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Improving the Thromboresistivity of Chemical Sensors via Nitric Oxide Release: Fabrication and in Vivo Evaluation of NO-Releasing Oxygen-Sensing Catheters

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The development and in vivo analytical performance of a nitric oxide (NO)-releasing amperometric oxygen sensor with greatly enhanced thromboresistivity are reported. Gas permeable coatings formulated with cross-linked silicone rubber (SR) containing NO-generating compounds (diazeniumdiolates) are shown to release NO for extended periods of time (>20 h) while reducing platelet adhesion and activation. Oxygen-sensing catheters prepared by dip-coating the NO-releasing films over the outer SR tubes of the implantable devices display similar analytical response properties in vitro (sensitivity, selectivity, response times) when compared to analogous sensors prepared without the NO release coatings. Superior analytical accuracy (relative to blood PO_2 values measured in vitro) and greatly reduced thrombus formation on the outer surface of the sensors are observed in vivo (in canine model) with the NO release PO_2 sensors compared to control sensors (without NO release) implanted simultaneously within the same animals. Based on these preliminary studies, the use of NO release polymers to fabricate catheter-style chemical sensors may be a potential solution to lingering biocompatibility and concomitant performance problems encountered when attempting to employ such devices for continuous intravascular measurements of blood gases and electrolytes.

The care of critically ill hospital patients in intensive or coronary care units mandates the frequent measurement of blood gases (pH, PO_2, PCO_2) and electrolytes (Na^+, K^+, Ca^{2+}) in undiluted whole blood.[1−6] Historically, these tests have been performed in centralized or satellite laboratories remote from the patient, resulting in infrequent measurement and long delays for obtaining vital data.[7] Among the many analytical technologies that could improve the quality of care for such patients, none is potentially more significant, yet technologically challenging, than the development of intravascular chemical sensors that function reliably for extended periods after implantation. Indeed, the availability of devices that could monitor blood gas, electrolyte, and metabolite levels accurately on a continuous basis could significantly change the way critically ill patients are treated.[8,9]

Despite technological advances resulting in the development of a number of prototype catheter-style commercial devices (both optical and electrochemical), implantable blood gas sensors have not found widespread use due to limitations in blood compatibility, including platelet adhesion on the sensor surface, as well as blood vessel constriction at the implant site (see Figure 1a).[10−15] Specifically, vasoconstriction can reduce blood flow at the implant site in certain patients leading to sensor output signals that do not correlate with true blood gas levels. Additionally, the deposition, aggregation, and activation of platelets and other cells on the sensor surface can ultimately lead to a thrombus, in which case measured pH, PO_2, and PCO_2 levels with the implanted sensor differ from levels in bulk blood due to cellular metabolic activity and perturbed mass transfer of analyte at the sensor/sample interface.[3,6]

To date, strategies for improving the blood compatibility of implanted devices include the use of more biocompatible hydro-

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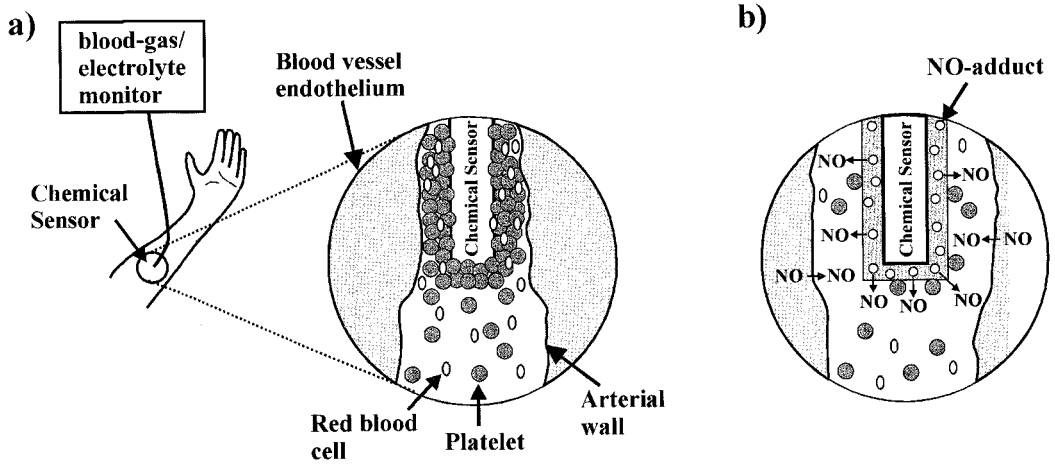

Figure 1. Schematic of (a) typical problems associated with current intravascular blood gas sensors and (b) an implantable sensor that measures PO_2 (and/or other analytes) while simultaneously releasing NO at the surface for several days.

phobic polymers (e.g., silicone rubber and polyurethane)[16] and surface modification, including albumin[17] and heparin[18,19] coatings, endothelial cell seeding,[20] and self-assembly.[21] However, none of these strategies have been completely successful in vivo. Furthermore, with respect to in vivo chemical sensing, the innate sensing chemistry of the device is not always compatible with the proposed biocompatible coating. For example, Telting-Diaz and co-workers[22] attempted to improve the biocompatibility of ion-selective electrode (ISE) catheter sensors (for CO_2 and pH) by impregnating the pH-sensing membrane/tubing material with the tridodecylmethylammonium (TDMA)−heparin ion pair. Unfortunately, such treatment destroyed the pH-sensing capabilities of the probe. Further, when compared to uncoated sensors, observations with the TDMA−heparin-treated sensors implanted within the carotid arteries of dogs revealed that significant clot formation occurred in vivo on both the treated and uncoated surfaces.

