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Technical Note

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Improved *in Vivo* Performance of Amperometric Oxygen (PO_2) Sensing Catheters via Electrochemical Nitric Oxide Generation/Release

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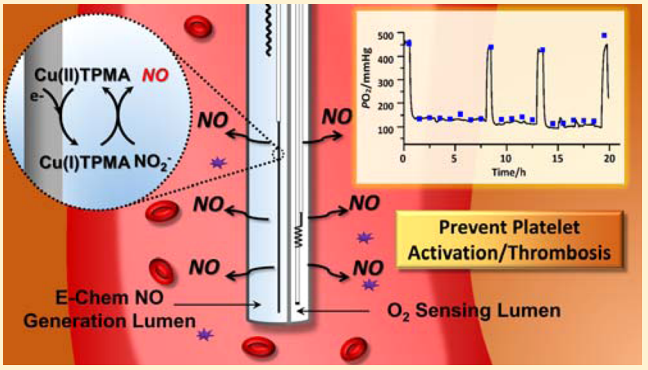
Supporting Information

ABSTRACT: A novel electrochemically controlled release method for nitric oxide (NO) (based on electrochemical reduction of nitrite ions) is combined with an amperometric oxygen sensor within a dual lumen catheter configuration for the continuous *in vivo* sensing of the partial pressure of oxygen (PO_2) in blood. The on-demand electrochemical NO generation/release method is shown to be fully compatible with amperometric PO_2 sensing. The performance of the sensors is evaluated in rabbit veins and pig arteries for 7 and 21 h, respectively. Overall, the NO releasing sensors measure both venous and arterial PO_2 values more accurately with an average deviation of $-2 \pm 11\%$ and good correlation ($R^2 = 0.97$) with *in vitro* blood measurements, whereas the corresponding control sensors without NO release show an average deviation of $-31 \pm 28\%$ and poor correlation ($R^2 = 0.43$) at time points >4 h after implantation in veins and >6 h in arteries. The NO releasing sensors induce less thrombus formation on the catheter surface in both veins and arteries ($p < 0.05$). This electrochemical NO generation/release method could offer a new and attractive means to improve the biocompatibility and performance of implantable chemical sensors.



Levels of chemical species in blood, including blood gases (pH, partial pressure of O_2 (PO_2), partial pressure of CO_2 (PCO_2)), electrolytes (Na^+, K^+, Ca^{2+}), glucose, and lactate, provide invaluable information for the diagnosis and treatment of hospitalized patients.[1,2] Currently, these analytes are intermittently measured *in vitro* with point-of-care devices using blood samples, which provides only periodic information, leaving large gaps in time between blood draws. Continuous monitoring of these species directly within blood vessels would greatly improve the quality of health care for critically ill patients.[3,4] Indeed, the development of intravascular devices that can monitor key physiological species in real-time is the "holey grail" in the field of chemical sensors. Despite extensive efforts over several decades, there are currently no sensing devices available that can achieve this goal, mostly due to poor biocompatibility of the devices once placed intravascularly (IV) within flowing blood.[5−7] One major complication is the formation of clots/thrombus, which occurs within hours after blood contact.[8] The thrombus can isolate the sensors from the bulk of the blood and cause unreliable analytical results.[9] Intravascular thrombus formation also has the intrinsic risk to embolize and affect vital organs in the patient.[10]

In the blood vessels, healthy endothelial cells generate nitric oxide (NO) at the flux from $(0.5-4.0) \times 10^{-10}$ mol·cm^{-2}·min^{-1}, and one of the functions of NO is to inhibit platelet activation/aggregation and prevent clotting at the surface of the endothelial cell layer.[11−13] Inspired by this knowledge, NO release/generation strategies have been adopted for the development of more biocompatible IV devices, including electrochemical sensors.[14−19] The traditional NO releasing sensors rely on coatings on the surface of the devices that contain NO donors (either by entrapping or covalent attachment) that decompose and generate NO spontaneously both *in vivo* and under storage in buffer solution.[20,21] Such passive NO release strategies are expensive and have shelf life issues due to the instability of many NO donors utilized to date. Further, there are concerns about leaching of NO donors and/or byproducts into the bloodstream. These issues have impeded their adaptation into clinical settings. Strategies based on NO generation from endogenous *S*-nitrosothiol (RSNO) species using immobilized catalysts on the surface of the sensors have also been pursued.[15] However, the levels of endogenous RSNOs are likely too variable from patient to patient to guarantee that enough surface NO can be generated to prevent platelet adhesion and clotting for each and every patient.

