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Antimicrobial efficacy of a nitric oxide-releasing ampicillin conjugate catheter lock solution on clinically-isolated antibiotic-resistant bacteria†

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Antibiotic lock therapy (ALT) is standard clinical practice for treating bacteremia linked with catheter-related bloodstream infections (CRBSIs). However, this strategy frequently fails against multi-drug-resistant bacteria in clinical settings. In this study, a novel approach to utilize a nitric oxide (NO) donor *S*-nitroso-*N*-acetyl-penicillamine (SNAP)-conjugated to ampicillin antibiotic (namely SNAPicillin) as a catheter lock solution is presented. The conjugate of two antimicrobial agents is anticipated to overcome the challenges of bacterial infection caused by antibiotic-resistant bacteria in ALT applications. Nitric oxide release from the SNAPicillin lock solution at varying concentrations was measured at 0 and 24 h time points in a catheter model system, which revealed tunable NO release at physiological levels. The clinical strains of *E. coli* (CDC AR-0089) and *S. marcescens* (CDC AR-0099) were screened using a zone of inhibition assay against standard antibiotics which confirmed the antibiotic resistance in bacteria. The minimum inhibitory concentration (MIC) testing of SNAPicillin unveiled the lowest MIC value for SNAPicillin against both *E. coli* and *S. marcescens* (1 and 2 mM of SNAPicillin, respectively) with an 8.24- and 4.28-log reduction in bacterial load compared to controls, respectively. In addition, while the ampicillin-treated biofilm demonstrated resistance toward the antibiotic, SNAPicillin led to >99% reduction in exterminating biofilm buildup on polymeric catheter surfaces. Lastly, the SNAPicillin lock solution was determined to be biocompatible *via* hemolysis and cell compatibility studies. Together, these results emphasize the promising potential of SNAPicillin lock solution with the dual-action of NO and ampicillin in overcoming bacterial challenges on medical devices like central venous catheters and other medical device interfaces.

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1. Introduction

Medical devices such as central venous catheters (CVCs) have become an indispensable part of the modern healthcare system. However, their usage often leads to complications such as catheter-related bloodstream infections (CRBSIs), including bacteremia and sepsis. These infections pose a significant burden on hospitalized patients globally, with the United States alone reporting approximately 250 000 cases annually with an annual cost of $2.3 billion.[1–3] For example, bacteria can attach to an indwelling catheter substrate, propagate, and travel from the site of catheter insertion to other locations in the body, severely impacting the function and lifetime of the medical device and patient health.[4–6] The formation of biofilms on medical devices exacerbates bacterial infections by creating a protective matrix called extracellular polymeric substance (EPS), which traps bacteria and shields them from antibiotics and host immune responses. This protective matrix, combined with factors such as limited antibiotic penetration, the release of sublethal antibiotic doses, antibiotic inactivation, or alterations in the physiochemical properties of the EPS matrix contribute to the development of antibiotic resistance in pathogens.[7,8] Eradicating bacterial biofilms often necessitates a dosage of traditional antibiotics that is 10 to 1000 times higher than what is needed to eliminate freely suspended planktonic bacteria.[9] Studies have shown that antibiotics exhibit greater efficacy in the initial stages of biofilm formation. However, bacterial biofilms can lead to chronic infections due to their ability to withstand harsh conditions and possess additional protective mechanisms. This resilience makes them resistant to antibiotics, thereby contributing to the emergence of multi-drug-resistant superbugs.

Bacteria on indwelling catheters can originate from diverse sources, including the infected catheter hub region, the patient's or caretaker's skin flora, inadequate sterility of skin or device

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prior to insertion, or hematogenous seeding from existing bacteria present in the patient's body. Current clinical guidelines recommend using antibiotic lock solutions along with anticoagulants to prevent and treat catheter infections.[10] These solutions are instilled within the catheter lumen when the catheter is not actively in use for drug or fluid administration to sterilize the catheter. In severe cases, treatment may involve surgically removing the catheter and administering high doses of systemic antibiotics, such as ampicillin, vancomycin, gentamicin, and cefazolin, with a risk of toxicity and emergence in antibiotic resistance.[9,11] However, a significant limitation of current antibiotic lock solution therapies is that they primarily target catheter infections within the inner lumen, while most catheter-associated infections occur on the external surface of the catheter.

To combat the CRBSIs caused by antibiotic-resistant bacteria, this study introduces a novel nitric oxide (NO)-releasing lock solution *via* covalent conjugation of NO donor to ampicillin antibiotic. Nitric oxide is a gasotransmitter molecule and a critical component of several important regulatory pathways in the body, such as vasodilation, prevention of platelet activation, pathogen eradication, anti-inflammation, and wound healing.[12] Previous research incorporating NO donors into polymers has demonstrated controlled release of NO, providing antibacterial activity on both the intraluminal and extraluminal surfaces of catheters, and improving biocompatibility. Importantly, this approach has shown effectiveness against bacteria without promoting bacterial resistance.[13] The antimicrobial and hemocompatible properties of NO make NO-releasing lock solutions an attractive choice for enhancing the function of indwelling catheters. Although NO serves multiple crucial roles, low concentrations of NO may not entirely eliminate bacteria. To address this challenge, previous literature has explored alternative approaches to antibiotic or ethanol-based lock solutions by incorporating NO-releasing donors that possess both antibacterial and hemocompatible properties.[14,15] These solutions have shown promising results in preventing bacterial viability on catheter surfaces, nevertheless, their impact on antibiotic resistant bacteria is yet to be explored.