Recently, Espadas-Torre and co-workers[23] reported the use of NO release for potentially improving the thromboresistivity of chemical sensors. Potentiometric polymeric membranes for pH and potassium ion sensing were prepared by incorporating (*Z*)-1-[*N*-methyl-*N*-[6-(*N*-methylammoniohexyl)amino]]diazen-1-ium-1,2-diolate (MAHMA/N_2O_2) as a uniform dispersion of solid particles into plasticized poly(vinyl chloride) (PVC) or Tecoflex polyurethane (PU) doped with an appropriate ionophore. Polymeric films prepared in this manner generated NO for several days after exposure to aqueous solution and exhibited markedly reduced platelet adhesion/activation when tested in vitro using platelet-rich plasma. Further, it was shown that the NO release chemistry could be incorporated within the ion-sensing membranes without impairing the analytical response characteristics of the potentiometric devices. However, no in vivo data were presented in this report to support the notion that localized NO release will actually improve the analytical performance of sensors implanted intravascularly.

Herein, we describe the fabrication and in vivo performance of a Clark-style[24] amperometric oxygen-sensing catheter that releases nitric oxide locally (at the sensor/blood interface). Sensors based on NO release may be a possible solution to the lingering biocompatibility problems (e.g., platelet adhesion and arterial constriction) associated with the use of these devices intravascularly.[25−27] This concept is illustrated schematically in Figure 1b. Indeed, the antiplatelet aggregation[28] and vasodilating properties[29] of NO, coupled with its short half-life (<1 s in blood),[30] make it an ideal species for attempting to improve the performance of in vivo sensors. It will be shown here that oxygen-sensing catheters prepared with NO release capability, and tested in vivo within the carotid arteries of mongrel dogs, yield more accurate analytical results compared to control oxygen sensors (without NO release) implanted within the same animals.

EXPERIMENTAL SECTION

Materials and Reagents. Phosphate-buffered saline (PBS, pH 7.4, Sigma), KCl (99.7%, Mallinckrodt), HCl (37.6%, Fisher), $FeCl_3 \cdot 3H_2O$ (97−100%, MCB Reagents), methocel 90 HG (Fluka), nitrite ion chromatography standard (EM Science), glutaraldehyde (25%, Sigma), hexamethyldisilazane (Sigma), tetrahydrofuran (THF; Fisher), dioctyl sebacate (Fluka), and *N*,*N*′-dimethyl-1,6-hexanediamine (98%, Aldrich) were used as received. MAHMA/N_2O_2 was a gift from the Laboratory of Comparative Carcinogenesis at the National Cancer Institute (Frederick, MD). Gases for preparing standard solutions of oxygen (5, 10, 21, and 50% O_2 (balance N_2)) were obtained from Cryogenic Gases (Detroit, MI).

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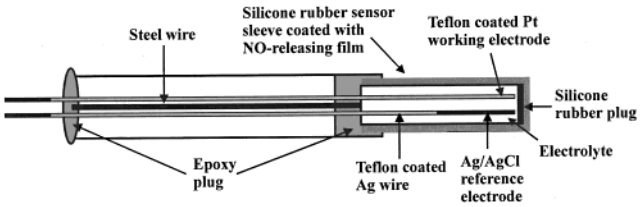

Figure 2. Schematic of catheter-style oxygen sensor modified with a thin silicone rubber polymeric coating containing the NO donor (diazeniumdiolate) molecule.

Solutions were prepared with reverse-osmosis, deionized water (Millipore Corp.). Teflon-coated Ag (99.9+%) and Pt (90% Pt, 10% Ir) wire were purchased from Medwire (Mt. Vernon, NY). Silicone rubber (SR; RTV-3140) and Silastic medical-grade tubing (0.51 mm i.d. × 0.94 mm o.d. and 0.91 mm i.d. × 1.2 mm o.d.) were obtained from the Dow Corning Corp. (Midland, MI). Epoxy gel (5 Minute, Devcon) was obtained from Ace Hardware (Ann Arbor, MI). Ultra four-way stopcocks and Angiocath catheter guides were products of Medex (Hilliard, OH) and Becton Dickinson (Sandy, UT), respectively.

Instrumentation. Amperometric measurements were performed using a Diamond Electro-Tech electrochemical analyzer (Ann Arbor, MI) interfaced to a Data Translations DT-2801-5716 analog/digital input/output board (Marlborough, MA) and a custom-built electrode interface module. Data acquisition was controlled with a PC and Labtech Notebook software (Wilmington, MA).

For in vivo studies, arterial blood flow was monitored with a Transonic flow meter and flow probes (Ithaca, NY). Briefly, the flow probes house two ultrasonic transducers and a fixed acoustic reflector. Ultrasonic signals are passed back and forth, alternatively intersecting the flowing blood in upstream and downstream directions. The flow meter measures the transit time for the ultrasonic wave to travel from one transducer to the other. The difference between the integrated upstream and downstream transit times is a measure of the volumetric flow (rather than velocity).

