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High-Performance Conducting Polymer Nanofiber Biosensors for Detection of Biomolecules

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1. Introduction

Sensitive detection of the physiologically important chemicals involved in brain function has drawn much attention for the diagnosis and treatment of brain diseases and neurological disorders.^[1-3] In particular, monitoring changes in extracellular glucose concentration that is indicative of glucose metabolism in the brain may improve diagnosis of and therapy for brain tumors^[4-6] and assist in understanding physiological changes following traumatic brain injuries.^[7] In addition to the significance of glucose monitoring in brain disease and neurological disorders, it is the key analyte for medical diagnostics and management of diabetes, which affects nearly 26 million Americans.^[8]

To date, the most common glucose biosensors achieve specific recognition of glucose by immobilization of the enzyme glucose oxidase (GOx) on the surface of electrodes. Although a number of methods have been developed for immobilization of GOx^[9-11] and detection of glucose^[12-14], achieving high sensitivity and longevity in these biosensors has remained a challenge. In biosensors using an enzyme as the biorecognition element, sensitivity and longevity are functions of the physical design and of enzyme stability over time.^[15] One challenge in developing chronic glucose biosensors arises from the inherent instability of GOx and the leaching of the enzyme from the electrode surface.^[15] In addition, GOx can be inactivated by hydrogen peroxide that is produced during the oxidation of glucose to

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gluconic acid.^[16] Micro-scale electrochemical sensors have several advantages for detection of biochemical signals compared to macroscopic counterparts: (1) they provide higher spatial resolution because of small geometric area (i.e. selectivity)^[15, 17]; (2) with small RC (R: resistance, C: capacitance) time constants due to the reduced double layer capacitance, they have higher temporal resolution and faster electron transfer^[18, 19]; and (3) and they have an increased mass transport rate due to nonplanar diffusion.^[3, 20] However, due to their small feature geometry, microelectrodes suffer from high impedance that increases thermal noise and signal loss (low signal-to-noise ratio).^[17, 21-23]

These challenges directed us toward developing a new glucose biosensor with improved sensitivity and longevity provided by microfabricated electrodes and conducting polymer nanofibers. The application of conducting polymer to bioelectronic surfaces^[24-26] has gained considerable attention as a means to address enzyme-based biosensor challenges by increasing the signal-to-noise ratio and serving as a suitable matrix for the immobilization and entrapment of enzymes.^[11, 27-31] It is not clear whether the conducting polymer “wires” the enzyme to the electrode, or the conductivity of the conducting polymer changes by hydrogen peroxide-induced oxidation.^[32-35] Among different conducting polymers, poly(3,4-ethylenedioxythiophene) (PEDOT) has been reported to exhibit superior chemical stability and electrical conductivity^[36-39], which has led to its successful use in amperometric biosensors.^[34, 40-42] In addition to the use of new electrode materials, the ability to fabricate nanostructures at the recording site allows for new physical designs of the biosensor. Nanostructured sensing elements provide higher sensitivity as a result of increased surface to volume ratio,^[15, 18] and PEDOT nanostructures have provided a decrease in impedance at recording sites by 77% compared to PEDOT film.^[43] Finally, PEDOT nanostructures have been shown to increase the percentage of sites having high-quality signals during chronic neural recording,^[44] making them excellent candidates for use in chronic glucose biosensors.

Here we report a novel method for fabrication of enzyme entrapped-conducting polymer nanofibers that offer higher sensitivity and increased lifetime compared to conducting polymer film counterparts. In the present work, GOx entrapped in PEDOT was prepared in films (PEDOT F-GOx) as well as in nanofibers (PEDOT NFs-GOx), and the electrical properties, sensitivity, and longevity of each type of biosensor were measured at polarization potentials of +300mV and +700mV vs. Ag/AgCl. It has been shown that conducting polymers could be utilized as mediator for amperometric detection of glucose at polarization potential lower than +700 mV.^[35] This is presumably due to direct electron transfer between glucose oxidase and the conducting polymer, which is an oxygen-independent detection.^[32, 35]

