Sensors and Actuators B 222 (2016) 425–432

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Enzymeless glucose sensor integrated with chronically implantable nerve cuff electrode for *in-situ* inflammation monitoring

Yi Jae Lee [a], Sung Jin Park [a,b], Kwang-Seok Yun [b], Ji Yoon Kang [a], Soo Hyun Lee [a,*]

[a] Center for BioMicrosystems, Korea Institute of Science and Technology, Seoul 136-791, Republic of Korea
[b] Department of Electronic Engineering, Sogang University, 1 Shinsoo-dong, Mapo-gu, Seoul 121-742, Republic of Korea

ARTICLE INFO

Article history:
Received 18 May 2015
Received in revised form 29 July 2015
Accepted 20 August 2015
Available online 21 August 2015

Keywords:
Enzymeless
Glucose sensor
Nerve cuff electrode
Black Pt
Nafion
Inflammation
Chronic implantation

ABSTRACT

Using glucose concentration as an inflammation responsive element, we newly established an enzymeless glucose sensor integrated with a chronically implantable peripheral nerve cuff electrode for continuous and *in-situ* monitoring of local inflammation. The glucose sensor integrated with a nerve cuff electrode was fabricated on a polyimide substrate side-by-side, then the glucose sensor and nerve cuff electrode were reversely folded, and were located inside and outside, respectively. The experimental results reveal that the electroplated black Pt working electrode of the glucose sensor shows an enhancive surface roughness factor of 16.41 and had a good distribution on the flexible polyimide surface, which exhibits distinctly enhanced electro-catalytic activity compared to that obtained with plain Pt. Amperometry and electrochemical impedance spectroscopy indicated that the fabricated sensor had a sensitivity of 7.17 μA/mM cm^2, an outstanding detection limit of 10 μM, significant selectivity, and excellent recovery performance for enzymeless glucose detection. In order to evaluate the feasibility for inflammation monitoring in the immediate vicinity of the implantable peripheral nerve cuff electrode, the association of an evoked nerve signal recording and glucose concentration was investigated through *ex-vivo* test using the sciatic nerve of a SD rat.

1. Introduction

Nerve cuff electrodes have been widely used for chronic nerve signal recording and stimulation [1–3]. However, much of the implanted device itself tends to induce a foreign body reaction and morphological changes such as the growth of surrounding connective tissue, nerve reshaping, epineurial fibrosis, and degeneration/regeneration of myelinated fibers [4]. In particular, fibrotic reaction is preceded by an important epineurial inflammation, which can alter the interface between the nerve and cuff electrode. Therefore, the development of inflammation suppression and ability to monitor in a local area is vital for chronically implanted devices.

Generally, an inflammatory reaction is the body's attempt to protect, maintain and try to remove something harmful or that is irritating in the body, specifically, acute inflammation reflects that the body's attempt to heal itself. Sometimes, long-term inflammation (chronic inflammation) can be a contributing factor to many serious and chronic diseases such as cancer, heart disease, diabetes, autoimmune diseases, and Alzheimer's disease [5]. Generally, when assessing the extent or activity of inflammation, serum C-reactive protein (CRP) level provides a rough guide to the amount of tissue involved in inflammation and to the integrity of the inflammatory response. The CRP method used in the laboratory is considered a more direct measure of the inflammatory process, while erythrocyte sedimentation rate (ESR) is a more indirect measure. ESR reflects the concentration of several plasma proteins including fibrinogen, α-globulins, β-globulins, immunoglobulins and albumin. Therefore, any condition (pathological or non-pathological) that affects any of the contributing proteins can alter the ESR.

Recently, several studies regarding the association of inflammation and glucose disorders have been reported [6,7]. The possibility that the inflammation affects the concentration of glucose in the immediate vicinity of a glucose sensor has also been suggested [8]. Specifically, a previous study reported increased glucose consumption that was double in wounded rat tissue compared to that of normal tissue [9]. Additionally, the degradation in the sensitivity of implanted devices may be caused by changes in the surrounding tissue due to an inflammatory response or from the formation of a fibrotic capsule that surrounds the implanted device.

