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A Conducting Polymer Nanojunction Sensor for Glucose Detection

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ABSTRACT

This paper presents a glucose sensor using conducting polymer/enzyme nanojunctions and demonstrates that unique features can arise when shrinking a sensor to the nanometer scale. Each nanojunction is formed by bridging a pair of nanoelectrodes separated with a small gap (20–60 nm) with polyaniline/glucose oxidase. The signal transduction mechanism of the sensor is based on the change in the nanojunction conductance as a result of glucose oxidation induced change in the polymer redox state. Due to the small size of the nanojunction sensor, the enzyme is regenerated naturally without the need of redox mediators, which consumes minimal amount of oxygen and at the same time gives very fast response (<200 ms). These features make the nanojunction sensor potentially useful for in vivo detection of glucose.

The ability to detect, quickly and reliably, the presence or absence of specific chemicals can be a matter of life or death. Leaks of toxic gases, monitoring of glucose in the bloodstream, testing for harmful compounds in foods, and early alert of chemical and biological warfare agents all require reliable and sensitive sensing devices. While the demand for such devices is ever more urgent, the capability of many relevant enabling technologies to build these devices is also unprecedented. One example is the rapid development of nanofabrication capabilities which allows one to develop sensors based on nanostructured materials and devices.[1−7] This approach is attractive because materials at the nanometer scale often exhibit unique physical and chemical properties that can be utilized to improve sensor performance. Here we demonstrate a conducting polymer nanojunction-based glucose sensor that possesses important features for potential in vivo glucose detection.

Due to the importance of glucose detection in diabetics, a plethora of glucose sensors have been proposed and developed over the past 20 years. However, the development of a miniaturized device that can quickly and reliably monitor glucose in vivo, independent of oxygen blood concentration, still faces a number of challenges. The normal clinical range for glucose in blood is between 3.5 and 6.1 mM, and abnormal glucose levels can reach as high as 20 mM. This concentration range can be easily monitored using an electrochemical sensor based on the redox properties of glucose oxidase (GOx).[8,9] The enzymatic reaction of GOx−glucose is followed electrochemically by measuring the regeneration enzymatic rate carried out either by the natural regenerator, oxygen,[10−13] or by artificial redox mediator molecules.[12,14,15] Both are not desirable for in vivo detection of glucose.

In addition to detecting electrochemical current, other methods have been reported.[16−23] For example, Bartlett et al. have introduced a strategy to detect different analytes using electrochemical transistors (∼20 μm gap) made of modified conducting polymers which undergo large conductivity changes upon oxidation or reduction of the polymer induced by biochemical reactions.[8] Good analytical performance was found for "in batch" glucose detection, using immobilized GOx and soluble redox mediators under anaerobic and aerobic conditions.[18,19]

In the present work we demonstrate a conducting polymer nanojunction sensor for glucose potentially useful for in vivo detection, since very specific and fast responses toward glucose can be detected in aerobic media without the need of using redox mediator molecules and consuming limiting oxygen (Figure 1A). Our sensor consists of an array of polyaniline nanojunctions. Each nanojunction is formed by electropolymerization[24] of polyaniline in the presence of poly(acrylic acid) (PAA) to bridge two nanoelectrodes separated with a nm-scale gap (20 to 60 nm). Poly(acrylic acid) allows us to maintain significant polyaniline conductivity near neutral pH.[18,25,26] Glucose oxidase (GOx) is immobilized onto the PANI−PAA to provide specific detection of glucose. Upon exposure to glucose, the GOx catalyzes

