of the cerebral environments, which becomes even more complicated in various physiological and pathological process such as cerebral ischemia/reperfusion, has made the effective monitoring of $O_2$ in vivo very challenging. While cerebral blood flow is tightly related to the level of cerebral $O_2$ and has been used in clinical for evaluating the changes of $O_2$ level during brain dysfunction, it is difficult to directly measure the $O_2$ concentration in the cerebral systems.[7] Some methods, such as fiber-optic fluorescence, near-infrared (NIR) light spectroscopy, positron emission tomography, nuclear magnetic resonance (NMR), and electron paramagnetic resonance (EPR),[8] have been reported for quantitative measurements of brain tissue $O_2$ in vivo. However, these methods were limited either by sophisticated instrumentation, or the requirement of an external probe, or the <10 mm detection depth from the surface of the body.

In vivo voltammetry that employs microsized microelectrode directly implanted into the brain regions can monitor the dynamic change of neurochemicals in the central nervous system in real time.[9] Despite the fact that carbon fibers (CFs) and carbon paste microelectrodes have been successfully used to probe neurochemicals (e.g., dopamine, $O_2$), they still suffer from the production of neurotoxic $H_2O_2$ during in vivo $O_2$ detection.[10] Platinum (Pt) is widely recognized as the most active metal for the electrochemical reduction of $O_2$, because it facilitates the reduction of $O_2$ through a four-electron process to produce water.[11] However, Pt microwire is difficult to implant directly into the brain and it must be sealed and insulated into a microdisk electrode with a diameter typically in the millimeter range, such as the Clark $O_2$ electrode.[12] To reduce brain tissue damage, the Pt microdisk electrode has been miniaturized, but this makes the method less sensitive. To achieve a high catalytic capability for $O_2$ reduction, Pt should be well-dispersed onto a support with a high surface area, while simultaneously bearing proper mechanical property to be implanted into the brain for in vivo measurements. In addition, besides dispersing the active phase, the support could also provide a porous structure, suppressing Pt dissolution and detachment during the $O_2$ reduction reaction. Carbon nanotubes (CNTs), as heterogeneous porous Pt catalyst scaffolds, have exhibited excellent properties to prevent Pt from electrochemical dissolving, agglomerating, and detaching; hence, they have largely improved the stability of Pt nanoparticles and facilitated the electrocatalytic activity toward $O_2$ reduction in comparison to conventional carbon black and carbon supports.[13] It is reasonable to envisage that CFs modified with platinized CNTs could offer a new way to fabricate microelectrodes for monitoring $O_2$ in vivo.

In this study, we demonstrate a new method for monitoring $O_2$ in vivo using platinized vertically aligned carbon nanotube (VACNT)-sheathed carbon fibers (Pt/VACNT-CFs). VACNT-CFs were generated via the pyrolysis of iron(II) phthalocyanine (FePc). In this way, VACNT-CF can be reproducibly fabricated with a high surface area, as well as good mechanical and electrochemical properties, for reproducible

electrochemical deposition of nanostructured Pt. Compared with the traditional Pt/CF microelectrodes, the Pt/VACNT-CF microelectrodes demonstrated here show fast overall kinetics and good stability for in vitro and in vivo $O_2$ monitoring in the cerebral systems. Therefore, this study provides a new, effective, and selective platform for in vivo measurements of $O_2$, which are important to various physiological and pathological studies.

## ■ EXPERIMENTAL SECTION

**Reagents and Solutions.** Dopamine (DA), ascorbic acid (AA), uric acid (UA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5% Nafion, 5-hydroxytryptamine (5-HT), and iron(II) phthalocyanine (FePc) were all purchased from Sigma and used as supplied. Artificial cerebrospinal fluid (aCSF) was prepared by mixing NaCl (126 mM), KCl (2.4 mM), $KH_2PO_4$ (0.5 mM), $MgCl_2$ (0.85 mM), $NaHCO_3$ (27.5 mM), $Na_2SO_4$ (0.5 mM), and $CaCl_2$ (1.1 mM) into doubly distilled water and the solution pH was adjusted to pH 7.4. Other chemicals were of at least analytical grade and used without further purification.

