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Nitric Oxide Release for Improving Performance of Implantable Chemical Sensors – A Review

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Abstract

Over the last three decades, there has been extensive interest in developing *in vivo* chemical sensors that can provide real-time measurements of blood gases (oxygen, carbon dioxide, and pH), glucose/lactate, and potentially other critical care analytes in the blood of hospitalized patients. However, clot formation with intravascular sensors and foreign body response toward sensors implanted subcutaneously can cause inaccurate analytical results. Further, the risk of bacterial infection from any sensor implanted in the human body is another major concern. To solve these issues, the release of an endogenous gas molecule, nitric oxide (NO), from the surface of such sensors has been investigated owing to NO's ability to inhibit platelet activation/adhesion, foreign body response and bacterial growth. This paper summarizes the importance of NO's therapeutic potential for this application and reviews the publications to date that report on the analytical performance of NO release sensors in laboratory testing and/or during *in vivo* testing.

Keywords

in vivo chemical sensors; real-time monitoring; biocompatibility; blood gases; glucose/lactate

1. Introduction

The real-time and accurate measurements of clinically important species, such as arterial blood gases (pH, PO_2 , PCO_2), electrolytes (Na^+ , K^+ , Ca^{2+}) and glucose/lactate levels in undiluted circulating whole blood of hospital patients via implantable chemical sensors continues to be of great interest to improve the quality of health care [1–3]. This is because intermittent *in vitro* blood measurements cannot provide real-time information on changing physiological conditions of critically ill patients, which can lead to life-threatening events for such patients. However, designing successful *in vivo* sensors that are accurate enough for such clinical use is usually complicated by adverse biological responses that lead to errant analytical results [4–6].

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The main biological response to implanted sensors depends largely on the location of implantation: blood or subcutaneous tissue [7]. When an implantable chemical sensor is in contact with flowing whole blood, the initial biological response is the nearly instant adsorption of plasma proteins (e.g., fibrinogen, fibronectin, vitronectin and von Willebrand's factor (vWF)) onto the foreign device surface, which leads to the adhesion, activation and aggregation of platelets that trap red blood cells and ultimately forms a thrombus (blood clot) within a matter of hours [8–11] (see Figure 1). Such biofouling on the surface of implantable sensors can restrict the mass transport of analytes to the sensing surface, leading to deterioration in sensor response/performance [12,13]. Further, the metabolic activities of adhered cells consume oxygen and glucose while producing carbon dioxide, thereby altering the local concentration of these species measured by the implanted chemical sensors, leading to inaccurate results [6].

Sensors implanted in the subcutaneous tissue (under the skin) are also subject to biocompatibility problems, primarily from inflammatory responses by the body's self-defense mechanism which can result in inaccurate results [3,13]. This acute inflammatory response rapidly takes place after sensor implantation as proteins and inflammatory cells migrate to the implant site and adhere to the sensor surface. Subsequently, local edema is created at the surface. Further, phagocytic cells (e.g., neutrophils, monocytes, and macrophages) form fibrous encapsulation around the sensors, creating a significant change of the analyte diffusion rate, resulting in frequent changes in the response curve/sensitivity of the sensor [14–16]. In addition, the injury to blood vessels caused by implanting chemical sensors in the subcutaneous tissue will facilitate the diffusion of the analyte from the blood to the subcutaneous fluids affecting the output accuracy of sensors [17]. To date, attempts to monitor in subcutaneous tissue have mostly been focused on glucose measurements, since the concentrations of glucose in this tissue closely relate to blood levels [18,19]. The critical parameters for animal models and data processing methods required to assess the reliability and usefulness of such subcutaneous glucose sensors have been studied [20].

Many strategies have been reported to reduce such biofouling on the surface of implanted chemical sensors by using various types of modified polymers [21–26]. Some examples include hydrogels [11,14], phospholipid-based biomimicry, flow-based systems, Nafion polymer coatings, surfactant-derived membranes, diamond-like carbons, use of polyurethane and silicone elastomers, and topology treatments [12,27–34]. Also, many efforts have been made to design “active” biocompatible materials, most often by immobilization of heparin, to prevent significant thrombus formation at the surface of intravascular devices [35–38]. However, platelet cell adhesion and activation can still occur, leading to localized changes in analyte levels. Furthermore, the use of immobilized heparin may be problematic for patients who suffer from thrombocytopenia, hemophilia or renal failure. Despite such extensive efforts, none of these methods have proven to be useful for fabrication of clinically effective implantable chemical sensors for use in critically ill hospital patients.