Catheter Electrode Fabrication. The sensor design first described by Mindt,[31] and currently the basis for at least one commercial in vivo PO_2 device,[32] was adopted to fabricate simple, catheter-style amperometric PO_2 sensors (see Figure 2). Clean Silastic medical-grade tubing (0.51 mm i.d. × 0.94 mm o.d.) was cut into ∼26 mm lengths, plugged at one end with silicone sealant (RTV-3140), and allowed to cure for at least 24 h. The sleeves were filled with 0.15 M KCl and 1.5% (wt) methocel electrolyte solution. Teflon-coated Ag and Pt wires ∼120 mm long and (0.076 mm o.d.) were stripped bare at one end (20 mm) to establish areas for electrical contact to the electrochemical analyzer. At the opposite end of the Ag wire, a Ag/AgCl reference electrode was prepared by stripping Teflon away (∼12 mm) and immersing the exposed wire into a 0.1 M HCl/1 M $FeCl_3$ solution for 15 s. The wire was then immersed in PBS solution for 3 s to remove excess acid solution. The opposite end of the Pt wire (unstripped, coated with Teflon except at the original cut) served as the working electrode. Thus, the cross-sectional area of the working electrode was proportional to the inner diameter of the cut Pt wire.

As shown in Figure 2, the Pt and Ag wires were first threaded through an ∼80 mm portion of a larger diameter Silastic medical-grade tubing (0.91 mm i.d. × 1.2 mm o.d.) and then into the plugged, smaller diameter sleeves containing electrolyte solution. Prior to connecting the proximal ends of the different Silastic tubings, the wire electrodes were fixed into the electrolyte-filled tubing with epoxy, thereby creating a sealed electrochemical cell. The epoxy also served as an adhesive for connecting the two pieces of Silastic tubing. Finally, a steel wire ∼60 mm in length (0.9 mm o.d.) was added to the larger Silastic tubing to make the entire sensor more rigid. The sensor was then mounted into a stopcock, thus, enabling a firm connection to a catheter guide used during in vivo implantation and testing. Current was monitored at an applied potential of −0.65 V vs Ag/AgCl. For benchtop experiments, sensor performance was evaluated by suspending the sensor in different tonometered solutions of oxygen (0, 5, 10, 21, and 50% O_2, the balance nitrogen.)

To assess the effect of using polymeric thin films containing NO-releasing diazeniumdiolates on the electrochemical performance of the catheters, the outer surfaces of certain SR catheters were coated with diazeniumdiolate-doped cocktail solutions consisting of 90% (w/w) silicone rubber (Dow Corning 3140 RTV coating), 8% (w/w) bis(2-ethylhexyl)sebacate (DOS), and 2% (w/w) diazeniumdiolate (MAHMA/N_2O_2) in 3 mL of THF. The SR tubing was dip-coated 7 times into the diazeniumdiolate-doped cocktail solution (for MAHMA/N_2O_2−SR films), followed by 3 times into a blank polymer solution (without diazeniumdiolate) to minimize donor leaching, and then dried overnight prior to insertion of the electrode wires. Control and blank catheter sensors were similarly prepared by doping with MAHMA (amine without diazeniumdiolate function) and without any doping, respectively.

NO Release Measurement. NO release profiles of coated silicone rubber tubes were determined by measuring the amount of NO generated indirectly as nitrite via the colorimetric Griess assay.[30] Due to the rapid reaction of NO with H_2O and O_2 to form almost exclusively NO_2^-,[33] the accumulated NO_2^- concentration is directly proportional to the amount of NO released from the polymer film. The nitrite concentration was determined spectrophotometrically at $\lambda_{max} = 540$ nm via calibration standards.[30]

In Vivo Testing. In vivo evaluation of NO-modified catheter sensors was conducted using large mongrel dogs (∼20−25 kg) to assess the effect of NO generation on analytical performance, thrombus formation at the sensor surface, and arterial blood flow distal from the implant site. The animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Michigan (A3114-01). Dogs were anesthetized intravenously with a sleep dose of sodium pentobarbitone (30 mg/kg). Pancuronium bromide was used as a muscle relaxant to suppress spontaneous breathing, thus allowing better control of desired oxygenation levels. Notably, *dogs were not systemically anticoagulated with heparin* so that a full assessment of the effectiveness of the NO release coatings in preventing platelet adhesion and improving sensor performance could be made.

Blood pressure of the animals was monitored continuously with a modular cardiovascular monitor (Hewlett-Packard) and place-

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ment of an intra-arterial blood pressure probe into the right femoral artery using a percutaneous 20 SWG intravenous cannula. The right femoral artery was also used for blood sample withdrawal and drug injection. After the trachea of the animal was intubated with an appropriate size endotracheal tube, the lungs were mechanically ventilated with 21% oxygen and 1% halothane (balance nitrogen) at 10 mL/kg. For typical experiments, two functional sensors and a nonfunctional sensor were implanted in both carotid and left femoral arteries, respectively, by cannulation of the proximal portion of arteries with a 14-gauge catheter guide. Sensors were inserted through the cannula so that ~9 mm of the sensor was exposed to flowing blood beyond the end of each cannula. This distance was chosen to minimize wall effects, a phenomenon affecting blood flow patterns at or near the inner arterial wall, total blood flow velocity within the artery,[8,14,15] and the sensing of false (low) oxygen values due to metabolism of the endothelia cells that line the wall of the artery.