**Preparation of Pt/VACNT-CF Microelectrodes.** The growth of VACNTs on CFs (T650-35, Fabric Development, Inc.) was performed as reported previously.[14] Briefly, the CFs were first treated in a high-temperature furnace at 1100 °C with a flow mixture of argon (300 mL min$^{-1}$) and $H_2$ (40 mL min$^{-1}$) carrying $SiCl_4$ for 20 min in the presence of trace $O_2$ under atmospheric pressure to form a thin layer of $SiO_2$ on the CFs. VACNTs were then grown on these preactivated CFs by pyrolysis of FePc under Ar/$H_2$ atmosphere at 800−1100 °C. The fabrication of VACNT-CF microelectrodes was quite similar to that of CF microelectrodes, as reported previously.[9j,k] More specifically, a single VACNT-CF was cut to a length of 1 cm and attached onto a copper wire with silver conducting paste. A glass capillary (outer diameter (od) of 1.5 mm, length of 100 mm) was pulled to a fine tip by a microelectrode puller (WD-1, Sichuan, China); the capillary tip then was trimmed to make the tip diameter 30−50 $\mu$m. Then, the VACNT-CF attached copper wire was carefully inserted into the capillary until the VACNT-CF extrude the fine open end of the capillary with extra Cu wire left at the other end of the capillary. Both open ends of the capillary were sealed with epoxy resin with 1:1 ethylendiamine as the hardener. The excess epoxy on the VACNT-CF was carefully removed with acetone to form microelectrodes with the pristine VACNT-CF electrode material. Thereafter, the VACNT-CF microelectrodes were dried at 100 °C for 2 h and the exposed VACNT-CF was carefully trimmed to 1.0 mm in length under a microscope. Prior to use, the VACNT-CFs thus fabricated were first cleaned by sequential sonication in acetone, 3 M $HNO_3$, 1.0 M KOH, and distilled water, each for 3 min and then subject to electrochemical treatment in 1.0 M NaOH at +1.5 V (vs KCl-saturated Ag/AgCl) for 80 s. Pt/VACNT-CF microelectrodes were prepared by electrochemical deposition of Pt nanoparticles onto the VACNT-CFs from 1.0 mM $H_2PtCl_6$ solution in 0.5 M $H_2SO_4$ by continuously scanning the microelectrodes within a potential range from +0.5 V to −0.7 V for consecutive 30 cycles at 100 mV/s. After that, the Pt/VACNT-CFs were taken out of solution, rinsed with distilled water, and then dried under ambient temperature. As a control, Pt/CF microelectrodes were prepared with the same procedure as that for the Pt/VACNT-CF microelectrodes.

**Apparatus and Measurements.** Electrochemical measurements were performed on a computer-controlled electro-

chemical analyzer (Model 660A, CHI Instruments, Shanghai, China). Pt/VACNT-CFs and Pt/CFs were used as working electrodes and a platinum wire was used as the counter electrode. For both in vitro and in vivo electrochemical measurements, a tissue-implantable microsized Ag/AgCl electrode was used as the reference electrode. The microsized reference electrode was prepared by first polarizing Ag wire (1 mm in diameter) at +0.6 V in 0.1 M hydrochloric acid for ca. 30 min to produce a Ag/AgCl wire and then inserting the as-prepared Ag/AgCl wire into a pulled glass capillary, in which aCSF was sucked from the fine end of the capillary and used as the inner solution for the reference electrode. The other end of the capillary with Ag wire exposed was sealed with epoxy. Calibration of the Pt/VACNT-CF microelectrodes both before and after in vivo experiments was performed in aCSF saturated with $N_2$, ambient air, or $O_2$, in which the $O_2$ concentration was taken to be 0, 200, and 1250 $\mu$M, respectively.[15] Scanning electron microscopy (SEM) used for characterization of VACNT-CF, and Pt/VACNT-CFs, using a Model S4300-F microscope (Hitachi, Inc., Tokyo, Japan).

