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Perspective—Electrospun Nanofibrous Structures for Electrochemical Enzymatic Glucose Biosensing: A Perspective

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Biosensing has capitalized on the excellent characteristics and properties of nanostructures for detecting glucose levels in diabetic patients. In glucose sensing systems, the fabrication of a suitable matrix for immobilizing glucose oxidase (GOx) has become more interesting for the application of nanofibers in enzymatic electrochemical biosensors. These nanofiber based electrochemical biosensors are superior in manufacturability and performance due to low cost, diversity of materials, ease of miniaturization, response time, durability, and structure versatility. This perspective highlights the latest material integration of various nanofibrous composite membranes of carbon nanotubes, carbon nanofibers, conductive nanoparticles and conductive polymers, that provide large matrix-like, porous surfaces to enhance the immobilization of enzymes, for the fabrication of glucose biosensors.

Many people around the world suffer from diabetes, which can result in damages to eyes, kidneys, nerves, gangrene, subsequent amputation and even death. Approximately 285 million people suffer from diabetes, and this number is expected to increase to 430 million by 2030. The monitoring and sensing of glucose (sugar) levels in the blood is very important for people with diabetes. The recent development of low-cost, highly sensitive, and highly stable electrochemical glucose biosensors has been given much attention due to promising results for point-of-care diagnoses and accurate monitoring of blood glucose levels in diabetic patients.

One important aspect in the fabrication of glucose sensors is the capability of keeping the glucose oxidase (GOx) enzyme on the surface of the electrode to electrochemically detect the active species. This has been accomplished by the use of suitable nanostructures for the immobilization of the enzyme. Among several configurations of nanostructures being used for glucose bio-sensing, electrospun nanofibrous structures are the most effective in achieving higher sensitivity, minimum limit of detection (LOD), better binding of enzyme with electrode, favorable porosity and thermal stability.

Figure 1 shows the representation of a nanofibrous structure-based glucose sensor. Nanofibrous structures offer configuration and integration advantages due to structural dimensions on the order of nanometers, small size, high surface area, porosity, enhanced optical and electrical properties, more efficient enzyme to electrode electron-transfer, and the capability of enhancing additional catalytic reactions; all of which, demonstrate significant benefits compared to macroscale materials. Electrospun nanofibrous membranes are tunable in terms of dimension; cost effective and safer in terms of fabrication; flexible in terms of orientation, scaling and bending; greener in terms of material processing; and versatile in terms of high surface-to-volume ratio. It is advantageous to fabricate bead free nanofibers, which has less variability in the fiber morphology and favor reproducibility of the sensing surface. Various nanofibrous composite structures, comprised of carbon nanotubes, carbon nanofibers, conductive nanoparticles, and conductive polymers, are suitable for the fabrication of glucose sensors due to the direct electron-transfer process between the electrode material surface and the GOx, which effectively immobilizes the enzymes and promotes the electrochemical response. Emphasis is placed on these composite structures due to the material conductivity, which in turn increases the sensitivity of the sensor. Researchers have begun to actively exploit these properties to improve the lifetime, efficiency, size, accuracy and usability of sensors for treating diabetes. Therefore, nanofibrous based glucose sensors are finally being considered for the initial stages of commercial and clinical implementation, which is predicted to offer better treatment in the future for diabetic patients.

In this perspective, we briefly review nanofibrous structures for electrochemical enzymatic glucose sensors and offer a future scope on a conductive polymer, poly (3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS), based electrospun nanofibrous membrane (ENFM) glucose sensor. The electrochemical analysis demonstrates enhanced sensitivity, good stability, reasonable repeatability, durability, and quick response time for glucose detection. This perspective will provide a reference source for researchers in biosensing, nanotechnology, materials science, and electrochemistry, attracting a wide range of diverse research audiences for future nanofibrous based biosensors for glucose monitoring.

