Fabrication of Nitric Oxide-Releasing Porous Polyurethane Membranes Coated Needle-Type Implantable Glucose Biosensors

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Abstract

The active release of pharmaceutical agents and the use of porous sensor membranes represent the two most promising strategies for addressing the poor tissue biocompatibility of implantable glucose biosensors. Herein, we describe the combination of these approaches to create nitric oxide (NO)-releasing porous fiber mat-modified sensor membranes. An electrospinning method was used to directly modify needle-type glucose biosensors with the NO donor-loaded fibers. The resulting NO-releasing fiber mat (540 ± 139 nm fiber diameter, $94.1 \pm 3.7\%$ porosity) released ~100 nmol NO per mg polyurethane over 6 h while maintaining a porous structure without leaching of the NO donor, even in serum. The porous fiber membrane did not influence the analytical performance of the biosensor when 50 μm thick.

Introduction

Percutaneously implanted electrochemical biosensors for continuous glucose monitoring (CGM) hold great potential for reducing complications of diabetes due to their ability to warn of hyperglycemia or hypoglycemia events.[1, 2] Limitations such as short sensor life (1 week), the need for frequent calibration (2–4 times/day), and unreliable accuracy in the data provided have prevented wide spread use of such devices to date.[1, 3, 4] Most shortcomings of CGM systems are due to the foreign body response (FBR).[5] Inflammatory cell response and collagen capsule formation, and the risk of infection due to percutaneous implantation result in poor analytical performance in vivo.[2] Recent work has focused on the development of outer membranes to mitigate the FBR and improve tissue integration and in vivo sensor performance.[2, 6]

Nitric oxide (NO)—an endogenously produced diatomic free radical—plays a number of physiological roles (e.g., angiogenesis, wound healing, and vasodilation) depending on release location and concentration.[7, 8] To utilize NO as a pharmaceutical agent, we and others have developed macromolecular NO-release scaffolds using *N*-diazeniumdiolate or *S*-nitrosothiol NO donors that enable controlled NO release.[8, 9] As biomaterials, NO-releasing interfaces have been shown to decrease the adhesion of inflammatory cell, reduce collagen capsule thickness, and increase blood vessel formation near the biomaterial.[10, 11] Additionally, the NO-releasing surfaces have proven to be highly effective at reducing bacteria adhesion, eradicating biofilms, and decreasing implant-associated infections.[12–15]

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Release of NO from glucose sensor membranes has been studied as an active strategy to mitigate the FBR and thus improve sensor performance in vivo by addressing the greatest shortcoming of such devices.[16] Nitric oxide release has been imparted to glucose sensors membranes by patterning NO-releasing xerogel array on top of a membrane[17] or physically entrapping NO donors (e.g., NO-releasing silica particles[18, 19] or low molecular weight molecules[20]) within polyurethane (PU) sensor membranes. Such NO-releasing membranes exhibited adequate analyte diffusion without compromising sensor performance.[18, 19] Gifford et al. reported that NO fluxes >0.83 pmol cm^{-2} s^{-1} for 18 h from a needle-type glucose biosensors were sufficient to reduce inflammation and enhance the sensor accuracy at 24 h post implantation compared to control sensors in a rodent model.[20]

Surface modifications based on porous architectures have also been developed and represent a passive strategy for improving tissue integration and decreasing collagen capsule formation.[21–24] Electrospun fibers appear promising as glucose sensor membranes because of their high surface to volume ratio and superior mechanical properties.[25] Additionally, electrospun fibers are able to be directly coated onto electrodes without impeding glucose oxidase activity since the polymer fibers solidify as the solvent evaporates during the electrospinning process.[26] Random oriented fibers with submicron diameter (~0.6 μm) have been reported to be effective at reducing secretion of proinflammatory molecules, decreasing the adhesion of foreign body giant cells, and promoting cell migration and proliferation—all resulting in reduced capsule formation.[25, 27–29] Wang et al. recently reported coating PU electrospun fibers on implantable glucose sensors using a dynamic collector, highlighting the ability to modify microsensors with porous membranes.[26] While electrospun fibers allow for the encapsulation and release of therapeutics in a controlled manner,[27, 30, 31] the use of drug-releasing electrospun fibers for implantable glucose sensors has not yet been reported.

Herein, we report the design of NO-releasing dendrimer-doped electrospun PU fibers as porous outermost glucose sensor membranes as depicted in Figure 1. The characterizations of needle-type glucose biosensors fabricated using such interfaces were demonstrated the ability to combine both active (i.e., NO-release) and passive (i.e., porous) strategies within a single outer sensor membrane coating.

