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S-Nitroso-*N*-acetylpenicillamine (SNAP) Impregnated Silicone Foley Catheters: A Potential Biomaterial/Device To Prevent Catheter-Associated Urinary Tract Infections

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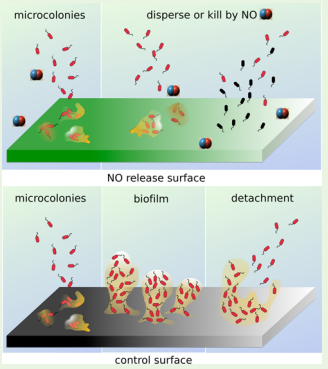
Supporting Information

ABSTRACT: Urinary Foley catheters are utilized for management of hospitalized patients and are associated with high rates of urinary tract infections (UTIs). Nitric oxide (NO) potently inhibits microbial biofilm formation, which is the primary cause of catheter associated UTIs (CAUTIs). Herein, commercial silicone Foley catheters are impregnated via a solvent swelling method with *S*-nitroso-*N*-acetyl-D-penicillamine (SNAP), a synthetic NO donor that exhibits long-term NO release and stability when incorporated into low water-uptake polymers. The proposed catheters generate NO surface-fluxes >0.7×10^{-10} mol min^{-1} cm^{-2} for over one month under physiological conditions, with minimal SNAP leaching. These biomedical devices are demonstrated to significantly decrease formation of biofilm on the surface of the catheter tubings over 3, 7, and 14 day periods by microbial species (*Staphylococcus epidermidis* and *Proteus mirabilis*) commonly causing CAUTIs. Toxicity assessment demonstrates that the SNAP-impregnated catheters are fully biocompatible, as extracts of the catheter tubings score 0 on a 3-point grading scale using an accepted mouse fibroblast cell-line toxicity model. Consequently, SNAP-impregnated silicone Foley catheters can likely provide an efficient strategy to greatly reduce the occurrence of nosocomial CAUTIs.



KEYWORDS: *nitric oxide, SNAP-impregnated Foley catheters, long-term NO release, antibiofilm, biocompatibility*

INTRODUCTION

The insertion of biomedical devices into the body, such as intravascular and urinary catheters, represents an indispensable component of modern medical care, especially for hospitalized patients. Nevertheless, the use of these medical devices is associated with a substantial risk of bacterial infections, with catheter-associated urinary tract infections (CAUTIs) and catheter-related bloodstream infections (CRBSIs) representing significant medical problems.[1,2] Specifically, Foley catheters, the most widely utilized indwelling urinary catheters, play a critical role in the relief of urinary retention, alleviation of urinary incontinence, and patient management during and after surgical procedures. Yet, because external bacteria can easily access the urinary tract, CAUTIs can result in 0.5−0.7 million nosocomial infections annually and account for 40% of hospital-acquired infections in the United States, representing the most common source of infection in clinical settings.[3−5] Because of the high incidence and potential clinical relevance of these infections, the cost of medical intervention for CAUTIs is quite significant, with $450 M spent annually in the United States alone.[6]

It is documented that microbial biofilms play a significant role in the pathogenesis of CAUTIs.[7] After the initial colonization of the abiotic surfaces, uropathogens form hydrated matrices of extracellular polymeric substances, dubbed as biofilms. These structures allow for the persistence of bacteria in the urinary tract by facilitating adhesion to the catheter's surface and the patient's epithelium, thus protecting the uropathogens from antibiotics and the host immune system.[8,9] Bacteria within biofilms can also detach and disseminate from the initial site of colonization to the upper urinary tract, thus being a potential cause of pyelonephritis.[10] Furthermore, uropathogenic bacteria can induce precipitation of calcium and magnesium phosphates, and form urinary stones (staghorn calculi),[11,12] which may obstruct urine flow through the catheter and lead to serious complications. Because 5 million patients each year receive urinary catheters in the U.S.,[3] and CAUTIs account for 80% of the total UTIs annually,[13] many infection control measures have been pursued. Though silver hydrogel coatings[14] or impregnation of urinary catheters with antiseptic chemicals reduce the severity of bacteriuria, no methods have yet been developed that effectively prevent biofilm-induced infections associated with the use of Foley catheters.[15,16]

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Nitric oxide (NO), a gas molecule endogenously produced by various NO synthase (NOS) enzymes, plays a critical physiological role in innate immunity by inhibiting proliferation of bacteria, fungi, and viruses.[17] For these reasons, polymeric materials that mimic endogenous NO release provide a potential solution against medical device-associated infections, and it has already been demonstrated that NO release can effectively prevent bacterial adhesion[18,19] and reduce biofilm development[20−22] on abiotic surfaces. Because gaseous NO is short-lived and highly reactive under physiological conditions, a vast array of NO donor molecules capable of efficiently storing and delivering NO locally have been studied for potential biomedical applications. Such molecules include *N*-diazeniumdiolates, *S*-nitrosothiols (RSNOs), nitrates, and metal-nitrosyl complexes, etc. Lipophilic diazeniumdiolates doped into polymer films,[21] RSNO-modified xerogels (where RSNOs are covalently bound to the matrix)[19] and endogenous RSNO catalyst-based coatings[18] all exhibit antimicrobial properties and reduced bacterial adhesion. Nitric oxide generation via electrochemical reduction of nitrite[22,23] or via reduction of sodium nitroprusside (SNP)[20,24] has also been shown to reduce bacterial cell viability within biofilm communities. Nevertheless, each of these NO-release strategies has some inherent drawbacks, in terms of storage stability, costly synthesis, or less than desirable NO release lifetimes.