Therefore, monitoring inflammation in the immediate vicinity of the implanted device is essential for the stable operation of

* Corresponding author. Tel.: +82 2 958 6755; fax: +82 2 958 6910.
E-mail addresses: shleekist@kist.re.kr, dream.ideal@gmail.com (S.H. Lee).

http://dx.doi.org/10.1016/j.snb.2015.08.091
0925-4005/

chronically implanted devices, *e.g.*, a nerve cuff electrode. Although there are many types of glucose sensors, such as a micro-needle, planar, and patch [10,11], most have only focused on monitoring body status through the glucose level in blood or interstitial fluid. The enzymeless glucose sensors are largely classified as potentiometric sensors, *e.g.*, polymer membrane including boronic acid derivatives coated sensor, voltammetric sensors, *e.g.*, sensor using ferrocene unit for voltammetric read-out-unit, and amperometric sensors using applying programmed potential pulsed or to monitor the current at a fixed potential [12]. More specifically in amperometric methods, enzymeless glucose sensors have been classified that use fixed potential amperometric analysis [13], medium fixed potential amperometry coupled with of flow injection analysis [14] and liquid chromatography, and variable potential amperometric methods such as potential sweep and pulsed voltammetry [15]. Amperometric analysis based on high catalytic nano-materials is one of the most widely used methods due to enhanced amperometric detection avoiding electroactive interference [16]. To our knowledge, this is the first study that directly applies a robust glucose sensor using black Pt integrated with an implantable nerve cuff electrode for inflammation monitoring of a peripheral nerve. The enzymeless glucose sensor with black Pt is operated by direct electro-oxidation of glucose *via* fixed potential amperometric analysis, which is considerably depending on the electrode material used.

Pt is one of the most studied noble metals in the field of sensors and catalysts. However, conventional Pt has drawbacks of low sensitivity, selectivity, often suffer from losing activity due to easily poisoned by adsorbed intermediates. While noble metal nanoparticles are the most widely used nanomaterials in electrochemical sensor configuration due to their enhanced electro-catalytic properties. Particularly, electroplated black Pt is an enhancive material for sensor sensitivity due to its improved oxidation rate by enlarged real surface area and has excellent biocompatibility [17,18]. The electro-catalytic oxidation of glucose on Pt nanoparticles is a kinetic controlled reaction, whereas the responses from the common interfering species such as ascorbic acid and uric acid will not be changed significantly since their oxidation are diffusion controlled [19].

In this study, an enzymeless glucose sensor integrated with a chronically implantable peripheral nerve cuff electrode was designed, fabricated and characterized for *in-situ* inflammation monitoring. For sensitive and selective detection of glucose that was stable and unaffected by the denaturing of an immobilized enzyme, black Pt was selectively electroplated onto a plain Pt working electrode of an enzymeless glucose sensor. Subsequently, it was deep-coated with Nafion to avoid anionic interfering species. Glucose sensitivity was compared with and without a Nafion coating. The electrochemical properties of a fabricated enzymeless glucose sensor integrated with a nerve cuff electrode were investigated by cyclic voltammetry, electrochemical impedance spectroscopy, and chronoamperometry. In addition, the fabricated nerve cuff electrode integrated with a glucose sensor was tested to confirm whether the nerve cuff electrode could receive a stable nerve signal transfer at various glucose concentrations using a rat sciatic nerve through *ex-vivo* test.

