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Improving blood compatibility of intravascular oxygen sensors via catalytic decomposition of *S*-nitrosothiols to generate nitric oxide *in situ*

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Abstract

Reliable, real-time, *in vivo* sensing (intravascular) of blood gases and electrolytes remains a difficult challenge owing to biocompatibility issues that occur when chemical sensors are implanted into the blood stream. Recently, local release of nitric oxide (NO) at the sensor/blood interface has been suggested as a potential solution to this problem. However, the lifetime of NO release from thin polymer films coated on implanted sensors is limited by the reservoir of NO donor loaded within the polymeric coating. To continuously produce NO at the sensor/blood interface, a novel approach to catalytically decompose endogenous *S*-nitrosothiols (RSNOs) in blood to generate NO *in situ* is reported herein. Metallic copper particles of two different sizes (3 μm and 80 nm) are embedded as catalysts in thin polymer coatings on the surface intravascular electrochemical oxygen sensing catheters. Oxygen levels (partial pressure of oxygen; PO_2) provided by the copper particle/polymer coated sensors are, on average, more accurate than values obtained from non-NO generating control sensors when both types of sensors are implanted in porcine arteries for 19–20 h. Upon termination of each *in vivo* study, catheters were explanted and examined for surface thrombosis via both visual image and lactate dehydrogenase (LDH) assay. The results indicate that the Cu^0-catalyst coatings significantly reduce the occurrence of surface thrombosis, likely from the ability to generate NO from endogenous RSNO species at the sensor/blood interface.

Keywords: Nitric oxide generation; *S*-Nitrosothiols; Copper catalyst; Intravascular electrochemical oxygen sensor

1. Introduction

Monitoring the cardiopulmonary function of critically ill patients mandates continuous measurements of blood gases and electrolytes. The development of implantable sensors (electrochemical and/or optical) capable of reliably measuring important physiological species, such as PO_2, PCO_2, pH, electrolytes, glucose and lactate *in vivo*, has remained a great challenge for several decades [1,2]. A variety of prototype catheter-style commercial devices have been developed for intravascular blood gas sensing purposes [3,4]. However, these devices have not found widespread use in the clinical arena, primarily due to the erratic results obtained when used for continuous *in vivo* measurements. Such errant results arise from the biological responses of living systems to foreign devices implanted in the blood stream. Proteins, such as collagen, fibrinogen and von Willebrand factor, adsorb onto the polymer surfaces of the implanted sensors within seconds [5,6]. The adsorbed protein layer mediates the adhesion and activation of metabolically active cells, e.g., platelets, that later arrive [7,8]. Platelets play a key role in blood coagulation since activated platelets keep recruiting circulating cells to the polymer surfaces which ultimately leads to the formation of blood clots [9]. The presence of active cells on the surface of such devices causes a localized change in pH, PO_2 and PCO_2 values due to cellular respiration compared to the bulk blood [1], yielding significant errors when results are compared to values obtained from *in vitro* measurements on discrete samples of blood.

Nitric oxide has been widely recognized as a potent vasodilator and inhibitor of platelet adhesion and activation [10–13]. It

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is produced from L-arginine by a class of enzymes known as nitric oxide synthases (NOS). The perfect thromboresistance of the human endothelium has been partly attributed to the low but continuous production of NO in this layer [14,15]. Endothelial cells that line the inner walls of healthy blood vessels produce NO with an estimated surface flux level of $0.5–4.0 \times 10^{-10}$ mol cm^{-2} min^{-1} [16]. Therefore, one approach to potentially resolve the hemocompatibility problem is to release or generate NO locally at the blood/sensor interface at or above the flux from normal endothelial cells in order to inhibit platelet adhesion and activation, and thus prevent gross thrombus formation. Biomedical devices (i.e., intravascular sensors, vascular grafts, extracorporeal circuits, etc.) coated with polymers containing diazeniumdiolate-type NO donors have already been shown to exhibit improved biocompatibility via various *in vivo* evaluations (in animal models) [17–19]. However, due to the limited reservoir of the NO donors in the polymer coatings, the NO-release approach is only suitable for biomedical devices that require short-term blood contact times, such as hemodialysis and extracorporeal circulation, but not for long-term (i.e., weeks or months) implantation. To maintain NO production for extended periods of time it may be possible to take advantage of endogenous species such as nitrosothiols (RSNOs) that are constantly produced in the body (from NO generated by NOS) to generate NO *in situ* at the polymer/blood interface.