This study investigates the application of a covalently conjugated NO-releasing antibiotic called SNAPicillin, as a catheter lock solution to increase the efficacy of conventional antibiotic lock therapies to prevent and treat CRBSIs. SNAPicillin consists of the antibiotic ampicillin covalently attached to a synthetic NO donor, *S*-nitroso-*N*-acetylpenicillamine (SNAP) (Fig. 1A). The SNAPicillin molecule was synthesized, characterized using NMR and FTIR, and evaluated for dry-state storage stability. The NO release from the SNAPicillin lock solution was evaluated for up to 24 h mimicking clinical applications where the lock solution would be replaced every 24 h. For *in vitro* biological evaluation, the SNAPicillin lock solution was studied for its minimum inhibitory concentration (MIC), antimicrobial efficacy towards antibiotic-resistant bacterial strains, cell compatibility, and hemolysis using International Organization for Standardization (ISO) standards. It is anticipated that the covalently bound combination of two antimicrobial agents (ampicillin and NO) will amplify the antimicrobial activity providing a new platform to eradicate CRBSIs (Fig. 1B).

2. Materials and methods

2.1 Materials

Acetic anhydride, chloroform, hydrochloric acid, magnesium sulfate anhydrous, methanol, *N*-acetyl-D-penicillamine (NAP), pyridine, thiazolyl blue tetrazolium bromide (MTT), and *t*-butyl nitrite (TBN), Mueller Hinton Broth and Agar (MHB and MHA), and sodium chloride, potassium chloride, sodium phosphate dibasic, potassium phosphate monobasic, sodium citrate dihydrate were purchased from Sigma Aldrich (St Louis, MO). *S*-Nitroso-*N*-acetylpenicillamine was purchased from PharmaBlock (Hatfield, PA). Calcium and magnesium-free PBS (1×) was purchased from Corning Incorporated (Manassas, VA). Drabkin's reagent was purchased from Ricca Chemical Company. Helixmark® silicone tubing (60-011-06) was purchased from VWR (Radnor, PA). All aqueous solutions were prepared using deionized water. Phosphate buffer saline (PBS) 0.01 M with 100 μM EDTA was used for all material characterization and NO analyzer studies. The clinical isolates of antibiotic-resistant bacterial strains, *E. coli* (CDC AR-0089) and *S. marcescens* (CDC AR-0099), were obtained from the Centers for Disease Control and Prevention (CDC, Atlanta, GA). Sodium ampicillin was purchased from Gold Biotechnology (St Louis, MO). Antibiotic discs of ampicillin, vancomycin, gentamicin, rifampicin, cefotaxime for screening and testing of antibiotic-resistant bacteria, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin–streptomycin (P/S, 5000 U mL^{-1}) were purchased from VWR (Radnor, PA). BJ (ATCC CRL-2522) human fibroblast cells for cell compatibility experiments were obtained from American Type Culture Collection (ATCC, Manassas, VA). Fresh porcine whole blood was acquired from the University of Georgia Swine Unit (Athens, GA).

2.2 Synthesis of SNAPicillin molecule

Conjugation of the NO donor compound *S*-nitroso-*N*-acetyl-D-penicillamine (SNAP) to ampicillin was achieved following a previous report with minor deviation.[16] First, a thiolactone derivative (NAPTH) of the acetylated penicillamine (NAP) precursor to SNAP was prepared following previous reports with slight modification.[17,18] In brief, NAP was dissolved in chilled anhydrous pyridine (250 mg mL^{-1}) with chilled acetic anhydride (~3.5 molar equivalent to NAP) added dropwise. The reaction mixture was stirred for 15 h, then condensed *via* rotary evaporation. The crude was dissolved in chloroform, washed 3× times with HCl (1 M), and dried over magnesium sulfate. The organic phase was further condensed and triturated in chilled hexanes overnight. NAPTH was obtained as a crystalline product *via* vacuum filtration and stored at −20 °C between uses.