Typically, in vivo experiments were performed on dogs for 6−24 h. During the experiments, blood samples were drawn from the animal at 30−60 min intervals and analyzed for PO_2, PCO_2, and pH using a Radiometer Medical ABL-505 blood gas analyzer (Copenhagen, Denmark). Similarly, the amperometric output of the implanted catheter sensors was recorded at 30−60 min intervals during the experiment, as well as more frequently when fluctuations in the output signal were observed. Blood flow was monitored using ultrasound flow probes placed distal to the arterial cannulation site. In addition, the rectal temperature and cardiac output of the dog were monitored throughout the experiment. Sensor calibration was accomplished by sampling blood 30 min after implantation and measuring PO_2 values in vitro. The sensor output current at that point was assigned the measured value, and zero current was assumed for zero oxygen levels, thus allowing the in vivo sensitivity of the implanted sensor to be determined.

Upon completion of the in vivo experiment, the animals were euthanized with a bolus injection of Beuthanasia-D Special (Schering-Plough Animal Health). The sensors were gently removed from the arteries in order to retain cellular adhesion, immersed gently in PBS for ~3 s to remove weakly adsorbed milieu, and fixed in 1% (w/v) aqueous glutaraldehyde solution overnight at room temperature. Samples were then dehydrated by immersion in serial dilutions of ethanol (30, 50, 70, 90, 95, 100, 100% v/v) and hexamethyldisilazane for 10 min each. After overnight solvent evaporation, the sensor tubing was sputtered with gold and examined for the presence of platelets, fibrin, etc., using a Hitachi S-3200N scanning electron microscope (SEM).

RESULTS AND DISCUSSION

The objective of these studies was to fabricate and characterize an implantable, catheter-style oxygen (PO_2) sensor modified with a thin polymeric film that slowly releases nitric oxide. The sensor design, first described by Clark[24] in 1956 and later miniaturized by Mindt,[31] is shown in Figure 2. Briefly, the sensor consists of narrow Silastic medical-grade tubing in which a cathode, anode, and inert electrolyte are separated from the sample medium by the walls of the silicone rubber tube. Oxygen diffuses across the silicone rubber tubing and is electrochemically reduced (e.g., $O_2 + 2H^+ + 4e^- \rightarrow 2OH^-$) at the Pt wire, polarized at −0.65 V versus Ag/AgCl. The current produced by this reaction (~0−300 nA) is

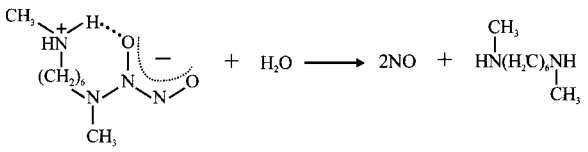

Figure 3. NO release reaction for the diazeniumdiolate MAHMA/N_2O_2 incorporated within plasticized silicone rubber films.

proportional to the partial pressure of oxygen. The purpose of the silicone outer tube is to prevent both fouling of the working electrode by biological molecules and electrochemical reduction of interfering species. However, despite this barrier, platelet adhesion/activation on the surface of this outer tubing coupled with vasoconstriction at the implant site can result in significant analytical accuracy problems,[10−15] thus limiting the use of intravascular catheter sensors in humans.[34,35]

The initial studies of NO-releasing catheter-style PO_2 sensors were based on SR coatings doped with the water-soluble diazeniumdiolate MAHMA/N_2O_2. This diazeniumdiolate is stable as a solid, but reacts spontaneously with water[36] to produce NO and a residual diamine, as shown in Figure 3. Since MAHMA/N_2O_2 is only soluble in the aqueous phase, it was dispersed uniformly as fine particles by sonnication into a cocktail solution consisting of plasticized SR and THF. Silicone rubber tubing was then dip-coated into this cocktail solution to create outer films capable of generating NO for extended periods when exposed to an aqueous solution or blood.

NO Release. To assess the feasibility of using NO donors for preparing thromboresistant amperometric oxygen sensors, initial experiments were conducted to measure the NO release rate from diazeniumdiolate-doped films. The NO release profiles for SR sensor sleeves fabricated by dip-coating SR tubing into MAHMA/N_2O_2-doped silastic cocktail solutions are shown in Figure 4. Such polymeric coatings release NO for several hours after the coated tubing is allowed to contact aqueous solution. When the diazeniumdiolate is incorporated into the polymeric phase, the rate of NO release is significantly reduced relative to dissolution of MAHMA/N_2O_2 directly in aqueous solution, where its half-life is 1 min.[36] Indeed, the rate of NO release from polymeric films is related to water uptake in the film.[37] Thus, the NO release rate depends on the composition of the membrane (e.g., polymer/plasticizer ratio, etc.). These parameters have been investigated and reported elsewhere.[23,37] In the present work with SR sleeves coated with SR-MAHMA/N_2O_2, the amount of NO release observed varies with the amount of diazeniumdiolate−polymer coated on the outer surface of the tubing as shown by the decrease in total NO released when the outer SR sleeves are dip-coated only 3 times vs 7 times (see Figure 4). Similarly, the amount of NO release can be varied by changing the mass of diazeniumdiolate dispersed within the polymer cocktail solution (data not shown).