Nitric oxide (NO) is a diatomic free radical and endogenously produced from L-arginine and oxygen via nitric oxide synthase (NOS) [39] enzyme within the endothelial cells that line the inner walls of all blood vessels. Nitric oxide is known to prevent platelet activation/adhesion and subsequent thrombosis, as well as serving as an inhibitor of bacterial cell proliferation

and biofilm formation [40–43]. Indeed, macrophage cells in our bodies that fight infection produce high levels of NO for this purpose. Further, it is known that NO at low levels is also a potent anti-inflammatory agent [44]. Hence, the prevention of thrombosis, infection, and foreign body response associated with implantable chemical sensors may be possible by incorporating them within platforms that can release low levels of NO at physiological flux levels of ca. $0.5 \sim 4.0 \times 10^{-10} \text{ mol}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ [45]. Nitric oxide concentrations can be best measured via a chemiluminescence method [46].

It is also widely known that NO can be highly reactive with *in vivo* species (oxyhemoglobin, oxygen, and thiols, etc.). Indeed, the lifetime of NO in blood is very short, $< 1 \text{ s}$. Nonetheless, its lifetime is still long enough to have important physiological activities at surfaces against bacteria and also to inhibit platelet activation (to prevent clotting when produced at flux rates that mimic those produced by endothelial cells). NO may also be considered as a potential interference for *in vivo* oxygen sensing. However, Hang et al. [47] showed that amperometric O_2 sensing is fully compatible with NO release, as no noticeable amperometric signal changes were observed for the O_2 sensor with NO generation at physiological flux levels. Indeed, Nichols et al. [48] evaluated the *in vivo* glucose recovery of subcutaneously implanted NO-releasing (from saturated NO solution) microdialysis probes in a rat model over 14 d period and reported that intermittent sustained NO release from implanted probe surfaces may improve glucose diffusion for subcutaneously implanted devices by mitigating the foreign body reaction. However, since NO has a short half-life, many NO donors have been studied for achieving prolonged and controlled NO delivery for potential clinical use, instead of using NO gas from a cylinder.

2. *N*-Diazoniumdiolates as NO donors

A *N*-diazoniumdiolate is an NO adduct with secondary amines [49], and one of the most widely investigated NO donors due to its ability to release NO spontaneously under physiological conditions via a proton-driven reaction [50,51]. One equivalent of amine reacts with two equivalents of NO to form the corresponding diazoniumdiolate under high NO(g) pressure (e.g., 80 psi). An initial study by Espadas-Torres et al. [52] reported the fabrication of classical polymer membrane type ion-selective electrodes (ISE) for H^+ and K^+ with a NO donor (*N,N'*-dimethylhexanediamine nitric oxide adduct, also known as DMHD/ N_2O_2) within to investigate whether low level of continuous NO release from the diazoniumdiolate species would impair the analytical performance of the sensors. As a result, ISE sensors with and without DMHD/ N_2O_2 showed a comparable potentiometric response: K^+ -detection (membrane composition: 66% Tecoflex polyurethane/30% dioctyl sebacate/0.6% potassium tertakis(4-chlorophenyl)borate/2.5% Valinomycin) exhibited slopes of 60.3 ± 0.1 and $60.6 \pm 0.2 \text{ mV/decade}$ and intercepts of -5.31 and -5.44 , respectively. For H^+ -detection (membrane composition: 33% tecoflex polyurethane/63% dioctyl sebacate/1.7% potassium tertakis(4-chlorophenyl)borate/2.5% tridodecylamine) the sensors exhibited slopes of 58.5 ± 0.9 and $59.7 \pm 0.6 \text{ mV/decade}$ with intercepts of -9.38 and -9.50 , respectively. The study also reports that the surfaces of the sensors exhibit a marked decrease in thrombogenic properties when measured by *in vitro* platelet adhesion studies using sheep plasma. This early study clearly suggested that their approach for enhancing biocompatibility of ion sensors by incorporation of a NO donor can be applicable

to a wide range of polymeric materials that can be used in implantable chemical devices. In another early study, Mowery et al. [53] prepared Clark style amperometric oxygen sensors and potentiometric sensors (pH and K^+) with three distinctly different types of diazeniumdiolate NO donors, DMHD/ N_2O_2 , linear polyethylenimine/ N_2O_2 (LPEI/ N_2O_2) and methoxymethyl piperazine PVC/ N_2O_2 (mompipPVC/ N_2O_2), and reported a significant decrease in platelet adhesion and activation for both *in vitro* and *in vivo* sensors fabricated with these materials.

