Published in final edited form as:

Biosens Bioelectron. 2011 July 15; 26(11): 4276–4282. doi:10.1016/j.bios.2011.04.026.

Intravascular Glucose/Lactate Sensors Prepared with Nitric Oxide Releasing Poly(lactide-co-glycolide)-Based Coatings for Enhanced Biocompatibility

Qinyi Yan¹, Terry C. Major², Robert H. Bartlett², and Mark E. Meyerhoff¹

Mark E. Meyerhoff: mmeyerho@umich.edu

¹Department of Chemistry, The University of Michigan, Ann Arbor, MI 48109-1055

²Department of Surgery, The University of Michigan, Ann Arbor, MI 48109-1055

Abstract

Intravenous amperometric needle-type enzymatic glucose/lactate sensors intended for continuous monitoring are prepared with a novel nitric oxide (NO) releasing layer to improve device hemocompatibility. To create an underlying NO release coating, the sensors with immobilized enzymes (either glucose oxidase or lactate oxidase) are prepared with a thin layer of poly(lactide-co-glycolide) (PLGA) loaded with lipophilic diazeniumdiolate species that slowly release NO via a proton driven reaction. An outer thin layer (ca. 30 μm) of PurSil (polyurethane/dimethylsiloxane copolymer) limits the flux of glucose and lactate to the inner layer of enzyme, to provide the desired linear amperometric response. A 30 μm coating of PLGA containing 33 wt% of the appropriate NO donor (*N*-diazeniumdiolated dibutylhexanediamine, DBHD/ N_2O_2) can release NO at a physiologically relevant rate $> 1 \times 10^{-10} \text{ mol min}^{-1} \text{ cm}^{-2}$ for at least 7 d without influencing the analytical performance of the glucose/lactate sensors. *In vitro*, the sensors exhibit relatively stable amperometric response over a one-week period with high selectivity over interferences (e.g., ascorbic acid) required for blood monitoring applications. Glucose sensors implanted in the veins of rabbits for 8 h exhibit significantly enhanced hemocompatibility for the NO release sensors vs. corresponding controls (without NO release in same animals), with greatly reduced thrombus formation on their surfaces. Further, the analytical performance of the NO release glucose sensors are superior to controls placed in the veins of the same animals, with a greater accuracy in measuring blood glucose levels as evaluated using a Clark error grid type analysis.

Keywords

intravascular glucose/lactate sensor; nitric oxide release; enhanced blood-compatibility

1. Introduction

There are more than 285 million people currently diagnosed with diabetes in the world, according to the statistics from the World Diabetes Foundation (<http://www.worlddiabetesfoundation.org/composite-35.htm>), and the number is increasing

© 2011 Elsevier B.V. All rights reserved.

Correspondence to: Mark E. Meyerhoff, mmeyerho@umich.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

every year. For the past few decades, the dominant method for monitoring blood glucose, whether at home or in the hospital environment, has been intermittent measurement of capillary blood glucose, usually coupled with finger pricking. However, such measurements only provide isolated single-time point measurements that cannot fully reflect variations throughout the day and night. While there has recently been great progress in the development of subcutaneous implantable electrochemical glucose sensors that provide real time monitoring capability (Waeger and Hummel, 2008), there remain some issues regarding the lag time in response between changes in blood glucose levels and subcutaneous fluid concentrations, and requirements for frequent recalibration with blood levels. For critically ill hospital patients, in particular, there is growing evidence that tight control of blood glucose levels can be of benefit not only to diabetic patients, but also non-diabetics (Bochicchio and Scalea, 2008; Kondepoti and Heise, 2007; Lonergan et al., 2006; Schetz et al., 2008; Vanhorebeek et al., 2007). In this environment, intravenous sensor placement would be the preferred mode to gain the most accurate assessment of real-time blood glucose levels.

Beyond glucose, the accurate monitoring of blood lactate levels is also of great biomedical importance in the critical care setting. Lactate plays a vital role in several biochemical processes that are involved in muscle movement, and it is the key metabolite of the anaerobic glycolytic pathway. In fact, levels of lactate in blood are now considered one of the most important indicators of the status of critically ill patients, with continuously elevated levels considered a key prognosticator of poor outcome. Hence, for such patients, the ability to monitor lactate continuously via an intravenous catheter at the patient's bedside would provide a means to assess whether prescribed clinical therapies are working to save the patient (Arnold et al., 2009; Holloway et al., 2001; Shapiro et al., 2010; Shapiro et al., 2005a; Shapiro et al., 2005b; Valenza et al., 2005).