SNAPicillin was prepared *via* the direct reaction of ampicillin with an equimolar amount of NAPTH in deionized water (~30 mg mL^{-1} with respect to the sodium salt of Amp) for 48 h at room temperature. The reaction vessel was then chilled, and *t*-butyl nitrite (TBN) was added dropwise in 6× times molar excess. **CAUTION:** The addition of TBN to deionized water can

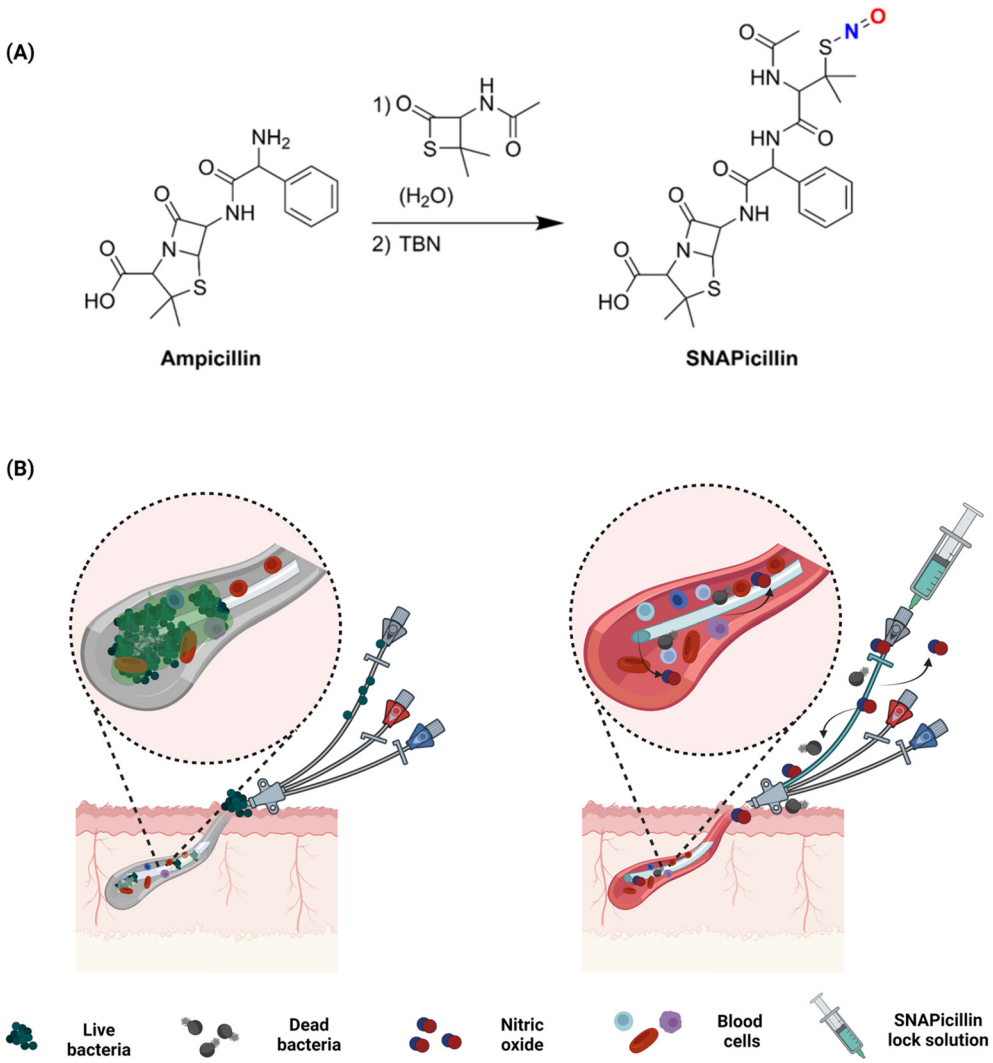

Fig. 1 (A) Synthesis route for generating NO-releasing ampicillin by conjugating thiolactone to ampicillin followed by organic nitrosation using *tert*-butyl nitrite (TBN) resulting in the new SNAPicillin molecule. (B) Biofilm formation and catheter-related bloodstream infections (CRBSIs) can be prevented and exterminated using NO-releasing SNAPicillin lock solution. The combination of antibiotic and NO can help in preventing and exterminating CRBSIs caused by opportunistic pathogens including antibiotic resistant strains of bacteria.

facilitate the evolution of nitrogen oxide (NO_x) gases from the reaction mixture. Only perform this step in a fully functioning fume hood operating at optimal capacity. Following the addition of TBN, SNAPicillin was obtained as a green precipitate, which was collected *via* vacuum filtration with 3× washing steps with deionized water and ethanol. The resulting light green crystalline product was dried under a vacuum overnight and stored at −20 °C with protection from light.

2.3 Characterization of SNAPicillin molecule

Characterization of the SNAPicillin molecule was performed using UV-vis spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and nuclear magnetic resonance spectroscopy (NMR). The FTIR spectra were recorded using a spectrum two spectrometer (PerkinElmer), collecting a total of 64 scans per sample with a resolution of 4 cm^{-1} using the KBr loading method. NMR spectra (^1H and ^{13}C) were collected using an Ascend 400 mHz magnet with an Advance III HD Nanobay console (Bruker). Spectra were collected in CD_3OD, with a total of 64 and 1024 scans for ^1H and ^{13}C runs, respectively. SNAPicillin λ_{max} (H_2O)/nm 340 (ε/mM^{-1} cm^{-1} 0.8546) and 207 (20.68); λ_{max} (KBR)/cm^{-1} 3313 (NH), 3063 (CH), 2974 (CH), 2934 (CH), 1780 (C═O), 1739 (C═C), 1646 (C═O), 1497 (N═O), 1373 (C–H), 1304 (C–H), and 1216 (O–H) (Fig. S1†); δ_H (400 MHz, CD_3OD) 9.00 (2 H, d, NH), 8.90 (1 H, d, NH), 7.33 (5H, s, CH), 5.63 (1 H, s, CH), 5.49 (1 H, s, CH), 5.43 (1H, s, CH), 4.70 (1 H, s, CH), 4.30 (1 H, s, CH), 2.14 (3 H, s, CH), 2.00 (3 H, s, CH), 1.91 (3 H, s, CH), 1.53 (3 H, s, CH), and 1.46 (3 H, s, CH) (Fig. S2†); δ_C (400 MHz, CD_3OD) 173 (NCO), 172 (NCO), 171 (NCO), 169 (COO), 136 (CH), 128 (CH), 127 (CH), 71 (CH), 67 (CH), 64 (C), 60 (CH), 59 (CH), 58 (CH), 45 (C), 30 (CH), 26 (CH), 24 (CH), and 21 (CH) (Fig. S3†). These results corrobo-