The effect of diazeniumdiolate species was further investigated for potential improvement of the biocompatibility and *in vivo* analytical performance of intravascular amperometric oxygen sensors by Schoenfisch et al. [54]. In this work, the authors fabricated NO-releasing silicone rubber-catheter-based amperometric PO_2 sensors via dip-coating the sensor with gas permeable coatings formulated with cross-linked silicone rubber containing DMHD/ N_2O_2 . They then demonstrated that there was greatly reduced thrombus formation on the outer surface of the sensors and superior overall sensor performance vs. control sensors when implanted, in the absence of systemic anticoagulation, within the carotid and femoral arteries of mongrel dogs for 6–24 h. The only caveat was leaching of *N,N'*-dimethylhexane-diamine and the corresponding toxic nitrosamine, a decomposition product of DMHD/ N_2O_2 , into aqueous soaking solutions or blood. Therefore, they concluded that NO donors such as diazeniumdiolates that possess a more lipophilic structure, or can be covalently attached to the outer polymer coating of the sensor would be more optimal.

Frost et al. [55] decreased the potential problem of leaching diamine and nitrosoamine by using a more lipophilic NO-releasing agent within the silicone rubber polymer that contains a diazeniumdiolate species (DACA-6/ N_2O_2), anchored to the cross-link sites of the rubber matrix. They further prepared Clarke-style amperometric oxygen-sensing catheters coated with an outer layer of the DACA-6/ N_2O_2 polymer that releases physiological levels of NO for >20 h, and showed significantly improved biocompatibility and analytical performances compared to control sensors without NO release when tested *in vivo* within the carotid and femoral arteries of swine for 16 h.

By coating a dual lumen silicone catheter with this DACA-6/ N_2O_2 -silicone layer, a potentiometric CO_2 sensor was also developed [56,57]. The wall between two lumens is doped with a proton ionophore, tridodecylamine, and a tetra-phenylborate type cation exchanger, functioning as a pH-selective layer. One lumen contains a bicarbonate/sodium chloride solution and a Ag/AgCl electrode. The pH of this lumen solution will be decreased in the presence of an increase in the PCO_2 level in solution (or blood) surrounding the device. This causes a potential change across the polymeric wall between the lumen filled with the bicarbonate solution and the other lumen filled with strong buffer (4-morpholinoethanesulfonic acid buffer, pH = 5.7). A second Ag/AgCl reference electrode in the buffered lumen provides a means to measure this potential change as a function of change in PCO_2 levels. The overall potential change of this two electrode system was found to be proportional to $\log(PCO_2)$ of the sample with a near-Nernstian slope (−60.1 to −57.8 mV/decade) even in the presence of $\sim 10 \times 10^{-10} \text{ mol}\cdot\text{cm}^{-2} \text{ min}^{-1}$ fluxes of NO from the surface of the device.

Given that the NO-releasing implantable PO_2 sensors showed promising *in vivo* results, Gifford et al. [58] developed the very first NO-releasing needle-type enzyme-based electrochemical glucose sensors for measurement of glucose in subcutaneous fluid. The outer membrane of this glucose sensor contained a diazeniumdiolate species, (Z)-1-[N-methyl-N-[6-(N-butylammoniohexyl)amino]]-diazen-1-ium-1,2-diolate (DBHD/ N_2O_2 ; see Figure 2 for chemical structure), that can release NO for ~16 h. The resulting sensor showed excellent analytical performance for both *in vitro* and *in vivo* tests, and inhibited the typical subcutaneous inflammatory response when tested in Sprague-Dawley rats for 3 d. Clarke error grid analysis indicated superior performance for the NO-releasing sensors when compared to controls on both day 1 and 3. Histological examination of the sites where the NO-releasing sensors were placed in the animals also showed significantly less inflammatory response over the first 24 h compared to non-NO release controls, and a significant decrease in the *in vivo* sensor signal stabilization time right after implantation. Yan et al. [59] and Wolf et al. [60] also developed the same type of implantable amperometric glucose sensors based on hydrogen peroxide detection with an outer polymer containing NO releasing DBHD/ N_2O_2 and found reduced thrombus formation and improved analytical performance compared to control sensors when implanted in the veins of rabbits for 7 h (see Figure 3). However, these studies found that NO release from a thin layer of DBHD/ N_2O_2 was too short and uncontrollable for ultimate clinical use and that NO could also be detected at an applied potential of +0.6 V and higher on the underlying platinum-iridium working electrode, required for electrochemical glucose sensors based on detection of hydrogen peroxide produced from glucose oxidase. Overall, these studies indicated that NO is compatible with subcutaneously and intravascularly-implanted electrochemical glucose sensors, but additional work on the best NO release chemistry was still necessary.

