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Study of Crystal Formation and Nitric Oxide (NO) Release Mechanism from *S*-Nitroso-*N*-acetylpenicillamine (SNAP)-Doped CarboSil Polymer Composites for Potential Antimicrobial Applications

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Abstract

Stable and long-term nitric oxide (NO) releasing polymeric materials have many potential biomedical applications. Herein, we report the real-time observation of the crystallization process of the NO donor, *S*-nitroso-*N*-acetylpenicillamine (SNAP), within a thermoplastic silicone-polycarbonate-urethane biomedical polymer, CarboSil 20 80A. It is demonstrated that the NO release rate from this composite material is directly correlated with the surface area that the CarboSil polymer film is exposed to when in contact with aqueous solution. The decomposition of SNAP in solution (e.g. PBS, ethanol, THF, etc.) is a pseudo-first-order reaction proportional to the SNAP concentration. Further, catheters fabricated with this novel NO releasing composite material are shown to exhibit significant effects on preventing biofilm formation on catheter surface by *Pseudomonas aeruginosa* and *Proteus mirabilis* grown in CDC bioreactor over 14 days, with a 2 and 3 log-unit reduction in number of live bacteria on their surfaces, respectively. Therefore, the SNAP-CarboSil composite is a promising new material to develop antimicrobial catheters, as well as other biomedical devices.

Keywords

Polymer-matrix composites (PMCs); Crystallization; Nitric Oxide (NO); *S*-Nitroso-*N*-acetylpenicillamine (SNAP); Antimicrobial catheters

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1. Introduction

Microorganisms commonly attach to living and nonliving surfaces, including those of a variety of indwelling medical devices [1]. After the initial colonization on the abiotic surfaces, bacteria form extracellular polysaccharide substance (EPS), also known as biofilm, that creates a physical and chemical barrier for the bacteria, preventing conventional antibiotic treatment or appropriate activation of the host defense system [2,3]. Infection due to bacterial adhesion and biofilm formation represents a major complication associated with the long-term use of indwelling biomedical devices, such as intravascular and urinary tract catheters. Intravascular catheters are utilized for management of hospitalized patients, where catheters are placed into the blood vessel for direct vascular access and used for infusion of fluids, drug administration, removal of blood samples, and patient monitoring, etc. Meanwhile urinary catheters serve an indispensable role in patient care, including preventing patient urine retention as well as providing a solution to chronic urinary incontinence. However, external bacteria can access the blood vessel or urinary tract despite the routine antiseptic procedures used when implanting these devices, and both catheter-related bloodstream infections (CRBSIs) and catheter-associated urinary tract infections (CAUTIs) cause lengthy hospital stays, increased healthcare costs and high mortality rates [4–6].

Current antimicrobial approaches for use with biomedical catheters include using catheters impregnated with antibiotics, employing antibiotic lock solutions, or utilizing catheters that possess a silver alloy coating that is capable of releasing Ag^+ ions to prevent bacterial infection [7–10]. Even though these antimicrobial catheters have been demonstrated in the laboratory to reduce bacterial adhesion and inhibit bacterial growth with some success [11–14], clinical results and efficiencies with such catheters have been less than satisfactory owing to the polysaccharide matrix that protects the bacteria and the increased level the microbial mutations (that prevents given antibiotic from proper functioning) within the biofilm [15,16]. Therefore, efforts aimed at devising novel solutions to effectively prevent biofilm-induced infections associated with the long-term use of indwelling catheters are still needed.

Nitric oxide (NO) was identified as the endothelium-derived relaxation factor (EDRF) by Ignarro, Furchgott, and Murad in 1987, and these scientists were awarded the Nobel Prize in Physiology or Medicine for their discovery in 1998 [17,18]. NO is an endogenous gas molecule synthesized from the amino acid L-arginine via three distinct isoforms of nitric oxide synthases (NOS): endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) [19–21]. The NO released by the endothelium cells that line the inner wall of all blood vessels helps to prevent platelet activation/adhesion and serves to control blood pressure, and the rate of release into the blood stream is typically at a surface flux rate of $0.5 - 4 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$ [19,22–24]. The human body also synthesizes greater amounts of NO from macrophages, upper airway epithelial cells, and other cells to serve as a potent antimicrobial agent to fight against bacterial infections *in vivo* [25,26]. In this regard, NO has been reported to kill both gram-positive and gram-negative bacteria [27–32]. It has also been reported that NO can modulate biofilm metabolism and make bacteria within biofilm more susceptible to antibiotics [33,34].