rate our prior characterization.[16] SNAPicillin purity was confirmed *via* NMR and a product of >90% purity was used for further studies.

2.4 Stability of SNAPicillin and degradation under physiological conditions

For storage stability studies, SNAPicillin powder was stored at −20 °C, 4 °C, room temperature (RT) – 23 °C, and 37 °C shielded from light. SNAPicillin powder was distributed among amber vials and placed in various temperature conditions. At each time point (7, 14, 21, and 28 d), SNAPicillin powder was dissolved in 10 mM PBS containing 100 μM EDTA (pH 7.4), and an Agilent Cary 60 UV-vis spectrophotometer was used to measure the absorbance of the solution at 340 nm (with the molar absorptivity of 0.8546 mM cm^{-1}). All samples were normalized by weight, and the stability of the SNAPicillin powder is presented as the percent of NO remaining over time compared to freshly prepared samples on day 0 since the detection was based upon the *S*-nitrosothiol bond peak at 340 nm. The final data is reported as the mean ± SEM (n = 3).

In order to measure the amount of SNAPicillin remaining in the lock solution over time, the 10 mM of SNAPicillin in 10 mM PBS containing 100 μM EDTA (pH 7.4) was prepared and tested for stability at 37 °C up to 72 h using UV-vis spectrophotometer. At each time point (0, 4, 6, 24, 30, 48, and 72 h), the absorbance of the SNAPicillin lock solution was recorded at 340 nm using an Agilent Cary 60 UV-vis. The stability of the SNAPicillin solution is presented as the percent of NO remaining over time compared to freshly prepared samples at hour 0. The final data is reported as the mean ± SEM (n = 3).

2.5 Preparation of SNAP and SNAPicillin lock solutions for nitric oxide release kinetics

Three concentrations (1-, 5-, and 10 mM) of lock solutions were prepared by dissolving SNAP and SNAPicillin in 1 mL of 10 mM PBS (pH 7.4) with 100 μM EDTA. Solutions were protected from light and vortexed until the powder dissolved, and a homogenous solution was obtained. Medical-grade silicone rubber (SR) tubing (Helixmark® 60-011-06) with 1.47 mm inner diameter, 1.96 mm outer diameter, and 0.23 mm wall thickness was used as a model catheter to evaluate the NO release kinetics. The two ends of the SR tubing were sealed with RTV silicone rubber and dried at room temperature for at least 24 h prior to injecting solutions. Approximately 20 μL of lock solution was filled into the SR tubing. Once the catheter tubing was filled with lock solutions, the samples were tested for NO release at 0 and 24 h.

2.5.1 Real-time nitric oxide release kinetics. The NO release from SNAP and SNAPicillin lock solution was quantified using the gold standard Zysense chemiluminescence nitric oxide analyzer (NOA) 280i (Frederick, CO) under a nitrogen atmosphere at physiological conditions. An amber glass sample cell was filled with 5 mL of 10 mM PBS, pH 7.4 with 100 μM EDTA and the baseline reading was recorded for 3–5 min at 37 °C. Then, SR catheter tubing filled with either SNAP or SNAPicillin lock solution was submerged into the sample cell, and NO release was measured until the release profile plateaued. The sample cell was continuously purged with nitrogen gas at a rate of 200 mL min^{-1}, which helped carry the NO gas to the reaction cell in NOA. The supply and cell pressure of the NOA were approximately 6 psi and 9.6 Torr, respectively. The amount of NO released from samples was normalized by the surface area of the SR tubing and is reported as NO flux with the units moles per min per cm^2. The final data are reported as the mean ± SD (n = 3).