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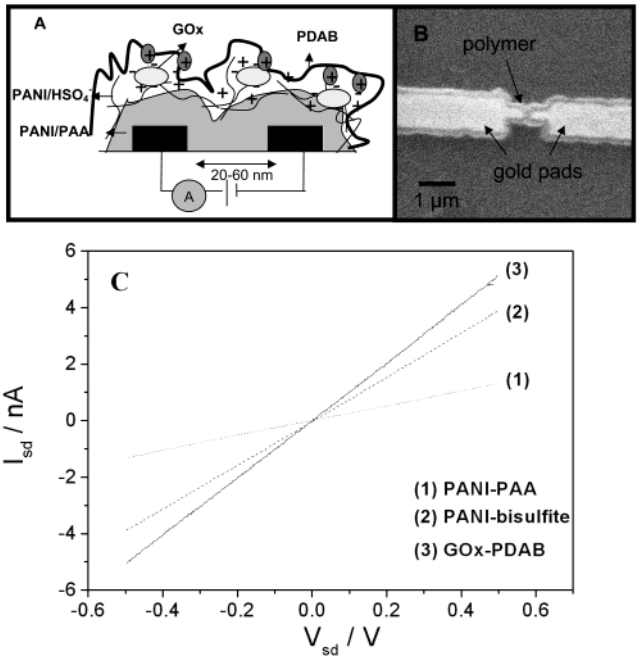

Figure 1. (A) The structure of the polymer nanojunction sensor. (B) SEM image of PANI−PAA/PANI−bisulfite/GOx−PDAB films deposited on gold pads with 20−60 nm gaps. (C) $I-V$ curves obtained in air after each nanogap modification step: (1) polymerization of PANI−PAA carried out in 0.4 M aniline + 150 mg mL^{-1} PAA (MW: 2000) solution with 0.5 M Na_2SO_4 and 0.5 M H_2SO_4 by potential sweep between −0.2 and 0.9 V vs SCE during the first cycle and between −0.2 and 0.78 V vs SCE during the following cycles at 0.05 V s^{-1}; (2) polymerization of PANI−bisulfite in a 0.4 M aniline + 0.5 M $NaHSO_4$ solution acidified to pH = 0 with H_2SO_4 by a single potential sweep from −0.2 to 0.9 V vs SCE; (3) immobilization of GOx−PDAB by exposing the polymer nanojunction to 0.5 M Na_2S0_4 + 25 mM 1,2-diaminobenzene + 167 μM glucose oxidase in a pH 5 citric acid /Na_2HPO_4 (McIlvaine) buffer solution for 15 min, and followed by electrodeposition of PDAB at +0.4 V vs SCE for 4 min.

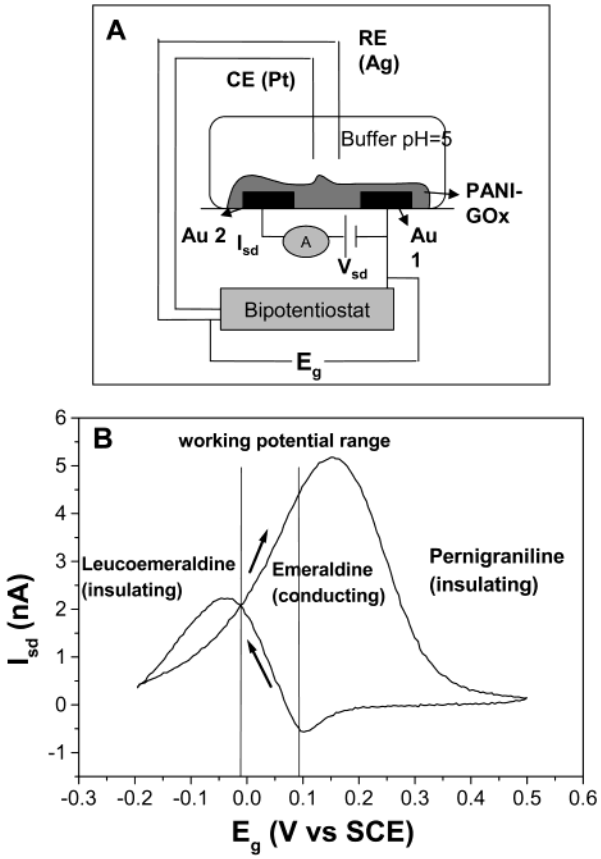

Figure 2. (A) Operation of the polymer nanojunction sensor, where Au 1 and Au 2 represent working electrodes 1 and 2 respectively, E_g is the gate potential referred to the reference electrode (RE), V_{sd} is the source-drain voltage biased between pads 1 and 2, CE is a Pt wire counter electrode. (B) Typical $I_{sd}-E_g$ curve of PANI−PAA/PANI−bisulfite/GOx−PDAB films deposited on gold pads with 20−60 nm gaps. Response obtained in aerated McIlvaine buffer (0.1 M citrate − 0.2 M phosphate), pH = 5 at 50 mV s^{-1} (V_{sd} = −20 mV). The PANI redox states are indicated as well as the typical working potential range used for hydrogen peroxide and/or glucose detection.