2. Results and discussion

Each biosensor (i.e. PEDOT F-GOx and PEDOT NFs-GOx) directly entrapped GOx in PEDOT during galvanostatic polymerization at the electrode site. The galvanostatic method was used for polymerization of PEDOT to control the rate of polymerization^[45], while a supporting electrolyte was included to facilitate GOx incorporation into the polymer. During polymerization, GOx became immobilized in the porous PEDOT by a combination of

physical entrapment and polymer charge balance.^[46] Entrapment directly during electropolymerization is a reproducible method that allows for spatially controlled deposition^[46] and can retain the enzyme's activity and stability compared to a covalent binding method.^[32] Enzyme entrapment also eliminates the use of harsh chemicals typically used to covalently bind enzymes to the surface of electrodes.^[32, 47] Other studies have shown that GOx retains its functionality after entrapment in conducting polymer and is actually more resistant to denaturation from changes in pH or temperature than soluble GOx.^[41, 48]

The GOx incorporated-PEDOT films (PEDOT F-GOx) were electrodeposited on the surface of platinum (Pt) microelectrode arrays ($1394 \mu\text{m}^2$) (**Fig. 1a, 1h, and 1i**) by electropolymerization from a solution containing 0.02 M EDOT monomer, 0.2 M poly(sodium-p-styrene sulfonate), and 1000 U ml^{-1} GOx (**Fig. 1b, 1e, and 1g**). To fabricate the GOx incorporated-PEDOT nanofibers (PEDOT NFs-GOx), poly(L-lactide) (PLLA) nanofibers were first directly elect spun on Pt microelectrode arrays (**Fig. 1c**). This electrospinning was followed by the electrochemical deposition of PEDOT on the Pt microelectrodes and around PLLA nanofibers in the same manner as the PEDOT F-GOx (**Fig. 1d, 1f, and 1g**). **Figures 1h and 1i** show the optical micrographs of Pt microelectrode arrays and PEDOT F-GOx and PEDOT NFs-GOx on the Pt sites, respectively. The total applied charge density during electrochemical deposition was controlled at 1.73 C cm^{-2} for all samples in room temperature (20°C). The temperature of electropolymerization was monitored using a digital probe thermometer that was placed next to the Pt microelectrode. While we did not detect any temperature change, we calculated that the change of temperature was about 0.1°C on the surface of Pt microelectrodes (see Supporting Information). Thus, we do not anticipate any possible change or destroy in GOx structure. The thickness of PEDOT film and PEDOT nanofiber mat was $327 \pm 10 \text{ nm}$ and $331 \pm 8 \text{ nm}$, respectively. PEDOT nanofibers had an outer diameter of $110 \pm 8 \text{ nm}$ with a core diameter of $80 \pm 8 \text{ nm}$, which represents the diameter of electrospun PLLA nanofibers (**Fig. 1j-1m**, see Supporting Information **Fig. S1**).

Fourier transform infrared spectroscopy was used to determine the effect of GOx on the PEDOT structures (**Fig. 2a**). The absorption bands at 1162 cm^{-1} , 1121 cm^{-1} and 1066 cm^{-1} are assigned to the stretching vibration of the ethylenedioxy group. The C-S vibration absorption can be seen at 947 cm^{-1} , 860 cm^{-1} and 712 cm^{-1} . The C-C and C=C absorption in the thiophene ring is at 1345 cm^{-1} and 1501 cm^{-1} . There is no significant difference between PEDOT nanofibers without GOx (PEDOT NFs) and PEDOT nanofibers with GOx (PEDOT NFs-GOx), showing that the polymer matrix is effectively PEDOT even when the GOx enzyme has been incorporated.^[42]

To determine the quantity of GOx enzyme in PEDOT film (PEDOT F-GOx) and PEDOT nanofiber (PEDOT NFs-GOx), electrochemical quartz crystal microbalance (EQCM) experiment was performed. The mass of entrapped enzyme was calculated by comparison between the mass of unmodified PEDOT-coated Pt electrodes (without GOx) and modified PEDOT-coated Pt electrodes (with GOx) assuming that at a constant electrodeposition charge density of 1.73 C cm^{-2} GOx does not impede the electrodeposition.^[42] EQCM results revealed that the amount of GOx incorporated in PEDOT nanofibers (PEDOT NFs-

