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Carbon-based Electrochemical Sensors for Neurotransmitter Detection: A review

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Mini Review

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Abstract

Neurotransmitter release and uptake dynamics such as Dopamine (DA) in the nucleus accumbens (Nac) in the mesolimbic pathway, dorsal striatum in the nigrostriatal pathway and prefrontal cortex in the mesocortical pathway have important implications for neurological and psychiatric disorders. A complete understanding of these diseases requires real-time monitoring of dynamic changes in neurotransmitter (NT) levels. This mini-review highlights the recent progress in the development of carbon-based electrochemical sensors for NT detection.

Keywords: Carbon materials; Electrochemical sensors; Neurotransmitters

Introduction

The central nervous system (CNS) consists of large networks of neurons that communicate with each other both electrically and chemically for information processing, analysis, and generation of outputs to achieve complex brain functions and behaviors. Dysregulated level of neurotransmitters (NTs) in the brain is believed to be the main cause of many neurological disorders, such as Parkinson's and Alzheimer's diseases, schizophrenia, and epilepsy [1-3]. *In vivo* or *in vitro* monitoring of NT dynamics offers important physiological or pathological insights underlying these brain diseases.

Conventional chemical sensing approaches usually use capillary electrophoresis (CE), microdialysis, or liquid chromatography (LC) for separation and fractionation of NTs, and laser-induced fluorescence spectroscopy, immunoassay, or mass spectrometry for detection. These approaches, however, suffer from poor temporal resolution due to the lag time between sample collection/treatment and analysis [4,5]. Additionally, sample treatments in microdialysis are found to cause loss of precursors tyrosine and L-DOPA and reduce the availability of dopamine (DA) *in vivo* [6,7]. While molecular imaging techniques have also been widely used in NT detection, it depends strongly on estimation, such as bound ligand concentration, kinetic parameters, and prior knowledge of NT release [8]. Compared to the above methods, electrochemical sensing approaches are favorable because of their cost effectiveness, simple design, millisecond dynamic response, and high spatial resolution [9,10].

A variety of electrochemical sensors has been developed for qualitative and quantitative detection of neuroelectrical and neurochemical signals. Traditional electrochemical sensors include patch clamp based on micropipettes, carbon fibers affixed A variety of electrochemical sensors has been developed for qualitative and quantitative detection of neuroelectrical and neurochemical signals. Traditional electrochemical

sensors include patch clamp based on micropipettes, carbon fibers affixed to pulled glass pipettes, push-pull probes, and microdialysis probes [11-16]. These devices, however, are difficult to manufacture and scale for high-density sensing applications. Microelectrodes made of metals, such as Au and Pt, greatly improve the spatial resolution for NT sensing, but they suffer from instability of sensing, corrosion, astrogliosis and fibrotic encapsulation [17-19]. Emerging carbon materials, such as carbon nanotubes [20-23], nanofibers [24], and micro/nanocrystalline diamonds (MCD/NCD) [25-28], have attracted an increasing attention recently, as new electrochemical sensing materials. In this mini-review, we highlight the carbon-based electrochemical sensors for NT detection developed over the last decade.

Electrochemical Sensing Techniques

Electrochemical techniques most commonly used for detection of neurotransmitter can be classified into several major categories: constant-potential amperometry (DC amperometry), differential-pulse voltammetry (DPV), and fast-scan cyclic voltammetry (FSCV). The sensing mechanism relies on the oxidation or reduction of the target neurotransmitters on a solid electrode made of conductive materials, such as carbon-based materials. Electrochemical sensing setup typically uses a three-electrode configuration with a reference electrode, a counter electrode, and a working electrode to be the sensing electrode. Two-electrode confirmation has also been used, which consists of a working electrode (a sensor) and a reference electrode. The following sections introduce each method briefly. In DC amperometry, the electrode is biased at a constant potential that is sufficient to oxidize or reduce the neurotransmitter of interest. The current, related to the amount of analyte electrolyzed, is recorded as a function of time. With a sampling rate in the kHz range, DC amperometry can detect signals on time scales below milliseconds [29]. However, DC amperometry provides limited chemical information as many molecules may be electroactive at the selected potential. Therefore, calibration of the sensor in a known analyte is usually required prior to *in vivo* measurements.

Compared to amperometry, DPV is more sensitive and selective. It uses square potential pulses of constant height superimposed on the potential linear sweep or stair steps. The currents in response to various analyte concentrations can be measured at the beginning and the end of each potential pulse, and plotted against the potential of the linear ramp. Whereas DPV has a higher sensitivity, it suffers from limited temporal resolution due to the slow response the scanning.

As an alternative, FSCV permits high temporal resolution without compromising good selectivity. In FSCV, a rapidly cycled triangular waveform is applied to the sensing electrode at a fast scan rate, typically higher than 100 Vs^{-1} [30]. This will result in rapid oxidation and reduction of electroactive species at the electrode surface. The current at the sensing electrode is plotted versus the applied voltage to give the cyclic voltammetry race. Despite its high spatiotemporal resolution, FSCV has large background current that arises mainly from the charging the electrical double layer. Signal processing methods, such as Hilbert transform or principal-components analysis, is usually used to eliminate the capacitive interference. The interested reader is referred to Refs. [29,30] for more detailed description of the electrochemical methods.