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Recently, a completely new electrochemical method has been reported to produce very controllable NO generation by electrochemical reduction of inorganic nitrite ions catalyzed by a copper(II)-ligand complex.[22] Not only can the NO generation/release be actively controlled "on" and "off", but also the flux of the NO release from the device surface can be readily modulated within the physiologically relevant range, by applying different voltages to an inner working wire electrode. This "on-demand" NO release method is highly desirable for implantable sensors for several reasons, including: (1) during the storage, the NO release can be turned "off" and thus the reservoir of NO precursor is preserved; (2) sodium nitrite as the NO donor is very stable and inexpensive compared to NO donors like diazeniumdiolates and *S*-nitrosothiols; and (3) the levels of NO release can be modulated *in vivo*, with low levels for most of the time to prevent clotting and higher levels turned on only periodically to better prevent/manage risk of infection.

In this study, we investigate, for the first time, the concept of combining electrochemical NO generation/release with intravascular chemical sensors to improve their *in vivo* analytical performance. Specifically, a dual lumen catheter-type amperometric PO_2 sensor (i.e., one lumen dedicated to electrochemical NO generation and the second lumen used for PO_2 sensing) is developed to demonstrate this concept. Such devices can be fabricated conveniently using commercial dual lumen silicone rubber catheter tubing without any NO releasing/generating coating. The performance of these sensors is further evaluated in rabbit and pig models for up to 21 h, both in veins and arteries. The sensors were exposed to a wide range of PO_2 *in vivo* from ~20 to ~480 mmHg, by changing ventilator levels of the fraction of inspired oxygen (FiO_2).

EXPERIMENTAL SECTION

Reagents and Instrumentation. Sodium nitrite, copper acetate, tris(2-pyridylmethyl)amine, sodium chloride, sodium bicarbonate, sodium carbonate, potassium chloride, and HEPES buffer were obtained from Sigma-Aldrich (St. Louis, MO). Teflon PFA-coated silver (0.127 mm OD) and platinum wires (0.125 mm OD) are products of A-M Systems (Sequim, WA). All solutions were prepared with Milli-Q water (Millipore Corp., Billerica, MA). Dual-lumen silicone catheters (7 Fr) were gifted from Cook Medical Inc. (Bloomington, IN). Silicone rubber adhesive (RTV-3140) was obtained from Dow Corning (Midland, MI). Tanks of gas with varying levels of O_2 balanced in N_2 were products from Cryogenic Gas Inc. (Detroit, MI).

All electrochemical experiments were performed using CH Instruments multichannel potentiostats (1000C, Austin, TX) and/or a BioStat potentiostat (ESA Biosciences Inc., Chelmsford, MA). Nitric oxide release from the catheters was measured using a Sievers Nitric Oxide Analyzer (GE Instruments, Boulder, CO). Blood gas values from blood samples drawn from the test animals were measured using a 700 series blood gas analyzer (Radiometer America Inc., Brea, CA).

Fabrication of Catheter-Type Electrochemical NO Releasing PO_2 Sensors. The procedures used were modified from those reported previously.[22,23] A long dual lumen catheter (see dimensions in Figure 1) was cut to 7 cm in length, and both lumens were sealed at one end with silicone rubber adhesive. The larger lumen was filled with a solution containing 4 mM CuTPMA, 0.4 M $NaNO_2$, 0.2 M NaCl, and 0.5 M HEPES (pH 7.2). A Teflon PFA-coated Pt wire (3 cm exposed) and a Ag/AgCl wire (5 cm exposed) were inserted into the lumen as working and reference electrodes, respectively. The smaller lumen was filled with 0.15 M KCl in 0.1 M bicarbonate/carbonate buffer (pH 10), and a PFA-coated Pt wire (only tip exposed) as well as a Ag/AgCl wire (3 cm exposed) were inserted for oxygen sensing. The openings of the lumens at the proximal end were then sealed (around the wires) with silicone rubber adhesives and left cured in water overnight.