GOx) was $31 \mu\text{g cm}^{-2}$ significantly more than the amount of GOx incorporated in PEDOT film (PEDOT FGOx, $1.3 \mu\text{g cm}^{-2}$) (**Fig. 2b**). The increase in amount of GOx in the PEDOT NFs-GOx compared to the PEDOT F-GOx could be due to a combination of two factors: (1) an increase in the effective surface area available to entrap GOx on the PEDOT nanofibers and (2) an electrostatic interaction between the positively charged PLLA nanofibers and the negatively charged GOx.^[49]

Once the fabrication of the glucose biosensors was characterized, the impedance of these biosensors was measured. It has been shown that conducting polymers could significantly decrease the impedance of implanted neural microelectrodes^[50-52]; in particular, we demonstrated that PEDOT nanotubes further reduced impedance of microelectrodes compared to PEDOT films.^[43] This impedance reduction enhanced the quality of neural recordings in vivo.^[44, 53] The reduction in impedance by PEDOT nanotubes compared to PEDOT films was due to the relative increase in effective electrode surface area provided by the nanotubes.^[22, 44] In order to confirm that PEDOT nanofibers-coated sites had less impedance than PEDOT film-coated sites and the inclusion of GOx in the biosensors would not affect this phenomenon, the impedance spectra of bare Pt electrode sites was compared to the impedance spectra of PEDOT F-GOx and PEDOT NFs-GOx microelectrodes. As shown in **Figure 2c**, while the impedance of PEDOT F-GOx and PEDOT NFs-GOx sites was significantly less than bare Pt sites, PEDOT NFs-GOx sites exhibited lower impedance than PEDOT F-GOx across all frequencies (from 10^{-2} Hz to 10^2 Hz). For example at a low frequency of 10^{-2} Hz, the impedance of PEDOT F-GOx sites was $111.2 \pm 8 \text{ M}\Omega$ while that of PEDOT NFs-GOx sites was reduced significantly to $19.3 \pm 5 \text{ M}\Omega$, representing an 83% reduction in impedance by the nanofiber morphology compared with film morphology. For comparison, the impedance at 0.01 Hz for a bare Pt electrode was $3660 \pm 23 \text{ M}\Omega$. These impedance changes are very comparable to those changes previously reported when electrode sites were modified with PEDOT film and PEDOT nanotubes without GOx.^[43]

The significant reduction in impedance for PEDOT NFs-GOx compared to PEDOT FGOx in combination with the increased entrapment of GOx in the PEDOT nanofibers (PEDOT NFs-GOx) was expected to directly increase the sensitivity of the PEDOT NFs-GOx biosensors. To measure current response, polarization potentials of +300mV or +700 mV vs. Ag/AgCl were applied to the each type of biosensor in a stirred solution of phosphate buffered saline solution (PBS, pH =7.0, 37 °C) while injections of increasing amounts of glucose were made (cumulative concentration ranging from 0.1 mM to 25 mM) (**Fig. 3a and 3b**). At both polarization potentials, the PEDOT NFs-GOx biosensors showed a larger amperometric response to the glucose injections than the PEDOT F-GOx biosensors. To quantify the amperometric response, calibration curves for each sensor were created relating the current response to the glucose concentration (**Fig. 3c**). While glucose concentrations ranging from 0.1 mM to 25 mM were examined at both +700 mV and +300 mV vs. Ag/AgCl (**Fig. 3c**), the biosensors were found to have a linear range of up to 5 mM of glucose (**Fig. 3d**). Other glucose sensors based on entrapping GOx within conducting polymers have achieved linear ranges of up to 10 mM^[41, 42, 54]; however, based on the biological range of glucose concentrations, a biosensor with a linear range of up to 5 mM is suitable for

measuring glucose in cerebrospinal fluid where glucose concentrations are typically two-thirds that of their concentration in the blood.^[55]