Because NO has a very short half-life, a wide variety of NO donors that are capable of releasing NO via different mechanisms/stimuli have been studied for therapeutic and targeted NO release [17,35–43]. One very promising NO release compound is *S*-nitroso-*N*-acetylpenicillamine (SNAP). SNAP is a tertiary *S*-nitrosothiol species, which is much more stable in storage than primary *S*-nitrosothiols (e.g., *S*-nitrosoglutathione) and it is also considered very biocompatible because NAP and its ultimate degradation product, penicillamine, have been used as chelators to treat heavy metal poisoning, such as Wilson's disease in humans, for many years [44–48]. Our group recently reported that SNAP is exceptionally stable when incorporated into CarboSil, a low water-uptake thermoplastic silicone-polycarbonate-urethane biomedical polymer [6]. The low water uptake nature of CarboSil enables minimized SNAP leaching when in contact with solution. Moreover, CarboSil itself has enhanced inherent biocompatibility over many previously studied polymer systems [35,49], such as poly(vinyl chloride)(PVC)-based materials. Solid state x-ray diffraction studies demonstrated that SNAP can partially dissolve in the CarboSil polymeric matrix (solubility: ca. 3.4–4.0 wt%), while the proportion exceeding this solubility limit crystallizes in its orthorhombic form. It was proposed that the hydrogen bonding between crystalline SNAP molecules contributes to its excellent shelf stability and slow dissolution of crystalline SNAP within the bulk of the CarboSil polymer determines the rate of NO release and ultimately leads to its long-term release under physiological conditions [6]. However, more detailed studies are needed to further understand the polymer/crystal composite formation and the NO release mechanism from the SNAP-doped CarboSil polymer matrix. Herein, we report our effort to prepare *S*-nitroso-*N*-acetylpenicillamine (SNAP)-doped CarboSil 20 80A polymer films and observe, in real time, the crystal formation process within the bulk of the polymer phase. The NO release mechanism of this composite material when it is in contact with physiological solutions and its correlation with respect to film surface area and polymer thickness are also carefully examined. SNAP/CarboSil catheters are also fabricated and evaluated for their long-term antimicrobial activity toward *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Proteus mirabilis* (*P. mirabilis*) in a CDC (Center for Disease Control) bioreactor for 14 days.

2. Materials and methods

2.1 Materials

N-Acetyl-D-penicillamine (NAP), sodium nitrite, L-cysteine, sodium chloride, potassium chloride, sodium phosphate dibasic, potassium phosphate monobasic, copper (II) chloride, ethylenediaminetetraacetic acid (EDTA), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, hydrochloric acid, sulfuric acid, Luria Bertani (LB) broth and LB agar were obtained from Fisher Scientific (Hampton, NH). CarboSil 20 80A was obtained from DSM Biomedical Inc. (Berkeley, CA). All aqueous solutions were prepared with 18.2 M Ω -deionized water using a Milli-Q filter from EMD Millipore (Billerica, MA). Phosphate buffered saline (PBS), pH 7.4, containing 138 mM NaCl, 2.7 mM KCl, 10 mM sodium phosphate, and 100 μ M EDTA was used for all *in vitro* experiments. *P. aeruginosa* ATCC 27853 and *P. mirabilis* ATCC 29906 was obtained from the American Type Culture Collection (ATCC) (Manassas, VA).