We have recently discovered that silicone rubber and other low water uptake biomedical polymers (e.g., CarboSil, a thermoplastic urethane copolymer with a mixed soft segment of poly(dimethylsiloxane) and hydroxyl-terminated polycarbonate with a hard segment of an aromatic diisocyanate, MDI (from DSM Biomedical Inc.), and E2As, a copolymer with a mixed soft segment of poly(dimethylsiloxane) and poly(hexamethylene oxide) with a methylene diphenyl isocyanate (MDI) hard segment (obtained from AorTech International, plc)) doped with *S*-nitroso-*N*-acetylpenicillamine (SNAP), a synthetic RSNO, exhibit remarkable NO release capability and storage stability.[25] However, preparing Foley catheters with SNAP present during the catheter extrusion process is not possible because of the instability of SNAP molecule at the elevated temperatures required for conventional catheter extrusion. To overcome this problem, we herein describe a simple solvent swelling/impregnation method to incorporate SNAP into FDA-approved silicone Foley catheter tubing. It has already been demonstrated that several organic solvents can swell silicone rubber;[26] thus, in this work, tetrahydrofuran (THF) is utilized to dissolve SNAP and also swell the silicone catheters to successfully impregnate them with SNAP. This technique, unlike the extrusion of polymers under high temperature control,[27] is conducted at room temperature and this reduces the decomposition of SNAP, the NO donor species. We also demonstrate that SNAP-impregnated silicone Foley catheter tubing steadily releases NO for one month at physiological and antimicrobial levels. The resulting SNAP-impregnated silicone catheter tubing are further shown to significantly decrease microbial biofilm formation after 14 d exposure to flowing media inoculated with *Staphylococcus epidermidis* (*S. epidermidis*) or *Proteus mirabilis* (*P. mirabilis*), two bacterial strains that most often induce CAUTIs.

EXPERIMENTAL DETAILS

Materials. *N*-Acetyl-D-penicillamine (NAP), sodium chloride, potassium chloride, sodium phosphate dibasic, potassium phosphate monobasic, L-cysteine, copper chloride, ethylenediaminetetraacetic acid (EDTA), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (St. Louis, MO), and used as received. Methanol (MeOH), hydrochloric acid (HCl), and sulfuric acid (H_2SO_4) were obtained from Fisher Scientific (Pittsburgh, PA). Luria−Bertani (LB) broth and LB agar were also obtained from Fisher Scientific Inc. Aqueous solutions were prepared with deionized water using a Milli-Q filter (18MΩ cm^{-1}; Millipore Corp., Billerica, MA). Phosphate buffered saline (PBS), pH 7.4, containing 10 mM sodium phosphate, 138 mM NaCl, 2.7 mM KCl, 100 μM EDTA was used for all in vitro experiments.

Silicone 2-way Foley Balloon Catheters, size 18 Fr (o.d. = 0.59 cm, i.d. = 0.30 cm), were purchased from Fortune Medical Instrument Corp (New Taipei City, TAIWAN). *S. epidermidis* ATCC 14990 and *P. mirabilis* ATCC 29906 were obtained from the American Type Culture Collection (ATCC) (Manassas, VA).

Synthesis of SNAP. SNAP was synthesized using a method previously described.[25] Briefly, an equimolar ratio of NAP and sodium nitrite was added to a 3:1 mixture by volume of methanol and water containing 2 M HCl and 2 M H_2SO_4. After 30 min of stirring, the reaction vessel was cooled in an ice bath to precipitate the green SNAP crystals. After 5 h, the crystals were collected by filtration, washed with ice-cold water, and allowed to air-dry for 48 h. The reaction and crystals were shielded from light at all times.

Preparation of SNAP Impregnated Silicone Foley Catheters. SNAP was first dissolved in THF at a concentration 125 mg/mL of solvent, for 15 min. Commercial FDA approved silicone Foley catheter tubing was cut either into 1 cm long sections (for NO release measurements, SNAP % loading and leaching assessments) or into 2 cm long sections (for biofilm studies). Such segments were completely immersed into SNAP-containing THF solutions, within glass vials, for 24 h in the dark. After this swelling/impregnation period, the catheter sections were dried in dark for 72 h within a fume hood to remove any residual solvent. Control Foley catheter segments (designated for biofilm experiments) were swollen in a vial containing only THF for 24 h, followed by a 72 h drying process within the fume hood.

NO Release Measurements. Nitric oxide release from the surfaces of 1 cm SNAP-impregnated Foley catheter segments (total surface area = 3.19 cm^2) was measured using a Sievers chemiluminescence Nitric Oxide Analyzer (NOA) 280i (Boulder, CO). Catheter segments were placed in an amber glass vessel (placed in a water bath at 37 °C) containing 4 mL of PBS (pH 7.4) with 100 μM EDTA. Nitric oxide released from the catheter segment was continuously purged from the buffer and swept from the headspace using an N_2 sweep gas and bubbler into the chemiluminescence detection chamber. When not being tested with the NOA, the SNAP-impregnated catheters were incubated in 10 mM PBS (pH 7.4) with 100 μM EDTA at 37 °C, avoiding exposure to light. All experiments were conducted in triplicate.