Carbon-based Materials used in Electrochemical Sensing

Carbon Fiber

Most commercial carbon fibers (CFs) are produced either from polyacrylonitrile (PAN) or from mesophase pitch. The basic structural units of CFs are randomly-organized graphite sheets or ribbons [31]. CFs was first introduced in 1979 for measuring the oxidation of neurotransmitters and is still one of the most popular carbon materials for electrochemical sensing applications [32]. CFs is suitable for implantation because of their biologic compatibility and small sizes with a typical diameter of less than 10μ. CFs also has good electrochemical properties, which make them an ideal material for rapid measurements using fast electrochemical techniques, such as high-speed chronoamperometry, and fast-scan cyclic voltammetry. To date, CF microelectrodes (CFMEs) have been widely used for sensing electro active neurotransmitters, such as dopamine, norepinephrine, and serotonin [24,33-38]. Polymers, such as Nafion [35], and carbon nanomaterials, such carbon nanotubes (CNTs) [38] and graphene [39], are often used to modify CF surfaces in order to improve their sensitivity and selectivity. For detection of non-electroactive compounds, enzymes can be attached to CFs using chemical treatments. Other methods, such as flame etching, can also enhance the sensitivity of the CFMEs by creating nanometer-scale surface structures [40]. Recently, new techniques have been reported, combining electrochemistry with electrophysiology at a single

microelectrode to enable a better understanding of *in vivo* neurotransmitter concentrations and neural activity [41,42].

Glassy Carbon

Glassy carbon (or known as vitreous carbon) is a non-graphitizing, carbon which combines glassy and ceramic properties. Glassy carbon can be produced by the slow pyrolysis of certain polymers at temperatures in the range 900-1000°C [31]. Glassy carbon containing both sp^2 and sp^3 bonded atoms has good electrochemical properties and biocompatibility. The glassy carbon has been used as an electrode material for detection of various biological molecules and metal ions. Similar to CFMEs, glassy carbon electrodes (GCEs) require surface modification to obtain desired sensitivity and selectivity for use in neurotransmitter measurements. In particular, GCEs modified with multi-wall CNTs [43-45] or graphene [46] have been used for dopamine measurement in the presence of ascorbic acid and serotonin. Other chemicals have been reported for GCEs treatments, such as cetylpyridine bromide/chitosan composite [47], calyx [4] arene crown-4 ether [48], and poly (3,5-dihydroxy benzoic acid) [49]. For organic molecule measurement, strong bio-fouling onto the GCEs has been observed, which requires special cleaning or mechanical polishing processes.

Diamond

Compared to other carbon-based materials, polycrystalline diamond has unique sp^3-bonded carbon microstructure, resulting in desired properties for electrochemical sensing, including excellent sensitivity, biocompatibility, and resistance to bio-fouling and chemical corrosion [50-54]. Boron doped polycrystalline diamond (BDD), with a high fraction of diamond-like (sp^3) bonds, also has a wider aqueous potential window (~3-4.6V) versus common metallic materials (1.2V for Pt) [27,53]. It is chemically and mechanically robust with a low background current owing to its lower double-layer capacitance (~3.8-5μF/cm^2) [28,53]. BDD can be grown using microwave plasma enhanced chemical vapor deposition (CVD) techniques on substrates. The electrical conductivity of BDD can be tuned precisely by controlling boron doping level (up to 1021 atom/cm^{-3}) [55] to achieve conductivity suitable for electrochemical sensing and neural recording (>10S/cm). Conventional diamond-based electrochemical sensors are constructed by selective coating of conductive diamond on the tip of sharpened and insulated metal wires [56,57]. The grain size of diamond films can range from a microcrystalline diamond structure (MCD 1–3 μm grain size) all the way to an ultra-nanocrystalline diamond (UNCD 3–7 nm grain size) structure. Diamond-coated sensors have been utilized for in vitro and in vivo detection of dopamine concentration, with a fine sensitivity of down to 27 nM [26]. Recently, advanced microfabrication techniques have been applied to the manufacturing of microscale diamond electrode arrays on either hard substrates [58] or soft polymer substrates [59,60]. Diamond microelectrode arrays offer great advantages over microwires, including high spatial resolution, high density, and scalability.

Other Carbon-based Materials

Other emerging carbon materials, such as graphene, have been studied for electrochemical sensing of neurotransmitters. For example, a nitrogen-doped graphene fiber microelectrode was developed for dopamine detection with a sensing limit of 30 nM [61]. Pyrolytic graphite electrode (EPPGE) was reported for the simultaneous determination of dopamine, serotonin, and ascorbic acid with detection limits of 90 nM, 60 nM and 200 nM [62], respectively. Polymer-carbon composite materials are also explored by many groups for electrochemical sensing applications. Examples of such materials include polypyrrole/graphene oxide composite films [63], sol–gel derived carbon nanotubes ceramic composite electrodes [64], nanoparticles–Nafion–modified carbon paste electrode [65].

Conclusion and Future Directions

Carbon-based microelectrodes are ideal electrochemical sensors for neurotransmitter detection. The recent research focuses on carbon material synthesis and modification to achieve high sensitivity and selectivity of the sensors. Future research will continue to develop new techniques for modification of device structures and surface properties. Advanced understanding of surface chemistry, materials science, and polymer chemistry will facilitate these endeavors to further enhance the sensitivity and selectivity of the sensors, reduce biofouling, and expand the number of neurotransmitters and neuromodulators that can be detected. Development of highly miniaturized, multi-channel electrodes is also a possible area of research, which will enable simultaneous, high-resolution measurement of multiple neurotransmitters from large-scale neural networks. Other areas of research may include developing electronic interfaces for signal readout, packaging devices, and testing sensor reliability and stability to facilitate long-term, *in vivo* measurements

of neurotransmitters in freely, behaving animals. Detailed insights into carbon-based electrochemical sensors can be obtained from several comprehensive review articles [66-68].

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