2.2 Study of SNAP decomposition reaction in solution

SNAP was synthesized as previously reported [6,50]. SNAP solutions (2 mM) in various solvents were prepared. The solvents chosen in this experiment were PBS with EDTA (100 μ M), PBS without EDTA, ethanol, THF and methanol-methyl ethyl ketone (30/70 v/v) solvent mixture. The decomposition experiments of SNAP in solution were conducted at 60 °C. The concentration of SNAP in solution were monitored by UV-Vis absorbance spectroscopy at 380 nm (in order to avoid any absorbance overlapping with solvents) using an Agilent 8453 UV-visible spectrophotometer from Agilent Technologies. Two hundred UV-Vis spectra were taken at 5 min intervals for each decomposition experiment. The absorbance data were fit into a first order reaction equation by KaleidaGraph 3.5 Synergy Software.

2.3 Real-time monitoring of SNAP crystallization process in CarboSil

An 8 wt% SNAP-CarboSil solution was prepared by dissolving 17.4 mg SNAP and 200 mg CarboSil in 2 mL THF. A clean glass cover slide was weighed. Three drops of solution were then dispensed on the glass slide and the total weight was measured immediately, followed by weight measurements every 10 min. Optical images of the sample were captured every 10 min by a Leica DMLP polarization microscope equipped with a N Plan 2.5 $\times$ objective under crossed polarizers in combination with a quarter-wave plate, and were then taken with a SPOT Flex Mosaic 15.2 camera using SPOT 5.2 Software from Diagnostic Instruments, Inc.

2.4 Preparation of SNAP-doped CarboSil films

Ten wt % SNAP was doped into CarboSil 20 80A polymer by solvent casting method using a Teflon plate [6]. After curing fully, small disks (0.7 cm diameter and 260 μ m thickness) were punched from the 10 wt% parent films. To actively control the surface area of the films that are in contact with buffer during NO release measurements, SNAP/CarboSil films were separated into two groups and sputter coated with a 400 Å thick of gold film by gold sputtering (Denton Vacuum Inc., Desk II) for 120 s on either the front or the back side of the film (see Figure 1), to reduce the film water-contacting surface area by 50 %. Meanwhile, another set of the cured films (all with 0.7 cm in diameter), but with different wt% levels of SNAP within (5 wt% or 10 wt%) or different thicknesses (260 μ m or 520 μ m thick) were also prepared for NO release measurements. Films with the following configurations (5 wt% and 260 μ m thick, 10 wt% and 260 μ m thick, and 10 wt% and 520 μ m thick) were coated with plain CarboSil polymer topcoats by dip-coating (45 μ m thick) to reduce SNAP leaching. The NO release from all polymeric films was measured in PBS buffer at 37 °C using a nitric oxide analyzer (NOA) (General Electric Analytical Instruments, Boulder, CO) via chemiluminescence measurements. The NO release profiles were recorded and compared between films with no gold coating, with only front side gold coating, and only back side gold coating, as well as between films with the same diameter but different SNAP concentration or thicknesses.

2.5 Evaluation of the antimicrobial activities of NO release CarboSil catheters

All SNAP catheters and control catheters were sterilized by ethylene oxide in the University of Michigan Central Sterile Processing Department [6] prior to testing. In order to determine

whether the bactericidal effect of SNAP-doped catheter is directly related to the nitric oxide that is being released, three control groups were used in the study. These included NAP-doped CarboSil catheters, plain CarboSil catheters, and a commercial silicone tubing. This combination allowed us to evaluate the whether the precursor NAP or the polymer CarboSil itself has any effect on bacterial adhesion. SNAP-doped, NAP-doped or plain CarboSil catheters were fabricated by a dip-coating method using a stainless steel mandrel [6] and the final catheters had dimensions of 2 mm i.d. and 4 mm o.d. (see Figure 2). The NO release from SNAP-doped catheters with CarboSil topcoats was measured using the NOA, as described in Section 2.4. All CarboSil catheter segments and commercial silicone tubing segments were mounted on coupon holders within a CDC bioreactor (see Figure 3) and the bioreactor was kept at 37 °C and supplemented with 10% Luria Bertani medium via a peristaltic pump with a continuous flow rate of 100 mL/h. First, the media in the bioreactor in contact with the catheter/tubing segments was inoculated with overnight bacteria culture ($\sim 1 \times 10^8$ colony forming unit/mL) for 1 h without stirring. Then the bacteria, *P. aeruginosa* ATCC 27853 or *P. mirabilis* ATCC 29906, respectively (for two different studies), were cultured and grown onto surfaces of catheters for 14 d, with stirring. Finally, all the catheter/tubing segments were removed from the bioreactor and cut into halves, and one half of each was homogenized in sterile PBS buffer, 10-folds serially diluted and then subjected to bacteria plate counting. The other half of all the segments were used for fluorescence imaging of live bacteria on the surface of the tubing. Briefly, control and SNAP impregnated catheter segments were stained with LIVE/DEAD BacLight Bacterial Viability kit (L7012, Invitrogen, Carlsbad, CA) according to the instructions, where green color indicates live bacteria and red color indicates dead bacteria. All fluorescence images were acquired with an inverted fluorescence microscope (Olympus IX71, 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.