Experimental

Materials

Glucose oxidase (type VII from *Aspergillus niger*), D-glucose anhydrous, acetaminophen (AP), L-ascorbic acid (AA), and uric acid (UA) were purchased from Sigma (St. Louis, MO). All salts and organic solvents were laboratory grade and purchased from Fisher Scientific (St. Louis, MO). Methyltrimethoxysilane (MTMOS) and phenol were purchased from Fluka (Buchs, Switzerland). Tecophilic (HP-93A-100) and Tecoflex (SG-85A) polyurethanes were gifts from Thermedics (Woburn, MA). Hydrothane (AL 25–80A) polyurethane was a gift from AdvanSource Biomaterials Corporation (Willmington, MA). Tecoplast (TP-470) polyurethane was a gift from Lubrizol (Cleveland, OH). Stainless steel wire (316L, 381μm diameter) was purchased from McMaster-Carr (Atlanta, GA). Polyterafluoroethylene (PTFE) coated-Pt/Ir (90% Pt) wire (177.8 and 246.4 μm bare and coated diameter, respectively) and silver wire (Ag 99.99%, 127 μm diameter) were purchased from Sigmund Cohn Corp. (Mount Vernon, NY). Nitrogen, argon, and nitric oxide calibration gases were purchased from Airgas National Welders Supply (Durham, NC). Anticoagulated whole blood with 5 U/mL heparin was obtained from healthy pigs at the Francis Owen Blood Research Laboratory (Chapel Hill, NC). Serum was obtained from this sample by centrifuging the blood for 15 min under 2000 ×*g* at 4 °C. Water was purified

(18.2 MΩ·cm; total organic content <6 ppb) using a Millipore Milli-Q Gradient A-10 purification system (Bedford, MA).

Nitric oxide-releasing dendrimer-doped polyurethane solution

1,2-Epoxy-9-decene (ED) functionalized fourth-generation of poly(amidoamine) (PAMAM) dendrimers (PAMAM G4-ED/NO) were synthesized as previously reported.[32] Subsequent *N*-diazeniumdiolation of the PAMAM G4-ED was performed as described previously.[32, 33] *N*-diazeniumdiolation of the 1,2-epoxy-9-decene (ED) functionalized fourth-generation poly(amidoamine) (PAMAM) dendrimers (PAMAM G4-ED) was performed under high pressure of NO (10 atm) for 3 d in the presence of sodium methoxide at room temperature with constant stirring. The NO donor-modified dendrimers were then stored in a methanol solution with sodium methoxide at −20 °C until use. The NO-release properties of the dendrimers and dendrimer-doped polyurethane electrospun fiber mat were then evaluated using both chemiluminescence and Griess assay. Nitric oxide release was measured using a Sievers Chemiluminescence Nitric Oxide Analyzer (NOA) Model 280i (Boulder, CO). To determine NO flux, electrospun samples were placed in a solution of deoxygenated PBS (0.01 M, pH 7.4) at 37 °C. The NO was carried to the NOA instrument by a stream of N_2 gas at a controlled rate (200 mL min^{-1} instrument collection rate). The NOA was calibrated using a 26.80 ppm NO gas (balance N_2) and air passed through a Sievers NO zero filter. Total amounts of NO released were determined indirectly by measuring the nitrite concentration via the Griess assay using a Labsystem Multiskan RC Microplate Spectrophotometer (at 540 nm; Helsinki, Finland). Of note, total NO concentrations measured with the Griess assay agreed with those obtained from chemiluminescence analysis indicating that these materials released NO and not nitrite.