The need for improved NO release coatings to enhance the *in vivo* performance of implantable chemical sensors led to the development of an NO-releasing hydrophilic platform. Marxer et al. [61] first developed an amperometric sol-gel derived oxygen sensor that releases NO via coating the platinum working electrode with an aminosilane/ethyltrimethoxysilane hybrid xerogel film doped with hydrophilic polyurethane (HPU) that are subsequently exposed to 5 atm of NO for 3 d (to form diazeniumdiolate groups on the amine sites). The sensors exhibited highly reproducible and linear sensitivities to oxygen with rapid response times and released NO at fluxes of $7.2 \times 10^{-10} \text{ mol}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$, effectively reducing platelet adhesion for 48 h. However, it was suggested that further work on improving the NO release layer was needed to evaluate the *in vivo* performance of such implantable chemical sensors. Indeed, Shin et al. [62] developed a macro glucose sensor based on a new type of hybrid sol-gel/polyurethane (PU), made by sequentially coating the platinum working electrode with the following four polymeric membranes: a sol-gel layer with immobilized glucose oxidase (GOx), PU for protection of the GOx, PU doped with NO donor (diazeniumdiolated sol-gel particles) and sol-gel particles, and then PU. The NO donor was prepared by exposing aminosilane-based sol-gel particles to 5 atm NO for 3 d to convert accessible amine groups to diazeniumdiolates. The design demonstrated good sensitivity, reproducibility and fast response time to glucose as well as a reduction of enzyme inactivation. However, the authors observed leaching of sol-gel particles even when the sensor was coated with a PU outermost layer and a drastic decrease in sensitivity of

sensor to glucose when coupled with this new NO-releasing sol-gel film. Also, a longer duration of higher fluxes of NO release was still needed for the miniaturized version of sensor to study the *in vivo* biocompatibility of such a design. Indeed, Oh et al. [63] reported a miniaturized glucose biosensor modified with NO-releasing xerogel micro-patterned array that released NO from 5 μm wide lines separated by 5 or 20 μm distances. The micro-patterned array allowed for an increase in glucose sensitivity since significant portions of sensor surface remain unmodified by the NO release coating. They found that generation of sufficient levels of NO from such a design reduced platelet adhesion (>40 % reduction) and *Pseudomonas aeruginosa* bacterial growth (70–80% reduction) without reducing enzymatic activity of GOx. However, since the NO release from sensor depleted after only 3 days, extension of NO release time would be necessary for longer-term *in vivo* testing of such miniaturized glucose biosensors.

Koh et al. [64] further investigated the fabrication of polyurethane-based implantable glucose sensors with a NO-releasing porous fiber mat-modified sensor membrane (540 ± 139 nm fiber diameter, $94.1 \pm 3.7\%$ porosity). The sensor released ~ 100 nmol of NO per mg of polyurethane over 6 h period without leaching of the NO donor, even in serum. However, to date, this new design has never been tested for analytical performance *in vivo*.

A diazeniumdiolate type NO donor was also used to provide NO release for film type fluorescent oxygen sensors. In initial efforts, the fluorescent oxygen indicator (pyrene/ perylene donor/acceptor pair) was doped into SR membrane with dispersed DMHD- N_2O_2 particles or DACA-6 modified SR membrane [65]. However, the oxygen response was found to become non-linear because of heterogeneity of the particle-doped film and/or a fluorescence quenching effect from the amine groups of the NO donor. This problem was solved by a two-layer configuration on a quartz slide, which consisted of an underlying DACA-6 modified SR membrane for NO release and an outer SR membrane doped with the oxygen indicator for fluorescence oxygen sensing. By using this configuration, only NO gas rather than the NO donor can interact with the oxygen indicator and the linearity and sensitivity toward oxygen was not perturbed. NO release at a flux of $>4 \times 10^{-10}$ mol·cm $^{-2}$ ·min $^{-1}$ was obtained for at least 24 h at room temperature and the sensor response is quite stable during this period.