Beyond NO release into the surrounding sample phase, NO can also permeate the walls of the SR tubing and be trapped as

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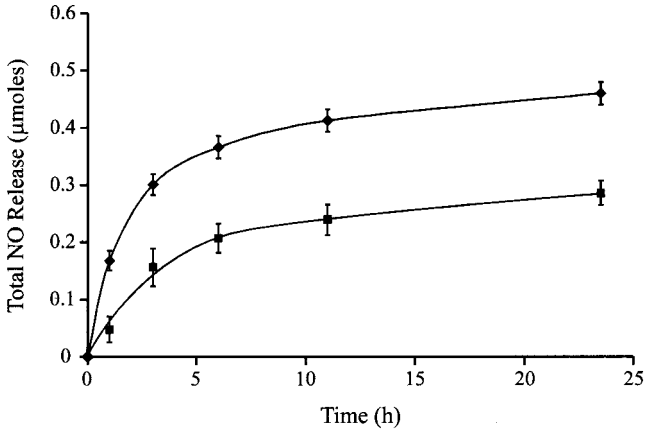

Figure 4. NO release profiles for 26.0 mm Silastic tubing coated with MAHMA/N_2O_2 polymer film incubated in PBS at 37 °C. The tubing was dip-coated 7 times (◆) or 3 times (■) into 3.0 mL of THF cocktail solution (90% SR/8% DOS/2% MAHMA/N_2O_2) with sufficient time to dry between each coating, followed by three dip-coatings in 3.0 mL of THF blank cocktail (92% SR/8% DOS). Each curve represents the average of at least five trials with representative standard deviations over the measurement period.

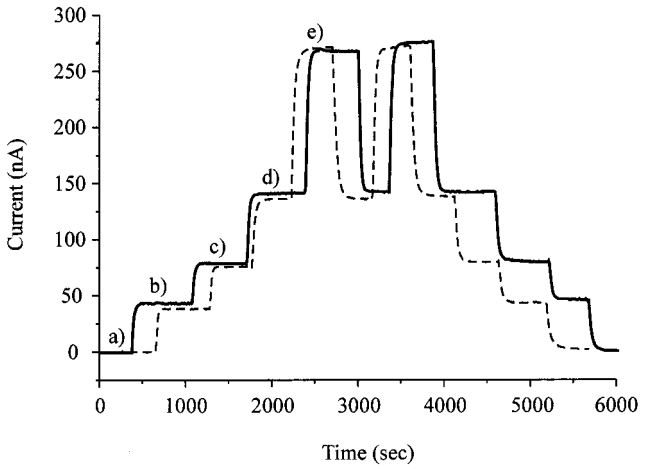

Figure 5. Benchtop sensor response profiles for control (dashed line) and NO releasing (solid line) catheter PO_2 sensors. The partial pressure of oxygen in the measuring solution (1.0 M KCl) was varied between (a) 0, (b) 36, (c) 72, (d) 151, and (e) 360 mmHg. The responses shown here are for the same sensor with different sleeves. Changes in PO_2 did not take place at similar time intervals for the two experiments. Response times are essentially identical for NO-releasing and control PO_2 sensors.

nitrite ions within the inner electrolyte solution of the oxygen catheter. Indeed, after a 24 h period, using a seven-layer coating, $\sim 5.8 \times 10^{-4}$ μmol of NO was measured (as nitrite) within the inner electrolyte solution. Consequently, the proposed NO release strategy would actually be detrimental for preparing thromboresistant amperometric sensors if NO and/or nitrite entering the inner electrolyte interferes with the electrochemical reduction of oxygen (see below).

Response Characteristics of Amperometric PO_2 Catheter Sensors. To assess the practical utility of NO-releasing polymers for eventual use as outer coatings for in vivo PO_2 sensors, benchtop experiments were conducted to examine whether amperometric PO_2 sensors with outer SR tubings coated with SR−MAHMA/N_2O_2 function reliably. For these feasibility studies, catheter-style PO_2 sensors were fabricated with blank, control (MAHMA), or MAHMA/N_2O_2-coated plasticized SR films to yield the configuration shown in Figure 2.

Prior to testing the sensors, newly fabricated catheter sensors were first preconditioned at −0.65 V vs Ag/AgCl in aerated PBS solution for 1 h to minimize signal drift. For MAHMA/N_2O_2-coated sleeves, this stabilization period involves the first wetting of the diazeniumdiolate-coated SR tubing, thereby initiating NO release. Typically, initial drift values ranged from 5 to 10% of the original current and ceased within ∼30−45 min after polarization of the Pt electrode.