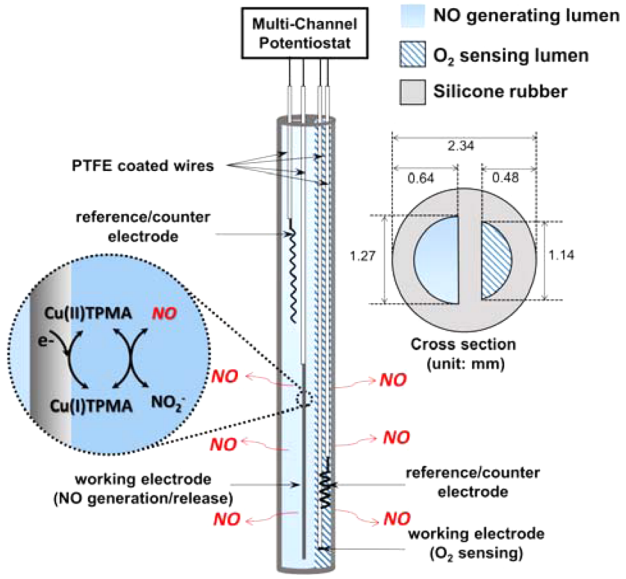

Figure 1. Schematic of dual-lumen catheter-type electrochemical NO generating/releasing PO_2 sensor with cross section geometry of catheter.

***PO_2* Sensor Calibration.** Catheter sensors were immersed in PBS buffer bubbled with different levels of O_2 (0%, 10%, 21%, 100%) at the flow rate of ~500 mL/min. The PO_2 sensing lumen of the catheter was polarized at −700 mV in PBS buffer with 0% O_2 for 1 h before calibration. At each level of PO_2, NO release was switched either from "on" to "off" or from "off" to "on", by applying −400 mV between the working and reference electrodes within the NO generating lumen. Note that the two lumens are separate electrochemical cells and the lead wires from each lumen are connected to different channels of a multichannel potentiostat. To determine response times, the sensors were switched between solutions presaturated with 0% and 21% O_2. The response time corresponds to the time needed to reach 90% of the steady-state current response after changing the oxygen level.

***In Vivo* Experiments.** The procedures used were in compliance with the University Committee on the Use and Care of Animals as well as federal regulations and were reported elsewhere.[15] Briefly, New Zealand white rabbits (~3 kg, $n = 5$) were placed under anesthesia for the 7 h experiments. Two catheter-type PO_2 sensors were placed in the jugular veins and connected to potentiostats with NO release lumen switched "on" for one of the sensors. No other anticoagulant or antiplatelet agents were administrated to the rabbits during the experiments. The initial fraction of inspired oxygen (FiO_2) was 100%. During the latter part of the experiment, the FiO_2 level was changed to 21% for ~1 h and then switched back to 100%. Venous blood was drawn every 30 min to test for PO_2 values using the blood gas analyzer as the reference method. To calibrate the sensors *in vivo*, the *ex vivo* data point at the 30 min time point after implantation was used as a one-point

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calibration, with intercept determined by a prior benchtop calibration of the oxygen sensing portion of the catheter. The continuous signal from the sensors was compared with the intermittent *in vitro* blood PO_2 values. The sensors with the blood vessels intact were explanted after systemic heparinization to prevent necrotic thrombosis during vessel harvesting; digital pictures were taken, and the red pixels were counted using ImageJ software to quantify clot area.[23,24]

Similar experiments were performed using a porcine model (~50 kg, n = 4) for 21 h. The sensors were placed in the femoral and carotid arteries via an open cut-down allowing for continuous blood flow past the sensors. The FiO_2 level was maintained at 21% and changed periodically to 100% for a 1 h period (ca. every 6 h). Arterial blood was drawn every hour to assess the accuracy of the PO_2 values provided by the implanted sensors. Similar to the experiments with rabbits, sensors and vessels were explanted at the end of the experiments to allow for quantification of clot burden.