**In Vivo Experiments.** Adult male Sprague–Dawley rats (300–350 g) were purchased from Health Science Center, Peking University. The animals were housed on a 12 h:12 h light–dark schedule with food and water ad libitum. Animal experiments were performed as reported previously.[5,9j,k] Briefly, the animals were anaesthetized with chloral hydrate (345 mg/kg, i.p.) and positioned onto a stereotaxic frame. The Pt/VACNT-CFs were implanted into rat hippocampus (AP = 5 mm, L = 5 mm from bregma, V = 4.5 mm from dura), using standard stereotaxic procedures. In order to reduce electrode fouling in tissue, the Pt/VACNT-CF microelectrodes were coated with Nafion by immersing the electrodes into a 0.5% Nafion solution for 2 s and dried at ambient temperature before in vivo measurement. The prepared microsized Ag/AgCl reference electrode was positioned into the dura of brain and secured with dental acrylic. Platinum wire embedded in subcutaneous tissue on the brain was used as the counter electrode. The surgery for global cerebral ischemia/reperfusion was performed using a method described previously.[5] Briefly, through a midline cervical incision, both common carotid arteries were exposed and isolated from surrounding connective tissue, with special care being taken to not damage the vagus or sympathetic nerves running closeby. The ischemia and reperfusion were achieved by first occluding and then opening both carotid arteries by pulling down and releasing the threads ligated to the carotid arteries. Mild hyperoxia and hypoxia were induced by exposing the animal for a short time to $O_2$ (60 s) and $N_2$ (40 s), respectively. $O_2$ or $N_2$ was contained in rubber bladders and delivered from a tube placed under the nose of the rat. With the animal under anesthesia (but still having some feeling to pinch), both hindfeet were clamped for 120 s with paper clip. Amperometric method with working electrode polarized at a constant potential of −0.5 V vs. Ag/AgCl (aCSF) reference electrode was employed for in vivo measurements of $O_2$ in rat brain.

## RESULTS AND DISCUSSION

**Electrochemical Properties of Pt/VACNT-CF Microelectrode.** Figure 1 displays scanning electron microscopy (SEM) images of the VACNT-CF (Figure 1A), Pt/CF (Figure 1B), and Pt/VACNT-CF (Figure 1C). Compared with the smooth surface of CF (outer diameter (od) = 7 $\mu$m), a cross-sectional view SEM image of the VACNT-CF taken after
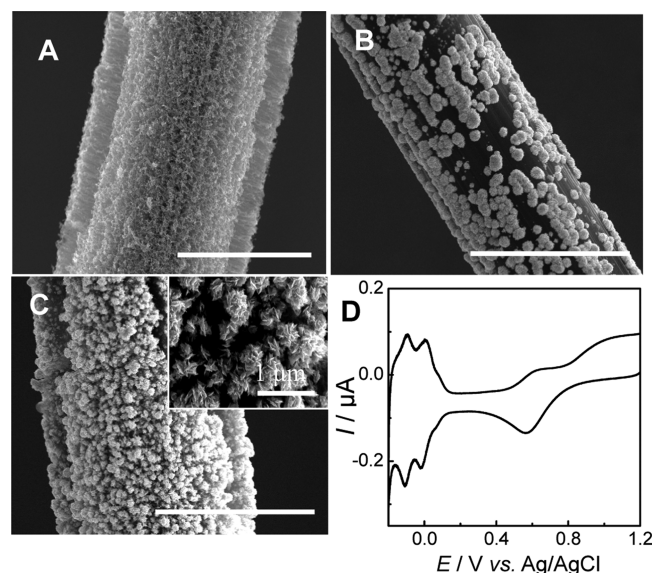