SNAP Loading Efficiency Test. Because the reaction of 2 RSNO → RSSR + 2 NO is catalyzed by light in the visible region of the spectrum,[28] the total loading of SNAP into the 1 cm catheter segments was determined by the total amount of NO (in moles) released over time in the presence of a high intensity white light source (until no additional NO could be detected above baseline by the NOA). By integrating the signal from the NOA over time, the total amount of SNAP-impregnated into each catheter segment can be calculated. Knowing the initial weight of the catheter segment, the wt % of SNAP within the catheter material can be quantitated. NO release was measured via the NOA, by immersing the catheter tubing sample in a clear glass vessel (kept inside a water bath at 37 °C) containing 50 mM $CuCl_2$, and 10 mM L-cysteine. The added L-cysteine catalyzes the reduction of Cu^{2+} into Cu^{+}, which then promotes the SNAP decomposition and the release of NO. A 100W halogen floodlight (GE model 17986) was placed 20 cm away from the sample, and was used to photoinitiate the NO release from the SNAP-impregnated catheter pieces. All experiments were conducted in triplicate.

SNAP Leaching from Surface of SNAP Impregnated Foley Catheters. One-centimeter-long catheter segments were completely immersed into 1 mL of 10 mM PBS (with 100 μM EDTA) inside individual amber glass vials and stored at 37 °C for the entire duration

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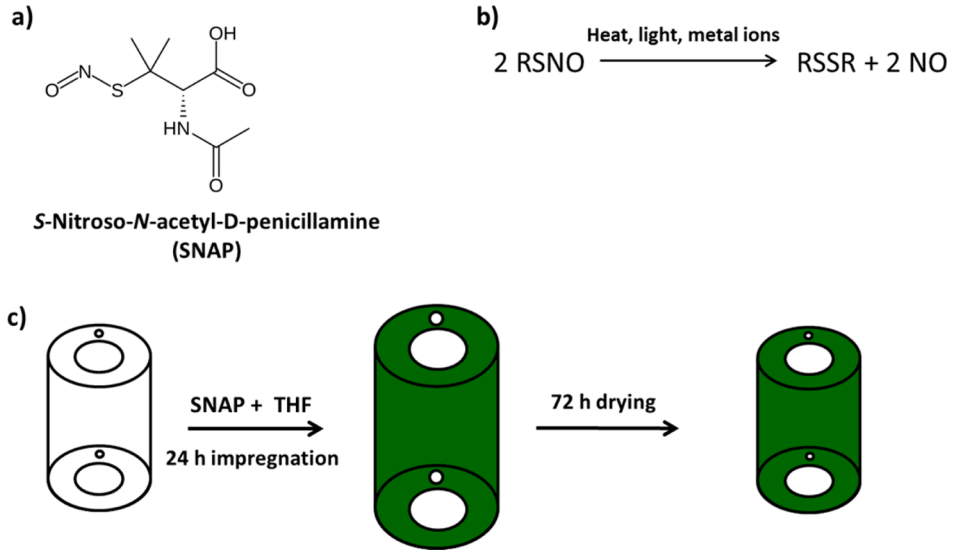

Figure 1. (a) Structure of *S*-nitroso-*N*-acetyl-D-penicillamine (SNAP); (b) scheme of *S*-nitrosothiol (RSNO) decomposition, where RSNO can release nitric oxide (NO) and yield the disulfide (RSSR) byproduct via light, heat or catalysis of metal ions (e.g., Cu(I)); (c) solvent swelling/impregnation method employed to load commercial Foley catheter silicone tubing with SNAP.