As shown in Figure 5, the analytical response properties of amperometric PO_2 catheter sensors are not compromised, even though the presence of MAHMA/N_2O_2 promotes continual NO release from SR films for several hours. The presence of the insoluble MAHMA/N_2O_2 particles dispersed in the outer coating does not reduce oxygen gas flux through the SR tubing, regardless of the PO_2 level. In addition, NO released into the sample and the internal electrolyte solution does not interfere with O_2 sensing at the working electrode. Differences in either the dynamic or analytical response of the catheter-type PO_2 sensor were not observed between gas sensors coated with the NO adduct and controls. Indeed, for multiple control and NO-releasing oxygen-sensing catheters prepared with different internal platinum working and reference electrode pairs, the mean sensitivities with respect to amperometric oxygen response are statistically equivalent (control, 0.76 ± 0.10 nA/mmHg; NO releasing, 0.83 ± 0.08 nA/mmHg; $N = 4$ pairs). These amperometric response properties clearly demonstrate that the presence of MAHMA/N_2O_2 and the concomitant generation of NO does not interfere with the diffusion and electrochemical reduction of O_2 required to generate analytically useful signals from sensors coated with diazeniumdiolate-doped films.

In Vivo Evaluation. While the oxygen-sensing catheters prepared with and without the NO release coatings display comparable analytical performance on the benchtop, the resulting sensors behave quite differently with regard to their potential thrombogenicity. Similar to previously reported results for NO-releasing PVC and polyurethane films,[37] preliminary in vitro thromboresistivity testing of MAHMA/N_2O_2-doped SR films using platelet-rich sheep plasma revealed a substantial decrease in both the number of adhered platelets and the extent of platelet activation for NO-releasing films compared to controls (images not shown). Such results have been observed repeatedly for a variety of polymeric films doped with MAHMA/N_2O_2.[23,37,38] However, to fully assess the utility of NO-releasing catheter sensors for continuous PO_2 monitoring, in vivo experiments were conducted by implanting functional sensors within the carotid and femoral arteries of mongrel dogs.

Analogous to benchtop experiments, newly fabricated catheter PO_2 sensors were first preconditioned in PBS solution for 1 h prior to testing. The catheter sensors were then implanted within the arteries of the dogs without *systemically anticoagulating the animal with heparin* as described in the Experimental Section. For each experiment, one control (functional sensor prepared with SR coating containing MAHMA) and one NO-releasing sensor were

(38) Mowery, K. A.; Schoenfisch, M. H.; Baliga, N.; Wahr, J. A.; Meyerhoff, M. E. *Electroanalysis* **1999**, *11*, 681.

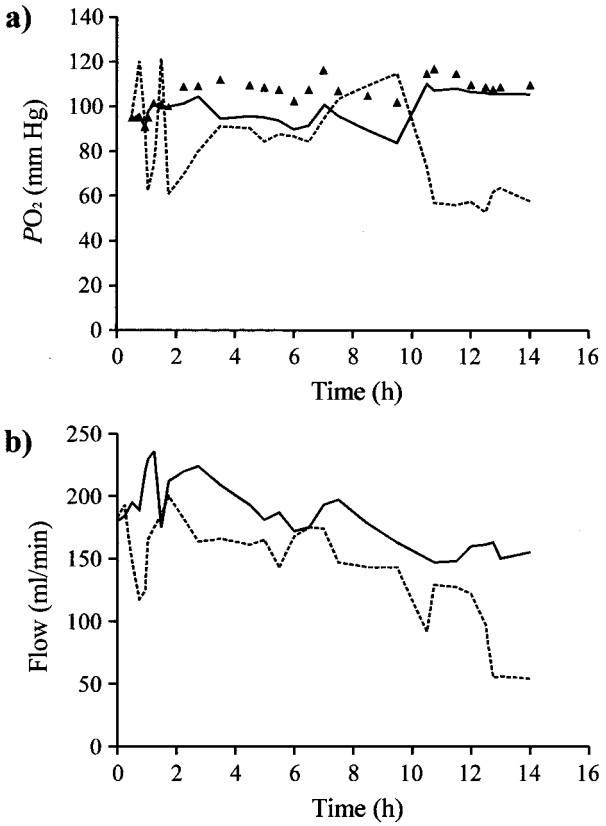

Figure 6. Data collected for a representative in vivo experiment with sensors implanted within the carotid arteries of a canine over 14 h: (a) oxygen levels and (b) blood flow. Blood oxygen values (▲) were monitored periodically on discrete blood samples with a conventional laboratory blood gas analyzer and electrochemically with calibrated control (- - -) and NO-releasing (—) catheter sensors. Blood flow was measured with a Transonic flow meter and probes placed on the artery immediately distal from the implanted sensors.

implanted in the left and right carotid arteries, respectively. An additional, nonfunctional sensor from the control group was also implanted in the remaining left femoral artery to further assess thromboresistivity.

Calibration of the sensors was performed ~30 min after implantation, once sensor and blood gas values stabilized, by correlating the electrochemical current (due to O_2 reduction at the cathode) to the PO_2 value obtained from in vitro blood gas measurements. Blood gas measurements were obtained periodically (at least every hour) on discrete samples throughout the duration of the experiment using a conventional laboratory blood gas analyzer. In addition to amperometric sensor output and arterial PO_2, several other parameters were monitored throughout the experiment including PCO_2, pH, blood pressure values, and rectal temperature. The blood flow in each carotid artery was monitored with flow probes placed distal from the sensor site.

