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Nitric Oxide-Releasing/Generating Polymers for the Development of Implantable Chemical Sensors with Enhanced Biocompatibility

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Abstract

The development of reliable *in vivo* chemical sensors for real-time clinical monitoring of blood gases, electrolytes, glucose, etc. in critically ill and diabetic patients remains a great challenge owing to inherent biocompatibility problems that can cause errant analytical results upon sensor implantation (e.g., cell adhesion, thrombosis, inflammation). Nitric oxide (NO) is a well-known inhibitor of platelet activation and adhesion, and also a potent inhibitor of smooth muscle cell proliferation. In addition, NO mediates inflammatory response and promotes angiogenesis. Polymers that release or generate NO at their surfaces have been shown to exhibit greatly enhanced thromboresistance *in vivo* when in contact with flowing blood, as well as reduce inflammatory response when placed subcutaneously, and thus have the potential to improve the biocompatibility of implanted chemical sensors. Locally elevated NO levels at the surface of implanted devices can be achieved by using polymers that incorporate NO donor species that can decompose and release NO spontaneously when in contact with physiological fluids, or NO-generating polymers that possess an immobilized catalyst that decompose endogenous *S*-nitrosothiols to generate NO *in situ*. The potential use of such NO-releasing/generating materials for preparing *in vivo* sensors implanted either intravascularly or subcutaneously, is examined in this review.

Keywords

Implantable Sensors; Biocompatibility; Nitric Oxide; Platelet Activity; Inflammatory Response

1. Introduction

The care of critically ill hospitalized patients requires frequent and accurate measurements of arterial blood gases (pH, PO_2 , PCO_2), electrolytes (Na^+ , K^+ , Ca^{2+}) and glucose/lactate levels in undiluted whole blood. To date, such values are usually obtained from *in vitro* tests using discrete blood samples drawn intermittently from patients and analyzed on benchtop instruments or smaller point-of-care devices [1–3]. However, intermittent sampling can miss events that can be life-threatening to the patient. Indeed, isolated measurements are not able to provide the desired real-time monitoring of minute-to-minute physiological changes that can occur in unstable critically ill patients [2]. Therefore, continuous intravascular sensing of clinically important species would be of great value to improve the quality of health care for such patients.

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At the same time, reliable *in vivo* monitoring of glucose concentrations in blood or the interstitial fluid could significantly enhance the quality of life for millions of non-hospitalized patients with diabetes mellitus. A successful implantable glucose sensor must: i) be small enough to be placed intravascularly or subcutaneously; ii) provide long-term stable analytical signals so that re-calibration can be minimized; iii) yield accurate measurements of glucose that correlate well with *in vitro* tests based on discrete blood samples so that therapeutic action can be taken based on the measured values (e.g., infusion of insulin); and iv) be compatible with the biological environment in which it is implanted (i.e., 'biocompatible').

Advances in the miniaturization of optical and electrochemical sensors for blood gases, electrolytes and glucose/lactate have made it possible to place such devices within blood vessels or under the skin. However, despite significant efforts toward developing implantable intravascular and/or subcutaneous chemical sensors suitable for continuous *in vivo* monitoring, the routine clinical use of such devices has not yet been realized. The major challenge to be overcome for success in this area is the erratic analytical results obtained from sensors implanted *in vivo*. Errant analytical results are generally attributed to the adverse biological responses to such implanted devices [2–5], which usually relates to the lack of biocompatibility of the implanted optical or electrochemical probes.

The biocompatibility of an implantable medical device refers to the ability of the device to perform its intended function, with the desired degree of incorporation within the host, without eliciting any undesirable local or systemic effects in that host [6]. The biological response to implanted sensors depends largely on the site of implantation. Intravascular placement is the most desirable for blood chemistry measurements. The biocompatibility problems associated with sensors implanted intravascularly have been reviewed previously [1–3,7]. When an intravascular sensor is in contact with blood, the initial biological response is the rapid adsorption of plasma proteins (e.g., fibrinogen, fibronectin and von Willebrand's factor (vWF)) onto the surface, followed by the adhesion and activation of platelets (see Fig. 1a) [8]. Once activated, platelets undergo shape change and secretion of the granular contents. The activated platelet is a key player in the coagulation cascade and will ultimately lead to the formation of a blood clot on the surface of the device [9]. Such biofouling of the sensing membrane can restrict the diffusion of analyte to the sensor surface [10]. Moreover, the metabolic activities of adhered cells consume oxygen and glucose while producing carbon dioxide, thus changing the local concentration of blood gases and glucose levels sensed by the implanted device [5]. Figure 1b illustrates the typical patterns of deviation for sensors implanted intravascularly. Even a monolayer of cells adhered onto the sensor surface is sufficient to produce the behavior illustrated in Fig. 1b.

