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Original article

Improved thromboresistance and analytical performance of intravascular amperometric glucose sensors using optimized nitric oxide release coatings

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ABSTRACT

In this work, nitric oxide (NO) release coatings designed for intravenous amperometric glucose sensors are optimized through the use of a polylactic acid (PLA) layer doped with a lipophilic diazeniumdiolated species that releases NO through a proton-driven mechanism. An Elast-Eon E2As polyurethane coating is used to both moderate NO release from the sensor surface and increase the sensor's linear detection range toward glucose. These sensors were evaluated for thromboresistance and *in vivo* glucose performance through implantation in rabbit veins. By maintaining NO flux on a similar scale to endogenous endothelial cells, implanted glucose sensors exhibited reduced surface clot formation which enables more accurate quantitative glucose measurements continuously. An *in vivo* time trace of implanted venous sensors demonstrated glucose values that correlated well with the discrete measurements of blood samples on a benchtop point-of-care sensor-based instrument. The raw measured currents from the implanted glucose sensors over 7 h time periods were converted to glucose concentration through use of both a one-point *in vivo* calibration and a calibration curve obtained *in vitro* within a bovine serum solution. Control sensors, assembled without NO release functionality, exhibit distinctive surface clotting over the 7 h *in vivo* implantation period.

1. Introduction

Accurate monitoring and control of blood glucose play critical roles in the treatment of patients with diabetes mellitus. Benchtop point-of-care devices are the current standard for many hospitals and medical care facilities, and finger prick glucometers are widely used for personal and home glucose measurements. While these devices yield discrete blood glucose values, trends of rapidly increasing/decreasing glucose concentrations often go unobserved when such devices are used. Some commercially available devices provide continuous glucose monitoring of interstitial fluid using implanted electrochemical sensors. However, due to the ~10–15 min lag time between changes in blood glucose concentration and changes in interstitial fluid glucose [1], in practice these devices can only supplement, rather than fully replace, discrete blood measurements.

Intravenous amperometric glucose sensors may provide a better alternative platform for continuous blood glucose measurements, especially within a hospital environment. Indeed, tight glycemic control is a requirement for many patients in intensive care units (ICUs) to achieve targeted treatment and better patient outcomes. The amperometric glucose sensors described in this work are designed to be implanted intravenously in critical care patients through existing IV port access or inserted into a catheter. This continuous measurement would provide medical staff with the ability to see trends in rising and falling blood glucose, allowing them to select better treatment options. Miniaturized electrochemical blood glucose sensors have been previously reported in literature; however, these sensors can quickly lose analytical accuracy due to thrombus formation on their surfaces when placed within the bloodstream of hospitalized patients [2,3]. *Via* the formation of thrombus with encapsulated platelets, the local glucose concentration near the surface of the sensor can be

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reduced by metabolic activity of platelets and other trapped cells, and therefore, the sensor reads a false glucose value compared to the level of glucose within the plasma phase of the blood. The well-known antithrombotic and anti-inflammatory properties of nitric oxide provide a useful method of potentially enhancing the hemocompatibility of a blood-contacting surface. Incorporating NO releasing donor molecules into a sensor's outer polymeric coatings allows it to mimic the functions of endothelial cells lining the inner walls of all blood vessels, which endogenously release NO at localized fluxes of $(0.5–4.0) \times 10^{-10}$ mol cm^{-2} min^{-1} [4]. This approach has previously been shown to reduce clot formation on the surface of intravenous glucose sensors and preserve their *in vivo* performance [5,6]. Herein, we extend this earlier work by further optimizing the NO release formulation and the outer glucose restriction layer used to prepare such devices to achieve more optimal linearity and *in vivo* performance.

2. Experimental

Glucose oxidase (Type VII, From *Aspergillus niger*), D-(+)-glucose, glutaraldehyde (25%), bovine serum albumin (BSA), bovine serum (sterile-filtered), sodium chloride, potassium chloride, sodium phosphate dibasic heptahydrate, potassium phosphate monobasic dihydrate, iron (III) chloride ($FeCl_3$), 37% hydrochloric acid (HCl), L-ascorbic acid, uric acid, Nafion (5 wt% solution in a lower aliphatic alcohols/H_2O mix), *m*-phenylenediamine, resorcinol, and tetrahydrofuran (THF) were purchased from Sigma–Aldrich (St. Louis, MO). An ester-end capped polylactic acid material (PLA, 100 DL 7E) was purchased from Evonik (formerly Lakeshore Biomaterials, Birmingham, AL). E2As Elast-Eon thermoplastic polyurethane was a gift from AorTech International, plc (Scoresby, Victoria, Australia). Diazeniumdiolated *N*,*N*′-dibutyl-1,6-hexanediamine (DBHD) or DBHD/N_2O_2 was synthesized by treating DBHD with 80 psi NO gas purchased from cryogenic gases (Detroit, MI) at room temperature for 24 h, as previously described [7]. All amperometric measurements were collected with a 4-channel ESA BioStat potentiostat instrument.

