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Attenuation of thrombosis and bacterial infection using dual function nitric oxide releasing central venous catheters in a 9 day rabbit model

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ABSTRACT

Two major problems with implanted catheters are clotting and infection. Nitric oxide (NO) is an endogenous vasodilator as well as natural inhibitor of platelet adhesion/activation and an antimicrobial agent, and NO-releasing polymers are expected to have similar properties. Here, NO-releasing central venous catheters (CVCs) are fabricated using Elast-eon™ E2As polymer with both diazeniumdiolated dibutylhexanediamine (DBHD/NONO) and poly(lactic-co-glycolic acid) (PLGA) additives, where the NO release can be modulated and optimized via the hydrolysis rate of the PLGA. It is observed that using a 10% w/w additive of a PLGA with ester end group provides the most controlled NO release from the CVCs over a 14 d period. The optimized DBHD/NONO-based catheters are non-hemolytic (hemolytic index of 0%) and noncytotoxic (grade 0). After 9 d of catheter implantation in the jugular veins of rabbits, the NO-releasing CVCs have a significantly reduced thrombus area (7 times smaller) and a 95% reduction in bacterial adhesion. These results show the promise of DBHD/NONO-based NO releasing materials as a solution to achieve extended NO release for longer term prevention of clotting and infection associated with intravascular catheters.

Statement of Significance

Clotting and infection are significant complications associated with central venous catheters (CVCs). While nitric oxide (NO) releasing materials have been shown to reduce platelet activation and bacterial infection *in vitro* and in short-term animal models, longer-term success of NO-releasing materials to further study their clinical potential has not been extensively evaluated to date. In this study, we evaluate diazeniumdiolate based NO-releasing CVCs over a 9 d period in a rabbit model. The explanted NO-releasing CVCs were found to have significantly reduced thrombus area and bacterial adhesion. These NO-releasing coatings can improve the hemocompatibility and bactericidal activity of intravascular catheters, as well as other medical devices (e.g., urinary catheters, vascular grafts).

1. Introduction

Infection and thrombus formation are the leading complications for blood-contacting devices in clinical settings today, and can result in extended hospital stays, increased healthcare costs, and even patient death [1,2]. Thrombus formation is currently treated through the systemic administration of heparin, increasing the risk for hemorrhage, thrombocytopenia, and thrombosis in patients [3]. Even with the use of heparin, venous thrombosis has been detected via Doppler imaging in 33% of intensive care unit patients [4]. Intravascular catheters are used in a variety of different settings, and range greatly in the length of use. Acute catheters used in operating rooms, emergency rooms, and intensive care units (ICUs) are typically used for up to 7 days, while more permanent catheters for cases such as long-term nutrition or dialysis can be used from months to years [2]. Preventing thrombus formation over these time periods is critical to maintain the functionality of the catheter. Long-term systemic administration of anticoagulants

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increase the patients risk for the complications stated above. Local administration of the anticoagulant, such as heparin lock solutions, are currently used clinically to prevent this local thrombus formation, but fail to address issues related to microbial infection [5].

Catheter associated infections are prevalent in clinical settings, where 1.7 million healthcare associated infections result in 99,000 deaths per year in the United States alone [6]. Infections associated with indwelling catheters are particularly common, where up to 40% of all indwelling catheters become infected [7]. The majority of serious catheter-related infections are associated with central venous catheters (CVCs), where more than 250,000 CVC-associated infections occur annually in the United States which results in $25,000 additional costs and a mortality rate of 12–25% per infection [8,9]. The treatment of these infections is typically done through the use of antibiotics, and has led to the development of antibiotic resistant strains of bacteria, such as methicillin resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* [10,11]. Once adhered to a surface, bacteria also have the ability to form biofilms, where an extracellular carbohydrate matrix encases communities of bacteria to protect the bacteria from bactericidal and bacteriostatic agents. This biofilm formation makes it difficult for antibiotics to penetrate, and has been shown to require up to 1000 times higher dose [12]. Bacteria that have adhered to a intravascular catheter can also detach from the surface and lead to bloodstream infections (80,000/year) and possibly death (28,000/year) [8].

The ability of bacteria or proteins (e.g., fibrinogen and von Willebrand Factor that stimulates activation of platelets to cause clotting) to adhere to the surface of catheter materials is influenced by a number of characteristics, including surface roughness and surface charge (hydrophobicity). Many approaches have been used to increase the biocompatibility of materials through limiting protein adsorption by decreasing surface roughness, varying hydrophobicity, zwitterionic surfaces, or grafting of hydrophilic polymers such as polyethylene glycol [13–19]. While these approaches can aid in limiting protein adsorption and bacteria adhesion, they do not address complications associated with platelet activation and adhesion, or respond to any bacteria that have adhered to the surface. Polymers with heparin immobilized to their surface have been shown to be effective at reducing thrombosis, and have reached the marketplace and clinical use [20–24]. However, these products still lack an active approach to prevent infection. Active catheter coatings containing antibacterial agents such as silver or antibiotics are available, but have nearly the same rate of infection as standard catheters [25]. In addition, both the silver and antibiotic containing catheters also do not address issues associated with platelet activation.