**Figure 1.** SEM images of the (A) VACNT-CF, (B) Pt/CF, and (C) Pt/VACNT-CF. [Scale bar in panels (A), (B), and (C) are 10 $\mu$m.] Inset in panel (C) shows many densely packed Pt nanoparticles homogeneously distributed on the surface of VACNTs. (D) Typical cyclic voltammogram (CV) obtained at the Pt/VACNT-CF in 0.5 M $H_2SO_4$ solution. Scan rate = 100 mV s$^{-1}$.

deliberately disturbing aligned CNTs along its length (Figure 1A) shows that VACNTs were densely packed on the CF surface to form a coaxial VACNT-CF microelectrode (od ≈ 13 $\mu$m). The VACNTs that were well-aligned around a CF can maintain a good porosity with a large surface area and excellent electrochemical properties, with the CF support providing mechanical stability and efficient electrical conduction to/from the VACNTs. Inspection of VACNTs after Pt deposition clearly shows that many densely packed Pt nanoparticles homogeneously distribute on the surface of VACNTs (Figure 1C). Although Pt nanoparticles could also be electrochemically deposited onto the pristine CFs under the same conditions, the resulting Pt particles distribute on the CFs more loosely (Figure 1B). This difference in morphologies of the Pt nanostructures electrodeposited on the CF and VACNT-CF may be attributed to the different electronic and porous properties of the CNT and CF.[16] Moreover, cyclic voltammograms (CV) obtained on the Pt/VACNT-CF in acidic media exhibits adsorption and desorption of hydrogen (Figure 1D), characteristic of clean electroactive surface of Pt.[13] As we shall see later, the VACNT-CFs are ideal Pt supports for in vivo monitoring of $O_2$.

Figure 2 compares the reduction process of $O_2$ at bare CF (Figure 2A), VACNT-CF (Figure 2B), Pt/CF (Figure 2C), and Pt/VACNT-CF (Figure 2D) microelectrodes. On the bare CF microelectrode, a steady-state current response was not obtained until −0.8 V, indicating a sluggish overall kinetics process for $O_2$ reduction. Clearly, the VACNT-CF exhibited a more positive onset potential and larger reduction current (Figure 2B), indicating a faster overall kinetics for $O_2$ reduction reaction on the VACNT-CF than on bare CF. This fast kinetics is commonly observed for $O_2$ reduction reaction at the CNT-based electrodes via a two-electron step to form the $H_2O_2$ intermediate, which is toxic to brain tissue, as demonstrated previously.[17] Interestingly, the onset potential of $O_2$ reduction reaction on the Pt/VACNT-CF occurs at ca. +0.2 V (Figure
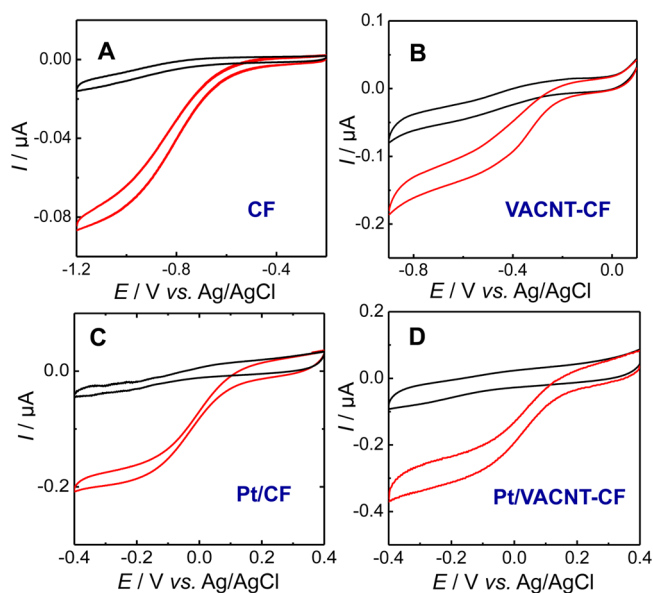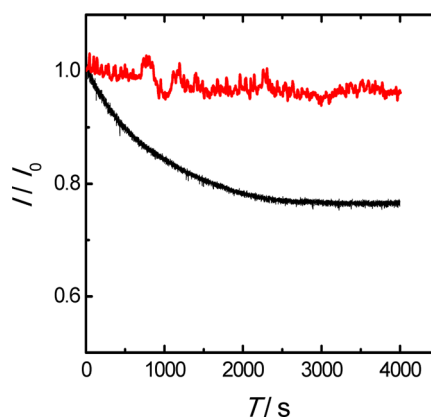
**Figure 2.** Typical CVs at the (A) bare CF, (B) VACNT-CF, (C) Pt/CF, and (D) Pt/VACNT-CF microelectrode in aCSF (pH 7.4) saturated with $N_2$ (black curve) or ambient air (red curve). Scan rate = 50 mV s$^{-1}$.