Glucose sensors were constructed based on previous designs [5,6,8,9] by first cutting a 1-mm length cavity in the Teflon coating of the platinum/iridium wire (outer diameter = 0.2 mm). A Nafion coating was applied to the cavity, and then *via* a CV electropolymerization process (cycling voltage between 0 and +830 mV at 2 mV s^{-1} for 18 h) a layer of polymerized resorcinol and *m*-phenylenediamine [10,11] was applied to the cavity to help reject electroactive interference species, such as ascorbic acid, uric acid, and acetaminophen from reaching the Pt/Ir surface. A silver/silver chloride (Ag/AgCl) wire electrode was tightly wrapped around the sensor to serve as an electrochemical reference, and heat-shrinkable polyester tubing was applied to secure the reference wire in place (Fig. 1a). Glucose oxidase was then immobilized within the cavity using glutaraldehyde. Outer layers were then applied to the sensor's surface using a wire loop: first, an ester-capped polylactic acid layer containing diazeniumdiolated dibutylhexyldiamine (2:1, wt/wt) (Fig. 1c) was applied to give the sensor NO releasing behavior, and then a 2% (wt/vol) Elast-Eon E2As polyurethane in THF (Fig. 1b) was applied as a top coat to modulate the NO release and also control the sensor's linear detection range for glucose by restricting glucose diffusion into the enzyme layer. E2As polyurethane was previously used with positive success as an NO release coating to prevent platelet activation and clotting within a rabbit model for extracorporeal circulation [12]. A diagram of the fully-assembled sensor is shown in Fig. 1a. The control sensors were prepared by similar procedure except that no diazeniumdiolated dibutylhexyldiamine was doped within the PLA coating.

3. Results and discussion

These sensors were soaked in phosphate buffered saline (PBS) or bovine serum at physiological conditions (pH 7.4, 37.5 °C) to hydrate the polyurethane layer for restricted glucose diffusion and

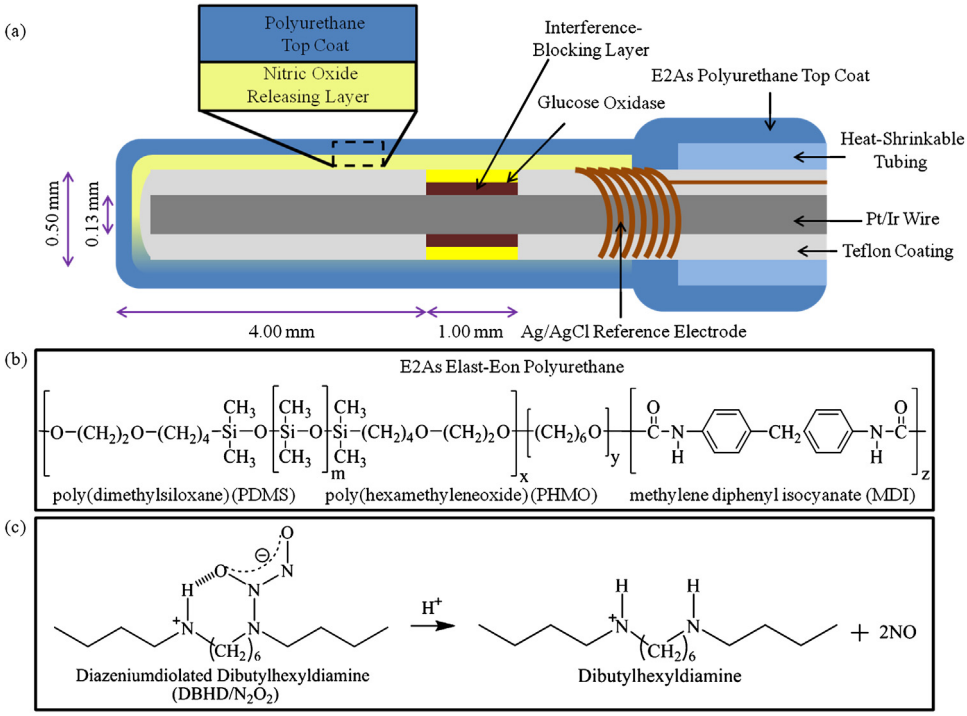

Fig. 1. (a) Needle/catheter type glucose sensor design; (b) E2As Elast-Eon polyurethane used for the sensor outer layer; (c) lipophilic diazeniumdiolated dibutylhexyldiamine and the proton-driven mechanism for nitric oxide release.