Using the linear response range, the sensitivity was determined by the slope of the calibration curve for each type of biosensor at each polarization potentials +300 mV and +700 mV. At +300 mV, the sensitivity of PEDOT F-GOx was $1.2 \pm 0.5 \mu\text{A cm}^{-2} \text{mM}^{-1}$, while that of the PEDOT NFs-GOx was $6.4 \pm 0.7 \mu\text{A cm}^{-2} \text{mM}^{-1}$, representing a 433% increase in sensitivity. Similarly, at +700mV, the sensitivity of PEDOT F-GOx was $3.6 \pm 0.8 \mu\text{A cm}^{-2} \text{mM}^{-1}$, while that of the PEDOT NFs-GOx was $9.2 \pm 1.1 \mu\text{A cm}^{-2} \text{mM}^{-1}$, representing a 156% increase in sensitivity. At both polarization potentials, the change in sensitivity from the PEDOT F-GOx samples compared to the PEDOT NFs-GOx samples was statistically significant ($p < 0.0001$). The increased surface area associated with PEDOT NFs-GOx rather than PEDOT F-GOx has a dual effect: (1) it decreases the impedance of the biosensor (**Fig. 2c**) and (2) it allows more GOx to be entrapped on the biosensor (EQCM data, **Fig. 2b**); both of these results contribute to the increased sensitivity of the PEDOT NFs-GOx biosensors compared to the PEDOT F-GOx biosensors.

In addition to improved sensitivity, the PEDOT NFs-GOx sensors also showed a lower limit of detection of glucose than the PEDOT F-GOx sensors. At +300 mV, the limit of detection of PEDOT F-GOx was 0.56 mM glucose, while that of the PEDOT NFs-GOx was 0.26 mM glucose. Increasing the potential to +700 mV resulted in the limit of detection of the PEDOT F-GOx to decrease to 0.45 mM glucose and that of the PEDOT NFs-GOx to decrease to 0.12 mM glucose. All of these limits of detection fall below the biological range of glucose concentrations, both in cerebrospinal fluid and in blood plasma.^[55] The +700 mV polarization potential resulted in statistically significant higher sensitivities and lower limits of detection than the +300 mV polarization potential for both PEDOT F-GOx and PEDOT NFs-GOx biosensors ($p < 0.0001$). However, the improvement in sensitivity for the nanofiber biosensors compared to the film biosensors at each potential was not equivalent (i.e. 433% increase compared to a 156% increase for +300mV and +700mV, respectively). It is possible that the higher polarization potential diminishes the increase in sensitivity of the PEDOT nanofiber sensor due to an increased reoxidation of hydrogen peroxide, which results in a loss of activity of GOx.^[16]

While using lower polarization potentials resulted in less sensitivity at the start of recording, lower polarization potentials could be desirable if it could increase the lifetime of the biosensor. The use of a lower polarization potential of +300 mV was based on the expectation that a lower polarization potential could possibly increase the lifetime of the sensor by oxidizing less hydrogen peroxide.^[35] The longevity of the electrodes was measured as the electrodes' sensitivities over the course of 30 days (**Fig. 4a**). Electrodes were stored at 4 °C in PBS between measurements. At +700 mV, the PEDOT F-GOx and PEDOT NFs-GOx biosensors showed a decrease in sensitivity of 99% (from $3.6 \pm 0.8 \mu\text{A cm}^{-2} \text{mM}^{-1}$ to $0.01 \pm 0.3 \mu\text{A cm}^{-2} \text{mM}^{-1}$) and 40% (from $9.2 \pm 1.1 \mu\text{A cm}^{-2} \text{mM}^{-1}$ to $5.5 \pm 0.5 \mu\text{A cm}^{-2} \text{mM}^{-1}$), respectively over the 30 days. Reducing the polarization potential to +300 mV improved the longevity of the sensors and resulted in a drop in sensitivity of 83% (from $1.2 \pm 0.5 \mu\text{A cm}^{-2} \text{mM}^{-1}$ to $0.2 \pm 0.2 \mu\text{A cm}^{-2} \text{mM}^{-1}$) for the PEDOT F-GOx and 28% (from $6.4 \pm 0.8 \mu\text{A cm}^{-2} \text{mM}^{-1}$ to $4.6 \pm 0.4 \mu\text{A cm}^{-2} \text{mM}^{-1}$) for the PEDOT NFs-GOx

biosensors (**Fig. 4a**). Interestingly, the PEDOT NFs-GOx biosensors experienced a smaller loss in sensitivity at both potentials than the PEDOT F-GOx biosensors.