2.6 Antibacterial efficacy of SNAPicillin lock solution

2.6.1 Screening of antibiotic-resistant bacterial strains. The screening of bacteria to confirm antibiotic resistance was done using a Kirby–Bauer disk diffusion susceptibility test against *E. coli* and *S. marcescens*. For this study, the individual isolated colonies of each strain of bacteria were inoculated in 10 mL of Mueller Hilton Broth (MHB) and grown overnight at 37 °C at 150 rpm. The optical density (OD) of bacterial culture was recorded using a UV-vis spectrophotometer (Cary 60, Agilent Technologies) at 600 nm wavelength. The final OD_{600} of the bacterial culture was adjusted to 0.1, and 50 μL of diluted culture was spread on the Mueller Hilton Agar (MHA) plate. Then, antibiotic discs (ampicillin, vancomycin, gentamicin, rifampicin, cefotaxime) were carefully placed on the agar plate using a sterile tweezer and gently pressed against the agar. Plates were allowed to dry at room temperature for 10 min before placing them in the 37 °C incubator for overnight incubation. The results from the zone of inhibition study were recorded by measuring the diameter (mm) of the zones defined by the absence of bacterial growth around the respective discs. Results from the study are reported as mean diameter ± standard deviation (SD, n = 3).

2.6.2 Determination of minimum inhibitory concentration (MIC). A range of drug concentrations of SNAP, ampicillin, and SNAPicillin was tested in nutrient-rich media to determine the effective values for killing and preventing the growth of bacteria. First, isolated colonies of *E. coli* or *S. marcescens* were grown in MHB media at 37 °C for 14–16 h at 150 rpm. The culture was then centrifuged at 3500 rpm for 7 min, washed in sterile PBS, resuspended in fresh MHB, and diluted to the desired concentration with MHB. The studies were completed in a 96-well plate, combining 100 μL of bacteria solution with 100 μL of the drug solution. Therefore, the solutions were prepared at twice the desired concentrations. The starting bacterial concentration was roughly 1×10^8 CFU per mL, and the SNAPicillin (or control) concentrations ranged from 7.63 nM to 8 mM. The prepared well plates were then incubated at 37 °C for 24 h at 150 rpm in the dark. After, the absorbance (abs) of each well was measured using a BioTek Cytation5 plate reader (Winooski, VT, USA) at 600 nm. The absorbance of each well was measured, and the relative growth of the treated wells was calculated compared to untreated bacteria. The relative growth of the treated bacteria was calculated relative to the

untreated bacteria (eqn (1)). Finally, MIC_{90} values were calculated based on the drug concentration needed to reduce relative growth to below 10%. The final data is reported as the mean ± SD (n = 3).

$$\text{Relative growth} = \frac{\text{abs(treated)} - \text{abs(blank)}}{\text{abs(untreated)} - \text{abs(blank)}} \quad (1)$$

Additionally, to help quantify the reduction of viable bacteria, the concentration that effectively prevented growth below a relative density of 0.1 was plated. At the end of the 24 h period and after absorbance readings, 10 μL of the bacterial suspension was taken from each well, serially diluted in PBS, and plated using a spiral plater (Eddy Jet 2W, IUL Instruments) using the Log mode 50 μL setting with 2 air purge cycles on MHA plates. The plates were incubated overnight at 37 °C, and the viable colony-forming units of bacteria were counted using a colony counter (Sphere Flash, IUL Instruments). Results for each concentration are reported as a percent reduction compared to untreated bacteria (eqn (2)). The final data is reported as the mean ± SD (n = 3).

$$\text{Reduction efficiency}(\%) = \left(\frac{\text{CFU per mL(treated)} - \text{CFU per mL(untreated)}}{\text{CFU per mL(untreated)}}\right) \times 100 \quad (2)$$

2.6.3 48 h biofilm eradication study. A robust biofilm experiment was used to determine the efficacy of the SNAPicillin catheter lock solution based on previously published reports.[19] For this study, antibiotic-resistant strains of *E. coli* and *S. marcescens* bacteria were grown following the same protocol as section 2.6.1. Once the bacteria reached the log phase, the cultures were diluted to 0.1 OD_{600} in MHB media, and 1 mL of the diluted culture was added to PDMS polymer samples (1 cm^2 of surface area to 1 mL of volume ratio) in a 48-well plate (n = 4). Samples were incubated at 37 °C at static conditions for 48 h to allow biofilm formation on polymer surfaces. The nutrient-rich MHB media was replenished every 24 h during the experiment. After 48 h of incubation, media from the samples was discarded, and samples were gently rinsed with sterile PBS, followed by the addition of 1 mL of 10 mM of either ampicillin or SNAPicillin solution. For control samples, fresh PBS was added. All samples were incubated at 37 °C in a bacterial incubator for 24 h in static conditions protected from ambient light. In order to determine the action of the solutions, samples were taken out of the wells, rinsed with fresh PBS to wash off any loosely attached cells, and transferred into a 15 mL Falcon tube with 1 mL of PBS. To extract the bacteria attached to the polymer samples, samples were homogenized and vortexed (1 min), and diluted bacteria suspension was plated on the MHA plates using the same methodology for bacteria plating as section 2.6.2. The viable colony-forming units (CFUs) of bacteria on the samples were normalized to the surface area of the samples, and the percent reduction in the viability of bacterial cells was determined using eqn (3) with respect to the control samples that received no treatment.

$$\text{Bacterial reduction}(\%) = \frac{\text{(control)} - \text{(test)}}{\text{(control)}} \times 100\% \quad (3)$$