A number of miniaturized electrochemical blood glucose and lactate sensors have been reported in the literature (Bindra et al., 1991; Hu et al., 1993; Yang et al., 1999). Both glucose and lactate measurements are based on immobilized enzymes (glucose oxidase and lactate oxidase, respectively) and the liberation of hydrogen peroxide from these enzymatic reactions with subsequent oxidation of H_2O_2 at an underlying electrode surface. The resulting anodic current is proportion to the concentration of glucose or lactate in the sample. Several commercial continuous glucose monitoring (CGM) systems based on subcutaneous devices have been developed for diabetic patients. Girardin, et al. recently reviewed both the biochemical perspectives and clinical applications of these designs (Girardin et al., 2009). However, the development of intravenous sensors that can function reliably for extended periods after implantation remains technologically challenging largely due to the biocompatibility issues that arise. When the sensor is implanted in the blood stream, especially within a readily available vein, thrombus formation can occur within minutes, triggered by platelet activation, adhesion and fibrin entanglement with blood cells. Similarly, implanted devices designed for subcutaneous measurements are also subject to biocompatibility issues, such as an inflammatory response that leads to a fibrotic encapsulation of the devices within a sheath of leukocytes, macrophages, fibroblasts, etc. Both situations disturb the physiological environment of the implantation site and may lead to false values of local glucose or lactate concentrations and/or decrease mass transfer of the analyte to the immobilized enzyme layer of the device. Either case can result in an unreliable analytical output which limits the lifetime and wide clinical use of such implantable sensors (Frost and Meyerhoff, 2002).

To overcome these hurdles, many researchers have focused on developing more biocompatible polymers for biomedical sensor applications. Several proposed approaches include the use of hydrogels, surfactants and others in the effort to minimize sensor

biofouling (Wisniewski and Reichert, 2000). Because protein adsorption is the first step in eventual formation of thrombus on the surface of intravascular sensors, decreasing protein adsorption should improve blood compatibility. Unfortunately, none of the approaches that are intended to prevent protein adsorption completely eliminate thrombus in low flow blood vessels or fully prevent subcutaneous inflammatory responses when tested *in vivo* (Frost and Meyerhoff, 2006).

The discovery of the anti-platelet and anti-inflammatory properties of nitric oxide (NO) has provided a new direction for research aimed at solving the fundamental biocompatibility problem of implanted sensors, as well as other blood contacting medical devices (Fleser et al., 2004; Frost et al., 2003; Frost and Meyerhoff, 2004; Frost et al., 2005; Frost et al., 2002; Gifford et al., 2005; Oh et al., 2005; Shin et al., 2004; Wu and Meyerhoff, 2008; Wu et al., 2007b). Indeed, NO is well-known as a potent inhibitor of platelet function (D'Atri et al., 2009; Riddell and Owen, 1999). In fact, NO is produced within endothelial cells (EC) that line the walls of all healthy blood vessels, and serves to prevent clotting by inhibiting platelet activation. This led to the idea of coating intravascular-implanted sensors with polymers that can release or generate NO at physiological concentrations so that adhesion of cells (platelets) and concomitant thrombus formation at the surface of the sensor can be reduced (Frost et al., 2005). Further, NO also inhibits inflammatory response (reduces neutrophil and macrophage migration to the implant site), and promotes angiogenesis (growth of new blood vessels). These are both important effects that could greatly improve the biocompatibility of sensors implanted subcutaneously.

Recent research in this laboratory (Fleser et al., 2004; Frost and Meyerhoff, 2004; Frost et al., 2005; Frost et al., 2002; Wu et al., 2007a; Wu et al., 2007c) and elsewhere (Major et al., 2010; Oh et al., 2005; Skrzypchak et al., 2007) has shown that polymers capable of releasing $1\text{--}15 \times 10^{-10} \text{ mol min}^{-1} \text{ cm}^{-2}$ of NO at the polymer/blood interface can effectively decrease the platelet adhesion and thrombus formation on the surfaces of implanted devices. Further, working in collaboration with Dr. Wilson's group at the University of Kansas, our laboratory demonstrated that electrochemical glucose sensors prepared with NO releasing outer polymeric membranes greatly decreased the *in vivo* inflammatory response during the initial subcutaneous implantation in rats (when compared to control sensors implanted in the same animals), without degrading the analytical performance of the sensor itself (Gifford et al., 2005). Schoenfisch and coworkers also modified miniaturized electrochemical glucose sensors with NO releasing xerogels and observed an improvement in biocompatibility over a 3 d period by analyzing the cell adhesion (Oh et al., 2005). To date, there have been no reports on the use of NO releasing glucose sensors for making improved intravascular measurements, or the adaptation of this concept for preparing lactate sensors with improved *in vivo* biocompatibility. Herein we report results of efforts in this direction that make use of a newly formulated NO release coating that consists of a lipophilic diazeniumdiolate species (*N*-diazoniumdiolated dibutylhexanediamine, DBHD/ N_2O_2) embedded within a layer of poly(lactide-*co*-glycolide) (PLGA), which is then covered by a outer layer of PurSil (polyurethane/dimethylsiloxane copolymer). DBHD/ N_2O_2 readily releases NO upon contact with water by a proton driven mechanism (Batchelor et al., 2003). PLGA undergoes a slow hydrolysis process to generate lactic acid and glycolic acid (Schwendeman, 2002). Therefore, the use of PLGA as a matrix for the diazeniumdiolate NO donor provides an added source of protons to promote extended NO release, without the need for using tetraphenylborate derivative additives as described in previous work (Batchelor et al., 2003), the presence of which was found herein to inhibit enzymatic activity of both glucose and lactate oxidases.