2.6 Statistical analysis

All experiments were conducted in triplicate. Data reported herein are all expressed as mean $\pm$ SEM (standard error of the mean). Comparison of means using student's t test was utilized to analyze the statistical differences between SNAP-doped catheters and control catheters. Values of $p < 0.05$ were considered statistically significant for all tests.

3. Theory

In solution, the decomposition of solubilized SNAP is a pseudo-first-order reaction proportional to the SNAP concentration, resulting in a relatively short half-life and rapid decomposition at room temperature. The stability of SNAP can be improved by doping SNAP into low water uptake polymers, such as CarboSil 20 80A, where SNAP will form crystals and embed within the polymer matrix. The intermolecular interactions between SNAP molecules in the crystal lattice stabilize this NO donor, and leads to long-term NO release via slow crystal dissolution process into the solid polymer. If this mechanism is correct, the NO release profile (such as NO release rates and longevity of the release) from SNAP-doped CarboSil polymer should be dictated by three factors: 1) the SNAP

concentration in the polymer matrix; 2) the water-contacting polymer surface area exposed to the test solution; and 3) the film thickness of the specific SNAP-doped CarboSil polymer formulation. In theory, NO release rates are controlled by SNAP concentration in the polymer and the water-contacting polymer surface area. The longevity of NO release, on the other hand, should be directly proportional to the SNAP loading/concentration and the film thickness of a specific SNAP-doped CarboSil polymeric material.

4. Results and discussion

4.1 NO release mechanism

It has been previously reported that SNAP is stable when doped into low-water uptake polymers such as CarboSil 20 80A [6], which is a polyurethane-based triblock copolymer. The elevated stability of SNAP in this polymer during storage has been previously ascribed to the strong intermolecular interactions between SNAP molecules that forms crystals in the bulk of the polymer phase when SNAP concentration exceeds its solubility limit in the polymer [6]. However, the process of how the polymer-crystal composites are formed during the casting and drying of the polymer phase as well as understanding what key parameters dictate the observed NO release rates from the polymer crystal composites still remains unclear.

We hypothesize that the NO release profile of the certain SNAP-doped polymer films are related to several factors: the film surface area in contact with aqueous phase, the film thickness, and the SNAP concentration within the polymeric film (see theory section above). In this work, to better understand the properties of this new composite material we first compared the NO release rates between 10 wt% SNAP-doped CarboSil films (all with 0.7 cm diameter) with thicknesses of 260 and 520 μm in PBS buffer. Of note, all the NO release data from CarboSil films were recorded until when the release rate was no longer above $0.5 \times 10^{-10} \text{ mol min}^{-1} \text{ cm}^{-2}$, which is the lower end of physiological NO release levels by the endothelial cells [19,22–24]. As shown in Figure 4A, the results demonstrate that the NO release levels are not significantly different between the 10 wt% films with 260 or 520 μm thickness, but the NO release lifetime is much longer for the films with twice the thickness. The overall thickness of the films tested is very small compared to their diameter, and increasing the thickness of films to 520 μm only increases the total surface area of the films by 6 % when compared to the original films, a relatively small increase. However, the NO release levels of the 5 wt% SNAP-doped films are nearly half of the levels observed for films doped with 10 wt% SNAP (see Figure 4B). Further, to understand whether the NO release rate is determined by the water-contacting surface area of the film or simply by the total surface area of the film, the NO release rates from three identical 10 wt% SNAP-doped CarboSil films were compared with no gold sputtering, one with 400 Å gold film sputtered on its front side, and one with 400 Å gold film sputtered on its back side. The gold coating on either side of the film will prevent direct water contact on that side of the films when they are immersed in solution. As shown in Figure 5, both of the gold sputtered CarboSil films exhibited half the NO release rate when compared to the non-coated films. This demonstrates that the NO release rate from SNAP-doped CarboSil films is controlled by the total water-contacting surface area of the polymer film. It also suggests that the water rich

