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Nanomaterial based self-referencing microbiosensors for cell and tissue physiology research

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ABSTRACT

Physiological studies require sensitive tools to directly quantify transport kinetics in the cell/tissue spatial domain under physiological conditions. Although biosensors are capable of measuring concentration, their applications in physiological studies are limited due to the relatively low sensitivity, excessive drift/noise, and inability to quantify analyte transport. Nanomaterials significantly improve the electrochemical transduction of microelectrodes, and make the construction of highly sensitive microbiosensors possible. Furthermore, a novel biosensor modality, self-referencing (SR), enables direct measurement of real-time flux and drift/noise subtraction. SR microbiosensors based on nanomaterials have been used to measure the real-time analyte transport in several cell/tissue studies coupled with various stimulators/inhibitors. These studies include: glucose uptake in pancreatic β cells, cancer cells, muscle tissues, intestinal tissues and *P. Aeruginosa* biofilms; glutamate flux near neuronal cells; and endogenous indole-3-acetic acid flux near the surface of *Zea mays* roots. Results from the SR studies provide important insights into cancer, diabetes, nutrition, neurophysiology, environmental and plant physiology studies under dynamic physiological conditions, demonstrating that the SR microbiosensors are an extremely valuable tool for physiology research.

1. Introduction

Electrochemical biosensors are transducers that convert biological information (such as analyte concentration) into electrical signals (such as current or voltage). Electrochemical biosensors are more efficient than conventional measurement techniques (including radioisotope tracing (Guillam et al., 2000; Hellman et al., 1974; Sweet et al., 1996; Zawalich and Matschinsky, 1977), NMR spectroscopy (Weiss et al., 1989), and microfluorometry assays (Moley et al., 1998; Passonneau and Lowry, 1993)) due to the high sensitivity, real-time monitoring capabilities and low cost, while the conventional techniques are complex, expensive and severely limited in terms of spatial and temporal resolution. Most electrochemical biosensors have two major components: the biorecognition element and the transduction element. Biorecognition elements include enzymes (for amperometric sensors (Gouveia-Caridade et al., 2008; Hrapovic et al., 2004; Kang et al., 2007; Salimi et al., 2004; Yao and Shiu, 2007; Zou et al., 2008)) and ionophores (for potentiometric sensors (McLamore and Porterfield, 2011; McLamore et al., 2009; Porterfield, 2007; Porterfield et al., 2009), and the transduction elements include the electrode and nanomaterials (Shi and Porterfield, 2011). Most biosensors function based on a two-step scheme: *biorecognition* and *transduction* (Shi and Porterfield, 2011). In *biorecognition*, the biorecognition element recognizes and binds to the target compound. The specificity associated with the binding ensures the selectivity of the biosensor. In *transduction*, a series of electrochemical reactions take place in the proximity of the transduction element(s), and sometimes the reactions are driven by an externally applied potential (working potential). The final outcome is an electrical signal (current or voltage) which is proportional to compound concentration.

2. Amperometric biosensor

Amperometric biosensors are used to measure the electroactive molecules such as H_2O_2 (Marc et al., 1997), NADH (Santos et al., 2006; Tsai et al., 2007) and indole-3-acetic acid (McLamore et al., 2010a). The target molecule is oxidized or reduced by the working potential, and a current proportional to concentration is generated. When coupled with enzymes (biorecognition element of amperometric sensor), amperometric biosensors can measure

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non-electroactive molecules such as glucose (Shi et al., 2011a), glutamate (McLamore et al., 2010b) and ethanol (Azevedo et al., 2005), because enzymes convert the target compound into an electro-active intermediate such as H_2O_2. Enzymes can be immobilized on the biosensor via a covalent linker such as glutaraldehyde (Shi et al., 2011b), or via adsorption by polymers (Shi et al., 2011b). Oxidase and dehydrogenase are the most commonly used enzymes. Biosensors based on oxidase rely on H_2O_2 as the electroactive intermediate, while those based on dehydrogenase rely on NADH as the intermediate. Take ethanol biosensors as an example. Alcohol dehydrogenase (ADH) (Santos et al., 2006; Tsai et al., 2007) and alcohol oxidase (AOx) (Gouveia-Caridade et al., 2008; Yildiz and Toppare, 2006) have been used for ethanol biosensing. ADH converts ethanol into acetaldehyde and NADH in the presence of NAD^+ as a cofactor. NADH is then oxidized:

$$\text{Ethanol} + NAD^+ \rightarrow CH_3CHO + NADH + H^+$$

$$NADH \rightarrow NAD^+ + H^+ + 2e^-$$

AOx converts ethanol into acetaldehyde and hydrogen peroxide. Hydrogen peroxide is then oxidized:

$$\text{Ethanol} + O_2 \rightarrow CH_3CHO + H_2O_2$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$$

The use of ADH or AOx has both advantages and disadvantages. Biosensors based on ADH require external NAD^+ for the production of NADH. The inefficient diffusion of NAD^+ towards the ADH immobilized on the biosensor complicates the measurement process and affects the biosensor sensitivity, detection limit and linear detection range (Azevedo et al., 2005). Biosensors based on AOx require O_2 to oxidize ethanol. When the ethanol concentration in the analyte is low, the O_2 from ambient air is adequate for the reactions to occur. However, when ethanol concentration increases, O_2 is gradually depleted and the oxidation of ethanol is constrained (although the oxidation of H_2O_2 also generates O_2). Therefore, the oxidation of ethanol becomes inadequate. This is reflected as a gradually attenuated current response when ethanol concentration increases. In other words, the current-concentration curve in the high concentration region is non-linear, where it is theoretically linear based on biosensing mechanism.