2. Experimental

2.1. Reagents and apparatus

HCPA (hexachloroplatinic acid hydrate, 99.9% purity, Aldrich), hydrochloric acid, and lead acetate (reagent grade, 95%) were prepared to make the Pt black electrode. The fabricated electrode was measured to check its surface roughness factor (RF) in a 1 M sulfuric acid (H_2SO_4, 95–98%, ACS, Sigma-Aldrich) solution using cyclic voltammetry. The 1 M H_2SO_4 was prepared by dilution in deionized water. The β-D(+) glucose (99.5%, Sigma) stock solution was prepared by diluting it in a 0.1 M PBS solution and allowing it to stand for 24 h before use, in order to create equilibration. The 0.1 M urea (98%, Sigma), uric acid (UA, 99%, Sigma), sucrose (99.5%, Sigma), and ascorbic acid (AA, 98%, Sigma) solution were prepared by diluting them in a 0.1 M phosphate buffered saline (PBS, pH 7.0) solution. Nafion (5 wt.% solution) as a coating material for selectivity was obtained from Sigma Aldrich, Korea.

The electrochemical experiments on the fabricated electrodes were performed by using Autolab (PGSTAT 302N, NOVA software, Ecochemie, Utrecht, The Netherlands) employing a three-electrode configuration with a fabricated black Pt for the working electrode (WE), a sputtered Pt as a counter electrode (CE), and an Ag/AgCl reference electrode (RE) at room temperature. The cyclic voltammograms of the fabricated sensor was recorded from −0.5 to +1.0 V in a range with a 50 mV/s scan rate. Chronoamperometry was performed in a 0.1 M PBS (10 mL volume) solution under continuous stirring to dilute the glucose with a constant concentration (0.5 M). The response current was recorded after stabilizing the background current at a nearly constant value and the prepared glucose were consecutively injected at a regular interval of 100 s. Surface morphology was characterized using a Hitachi S-2300 scanning electron microscope (SEM).

2.2. Fabrication of an enzymeless glucose sensor integrated with a nerve cuff electrode

Fig. 1 provides conceptual drawings of an enzymeless glucose sensor integrated with a nerve cuff electrode for inflammation monitoring in a peripheral nerve. As shown in Fig. 1(a), the nerve cuff electrode consisted of four Pt line electrodes (0.25 mm × 1 mm) and the glucose sensor consisted of black Pt as a WE (geometry area: 4 mm^2), sputtered Pt as a CE, and Ag/AgCl as a RE. The fabricated glucose sensor integrated with a nerve cuff electrode was reversely folded to the nerve cuff electrode (Fig. 1b). Fig. 1(c and d) shows a wrapped glucose sensor (outside) integrated with a nerve cuff electrode (inside) on a peripheral nerve and local inflammation monitoring of glucose sensor *via* fluctuation of glucose concentration by the inflammatory activation.

The fabrication process for the enzymeless glucose sensor integrated with a nerve cuff electrode is described in Supplementary data Fig. S1 and in our previous research [20].

The first polyimide (VTEC 1388, Richard Blaine International, Inc., Philadelphia, PA, USA) layer of a 20 μm thickness was formed on a 4-in. silicon wafer by spin-coating (Supplementary data Fig. S1a). After curing the wafer at 90 °C for 10 min, 110 °C for 10 min, and 220 °C for 60 min in a convection oven, sputtered Ti/Pt (50/300 nm) layers on top of the 1st polyimide layer were formed by using a lift-off process (Supplementary data Fig. S1b). Next, the 2nd polyimide (5 μm thickness) layer for insulation was formed by spin-coating. In order to selectively open the electrode site and the connector pads, a positive photoresist (AZ 9260, AZ Electronic Materials, NJ, USA) was patterned on the 2nd polyimide layer and the exposed polyimide patterns were etched by reactive ion etching (RIE) (Plasma Therm, St. Petersburg, FL, USA) (Supplementary data Fig. S1c). A laser dicing machine (M-2000, Exitech, Oxford, UK) was used to cut the perimeter of the electrode substrate (Supplementary data Fig. S1d). To achieve a cuff shape with a 1 mm diameter capable of wrapping around the sciatic nerve of a rat, the flat nerve electrode and glucose sensor electrode were reversely folded and reformed using a metal rod, and then cured in a convection oven at 220 °C for 2 h to permanently fix the shape of the cuff (Supplementary data Fig. S1e). Then the black Pt was selectively electroplated on the sputtered Pt WE using a potentiostatic mode (−200 mV, 100 s).