the oxidation of glucose and becomes reduced. The reduced form of GOx is regenerated via reoxidization by O_2 in the solution, which produces H_2O_2. H_2O_2 then oxidizes polyaniline and triggers an increase in the polyaniline conductivity because of the sensitive dependence of polyaniline conductivity on its redox state.[27] These reaction steps are summarized as

$$\text{glucose} + \text{GOx(FAD)} \rightarrow \text{gluconolactone} + \text{GOx(FADH}_2\text{)} \quad (1)$$

$$\text{GOx(FADH}_2\text{)} + \text{O}_2 \rightarrow \text{GOx(FAD)} + \text{H}_2\text{O}_2 \quad (2)$$

$$\text{H}_2\text{O}_2 + \text{PANI}_{\text{red}} \rightarrow \text{H}_2\text{O} + \text{PANI}_{\text{ox}}\ \text{(more conducting)} \quad (3)$$

where GOx(FAD) and GOx($FADH_2$) represent the oxidized and reduced forms of the enzyme. Because our nanojunction is extremely small, we have achieved fast glucose responses without consuming appreciable amounts of O_2 so that O_2 concentration does not limit the enzymatic reaction rate.

Our nanoelectrode arrays (Au) were fabricated on oxidized Si substrate using electron beam lithography (Figure 1B) (see ref 6 for details). The nanoelectrodes were coated with Si_3N_4 except for a small portion near the end of each nanoelectrode to minimize leakage current due to ionic conduction through electrolyte. We bridged the gap between each pair of nanoelectrodes with polyaniline/poly(acrylic acid) (PANI/PAA) by cycling the potential of the nanoelectrodes between −0.2 and 0.9 V vs SCE during the first cycle and between −0.2 and 0.78 V vs SCE during the following cycles at 0.05 V s^{-1} in 0.4 M aniline + 150 mg mL^{-1} PAA (MW: 2000) solution with 0.5 M Na_2SO_4 and 0.5 M H_2SO_4 (step 1). The potential cycling polymerized the monomers and deposited the polymer onto the electrodes. A large increase in the current was observed once PANI/PAA bridges the gap, which occurred typically after 10−24 potential cycles. After this initial step, the polymer was modified by electrodeposition of a thin layer of PANI doped with small anions (HSO_4^-) (step 2) followed by glucose oxidase adsorption and immobilization within poly(1,2-diaminobenzene) (PDAB) at pH 5 (step 3). The second step was carried out to induce electrostatic adsorption of GOx (isoelectric point = 4.2) on

PANI/HSO_4^-. PDAB was used in the last step to both immobilize glucose oxidase and to reject possible interference from ascorbate that typically exists in large quantity in physiological fluid.[28] The last step was performed according to the procedure previously described by Bartlett et al.[20] The current−voltage ($I-V$) curves recorded after each of the above three steps are shown in Figure 1C. After step 2, the current increases by a large amount, which is expected because more polyaniline is deposited into the gap during the step. A slight increase in the current is also observed after step 3, which is not yet understood. However, since this behavior was observed both in the presence and absence of GOx, it is likely due to a conformational change in the PANI upon PDAB deposition. The successive electropolymerization steps produce a homogeneous polymer deposit (Figure 1A) and result in a densely packed polymer bridge between the two nanoelectrodes (Figure 1B).