The small loss in sensitivity in the PEDOT NF-GOx biosensors could be due either to better retention of the GOx enzyme and/or less loss of electroactivity over time than in the PEDOT F-GOx sensors. Since the observed sensitivity decrease is dependent on the applied polarization potential, the cyclic voltammetry (CV) behavior of PEDOT F-GOx and PEDOT NFs-GOx potentiostated at +700 mV and +300 mV was investigated (**Fig. 4b-e**). The electroactivity change over 280 minutes was measured, which corresponds to the total time the biosensors were potentiostated for sensitivity measurements over 30 days. The variation of the CV for the PEDOT F-GOx and PEDOT NFs-GOx microelectrodes after they were potentiostated at +700 mV and +300 mV for 0, 20, 60, 200, and 280 min is shown in **Figure 4b-e** (0, 20, 60, 200, and 280 min corresponds to before applying voltage, day 1, day 3, day 10, and day 30, respectively). To characterize the electroactivity loss of PEDOT, the charge storage capacity density (CSCD) was calculated. The surface area under the CV curve is proportional to the charge storage capacity of a material that can transfer or store during one cycle of CV. The CSCD is calculated by dividing surface area under the CV curve by the product of the scan rate and surface area of the microelectrode.^[51, 56]

As shown in **Figure 4f**, at +700 mV, the CSCD of PEDOT F-GOx at 280 min (day 30) was $35.8 \pm 3.8 \text{ mC cm}^{-2}$, showing a 71 % drop compared with the CSCD at 0 min (before applying polarization potential) ($121.8 \pm 15.2 \text{ mC cm}^{-2}$). For the PEDOT NFs-GOx, the CSCD at 280 min was $35.6 \pm 4.1 \text{ mC cm}^{-2}$, indicating a 74% drop compared with the CSCD at 0 min ($139.2 \pm 14.8 \text{ mC cm}^{-2}$). At +300 mV, the PEDOT F-GOx has CSCD value of $50.0 \pm 3.9 \text{ mC cm}^{-2}$, showing 59% decrease compared with the CSCD at 0 min ($121.6 \pm 11.3 \text{ mC cm}^{-2}$). For the PEDOT NFs-GOx, the CSCD at 280 min was $66.8 \pm 3.6 \text{ mC cm}^{-2}$, indicating 30% decrease compared with the CSCD at 0 min ($95.1 \pm 9.1 \text{ mC cm}^{-2}$) (**Fig. 4f**). These results are in accordance with the abovementioned loss of sensitivity study and demonstrate that +700 mV groups lead to more electroactivity loss than +300 mV groups. The decrease in electroactivity of all biosensor groups might be explained by nucleophilic attack by water or anions^[57] and chemical oxidation by strong oxidizing agents such as hydrogen peroxide.^[13,26,33] Ring opening and loss of conjugation, have also been proposed to explain the loss of electroactivity^[58]. Evidently, this led to a decrease in the conductivity of PEDOT.

3. Conclusions

The presented strategy for development of GOx entrapped-PEDOT nanofiber on the surface of Pt microelectrode biosensors has four significant advantages: (1) it offers a suitable nano-scale matrix for entrapment of GOx; (2) it reduces the impedance of Pt microelectrodes; (3) it increases the entrapment of GOx within the PEDOT; and (4) it detects glucose at lower polarization potential which results in less electroactivity loss of PEDOT. Consequently, the PEDOT nanofiber-based biosensor demonstrates a significant improvement on sensitivity, limit of detection, and longevity compared to PEDOT film counterpart. We envision the extension of the strategy reported here as a proof of concept to develop conducting polymer nanofiber-based biosensors for detection of other neurochemicals and neurotransmitters. With enormous potential for long-term sensitive detection of other biomolecules and

chemical substances, we believe this technique can be used for different applications in food science, homeland security, and biomedical areas.

Experimental Section

Materials

Poly (L-lactide) (PLLA, RESOMER® L 210) with inherent viscosity 3.3-4.3 dl g⁻¹ was purchased from Boehringer Ingelheim Pharma GmbH & Co. (KG, Germany). 3, 4-ethylenedioxythiophene (EDOT), with molecular weight 142.17 g mol⁻¹ was received from Sigma Aldrich. Poly(sodium-p-styrene sulfonate) (PSS) (Mw 70,000) was purchased from Acros Organics). Glucose oxidase (GOx) type X-S from *Aspergillus niger* (129,900 U g⁻¹) was purchased from Sigma-Aldrich.