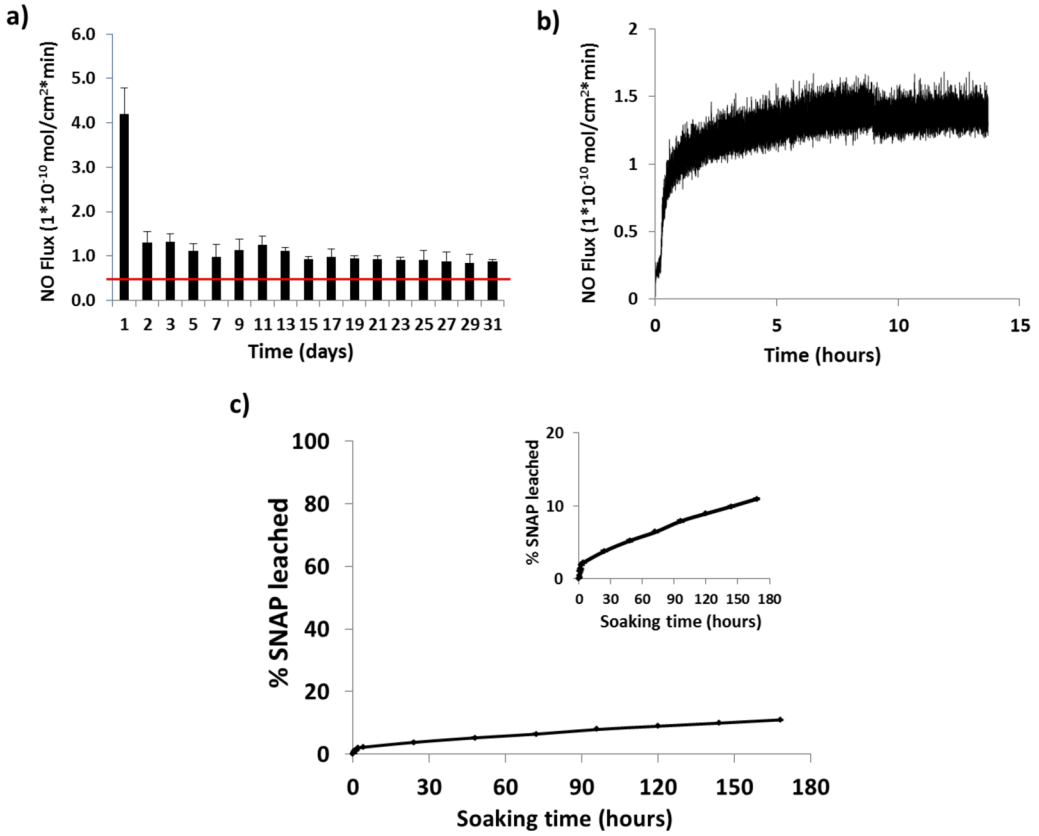

Figure 2. (a) Release of NO from SNAP-impregnated silicone Foley catheter tubings over a 30 d period (n = 3); (b) NO release profile of one SNAP-impregnated catheter segment after 3 d of soaking in PBS; (c) percentage of total loaded SNAP that leaches from the surface of the silicone tubing during the first 7 days of soaking in PBS at 37 °C. Data are the mean ± SEM (n = 3).

of the experiment. At various time points, each of the soaking solutions, in which catheter segments had been stored, were tested by UV−Vis to detect the SNAP characteristic absorbance band. By using the Beer−Lambert law ($A = \varepsilon lc$), the moles of SNAP leached out at every time point was calculated. Since the weight percentage of total SNAP loading was determined from total NO release (see above), the percentage of leached SNAP can be quantitated. All UV−Vis spectra were recorded in the wavelength range of 250−650 nm using a UV−Vis spectrophotometer (Lambda 35, PerkineElmer, MA). The presence of the S-NO group of SNAP provides characteristic absorbance maxima at 340 and 590 nm, corresponding to the $\pi \rightarrow \pi^*$ and $n_N \rightarrow \pi^*$ electronic transitions.[29,30] The molar absorption coefficient for SNAP in PBS at 340 nm was determined to be ε_{SNAP} = 1075 M^{-1} cm^{-1}. PBS was used as the blank and all experiments were conducted in triplicate.

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Biofilm Growth Conditions and Plate Counting. *S. epidermidis* and *P. mirabilis* organisms were grown onto surface of control and NO release catheters for 3, 7, and 14 days using a CDC bioreactor. The methods used were similar to those reported previously.[23,31] Details of these microbiology procedures in relation to the studies reported here are provided in the Supporting Information file.

Biofilm Imaging. Control and SNAP-impregnated catheter segments designated for fluorescent imaging were stained with LIVE/DEAD BacLight Bacterial Viability kit (L7012, Invitrogen, Carlsbad, CA) according to its instructions. Fluorescent images were acquired with an inverted fluorescence microscope (Olympus IX 71, Center Valley, PA) equipped with Fluorescence Illumination System (X-Cite 120, EXFO) and filters for SYTO-9 (excitation = 488 nm/emission = 520 nm) and Propidium Iodide (excitation = 535 nm/emission = 617 nm). Images were obtained using an oil immersed 60× objective lens. Only the fluorescent micrographs of control Foley catheters surfaces upon which *P. mirabilis* had been grown for 14 days were taken with a 10× objective lens because of the high biofilm biomass on these pieces of tubing (note: the use of the 60× objective lens would have impeded to obtain a clear focus of the biofilm layer on the catheter surfaces). All experiments were conducted in triplicate, and different surface areas of the catheter segments were randomly chosen for imaging.

Statistical Analysis. Data for all experiments are expressed as mean ± SEM (standard error of the mean). Comparison of means using student's *t* test was utilized to analyze whether there was a statistical difference between the data for SNAP-impregnated vs control silicone Foley catheter materials. Values of $p < 0.05$ were considered statistically significant and graphically illustrated with a *.

RESULTS

NO Generation of SNAP-Impregnated Silicone Foley Catheters. The total SNAP amount loaded into Foley catheter segments by the solvent impregnation method was found to be 5.43 ± 0.15 wt % ($n = 3$) using photolysis to emit the entire payload of NO in a reasonable time frame (see Experimental Section). Upon placing the dried tubing segments loaded with SNAP into PBS buffer at 37 °C, SNAP decomposition within the walls of the tubing commences owing to the combination of both the presence of moisture and elevated temperature. NO release data clearly show that the SNAP-impregnated catheter tubing can release NO at a flux between 1.4 and 0.8 × 10^{-10} mol min^{-1} cm^{-2} for at least 30 d (see Figure 2a) (note: both outer and inner wall surfaces of the pieces of tubing are utilized to calculate flux levels). As shown in Figure 2b, the steady-state NO release profile, at any given time point for testing the silicone tubing, is relatively stable after ca. 30 min when studying the NO release in the nitrogen-purged solution phase using the NOA. It should be noted that the catheter tubing displays an initial burst of NO during the first few hours of soaking on day 1 (Figure 2a). This is likely due to the rapid reaction and leaching of SNAP present in the outermost surface layers of the silicone tubing. In addition, because it is known that the pH of urine can vary widely (pH 6.5–8.0), the rate of NO release from separate SNAP-impregnated catheter pieces was examined over this pH range during the initial week of NO release testing. It was found that when the pH of the soaking/test buffer is pH 6.5 or pH 8.0 (using PBS buffers at these pH values), the rate of NO release from the catheters is not statistically different than observed when soaking and testing in pH 7.4 PBS (see Figure S1).