We have studied the charge transport properties of the modified polymer nanojunctions under mild pH conditions in a field effect transistor configuration, in which the two Au nanoelectrodes serve as source and drain electrodes, and a reference electrode (Ag wire)[29]together with a counter electrode (Pt wire) in the electrolyte provide gate control (Figure 2A). The gate potential (E_g) and the source-drain bias (V_{sd}) were controlled with a homemade bipotentiostat. The gate potentials are quoted in terms of more widely used SCE reference. Figure 2B shows a typical source-drain current (I_{sd}) vs E_g with $V_{sd} = -20$ mV in a McIlvaine buffer + 0.5 M Na_2SO_4 solution (pH 5). The curve is similar to what has reported for unmodified conductive PANI films.[24] A small negative current is observed when the gate potential is scanned backward, which is due to leakage current. The leakage current is much smaller than the current through the polymer nanojunction and decreases when the potential scan rate decreases. So leakage current is negligible when we fix the gate potential at a value ([−20;80] mV vs SCE) where the conductance of the polymer nanojunction is large.

Since one of the goals of this work is a sensor for potential in vivo monitoring of glucose, we must avoid the use of redox mediator molecules (which can be toxic) for GOx regeneration. This is achieved here by relying on the natural GOx regeneration by dissolved oxygen molecules and monitoring the regeneration product, hydrogen peroxide, which changes the conductance of the polymer nanojunction. So it is necessary to study the response of the polymer nanojunction (PANI−PAA/PANI-bisulfite/PDAB) conductance to hydrogen peroxide. Figure 3A shows the conduction current response of a polymer junction upon hydrogen peroxide at a constant gate potential of 20 mV vs SCE and bias potential of −10 mV in McIlvaine buffer + 0.5 M Na_2SO_4 (pH 5). I_{sd} increases with hydrogen peroxide concentration. This current increase is not due to electrochemical oxidation of hydrogen peroxide because the potential, 20 mV, is well below the oxidation potential of hydrogen peroxide. Instead, we believe that the current increase is due to a change in the redox state of the polyaniline by hydrogen peroxide, an oxidizing agent. Any change in conductivity due to local pH change can be ruled out because we used a high buffer capacity solution prepared from a mixture of 0.2 M Na_2PO_4 and 0.1 M citric acid solutions. I_{sd} change vs hydrogen peroxide concentration is plotted in Figure 3B, which can be used as a calibration curve. We note that the conductivities of polypyrrol films[30] and PANI-bisulfite films reported in the literature deteriorate in the presence of hydrogen peroxide.[20] In contrast, the conductivity of our polymer nanojunctions is enhanced by hydrogen peroxide, which allows us to directly detect the natural regeneration process of GOx.