Current Status of Glucose Sensors Using Electrospun Nanofibers

Since the first reported GOx enzymatic electrode in 1967, electrochemical glucose sensor structures have evolved from thin film to 2D and 3D structures. The 2D and 3D structures (tubes, particles, fibers, etc) have characteristics of high porosity and large specific surface area. By embedding 2D and 3D structures into electrospun nanofiber composites and using conductive polymers, researchers have been able to offer customized loading of enzymes, better binding of GOx with the electrode and excellent ability to immobilize enzymes. Electrospun nanofibrous structures have a critical impact on glucose sensor reliability, surface sensitivity and real-time responsiveness for detecting changes in glucose levels. Enzyme based detection requires a stable and highly sensitive sensing electrode matrix. This matrix, fabricated by electrospinning, can have a dispersion or attachment of functional nanostructures in the polymeric solution and offers efficient binding of functional particles. The nanofibrous matrix can also encapsulate or protect the enzymes from denaturation when exposed to changes in pH or temperature, leading to enhancements in sensing performance and long-term stability (addressing issues plaguing enzymatic sensors). In addition, nanofibrous membranes allow for increased stabilization of the enzymatic activity and limit leaching of enzyme molecules in the electrolyte solution. A favorable surface for effective enzyme loading is created, and direct electron-transfer between electrode and enzyme is facilitated using nanofibrous composites of carbon nanotubes, carbon nanofibers, conductive nanoparticles and conductive polymers.
Carbon nanotube (CNT) based electrospun nanofibers.—
Carbon nanotubes (single and multi-walled) have been found to promote catalytic behavior, electrical conductance and reduce fouling in an immobilization matrix.26 These carbon-based materials can improve the performance and lifetime of glucose sensors.23 Poly(acrylonitrile-co-acrylic acid) (PANCAA) nanofibrous membranes (NFMs), filled with multi-walled carbon nanotubes (MWCNTs), were electrospun and deposited onto platinum (Pt) electrodes. MWCNTs are used to enhance the activity of GOx enzyme for glucose sensing.24 The experimental results indicated that MWCNTs did indeed enhance the sensitivity of immobilized GOx and that the sensor could be used more than 6 times with little decrease in current.24 Nylon 6,6 nanofibers were electrospun and MWCNTs were incorporated and encapsulated with conducting polymer, (poly-4-(4,7-di(thiophen-2-yl)-1H-benzol[d]imidazol-2-yl)benzaldehyde) (PBIBA), to form a nanofibrous matrix. These nylon 6,6 nanofibers are bead free (Fig. 2a), which helps with the formation of a homogenous distribution of a nanofibrous membrane on the electrode surface and promotes good stability. It is highlighted that with an increase inCNTs, there is an increase in electron transfer and an increase in morphological porosity due to CNTs. The matrix has a high surface to volume ratio coating of PBIBA, which enhanced the covalent loading of the GOx (Fig. 2b) to the nanofibrous surface for effective immobilization.25 Nitrogen-doped carbon nanotubes (NCNTs) were also presented as ideal carbon nanomaterials for immobilizing and maintaining high electrochemical activity of enzymes, due to its biocompatibility and multiple active sites.25 For more timely point-of-care diagnosis of glucose levels, researchers should investigate nanofibers filled with single-walled carbon nanotubes (SWCNTs) to fabricate glucose biosensors. As a comparative, SWCNTs have been shown to have lower charge transfer resistance than MWCNTs, yielding a higher electron-transfer rate at the electrode-enzyme interface and better glucose detection.26

Carbon nanoﬁber (CNF) based electrospun nanofibers.—In considering CNFs, nitrogen-doped carbon nanofibers (NCNFs) were prepared by electrospinning polyacrylonitrile (PAN) nanofibers and performing subsequent carbonization, as shown in Fig. 2c.27 GOx and NCNFs were utilized to modify a glassy carbon electrode (GCE) of a biosensor for detecting glucose. Incorporating nitrogen increases the electron-transfer rate and enhances oxygen reduction for a highly stable and quickly responsive glucose sensor. Due to the unique free-standing NCNF structure, the glucose sensor demonstrated enhanced stability and high sensitivity.27 Highly dispersive nickel (Ni)/cobaltous oxide (CoO) carbon nanofibers were fabricated by electrospinning and exhibited excellent sensitivity and catalytic activity for determining levels of glucose. The amount of surfactant had a significant impact on the material morphology, which further affected the current response sensitivity as glucose levels changed.16 Another glucose sensor was fabricated based on hybrid nanostructures of palladium-helical carbon nanofibers (Pd-HCNFs), and immobilization of GOx with nan was conducted on a glassy carbon electrode (GCE). The resulting biosensor exhibited good sensitivity and responded to a wide range of changes in glucose levels.28 Prussian blue (PB) nanostructures were grown on carboxylic group-functionalized carbon nanofibers (FCNFs) to form low cost nanocomposites with good stability and selectivity. Results supported PB–FCNF biosensors being effectively applied for glucose measurements.29 Therefore, the sensing performance and

Figure 1. Representation of the electrospun nanofibrous structure based glucose sensor.

Figure 2. (a) SEM images of Nylon 6,6/MWCNT nanofibers (b) SEM image of PBIBA coated Nylon 6,6/MWCNTs nanofibers with GOx.32 Reprinted with permission from Copyright (2014) American Chemical Society. (c) SEM image of NCNFs27 Reprinted with permission from Copyright (2014) American Chemical Society.
enzymatic glucose response of electrospun carbon nanofibers will continue to improve as researchers investigate nanofiber-hybrids (incorporation of nanostructures, particularly nanoparticles).