SNAP Leaching from the Catheter Surface. To ensure that SNAP leaching from the catheter polymeric surface is primarily limited to the first day of soaking because of rapid water uptake at the outermost surface of the silicone rubber, we conducted leaching studies using UV–vis absorbance spectroscopy over the first 7 days of soaking. At various time points, the UV–vis spectra of aliquots of the PBS soaking solution were assayed for SNAP concentration. Since the total SNAP amount loaded into these 1 cm long catheter sections had previously been quantified, it was determined that only 3.51 ± 0.04% of the total loaded SNAP leached from the catheter segment on the first day of soaking (see Figure 2c). Minimal amounts of SNAP continued to leach from the Foley catheters during the subsequent days of soaking. Indeed, only 10.92 ± 0.05% of the total SNAP was lost during the first week (see Figure 2c). It should be noted that NAP and/or NAP disulfide leaching from the catheter surface is not considered a major concern, because *N*-acetyl-penicillamine has been used clinically to treat mercury and other heavy metal poisoning for many years.[32]

SNAP Impregnated Foley Catheters Antibiofilm Properties against *S. epidermidis*. The total viable *S. epidermidis* adhered on the catheter tubing surface was determined after growing biofilms for either 3, 7, or 14 days at 37 °C. Plate count data for 3 day old *S. epidermidis* biofilms show that the viable bacteria attached on the surface of the SNAP-impregnated catheter tubing is ca. 50% less than on controls (Figure 3a; $n = 3$, p value = 0.014). Plate count data for 7 day *S. epidermidis* biofilms exhibited a significant 2.5 log units difference in viable bacteria between control and NO releasing catheter segments (Figure 3a; $n = 3$, p value = 0.015). Similarly, when *S. epidermidis* biofilms were grown for 14 days and

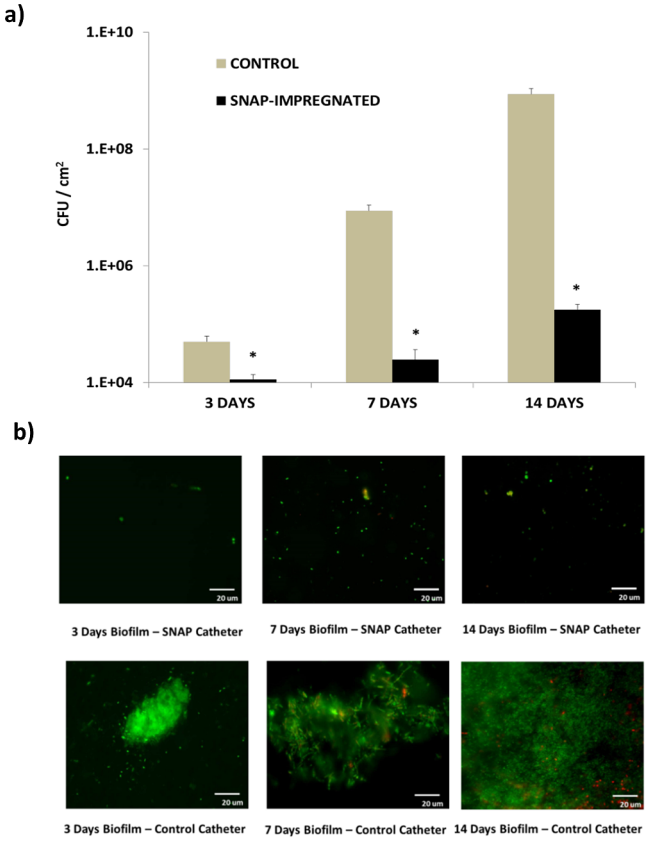

Figure 3. *S. epidermidis* biofilms developed on Foley catheters segments in a CDC biofilm reactor for 3, 7, and 14 days. (a) Plate count of the number of viable bacteria adhered to the catheter surface; (b) representative fluorescence images with oil immersion 60× objective lens of 3, 7, and 14 day *S. epidermidis* biofilms on the surface of the catheter tubing.

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reached a matured growth stage, the reduction in adhered viable bacteria on SNAP-impregnated catheters compared to controls differs by 3.7 logarithmic units (Figure 3a; n = 3, p value = 0.013). Fluorescence imaging data of control and SNAP-impregnated catheter segments after 3, 7, or 14 days of growth were also collected. As shown in Figure 3b, it is observed that although biofilm thickness and surface coverage increases with incubation time on control catheters, they remain relatively low over time on the surfaces of the SNAP-impregnated catheters.

SNAP Impregnated Foley Catheters Antibiofilm Properties against *P. mirabilis*. Results for 3 day *P. mirabilis* biofilms show a 3 logarithmic units difference in cell viability between the SNAP-impregnated and control catheter segments (Figure 4a; n = 3, p value = 0.02). Cell counts for 7 and 14 day