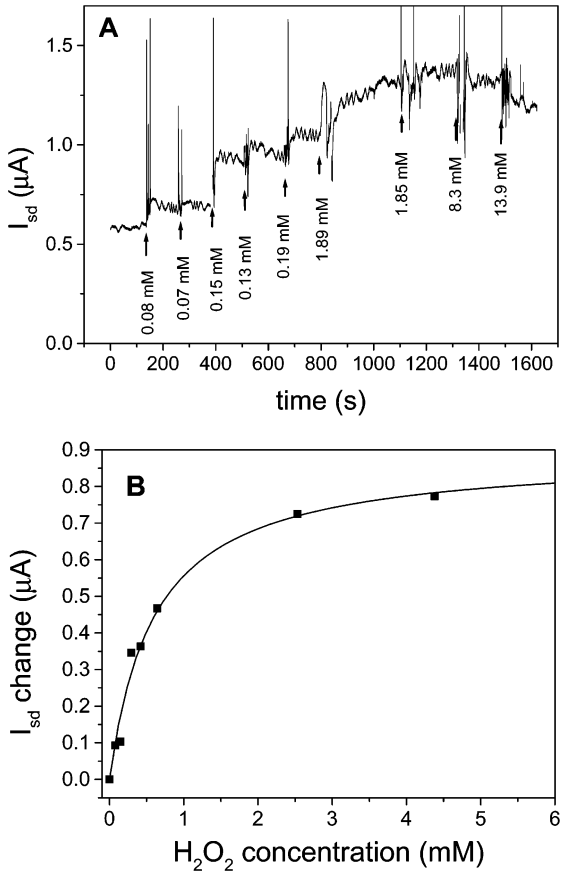

Figure 3. (A) Time course of drain current at $E_g = 20$ mV vs SCE ($V_{sd} = -10$ mV) recorded on a PANI−PAA/PANI−bisulfite/PDAB (without GOx) modified electrodes (3-μm gap) in 20 uL McIlvaine buffer, 0.5 M Na_2SO_4 pH 5 upon H_2O_2 successive additions. The added hydrogen peroxide concentrations are indicated. (B) Corresponding calibration plot of drain current change vs H_2O_2 concentration.

We have monitored the conductance changes of the polymer nanojunctions (coated with GOx) as various amounts of glucose are added into the buffer (Figure 4A). Upon each addition of glucose, the current (conductance) of the polymer nanojunction increases abruptly and then reaches a stable value. The response time is less than 1 s, which is a direct result of the small size of our polymer nanojunctions. The response time of electrochemical transistors with 10 μm gap used under similar electrochemical setup conditions is typically 10 min.[17] Fast response is an important feature for real-time in vivo monitoring of glucose. The sensitivity of

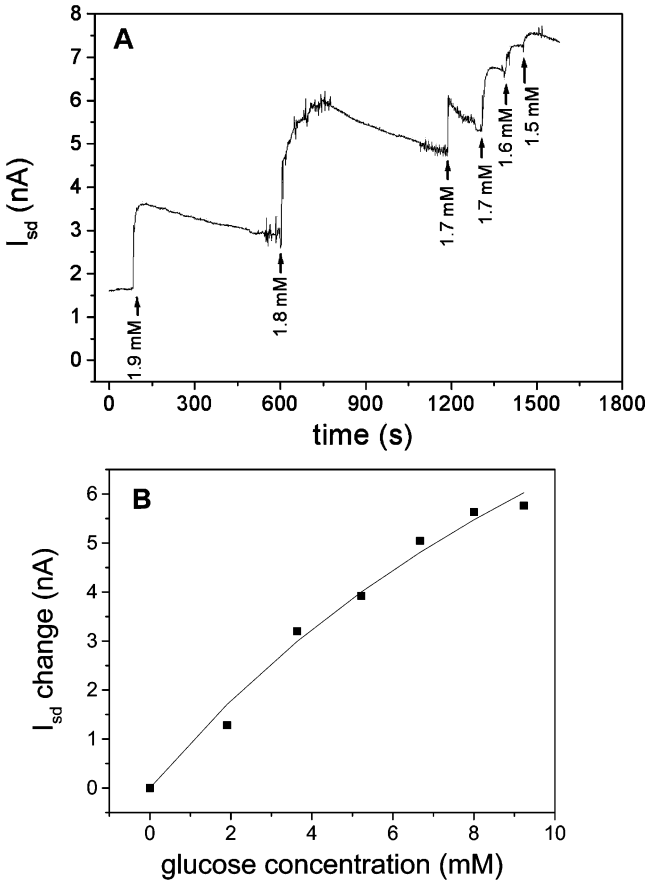

Figure 4. (A) Time course of drain current at E_g = 35 mV vs SCE (V_{sd} = −20 mV) for a PANI−PAA/PANI−bisulfite/GOx−PDAB nanojunction (20−60 nm) in 20 μL McIlvaine buffer, 0.5 M Na_2SO_4 pH 5 upon 1 μL successive additions of 40 mM glucose. (B) Corresponding calibration plot of drain current change vs glucose concentration.

our polymer nanojunction sensor is 1 nA/mM (Figure 4B). For a current noise level of a few pA of the current amplifier, the detection limit is μM-scale. The sensitivity is adequate since clinical glucose concentration is greater than 3.5 mM.

In summary, we have demonstrated a conducting polymer nanojunction sensor that can detect glucose without using redox mediator. The small junction size not only promises high degree of integration of the device but also results in fast response time and minimal consumption of oxygen needed to regenerate GOx. The features are essential for an in vivo device for real-time monitoring of glucose levels.

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