



Electronic hydroxyl radical microsensors based on the conductivity change of polyaniline



Jung-Ying Fang^a, Chia-Ho Chu^a, Indu Sarang^a, Kuan-Chung Fang^a, Chen-Pin Hsu^a, Yu-Fen Huang^b, Chia-Hsien Hsu^c, Chih-Chen Chen^a, Sheng-Shian Li^a, J. Andrew Yeh^a, Da-Jeng Yao^a, Yu-Lin Wang^{a,*}

^a Institute of Nanoengineering and Microsystems, National Tsing Hua University, Hsinchu 300, Taiwan, ROC

^b Department of Biomedical Engineering and Environmental Science, National Tsing Hua University, Hsinchu 300, Taiwan, ROC

^c Division of Medical Engineering, National Health Research Institutes, Miaoli, Taiwan, ROC

ARTICLE INFO

Article history:

Received 30 July 2014

Received in revised form 15 October 2014

Accepted 4 November 2014

Available online 13 November 2014

Keywords:

Polyaniline (PANI)

Hydroxyl radical

Disposable sensors

Fenton reaction

Reactive oxygen species (ROS)

ABSTRACT

In this study, conductive polyaniline (PANI) was coated on Si₃N₄/Si substrates and reacted with hydroxyl radicals created by mixing ferrous ions and hydrogen peroxide in a buffer solution, as known as the Fenton reaction. The hydroxyl radicals reacted with PANI, resulting in the decreased conductivity of PANI. The reduction in the conductivity of PANI was found to be proportional to the concentration of hydroxyl radicals in the buffer solution. The concentration of hydroxyl radicals was calibrated by comparing the fluorescence intensities of the Amplex ultrared molecules oxidized by radicals and hydrogen peroxide via the catalysis of horseradish peroxidase (HRP), respectively. The conductivity change of the PANI shows a linear relationship to the concentration of hydroxyl radicals, ranging from 0.2 μM to 0.8 μM. The conductivity of PANI does not respond to either ferrous ions or hydrogen peroxide alone. Only when both ferrous ions and hydrogen peroxide are present in the solution, the conductivity of PANI decreases, indicating that the conductivity change of PANI is attributed to the existence of hydroxyl radicals. The simple process for the sensor fabrication allows the sensor to be cost-effective and disposable. This electronic sensor can detect one of the most important reactive oxygen species (ROS), the hydroxyl radical, and therefore is promising for studying oxidative stress.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Oxidative stress is an important topic and is believed to be a contributor to tumors, cancers, Parkinson's disease, and aging [1]. Oxidative stress is induced by excessive reactive oxygen species (ROS), including hydrogen peroxide H₂O₂, hydroxyl radical OH[•], superoxide anion O₂^{•-}, and singlet oxygen ¹O₂. Hydroxyl radical was reported to be the most reactive free radical [2,3] and can be generated through the Fenton reaction or Haber–Weiss reaction in vivo [3,11]. Hydroxyl radical was found to be able to damage DNA [4], RNA [5], proteins [6] and lipids [7], leading to abnormal cell response and apoptosis [8] in both physiological and pathological environments [9,10]. However, detecting radicals, especially the hydroxyl radical, is challenging due to its very short lifetime (~μs) [1]. Several techniques are utilized to detect hydroxyl radicals. Electron spin resonance (ESR) spectroscopy is a typical technique

for detecting molecules with unpaired electrons by characterizing the electron paramagnetic spectrum. However, the lifetime of hydroxyl radicals is too short to be detected directly by ESR. Instead, a spin trap molecule, such as 5,5-dimethyl-1-pyrroline N-oxide (DMPO), is employed to bind with hydroxyl radicals to form a radical complex whose half-life time is long enough to make it detectable by ESR [1,12]. Chemiluminescence and fluorescence spectroscopy have also been frequently used to quantify hydroxyl radicals. The oxidation of chemiluminogenic or fluorescent probes by hydroxyl radicals may either enhance or quench their signals [2,12–15]. Hydroxyl radicals have also been measured by monitoring the ultraviolet–visible (UV–vis) absorption spectra of probe molecules, such as Br⁻ [16], crocin [17], ferrocyanide [18], and Rhodamine B [19]. High pressure liquid chromatography (HPLC) has also been employed for hydroxyl radical detection [20]. The above mentioned techniques are currently the most commonly used methods for detecting hydroxyl radicals. However, these techniques all require extensive instruments, and face a problem that is the degradation of probe molecules during the measurement. Electronic miniaturized microsensors might be good candidates

* Corresponding author. Tel.: +886 3 5162405.

E-mail address: yliwang@mx.nthu.edu.tw (Y.-L. Wang).

to effectively reduce the high cost of hydroxyl radical detection. Electrochemical sensors [21] and quartz crystal microbalance (QCM) sensor [22] were reported to detect hydroxyl radicals. Compared to the typical techniques, including ESR, fluorescence, chemiluminescence, UV–vis, and HPLC, electronic microsensors were seldom reported for free radical detection. However, due to the great advance in microfabrication techniques, electronic microsensors can be very cost-effective, and in the meantime, with comparable sensitivity and limit of detection (LOD). Thus, there is a great interest and demand to develop simple, cost-effective, and highly sensitive electronic microsensors for hydroxyl radical detection.