Fabrication of needle-type glucose sensors

Needle-type glucose sensors were fabricated by adapting a procedure described previously.[20, 34–36] A silver/silver chloride (Ag/AgCl) wire electrode was fabricated by winding silver (Ag) wire on stainless steel wire (2.5 cm length). The wire was converted to AgCl via galvanometry by soaking in saturated ferric chloride (FeCl$_3$) solution for >2 h. After sonication in water and ethanol for 15 min each, the Ag/AgCl wire was removed from the stainless steel and wrapped around a polytetrafluoroethylene (PTFE)-coated Pt/Ir wire (3.5 cm length). A section of the PTFE coating (~5 mm) was then stripped from the end of wire to expose the sensing cavity. The end of the sensor was sealed with epoxy, resulting in a ~2 mm sensing cavity (surface area ~0.017 cm^2). As shown in Figure 1, the sensing cavity consisted of four functional layers: (1) an inner-selective membrane, that excludes electroactive interferents such as ascorbic acid (AA), uric acid (UA), and acetaminophen (AP); (2) a glucose oxidase layer, where glucose is converted to gluconolactone and oxygen to hydrogen peroxide; (3) a diffusion limiting layer that controls the diffusion and partitioning of glucose and oxygen to overcome the oxygen deficit in vivo; and, (4) a porous NO-releasing electrospun fibrous membrane designed to mitigate the FBR. The inner-selective polyphenol layer was applied under control of self-limiting electropolymerization (film thickness of ~10–100 nm) to prevent interfering species from reaching or fouling the electrode surface, affording a great improvement in biosensor selectivity over acetaminophen in particular (see SI Figure 4). The electrochemical polymerization of the permselective polyphenol films was carried out potentiostatically at +900 mV (vs. Ag/AgCl) for 15 min in 40 mM phenol solution in phosphate buffered saline (PBS, pH 7.4) after degassing with nitrogen for 20 min. The charge passed during electropolymerization was −0.53 ± 0.27 μC. A glucose oxidase (GOx) layer was then applied on top of the inner-selective membrane by immobilizing GOx within a sol-gel matrix. The enzyme-containing sol was prepared as described previously.[18, 19] The needle-type electrodes were dip-coated 15 times (with a single cycle consisting of a 5 s dip with 10 s drying under ambient

conditions). The enzyme layer contained an excess of glucose oxidase so that the rate of glucose conversion was only dependent on glucose concentration. The diffusion-limiting layer was added by dip-coating the electrodes twice in a polyurethane (PU) polymer solution consisting of 80 mg mL^{-1} 50% (w/w) Tecoflex and Hydrothane dissolved in 50% (v/v) THF/EtOH solution. Each layer was dried under ambient conditions for 30 min before casting the subsequent layer. The thickness of the diffusion-limiting PU layer was 28.2 ± 13.0 μm, allowing for a dynamic glucose response range over the entire physiological range of blood glucose concentrations (1–30 mM). The outermost NO-releasing electrospun porous polyurethane membranes were coated onto the needle-type glucose sensors using a custom electrospinning apparatus consisting of a Series 205B High Voltage Power Supply (Bertan Associates, Inc.; Hicksville, NY), a Kent Scientific Genie Plus syringe pump (Torrington, CT), and a rotating collector.[31] A schematic of the set-up and the electrospinning parameters employed are provided in Supporting Information (SI Figure 1 and SI Table 1). Polyurethane solutions containing NO-releasing dendrimers were prepared by first dissolving the PU in 1.6 mL of a 3:1 (v/v) tetrahydrofuran (THF):*N,N*-dimethylformanide (DMF) mixture, followed by the addition of PAMAM G4-ED/NO solution (400 μL dissolved in methanol). The final concentration of polymer in solution was 12–16% (w/v). Dendrimer concentration is reported as wt% of the polymer mass.

Electrochemical sensor performance

The analytical performance of the fabricated glucose biosensor was electrochemically evaluated via constant potential amperometry using a CH Instruments 1030A potentiostat (Austin, TX). Sensor performance was evaluated in 0.01 M PBS (pH 7.4) or porcine serum at room temperature or 37 °C using an integrated Ag/AgCl wire as a pseudo counter and reference electrode. After the background current was polarized, the sensitivity, response time, and selectivity of the biosensors were evaluated by sequentially injecting aliquots of glucose or interferent solutions into PBS or serum with constant stirring. A working electrode potential of +600 mV vs. Ag/AgCl was employed. This applied potential allows adequate glucose sensitivity and minimal current interference from NO oxidation (+900 mV vs. Ag/AgCl).[18] The sensitivity to glucose was determined based on the slope of the linear regression between the measured oxidation current and glucose concentration. The amperometric selectivity coefficient ($logK_{glu}^{amp}$) was calculated according to equation (1) where ΔI_{glu} and ΔI_j are the changes in measured current responses to predetermined concentrations of glucose (c_{glu}) and interference species (c_j; j=AP, AA, and UA), respectively.

$$\log K_{glu}^{amp} = \log\left(\frac{\Delta I_j / c_j}{\Delta I_{glu}/c_{glu}}\right) \quad (1)$$

Characterization of NO-releasing dendrimer-doped electrospun fiber membrane-coated needle-type glucose sensors