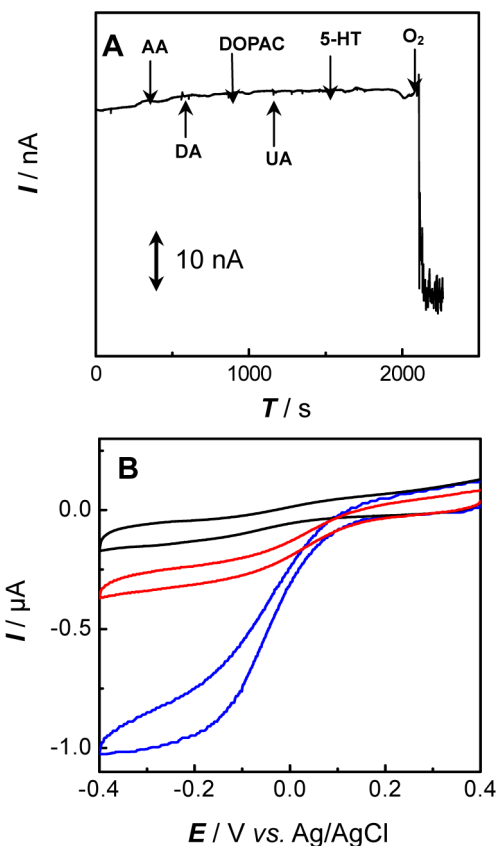**Figure 3.** Amperometric response for air-saturated $O_2$ recorded with the Pt/VACNT-CF (red curve) and Pt/CF microelectrodes (black curve) in aCSF. $I_0$ and $I$ were current values of starting and given time, respectively. The electrodes were polarized at $-0.5$ V vs Ag/AgCl (aCSF).

2D), which is ~0.35 V and ~0.05 V more positive than those on the VACNT-CF (Figure 2B) and Pt/CF (Figure 2C), respectively. The more positive onset potential obtained at Pt/VACNT-CF suggests the VACNT-CF support plays an important role in enhancing the catalytic activity toward $O_2$ reduction.[13a−d] In addition, we found that the steady-state reduction current at the Pt/VACNT-CF (0.37 $\mu$A) is almost twice of that at the VACNT-CF (0.16 $\mu$A). These results demonstrate that a four-electron $O_2$ reduction process occurs at the platinized VACNT-CF electrode prepared in this study. The Pt/VACNT-CF electrode with the demonstrated good electrocatalytic properties would offer an opportunity for in vivo $O_2$ detection (vide infra).

The efficient $O_2$ reduction at the VACNT-CF electrodes was attributed to the high porosity and electronic properties of CNTs, facilitating the diffusion of the reactant and its interaction with the electrode surface.[13,16] As reported previously,[11] deposition of nanostructured Pt onto CNTs could also enhance the electrocatalytic stability of Pt for $O_2$ reduction reaction by minimizing the substrate-detachment and agglomeration of Pt, which often occur with conventional carbon black or carbon fiber supports. Indeed, the Pt/VACNT-CF microelectrode used in this study exhibits a good stability with only a 3.0% decrease in the current after continuously measuring air-saturated $O_2$ in aCSF at $-0.5$ V for more than 1 h (Figure 3, red curve). This value compares very favorably with that of the Pt/CF microelectrode (i.e., 24%, Figure 3, black curve). These results provide validation for the use of the Pt/VACNT-CFs for in vivo electrochemical monitoring of $O_2$, as will be described below.