2. Materials and methods

2.1 Materials

Glucose oxidase (Type VII, From *Aspergillus niger*), d-(+)-glucose, glutaraldehyde, bovine serum albumin (BSA), sodium chloride, potassium chloride, sodium phosphate dibasic, potassium phosphate monobasic, iron (III) chloride (FeCl_3), 37 % hydrochloric acid (HCl), cellulose acetate, methanol, L-lactate, L-ascorbic acid, uric acid, Nafion (5 wt % solution in a lower aliphatic alcohols/ H_2O mix), 1, 3-diaminobenzene, resorcinol, tetrahydrofuran (THF), dimethylacetamide (DMAc), *N,N'*-dibutyl-1,6-hexanediamine (DBHD), polyethylenimine (PEI) and poly(DL-lactide-*co*-glycolide) 50:50 (PLGA, RESOMER[®] RG 502 H) were purchased from Sigma-Aldrich (St. Louis, MO). Platinum/iridium (Pt/Ir) and silver (Ag) wires were products of A-M Systems (Sequim, WA). Lactate oxidase was obtained from Genzyme (Cambridge, MA). Potassium tetrakis(4-chlorophenyl)borate (KTPCIPB) was from Fluka (Ronkonkoma, NY). PurSil 20 80A was from the Polymer Technology Group (Berkeley, CA). 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 (Batchelor et al., 2003).

2.2 Fabrication of NO releasing glucose/lactate sensors

The design of the needle-type glucose sensor was based on previous publications (Bindra et al., 1991; Gifford et al., 2005). Briefly, a 1-mm cavity was cut at 4 mm from one tip of a 10-cm-long Teflon-coated Pt/Ir wire of 0.2 mm outer diameter (see Fig. 1). A 15-cm, 0.1-mm o.d. silver/silver chloride (Ag/AgCl) wire was tightly wrapped around the sensor starting 1.5 mm above the opening covering a length of 4 mm. The Ag/AgCl wire was prepared by dipping the Ag wire into the FeCl_3/HCl solution. The straight section above the wrapped Ag/AgCl wire was covered with a 5-cm long, 0.4-mm o.d., heat shrinkable polyester tubing (Advanced Polymers, Salem, NH). The 1-mm opening of the sensor was dip-coated with a thin layer of Nafion. Then, an in-situ electropolymerization coating was applied using a Voltammograph potentiostat (Bioanalytical Systems Inc., W. Lafayette, IN) cycling voltage between 0 to +830 mV at 2 mV/s for 18 h with 1,3-diaminobenzene and resorcinol (Geise et al., 1991) to enhance selectivity over interferences that are electroactive, e.g., ascorbate, urate, and acetaminophen. One μL of glucose oxidase solution with BSA was then dropped in the cavity along the wire. This layer was dried for 30 min and then crosslinked by adding 1 μL of 2% (vol/vol) glutaraldehyde solution and allowing it to cure in air for 1 h. The sensor was then rinsed with water, and allowed to dry for 1 h. NO release sensors were first loop cast with 2:1 (wt/wt) PLGA and DBHD/ N_2O_2 in THF and then ca. 4% (wt/vol) PurSil solution in 1:1.5 DMAc and THF. Control sensors were first coated with PLGA solution in THF without the diazeniumdiolate added, and then coated with the outer PurSil solution layer.

The fabrication of lactate sensor was the same to that of glucose sensor, except that the enzyme solution was lactate oxidase stabilized with 0.7% of PEI, and the crosslinking time was 2 h instead of 1 h.

Glucose and lactate sensors were calibrated on a 4-channel potentiostat BioStat (ESA Biosciences Inc., Chelmsford, MA). The sensors were tested at an applied potential of +600 mV vs. Ag/AgCl reference in 0.1 M phosphate buffer saline (PBS), pH 7.4 at 37°. The buffer was purged with 10 % oxygen in nitrogen (Cryogenic Gases, Detroit, MI) to ensure that the sensors can provide a linear response to 15 mM glucose under such low oxygen tension (levels closer to *in vivo* conditions).

2.3 Nitric oxide release *in vitro*

Nitric oxide released from both glucose and lactate sensors was monitored via chemiluminescence with a Seivers Nitric Oxide Analyzer (NOA) 280 (Boulder, CO). The sensors were immersed into 0.1 M PBS, pH 7.4, at 37° and the NO flux data was collected. Long-term NO release was monitored over 7-d period by collecting data for one hour each day. The sensors were soaked in PBS at 37° with continuous nitrogen purging to ensure that all sensors release NO under the same conditions as when measured NO flux using the NOA.