The electrochemical response of control and NO-releasing catheter PO_2 sensors, arterial blood gas values (measured with a benchtop blood gas analyzer), and blood flows for a representative in vivo experiment are shown in Figure 6. In this particular 14 h experiment, a significant discrepancy between the control sensor and in vitro PO_2 values is observed immediately following implantation and again toward the end of the experiment. As shown in Figure 6a, the control sensor output fluctuates severely (>50%) during the first few hours after implantation. Similar

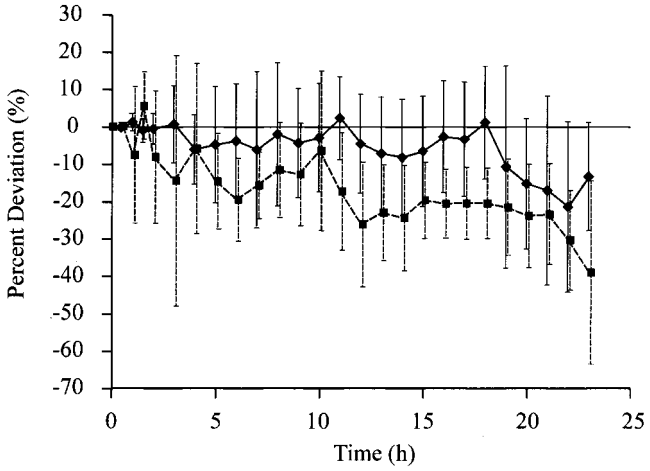

Figure 7. Average sensor deviation from true PO_2 values for control (■) and NO-releasing (◆) PO_2 sensors for seven different animal experiments. Although the length for each experiment varied from 12 to 23 h, each data point represents the average ± sd for at least three sensors.

behavior was observed in four of the seven control PO_2 sensors, as opposed to only one of the seven NO-releasing experimental sensors in seven completely separate animal experiments with the SR−MAHMA/N_2O_2 coating. Such early phase deviation from actual PO_2 levels is hypothesized to result from blood vessel constriction (spasms) around the sensor, as evidenced by the accompanying decrease in the blood flow shown in Figure 6b. Furthermore, poor performance for control sensors is observed at longer implant times, suggesting thrombus formation on the sensor surface. Notably, the output of the NO-releasing PO_2 catheter sensor followed the actual blood PO_2 values within 10% throughout the experiment.

The average sensor performance for functional control and NO-releasing sensors from the seven separate animal experiments is summarized in Figure 7. A performance trend is evident when the percent deviation from true PO_2 values for control and NO-releasing sensors is plotted as a function of implant time. Indeed, the performance of sensors that release NO appears to be improved when compared to control sensors. The blood flow data (not shown) does not reveal a statistical difference in flow distal from the implant site for NO-releasing and control sensors when all of the data from the seven animal experiments are averaged. This observation is not surprising given the large differences between the animals (e.g., artery size and coagulation variability). However, as shown in Figure 6b, in individual experiments there was a clear correlation between aberrant PO_2 values measured with the control sensor and low or varying blood flow in the artery in which this sensor was placed.

The data shown in Figure 7 were also analyzed using a paired *t*-test to assess the statistical difference in the mean values for the oxygen sensor measurements over the time course of the in vivo experiments. Ten out of the 20 data sets between 1 and 18 h show a statistical difference in the mean PO_2 levels for the two sensors at the $p \leq 0.2$ level (≥80% confidence), and 5 of the 20 data sets show a statistical difference at the $p \leq 0.1$ level (≥90% confidence). Data sets beyond 18 h represent fewer number of experiments (since not all in vivo experiments lasted 23 h), and as shown in Figure 4, NO release rates at these later times have

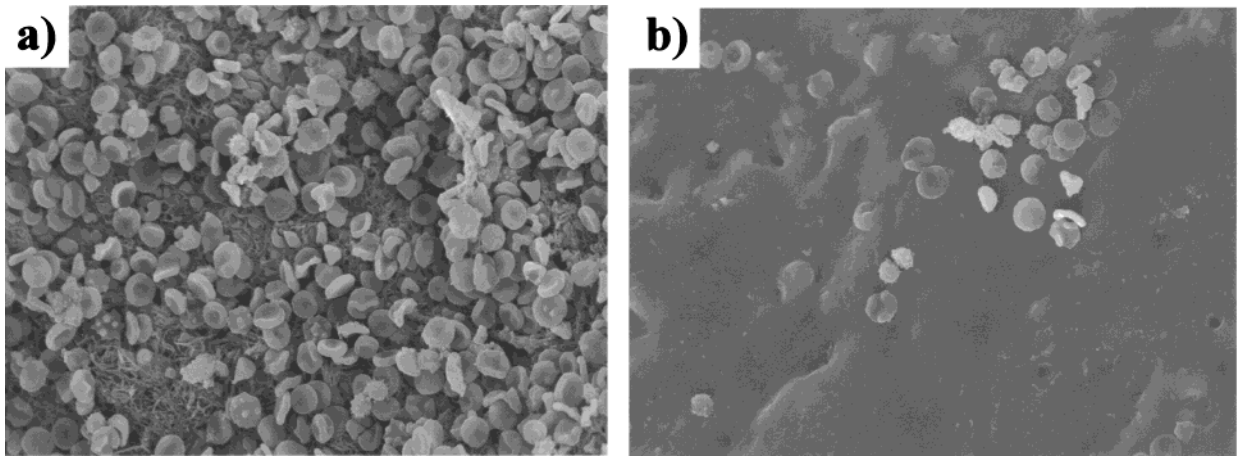

Figure 8. Scanning electron microscope images of surfaces of (a) control and (b) SR−MAHMA/N_2O_2-coated sensors after placement within carotid artery of a canine for 14 h. Each image is a 1000-fold magnification of the sample.