Similar patterns of deviation can also be observed when blood flow is reduced in the blood vessel where the sensor is placed, or when the sensor touches the wall of blood vessels where inherent metabolism of endothelial cells can affect sensor performance in the same way as adhered blood cells ("wall effect") [2,5]. Moreover, thrombus formation in patients can occur randomly since it also depends on factors such as the native coagulation propensity of a particular patient and the velocity of blood-flow within the lumen of the vessel in which the sensor is placed. The unpredictability of intravascular sensor performance due to these biological factors makes the resulting measurements unreliable, preventing timely therapeutic intervention.

Sensors implanted under the skin (subcutaneous measurements), such as those often developed for monitoring glucose levels, do not encounter the same issues of platelet activation and thrombus formation. However, such sensors are subject to their own biocompatibility problems, primarily from inflammatory responses that can affect their accuracy. The size, shape and physical/chemical properties of the implanted devices contribute to variations in the

intensity and time duration of the inflammatory and wound-healing process [11]. Acute inflammatory response starts immediately after sensor implantation as fluids, plasma proteins and inflammatory cells migrate to the implant site [12]. The first event that occurs is protein adsorption, followed by the attack of phagocytic cells (neutrophils, monocytes and macrophages) to the implanted sensor, attempting to destroy it. However, since the sensor is large, only 'frustrated phagocytosis' occurs [11,12]. Acute inflammatory response (24 – 48 h) is characterized by the predominance of neutrophils, which are later replaced by macrophages, monocytes and lymphocytes during the chronic inflammation stage [12]. Ultimately, fibrous encapsulation of the subcutaneously implanted device by primarily macrophages and fibrin indicates the end-stage of the wound-healing process.

The fibrous capsule can change the analyte levels in the vicinity of the sensor through metabolic activities (e.g., faster glucose consumption). It also can affect the transport of analyte to the sensor surface as well as that between the blood and the interstitial fluid. This can lead to significant changes in the calibration curve (i.e., lowering the sensitivity) as well as affecting the lag time of the sensor's response to the fluctuations of glucose levels in bulk blood for both optical [13,14] and electrochemical sensors [4,11,15]. Angiogenesis around the site of tissue injury caused by implanted sensor is also part of the wound-healing process. Since more blood vessels around the implanted sensors will facilitate the diffusion of the analyte from blood to the subcutaneous fluids (so interstitial fluid levels of the analyte more closely resemble blood levels), the degree of angiogenesis after implantation will also influence the output of sensors placed subcutaneously [16].

2. Strategies Toward Improving Biocompatibility

2.1. Surface modifications

The surface of an implanted device is thought by many to be of critical importance in determining the *in vivo* performance of any implant. Surface modification strategies employed to address biocompatibility issues have been reviewed by Wisniewski et al. [10]. These approaches include hydrogel overlays, phospholipid-based biomimicry, flow-based systems, Nafion coatings, surfactants, covalent attachments, diamond-like carbons, and topology treatments. Surfaces with high water content are found to be more inert to protein adsorption and cell adhesion as a result of low interfacial energy [17]. Hydrogels and other hydrophilic surface modifications are aimed at minimizing protein adsorption, since such adsorption initiates a series of biological responses [8,12]. Biomaterials with the surface immobilized biological molecules, such as albumin [18], heparin [19], thrombomodulin [20], prostacyclin [21] and hyaluronic acid [22] have all been shown to exhibit some effectiveness in enhancing biocompatibility.