Fabrication of PEDOT Biosensors

A solution containing 0.02 M EDOT monomer, 0.2 M PSS, and 1,000 U ml⁻¹ GOx was prepared in DI water. Electrochemical polymerization was carried out galvanostatically at current density 0.5 mA cm⁻² for 60 s (1.73 C cm⁻²) in room temperature (20 °C) using an Ag/AgCl reference electrode and Pt counter electrode on platinum sites (1394 μm²) of microelectrode arrays. For PEDOT NFs-GOx, PLLA nanofibers were first spun on the surface of Pt microelectrode arrays from 7.8% (w/v) PLLA in chloroform. The solution was stirred at a temperature of 50 °C for 10 hr prior to electrospinning in order to produce a homogenous solution. The electrospinning process was carried out in an electrical field of 0.6 kV cm⁻¹ with a flow rate of 0.25 mL hr⁻¹ for 30 s. The microelectrodes arrays were held at a distance of 10 cm from the syringe needle during this process. Electrochemical polymerization was then carried out as described above.

Shape, size and surface characterization

The surface morphology of PEDOT F-GOx and PEDOT NFs-GOx was characterized using Field Emission Scanning Electron Microscopy (FEI NanoSEM 630 FESEM). Later, the size and thickness of PEDOT film and PEDOT nanofibers mat were assessed by analyzing the SEM micrographs with Image J analysis software (NIH).

Electrochemical Quartz Crystal Microbalance (EQCM) Measurements

EQCM experiments were done on a 0.33 cm² crystal (6 MHz, Pt/TiO₂, polished, Metrohm, USA) using Autolab PGSTAT 302N at 20°C. The frequency change can be related to the mass change by the Sauerbrey equation:

$$\Delta f = C_f \cdot \Delta m$$

f is the frequency change (Hz), C_f is the sensitivity factor (0.0815 Hz·ng⁻¹·cm² for this 6 MHz crystal at 20°C).

FTIR Spectroscopy

FTIR spectra was recorded on a computer controlled Bruker Vertex70 FTIR spectrometer with “MVP-Pro” Attenuated Total Reflectance (ATR) accessories (resolution = 4 cm^{-1} , scan time = 100). The wavenumber range was $400\text{--}4000\text{ cm}^{-1}$.

Impedance Spectroscopy

An Autolab PGSTAT-302N (Metrohm Autolab, Netherlands) was used to record impedance spectra of bare platinum electrode (Pt) sites as well as Pt sites coated with PEDOT F-GOx and PEDOT NFs-GOx. A solution of phosphate buffered solution (PBS, 0.1M, pH=7) was used as an electrolyte in a three-electrode cell configuration. The counter electrode was platinum foil and an Ag/AgCl reference electrode was used. An AC sinusoidal signal with 5 mV amplitude was used to record impedance of a frequency range of $10^{-2}\text{--}10^4\text{ Hz}$.

Cyclic Voltammetry (CV)

CV was measured using Autolab PGSTAT 302N instrument (Metrohm Autolab, Netherlands). A solution of phosphate buffered solution (PBS, 0.1M, pH=7) was used as an electrolyte in a three-electrode cell configuration. The potential applied to the working electrode was swept from -1.0 to 1.6 V vs. Ag/AgCl with scanning rate of 100 mV s^{-1} . The charge storage capacity (CSCD) was calculated using Origin Pro software (OriginLab). The CV measurement was performed for the electroactivity loss study immediately after fabrication of biosensors and applied bias voltage of +300 mV and +700 mV vs. Ag/AgCl.

Amperometric Response Measurements

A QuadStat E164 and e-corder 821 (eDAQ, Australia) was used to record the amperometric response of PEDOT F-GOx and PEDOT NFs-GOx biosensors to injections of glucose. A 20 ml stirred solution of PBS (0.1M, pH = 7, $37\text{ }^{\circ}\text{C}$) was used as an electrolyte in a three-electrode cell configuration. The counter electrode was platinum foil and an Ag/AgCl reference electrode was used. Glucose stock solutions were left 24 h at room temperature prior to use. A polarization potential of either +300 mV or +700 mV was applied to the working electrode. Once the background current stabilized, increasing amounts of glucose solution was injected to the stirred PBS solution to create glucose concentrations ranging from 0.1 mM to 25 mM. The resulting current measurements were used to calculate the sensitivity and limit of detection. The PEDOT F-GOx and PEDOT NFs-GOx electrodes were stored at $4\text{ }^{\circ}\text{C}$ after glucose detection experiments.