Obviously, the quartz slide-based sensor is not ideal for *in vivo* use. Rather, optical fiber sensor technology has been used successfully for *in vivo* diagnostic applications. Therefore, the two-layer configuration was expanded to an optical fiber type sensor. Dobmeier et al. [66] developed a NO-releasing fiber optical pH sensor. An aminoalkoxysilane-based xerogel membrane with covalently bonded N-diazeniumdiolates as the NO donor was deposited on the tip of optical fibers. Then the same type of xerogel doped with fluorescent pH indicator, seminaphthorhodamine-1 carboxylate, was coated on the top of the NO donor layer. NO release at $>0.24 \times 10^{-10}$ mol·cm $^{-2}$ ·min $^{-1}$ flux was obtained for up to 16 h, indicating the good permeability of NO through the pH-sensitive coating. The underlying NO-releasing layer allows transmission of excitation and emission light and doesn't interfere with the fluorescent pH detection. Response to physiologically relevant pH (7.0–7.8) was obtained by this sensor with a short response time, good linearity, and high precision. Also, *in vitro*

platelet adhesion studies confirmed the effectiveness of NO released from such sensor device to inhibit of adhesion of platelets from porcine serum samples.

3. Nitrite for NO Generation

To avoid the leaching of NO donors and/or byproducts into the bloodstream, and to produce very controllable NO generation over prolonged time, Ren et al. [67] reported improvement in *in vivo* analytical performance of intravascular analytical sensors by coupling them with a new electrochemical method of NO generation. This method involves electrochemical reduction of inorganic nitrite ions catalyzed by a copper(II)-ligand complex, copper(II)-tri(2-pyridylmethyl)amine (Cu(II)TPMA; see structure in Figure 2). A dual lumen catheter-type amperometric PO_2 sensor design was developed (with electrochemical NO generation in bigger lumen and PO_2 -sensing platinum-iridium electrode in smaller lumen; see Figure 4) and these devices were implanted in both veins and arteries of rabbits and pigs for up to 21 h. The NO generating sensors exhibited less clot formation and much more accurate analytical results compared to controls. The advantage of this sensor design is that the amount NO generation can be easily controlled by different applied potentials of between -0.2 and -0.4 V to the working electrode of the NO generating lumen, and be turned “on” and “off” merely by disconnecting the electrode leads from that lumen to the potentiostat [68]. Furthermore, the duration of NO generation can be readily extended by increasing the volume or concentration of nitrite within the reservoir. Overall, the study showed that this method could significantly improve the biocompatibility of a wide variety of implantable chemical sensors and provides a potential tool for longer-term *in vivo* investigations into the interplay between long-term NO release and extended analytically useful *in vivo* sensor performance.

4. S-Nitrosothiols (RSNOs) as NO Donors

S-Nitrosothiols (RSNO) are another class of NO donors that could be used to improve the biocompatibility of implantable chemical sensors [69]. They are transporters and/or storage vehicles of NO in the body and can be rapidly decomposed to release NO via a several different pathways [70–73], including thermal and photolytic cleavage of the S-N bond of the RSNO to release NO. In addition, Cu^+ species (formed from reduction of Cu^{2+}) can catalyze the reaction of RSNOs to liberate NO. Furthermore, ascorbate-mediated reactions can occur in which ascorbate at low concentration can reduce trace copper ion impurities in solution (i.e., Cu^{2+}) to generate Cu^+ that can then react with RSNOs to produce NO.

Soto et al. [45] were the first to develop implantable chemical sensors with an RSNO as the NO donor and reported the first long-term investigation on the *in vivo* analytical performance of percutaneously implanted NO-releasing amperometric glucose sensors in swine. The sensors were implanted for 10 d and analytical results were obtained comparing the NO-releasing sensors with two different NO-releasing rates with the same total amounts of NO released ($3.1 \mu\text{mol}\cdot\text{cm}^{-2}$): rapid NO release (16.0 ± 4.4 h) from N-diazeniumdiolate NO donor (MAP3/NO) [74] and slower NO release ($>74.6 \pm 16.6$ h) from S-nitrosothiols-modified silica nanoparticles (MPTMS-RSNO). It was shown that the sensors with slower NO-release rate for extended durations exhibited a significantly lower mean absolute relative