2.2 Potential for Utilizing Nitric Oxide (NO) with In Vivo Sensors

Unfortunately, hydrophilic surfaces by themselves may retard, but do not completely prevent the adsorption of proteins on surfaces *in vivo*. Eventually, the proteins that can initiate platelet adhesion/activation or those that mediate inflammatory responses will still adsorb onto the surface of the implanted sensor, leading to thrombus formation and/or inflammation. The biological environments in which the sensors are implanted involve a myriad of activities besides the cell surface expression of certain molecules. In the case of the vascular endothelium, that lines the inner walls of all blood vessels (and is known to be the best example of a completely non-thrombogenic surface), a number of active molecules such as thrombomodulin, prostacyclin, heparan sulfate, and nitric oxide (NO) are either expressed at the surface or are continuously being secreted to mediate the biological responses underlining biocompatibility [9].

Over the past twenty years, NO has received considerable interest from the pharmacological and biomedical research communities owing to its diverse physiological functions as a protective, regulatory and signaling molecule involved in the regulation of blood pressure, clotting, neurotransmission and immune response. Indeed, NO is widely recognized as a key anti-platelet, anti-inflammatory and vasodilating agent [23–27]. In addition, NO is also known to inhibit bacterial growth [28,29] and promote angiogenesis [30].

Nitric oxide is produced endogenously from L-arginine and oxygen by enzymes known as nitric oxide synthases (NOS) [31]. NO exerts its vasodilation and anti-platelet function by binding to the heme iron of soluble guanylate cyclase and subsequently increasing the production and intracellular concentrations of cyclic guanosine monophosphate (cGMP), a secondary messenger [32]. The endogenous concentration of NO as a dissolved gas is very low (~3 nM) [33]. Under physiological conditions, oxidized NO (in the form of NO^+ or N_2O_3) reacts with thiol groups in cysteine or cysteine-containing peptides and proteins (e.g., glutathione, albumin and hemoglobin) to yield S-nitrosothiols (RSNOs) [34,35]. RSNOs act as carriers of NO in the body and are thought to be responsible for the storage and bioavailability of NO [33,36].

Vasoconstriction can occur at the site where the intravascular sensor is implanted as a biological response to reduce bleeding at the implant site. Meanwhile, platelets are activated and recruited to repair the vascular injury [37]. NO is synthesized in the endothelial cells that line the wall of healthy blood vessel and diffuses in all directions. When NO reaches the underlying vascular smooth muscle cells, NO stimulates cGMP production and, in turn, decreases the Ca^{2+} levels in the smooth muscle cells, leading to vascular relaxation [38]. Muscle relaxation allows the blood vessel to dilate and lowers the blood pressure. NO also diffuses into circulating platelets, especially when they approach the surface of the endothelium. Platelet activation is also Ca^{2+} dependent [39] and is thus inhibited by NO [27,40]. The lifetime of NO in whole blood is very short (< 1 sec) due to its reaction with heme-containing compounds such as oxyhemoglobin [41]. Therefore the anti-platelet effect occurs only locally when platelets are in close proximity to the endothelium, as the presence of red blood cells would block the NO transfer to platelets. It has been estimated that the endothelial derived NO flux is in the range of $0.5\text{--}4.0 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$ [42]. Therefore, if the surface of an intravascular sensor can release or generate NO at or above this range of fluxes, it is expected to display enhanced thromboresistance. Furthermore, the released NO will be effective only locally at the blood/polymer interface, which is ideal for biocompatibility purposes. Owing to the low NO fluxes, the small dimension of the intravascular sensor, and the very short half-life of NO in blood, the locally released NO is unlikely to cause any systemic effect that may lead to cytotoxicity or hemorrhage.

Nitric oxide also is involved in virtually every step of the inflammatory process [43]. In fact, NO plays a crucial role in wound healing and down regulates mediators of inflammatory response. Low concentrations of NO can inhibit adhesive molecule expression, cytokine and chemokine synthesis as well as leukocyte adhesion and transmigration [43]. Nitric oxide also promotes angiogenesis and disruption of the NOS-based NO generation pathway is known to impair neovascularization [30]. Subcutaneous implantation also brings the risk of infection, which can also be inhibited by NO [29]. Therefore, an active surface that can continuously release or generate NO in the interstitial fluid may also benefit greatly from a locally enhanced level of NO, with respect to reducing inflammatory cell migration and promotion of neovascularization that could increase the exchange of glucose and other analytes into the subcutaneous fluid adjacent to the implanted sensor.