An alternate approach to improving the biocompatibility of materials is to mimic the natural endothelium. It has been shown that nitric oxide (NO) is the primary regulator in inhibiting platelet activation and adhesion, and is naturally released from vasculature at an estimated surface flux of $0.5–4 \times 10^{-10}$ mol cm^{-2} min^{-1} [26,27]. Along with inhibiting platelet adhesion, NO has also been shown to have broad-spectrum antimicrobial properties, killing both gram-positive and gram-negative bacteria [28–32]. Many NO donors such as *S*-nitrosothiols [33,34] and N-diazeniumdiolates [35,36] have been developed and studied for their potential to locally release NO from polymer surfaces. The addition of NO donors into polymeric materials has been shown to be non-cytotoxic and non-hemolytic, while maintaining the mechanical properties of the base polymer [37].

A major requirement for long-term applications is the ability extend the NO release lifetime to match the intended usage lifetime of the biomedical device. Methods for controlling the release of NO from materials have been the applications of additional polymer top coats in order to prevent leaching of the NO donor molecules, the use of polymers with low water uptake, as well as chemical additives. Prior work has shown diazeniumdiolated dibutylhexanediamine (DBHD/NONO) (Fig. 1A) releases physiological levels of NO when incorporated into hydrophobic polymer films [38–41]. However, the proton driven release mechanism of NO from this molecule creates free lipophilic amine species, increasing the pH within the material which stops the NO release before the total available NO is released [42]. Initially Batchelor et al. utilized lipophilic anionic species (e.g., tetraphenyborate derivatives) as additives that slightly extended the NO release from films by buffering the proton activity within the polymer [38,42]; however, there were concerns related to its cytotoxicity toward endothelial and smooth muscle cells [43]. The addition of poly (lactide-co-glycolide) (PLGA) has been one of the most promising methods reported to date of extending NO release from diazeniumdiolate-based materials. The NO release from DBHD/NONO-based materials can be modulated using PLGA additives, which hydrolyzes to produce lactic/glycolic acid species that can balance the production of the lipophilic DBHD amine byproduct of the NO release reaction and continue to promote NO release (Fig. 1B) [40,41,44]. The specific properties (i.e., acid or ester end groups) of the PLGA additives were previously studied in terms of their initial NO burst release effects, where PLGAs with acid end groups were found to produce an unwanted burst release from DBHD/NONO [40,41]. In these prior studies films prepared with PLGAs with ester end groups avoided this burst release and had extended NO release profiles.

Herein, we report the use of PLGA and DBHD/NONO as additives within Elast-eon E2As polyurethane to fabricate NO-releasing central venous catheters (CVCs) in order to combat issues of both thrombosis and infection. The release rates of NO were measured from the formulations utilizing PLGAs with ester end groups in order to avoid any burst release and optimize the NO release profile over a 14 day period. The optimized NO-releasing CVCs were evaluated in long-term (9 d catheter) intravascular rabbit studies to observe the NO effects on prevention of clotting and bacterial adhesion, in comparison to E2As control catheters.

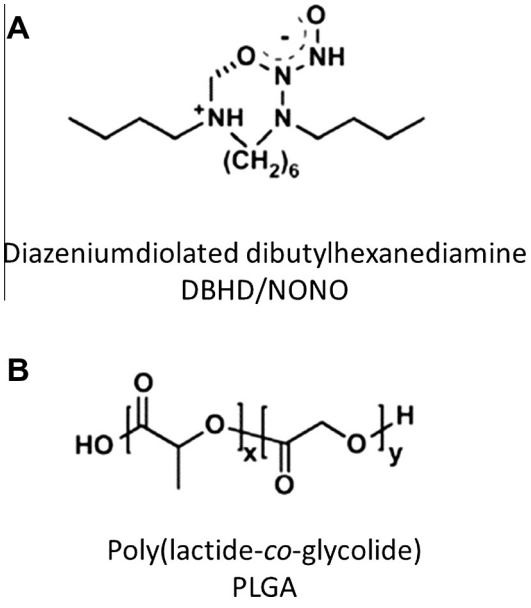

Fig. 1. Structure of (A) diazeniumdiolated dibutylhexanediamine and (B) poly (lactide-*co*-glycolide).