Signal Processing and Statistics. The *in vivo* data from the sensors were recorded every second and averaged every 30 s to reduce the electronic noise as well as the size of the data set. A Student's t test (two-tail, paired) was used to evaluate the significance of the data sets. Linear regression and R^2 were used to evaluate the accuracy and correlation, respectively.

RESULTS AND DISCUSSION

Rationale for Sensor Design. A commercial dual-lumen silicone catheter (cross section geometry shown in Figure 1) was used to fabricate the electrochemical NO releasing PO_2 sensors. Silicone rubber is preferred because it is highly permeable toward both the analyte, O_2, and the anticlotting agent, NO, while impermeable toward the inner solutions of reservoir ions.[22,25] One lumen of the catheter is dedicated to O_2 sensing, using the cross-section distal tip of a PFA-coated Pt wire working electrode. It is held at a cathodic potential (−0.7 V vs Ag/AgCl) where reduction of O_2 occurs to yield a steady-state current proportional to PO_2 levels. The other lumen is dedicated to NO generation/release and contains a reservoir of sodium nitrite (0.4 M) and CuTPMA (4 mM) catalyst. Note that, although the cross-section geometry of the dual lumen catheter is not symmetric, it can be shown by finite element analysis (via Comsol Multiphysics software) that the NO distribution is symmetrically enhanced around the entire dual lumen catheter assembly because of the high diffusivity and solubility of this neutral lipophilic gas molecule in the silicone rubber (see Figure S1).

Sensor Performance on the Benchtop. The first study involved assessing the compatibility of the O_2 sensing with the electrochemical NO generation process. The design of the sensor facilitates such investigation, since the two electrochemical systems reside within the two separate lumens of the same catheter device and the NO generation lumen can be easily turned "on" and "off" by applying −0.4 V to the Pt working electrode within that lumen. Thus, the exact same sensor can be studied with and without NO release, merely by disconnecting the electrode leads from the NO generating lumen to the potentiostat. The O_2 sensing was found to be fully compatible with NO release, as no noticeable amperometric signal changes were observed for the O_2 sensor when NO generation was switched "on" or "off" at each O_2 level during the calibration (see Figure S2). This is expected as the reaction between NO and O_2 is second order with respect to NO, implying that the reaction is slow when the concentration of NO is low.[26] This is true for the catheters under investigation, as they generate a relatively low flux of NO, $\sim 1.5 \times 10^{-10}$ mol·cm^{-2}·min^{-1} (see below). The high solubility of O_2 in silicone rubber also sufficiently supplies O_2 to the electrode surface even if a portion of the O_2 does react with NO.

In benchtop studies, the sensors exhibited stable amperometric calibrations during storage at 37 °C over 3 d with continuous NO generation/release (Figure S3). The response time of the PO_2 sensors were ca. 7 min (Figure S4), primarily determined by the dimensions (e.g., wall thickness) of the dual lumen catheters employed in these studies. Although not ideal, this response time is sufficient to be clinically useful, especially compared to the current situation where PO_2 can only be measured *ex vivo* using samples of fresh blood. The response time for standard Clark-type O_2 sensor also depends on the dimension of the sensor (membrane thickness, distance between the electrode to the membrane, etc.), but since the membranes can be very thin for *ex vivo* sensors, the response times of these devices are generally 1 min or so. This is not possible when the wall of the catheter is being used as the gas permeable membrane, since wall thickness needs to be large enough to provide the catheter mechanical strength to be placed within a blood vessel.

The NO release of such devices was also examined. The sensing catheters released NO, as measured by chemiluminescence measurements,[27] at an average surface flux of $>1.0 \times 10^{-10}$ mol·cm^{-2}·min^{-1} (based on the area inserted in blood vessels) for 72 h, which is more than sufficient for the short-term proof-of-concept studies reported here (see Figure S5). The duration of the NO release in these particular devices is mainly limited by the small volume of the NO generation reservoir solution containing nitrite ions (~30 μL). The duration of NO generation/release can be readily extended, if necessary, by increasing the volume or concentration of nitrite within the reservoir. It has been shown that devices that have a larger reservoir (using longer catheters) can exhibit NO release at relevant fluxes for >7 d.[22] This provides a simple solution for extending the NO release duration since only a relatively small portion of the device needs to reside within the blood vessels. The surface region of NO release can be controlled by situating the active NO generating electrode near the distal end where the catheter is implanted within the blood vessel.[22] Increasing the concentration of nitrite in the reservoir without changing the volume, though effective for longer-term NO release, is limited by the further increase in osmotic pressure, which could potentially compromise the stability of the device over longer-term use.