3. Implantable Sensors with NO-Releasing/Generating Coatings

3.1 NO-releasing materials

Since the discovery of the anti-thrombogenic role of NO, the possibility of improving biocompatibility by incorporating NO-releasing/generating function into biomedical polymers has been explored by several research groups. Ramamurthi and Lewis [40] used the direct infusion of gaseous NO_(g) (0.1 ppm) through a semi-permeable membrane to produce surface NO fluxes ranging between $3\text{--}6.6 \times 10^{-8} \text{ mol cm}^{-2} \text{ min}^{-1}$. These low levels of NO were shown to inhibit platelet adhesion by as much as 87%. However, it would be quite difficult to deliver gaseous NO (from reservoir of NO gas) to the surface of an implanted sensor, once it was placed in an artery/vein or under the skin. Fortunately, a number of NO donor compounds have been developed to deliver NO *in vivo* and study its biological effects [44]. For implantable sensor applications, the NO donors must be incorporated in the polymer either as a thin outer coating or into the bulk polymer from which the medical sensor is made (e.g., polymeric tubing for certain catheter style sensors). The duration of NO release should match the implant lifetime of the device itself. In addition, it is critical that the NO release rate be controllable, as excess exposure to the NO donor, NO and any reaction products can be cytotoxic, mutagenic or carcinogenic.

To date, the two most widely investigated NO donors for biomedical applications are *S*-nitrosothiols (RSNO) and *N*-diazoniumdiolates (so-called NONOates). *S*-Nitrosothiols can be prepared by reacting thiols with nitrous acid. *S*-Nitrosoglutathione (GSNO) and *S*-nitroso-*N*-acetyl-cysteine (SNAC) have been blended into various polymers (e.g., poly(vinyl alcohol), poly(vinyl pyrrolidone), poly(ethylene glycol)) by de Oliveira and coworkers for targeted delivery of NO and parent RSNOs [45–47]. Bohl and West demonstrated that *S*-nitrosocysteine (CysNO) immobilized within a poly(ethylene glycol) hydrogel reduced platelet adhesion and smooth muscle cell proliferation *in vitro* [48].

A *N*-diazoniumdiolate is an NO adduct with secondary amines [49]. One equivalent of amine reacts with two equivalents of NO to form the corresponding diazeniumdiolate under high NO_(g) pressure (e.g., 80 psi). The NO release from diazeniumdiolates is proton-driven and follows pseudo-first order kinetics at physiological pH [50]. The chemistry of NO release from dibutylhexyldiamine diazeniumdiolate (DBHD/N₂O₂) is depicted schematically in Fig. 2a. Three general procedures have been used in the preparation of diazeniumdiolate-based NO-releasing polymers: 1) dispersion of small amine-based diazeniumdiolate molecules into polymer matrices (e.g., DBHD/N₂O₂) [51]; 2) diazeniumdiolation of covalently linked amine sites on pendant polymer side chains, or on the polymer backbone [52,53]; and 3) covalent binding of diazeniumdiolate groups to micro- or nano-particles, and use of such particles as polymer fillers [54]. The half-life of NO release can be modulated by selecting different secondary amine structures [55] and the NO fluxes can be tuned by changing the diazeniumdiolate dopant amount, hydrophobicity of the polymer matrices or by applying topcoats of polymers to control the diffusion of water into the layer containing the NONOate. The leaching of non-covalently bound small molecule diazeniumdiolates can be greatly reduced by using highly lipophilic diazeniumdiolates blended into the polymer matrix [51].

A key assumption with respect to potentially using NO-release polymers to prepare implantable sensors is that the NO release chemistry does not interfere with the sensing chemistry of the device. Since NO is a redox active species, it is necessary to establish that the initial NO donors and the released NO from the outer coating does not interfere with the analyte measurement by significantly changing the sensitivity, selectivity or lifetime of the implantable chemical sensor, especially electrochemical devices.

