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Improved hemocompatibility of silicone rubber extracorporeal tubing via solvent swelling-impregnation of *S*-nitroso-*N*-acetylpenicillamine (SNAP) and evaluation in rabbit thrombogenicity model

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ABSTRACT

Blood-contacting devices, including extracorporeal circulation (ECC) circuits, can suffer from complications due to platelet activation and thrombus formation. Development of nitric oxide (NO) releasing polymers is one method to improve hemocompatibility, taking advantage of the ability of low levels of NO to prevent platelet activation/adhesion. In this study a novel solvent swelling method is used to load the walls of silicone rubber tubing with the NO donor *S*-nitroso-*N*-acetylpenicillamine (SNAP). This SNAP-silicone rubber tubing exhibits an NO flux of ca. 1×10^{-10} mol cm^{-2} min^{-1}, which mimics the range of NO release from the normal endothelium, which is stable for at least 4 h. Images of the tubing before and after swelling, obtained via scanning electron microscopy, demonstrate that this swelling method has little effect on the surface properties of the tubing. The SNAP-loaded silicone rubber and silicone rubber control tubing are used to fabricate ECC circuits that are evaluated in a rabbit model of thrombogenicity. After 4 h of blood flow, the SNAP-loaded silicone rubber circuits were able to preserve the blood platelet count at 64% of baseline (vs. 12% for silicone rubber control). A 67% reduction in the degree of thrombus formation within the thrombogenicity chamber was also observed. This study demonstrates the ability to improve the hemocompatibility of existing/commercial silicone rubber tubing via a simple solvent swelling-impregnation technique, which may also be applicable to other silicone-based blood-contacting devices.

Statement of Significance

Localized nitric oxide (NO) release can be achieved from biomedical grade polymers doped with *S*-nitroso-*N*-acetylpenicillamine (SNAP). Despite the promising *in vitro* and *in vivo* biocompatibility results reported for these NO releasing polymers, many of these materials may face challenges in being translated to clinical applications, especially in the areas of polymer processing and manufacturing. In this study, we report a solvent swelling-impregnation technique to incorporate SNAP into extracorporeal circuit (ECC) tubing. These NO-releasing ECCs were able to attenuate the activation of platelets and maintain their functionality, while significantly reducing the extent of thrombus formation during 4 h blood flow in the rabbit model of thrombogenicity.

1. Introduction

Extracorporeal circulation (ECC) circuits are critical to several medical procedures including hemodialysis, open heart surgery, and extracorporeal membrane oxygenation. During these medical procedures blood is pumped through tubing and is also exposed to devices for gas exchange or filtration. Exposure of blood to these foreign surfaces results in a complex response that initiates the coagulation cascade, surface fouling, and inflammation (involving neutrophils, monocytes, and complements) that can potentially lead to adverse medical outcomes. In the coagulation cascade proteins, such as fibrinogen and fibronectin, rapidly adsorb to the

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foreign surface [1]. These adsorbed proteins interact with platelets leading to the exposure of the platelet glycoprotein GPIIb/IIIa receptor that binds platelets to fibrinogen, as well as conformational changes that further induce the activation and aggregation of more platelets (e.g., excretion of P-selectin) [2]. This surface contact (intrinsic pathway) converges with the extrinsic pathway at the common pathway of coagulation where thrombin converts fibrinogen to fibrin, which ultimately results the entrapment of platelets/red blood cells forming thrombus on the surface [2]. Systemic anticoagulation is necessary to preserve the patency of the ECC circuit; however, platelet consumption is still observed. The platelet count typically drops to <40% of normal during the initial 1–2 h of blood flow through ECC circuits [3]. According to a report by the Extracorporeal Life Support Organization (ELSO), bleeding and thrombosis still occur with a significant number of patients (7–34%) on extracorporeal life support (ECLS) devices [4]. Despite systemic anticoagulation, thrombus formation, hemorrhage, and infection are some of the most adverse side effects that occur during these ECC procedures which can lead to catastrophic complications and mortality [5–7].

The development of nitric oxide (NO) releasing polymers is an approach to improve hemocompatibility of ECC and other blood-contacting devices that has been reported in the literature [8–10]. Nitric oxide has a number of important physiological roles, including its ability to attenuate platelet activation. The normal endothelium releases NO at a flux in the range of $0.5–4 \times 10^{-10}$ -mol cm^{-2} min^{-1} that prevents the activation of platelets that come in close proximity of this surface [11]. In addition, NO serves other critical physiological roles including prevention of infection, due to its broad spectrum antimicrobial properties [12], which is also important for ECC applications. Therapeutic delivery of NO from polymer surfaces is a promising approach to improve hemocompatibility and reduce device-related infections for ECCs [13–16], as well as other application (e.g., *in vivo* biosensors [17–21], catheters [22–25] grafts [26]).