**Selectivity, Linearity, and Reproducibility.** The excellent electrochemical properties of the Pt/VACNT-CF microelectrode make it possible for selective detection of $O_2$ even with other electroactive species coexisting in the cerebral systems. As shown in Figure 4A, compared to 30 $\mu$M $O_2$, the presence of other electrochemically active species at their physiological levels in the cerebral system, including 400 $\mu$M





**Figure 4.** (A) Typical amperometric response of 400 $\mu$M AA, 10 $\mu$M DA, 20 $\mu$M DOPAC, 50 $\mu$M UA, 10 $\mu$M 5-HT, and 30 $\mu$M $O_2$ at the Pt/VACNT-CF. The electrode was poised at $-0.5$ V vs Ag/AgCl (aCSF). (B) Typical CVs at the Pt/VACNT-CF microelectrode in aCSF (pH 7.4) saturated with $N_2$ (black curve), ambient air (red curve), or $O_2$ (blue curve). Scan rate = 50 mV s$^{-1}$.

AA, 10 $\mu$M DA, 20 $\mu$M DOPAC, 50 $\mu$M UA, and 10 $\mu$M 5-HT did not produce any observable current response when the electrode was poised at $-0.5$ V, suggesting that these species do not interfere with the monitoring of $O_2$. It is well-known that $H_2O_2$ can also be electrochemically reduced on a Pt surface at potentials similar to that for $O_2$ reduction.[13] However, the physiological level of $H_2O_2$ (1 $\mu$M) was much lower than that

of $O_2$ (30−80 $\mu$M); thus, the interference from $H_2O_2$ was also negligible.[12,15,18] These observations ascertain the high selectivity for $O_2$ measurements with the Pt/VACNT-CF microelectrode, and further ensure our newly developed analytical approach for selective measurements of $O_2$ in rat brain.

In addition to the high selectivity toward $O_2$, the Pt/VACNT-CF microelectrode also shows good linearity for the measurement of $O_2$. As could be seen in Figure 4B, the steady-state currents increased proportionally in three standard solutions ($N_2$-purged, air-saturated, and $O_2$-saturated aCSF) at the as-prepared Pt/VACNT-CF microelectrode with good linearity ($\gamma$ = 0.9951) and sensitivity (0.91 nA $\mu$M$^{-1}$) toward $O_2$. We have also investigated the reproducibility of the Pt/VACNT-CF microelectrodes by comparing the current responses to $O_2$ on different microelectrodes. We found that, for all of the Pt/VACNT-CF microelectrodes, a well-defined sigmoid-shaped voltammogram was obtained for the $O_2$ reduction with almost the same current response (data not shown), suggesting that the Pt/VACNT-CF microelectrodes could be easily and reproducibly fabricated by our method. The unique electrochemical property of the Pt/VACNT-CF microelectrodes, together with their demonstrated selectivity, stability, and linearity, makes the Pt/VACNTs-CF particularly attractive for in vivo monitoring of $O_2$.