layer in the outermost surfaces of the polymers is the likely site where most of the NO is liberated from soluble SNAP within the polymer.

From the results described above, the factors that control the NO release levels and longevity of release are summarized as follows. Firstly, the results indicate that NO release levels do not increase as the thickness of the film increases, because the water-contacting surface area and the SNAP concentration in the film are the rate determining factors dictating the observed NO release kinetics. Secondly, if the water-contacting surface area remains the same, the NO release rate decreases as the SNAP concentration decreases. Thirdly, the longevity of NO release from a given film is determined by the SNAP concentration within the film, as well as the thickness of SNAP-doped polymeric film.

4.2 SNAP decomposition in solution

We recently reported that when SNAP is doped into CarboSil 20 80A, 3.4–4.0 wt% of SNAP is dissolved in the polymer phase while any additional SNAP above this solubility threshold exists as crystals embedded within the polymer matrix [6]. It appears that the SNAP that is solubilized in the polymer matrix, analogous to SNAP dissolved in a solvent, is more reactive and more likely to decompose and releases NO, than crystalline SNAP that is stabilized by strong hydrogen bonding [6] as well as the low water uptake nature in the bulk region of the polymeric phase. SNAP decomposition in liquid solution is investigated herein to determine SNAP stability in various solvents. Such, solution phase decomposition experiments of SNAP in various solvents were conducted at 60 °C, in order to accelerate the decomposition and observe obvious signal changes over a short time period. The SNAP concentrations in various solvent solutions were monitored by UV-Vis absorbance spectroscopy. As illustrated in Figure 6, all decomposition reactions exhibited pseudo-first-order behavior relative to SNAP levels at low concentrations. All experimental data fit into first-order reaction equation $A_t = A_0 e^{-kt} + c$, where A_t is the concentration of reactant at time t , A_0 is the initial concentration, k is the first-order reaction rate constant, and c is the instrumental system error.

In agreement with previously reported results, in PBS without EDTA, the decomposition of SNAP is accelerated, due to the presence of trace levels of metal catalysts, such as Cu^{2+} ions [51,52]. In PBS-EDTA solution, EDTA chelates any copper ions, and as a result, the rate constant of SNAP decomposition in PBS-EDTA is comparable to those in the other selected organic solvents, with half-lives all within a 3 h period (see Table 1). When SNAP is in the CarboSil polymer “solid solution”, the SNAP decomposition is primarily through thermal and photochemical decomposition, instead of metal ion catalysis [53].

4.3 SNAP crystallization process in CarboSil polymer

It was proposed in our previous work [6] that during solvent evaporation, as the amount of the solvent decreases, SNAP eventually reaches to its saturation in CarboSil and then crystallizes. As a follow-up, the crystallization of SNAP within CarboSil polymer was observed *in situ* by optical microscopy while the evaporation of the THF solvent was monitored by weight measurements. Significant loss of weight was observed in the first half hour due to the THF evaporation (see Figure 7). Formation of SNAP crystals was first

visually detected after 40 min, when most of THF has evaporated, followed by rapid crystal growth. Such behavior is analogous to crystallization in a supersaturated liquid solution, except that the “solvent” for SNAP is the solid CarboSil polymer, once most of the THF has evaporated. As THF evaporates, SNAP reaches its solubility limit in CarboSil and then forms a supersaturated solid “solution”. Once crystalline seeds form, crystal growth is relatively fast, as shown in Figure 8, which can stem from the mobility of SNAP molecule in the presence of any remaining THF solvent in the polymer [54–56]. This crystallization process observed experimentally in real time helps to verify our previous theory [6].