Initial work with NO releasing chemical sensors focused on using a small molecule NO donor, dimethylhexane diamine diazeniumdiolate (DMHD/N₂O₂), dispersed into plasticized poly (vinyl chloride) (PVC) and polyurethane (PU) membranes for the purpose of preparing NO releasing potentiometric ion-selective sensors. Indeed, Espadas-Torre et al. reported that pH and K⁺ sensors prepared with the NO-releasing polymer membranes (doped also with the appropriate ion-selective ionophores (tridodecylamine (TDDA) or valinomycin) for sensing purposes) exhibit similar potentiometric response sensitivities and selectivities towards H⁺ and K⁺ as control sensors without NO release [56]. The NO-releasing ion-selective polymer films also exhibit greatly reduced platelet adhesion and activation compared to control films without NO release when tested *in vitro* using platelet-rich sheep plasma. Mowery et al. further demonstrated that two other NO donors, diazeniumdiolated linear polyethylenimine and diazeniumdiolated methoxymethylpiperazine PVC, can also be used to prepare potentiometric sensing membranes [57]. They also extended the application of (DMHD/N₂O₂) to a Clark-style amperometric oxygen sensor and proved that NO release does not affect the sensor's amperometric response [57]. Frost et al. utilized the diazeniumdiolated diaminoalkyltrimethoxysilane (DACA/N₂O₂) crosslinked polydimethylsiloxane (PDMS) to coat the outer walls of a potentiometric CO₂ sensing catheter [58]. The NO-releasing CO₂ sensors exhibited comparable Nernstian response slopes as the control sensors without NO release chemistry. Apparently, the low fluxes of NO released from such coatings do not have a significant impact on the analytical performance of such devices.

Nitric oxide release has also been employed to enhance the biocompatibility of optical sensors. Schoenfisch et al. reported a dual-layer fluorescence-based oxygen sensor configuration with the underlying NO-releasing layer made from DACA/N₂O₂ crosslinked silicone rubber (SR) covered by a second polymeric layer containing the pyrene/perylene fluorescent indicators [59]. The NO-releasing sensors yielded identical responses to oxygen as conventional sensors without NO release. Further, Schoenfisch and coworkers developed a NO-releasing xerogel-based optical pH sensor and demonstrated its improved blood compatibility through an *in vitro* platelet adhesion study [60].

To further confirm that NO-releasing polymers can improve biocompatibility, separate *in vitro* platelet adhesion studies using platelet-rich plasma have been carried out [56,61]. More quantitative studies on the reduction of *in vitro* platelet adhesion on plasticized PVC with varying fluxes of NO was recently conducted using a novel lactate dehydrogenase (LDH) assay [62]. Reduced platelet adhesion was correlated with the increase in NO fluxes. Platelet adhesion was effectively reduced from $14.0 \pm 2.1 \times 10^5$ cells cm⁻² on the control polymer, to $2.96 \pm 0.18 \times 10^5$ cells cm⁻² on polymers with an NO flux of $7.05 \pm 0.25 \times 10^{-10}$ mol cm⁻² min⁻¹.

The initial proof-of-concept that NO release can improve the biocompatibility of devices implanted intravascularly were performed by implanting sham catheters coated with diazeniumdiolated methoxymethylpiperazine PVC into canine carotid arteries for 6 h [57]. The catheters coated with NO-releasing polymer showed greatly reduced platelet activation and adhesion compared to conventional PVC implanted in the same animal. These experiments indicated that low levels of NO release did in fact inhibit thrombus formation on the surface of implanted intravascular devices within the complex and dynamic environment of flowing blood.

The first *in vivo* evaluation of NO-releasing intravascular chemical sensors was reported by Schoenfisch et al. [63]. In this study, DMHD/N₂O₂ dispersed in cross-linked silicone rubber (SR) was coated over the outer SR gas-permeable tubing of a Clark-style amperometric oxygen-sensing catheter to impart NO release. These sensors were implanted in canine arteries for up to 23 h without systemic anticoagulation. During the course of the experiments, the oxygen levels monitored by the NO-releasing sensors tracked more closely with *in vitro* blood

gas analysis of PO_2 levels performed on discrete blood samples drawn from the animal, as compared to non-NO release control oxygen sensors implanted within the same animal (e.g., more accurate results). At the end of each animal study, the sensors were carefully explanted from the arteries and examined by scanning electron microscopy (SEM). The NO-releasing catheters consistently showed a marked decrease in platelet adhesion under SEM, with no gross thrombus formation. On the other hand, gross thrombus formation was routinely observed on the control catheters which were covered by adhered platelets, fibrin and entrapped red cells.