deviation (MARD) on days 1 and 3 (26.0 ± 5.1 and $23.9 \pm 8.6\%$, respectively) versus controls (34.3 ± 10.9 and $38.8 \pm 10.4\%$, respectively), and were characterized by shorter sensor lag time (< 4.2 min) in response to intravenous glucose tolerance tests when compared to rapid NO-releasing and controls sensors (>5.8 min) at 3, 7, and 10 d. Overall, it was shown that both rapid and slower NO-release sensors exhibited improved accuracy vs. controls and it was suggested that the materials that are capable of releasing a large amount of NO with even longer duration (i.e., several weeks) would be necessary to create the ultimate NO-release strategy for long-term implantable chemical sensing technology. Indeed, Soto et al. [75] further investigated fabrication of an implantable glucose sensor using an optimized HP-93A-100 PU membrane doped with RSNO-modified silica nanoparticles as the outer, glucose diffusion-limiting layer. They showed that the sensor yielded a linear glucose calibration between 1–21 mM over a 2 week period while incubating in PBS. Further, the sensing device released NO at $>0.8 \text{ pmol}\cdot\text{cm}^{-2}\cdot\text{s}^{-1}$ for up to 6 d with negligible particle leaching ($<0.6\%$). However, *in vivo* analytical performance of this new design has not yet been reported in the literature.

Of many RSNOs available for use in developing NO release implantable sensors, SNAP (S-nitroso-*N*-acetyl-penicillamine; see chemical structure shown in Figure 2) has been intensively investigated to prevent donor leaching and stabilize release of NO for improvement in the biocompatibility of various implantable medical devices, including intravascular chemical sensors. Cha et al. [76] demonstrated the compatibility of NO with implantable enzymatic amperometric glucose sensors based on the osmium (III/II) mediated electro-chemistry and showed that amperometric glucose response is preserved in the presence of continuous NO release from the surrounding SNAP-doped silicone tubing when tested in both phosphate buffer (pH 7.4) and *in vitro* within heparinized whole porcine blood at 37 °C. As shown in Figure 4, an additional layer of copper nanoparticles (5 wt. % and 40–60 nm) in Carbosil (Polyurethane-based polymer) between the Teflon coating on the platinum-iridium working electrode and the SNAP-impregnated silicone tubing facilitates physiological levels of NO release ($>1 \times 10^{-10} \text{ mol}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$) for at least 3 d. Furthermore, the sensor requires a low applied potential, +0.2 V, that avoids any additional background current from oxidation of NO at the underlying working electrode, which is normally observed from NO-releasing glucose sensors based on conventional hydrogen peroxide detection method that require a much higher applied potential, +0.6 V or higher. Overall, it was shown in this work, for the first time, that implantable amperometric glucose sensors prepared using an osmium(III/II)-bipyridine-polyvinylimidazole complex to mediate electron transfer to the working electrode from the glucose oxidase enzyme (i.e., sensor not based on hydrogen peroxide detection), can maintain continuous analytical functionality and measurement accuracy in the presence of NO release.

5. Endogenous RSNOs for NO generation

One common limitation of various NO release systems is the finite amount of NO donors that can be held within the sensor device. The NO donor will be depleted typically within a time duration of one day to one month and the biocompatibility benefit will no longer exist once the NO release stops. To overcome this issue, generation of NO at the surface of biomedical implants from endogenous NO donors such as *S*-nitroso-albumin, *S*-

nitrosoglutathione, *S*-nitrosocysteine, and nitrite has been suggested. Duan and Lewis et al. [77] modified biomedical polymers with L-cysteine and found that such surfaces can generate NO from S-nitroso-albumin via a NO transfer and release mechanism. However, the decomposition of nitrosated cysteine may convert L-cysteine to its disulfide dimer product that is unable to generate NO. Other researchers have examined a series of metal ion-based catalysts embedded within or tethered to a polymer coating of biomedical implants for *in situ* generation of NO from endogenous RSNOs [78–84]. For example, copper complexes, organoselenium species, organoditelluride species, and copper particles on the implants have been found to catalyze decomposition of various endogenous NO donors in the presence of reductants such as ascorbic acid or glutathione.