2.7 Biocompatibility assessment of SNAPicillin lock solution

2.7.1 Hemolysis assay. The hemolytic response of the SNAPicillin and control lock solution was assessed according to the ASTM International F756 protocol using modifications to be applicable for the biological evaluation of lock solutions with blood interaction.[20] Briefly, porcine whole blood was diluted with calcium and magnesium-free PBS (CMF-PBS) to a total hemoglobin concentration of 10 ± 1.0 mg mL^{-1}. Next, 1 mL of porcine whole blood was diluted with 7 mL of sterile deionized water, as the positive control, or with 7 mL CMF-PBS, as the blank control, and incubated. Sample lock solutions with starting concentration of 10 mM with the drug dissolved in CMF-PBS were incubated in dilute whole blood at varying blood/lock solution ratios (*i.e.* 0.06, 0.09, 0.2, and 0.5% lock solution leakage),[15] tested and calculated to simulate leakage events using eqn (4). All samples were incubated at 37 °C for 3 h with manual rocking every 30 min. After incubation, the samples were centrifuged at 800*g* for 15 min; each supernatant was then combined with a 1 : 1 ratio of Drabkin's reagent and allowed to sit for 15 min at room temperature. The absorbance was then quantified at 540 nm. Percent hemolysis was calculated using eqn (5).

$$\text{Lock solution}(\%) = \frac{\text{volume of lock solution used}}{\text{volume of dilute whole blood}} \times 100 \quad (4)$$

$$\text{Hemolysis}(\%) = \frac{\text{abs(sample)} - \text{abs(blank)}}{(0.844) \times \text{abs(dilute whole blood)} - \text{abs(blank)}} \times 100 \quad (5)$$

2.7.2 *In vitro* cytocompatibility. The cellular cytocompatibility of SNAPicillin lock solutions was assessed through 24 h *in vitro* assays with human fibroblasts (BJ) following our previously reported method for lock solution screening with minor deviation.[15] Cryopreserved stocks of BJ cells were revived and subcultured in DMEM supplemented with FBS (10% v/v) and P/S (1% v/v) under a 5% CO_2-humidified atmosphere at 37 °C. Cells were subcultured up to 15 passages between experiments. Subconfluent cells were trypsinized (0.05% v/v with 5 mM EDTA) and collected *via* centrifugation (200 rcf, 5 min). Resuspended cells were further counted for the preparation of culture plates. For cytotoxicity experiments, cells were seeded onto 96-well tissue culture plates (5000 cells per well) and grown for 24 h before the screening.

Dilutions of lock solutions (10 mM stock concentration) were prepared from PBS (1×) in complete media to mimic any fluid leakage from catheters. In this study, we evaluated 0.06, 0.09, and 0.20% v/v dilutions of the SNAP, ampicillin, and SNAPicillin lock solutions based on the clinical usage of 5–10 mL of lock solution for venous catheters as well as our prior work with NO-releasing lock solutions.[15,21] A further

0.50% v/v dilution (corresponding to an excess of 25 mL of leaked lock solution into 5 L of blood) was also evaluated to showcase the effects of a potential worst-case scenario. After the initial seeding period, subconfluent cells were exposed to the lock solution dilutions for an additional 24 h. Afterward, media was aspirated from wells and replaced with clean media supplemented with MTT (0.5 mg mL^{-1}). Plates were incubated for an additional 2.5 h, with media aspirated and residual formazan precipitate dissolved in DMSO and read for absorbance (λ = 570 nm, with λ_{ref} = 690 nm). The relative percent viability of cells was calculated with respect to a control group treated with clean media using eqn (6). Final data is reported as mean relative cellular viability ± SD (n = 3).

$$\text{Relative cell viability}(\%) = \frac{\Delta OD_{\text{lock solution dilution}}}{\Delta OD_{\text{control group}}} \times 100 \quad (6)$$

2.8 Statistical analysis

All data in the manuscript are reported as mean ± standard deviation (SD) with a sample size of $n \geq 3$. A two-tailed Student's t-test with a hypothesis of unequal variance and α = 0.05 was used to ascertain the statistical significance between the test groups and the controls. For antibacterial MIC plating studies, one-way ANOVA analysis was performed on the logarithmic calculations. Values of $p < 0.05$ were deemed significant.

3. Results and discussion

3.1 Dry-state storage stability of SNAPicillin

The clinical application of NO-releasing compounds is highly dependent on their storage conditions. Irrespective of their amount or capacity, donors should be stored effectively. If proper protocols for storage conditions are not followed, the donors could be exposed to changes in the environment, thereby prematurely losing their effectiveness and potency. In order to determine the best storage conditions for SNAPicillin, the stability analysis of SNAPicillin was determined using UV-vis spectroscopy. The SNAPicillin powder was stored at −20 °C, 4 °C, RT, and 37 °C, and the stability of its *S*-nitrosothiol bond was measured periodically for 28 d determined by the molar absorption coefficient ε_{340} = 0.8546 mM^{-1} cm^{-1} (Fig. S4†). At each time point (0, 7, 14, 21, and 28 d), the NO content detected was compared to the initial NO content. The *S*-nitrosothiol bond was stable at lower temperatures, with 92.46 ± 0.94% and 91.07 ± 2.08% of NO remaining at −20 °C and 4 °C, respectively, after 28 d. On the other hand, significant NO was lost at higher temperatures. After 28 d at RT, only 50.39 ± 0.44% NO remained, and at 37 °C, merely 35.56 ± 1.34% of NO was observed (Fig. 2A). Storage stability of NO donors is challenging as the *S*-nitrosothiol bond is degraded by heat along with light, metal ions, and hydrolysis.[22] Consequently, it logically follows that storing SNAPicillin powder in higher temperature conditions leads to NO degradation over time. This trend is similar to what is seen with other *S*-nitrosothiols[23,24] and significantly better than cysteine-derived NO-donors,[24,25] which have short half-lives and limited stability even at −20 °C. This data demonstrates that SNAPicillin can be efficiently stored in cold temperatures to provide the best stability and potency in potential clinical applications.