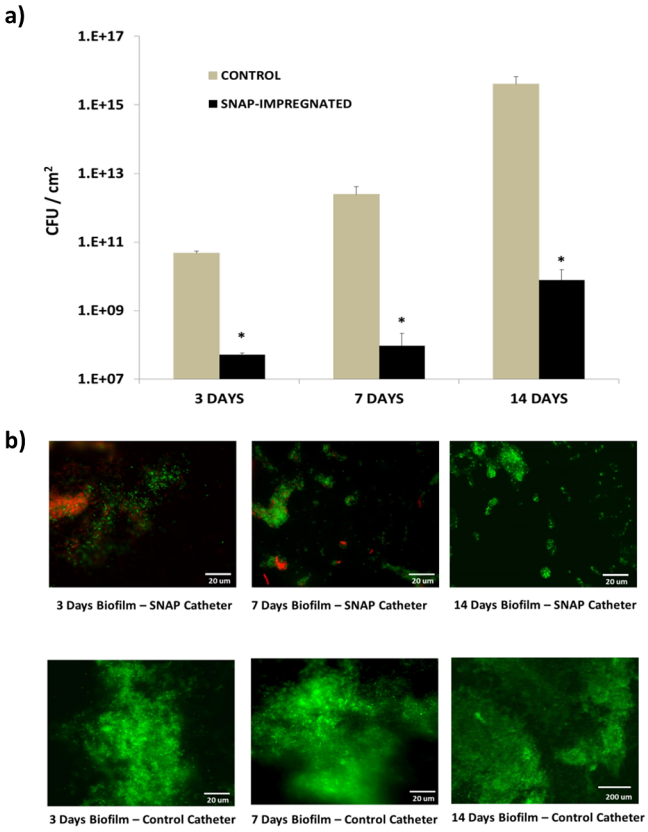

Figure 4. *P. mirabilis* biofilms developed on Foley catheter segments in a CDC biofilm reactor for 3, 7, and 14 days. (a) Plate count of the number of viable bacteria adhered to the catheter surface; (b) representative fluorescence images with oil immersion 60× objective lens of 3, 7, and 14 day *P. mirabilis* biofilms (note: for control catheters at day 14, because of high biofilm biomass, images were taken with a 10× objective lens).

P. mirabilis biofilms grown on the surface of SNAP-impregnated catheter tubing are 5 (Figure 4a, n = 3, p value = 0.02) and 6 (Figure 4a, n = 3, p value = 0.007) log units lower than those for controls, respectively. These findings are further corroborated by the fluorescent imaging data, which shows that bacterial surface coverage on the SNAP-impregnated catheter segments is noticeably less than controls (see Figure 4b). These results demonstrate that NO releasing SNAP-impregnated Foley catheters could provide a novel approach to prevent catheter surface encrustation by crystalline biofilm-forming bacterial species.

***P. mirabilis* Antibiofilm Study after Presoaking SNAP Catheters for 24 h.** Chemiluminescence data from the NOA shows that the impregnated catheters display an initial burst of NO during the first day of soaking at 37 °C, which is due to the thermal decomposition and the diffusion of SNAP out of the polymeric surface of the catheters. The bactericidal efficacies of NO releasing materials improve with increasing initial NO flux.[32,33] To ensure that SNAP-impregnated catheters' antibiofilm properties were not due to the bactericidal effect of an initial high NO flux (4.5 mol min^{-1} cm^{-2}) during the initial hours of antibacterial studies in the bioreactor, we conducted additional experiments in which the SNAP catheters were first presoaked in PBS at 37 °C for 24 h. After this presoaking step, 3 days *P. mirabilis* antibiofilms studies were conducted exactly as described above. Plate count data of viable bacteria adhered on the catheter surfaces clearly illustrates that there is more than 3 logarithmic units difference between controls and presoaked SNAP-impregnated catheters (Figure 5a, n = 6, p value = 0.010). Fluorescent images also show that bacterial surface coverage of presoaked SNAP-impregnated catheters is noticeably less than controls (see Figure 5b).

SNAP Impregnated Foley Catheters NO Releasing Properties Post *P. mirabilis* Antibiofilm Experiment. The NO releasing profile of three SNAP catheter segments, presoaked in PBS prior to the beginning of a 3 day *P. mirabilis* biofilm experiment, were tested via the NOA at the end of the biofilm growth period. As shown in Figure S2, the SNAP-impregnated catheter segments can still release NO at a flux >1 × 10^{-10} mol min^{-1} cm^{-2} at the end of the biofilm experiment (96 h post initial soaking step) (Figure S2, n = 3). Further, the NO releasing properties of the same catheter segments were then also tested on the following day, and the NO flux remained relatively constant (120 h post initial soaking step) (Figure S2, n = 3).

DISCUSSION

The long-term NO release data confirms that this impregnation method, conducted at room temperature, allows for the successful incorporation of SNAP within the polymer, and that NO release can last for over 1 month at relatively constant flux levels, with minimal leaching of the total loaded SNAP after the first day of soaking (Figure 2a, c). This illustrates the advantage of using the simple solvent impregnation approach over extruding the polymer with the NO donor present, as the latter would degrade the SNAP (loss in NO) because the elevated temperatures required.[27]

Because stable NO releasing devices can abate bacterial adhesion and colonization associated with catheterization, we examined the antimicrobial properties of the SNAP-impregnated silicone Foley catheter segments with bacterial strains associated with CAUTIs. Initially, *Staphylococcus epidermidis*, a species responsible for both intravascular and urinary infections,[35] was studied. As the CDC biofilm reactor represents a standardized model to replicate in vivo urinary tract conditions,[31] this methodology was utilized to simulate the development of bacterial biofilms on the surface of urinary Foley catheters. Remarkably, the viable *S. epidermidis* adhered on the surface of SNAP catheter tubing after growing biofilms for 7 and 14 days was reduced by 2.5 and 3.5 log units, respectively, relative to the controls (see Figure 3a). However, it should be noted that reduction of bacterial viability for 3 d

