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Inhibition of bacterial adhesion and biofilm formation by dual functional textured and nitric oxide releasing surfaces

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ABSTRACT

In separate prior studies, physical topographic surface modification or nitric oxide (NO) release has been demonstrated to each be an effective approach to inhibit and control bacterial adhesion and biofilm formation on polymeric surfaces. Such approaches can prevent biomaterial-associated infection without causing the antibiotic resistance of the strain. In this work, both techniques were successfully integrated and applied to a polyurethane (PU) biomaterial surface that bears ordered pillar topographies (400/400 nm and 500/500 nm patterns) at the top surface and a *S*-nitroso-*N*-acetylpenicillamine (SNAP, NO donor) doped sub-layer in the middle, via a soft lithography two-stage replication process. Upon placing the SNAP textured PU films into PBS at 37 °C, the decomposition of SNAP within polymer film initiates NO release with a lifetime of up to 10 days at flux levels >0.5 × 10^{-10} mol min^{-1} cm^{-2} for a textured polyurethane layer containing 15 wt% SNAP. The textured surface reduces the accessible surface area and the opportunity of bacteria-surface interaction, while the NO release from the same surface further inhibits bacterial growth and biofilm formation. Such dual functionality surfaces are shown to provide a synergistic effect on inhibition of *Staphylococcus epidermidis* bacterial adhesion that is significantly greater than the inhibition of bacterial adhesion achieved by either single treatment approach alone. Longer term experiments to observe biofilm formation demonstrate that the SNAP doped-textured PU surface can inhibit the biofilm formation for >28 d and provide a practical approach to improve the biocompatibility of current biomimetic biomaterials and thereby reduce the risk of pathogenic infection.

Statement of Significance

Microbial infection remains a significant barrier to development and implementation of advanced blood-contacting medical devices. Clearly, determining how to design and control material properties that can reduce microbial infection is a central question to biomaterial researchers. In separate prior studies, physical topographic surface modification or nitric oxide (NO) release has been demonstrated to each be an effective approach to inhibit and control bacterial adhesion and biofilm formation on polymeric surfaces. Such approaches can prevent biomaterial-associated infection without causing antibiotic resistance of the bacterial strain. However, efficiency of antimicrobial properties of each approach is still limited and far from sufficient for widespread clinical use. This work successfully integrates both techniques and applies them to a polyurethane (PU) biomaterial surface that bears dual functions, surface topographic modification and NO release. The former reduces the surface contact area and changes surface wettability, resulting in reduction of bacterial adhesion, and NO release further inhibits bacteria growth. Such dual functionalized surfaces provide a synergistic effect on inhibition of *Staphylococcus epidermidis* bacterial adhesion that is significantly greater than the inhibition of bacterial adhesion achieved by either single treatment approach alone. Furthermore, longer-term experiments demonstrate that the dual functionalized surfaces can inhibit biofilm formation for >28 days. The success of this work provides a practical approach to improve the biocompatibility of current biomaterials and thereby reduce the risk of pathogenic infection.

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1. Introduction

The long-term use of biomaterials designed for fabricating implantable medical devices such as intravascular catheters, urinary catheters, indwelling blood pumps, vascular assist devices, and orthopedic implants is complicated by the potential for microbial infection due to pathogenic bacterial adhesion and biofilm formation on biomaterial surfaces [1–3]. Due to the difficulty in treating bacterial biofilms via antibiotics and the increasing levels of antibiotic resistance of pathogens, surgical removal and replacement of the implanted devices is often the only treatment for device-centered infections, causing a significant increase in morbidity and cost [4]. Combatting device-associated infections has been a great challenge in the field of implant-associated health care [5].

Bacterial adhesion to the device surface is the first and critical step in the pathogenesis of implant related infections. As an alternative to traditional methods in which antibiotics or biocides are released or infused (e.g., catheter lock solutions), methods to design and control material properties to reduce/control bacterial adhesion and thereby reduce microbial infection are more attractive approaches [6,7]. One promising method is the generation of functional surfaces through topographic modifications (e.g., surface texturing) that significantly reduce the initial attachment of microorganisms and the number of persistent pathogens to the surfaces of the implant [8]. By analogy to natural antifouling surfaces such as shark skin, shell or lotus leaf, etc., the physical topographic surface modification with micro- or nano-size features reduces the surface contact area and changes the surface energy, and this approach has been shown to be effective in controlling bacterial adhesion and biofilm formation [9–12]. With interest in this area and the related research increasing, over the past two decades biomimetic or bio-inspired materials have been developed to prepare the sterile biomaterials for the use in medical devices [13,14]. For example, the topographical features mimicking the shark's skin were applied into polydimethylsiloxane (PDMS) elastomer and it was found that such surfaces disrupt biofilm formation of *Staphylococcus aureus* (*S. aureus*), suggesting the possibility for application of this approach in biomedical devices [15]. Furthermore, an *in vitro* study showed that the micropatterned surface inhibited the bacterial colonization and migration of uropathogenic *Escherichia coli (E. coli)*; thereby it could be applied to reduce the risk of catheter-associated urinary tract infection [16]. Our group has previously developed submicron-textured polyurethane biomaterial surfaces featuring patterns with pillars of diameter and spacing of 400/400 nm and 500/500 nm, and demonstrated that both textured surfaces decreased the adhesion of *Staphylococcal epidermidis* (*S. epidermidis*) and *S. aureus* and inhibited biofilm formation under shear and static conditions [17]. Such submicron-textured surfaces have also been shown to reduce platelet adhesion, thereby reducing the potential of implant associated blood clotting/thrombosis of the device if implanted in the blood stream [18]. These *in vitro* successes of textured materials have demonstrated the great potential for their use in clinical applications to combat health-care infections and thrombosis caused by catheters and other implanted devices.