Fiber diameter and percent porosity were determined using an environmental scanning electron microscope (ESEM; Quanta 200 field emission gun; FEI company; Hillsboro, OR) without additional metal coating. Fiber diameters were determined using NIH ImageJ software (Bethesda, MD). The percent porosity of the fibrous membrane was calculated according to the following equation where ρ is the density of the electrospun fiber mats, and ρ$_0$ is the density of the bulk polymer.[23, 37, 38]

$$\text{Porosity } (\%) = \left(1 - \frac{\rho}{\rho_0}\right) \times 100\% \quad (1)$$

Nitric oxide release from the fabricated electrospun fiber mat was evaluated as described above.[31, 39] The stability and distribution of the dendrimers within the PU fibers was assessed using fluorometry and confocal microscopy.[33] Before modifying the PAMAM dendrimers with ED functional groups, dendrimers were tagged with rhodamine isothiocyanate (RITC) in a 1:1 molar ratio so that on average only one of the 64 primary amines on the dendrimer surface was modified. After fluorescently labeled dendrimer-doped fiber membranes were incubated in PBS for 7 d at 37 °C, the fluorescence of the soak solution was measured using Cary Eclipse fluorescence spectrometer (Varian Inc., Palo Alto, CA). The fluorescence intensity represented the degree of dendrimer leaching. In addition to this stability test, confocal fluorescence microscopy was used to image the distribution of fluorescently labeled PAMAM G4-ED/NO dendrimer in the electrospun fibers with an LSM 510 laser scanning confocal microscope (Carl Zeiss, Thornwood, NY).

Results and Discussion

Characterization of NO-releasing dendrimer-doped electrospun polyurethane fibers

Non-porous PU membranes have been employed to fabricate implantable glucose sensors in order to balance the diffusion ratio of glucose and oxygen, and prevent an oxygen deficit.[20, 40, 41] Generally, such polymers are also considered tolerable by the body. Nevertheless, the immune response and subsequent collagen encapsulation that forms around the sensor and PU membrane still negatively impacts in vivo glucose sensor performance.[42] To potentially reduce the foreign body response (FBR) and improve the analytical performance of CGM sensors,[2, 6] NO donor-containing PU fibers were electrospun onto enzyme-based needle-type glucose sensors as an additional outermost porous membrane on top of the standard diffusion-limiting PU layer to create a porous interface capable of releasing a therapeutic agent known to mitigate the FBR (Figure 1).

Prior to bench top sensor testing, the *N*-diazeniumdiolated dendrimer-doped electrospun fibrous PU membranes were characterized with respect to their physical properties (e.g., fiber diameter and porosity), NO release, and stability (i.e., dendrimer leaching). Scanning electrochemical microscopy imaging of the cross section of the fully fabricated glucose sensor confirmed a tree dimensional outer membrane, highlighting the ability to successfully coat porous electrospun fibers onto needle-type biosensors (Figure 2B). As shown in Figure 2, the electrospun membranes exhibited a random open pore structure with interconnected fibers and no bead formation over the entire electrode surface (1.4 ± 0.7 mg/sensor). Water uptake by the fibers, important for both glucose response and NO release, varied based on the type of PU (4.7 ± 1.0, 1.6 ± 0.2, and 0.8 ± 0.5 mg of water/mg of PU fiber for Tecophilic, Tecoflex and Tecoplast, respectively). Fiber diameters ranging from 400–600 nm were achieved by varying the concentration of PU solution (12–16% (w/v)). Of note, such diameters were previously shown to reduce both the adhesion and activation of macrophage cells, suggesting a lessened initial FBR.[28] The presence of the NO donor-modified dendrimer did not significantly affect fiber diameter. The percent porosity of the fibrous membranes for fiber diameters ranging from 400–600 nm was 67.3 ± 4.4, 57.6 ± 2.5, and 94.1 ± 3.7 % for the Tecophilic, Tecoflex, and Tecoplast fibers, respectively. Such porosity is in line with prior reports describing the use of highly porous interfaces (>90% porosity) to increase cell infiltration and reduce collagen capsule formation (i.e., thickness).[23, 29] Based on the above data and the prior tissue biocompatibility literature, the Tecoplast fiber mat would likely be most effective at reducing the FBR.