The stability of the SNAPicillin lock solution was also monitored under physiological conditions using a UV-vis spectrophotometer to estimate the dwelling period of the SNAPicillin lock solution in catheters to prevent infections. This was done by preparing 10 mM SNAPicillin lock solution in 10 mM PBS containing 100 μM EDTA (pH 7.4) and measuring the absorbance at 340 nm at each time point (0, 4, 6, 24, 30, 48, and 72 h). The stability of the lock solution was calculated as a percent of NO-releasing SNAPicillin remaining over time compared to absorbance obtained for the freshly prepared solution at 0 h. The data obtained from the study shows that the gradual degradation of SNAPicillin solution occurs at 37 °C as NO is constantly being released in the solution. SNAPicillin displayed a half-life ($t_{1/2}$) of ∼30 h in PBS (10 mM, pH 7.4) with 100 μM EDTA at 37 °C (Fig. 2B). It is widely understood that the RSNO form of NO donors are relatively less stable in solution than in dry-state.[26] Therefore, from a commercial standpoint, it is more feasible to prepare and store the SNAPicillin in a dry state and reconstitute it as a solution in the desired solvent before administrating it as a lock solution. In clinical applications, lock solutions could be instilled within catheters every *ca.* 24 h to retain physiological levels of NO during catheter usage.

3.2 Real-time nitric oxide release

In order to identify the appropriate concentration of SNAPicillin lock solution for physiological levels of NO release, three different concentrations were chosen for the study. Catheters with SNAP and SNAPicillin lock solutions were prepared by filling the Helixmark SR-06 silicone rubber tubing with 50 μL of 1, 5, and 10 mM SNAP or SNAPicillin solution. Prepared catheter samples were placed in an amber glass chamber with 5 mL of PBS–EDTA at physiological conditions (pH 7.4, 37 °C) and tested for NO release using a chemiluminescence nitric oxide analyzer. Results from the study demonstrate a positive correlation between NO-donor concentration and NO release measured. It was observed that increasing the lock solution concentration increased the NO release level. Notably, at 0 h, the 1, 5, and 10 mM of SNAPicillin lock solution released 0.08 ± 0.01, 0.37 ± 0.06, and 0.58 ± 0.09 × 10^{-10} mol min^{-1} cm^{-2} of NO from catheter tubing, respectively. At the end of 24 h, the levels of NO release dropped to 0.05 ± 0.01, 0.23 ± 0.04, and 0.44 ± 0.09 × 10^{-10} mol min^{-1} cm^{-2} of NO for 1, 5, and 10 mM concentration of SNAPicillin lock solution, respectively (Fig. 2C). Since 10 mM samples showed the highest NO release behavior, studies for antibacterial and biocompatibility were conducted with 10 mM lock solution due to its ability to emulate physiologically relevant levels of NO from endothelial cells (0.5–4 × 10^{-10} mol min^{-1} cm^{-2}). The amount of NO release from the SNAP lock solution was also measured as a control. Results indicate that 1, 5, and 10 mM of SNAP lock solution released 0.14 ± 0.08, 0.64 ± 0.16, and 1.44 ± 0.14 × 10^{-10} mol min^{-1} cm^{-2} of NO from catheter tubing, respectively, at 0 h. It was observed that lower concentrations of both