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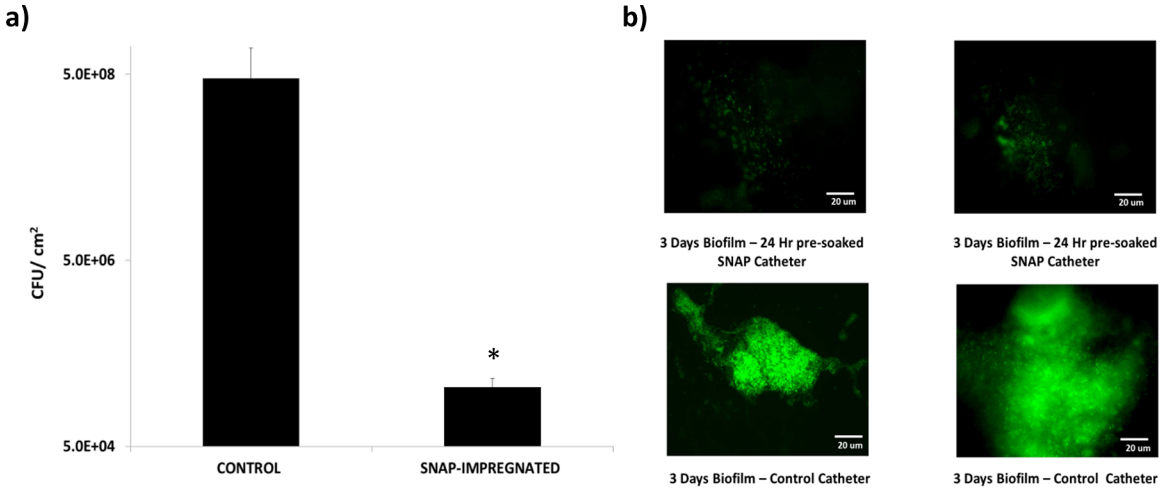

Figure 5. *P. mirabilis* biofilms developed on Foley catheter segments in a CDC biofilm reactor for 3 days. SNAP-impregnated catheters presoaked in PBS buffer for 24 h prior to the beginning of biofilm/bioreactor experiment. (a) Plate count of the number of viable bacteria adhered to the catheter surface. (b) Representative fluorescence Images with oil immersion 60× objective lens of catheter surfaces after 3 days of growth of *P. mirabilis* biofilms.

old *S. epidermidis* biofilms was moderate, only 50% less than on control surfaces. A plausible reason for this finding may be that the bacteria had not yet developed into a mature biofilm, but were still in a microcolony growth stage. Because bacterial counts on the control surfaces were also low, this fact did not allow a high percentage difference between the SNAP-doped and control catheters to be observed. Thus, from the analysis of plate count data (Figure 3a), it is evident that the SNAP-impregnated catheter segments can progressively reduce formation of *S. epidermidis* biofilms. This finding is substantiated by the fluorescence imaging data (Figure 3b).

Although *S. epidermidis* represents a commensal inhabitant of healthy mucosal microflora and displays lower pathogenic potential than *S. aureus*, *P. mirabilis*, and *P. aeruginosa*,[36] its principle virulence factor is the capacity to form high-biomass biofilms and colonize biomaterials.[37−39] It has been reported that biofilm-forming clinical strains of *S. epidermidis*, isolated from urinary tract infections, are significantly more resistant to a wide array of antibiotic treatments (e.g., ampicillin, ciprofloxacin, gentamicin, levofloxacin) than nonbiofilm producing strains.[40,41] It has been suggested that the biofilm extracellular polysaccharide substance (EPS) represents a physical and chemical barrier to antibiotics,[42] and may thus retard the penetration rates enough to induce the expression of genes that mediate antibiotic resistance.[43] Low susceptibility to antibiotics may also be related to the metabolic state of bacteria within biofilms.[44] As the cells located within the biofilm experience nutrient limitation, this condition can result in a stationary phase-like dormancy, thus promoting biofilm resistance to antibiotics as compared to planktonic stage-bacteria.[45,46] Hence, the findings that SNAP-impregnated Foley catheters significantly limit *S. epidermidis* viability may represent a future strategy to prevent nosocomial urinary infections.

Because the plate count and fluorescent imaging data for *S. epidermidis* biofilms exhibit a significant difference between SNAP-impregnated and control catheters, the same experiments were conducted with *P. mirabilis*, the primary bacterium associated with complicated urinary tract infections.[47,48] This urease positive strain is capable of establishing mature crystalline biofilms,[11,48] thus complicating the management of patients relying on long-term catheterization. Indeed, this bacterium, by catalyzing the conversion of urea into ammonia, elevates urine pH and causes calcium/magnesium phosphates to precipitate and become incorporated into biofilms.[49] As this condition leads to severe clinical sequelae, prevention strategies are sorely needed.

We demonstrate that SNAP-doped catheter tubing substantially decreases bacterial viability at each stage of biofilm maturation (see Figure 4a, b), and that the difference in viable *P. mirabilis* cell counts between SNAP and control catheters increases with biofilm growth time. This indicates that the SNAP-doped catheters, by releasing NO, progressively reduce biofilm development. Further, it should be noted that *P. mirabilis* biofilms developed to a mature stage after only 3 d of growth, and that average biofilm thickness progressively increased at later time points. Indeed, the plate count data of control catheters for the *P. mirabilis* experiments are significantly higher compared to *S. epidermidis* biofilms, which is supported by fluorescent micrographs that show significantly greater biomass (see Figure 4b). One explanation may be that *P. mirabilis*, by expressing mannose-resistant/*Proteus*-like (MR/P) fimbriae and *P. mirabilis* fimbriae (PMF), can rapidly adhere/colonize onto the abiotic surfaces.[50,51]