Nitric oxide release from PAMAM G4-ED/NO dendrimer-doped electrospun fiber membranes was measured in PBS (pH 7.4) at 37 °C to mimic physiological conditions (SI Figure 2). The Tecoplast electrospun fiber membrane doped with 5 wt% PAMAM G4-ED/NO dendrimer exhibited a maximum NO flux of 19.8 ± 6.4 pmol mg^{-1} s^{-1} with 0.10 ± 0.08 μmol mg^{-1} total NO released over ~7 h. The time to reach maximum NO flux was 2.2 ± 0.3 min. As expected, the total NO released was proportional to the amount of dendrimer embedded within the fibers. For example, the total amount of NO released from the Tecoplast PU fibers was increased from 0.10 ± 0.08 to 0.27 ± 0.12 μmol mg^{-1} upon altering the NO donor (PAMAM G4-ED/NO) concentration from 5 to 10 wt%. Surprisingly, the NO release (kinetics) was not altered based on water uptake by the PU. Although the use of more hydrophobic PU (i.e., Tecoplast) resulted in a slightly suppressed maximum NO flux, the duration of NO release was similar to the NO-releasing dendrimer alone (Table 1). Such behavior is attributed to the porous structure of the electrospun fiber mat and/or the distribution of dendrimers within the fibers.[31] Although the duration of release was not significantly extended in this case, co-axial electrospun fibers consisting of an NO-releasing core and distinct water-restricting hydrophobic polymer sheath may further extend the NO-release duration. Such studies are currently in progress. Based on the most ideal fiber characteristics (i.e., diameter, porosity, and NO release), Tecoplast was chosen as the polymer system for studies demonstrating the ability to directly modify already fabricated glucose biosensors with a porous outer membrane.

Rhodamine isothiocyante (RITC) fluorophore-tagged dendrimers were used to evaluate the distribution and stability of NO donor. Confocal microscopy confirmed that the fluorescent NO-releasing PAMAM G4-ED dendrimers were homogeneously dispersed within individual fibers (Figure 2D) and throughout the entire fiber mat. Stability of the NO-releasing dendrimer within the fibers was determined using a leaching assay. Fibers doped with fluorescently modified dendrimers were immersed in PBS (pH 7.4 at 37 °C) for 7 d. Fluorescence emission (at 570 nm) of the soak solution was then measured using the fluorometer. These soak solutions did not fluorescence indicating that the leaching concentrations were less than the limit of detection (i.e., 0.03 μg/mL). Indeed, leaching of the dendrimer NO-release vehicles from the fibers were negligible (<0.5%).

To assess the structured stability of the three-dimensional porous membranes, the electrospun fiber-coated glucose biosensors were soaked in PBS and porcine serum for 7 d at 37 °C. The Tecoplast electrospun fiber membrane (characterized by the lowest water uptake) maintained its porous structure after soaking in PBS for >7 d. In contrast, the Tecophilic membrane (highest water uptake) exhibited reduced porosity due presumably to the swelling of the fibers (data not shown). The topography of the Tecoplast electrospun fiber mat-modified sensor was constant even after 1 week incubation in porcine serum (SI Figure 3). Of note, the structural stability of the porous membranes suggests the potential for in vivo use.[43]

Electrochemical performance of electrospun membrane coated glucose biosensor

The analytical response of the fabricated NO-releasing electrospun fiber-modified needle-type glucose sensors was characterized in vitro as a function of PU composition to assess the impact of the fiber mat (Table 1). Neither the electrospinning process nor NO release affected the sensor performance. Sensor response was studied as a function of the electrospun membrane thickness using the most hydrophobic PU (i.e., Tecoplast). Adjusting the feed volume of the polymer solution during the electrospinning process allowed us to vary the fiber mat thickness. Greater feed volumes resulted in thicker fiber mats (44.5 ± 12.2, 71.5 ± 19.1, and 241.5 ± 84.3 μm for 1, 2, and 3 mL, respectively) that were no longer useful as sensor membranes (Figure 3). While the addition of 50 μm thick electrospun fiber outer membranes enables both a rapid glucose response (~1 min response time; $t_{95\%}$) and

suitable dynamic range, thicker (>50 μm) fiber mat membranes exhibited response similar to non-porous hydrophobic PU sensor membranes ($t_{95\%}$= ~125–250 s). Subsequent sensor performance testing was thus carried out using biosensors fabricated with thin (~45 μm) electrospun fiber mat membranes.