2.4 *In vivo* protocol of evaluation of both sensor performance and biocompatibility

A total of 15 white rabbits (Myrtle's Rabbitry, Thompson's Station, TN) were used in this study to test the glucose sensors only. A protocol as described elsewhere in detail was followed for the *in vivo* experiments except with the maintenance fluid rate adjusted to 3.3 mL/kg/min (Major et al., 2010). One control (without NO release) and two NO release glucose sensors were implanted in the anesthetized rabbit veins for 8 h. The electrochemical response of each sensor to blood glucose was monitored via the current output of the sensors. To calibrate the sensors and evaluate the analytical performance regarding sensing glucose, 0.6 mL blood was drawn every 30 min to test the blood glucose level using a 700 Series Radiometer blood analyzer (Radiometer America Inc., Westlake, OH). The blood glucose level measured *in vitro* on the Radiometer instrument after 1 h of sensor implantation was used to set a one point calibration for each of the implanted sensors (Choleau et al., 2002a, b), and the continuous output thereafter was compared to the other intermittent blood glucose values. After 8 h, the sensors were explanted and the thrombus formation on each device was documented via digital photography. For a quantitative analysis of the thrombus formation, Image J software (<http://rsbweb.nih.gov/ij/index.html>) was used to measure the area of the red pixels (thrombus) from the photos, and the results of the control and NO release sensors were compared.

3. Results and discussion

3.1 Prolonged NO release using PLGA

Initially, diazeniumdiolated dibutylhexanediamine (DBHD/N₂O₂) was doped as the NO donor in the outer polymeric PurSil layer of the glucose sensor, similar to the approach reported previously for subcutaneous glucose sensors experiments (Gifford et al., 2005). Potassium tetrakis(4-chlorophenyl)borate (KTPCIPB) was first used as a lipophilic counter ion to stabilize the pH level within the NO release polymers as free amines are produced after NO release. This increase in the basic environment further slows the decomposition of the remaining diazeniumdiolates to release NO; however, as shown previously, the addition of the borate derivative helps buffer the organic phase, keeping the pH lower (Batchelor et al., 2003) (via proton exchange with potassium ion of the borate salt, and borate then becomes the counteranion to the protonated amine of DBHD). Unfortunately, when either glucose oxidase or lactate oxidase were in contact with the membrane containing the borate derivative for some time (days), both enzymes lost essentially all activity. Control experiments were performed by fabricating glucose sensors that were coated with polymers containing only DBHD/N₂O₂, only borate and both. Results showed that glucose sensors with only DBHD/N₂O₂ maintained their catalytic activity, while the sensors with borate or with both species lost response to glucose. This result suggests that the borate derivative and not the NO releasing diazeniumdiolate deactivated the enzyme preventing the response of the glucose sensor when using outer NO release membranes containing these species.

To overcome the borate deactivation effect on the enzyme activity we attempted to coat the sensors with an NO releasing PurSil layer, but excluding the sensing area where the

enzymes are immobilized, with the hope that the NO release from surrounding area could prevent thrombus formation on this small opening. However, a preliminary *in vivo* study showed that thrombus formation still occurred precisely on the 1-mm opening not covered by NO releasing material after 8 h of implantation in rabbit veins. As a result, a substitute for the borate, which can preserve the enzyme activity, was needed to prolong the NO release time while also still exhibiting a similar function to control the pH of the organic layer containing DBHD/N₂O₂, that would prolong the NO release from the coating.

PLGA is a widely used biodegradable and biocompatible polymer which has been approved by the U.S. Food and Drug Administration (FDA) in numerous products, especially in drug delivery systems (Schwendeman, 2002). PLGA is a candidate to substitute for the toxic tetraphenylborate species to create NO release coating because PLGA undergoes a slow hydrolysis to produce lactic acid and glycolic acid, and thus can provide an acidic micro-environment ideally suited for proton driven NO release from diazeniumdiolates. As the PLGA is hydrolyzed, the lactic and glycolic acids can protonate the free amines produced in the NO release process from the DBHD/N₂O₂ species; thus long-term NO release can be ensured without influencing enzyme activity. As a result, PLGA was examined as the base material to create a new NO releasing inner polymer coatings for both glucose and lactate sensors. As hypothesized, PLGA did not have any negative influence in either the glucose or lactate oxidase activities.

Figure 2 (a) shows the NO release of glucose/lactate sensors over one week period of time using PLGA as the matrix in which the DBHD/N₂O₂ is embedded. The NO flux reached an average maximum of $24 \times 10^{-10} \text{ mol min}^{-1} \text{ cm}^{-2}$ approximately 30 min after immersion in PBS. The NO release then decayed exponentially, but continued for more than 7 d at a rate $> 1 \times 10^{-10} \text{ mol min}^{-1} \text{ cm}^{-2}$, a flux similar to the NO flux produced by endothelial cells in cell culture (Vaughn et al., 1998). This NO release result is similar to that observed when using tetraphenylborate derivatives as an additive in the PurSil polymer matrix (Fig. 2(b)). On the other hand, with the same amount of DBHD/N₂O₂ alone doped into the PurSil polymer without the tetraphenylborate or PLGA, the maximum flux was only $3 \times 10^{-10} \text{ mol min}^{-1} \text{ cm}^{-2}$ and the NO flux diminished to $< 1 \times 10^{-10} \text{ mol min}^{-1} \text{ cm}^{-2}$ within several hours (Fig. 2 (b)). Hence, using a matrix that contained all PLGA for DBHD/N₂O₂ and a top coating of PurSil, provides a bilayer coating that releases NO for more than one week without influencing the enzyme activity. This is a much longer period than the previously proposed NO release glucose sensors (Gifford et al., 2005; Shin et al., 2004). It should be noted that both the inner PLGA/DBHD/N₂O₂ coatings and the top PurSil layer were estimated to be ca. 30 μm thick, respectively, based on a cross-sectional image obtained by a Hitachi S-3200N Scanning Electron Microscope (SEM).