**Conductive nanoparticle based electrospun nanofibers.**—Conductive nanoparticles have sensitive electrochemical properties based on their size, dispersion, atomic configuration, and level of conductivity. For high electrochemical activity to occur in functional materials, there needs to be a high dispersion of nanoparticles in the material, but there is a tendency for nanoparticles to aggregate, lowering the catalytic activity and reuse lifetime. Therefore, there is a trade-off with the amount of nanoparticles that can be dispersed, in situ or ex situ, to the nanofibers via covalent bonding, adsorption, or encapsulation. A polymeric nanofiber matrix with nanoparticles tends to offer a stable support structure for the interface of the enzyme on a solid surface for immobilization. In recent fabrications of nanofibrous composite sensors, the nanoparticle of choice has been gold nanoparticles, where gold is one of the highly conductive materials at $4.10 \times 10^7 \text{ s}^{-1}$ (20 $^\circ\text{C}$). A nanofibrous surface of a poly(vinyl alcohol) (PVA) and graphene oxide (GO) electrospun composite was coated with gold nanoparticles (AuNPs) and then exposed to a copper (Cu) nanoflower (nf) -GO$_x$ and horseradish per-oxidase. The electrochemical analysis of the PVA/GO$_x$-AuNP-Cu-nf nanofibrous glucose sensor was enhanced by the addition of AuNPs and Cu-nf, which increased the overall conductivity of the sensing electrode. Poly(ethyleneimine) (PEI)/PVA/GO$_x$ were electrospun on gold electrodes, and the surface of the nanofibers were post treated with AuNPs. The adhesion of gold electrodes with the nanofibrous membrane and the nanofiber water stability was improved by modifying the surface of the glucose sensor. In another study, gold nanoparticles, poly(acrylonitrile) (PAN) electrospun nanofibers, and CNTs were used to fabricate a glucose sensor. The PAN nanofibers were decorated with gold nanoparticles, and carboxylated CNTs were further coated.

Figure 3. (a) Chronoamperometric measurements of an ENFM electrode for a successive increase of glucose concentration. Inset: shows the plot of the concentration of the glucose with current. (b) SEM image (i) and AFM image (ii) of electrospun PEDOT: PSS nanofibers without GO$_x$. SEM image (iii) and AFM image (iv) of swollen electrospun PEDOT: PSS nanofibers with GO$_x$. (c) Durability test of an ENFM electrode for 60 days at 5 mM glucose concentration.
Table I. Comparison of performance with prior state-of-the-art electrospun nanofibrous membrane based electrochemical enzymatic glucose sensing electrodes.

| References | Electrospun Nanofibers | Nano materials | Glucose Sensing Electrodes | AFD (nm) | LOD (μM) | Durability (days) |
|------------|------------------------|----------------|-----------------------------|----------|----------|-------------------|
| 14         | PVA-GOx                | Cu-nf/AuNPs    | Au/PVA-GOx/Cu-NFs/AuNPs     | ~168     | 0.018    | 20                |
| 34         | PVA/PEI/GOx            | AuNPs          | Au-4ATPSAM/PAV/PEI/GOx/AuNPs | 240 ± 20 | 0.9      | 4                 |
| 32         | Nylon 6,6/PBIBA        | MWCNTs         | Graphite/Nylon6,6/PBIBA/MWCNTs GOx | 60 ± 10 | 9        | 44                |
| 38         | PVA-SbQ                | MWCNT-COOHs    | Au/PVA-SbQ/MWCNT-COOHs/GOx | 250 ± 50 | 2        | 5                 |
| This Work  | PVDF/PEDOT:PSS         | No             | Au/PVDF/PEDOT:PSS/GOx       | 100 ± 50 | 5        | 60                |
onto the nanofibers by electrophoretic deposition, which helped to facilitate direct electron transfer and the GOx immobilization with this nanofibrous electrode surface.\textsuperscript{17} Hence, the conductive properties of the glucose sensor electrode were significantly enhanced by decorating the nanofibers with gold nanoparticles and CNTs and thus the sensitivity of the sensor. The glucose biosensors exhibited good operational stability and repeatability with low LOD. It should also be noted that a dispersion of nanoparticles into nanofibers may have a mass transfer rate limit and are less likely to be recyclable (impacting sustainability), and the nanofibers may tend to aggregate causing the sensor to lose detection functionality. Therefore, further exploration of conductive polymers, as another alternative for nanofibrous glucose sensing, is needed.