**In Vivo Monitoring of Hippocampus $O_2$ during Various Physiological Process.** To reduce electrode fouling after electrode implantation into brain region, a thin Nafion film was coated on the surface of the Pt/VACNT-CF microelectrode. As demonstrated previously,[12,15a] the Nafion overcoating could significantly reduce the electrode fouling. We found that the response of $O_2$ reduction did not change obviously at the Pt/VACNT-CF after Nafion coating, which was probably due to the high diffusion coefficient of $O_2$ in this thin film.[10a,12] Thereafter, we investigated in vivo stability of the Nafion-coated Pt/VACNT-CF microelectrode in rat hippocampus. As shown in Figure 5, after a few minutes of
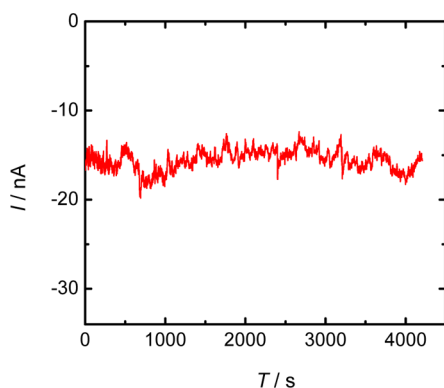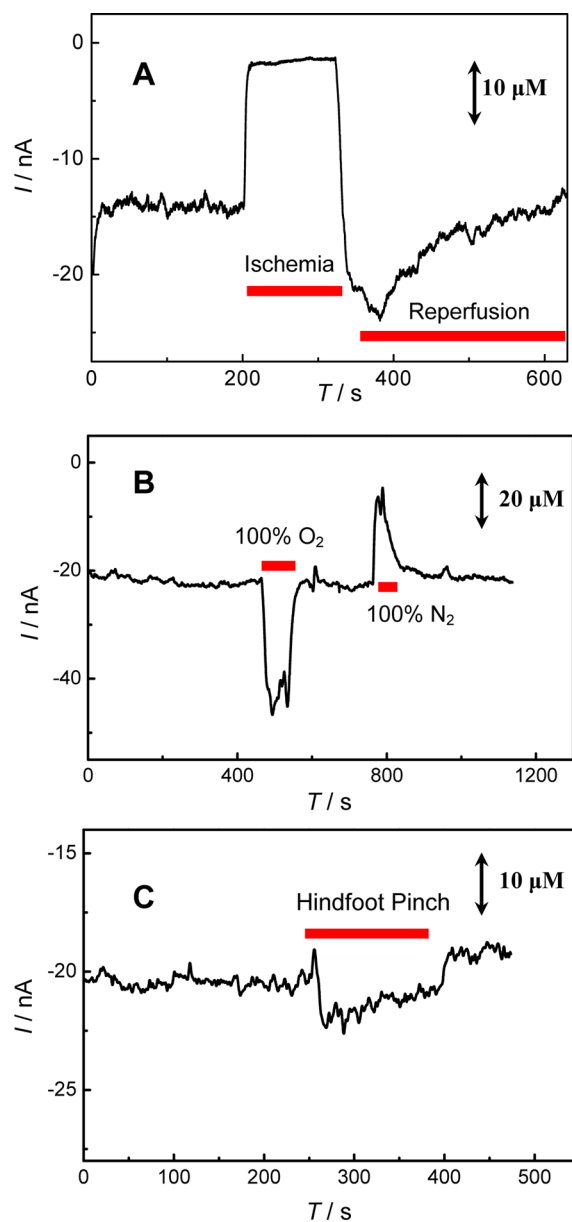
**Figure 5.** Amperometric response for $O_2$ recorded in vivo with the Nafion-coated Pt/VACNT-CF microelectrode implanted into hippocampus with working electrode polarized at −0.5 V vs Ag/AgCl (aCSF).

stabilization, the current response evidently did not change after 1 h of measurements. This value is comparable with those reported previously for other electrodes.[10a,15e] Moreover, the Nafion-coated Pt/VACNT-CF microelectrode shows good reproducibility for in vivo measurement of $O_2$; the relative standard deviation was calculated to be 5.7% ($n$ = 7). Besides,

the basal level of hippocampus $O_2$ of the anesthetized rats was estimated to be 30.8 ± 7.1 $\mu$M ($n$ = 3) for the Pt/VACNT-CF microelectrode, which was in good agreement with the reported values.[8a,b,15d,e]

Finally, the Pt/VACNT-CF microelectrode was used to investigate the $O_2$ activities in rat brain under ischemia, respiring $O_2$ or $N_2$, and hindfeet pinch conditions. Figure 6