Glucose sensor performance was also evaluated as a function of PU fiber composition and water uptake. Regardless of PU fiber composition, the dynamic response range of all sensors was 1–30 mM glucose as would be expected due to the diffusion-limiting non-porous PU membrane layer beneath the porous fiber mat. This diffusion-limiting layer also dictated the glucose sensitivity of the biosensors. The use of a lower water uptake PU (i.e., Tecoplast) fiber mat as the outermost porous membrane resulted in decreased sensor response to glucose and a longer response time (Table 1). While Tecophilic PU had a negligible effect on sensitivity and response time, the more hydrophobic PU (i.e., Tecoplast) reduced sensor response to glucose by ~30% while increasing response time ($t_{95\%}$) from 15.9 ± 9.0 to 76.4 ± 21.7 s. Of note, the addition of the more hydrophobic porous membrane neither compromised the selectivity nor dynamic range.

Despite the diminished sensor performance, the sensitivity of the resulting glucose biosensor coated with the dendrimer-doped Tecoplast electrospun fiber (16% (w/v) with 5 wt% PAMAM G4-ED/NO dendrimer) was 2.4 ± 1.6 nA/mM (Figure 4), a response comparable to commercially available and previously published implantable glucose biosensors.[40, 44] The amperometric selectivity coefficients of the biosensors were –0.73 ± 0.85, 0.96 ± 0.10, and 0.28 ± 0.14 for ascorbic acid, acetaminophen, and uric acid, respectively, indicating that the fiber mat-modified biosensors were sufficiently selective for glucose over interferences at physiologically relevant concentrations. To mimic a more physiologically relevant solution, the sensor response was also evaluated in porcine serum at 37 °C, resulting in sensitivity of 2.15 ± 0.60 nA/mM. In addition to a comparable sensitivity to the PBS studies above, the biosensors remained functional for at least a week in serum at 37 °C (data not shown). While bench-top evaluation of the sensor performance suggests a viable device, additional in vivo experiments are obviously necessary to understand the benefits of porosity and NO release on analytical performance. Planning of such studies is currently underway.

Conclusion

Porous fiber mats capable of NO release may reduce the undesirable FBR associated with sensor implantation in subcutaneous tissue. Herein, a facile electrospinning method was developed to modify needle-type electrochemical biosensors with thin, NO-releasing porous PU membranes. Importantly, sensor performance was not diminished with the additional NO-releasing membrane, suggesting similar fibrous coatings could be applied to other tissue-contacting devices with minimal impact to device utility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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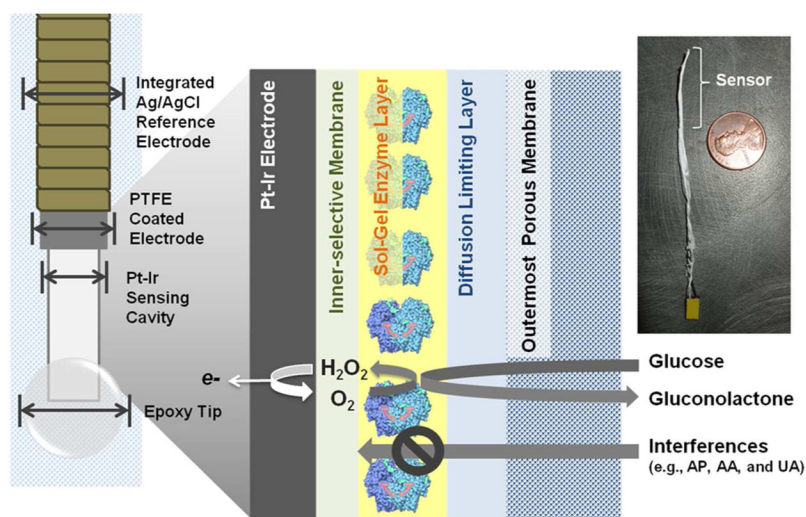44. Klonoff DC. Diabetes Care. 2005; 28:1231–1239. [PubMed: 15855600]