These encouraging results suggested the potential of using NO-releasing polymers to enhance the biocompatibility and concomitant analytical performance of intravascular chemical sensors. However, it was found that the small molecule NO donor (DMHD/ N_2O_2) could leach out of the polymer coating and a significant amount of NO was released from by the decomposition of the donor outside of the polymer coating matrix [61]. This poses potential risks as the decomposition product, free dimethylhexane diamine, could react with reactive nitrogen oxide species and form a *N*-nitrosamine, which is carcinogenic. In addition, the leaching of NO donor into the aqueous phase would reduce the effectiveness of locally released NO to inhibit platelet adhesion/activation at the polymer/blood interface (i.e., less NO being locally released at sensor/blood interface).

To address the leaching problem, Zhang et al. developed the DACA-SR/ N_2O_2 material where the diazeniumdiolate groups are anchored on a diamine silane crosslinker and thus the leaching of the parent diamine from the polymer matrix is eliminated [64]. Frost et al. applied this covalently attached diazeniumdiolated DACA-SR/ N_2O_2 coating ($\sim 100\ \mu\text{m}$ thickness) on the surface of Clark-style oxygen-sensing catheters and evaluated the performance of these devices in porcine carotid and femoral arteries for 16 h [65]. The blood oxygen levels determined by the NO-releasing sensors ($N = 9$) were statistically identical to the standard *in vitro* blood gas measurements, while the control sensors ($N = 9$) implanted in the same animals showed statistically significant deviations (negative values) at 95% confidence interval in oxygen measurements after 10 h of implantation. Furthermore, the NO-releasing catheters were found to be relatively thrombus-free after explantation, while the control catheters without NO-release exhibited largely varied degrees of biological response, ranging from very few adhered platelets to a complete coverage of the surface by blood clots. Such variation in biological response is typically observed with implanted sensors, and it is precisely this variability that makes measurements with conventional sensors so unreliable.

As mentioned above, another approach to create NO release polymeric coatings is to use a more lipophilic parent diamine compound with longer alkyl side-chains so that extraction into aqueous phase is minimized [51]. Replacing the methyl side groups in DMHD with butyl groups enhances the partition coefficient of the diamine into the organic polymer phase by 10^4 . In another study, the resulting diazeniumdiolated dibutylhexyldiamine (DBHD/ N_2O_2) was dispersed into SR, together with potassium tetrakis(4-chlorophenyl) borate (KTpCIPB) which buffers the pH in the organic phase to maintain a more constant longer term NO release. Biocompatibility evaluations of NO-releasing catheters with outer SR coatings containing DBHD/ N_2O_2 in the porcine artery model yielded similar results to other NO-releasing sensor studies, with more accurate blood oxygen measurements and less platelet adhesion and activation observed [58].

NO-releasing polymers containing DBHD/ N_2O_2 have also shows improved biocompatibility when used as outer coatings for preparation of needle-type subcutaneous glucose sensors. Gifford et al. prepared glucose sensors with a thin PU/PDMS coating ($\sim 10\ \mu\text{m}$ thick) doped with the DBHD/ N_2O_2 NO donor and implanted them in rats together with control sensors [66]. Histological evaluation of neutrophil infiltration revealed that inflammatory response was greatly reduced during the first 24 h at implant sites where the NO release sensors were placed

vs. control sites in the same animals. However, the NO release from the polymer coating could only last ca. 16 h, owing the very thin coating necessary to prevent a dramatic decrease in the flux of glucose to the underlying glucose oxidase enzyme layer of the sensor. Therefore, although the sensors remained functional *in vivo* for several days, the reduction of inflammatory response was not maintained for an extended implant period (3 d). Increasing the coating thickness is not an option as it would change the membrane permeability and adversely affect the sensitivity and response time of the glucose sensor. To circumvent the limitation of NO donor reservoir in the NO-releasing coating, alternative NO-generating approaches have been pursued to immobilize catalysts onto the polymer surface that can decompose endogenous RSNOs, an NO carrier, to generate NO *in situ* at the polymer/blood interface.