**Future Scope of Conductive Polymer Based Electrospun Nanofibers for Glucose Sensors**

The present work focuses on the simple cost-effective process (electrospinning) for fabricating a nanofibrous membrane glucose biosensor with high sensitivity, repeatability and durability. Nanofibrous membranes have attracted much attention in the development of biosensors because of the physical entrapment of GOx within their porous structure. Using the conductive polymer, poly(3,4-ethylenedioxythiophene):poly(styrene sulfonate) (PEDOT:PSS),\textsuperscript{35} to electrospin a membrane-based sensor electrode created better binding of the bioenzyme (glucose oxidase). The conductivity of PEDOT is enhanced significantly by doping with poly (styrene sulfonate) (PSS), and PEDOT: PSS thin-films have been studied widely, due to higher enzyme immobilization capability, good electrical conductivity, better processability, excellent electrochemical stability, reliability, and biocompatibility,\textsuperscript{6,36,37} and this is some of the first work reported on a PEDOT: PSS nanofiber glucose biosensor. The electrochemical behavior of a PEDOT: PSS nanofiber membrane electrode was studied for the detection of glucose. The results show the potential advantages of the nanofiber electrodes for biosensing and an effective measurement of glucose concentration. Chronoamperometric analysis is used to demonstrate the relationship between the current and glucose concentration as shown in Fig. 3a inset.

Electrospun nanofibrous membranes were obtained by electrospinning a homogeneous polymeric solution of polyvinylidene difluoride (PVDF)/Tetrahydrofuran (THF)/PEDOT:PSS. A fibrous morphology was depicted in scanning electron microscopy (SEM) scans and atomic force microscopy (AFM) micrographs (Fig. 3b (i) and (ii)). The average fiber diameters (AFD) were in the range of 100–150 nm with minimal abnormal features, such as beads, in the fibers. The fibers had a random orientation and were several micrometers in length. A high quality nanofibrous morphology for a PVDF/PEDOT:PSS membrane created a high surface area to volume ratio for the immobilization of GOx. Figure 3b (iii) and (iv) show the SEM and AFM micrograph images after loading the enzyme onto the PVDF/PEDOT:PSS nanofibrous membrane, where the fiber-like morphology of PVDF/PEDOT:PSS shows signs of swelling. The electrospun conductive polymer membrane helps in binding the GOx enzyme with the gold electrode, which helps the durability and sensitivity of the glucose sensor. The conductive polymer membranes reduce the leaching of enzyme molecules from the sensing electrode and act as a mediator for immobilization. The porous nanofibrous matrix of PVDF/PEDOT:PSS was completely covered by a layer of GOx. The porous large surface area enabled diffusion of the GOx to the surface of the nanofibrous membrane and further enhanced the detection mechanism of this nanofibrous sensor.

Focusing on electrospun nanofibrous composite membranes, the results in comparison Table 1 were selected based on the use of CNTs, CNFs, conductive NPs and conductive polymers for enzymatic glucose sensors. All of the nanofibrous glucose sensors demonstrate electrochemical enzymatic glucose sensing promoted by the use of conductive materials. Limited data on the durability of these nanofibrous composite membranes for glucose sensing will prolong the commercialization of these devices. The significance of the current work on the conductive polymer based glucose sensing electrode using PVDF/PEDOT:PSS shows an approximate 11% change in current response over 60 days (Fig. 3c), compared to 4-44 days for the comparative studies. Therefore, as new nanomaterials are investigated for electrospun nanofibrous membranes, the testing of these sensors should include extensive electrochemical analysis to predict sensor durability. In addition, recent progress in ENFM based glucose sensors encourages the application of conductive nanoparticles\textsuperscript{14,34} and versatile configurations e.g. yarnd fibers, coaxial fibers, and hollow core/shell fibers.\textsuperscript{13} Therefore, the promising result using PVDF/PEDOT:PSS ENFM based sensing electrodes can be enhanced by adding nanoparticles with different configurations and characterizations, in terms of Raman spectroscopy, X-ray diffraction (XRD), and fluorescence, in future work.

**Conclusions**

In this perspective paper, we have reported the various nanofibrous structured based glucose sensors and the potential of these structures to enhance the electrochemical response for glucose detection. A fibrous morphology of a conductive polymer indicated high distribution of GOx onto the electrospun nanofibrous matrix. The electrospun nanofibers used as a unique sensing electrode matrix to facilitate GOx immobilization. The conductive polymer-based nanofibers, presented as a membrane, greatly promoted the electron-transfer between GOx and the sensor electrode. The very high surface area of the nanofibers, together with many active binding sites, creates a favorable environment for the adsorption of enzymes. Nanofibrous membranes show increased stabilization of enzymatic activity and limit leaching of enzyme molecules in the electrolyte solution during electroactive analysis. The improved durability, repeatability and stability of electrospun nanofibrous based sensing electrodes suggests continued research on nanofiber structures for effective miniaturization of glucose biosensors. Looking ahead, coaxial (core–shell) nanofibrous composite structures for enzymatic activity may expand the sensor functionality, reduce leaching due to controlled material environment, and enable further integration of nanofibrous enzymatic biosensors. In addition, the development of batch fabrication for scaling-up of electrospun nanofibrous glucose sensors will be an important challenge to address in the commercialization of these devices.

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