Statistical Analysis

The statistical significance of the difference in sensitivity for various sample conditions (sensor morphology and polarization potential) was determined using a two-way ANOVA analysis in Prism software. Sensitivity was measured for ten samples of each condition for this analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Schematic of fabrication process of GOx-incorporated PEDOT on microelectrode array: a) Pt microelectrode array. b,c) electrodeposition of GOx-incorporated PEDOT film (PEDOT F-GOx). c) electrospinning of PLLA nanofibers on the microelectrode array. d,f) electrodeposition of PEDOT around the PLLA nanofibers to form GOx-incorporated PEDOT nanofibers (PEDOT NF-GOx). g) schematic of entrapment of GOx within PEDOT structure. h) optical micrograph of entire electrode array. i) optical micrograph of microfabricated electrodes showing two uncoated Pt sites and four GOx-incorporated

PEDOT sites. j) scanning electron micrograph of PEDOT F-GOx. k) higher magnification SEM of PEDOT F-GOx. l) scanning electron micrograph of PEDOT NFs-GOx. m) higher magnification SEM of PEDOT NFs-GOx

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Figure 2.

a) ATR-FTIR spectra of PEDOT nanofibers without GOx (PEDOT NFs, black line) and PEDOT nanofibers with GOx (PEDOT NFs-GOx, red line) showing PEDOT is the main component. b) EQCM data showing the mass of GOx incorporated into PEDOT NF-GOx ($31 \mu\text{g cm}^{-2}$) was significantly greater than the mass of GOx incorporated into PEDOT F-GOx ($1.3 \mu\text{g cm}^{-2}$). c) Impedance spectroscopy of bare Pt (black squares), PEDOT F-GOx (red circles), and PEDOT NFs-GOx (blue triangles) demonstrating that the impedance of PEDOT NF-GOx was less than that of PEDOT F-GOx across all frequencies.

Figure 3.

a, b) the current response of the PEDOT F-GOx (red line) and PEDOT NF-GOx (blue line) coated microelectrodes arrays (Pt surface area $1394 \mu\text{m}^2$) to increasing concentrations of GOx at polarization potential +300 mV and +700 mV vs. Ag/AgCl, respectively. c) calibration curves of PEDOT modified microelectrodes: PEDOT NFs-GOx at +700 mV (black squares), PEDOT F-GOx at +700 mV (green triangles), PEDOT NFs-GOx at +300 mV (red circles), and PEDOT F-GOx at +300 mV (blue diamonds). d) linear regression of the dynamic range (0.1 mM- 5 mM) for PEDOT NFs-GOx at +700 mV, all samples (PEDOT NFs-GOx and PEDOT F-GOx) had linear region from 0.1 mM to 5 mM glucose.

Figure 4.

a) sensitivity of PEDOT F-GOx and PEDOT NFs-GOx biosensors at +700 mV and +300 mV vs Ag/AgCl over the course of 30 days, PEDOT NFs-GOx at 700mV (black squares), PEDOT NFs-GOx at 300 mV (red circles), PEDOT F-GOx at 700 mV (green triangles), and PEDOT F-GOx at 300 mV (blue diamonds). b-d) CVs for difference biosensors showing the variation in electroactivity after the electrodes were held for 0, 20, 60, 200, 280 min at +700 mV for PEDOT F-GOx (0, 20, 60, 200, and 280 min corresponds to before applying voltage, day 1, day 3, day 10, and day 30, respectively) (b), +700 mV for PEDOT NFs-GOx (c),

+300 mV for PEDOT F-GOx (d), and +300 mV for PEDOT NFs-GOx. f) charge storage capacity density (CSCD) for each type of biosensors after they were potentiostated at +700 mV and +300 mV for 0, 20, 60, 200, and 280 min, PEDOT NFs-GOx at 700mV (black squares), PEDOT NFs-GOx at 300 mV (red circles), PEDOT F-GOx at 700 mV (green triangles), and PEDOT F-GOx at 300 mV (blue diamonds). Data are shown for $\pm$ standard deviation (n = 10)