Sensor Performance *in Vivo*. The catheter-type PO_2 sensors were first studied in rabbit veins over a 7 h period. The sensors were purposely challenged with lower venous PO_2 levels during the latter period of the experiment, by switching the FiO_2 from 100% to 21%. The NO releasing sensors measured the PO_2 levels accurately and were able to follow both the decrease of PO_2 at the ~4 h time point and the recovery of PO_2 at the ~5.5 h time point (see Figure 2a as representative example). In contrast, the signal from the control sensors started to deviate negatively during the latter time period of the experiment, and although the sensors responded to a decrease of PO_2 at ca. the ~4 h mark, the levels measured were not accurate (negative deviation from *in vitro* blood-gas instrument values) and the responses were not able to fully recover when the PO_2 is changed to the higher level via increasing the FiO_2 level. This was due to the formation of the

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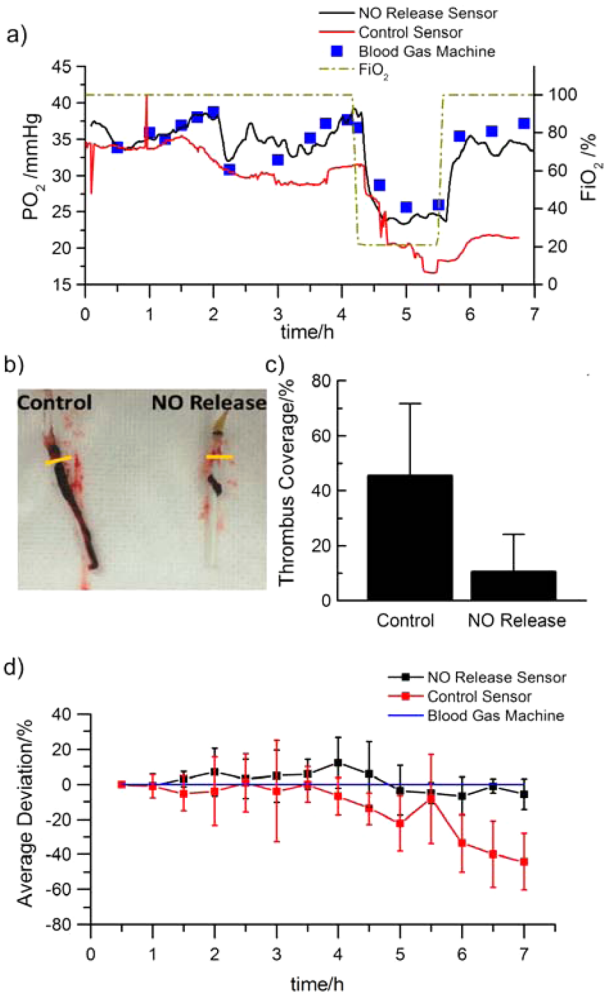

Figure 2. Performance of electrochemical NO generating/releasing PO_2 sensors implanted in rabbit veins for 7 h: (a) representative sensor response for a NO releasing sensor (black) and a control sensor (red) compared with blood draw *in vitro* test values (blue square); the FiO_2 levels were changed purposely between 100% and 21% (dash dot) to vary venous PO_2; (b) representative photo illustrating the degree of clot formation on the surface of the control and the NO releasing sensors after being explanted; (c) average thrombus coverage percentage on NO releasing sensors vs control sensors (n = 5 rabbits, $p < 0.05$); (d) average deviation of the NO releasing sensors (black) and control sensors (red) from the reference method (blue). Error bars indicate standard deviation.

large blood clots on the control sensor (see Figure 2b, as an example). Thrombus formation around the catheter surface can create a local environment that has lower O_2 level because of the consumption of oxygen by platelets and other cells within the clot.[9] Overall, the NO releasing sensors induced less clot formation than the control sensors as measured by imaging the surface of the catheters after explantation from the rabbit after 7 h (see Figure 2c for data for n = 5 rabbits, $p < 0.05$).

