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Fluorinated Nanocarbon Film Electrode Capable of Signal Amplification for Lipopolysaccharide Detection

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ABSTRACT

We describe a current amplification system that employs a fluorinated nanocarbon (F-nanocarbon) film electrode formed by unbalanced magnetron (UBM) sputtering with a short CF_4 plasma treatment. The F-nanocarbon film exhibited the typical electrochemical reaction of a ferrocene-based mediator while strongly suppressing the electrochemical oxidation of Fe^{2+} ions. This selectivity provided the current amplification of ferrocene mediators with Fe^{2+} ions solely by using the F-nanocarbon film electrode without interference from the direct oxidation current of Fe^{2+} ions. The current amplification system was used to realize an electrochemical biosensor with superior performance for detecting lipopolysaccharides. A detection limit of 2 ng mL^{-1} with good reproducibility (RSD of 4.2%) was achieved thanks to the very low noise made by possible by the ultraflat and hydrophobic surface.

1. Introduction

The electrochemical performance of carbon film electrodes can be widely controlled by controlling the surface termination/doping/modification of other atoms [1]. Various surface terminations on carbon-based electrodes have been reported including hydrogen [2,3], oxygen [4], nitrogen [5], fluorine [6–15] and metals [16,17] for modulating electron transfer rates, which are dependent on analytes. Fluorine is a fascinating atom because it has high hydrophobicity and the highest electro-negativity in the periodic table. Fluorination has been reported for various carbon electrodes including graphite, glassy carbon (GC), carbon nanotubes (CNT), carbon nanofiber, graphene and boron-doped diamond (BDD) [6–15]. Although these fluorinated carbon electrodes provide unique characteristics such as improved hydrophobicity and a different electron transfer rate from those of original carbon electrodes, the property of a fluorinated GC electrode is frequently lost because fluorine atoms are often removed from the GC electrode during electrochemical measurement [7,15]. Furthermore, the fluorinated GC electrode is unstable as regards high potential polarization or continuous measurement because its surface is easily oxidized [15,18]. In contrast, fluorinated BDD electrodes exhibit better long-term stability [12,14], suggesting that a fluorinated surface containing sp^3 carbon exhibits less oxidization and damage under anodic polarization than GC.

We previously reported electrochemically stable fluorinated nanocarbon (F-nanocarbon) film electrode formed by electron cyclotron resonance (ECR) sputtering with a short CF_4 plasma treatment [15]. The nanocarbon film electrode has a nanocrystalline sp^2 and sp^3 mixed-bond structure with an atomically flat surface. The fluorinated surface is easily prepared without losing the surface conductivity and surface flatness of the nanocarbon film electrode. The F-nanocarbon film electrode also exhibits high electrochemical selectivity for some species. For example, the F-nanocarbon film electrode suppresses the electrochemical oxidation of hydrophilic and inner-sphere species such as $Fe^{2+/3+}$ and $Fe(CN)_6^{3-/4-}$ [15], thanks to its hydrophobic surface. In contrast, the responses of hydrophilic and outer-sphere $Ru(NH_3)_6^{3+/2+}$ are almost unchanged. The F-nanocarbon film has a very stable surface compared with fluorinated GC in terms of continuous electrochemical measurements [15]. In fact, the slow electron transfer rates for $Fe^{2+/3+}$ and $Fe(CN)_6^{3-/4-}$ at the F-nanocarbon film electrode still remain after 20–50 potential cycles, whereas these slow electron transfer rates are easily recovered for fluorinated GC under the same conditions owing to the desorption of fluorine containing groups from the surface. We also employed the

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F-nanocarbon film electrode to selectively detect hydrophobic antioxidants in foods and drinks [19]. The F-nanocarbon film electrode exhibited fast electron transfer for hydrophobic α-tocopherol (vitamin E). In contrast, the electrochemical responses for hydrophilic antioxidants such as ascorbic acid (vitamin C) were effectively suppressed at the F-nanocarbon film electrode [19]. These properties allowed us to achieve selective and quantitative measurements of hydrophobic antioxidants while suppressing the responses of hydrophilic antioxidants in the analyte solution. We expect this selectivity to enable us to construct a current amplification system in combination with a relatively hydrophobic and outer-sphere ferrocene mediator and hydrophilic and inner-sphere $Fe^{2+/3+}$ as a reductant. Current amplification systems for electron transfer mediators have been widely studied by using an enzyme-modified electrode to improve the sensitivity and detection limit of various biomolecules [20,21]. If we achieve a current amplification system by redox cycling with an F-nanocarbon film electrode as shown Fig. 1(a), we can expect to use this system for bioelectroanalysis with a lower concentration and a high S/N ratio because the electrochemical inactivity of the F-nanocarbon film suppresses the direct oxidation of Fe^{2+} ions at a fluorinated surface.

Here we describe a current amplification system that uses an F-nanocarbon film electrode, which is unlike enzymatic amplification. We employed F-nanocarbon film to obtain the selective electrochemical reaction of ferrocene-based mediator against an Fe^{2+}/Fe^{3+} redox couple. Our aim is to apply this approach to an electrochemical biosensor for detecting lipopolysaccharides (LPS) thus achieving superior performance to that reported in our previous studies [22–24].