**Figure 6.** Amperometric response for the hippocampus $O_2$ recorded in anesthetized rats during (A) global ischemia/reperfusion, (B) exposing to pure $O_2$ and $N_2$, and (C) hindfoot pinch, with the Nafion-coated Pt/VACNT-CF microelectrode as working electrode. The electrode was polarized at −0.5 V vs Ag/AgCl (aCSF).

shows typical dynamic current response recorded with the Nafion-coated Pt/VACNT-CF microelectrode at −0.50 V in the rat hippocampus during global ischemia/reperfusion (Figure 6A), respiring $O_2$ or $N_2$ (Figure 6B), and hindfeet pinch (Figure 6C). As seen in Figure 6A, when the animal was administrated with global ischemia by occluding the bilateral common carotid arteries, the $O_2$ level was quickly decreased by

93%. This low level was gradually restored to the basal level after reperfusion. More interestingly, at the very beginning of reperfusion, the $O_2$ level even transiently abounds to exceed pre-ischemia levels, indicating the existence of post-ischemic hyperperfusion that has been documented in animal stroke models or a low $O_2$ consumption at the early stage of reperfusion because of some cell are damaged.[19] The results obtained with our electrode were consistent with the previously reported ones using other methods.[8b,c,15e,20] On the other hand, we observed a very small current response after ischemia, suggesting that the level of $O_2$ is very low and that the $H_2O_2$ produced at the early stage of brain ischemia process does not greatly interfere with the $O_2$ measurement in this study.

As seen in Figure 6B, relative to the $O_2$ level obtained with the animal under normal conditions (i.e., spontaneously breathing air), breathing pure $O_2$ gas (i.e., hyperoxia) rapidly increased the $O_2$ level by ca. 91%, while breathing pure $N_2$ (i.e., hypoxia) decreased the $O_2$ level by ca. 68%. The current responses under both hyperoxia and hypoxia conditions returned to the basal level with spontaneous air breathing. These results demonstrated the fast response of our electrode toward $O_2$ reduction and the immediate transport of inhaled gases to the brain, which was consistent with those reported previously.[15d−f] Figure 6C illustrated the $O_2$ change a few seconds after the paper clip application to the hindfeet of the animal. We found that the $O_2$ level was increased slightly (ca. 7.8%), compared with the basal level. This subtle increase was due to the increase in the neutral activity and the regional cerebral blood flow caused by physiological stimuli.[15d−f] These results demonstrated that the Pt/VACNT-CF microelectrode could be used for effectively monitoring the change, even subtle change, in the $O_2$ level in the brain during various physiological processes and thus facilitate future studies on the neuro-chemical processes involved in various brain functions.

## CONCLUSION

We have successfully developed a new electrochemical method using the platinized vertically aligned carbon nanotube-sheathed carbon fiber (Pt/VACNT-CF) microelectrodes for in vivo measurements of $O_2$ following the global cerebral ischemia/reperfusion, respiring $O_2$ or $N_2$ and hindfoot pinch. The use of the VACNT-CF as the Pt support greatly improved electrocatalytic activity and stability toward the reduction of $O_2$, and minimized the person-to-person and electrode-to-electrode deviations intrinsically associated with manual mounting carbon nanotubes (CNTs) onto carbon fiber (CF). Compared with the existing microelectrodes for detecting brain $O_2$, such as Au, Pt, CF, and carbon paste microelectrodes, the Pt/VACNT-CF microelectrodes demonstrated here show a high catalytic efficiency toward four-electron reduction of $O_2$ without the formation of toxic $H_2O_2$. Both in vitro and in vivo experiments demonstrated that the Pt/VACNT-CF microelectrodes possess a high selectivity, good reproducibility, and stability useful for reliable measurements of $O_2$ in rat brain. This study offers a new analytical platform for in vivo $O_2$ measurements of great importance in understanding of various physiological and pathological processes associated with $O_2$.

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### Notes
The authors declare no competing financial interest.

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