4.4 Study of antimicrobial efficiencies

To evaluate the potential of NO releasing catheters being used as antimicrobial devices, including both intravascular and urinary catheters, we examined the antimicrobial properties of CarboSil catheters (2 mm i.d. and 4 mm o.d.) containing 20 wt% SNAP against two common infection-causing bacteria: *P. aeruginosa* and *P. mirabilis*. *P. aeruginosa* has been reported to be one of the leading bacterium that causes catheter-related blood stream infections, and represents 16% of all the CRBSI cases [57,58]. Infections caused by *P. aeruginosa* also often occur in patients already battling with more severe illness, e.g., extensive trauma, burns, or malignancy [59]. It is very important to examine the antimicrobial effect of NO against this particular strain of bacteria because *P. aeruginosa* possesses NO reductase enzyme that may enable the bacteria cells to metabolize and deactivate NO, by converting NO to nitrous oxide (N₂O) and ultimately to nitrogen [60–62]. On the other hand, the primary bacterium associated with persistent and complicated catheter-associated urinary tract infections (CAUTIs) is *P. mirabilis* [3,63,64], which is also known for its ability to elevate urine pH, induce calcium/magnesium phosphate precipitation and produce mature crystalline biofilms [3,63,65]. The CDC biofilm reactor, a laboratory biofilm reactor system, was used in this study since it provides a good model system that offers an environment that mimics the bacterial growth on the polymer surface under moderate fluid shear stress [66]. The 20 wt% SNAP-doped CarboSil catheters can release NO at levels $> 0.5 \times 10^{-10} \text{ mol cm}^{-2} \text{ min}^{-1}$, the lower end of endothelial NO flux levels, for 3 weeks under physiological conditions. Such long-term (21 d) continuous and stable NO release can greatly inhibit the development of mature biofilm on the surface of NO release catheters.

As shown in Figure 10, for *P. aeruginosa*, there was a 2 log-unit reduction in the number of viable bacteria adhered on the surface of the SNAP catheters after 14 d compared with other groups and there was no significant difference in the adhesion of bacteria on the three control groups, including NAP-doped catheters, indicating the bactericidal effect of the SNAP CarboSil catheter is solely related to the NO being released during the experiments (Figure 9). These findings were substantiated by the fluorescence imaging data, in which all three control catheter surfaces were covered by high biomass mature biofilm formed by live bacteria (green) while the SNAP catheter surfaces had noticeably less bacterial coverage and most of bacteria on SNAP catheter surfaces were either single or dead (red) bacteria cells. It should be noted that during the process of forming mature biofilm, bacteria cells that are located within the biofilm experience nutrient limitation, which can result in a stationary phase-like dormancy [67,68] or irregular shapes [69].

Results for the 14 d *P. mirabilis* biofilm culture experiments exhibited a 3 log-unit difference in adhered bacterial cell viability between SNAP catheters and the three different groups of control catheters (see Figure 11). The fluorescence imaging data also show that bacterial coverage on the NO releasing catheter is noticeably less than the three controls. Clearly, these results demonstrate that overcoming bacteria colonization and biofilm formation on the surfaces of NO releasing catheter through continuous NO release represents an innovative and highly desirable approach to prevent bacterial biofilm formation, reduce risks associated with catheter-related bloodstream infections and urinary catheter infections. Hence, the use of SNAP-doped CarboSil catheters should ultimately lead to improved device performance and patient outcomes.