3.2 *In vitro* performance of NO releasing glucose/lactate sensors

In addition to biocompatibility, any enzyme-based sensor aimed at real biological applications must have reasonably stable sensitivity, a wide enough linear range for blood measurements, good response times and an acceptable selectivity over naturally existing interferences. The *in vitro* normalized sensitivity of NO releasing glucose sensors (n=4) over one week period of time is shown in Figure 3 (a). The sensitivity percentage was calculated from dividing the sensitivity on each day by that on day 7. As is shown in Fig. 3 (a), the sensitivity increased during the first 3 d because of the conditioning of outer polymeric coatings (water uptake), and remained relatively stable thereafter. The NO releasing glucose sensors showed a stable sensitivity from 1.9 to 3.7 nA/mM, had a linear range from 0 to over 15 mM under a 10% level of oxygen ($p\text{O}_2$ of 70 mmHg) which is close to the venous blood oxygen levels, and the steady-state current was reached within 5 minutes. The repeatability of the NO release glucose sensors was tested by inserting the device into 5 mM of glucose solution 5 separate times, with washing and stabilizing the baseline in between these

multiple measurements. The glucose concentrations were back-calculated using the calibration curve plotted earlier. The sensor showed an acceptable repeatability with an average of 5.19 ± 0.53 mM measured for the 5 measurements.

Figure 3 (b) shows the stability of NO release lactate sensors ($n=3$) over one week in terms of the normalized sensitivity. The sensitivity decreased slightly over time, which is possibly due to the gradual loss in enzyme activity. Indeed, lactate oxidase is known to be a less stable enzyme than glucose oxidase (Lillis et al., 2000). NO releasing lactate sensors exhibited a stable sensitivity range from 4.9 to 7.2 nA/mM, had a linear range from 0 -10 mM lactate and the response time was less than 2 minutes. Both NO releasing glucose and lactate sensors showed acceptable *in vitro* analytical performance over 7-day period, similar to the designs proposed earlier (Bindra et al., 1991; Hu et al., 1993), and were viewed as potentially useful for *in vivo* measurement.

When NO release was first introduced to create an implantable glucose sensor, there was some concern about an increase in the background current (due to oxidation of NO at the platinum anode) that could add inaccuracy to the glucose measurement, especially in the case of using a one-point calibration for the *in vivo* application. From the *in vitro* calibration results, NO releasing glucose sensors that possess a flux of ca. 4×10^{-10} mol min⁻¹ cm⁻² had an average background current of 2.3 ± 0.9 nA ($n=4$) which was only slightly higher than the control sensors without NO release, that had an average of 1.2 ± 0.7 nA ($n=4$). However, NO releasing sensors had a sensitivity range from 1.9 to 3.7 nA/mM, so the signal-to-background ratio at normal glucose levels (5.5 mM) was between 5 – 10. Furthermore, the background of NO releasing glucose sensors in the blood stream was expected to be lower than tested *in vitro*, as there are many biological NO scavengers, such as oxyhemoglobin that exist in the blood, that will decrease the flux of NO toward in the inner platinum working electrode of the intravascular sensor. As a result, the background current contributed from NO release is negligible for intravascular applications, especially for the glucose sensor with sensitivity > 2 nA/mM. The NO contribution to the lactate sensor background was greater than glucose sensor because normal lactate levels can be < 0.5 mM. Nevertheless, for real measurements, especially for patients in the ICU with an elevated lactate levels > 2 mM, the current from the NO background is also < 20% of the signal that would occur at these levels of lactate. Although undesirable, a two-point calibration can be performed when the sensor background from NO release is contributing significantly to the sensor signal.

When PLGA was first applied to the NO releasing lactate sensor, there was a concern that the lactic acid produced would also increase the background signal. However, the results showed that the increase in background signal was from NO rather than lactate, as the background was similar to that of the NO releasing glucose sensor with the same level of NO release. Indeed, the degradation timeframe of the PLGA 50:50 with an internal viscosity of 0.16 – 0.24 dL/g is known to be 2 to 4 weeks (<http://www.surmodicsbiomaterials.com/products-stock-polymers.html>), so the hydrolysis of the PLGA layer coated onto sensors under the PurSil layer occurs at a very slow rate producing very low steady-state levels of lactic acid (likely in the nanomolar range) in the sensing layer of the device, while the physiological levels of lactic acid are in the millimolar range. As a result, PLGA is suitable to use to prolong NO release on lactate sensors without adding inaccuracy to the analytical performance.