3. Nanomaterials and biosensing

In order to apply biosensors to physiological research, miniaturization of sensors is required for a high spatial resolution, because the sensors are often operated at cell or tissue level. Miniaturization increases the resistance of the sensor, which significantly decreases the maximum attainable sensitivity (Bard and Faulkner, 2000). The sensitivity issue affects not only the limit of detection, but also the capability of measuring very small changes in concentration over time (Shi et al., 2011a), while the small changes can be key to exploring important physiological phenomena, such as β cell glucose consumption during insulin secretion (Jung et al., 2000). One effective way to solve the low sensitivity problem is to enhance electrochemical transduction via incorporating nanomaterials. Carbon nanotube (CNT) (Claussen et al., 2011; McLamore et al., 2010a; McLamore et al., 2010b; McLamore et al., 2011; Shi et al., 2011a; Shi et al., 2011b; Shi et al., 2011c), graphene (Kang et al., 2009; Shao et al., 2010; Zhang et al., 2011), graphene oxide (Shi et al., in press) and metal nanomaterials (Claussen et al., 2011; McLamore et al., 2010a; McLamore et al., 2010b; McLamore et al., 2011; Shi et al., 2011a; Shi et al., 2011b; Shi et al., 2011c) are the most commonly used nanomaterials for biosensing enhancement. Carbon nanomaterials increase electrochemical transduction partially due to the unique structure and the different local density of states, which increase the electronic interaction width and decrease the activation energy for redox reactions (Nugent et al., 2001), and partially due to the defect sites which facilitate the chemisorption of molecules and lower the activation energy (Chakrapani et al., 2003). Metal nanomaterials possess electrocatalytic activities due to the multiple oxidation states, enabling reactants such as H_2O_2 to form intermediates at the surface, and lowering the activation energy of reactions such as H_2O_2 oxidation (Li et al., 2005).

The combination of metal and carbon nanomaterials for biosensor enhancement has proved to be feasible and more effective than using a single material (Hrapovic et al., 2004; Kang et al., 2007, 2008; McLamore et al., 2010a; McLamore et al., 2010b; McLamore et al., 2011; Shi et al., 2011a; Shi et al., 2011c; Shi and Porterfield, 2011; Zou et al., 2008). For example, CNT can act as the molecular template for Pt black electrodeposition, and the resultant hybrid nanocomposite is more effective in

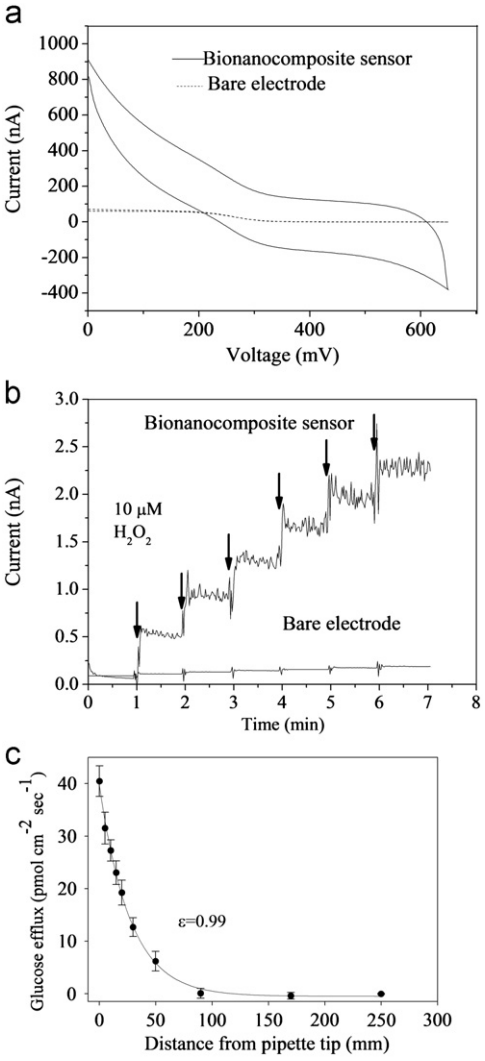

Fig. 1. (a) CV in 4 mM Fe(CN)63-/1 M KNO3 for a bare micro electrode and a bionanocomposite sensor at a scan rate 20 mV/s. (b) Representative current response to H_2O_2 for a bionanocomposite sensor and a bare electrode. (c) Abiotic step back experiment from pulled micropipette containing 3 mM glucose and 0.5% agar in PBS at 37 °C. Correlation coefficient (ε) between measured (●) and predicted flux (solid line) was 0.99. All error bars represent the standard error of the arithmetic mean. (Reprinted with permission from (Shi et al. 2011c)).