5. Conclusion

In summary, in this work we have provided the first real-time observation of the formation of SNAP crystallization within a polymer phase that creates a unique composite material for potential biomedical applications. SNAP crystals form and become embedded within CarboSil polymer when the SNAP concentration increases and exceeds its solubility during organic solvent (e.g., THF) evaporation. It is also clear that in addition to the SNAP concentration in the polymer matrix, the NO release profile (level of NO release and longevity of NO release) from such polymers is directly related to the water-contacting polymer surface area and the thickness of specific SNAP-doped CarboSil polymeric material. The decomposition of solubilized SNAP is a pseudo-first-order reaction proportional to the SNAP concentration. The SNAP-doped CarboSil catheters were also shown to exhibit significant antimicrobial properties against the bacteria *P. aeruginosa* and *P. mirabilis*, which are known to be associated with high rates of hospital infections for intravascular and urinary catheters, respectively. Such an NO release composite material provides a cost-effective strategy to potentially improve the antimicrobial properties of indwelling intravascular and urinary catheters, as well as other implantable medical devices.

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Figure 1. The images of 10 wt% SNAP-doped CarboSil film with no gold sputtering (left), front side gold sputtering (middle) and back side gold sputtering (right).

Figure 2.
The images of SNAP-doped (top), NAP-doped (middle) and plain control CarboSil catheters, all with blank CarboSil topcoats.

Figure 3.
Schematic of the CDC bioreactor in which the different catheters were tested for bacterial biofilms formation. [70]

Figure 4.

The NO release comparison between 10 wt% SNAP-doped CarboSil films with thickness of 260 or 520 μm (A); and 260 μm thick SNAP-doped films with 5 wt% or 10 wt% SNAP concentration (B).

Figure 5. The NO release comparison between 10 wt% SNAP-doped CarboSil films with no gold film coating (square), front side 400 Å gold coating (triangle) and back side 400 Å gold coating (circle).

Figure 6. SNAP decomposition (formation of NO) in various solvents at 60 °C represented by absorbance of SNAP at 380 nm versus time (s). The decomposition of SNAP in solvent is a pseudo-first-order reaction. All decomposition reactions were fit into the equation $A(t) = A(0) \exp(-kt) + c$, where $A(t)$ represents the concentrations of SNAP at time t , $A(0)$ represents the initial concentration, k is the reaction rate coefficient, and c is the correct constant for instrumental system error.

Figure 7. The weight of 8 wt% SNAP-CarboSil (dissolved in THF) droplet (mg) decreases over time, as the polymer-crystal composite forms during the solvent evaporation.

Figure 8. Optical images of SNAP crystallization in CarboSil by evaporation. The first image was the one taken after 25 min of solvent evaporation, followed by images captured at 10 min interval.

Figure 9. NO release from 20 wt% SNAP-doped CarboSil catheters with CarboSil topcoats in PBS at 37 °C for 21 d. Data are mean $\pm$ SEM (n=3).

Figure 10.

Upper image: Bar graph of plate count data for adhesion of viable *P. aeruginosa* bacteria to the surfaces of silicone/CarboSil catheters run in CDC bioreactor for 14 d. Lower images: fluorescence microscopic images of surface live (green) and dead (red) bacteria on different catheters, silicone catheters (A), CarboSil catheters (B), NAP-doped CarboSil catheters (C), and SNAP-doped CarboSil catheters (D) (n=4 for each condition) and their representative fluorescence images; scale bar 20 μ m.

Figure 11.

Upper image: Bar graph of plate count data for adhesion of viable *P. mirabilis* bacteria to the surfaces of silicone/CarboSil catheters run in CDC bioreactor for 14 d. Lower images: fluorescence microscopic images of surface live (green) and dead (red) bacteria on different catheters, silicone catheters (A), CarboSil catheters (B), NAP-doped CarboSil catheters (C), and SNAP-doped CarboSil catheters (D) (n=4 for each condition) and their representative fluorescence images; scale bar 20 μ m.

Table 1

The reaction rate constants of SNAP decomposition in various solvents at 60 °C.

Solvent	Reaction rate constant (10^{-7} s^{-1})	Half-life (min)
PBS (without EDTA)	1389 ± 3	83 ± 0
PBS with EDTA	721 ± 0	160 ± 0
EtOH	852 ± 7	136 ± 1
THF	845 ± 11	137 ± 2
MeOH-MEK	820 ± 2	141 ± 3