The NO release sensors also need to have excellent selectivity over naturally existing interferences such as ascorbic acid, uric acid and other neutral molecules such as acetaminophen, in order to be considered for real clinical application. The electropolymerization of 1, 3-diaminobenzene and resorcinol was applied as the inner layer

onto the sensing area and the selectivity results over such molecules are shown in Table 1. Using the NO releasing glucose sensor for an example, the % error was calculated by dividing the current from the maximum possible *in vivo* level of interferences (0.5 mM for ascorbic acid and 0.2 mM for acetaminophen) by the signal from 5 mM of glucose. Even though the selectivity decreased within a one-week period, the % error remained < 3% for ascorbic acid and < 7% for acetaminophen. This indicated that the sensor could retain a reasonable selectivity over major interferences for at least one week during the NO release time.

3.3 Blood-compatibility and surface thrombus evaluation

All the control and NO releasing glucose sensors were conditioned in the PBS buffer for 3 days before the implantation in rabbit veins to ensure stable sensitivity of glucose sensors during the *in vivo* experiments. For NO releasing sensors, the NO flux was checked by the NOA both before and after the *in vivo* implantation, and the results were similar to the *in vitro* NO release profile, as shown in Fig. 2 (a). The NO releasing glucose sensors were capable of maintaining a steady-state surface flux of $> 4 \times 10^{-10} \text{ mol min}^{-1} \text{ cm}^{-2}$ during the entire 8 h of the experiment. Figures 4 (a) and (b) show photos of control and NO releasing glucose sensors explanted from rabbit veins after 8 h of implantation. Photos (a) and (b) were two individual rabbits with 3 sensors implanted in each. As can be seen from the photos, the bottom 2 NO releasing sensors in both (a) and (b) showed minimal thrombus formation on the surface, while the one control sensor (top) without NO release formed obvious thrombus on the surface. The copolymer of polyurethane and siloxane, PurSil, with blank underlying PLGA layer, is clearly thrombogenic. Using the ImageJ software, a more detailed statistical analysis of thrombus formation was performed. Figure 4 (c) shows the overall thrombus formation result comparison between NO releasing (n=30) and control glucose sensors (n=15). Although the deviations were comparatively large, the mean values are statistically different, with NO releasing glucose sensors forming considerably less thrombus than control sensors without NO release ($p < 0.05$). These results confirm the expected antithrombus function of NO being emitted from the surface of the glucose sensors at a surface flux of $> 4 \times 10^{-10} \text{ mol min}^{-1} \text{ cm}^{-2}$. This new NO release system using PLGA can be further extended to other device applications in the biomedical field.

3.4 *In vivo* glucose sensor performance

The implanted glucose sensors were calibrated by a bench top commercial Radiometer instrument (blood-gas/electrolyte/metabolite analyzer) using a one-point calibration method described earlier. The calculated blood glucose values of the implanted glucose sensors were also correlated to the Radiometer readings. There was a strong correlation between readings from the *in vivo* and *in vitro* devices, with the correlation coefficient between the glucose readings from the sensors and that from the Radiometer being 0.7742 ($p < 0.05$) (n=30) for 15 separate *in vivo* sensor experiments.

From the Clarke error grid (see Fig. 5), the NO releasing glucose sensors exhibited more accurate detection of glucose in the blood stream than the control sensors. NO releasing sensors had 97.5% of data points in Zone A (clinically accurate zone) and Zone B (benign error zone with no clinical consequences) (Fig. 5 (a)), where measurement results are accepted as accurate, while control sensors had 86.7% (Fig. 5 (b)). This could be due to the fact that the thrombus formation on the implanted control sensors disturbed local glucose diffusion, leading to false current output for a given level of glucose in the blood stream. The NO releasing sensors remained relatively accurate in reporting blood glucose levels as a result of NO down-regulating the platelet activity, thus eliminating thrombus formation. Furthermore, the thrombus formed on the surface of the implanted control devices could

block blood flow within the vessel, which would have a negative effect on the local implant site, and ultimately dangerous for the patient.

4. Conclusions

Nitric oxide releasing glucose and lactate sensing catheters have been successfully fabricated that exhibit NO release for more than one week at levels that attenuate thrombus formation using a new PLGA-based coating containing a lipophilic diazeniumdiolate NO donor species. The analytical performance of both the glucose and lactate sensors is not influenced significantly by the NO release or PLGA degradation products. The sensors exhibit relatively stable amperometric response over a one-week period with high selectivity over interferences, making them suitable for blood monitoring applications. An *in vivo* study (intravenous) of glucose sensors prepared in this manner showed that this coating exhibits very good anti-thrombus function for the implanted sensors, while not disturbing the glucose sensing function. NO release glucose sensors implanted in the veins of rabbits for 8 h showed greatly reduced thrombus formation on their surfaces compared to corresponding controls. Further, improved accuracy in reporting blood glucose levels of NO release glucose sensors is achieved vs. controls as evaluated using a Clark error grid type analysis. Although the lactate sensor reported here has not yet been evaluated for *in vivo* performance, the data on this sensor reported herein marks the first time that a lactate sensor has been formulated with an NO release coating. Thus the data presented in this paper for the lactate sensor demonstrates that NO release chemistry does not interfere with the lactate oxidase chemistry. While used here only for preparing glucose and lactate catheter style electrochemical sensors, the new NO release coating using PLGA has prospective applications as a coating for many other blood-contacting biomedical devices.