Previously we have reported an ultra-sensitive hydrogen peroxide microsensor using horseradish peroxidase (HRP)-immobilized polyaniline (PANI) [23]. Hydrogen peroxide oxidizes PANI via the catalytic effect of HRP. The oxidized PANI shows decreased conductivity compared to the reduced PANI. The limit of detection of the hydrogen peroxide sensor is as low as 0.7 nM, which is suitable for trace ROS detection. The PANI film shows no response to hydrogen peroxide if the HRP is not present, indicating that hydrogen peroxide does not interact with PANI and therefore does not change the conductivity of the PANI thin film without the HRP enzyme. On the other hand, PANI was reported as a good radical scavenger [24]. Hydrogen proton-doped PANI was proved to be able to prevent red blood cells from the attack by hydroxyl radicals, which was generated through the Fenton reaction in the presence of hydrogen peroxide [25]. Accordingly, it is possible to fabricate electronic hydroxyl radical microsensors by utilizing highly doped PANI and measuring the conductivity change of the doped-PANI after it has reacted with hydroxyl radicals. It is believed that the doped-PANI can be oxidized by hydroxyl radicals and the oxidation of doped-PANI will result in the decreased conductivity, which can be related to the concentration of hydroxyl radicals in solutions.

In this study, we investigate the conductivity decrease of doped-PANI due to the oxidation of doped-PANI by hydroxyl radicals. The doped-PANI was spin-coated and baked on a Si₃N₄/Si substrate. The hydroxyl radicals were generated through the Fenton reaction in which ferrous ion is oxidized by hydrogen peroxide. The decreased conductivity of the doped-PANI was found to be linearly dependent on the concentration of hydroxyl radicals. The concentration of the hydroxyl radicals was estimated and calibrated by comparing the fluorescence intensity of a dye, Amplex ultrared, oxidized by hydroxyl radicals with that of the dye oxidized by hydrogen peroxide via the catalytic effect of HRP. The limit of detection (LOD) of this hydroxyl radical sensor is around 0.2 μM, which is comparable to those of typical techniques such as ESR or fluorescence measurement, which LOD ranges typically around μM. In addition to the good LOD, the sensor only requires a small sample volume. The developed hydroxyl radical microsensor has advantages including low cost, small size, and ease of operation, which make it an ideal candidate for detecting hydroxyl radicals for oxidative stress studies.

2. Materials and methods

2.1. Preparation of PANI thin film on substrate

The microchip consists of two metal electrodes made of 200 Å Ti and 1000 Å Au layers deposited with an e-beam evaporator on a Si₃N₄/Si substrate. The length and the width of the Au electrodes are 500 μm and 100 μm, respectively. The gap between the two metal electrodes is 10 μm. Polyaniline emeraldine base was purchased from Headway Advanced Materials Inc. (cat. # ICP-723). Polyaniline was centrifuged until a clear aqueous layer was observed.

The aqueous layer was carefully removed. 2 mg of the remaining polyaniline was then mixed with 250 μl of de-ionized water thoroughly. The prepared polyaniline solution was coated onto the chip and covered the gap between electrodes. The PANI-coated chip was baked on a hotplate at 100 °C for 20 min. The thickness of the PANI thin film is 1.5 μm, obtained with a surface profiler. The PANI/Au interface was confirmed to be an ohmic contact by measuring the current–voltage characteristics of the device. Fig. 1(a) shows that the process steps for the reaction between PANI and hydroxyl radicals. Fig. 1(b) shows the top-view photograph of the PANI-coated microchip.

2.2. Fenton reaction and hydroxyl radicals

The hydroxyl radical was generated through the Fenton reaction as shown below in reaction (1):



Both iron (II) sulfate heptahydrate (cat. # 7782-63-0) and hydrogen peroxide (cat. #7722-84-1) were purchased from Sigma. The iron (II) sulfate heptahydrate was dissolved in 10 mM citric acid/20 mM disodium phosphate buffer solution (pH = 7.4) to generate ferrous ion (Fe²⁺). The hydrogen peroxide was also prepared in the same buffer solution.

2.3. Microsensor measurement

The current of the sensor was measured at a dc bias of 0.1 V at room temperature using an Agilent B1500 parameter analyzer. First, 10 mM citric acid/20 mM disodium phosphate buffer solution was applied onto the pristine sensor three times until a stable baseline current was reached. Hydrogen peroxide solutions prepared in the 10 mM citric acid/20 mM disodium phosphate buffer solution were directly dispensed on the surface of PANI layer, followed by adding ferrous ion solutions. Different target concentrations of hydrogen peroxide, including 1.56 mM, 3.13 mM, 4.69 mM, 6.25 mM, 7.81 mM, and 9.38 mM at a fixed ferrous ion concentration (Fe²⁺ = 1.25 mM) were investigated. The significant current changes of the sensors were observed when the ferrous ion solution was added, indicating that the signal resulted from the appearance of hydroxyl radicals generated through the Fenton reaction.

2.4. Fluorescence measurement of Amplex ultrared oxidized by hydrogen peroxide via HRP

The Amplex ultrared can react with hydrogen peroxide in the presence of HRP to form highly fluorescent resorufin (3H-phenoxazin-3-one, 7-hydroxy) [12]. Both Amplex ultrared and HRP were prepared in the 10 mM citric acid/20 mM disodium phosphate buffer solution. 50 μM Amplex ultrared was mixed with different concentrations of hydrogen peroxide in the presence of 2.95 units/mL HRP. The concentration of hydrogen peroxide ranged from 0.1 to 1 μM. The excitation and emission wavelength of Amplex ultrared are 540 nm and 590 nm, respectively. The fluorescence intensities of the oxidized Amplex ultrared were measured with plate reader after 25 minute reaction. Because the amount of the dye was excess compared to that of hydrogen peroxide, hydrogen peroxide was fully consumed, thereby generating an equal number of oxidized dye molecules. The fluorescence intensity versus the concentration of hydrogen peroxide is shown and the calibration curve is used to quantify the concentration of the oxidized dye.

Download English Version:

<https://daneshyari.com/en/article/750871>

Download Persian Version:

<https://daneshyari.com/article/750871>

[Daneshyari.com](https://daneshyari.com)