Wu et al. [84] adapted the concept of NO generation to intravascular chemical sensors. In this work, 3 μm and 80 nm Cu^0 particles were doped into a polyurethane or polyurethane/silicone rubber layer coated on a silicone rubber catheter type amperometric oxygen sensor. Slow corrosion of the Cu^0 particles generates copper ions, which catalyzes decomposition of GSNO (a naturally present RSNO in blood) in the presence of GSH (also present in blood). The NO-generating oxygen sensors were implanted into porcine arteries for 19–20 h. A lower chance of thrombus formation and an enhanced oxygen sensing accuracy were observed on such sensors compared to control sensor fabricated without the Cu^0 particles coating. However, it was found that the effectiveness of this NO generation method depends on animals. Some animals show significant clots on the Cu^0 particle-modified catheters, which suggests that these animals may have a lower amount of circulating endogenous RSNOs (probably GSNO) in blood stream and are unable to generate high enough NO levels at the surface of implantable sensors to completely prevent thrombus formation.

6. Conclusions

The prospect of developing NO-releasing or generating real-time continuous monitoring sensors that respond to clinically important ions, gases and molecules in blood or subcutaneous fluid is an ambitious effort to solve a very challenging analytical problem. A variety of NO release/generation strategies have been shown to release or generate sufficient amounts of NO to enhance the biocompatibility and *in vivo* analytical performance of both intravascular and subcutaneous chemical sensors. However, additional efforts are still needed to devise a platform that can achieve stable release or generation of suitable NO levels for 1–2 weeks, and also be compatible with the several different types of sensing chemistries and sensor designs required to continuously monitor many of the clinically important analytes in blood. If such sensors can ultimately be realized through continued research efforts, then commercial *in vivo* sensors could finally become a reality for routine *in vivo* monitoring of patients in intensive care units around the world.

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Highlights

- The importance of nitric oxide's role in improving biocompatibility of implantable chemical sensors
- Recent progress on various types of nitric oxide releasing and generation platforms
- Analytical performance of nitric oxide releasing implantable chemical sensors in laboratory testing and/or during *in vivo* testing.

Figure 1.
Sequential process that ultimately leads to thrombus formation on the foreign device surface.

Figure 2.

The structures of some NO donors/generators: (a) (*Z*)-1-[*N*-butyl-*N*-(6-(*N*-methylammoniohexyl)amino)]-diazen-1-ium-1,2-diolate (DBHD/N₂O₂), (b) *S*-nitroso-*N*-acetyl-D-penicillamine (SNAP), and (c) Copper(II)-tri(2-pyridylmethyl)amine (Cu(II)TPMA), a catalyst used for electrochemical generation of NO from an inorganic nitrite reservoir.

Figure 3.

(a) NO-releasing needle/catheter type glucose sensor with DBHD/ N_2O_2 as NO donor; (b) photos of the control (top) and NO release (bottom) glucose sensors after the *in vivo* experiment. The portions of the sensors to the left of the dashed lines were actually inside the veins; (c) Comparison of glucose concentration values obtained from benchtop blood gas analyzer and the converted current values measured by the continuous sensor. One conversion of current to glucose concentration ($\text{mmol}\cdot\text{L}^{-1}$) was made with the calibration curve in bovine serum; the other conversion was a one point calibration, adapted from [60].

Figure 4. (a) Schematic of dual-lumen catheter-type electrochemical NO generating/releasing PO₂ sensor with cross section geometry of catheter; (b) representative photo illustrating the degree of clot formation on the surface of the control and the NO releasing sensors implanted in rabbit veins for 7 h; (c) average thrombus coverage percentage on NO releasing sensors vs. control sensors. (n = 5 rabbits, p < 0.05); (d) representative current response for a NO releasing sensor (black) and a control sensor (red) (implanted in pig arteries for 21 h) compared with blood draw in vitro test values (reference method, blue squares). Arrows indicate where fraction of inspired oxygen (FiO₂) changes from 21% to 100%. Figure adapted from [68].

Figure 5.

(a) Designs of implantable NO-releasing osmium-mediated glucose sensor; (b–d) *In vitro* comparison of intermittent glucose values obtained from a commercial glucometer and continuous glucose measurements obtained from a NO-releasing osmium-mediated glucose sensor as well as a control (No NO release) osmium-mediated glucose sensor poised at 200 mV in heparinized whole porcine blood for 3 days. On each day, glucose values were back-calculated using calibration data obtained prior to the *in vitro* blood testing (adapted from [76]).