Acknowledgments

This work was supported by a grant from the National Institutes for Health (Grant EB-000783).

References

- Arnold RC, Shapiro NI, Jones AE, Schorr C, Pope J, Casner E, Parrillo JE, Dellinger RP, Trzeciak S. Emergency Med Shock Res Network, E.M. Shock. 2009.; 32(1):35–39. [PubMed: 19533847]
- Batchelor MM, Reoma SL, Fleser PS, Nuthakki VK, Callahan RE, Shanley CJ, Politis JK, Elmore J, Merz SI, Meyerhoff ME. J. Med. Chem. 2003; 46(24):5153–5161. [PubMed: 14613318]
- Bindra DS, Zhang YN, Wilson GS, Sternberg R, Thevenot DR, Moatti D, Reach G. Anal. Chem. 1991; 63(17):1692–1696. [PubMed: 1789439]
- Bochicchio GV, Scalea TM. Serono. Sym. 2008; 42:261–275.
- Choleau C, Klein JC, Reach G, Aussedat B, Demaria-Pesce V, Wilson GS, Gifford R, Ward WK. Biosens. Bioelectron. 2002a; 17(8):641–646. [PubMed: 12052349]
- Choleau C, Klein JC, Reach G, Aussedat B, Demaria-Pesce V, Wilson GS, Gifford R, Ward WK. Biosens. Bioelectron. 2002b; 17(8):647–654. [PubMed: 12052350]
- D'Atri LP, Malaver E, Romaniuk MA, Pozner RG, Negrotto S, Schattner M. Curr. Med. Chem. 2009; 16(4):417–429. [PubMed: 19199914]
- Fleser PS, Nuthakki VK, Malinzak LE, Callahan RE, Seymour ML, Reynolds MM, Merz SI, Meyerhoff ME, Bendick PJ, Zelenock GB, Shanley CJ. J. Vasc. Surg. 2004; 40(4):803–811. [PubMed: 15472611]
- Frost M, Meyerhoff ME. Anal. Chem. 2006; 78(21):7370–7377. [PubMed: 17128516]
- Frost MC, Batchelor MM, Lee YM, Zhang HP, Kang YJ, Oh BK, Wilson GS, Gifford R, Rudich SM, Meyerhoff ME. Microchem. J. 2003; 74(3):277–288.
- Frost MC, Meyerhoff ME. Curr. Opin. Chem. Biol. 2002; 6(5):633–641. [PubMed: 12413548]
- Frost MC, Meyerhoff ME. Oxygen Sensing. 2004:704–715.