2. Experimental

2.1. Carbon film preparation and CF_4 plasma treatment

In this study, nanocarbon film electrodes were deposited with the unbalanced magnetron (UBM) sputtering method [24,25]. The film has relatively good electrochemical properties similar to those of our previously reported nanocarbon film formed using ECR sputtering [26–33]. Briefly, the nanocarbon films were deposited on highly doped silicon (100) substrates with UBM sputtering equipment (Universal Systems, Japan) at room temperature (without substrate heating). The DC voltage applied to the carbon target was 480 V. The argon gas pressure used for the sputtering was 6.0×10^{-1} Pa. During deposition, the irradiation ion current density was 3.0 μA cm^{-2} and the ion acceleration voltage was 100 V. The nanocarbon films were obtained in about 50 min and were typically 40 nm thick. The carbon electrodes were fluorinated by using reactive ion etching (RIE) equipment (Model RIE-200L, SAMCO, Inc., Japan) in accordance with previous reports [15,19]. The radio frequency power was 40 W and the CF_4 gas pressure and flow rate were 10 Pa and 10 sccm, respectively. The plasma treatment was performed for 30 s under the above conditions.

2.2. Film characterization

X-ray photoelectron spectroscopy (XPS) was conducted with a Shimadzu/Kratos model AXIS Ultra (Al Kα 1486.6 eV) spectrometer to determine the elemental composition of the film surface. The F/C and O/C ratios were calculated from the intensities of C1s, F1s and O1s (n = 2). The water contact angle was measured with a Drop Master DM 300 (Kyowa Interface Science Co., Ltd.). Milli-Q water droplets were used to characterize the surface hydrophobicity of the nanocarbon film electrode.

2.3. Chemicals

Poly-ε-lysine (ε-PL) (see Fig. 3(a)) was supplied by JNC Corporation (Japan). Ferrocene-attached polymyxin B (FcPMB) (Fig. 3(b)) was synthesized as previously reported [23]. The LPS used in this study was Japanese pharmacopoeia reference standard endotoxin purchased from the Pharmaceutical and Medical Device Regulatory Science Society of Japan. BS^3 (bis(sulfosuccinimidyl) suberate), a homobifunctionally water-soluble crosslinker for amines was purchased from Thermo Scientific Pierce (USA). All other chemicals were of analytical grade.

2.4. Electrochemical experiments

All the electrochemical experiments were performed using an ALS/CHI 760B electrochemical analyzer (CH Instruments, Inc. USA). A platinum wire and an Ag/AgCl (3 M NaCl) electrode were used as auxiliary and reference electrodes, respectively. A nanocarbon film was used as the working electrode. After depositing the nanocarbon film on the Si wafer, we cut the film into rectangles, and then fixed masking tape with a 2 mm diameter hole in it onto these rectangles to form disk electrodes. A 50 mM acetate buffer (pH 5.0) was used as the electrolyte solution for the electrochemical measurements. For electrochemical LPS measurement, we fabricated, as outlined below, an F-nanocarbon film electrode modified with ε-PL with a high affinity for LPS, which was similar to that described in our previous report [24]. A mixture solution (5 μl) containing 12.5 v/v% ε-PL, 4 w/w% bovine serum albumin (BSA) and 2 wt% BS^3 was placed on the electrodes, and then allowed to dry overnight. In this way, a crosslinked ε-PL membrane was formed on the surface of the F-nanocarbon film electrode. We also measured the LPS concentration by using a conventional LAL measurement system (Endosafe®-PTS™, Charles River Laboratories International (USA)).

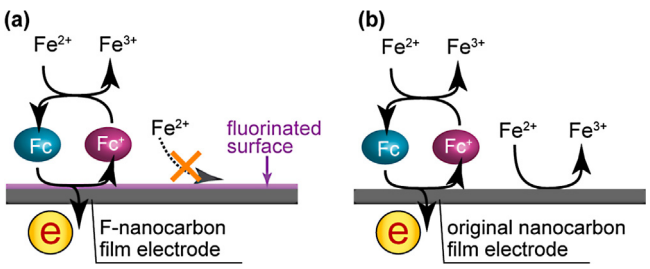

Fig. 1. Schematic illustration of a current amplification system using an F-nanocarbon film electrode (a) and the original nanocarbon film electrode as a comparison (b).

Table 1
Surface properties of the nanocarbon film before and after fluorination.

fluorination		nanocarbon	
		before	after
C 1s (%)[a]	sp^2 content	50.1	34.6
	sp^3 content	49.9	54.3
F/C[a]		–	0.15
O/C [a]		0.02	0.02
Contact angle/°[b]		75.6 ± 0.4	90.8 ± 0.9
C^0/μF cm^{-2}		9.31	2.63

[a] The chemical components of C, F and O were obtained and analyzed using XPS analysis.
[b] The contact angle was the average value (±S.D.) obtained from 10 measurements.

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