Nitric oxide has very short lifetime in blood [20] due to its reactivity with various blood components [21]. In contrast, a more abundant (i.e., micromolar concentrations) and stable form of NO in blood are *S*-nitroso adducts with thiol groups (RSNOs) [22], such as *S*-nitrosoalbumin (AlbSNO), *S*-nitrosocysteine (CysNO) and *S*-nitrosoglutathione (GSNO) [22–24]. One well-known mechanism of RSNO decomposition to yield NO is catalyzed by Cu^+ [25], in which Cu^+ is produced from the reduction of Cu^{2+} by thiolates or other reducing equivalents that exist in the physiological environment (e.g., ascorbate). We have already demonstrated that polymer films doped with lipophilic cyclen-type Cu(II) complexes [26] and Cu(II)–cylen complex covalently linked to poly(2-hydroxyethyl methacrylate) hydrogels [27] are capable of generating significant NO fluxes ($>1 \times 10^{-10}$ mol cm^{-2} min^{-1}) in the presence of physiologically relevant concentrations of GSNO and appropriate reducing agents. However, our preliminary studies indicate that such polymer films based on these Cu(II)–cylen complexes are not ideally suited for long-term NO-generation *in vivo* due to the loss of NO-generating functionality after extended storage in plasma. In this paper, we report the use of polymer films doped with small metallic copper particles as the catalytic coatings on the surface of intravascular electrochemical oxygen sensing catheters. Such coatings can generate NO *in situ* at the sensor/blood interface via a slow corrosion of the copper particles to produce copper ions. When placed in porcine arteries, the oxygen sensing catheters with NO-generating capability are shown to exhibit improved blood compatibility and more accurate PO_2 measurements when compared to corresponding control oxygen sensing catheters implanted within the same animals.

2. Experimental

2.1. Materials

Sodium chloride, potassium chloride, sodium nitrite, reduced L-glutathione (GSH), sulfuric acid (95–98%), bicinchoninic acid (disodium salt), dibutyltin dilaurate (95%), Triton X-100, bovine serum albumin and 3-μm copper powder were purchased from Sigma–Aldrich (St. Louis, MO). Copper nanoparticles (80 nm size) were from Inframat Advanced Materials (Farmington, CT). Ethylenediaminetetraacetic acid (EDTA) was obtained from Mallinckrodt (Paris, KY). Methocel 90 HG and 3-aminopropyltrimethoxy-silane were from Fluka (Milwaukee, WI). The silicone rubber tubing (0.51 mm i.d. ← 0.94 mm o.d.) used to construct catheters was obtained from Helix Medial, Inc. (Carpinteria, CA), and Silastic medical grade tubing (0.94 mm i.d. × 1.29 mm o.d.) was received as a gift from Medtronic (Minneapolis, MN). Silicone rubber (RTV-3140) was the product of Dow Corning (Midland, MI). Tecophilic SP-60D-60 and Tecoflex SG-80A polyurethanes were from Noveon (Cleveland, OH). A quick-setting two-part epoxy was the product of Super Glue Corp. (Rancho Cucamonga, CA). The PTFE-coated Pt/Ir and Ag wires employed to fabricate the oxygen sensing catheters were products of Medwire Corp. (Mt. Vernon, NY). Ultra 4-way stopcocks and Angiocath catheter guides (16 g × 1.16 in.) used for catheter implantation in the arteries of pigs were from Medex (Hillard, OH) and Becton Dickinson (Sandy, UT), respectively.

2.2. Fabrication of NO-generating oxygen sensors

The Clark-type amperometric oxygen sensing catheters employed in this work were fabricated as previously reported [17,19]. However, instead of applying an NO-release polymer coating on the surface of the silicone rubber catheter, a thin layer of a polymer doped with Cu^0 catalyst (in the form of either micron- or nano-sized Cu^0 particles) was coated on the surface of the silicone rubber tubing (see Fig. 1). Briefly, the

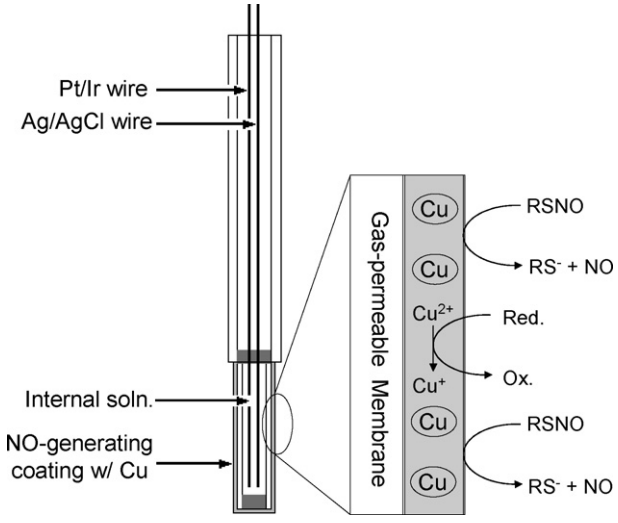

Fig. 1. Schematic of intravascular oxygen sensor design and the NO generation mechanism.