Since the PO_2 level is different within each individual animal at different time points, the accuracy of the sensors was evaluated by quantitating the deviations (Dev) of the PO_2 values provided by the sensors vs those provided from the *in vitro* blood sample PO_2 measurements at the same time point, assuming the *in vitro* measured values are 100% accurate. The deviation can be calculated as

$$\%\mathrm{Dev} = ((PO_{2\,\mathrm{sensor}} - PO_{2\,\mathrm{reference}})/PO_{2\,\mathrm{reference}}) \times 100$$

where $PO_{2\ \mathrm{sensor}}$ is the measured PO_2 from catheter-type sensors and $PO_{2\ \mathrm{reference}}$ is the measured PO_2 from the blood gas analyzer using the discrete blood samples. In general, the NO releasing sensors showed deviations within ±15% and the differences are not significant at each time point ($p > 0.2$, n = 5 rabbits, Figure 2d), while the control sensors exhibited significant negative deviations at time points >4 h after they were implanted within veins ($p < 0.05$, n = 5 rabbits, Figure 2d). It should be noted that venous PO_2 values are typically much lower and encompass a relatively narrow range (25−50 mmHg) compared to arterial blood (see below for porcine experiments). Small changes in the venous PO_2 values provide information about tissue perfusion.[28] The effective functionality of NO releasing PO_2 catheter sensors within veins has not been evaluated previously.

For testing the proposed sensors *in vivo* over longer time periods and over a wider range of PO_2 levels, the catheter sensors were further implanted within pig arteries for 21 h. Again, the FiO_2 was varied between 100% and 21% to challenge the sensors with different arterial PO_2 levels *in vivo*. As in the rabbit experiments, the sensors with electrochemical NO release followed the changes in PO_2 more accurately and reversibly while the control sensors without NO generation turned "on" start to exhibit negative deviations from *in vitro* measured PO_2 levels after 6 h (Figure 3a). Overall, the NO releasing sensors provided more reliable PO_2 values for the entire 21 h *in vivo* experiments (n = 4 pigs, $p > 0.1$ at each time point) while the controls sensors, after 6 h of implantation,

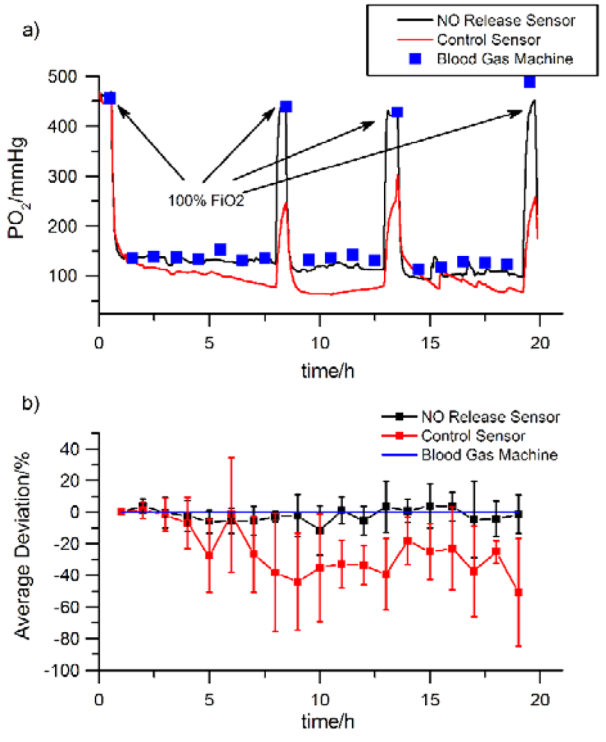

Figure 3. Performance of electrochemical NO generating/releasing PO_2 sensors implanted in pig arteries for 21 h: (a) representative current response for a NO releasing sensor (black) and a control sensor (red) compared with blood draw *in vitro* test values (reference method, blue square). Arrows indicate where FiO_2 changes from 21% to 100%; (b) average deviation of the NO release sensors (black) and control sensors (red) from the reference method (blue). Error bars indicate standard deviation.