Our findings with *P. mirabilis* are significant because, to the best of our knowledge, this is the first report of a synthetic NO-donor with extremely stable NO release at low/nontoxic fluxes, that is capable of preventing *P. mirabilis* mature biofilm formation. Earlier, Deupree et al. demonstrated that a synergistic activity of diazeniumdiolate-modified proline (PROLI/NO) and sulfadiazine (AgSD) exerts antimicrobial activity against *P. mirabilis* in vitro.[52] Yet, since in that work, overnight bacterial cultures were utilized in an acute (2 h) time-kill viability assay, the bactericidal properties of the chemicals were only investigated on planktonic cells. The antimicrobial efficacy of antibiotics and antiseptics coated devices has also been previously evaluated.[53,54] Nevertheless, though in vitro testing of Foley catheters coated with the antiseptic gendine significantly reduced both Gram-positive and Gram-negative bacterial cell counts, those biofilms were only cultured for 24 h.[53] Furthermore, although preliminary clinical trials assessing the antimicrobial properties of urinary catheters impregnated with antibiotics have been conducted and catheter-associated

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Gram-positive bacteriuria was significantly reduced, no protection against Gram-negative bacteria, the leading cause of CAUTIs, was achieved.[54] Moreover, although initial antibiotic dosages can be effective, over time, they induce tolerance and resistance.[55−57] Instead, many bacteria have not developed efficient strategies to combat the local bactericidal/biofilm dispersal effect of NO. Indeed, though some bacterial species (e.g., *P. aeruginosa*) express NO reductase (Nor) enzymes, that catalyzes NO conversion to N_2O, biofilm development by wild-type *P. aeruginosa* can still be abated by NO donors.[20,24] Thus, NO releasing SNAP-impregnated Foley catheters may provide a useful strategy to prevent nosocomial CAUTIs by various bacterial strains.

In addition, our results are corroborated by the fact that the antimicrobial properties of our new NO releasing materials are independent of really high NO flux displayed during the first hours of soaking at 37 °C. Indeed, even when SNAP catheters are presoaked for 24 h before the start of the biofilm experiment, viable *P.mirabilis* cell counts for NO releasing catheters are significantly lower than controls (Figure 5a, b). It can thus be concluded that the release of high NO levels during day 1 was not the major factor contributing to the potent antibiofilm properties of SNAP-impregnated catheters observed in the initial experiments.

It should be noted that previous studies have demonstrated how high initial NO fluxes augment the bactericidal efficacy and further inhibit bacterial adhesion on polymeric material surfaces.[33,34] However, because studies investigating nontoxic NO flux thresholds in urinary tract tissues have yet to be conducted, in this work, we aimed for SNAP-impregnated catheters to generate NO within ranges similar to those released by healthy endothelial cells (0.5−4.0 mol × 10^{-10} mol min^{-1} cm^{-2}).

Finally, as shown in Figure S2, we demonstrate that SNAP-impregnated catheter segments, presoaked in PBS prior to the beginning of a 3 day *P. mirabilis* biofilm experiment, can still release NO at a flux >1 × 10^{-10} mol min^{-1} cm^{-2} at the end of the biofilm experiment (96 and 120 h post the initial soaking step). These results provide further evidence that the antibiofilm activity of SNAP-impregnated catheters is due to the steady long-term NO release properties of these newly proposed biomedical devices.

CONCLUSIONS

In summary, we have demonstrated that an FDA approved silicone Foley catheter material can be impregnated with SNAP via a solvent swelling method and that the resulting catheters can release a relatively steady NO flux at their surfaces for 30 days. Because no extrusion process is required for the incorporation of the NO donor/antimicrobial agent into the catheter walls with little or no chemical degradation of the SNAP, the catheters should maintain functionality with stable NO release capability. The SNAP-impregnated catheter tubings were also shown to exhibit significant antibiofilm properties using two bacteria strains (*S. epidermidis* and *P. mirabilis*) that are responsible for high rates of nosocomial urinary catheter associated infections. Furthermore, a preliminary assessment of the toxicity of SNAP-impregnated catheter tubing was provided by Wuxi AppTec Inc. (St. Paul, MN) using ISO-based GLP biocompatibility studies. The catheter tubing received the safest scores possible (0) for in vitro toxicity testing (0−1 = safe, 3−4 = toxic) on L-929 Mouse Fibroblast Cells (extracts were taken from the catheter pieces stored for 24 h at 37 °C in Eagle's Minimal Essential Media), as well as for in vivo testing in mice (extracts were taken from the catheter pieces stored for 72 h at 37 °C in both saline and sesame oil). Hence, it is likely that SNAP-impregnated Foley urinary catheters could provide an inexpensive and safe approach to dramatically reducing the frequency of nosocomial CAUTIs, and studies are now planned to evaluate this new approach in vivo via long-term animal testing.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsbiomaterials.5b00032.

Details on biofilm growth conditions and plate counting for *S. epidermidis* and *P. mirabilis*; the effect of soaking and test pH values on NO release fluxes from SNAP-impregnated silicone Foley catheters (Figure S1); and the NO release properties of SNAP-impregnated Foley catheters post *P. mirabilis* antibiofilm experiments (Figure S2) (PDF)

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Notes

The authors declare the following competing financial interest(s): Mr. Michael Kappler and Dr. Hao Chen are employees of Biocrede Inc. Dr. Hao Chen has an equity position in this start-up company. The company received the STTR grant cited from the NIH. The remaining authors have no equity position in Biocrede Inc. and work solely for the University of Michigan. A subaward from Biocrede to the University of Michigan funded most of the research described in this manuscript.

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