Figure 1.
Schematic of the layer-by-layer structure of NO-releasing porous electrospun fiber membrane coated needle-type glucose sensors with actual fabricated electrode shown on the right.
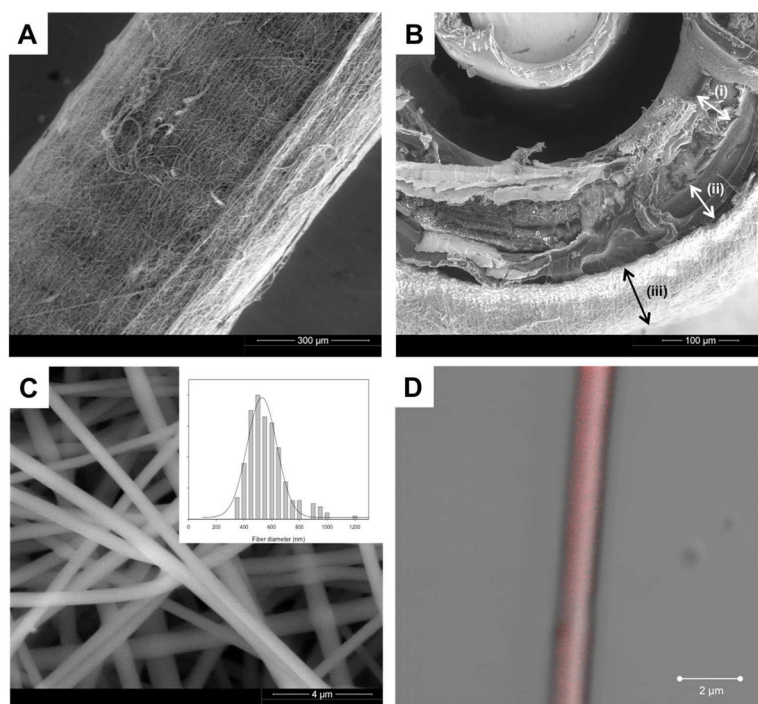
Figure 2.
Environmental scanning electron microscope (ESEM) images of (A) the surface of the NO-releasing dendrimer-doped electrospun polyurethane membrane-modified needle-type glucose sensors; with (B) cross-sectional view indicating (i) sol-gel/glucose oxidase enzyme layer, (ii) the diffusion-limiting non-porous polyurethane layer, and (iii) the electrospun fiber porous membrane (mat); (C) higher magnification view of the electrospun fiber mat with inset indicating fiber diameter size distribution (540 ± 139 nm); and (D) confocal image of a single fiber containing RITC-labeled dendrimer.
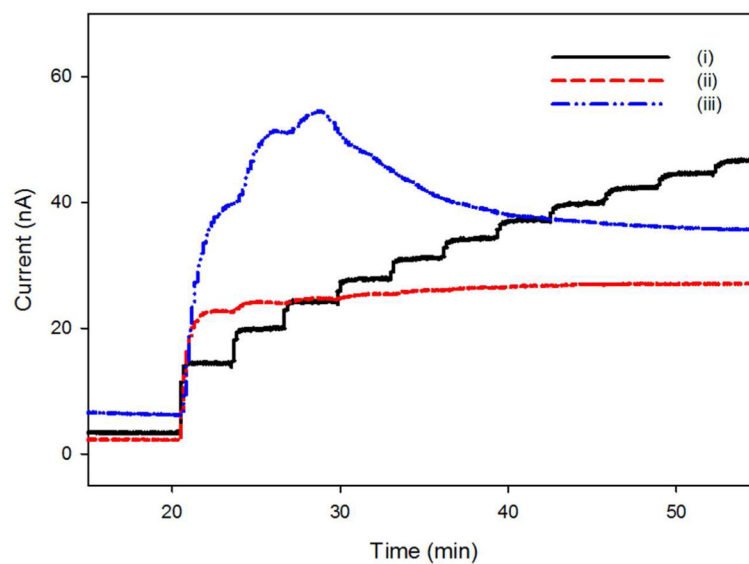
Figure 3.
Amperometric response of needle-type glucose sensors coated with NO-releasing dendrimer (PAMAM G4-ED/NO)-doped membranes at a fiber mat thickness of (i) 45 to (ii) 72 and (iii) 242 μm. The glucose concentration was changed at 3 mM increments.
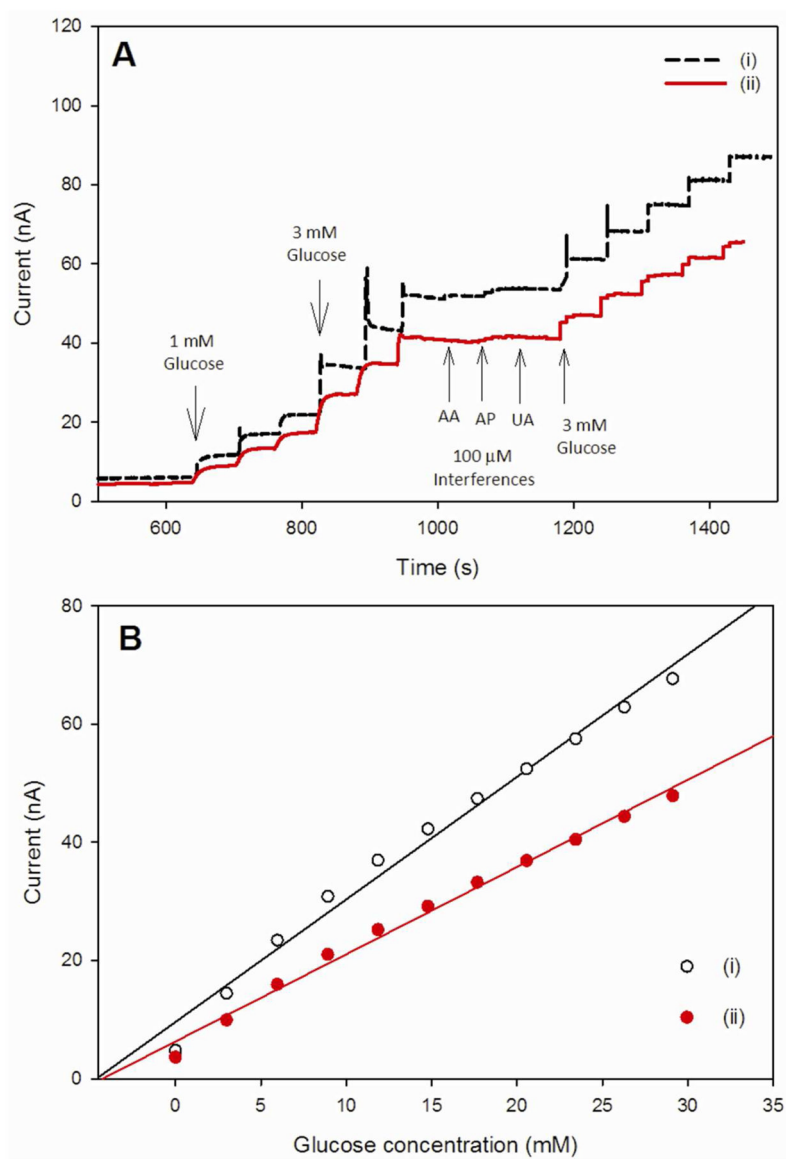
Figure 4.
(A) Amperometric response of a needle-type glucose sensor for (i) control (i.e., without) and (ii) 5 wt% NO-releasing dendrimer-doped 16% (w/v) Tecoplast electrospun fiber-modified outermost membrane. Glucose, ascorbic acid (AA), acetaminophen (AP), and uric acid (UA) were sequentially injected at relevant in vivo concentrations. (B) Calibration curves of (i) control and (ii) porous electrospun fiber membrane-modified glucose sensors in pH 7.4 PBS. The applied electrode potential was +600 mV vs. Ag/AgCl.