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showed a significant negative deviation of >20% at almost every time point (n = 4 pigs, $p < 0.05$ at each time point except for the 16th h; see Figure 3b). Note that the ability to follow active modulation of PO_2 in both veins and arteries were demonstrated for the first time for these new NO releasing PO_2 sensors.

The measurements from both rabbit veins and pig arteries can also be combined and compared with the reference method to assess their overall analytical performance *in vivo* (see Figure 4). Data points after the 4 h time point in the rabbits and after the 6 h time point in pigs were included in the comparison, since it generally takes time to observe the lowered analytical results for the control sensors (accumulation of clots/thrombus takes time). The NO releasing sensors exhibited good correlation (R^2 = 0.97) and accuracy for PO_2 measurements with an average deviation of −2 ± 11%, whereas the control sensors yielded much poorer correlation (R^2 = 0.43) and a deviation of −31 ± 28%. On the basis of the periodic blood sample tests as reference method (n = 84), 96% of the measurements from NO generating/releasing sensors were within ±20% error, while only 32% of the measurements from control sensors were within ±20% error (Figure 4). Linear regression was performed on these measurements to obtain slopes for the results from the electrochemical PO_2 sensors vs those from the reference *in vitro* measurement method. The electrochemical NO releasing sensors yielded a slope of 0.90 (not shown in Figure 4 for clarity), indicating good overall accuracy. The control sensors, in contrast, exhibited a slope of 0.51, indicating an overall 49% suppression of the signals. Again, this is most likely due to thrombus formation and concomitant entrapped metabolically active cells on the control catheters.

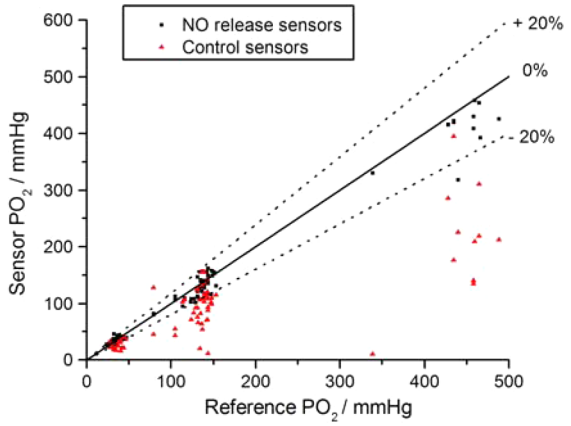

Figure 4. Comparison of measured PO_2 from catheter-type sensors *in vivo* vs the reference method from blood samples. Data contain all the measurements >4 h time point in rabbit experiments and >6 h time point in pig experiments. Black squares represent results from the NO generating/releasing sensors. The red triangles represent the measurements from the control sensors. Dash lines and the solid line indicate ±20% error and 0% error, respectively.

CONCLUSIONS

Catheter-type amperometric PO_2 sensors incorporating a novel electrochemical NO generating/release system have been developed. These NO releasing sensors were implanted in both veins and arteries of animal models for up to 21 h and yield less clot formation and more accurate analytical results. This method could provide a general strategy for improving the hemocompatibility of a wide variety of blood contacting sensors/devices. Further, owing to the potent antimicrobial properties of NO,[29] such electrochemical NO generating devices could also greatly lower the risk of infection, which is another major issue with intravascular sensors and other devices, especially when the dwelling time is longer than 24 h. We envision that a low level of NO can be used to prevent clotting but can be increased for short periods (3 h per day etc.) to better kill bacteria.[21] This can be readily achieved by the new electrochemical NO release system investigated in this study. A longer-term (7 d) *in vivo* investigation of the new IV-PO_2 sensor design in freely moving animals is currently being planned.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.5b01590.

Data relating to simulation of NO distribution around the sensing catheter, calibration of the PO_2 sensors with and without NO generation, stability of the sensors, response time, and NO release measurements. (PDF)

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Notes

The authors declare no competing financial interest.

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