To date, NO releasing polymers have been prepared utilizing common NO donor molecules such as *N*-diazeniumdiolates [13,16,18,26–28] and *S*-nitrosothiols (RSNOs) [15,23,29–32], including *S*-nitrosoglutathione (GSNO) and *S*-nitroso-*N*-acetylpenicillamine (SNAP). These NO donor molecules have been incorporated into biomedical polymers via synthetic modifications [31–33] and non-covalent dispersal [14,15,29], where ultimate exposure to physiological conditions can initiate the release of their NO payload. The advantage of this approach is that the NO delivery can be adjusted for a specific medical application by modifying variables such as the concentration of NO donor within the polymer matrix or utilizing polymer topcoats (which also can serve to reduce leaching of the NO donor). Heat, light, and metal ions (e.g., Cu^{+}) are the major catalysts for RSNO species that initiate NO release [34]. For RSNO-based polymers, the major limitations have been low synthetic yields, leaching of the RSNO (which delocalizes the NO delivery and can cause effects downstream), and instability of the RSNO functionality during storage. Incorporating the NO donor SNAP into hydrophobic polymers, such as silicone rubber, has been reported to reduce leaching, prolong NO release, and retain NO functionality during storage [15]. Despite the promising *in vitro* and *in vivo* biocompatibility results reported for these NO releasing polymers, many of these materials may face challenges in being translated to clinical applications, especially in the areas of polymer processing and manufacturing.

Silicone rubber has been widely used in various biomedical applications including intravascular and urinary (Foley) catheters, drains, and insulators to pacemaker leads as well as in ECC procedures (e.g., tubing, blood oxygenators, peristaltic pump chambers, etc.) [35]. Most catheters and medical grade tubing are manufactured using an extrusion process. For example, silicone rubber tubing formed using liquid injection molding undergoes temperatures of 150–200 °C during typical extrusion to ensure proper curing of the polymer [36]. Many of the NO donor compounds currently being studied are heat sensitive and their NO functionality could be severely affected by these extreme temperatures. Bainbridge et al. reported that clean decomposition of SNAP and GSNO occurs at 148 °C and results in the release of NO [37]. This suggests that a significant loss of the NO payload could occur during the silicone rubber extrusion process if the NO donors are present in the extrusion mixture. Therefore, alternative methods are needed in order to incorporate NO donors within silicone-based biomedical devices. Recently, it was demonstrated that SNAP could be loaded into pre-existing silicone rubber urinary catheters by a solvent swelling-impregnation technique to reduce risk of infection [23]. In this study, we investigate this novel solvent swelling-impregnation approach to incorporate SNAP into silicone rubber ECC circuits. It is known that NO has excellent diffusion properties in silicone rubber [38] and also the leaching of SNAP from silicone rubber can be reduced because of silicone rubber's low water uptake and cross-linked polymer network [15,23]. Hence, for this work, SNAP-loaded silicone rubber tubing is investigated to prepare extracorporeal circulation (ECC) loops, and the resulting loops are evaluated for their effects on hemocompatibility in an ECC rabbit model of thrombogenicity.

2. Materials and methods

2.1. Materials

N-Acetyl-D-penicillamine (NAP), sodium chloride, potassium chloride, sodium phosphate dibasic, potassium phosphate monobasic, ethylenediaminetetraacetic acid (EDTA), tetrahydrofuran (THF), sulfuric acid and *N*,*N*-dimethylacetamide (DMAc) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, hydrochloric acid, and sulfuric acid were obtained from Fisher Scientific (Pittsburgh, PA). Saint-Gobain™ Tygon™ Formula 3350 Silicone Tubing was purchased from Fisher Scientific (Pittsburgh, PA). Dow Corning RTV 3140 silicone rubber sealant was purchased from Ellsworth Adhesives (Germantown, WI). All aqueous solutions were prepared with 18.2 MΩ deionized water using a Milli-Q filter (Millipore Corp., Billerica, MA). Phosphate buffered saline (PBS), pH 7.4, containing 138 mM NaCl, 2.7 mM KCl, 10 mM sodium phosphate, 100 μM EDTA was used for all *in vitro* experiments.

2.2. SNAP synthesis protocol

SNAP was synthesized using a modified version of a previously reported method [39]. Briefly, an equimolar ratio of NAP and sodium nitrite was dissolved in a 1:1 mixture of water and methanol containing 2 M HCl and 2 M H_2SO_4. After stirring, the reaction vessel was cooled in an ice bath to precipitate the green SNAP crystals. The crystals were collected by filtration, rinsed with water, and dried under ambient conditions. The reaction mixture and resulting crystals were protected from light at all times.

2.3. Preparation of SNAP impregnated tubing and ECC loops

The SNAP swelling solution was prepared by dissolving SNAP in THF using concentrations of 15, 25, and 35 mg/mL. The Saint-Gobain™ Tygon™ silicone rubber tubing was soaked in the SNAP swelling solution for 24 h. The tubing was removed, briefly rinsed with PBS, and dried for 48 h under ambient conditions to allow the excess THF to evaporate. The tubing and swelling solutions were protected from light throughout the swelling process.