deteriorated to an extremely low level; hence, none of the differences in mean values for these latter data sets are statistically different at the ≥80% confidence level.

Following in vivo evaluation, the oxygen sensors were carefully removed and examined for thrombus formation with a scanning electron microscope. As shown via the representative SEM images in Figure 8, the extent of platelet adhesion and aggregation is significantly greater on the surface of the controls compared to NO-releasing sensors. The prevalence of other cells, in particular red blood cells, on the surface of the control sensor represents thrombus formation, in which activated platelets initiate the formation of a fibrin network entrapping red blood cells. Although the specific images presented in Figure 8 provide only a snapshot of the difference consistently observed between control and NO-releasing sensors, they depict the average surface features observed in many in vivo experiments. Such differences in cell adhesion were routinely observed visually after explanting the sensors; however, microscopic examination by SEM also clearly indicates the formation of an underlying fibrin network of the type normally associated with platelet-mediated clot formation. Thus, these images clearly illustrate the efficacy of NO release polymer films in preventing thrombus formation on the surface of implanted sensors in the absence of systemic anticoagulation.

Although the average overall analytical performance for NO-releasing PO_2 sensors also diminished slightly beyond the 18 h point (Figure 7), platelets were seldom observed on the surfaces of NO-releasing sensors implanted for extended periods. This behavior implies that platelets might begin to adhere to NO-releasing sensors once the NO release rate diminishes below a given level, resulting in the poor performance patterns observed for control sensors. However, the coating of cells is not yet rugged enough to survive the explantation and subsequent rinsing procedures, and hence the monolayer of platelets is lost and not observed by SEM (image not shown).

It is important to note that a significant deviation in control sensor output and/or thrombus formation was not observed in each experiment. On two occasions the output for the control sensors followed in vitro blood gas measurements as well as the NO release sensor. Again, performance for implanted sensors without any thromboresisitive coatings will always vary from animal to animal due to differences in the artery size, coagulability of the given animal, etc. However, on the same note, significant platelet adhesion and signal deviation was never observed for any of the NO-releasing PO_2 sensors, clearly demonstrating superior overall sensor performance vs the control group. Certainly, a much larger number of animal studies will be required to obtain definitive data that show a consistent statistical difference (with a greater confidence level; see above) in the performance of NO-releasing vs control sensors.

As a caveat, while oxygen-sensing catheters coated with SR−MAHMA/N_2O_2 exhibit improved in vivo performance, MAHMA/N_2O_2 and its decomposition products (*N*,*N*′-dimethylhexanediamine and the corresponding nitrosamine) have been shown to leach from polymer films into aqueous soaking solutions.[37] Although administration of diazeniumdiolates directly in blood as systemic drug agents for controlling platelet activity has been suggested and patented by Keefer and Hrabie,[39] it may be more desirable if none of the original donor species or corresponding byproducts could enter the blood. Toward this end, a number of new polymeric diazeniumdiolates (more lipophilic NO donors and NO donors bound covalently to the polymer backbone) are currently being prepared and investigated in our laboratory.[37,40,41] The fabrication and biocompatibility testing of catheter sensors based on such diazeniumdiolates are underway and will be reported elsewhere.

CONCLUSIONS

The approach described herein is a new strategy for improving the thromboresistivity and performance of catheter-type chemical sensors that have heretofore performed erratically when implanted intravascularly. Sensors that release NO may eliminate the need for systemic anticoagulation by reducing thrombus formation, resulting in more accurate and precise real-time blood gas measurements. Further characterization of alternate NO-releasing materials in which the diazeniumdiolate is covalently attached to the polymer backbone[37] or fumed silica particles doped within given polymers[40] are currently in progress. In addition, such polymeric materials may also prove valuable in the development

(39) Keefer, L. K.; Hrabie, J. A. U.S. Patent 5 155 137, 1992.
(40) Zhang, H.; Schoenfisch, M. H.; Meyerhoff, M. E. *218th Am. Chem. Soc. Natl. Mtg.*, New Orleans, LA, August 1999; Paper 190.
(41) Schoenfisch, M. H.; Zhang, H.; Meyerhoff, M. E., in preparation.

of more thromboresistant optical sensors[41] and extracorporeal tubings[42] (for cardiopulmonary bypass surgery, ECMO, etc.). In the latter case, use of such materials could potentially reduce and/or eliminate the need for systemic heparin anticoagulation for patients undergoing such procedures. Studies in this direction are in progress with collaborators at the University of Michigan School of Medicine.

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