Table 1

Characterization of NO release and glucose sensor performance for the porous NO-releasing electrospun fiber-modified needle-type glucose sensors as a function of the fiber. All porous membranes were doped with 5 wt % *N*-diazeniumdiolated dendrimers (PAMAM G4-ED/NO).

Type of polyurethane[a]		Tecophilic (HP-93A-100)	Tecoflex (SG-80A)	Tecoplast (TP-470)
PU solution concentration[b] (% (w/v))		12	12	16
Physical properties of outermost membrane	Water uptake (mg, H_2O/mg, Fiber mat)	4.7 ± 1.0	1.6 ± 0.2	0.8 ± 0.5
	Percent porosity (%)	67.3 ± 4.4	57.6 ± 2.5	94.1 ± 3.7
	Fiber diameter (nm)	567 ± 288	404 ± 190	540 ± 139
Sensor performance	Glucose Sensitivity (nA mM^{-1})	4.1 ± 3.8	3.0 ± 2.0	2.4 ± 1.6
	Response time ($t_{95\%}$; s)	32.1 ± 7.2	43.3 ± 12.7	76.4 ± 21.7
NO release properties	$[NO]_{max}$ (pmol mg^{-1}s^{-1})	52.0 ± 26.1	24.4 ± 8.2	19.8 ± 6.4
	Total NO released[c] (μmol mg^{-1})	0.15 ± 0.02	0.14 ± 0.03	0.10 ± 0.01
	t_d (h)[d]	7.3 ± 3.6	8.9 ± 1.6	6.7 ± 1.0

[a] The water uptake of polyurethanes was described previously.[18]

[b] Concentration of polymer solution was optimized to achieve 400–600 nm fiber.[28]

[c] Total NO released as determined using the Griess assay.

[d] Experimentally defined at time point of 99% NO released.