- Frost MC, Reynolds MM, Meyerhoff ME. *Biomaterials*. 2005; 26(14):1685–1693. [PubMed: 15576142]
- Frost MC, Rudich SM, Zhang HP, Maraschio MA, Meyerhoff ME. *Anal. Chem*. 2002; 74(23):5942–5947. [PubMed: 12498188]
- Geise RJ, Adams JM, Barone NJ, Yacynych AM. *Biosens. Bioelectron*. 1991; 6(2):151–160.
- Gifford R, Batchelor MM, Lee Y, Gokulrangan G, Meyerhoff ME, Wilson GS. *J. Biomed. Mater. Res. A*. 2005; 75A(4):755–766. [PubMed: 16138325]
- Girardin CM, Huot C, Gonthier M, Delvin E. *Clin. Biochem*. 2009; 42(3):136–142. [PubMed: 18951887]
- Holloway P, Benham S, St John A. *Clin. Chim. Acta*. 2001; 307(1–2):9–13. [PubMed: 11369330]
<http://www.surmodicsbiomaterials.com/products-stock-polymers.html>.
<http://www.worlddiabetesfoundation.org/composite-35.htm>.
- Hu YB, Zhang YN, Wilson GS. *Anal. Chim. Acta*. 1993; 281(3):503–511.
- Kondepati VR, Heise HM. *Anal. Bioanal. Chem*. 2007; 388(3):545–563. [PubMed: 17431594]
- Lillis B, Grogan C, Berney H, Lane WA. *Sensor. Actuat. B-Chem*. 2000; 68(1–3):109–114.
- Loneragan T, Le Compte A, Willacy M, Chase JG, Shaw GM, Wong XW, Lotz T, Lin J, Hann CE. *Diabetes Technol. The*. 2006; 8(2):191–206.
- Major TC, Brant DO, Reynolds MM, Bartlett RH, Meyerhoff ME, Handa H, Annich GM. *Biomaterials*. 2010; 31(10):2736–2745. [PubMed: 20042236]
- Oh BK, Robbins ME, Nablo BJ, Schoenfisch MH. *Biosens. Bioelectron*. 2005; 21(5):749–757. [PubMed: 16242614]
- Riddell, DR.; Owen, JS. *Vitamins and Hormones - Advances in Research and Applications*. Vol. Vol 57. San Diego: Academic Press Inc; 1999. p. 25-48.
- Schetz M, Vanhorebeek I, Wouters PJ, Wilmer A, van den Berghe G. *J. Am. Soc. Nephrol*. 2008; 19(3):571–578. [PubMed: 18235100]
- Schwendeman SP. *Crit. Rev. Ther. Drug*. 2002; 19(1):73–98.
- Shapiro NI, Fisher C, Donnino M, Cataldo L, Tang A, Trzeciak S, Horowitz G, Wolfe RE. *J. Emerg. Med*. 2010; 39(1):89–94. [PubMed: 19717267]
- Shapiro NI, Howell MD, Talmor D. *Ann. Emerg. Med*. 2005a; 46(6):562–562.
- Shapiro NI, Howell MD, Talmor D, Nathanson LA, Lisbon A, Wolfe RE, Weiss JW. *Ann. Emerg. Med*. 2005b; 45(5):524–528. [PubMed: 15855951]
- Shin JH, Marxer SM, Schoenfisch MH. *Anal. Chem*. 2004; 76(15):4543–4549. [PubMed: 15283600]
- Skrzypchak AM, Lafayette NG, Bartlett RH, Zhou ZR, Frost MC, Meyerhoff ME, Reynolds MM, Annich GM. *Perfusion-Uk*. 2007; 22(3):193–200.
- Valenza F, Aletti G, Fossali T, Chevillard G, Sacconi F, Irace M, Gattinoni L. *Crit. Care*. 2005; 9(6): 588–593. [PubMed: 16356243]
- Vanhorebeek I, Langouche L, Van den Berghe G. *Chest*. 2007; 132(1):268–278. [PubMed: 17625087]
- Vaughn MW, Kuo L, Liao JC. *Am. J. Physiol.-Heart Circul. Physiol*. 1998; 274(6):H2163–H2176.
- Waeger P, Hummel M. *Diabetes Stoffwech. H*. 2008; 17(5):385–391.
- Wisniewski N, Reichert M. *Colloid Surf. B-Biointerfaces*. 2000; 18(3–4):197–219.
- Wu B, Gerlitz B, Grinnell BW, Meyerhoff ME. *Biomaterials*. 2007a; 28(28):4047–4055. [PubMed: 17597201]
- Wu YD, Meyerhoff ME. *Talanta*. 2008; 75(3):642–650. [PubMed: 18585126]
- Wu YD, Rojas AP, Griffith GW, Skrzypchak AM, Lafayette N, Bartlett RH, Meyerhoff ME. *Sensor. Actuat. B-Chem*. 2007b; 121(1):36–46.
- Wu YD, Zhou ZR, Meyerhoff ME. *J. Biomed. Mater. Res. A*. 2007c; 81A(4):956–963.
- Yang QL, Atanasov P, Wilkins E. *Biosens. Bioelectron*. 1999; 14(2):203–210. [PubMed: 10101841]

Figure 1.
NO releasing glucose/lactate sensors configuration.

Figure 2.

(a) NO release of glucose/lactate sensors for over 7 days (n=4). (b) NO release comparison of the same amount of DBHD/N₂O₂ in PLGA, PurSil with KTpCIPB and PurSil only.

Figure 3. Normalized sensitivity of (a) NO releasing glucose sensors (n=4) and (b) NO releasing lactate sensors (n=3) over one week period of time. The percentage was calculated from dividing the sensitivity values on each day by the sensitivity on day 7.

Figure 4.

Glucose sensors explanted after 8-hour implantation in rabbit veins. Photos (a) and (b) demonstrate the drastic difference in thrombus formation between control (top) and NO releasing (middle, bottom) glucose sensors in two example rabbits. (c) Red pixel area in terms of thrombus formation of both control (n=15) and NO releasing glucose sensors (n=30).

Figure 5.
Clarke error grid comparison of (a) control (n=15) and (b) NO releasing glucose sensors (n=30) when implanted in rabbit veins for 8 h.

Table 1Selectivity of NO releasing glucose sensors over a one-week period^a (n=4).

Day	3	5	6	7
Ascorbic Acid (%)	0	1.10±1.01	2.08±1.09	2.88±1.53
Acetaminophen (%)	1.86±1.38	3.31±1.09	5.35±1.66	6.76±1.67

^a selectivity was measured by dividing the current from the maximum possible physiological level of interferences (0.5 mM for ascorbic acid and 0.2 mM for acetaminophen) by the signal from 5 mM of glucose.