Nitric oxide (NO) releasing biomaterials represent another biomimetic strategy with great potential for clinical use. NO is an endogenous gas molecule and its continuous release from the endothelial cells that line all blood vessels can effectively prevent the adhesion/activation of platelets on normal blood vessel walls [19]. NO also plays an important role in the immune response as an antimicrobial agent and host defense against pathogenic bacteria [20]. As a diatomic free radical, NO can cross the membranes to enter the microbial cell readily and kill the microbe by directly damaging DNA, proteins, and lipids through production of potent nitrosating species or by combining with reactive oxygen species (e.g., superoxide, peroxide) and oxidizing the same targets [21,22]. Furthermore, the rapid reduction of microbial loads reduces the pressure for the evolution and spreading of variant bacteria and limits the possibility of promoting NO resistant strains [23]. For these reasons, polymeric materials that mimic endogenous NO release provide a potential solution against medical device-associated microbial infection and also can prevent platelet activation and thereby reduce risk of thrombus formation. Extensive studies have already demonstrated that NO release can effectively inhibit bacterial adhesion and reduce biofilm development on material surfaces [24–27].

Since NO is highly reactive and short-lived under physiological conditions, NO donor molecules with functional groups that can store and release NO are necessary. These NO donors are incorporated into materials either by blending discrete NO donors within polymeric films, or by covalently attaching them to polymer backbones and/or to the inorganic polymeric filler particles that are often employed to enhance the strength of biomedical polymers (e.g., fumed silica or titanium dioxide) [28]. *N*-Diazeniumdiolates [26,29,30] and *S*-nitrosothiols [30–32] are commonly used to prepare NO releasing polymeric matrices for improved biocompatibility of blood-contacting medical devices. The *N*-diazeniumdiolates are generally synthesized by reaction of amines with NO gas to form relatively stable compounds that release NO when in contact with bodily fluids through proton or thermally driven mechanisms. The *S*-nitrosothiols are generally formed by reaction of nitrous acid with the parent thiol, and undergo thermal decomposition to release NO, which are generally catalyzed by metal ions (e.g., copper), and light [30]. Nitric oxide can also be generated via other techniques, such as electrochemical reduction of nitrite [33], or reduction of sodium nitroprusside [34]. However, the current NO release strategies have the challenges in terms of storage, stability, costly synthesis or short NO release lifetime. Among the NO donors reported to date, *S*-nitroso-*N*-acetylpenicillamine (SNAP) is one of the most attractive ones in terms of its long-term NO release capability and enhanced stability when incorporated into low water uptake biomedical polymers. Indeed, SNAP has already been incorporated into a number of low water uptake polymers to yield promising new biomaterials. For example, it was reported that SNAP-doped polyurethane Elast-eon E2As polymer retained 82% of the initial SNAP after 2 month storage at 37 °C [35] and released NO slowly at the physiological flux level for 3 weeks. Similarly, SNAP in CarboSil 20 80A also exhibits a stability at 88.5% ± 4.3% of the initial amount after 8 months storage in the dark at 37 °C [36]. The increased stability of SNAP within these polymers is believed to be due to the intermolecular hydrogen bonds between crystallized SNAP molecules. For example, long-term storage stability of SNAP in the CarboSil polymer was found to originate from the formation of a polymer-crystal composite during the solvent evaporation. This composite led to sustained NO release at the physiological flux levels that can last for >3 weeks with cross-linked silicone rubber as a topcoat [36].

The SNAP-doped polymers exhibit good blood compatibility. The E2As catheters doped with SNAP significantly reduced the amount of thrombus and bacterial adhesion compared the E2As control catheters when they were implanted in sheep veins for 7 d [37]. An *in vitro* experiment of *S. aureus* biofilm formation over a 7 d period showed that SNAP-doped CarboSil 20 80A intravascular catheters had 5 log units reduction of viable cell count on their surfaces [36]. All results reported to date suggest that SNAP-doped low water uptake polyurethane copolymers could be attractive for