3.2 NO-generating materials

As discussed above, polymer coatings containing NO donors on the surfaces of implantable sensors will eventually be depleted, and thus these sensors are likely to eventually lose the biocompatibility advantage offered by NO release. The concept of “NO generation” coatings is to utilize the endogenous RSNO species that exist in the physiological fluids as a continuously replenishing NO donor to generate enhanced NO levels at the blood/polymer interface. RSNOs are regarded as a reservoir and carrier of NO in the body. The most abundant endogenous RSNOs are S-nitrosoalbumin (AlbSNO) and GSNO. Transnitrosation reactions occur *in vivo* so that the nitroso-group can be transferred from one thiol to another [34,36]. The cleavage of S-NO bond in RSNOs to release NO can occur by three well-established mechanisms [67]. First, copper ion mediated catalytic decomposition requires Cu^{2+} to first be reduced to Cu^+ . Copper(I) reacts with RSNO to release NO by transferring an electron, and thus forms a thiolate anion and regenerates Cu^{2+} . Under physiological conditions, ascorbate and thiolate anions are sufficient reducing agents to convert Cu^{2+} to Cu^+ . The second RSNO decomposition pathway is through the reaction with high concentrations ($> 1 \text{ mM}$) of ascorbate to release NO and produces thiolate and dehydroascorbate [68]. The third decomposition mechanism of RSNOs is the hemolytic cleavage of the S-NO bond by light at characteristic absorbance bands of 330–350 nm or 550–600 nm, yielding NO and the disulfide of the parent thiols [69]. Further, Hou et al. reported that glutathione peroxidase (GPx) and other seleno compounds (e.g., selenocystamine) can also catalyze RSNO decomposition.[70] Recent research using organotellurium compounds indicate that they also possess similar catalytic activities towards RSNO decomposition [71].

The potential advantage of the NO-generation approach *in vivo* is the utilization of circulating RSNO as an unlimited supply of NO at the polymer/blood interface. Duan et al. explored the possibility of generating NO by transnitrosation from endogenous RSNOs to L-cysteine immobilized on polymer surfaces [72]. They covalently attached L-cysteine to PU and polyethylene terephthalate (PET) and these L-cysteine modified polymers reduce *in vitro* platelet adhesion by more than 50% when tested in plasma, but not with platelet suspension in phosphate buffer. The suggested mechanism was through the transnitrosation of NO^+ from endogenous RSNOs (e.g., AlbSNO) to the cysteine moiety on the polymer surface to yield immobilized CysNO. CysNO is relatively unstable and can thus decompose to release NO locally at the polymer interface.

Oh et al. developed a lipophilic Cu(II)-cyclen type complex to generate NO from RSNO and nitrite under physiological conditions [73,74]. PVC and PU films doped with this Cu(II) complex generated NO in the presence of reducing agents (e.g., ascorbate, cysteine, glutathione). It was demonstrated such polymers can generate NO with an apparent surface flux as high as $8 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$ from physiological levels ($1 \text{ }\mu\text{M}$) of GSNO. The same polymer was also shown to generate NO from nitrite under physiological conditions in the presence of ascorbate. A material that is capable of locally and continuously generating

NO from endogenous RSNOs and nitrite could be used to tackle the biocompatibility problems for not only implantable sensors, but even long-term implants, such as stents and vascular grafts.

Wu et al. recently evaluated the biocompatibility of NO-generating polymeric coatings *in vivo* on intravascular oxygen-sensing catheters using the aforementioned porcine artery model for up to 20 h [75]. The NO-generation was achieved using small metallic copper particles of two different sizes (3 μm and 80 nm), doped within a thin outer coating of an appropriate polymer applied to the surface of the oxygen sensing catheter. Corrosion of metallic copper provides the copper ions required for RSNO decomposition. (See Fig. 2b) The 3 μm Cu^0 particles were dispersed in a polymer blend of RTV-3140 SR and Tecophilic SP-60D-60 hydrophilic PU (SR:PU = 2:1), while the 80 nm Cu^0 particles were dispersed in Tecoflex SG-80A PU with an underlying adhesion promoter layer to ensure tight adhesion to the SR tubing used to construct the catheter. It was demonstrated that such coatings can generate NO surface fluxes of more than $2 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$ at physiological GSNO levels. Further, the incorporation of Cu^0 particles does not alter the sensitivity and stability of the sensor, nor does it cause any significant leaching of Cu ions due to corrosion. On average, the NO-generating sensors (N=12 for the 3- μm group and N=6 for the 80-nm group) showed improved biocompatibility along with more accurate PO_2 measurements when compared to control sensors without Cu^0 particles. The average percent deviations of PO_2 measured by NO-generating and control sensors as compared with standard blood gas machine results (considered 100% accurate) are plotted in Fig. 3. On average, values from NO-generating sensors showed closer agreement with *in vitro* blood gas measurements, while control sensors yielded consistent false-negative values due to thrombus formation. Nine out of 12 control sensors in the 3- μm group developed mature blood clot as determined after explantation, while only 3 out of the 12 NO-generating sensors had evidence of surface clot. Similar results were obtained with the 80-nm particle group – 3 out of the 6 control sensors had surface clot and only 1 out of the 6 NO-generating sensors had thrombus formed over the surface.

Figure 4 shows the representative images of NO-generating and control sensors from each group after explantation. It is clear that the NO generating sensor has less thrombus at the sensing tip. An LDH assay [62] was used to quantify the degree of thrombus formation on the sensor surfaces. The average LDH content in the biolayer on the control sensor surfaces is ~ 8 times greater than the LDH content of the biolayer covering the NO-generating sensors, further indicating the presence of more adhered cells on control sensors. The thrombus formation on some of the NO-generating sensors might be attributed to the low endogenous RSNO levels in particular animals so that there might be insufficient NO generated at the surface. Indeed, the question of actual RSNO levels in blood is still under debate and the reported values in the literature range from 15 nM to 7 μM [33,76]. There might also be a considerable animal-to-animal variation in endogenous RSNO concentrations, giving rise to the difference in biological response seen in the animals. To overcome the limitation of basal RSNO levels, it might be necessary to provide a bolus of naturally occurring RSNO each day, to ensure that blood levels are adequate to create elevated levels of NO at the sensor/blood interface. Use of newly developed electrochemical RSNO sensors [77,78] to monitor blood RSNO concentrations will likely to help sort out whether such action would be necessary to implement the NO generation approach to enhance the biocompatibility of the implanted sensors.

4. Future Prospects

The routine use of implantable chemical sensors is highly dependent on the reliability of their analytical results. To date, the analytical functionality of such devices is still hampered by the adverse biological responses which render the analytical results not reliable enough to make

therapeutic/clinical decisions. As outlined above, it has been recently shown that the local release/generation of biologically active species such as NO is capable of reducing thrombus formation as well as inflammatory responses for intravascular and subcutaneous sensors, making the output signals from such devices potentially more useful. However, NO alone is unlikely to be the solution of the entire biocompatibility problem. Indeed, the vascular endothelium is a perfectly non-thrombogenic surface due to a number of molecules working synergistically to maintain vascular homeostasis. A biomimetic approach to create an 'artificial endothelium' will likely provide the most promising approach for the development of truly biocompatible sensors. NO release/generating polymers are the first step in this direction. For example, surface immobilization of heparin and thrombomodulin, used in conjunction with continuous release of a variety of naturally occurring antithrombogenic species (including NO and prostacyclin), may render implanted sensors even greater biocompatibility than NO release/generation alone. A dual-functional polymeric coating with surface-immobilized active heparin and NO-release has recently been reported [79]. In addition, it has been found that surface-bound thrombomodulin on NO-releasing polymers retains its biological function and might be a promising candidate for future development of implantable sensors (unpublished results).

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Figure 1.

(a) Sequence of events leading to thrombus formation on surface of intravascularly implanted sensor; (b) subsequent deviations in implanted sensor measurements of blood gases/pH due to cell adhesion and/or low blood flow at implant site.

Figure 2.
Schematic of (a) NO-release from DBHD/N₂O₂; (b) NO generation by immobilized Cu catalyst

Figure 3.

Plots of the average percent accuracy of: (a) NO-generating sensors based on 3- μm Cu^0 particles and control sensors (N = 12 each), and (b) NO-generating sensors based on 80 nm Cu^0 particles and control sensors (N = 6 each), as compared to *in vitro* blood gas analyzer results. (From Ref.75, with permission)

Figure 4.

Images of two representative pairs of NO-generating (left) and control sensors (right) after *in vivo* studies. The portions within the dotted boxes were exposed to blood. (From Ref.75, with permission)