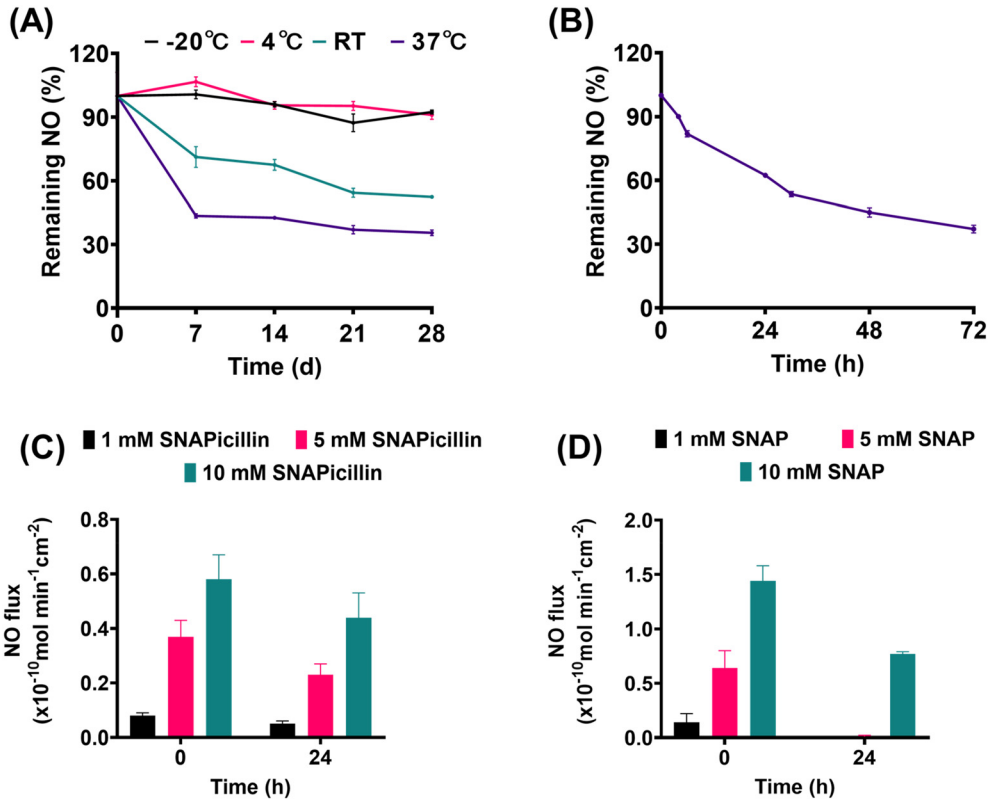

Fig. 2 (A) Storage stability of SNAPicillin powder at four different temperature conditions (−20 °C, 4 °C, room temperature (RT), and 37 °C). The decomposition of SNAPicillin is presented in terms of percent nitric oxide (NO) remaining on 0, 7, 14, 21, and 28 d with respect to absorbance on day 0. (B) Decomposition of 10 mM SNAPicillin lock solution in PBS (10 mM, pH 7.4) containing 100 μM EDTA at 37 °C. (C) Realtime NO release kinetics from the SNAPicillin and (D) SNAP lock solution at different concentrations as detected by the gold-standard nitric oxide analyzer (NOA) in PBS (10 mM, pH 7.4) with 100 μM of EDTA at physiological conditions (37 °C). All data are presented as mean ± SD ($n \geq 3$).

SNAP lock solutions (1 and 5 mM) had faster declination of NO compared to higher concentrations (10 mM). The levels of NO recorded with 1 and 5 mM were almost 0 at 24 h while 10 mM still had NO levels of 0.77 ± 0.02 × 10^{-10} mol min^{-1} cm^{-2} (Fig. 2D). The solubility of SNAPicillin before and after modification was assessed in water, PBS–EDTA, and HBSS conditioning media by dissolving SNAP, ampicillin, and SNAPicillin at varying concentrations (Tables S1–S9†). Both ampicillin and SNAP show >250 mM and 25–45 mM solubility in all three solvent conditions, respectively. Nevertheless, the conjugation of SNAP to ampicillin was seen to reduce the solubility of the conjugated compound, making it more hydrophobic in nature. The SNAPicillin had a solubility of 1, 10, and 5 mM in DI water, PBS–EDTA, and conditioning media, respectively. Therefore, no further concentrations of solutions were tested due to the solubility limit of SNAPicillin in PBS–EDTA. For both SNAP and SNAPicillin lock solutions, the equimolar concentration of solutions was tested for their corresponding real-time NO release. However, based on the results obtained, it can be said that the RSNO moiety of SNAPicillin is relatively less reactive than the SNAP molecule in the given test conditions. This trend of NO release corroborates with other NO release reports published in the literature where bulky RSNO molecules have elevated intramolecular bonding that results in a slower degradation rate and thereby increased stability of the molecule.[27] In clinical settings, the SNAPicillin lock solution can easily be replenished every 24 h to maintain the antibacterial properties of NO and ampicillin over time. These results imply that a catheter lock solution containing SNAPicillin molecule has the tendency to release NO for at least 24 h, making it a suitable choice for catheter lock solution applications.

3.3 Antibacterial efficacy of lock solution

3.3.1 Screening of antibiotic-resistant bacteria strains. To select the antibiotic-resistant bacterial strains for further antibacterial experiments, clinical strains of bacteria were screened for antibiotic resistance using a Kirby–Bauer disk diffusion susceptibility test in a 24 h zone of inhibition study.[28] Five different antibiotic discs (ampicillin, vancomycin, gentamicin, rifampicin, and cefotaxime) were placed on agar plates with 50 μL of bacterial culture (*E. coli* (AR-0089) and *S. marcescens* (AR-0099)). Plates with bacteria and antibiotic discs were incubated overnight at 37 °C before measuring the diameter of zones in mm. Results from the antibiotic susceptibility test are reported as mean ± SD (n = 3) and tabulated in Table 1. Both *E. coli* and *S. marcescens* were found to be resistant to ampicillin and vancomycin antibiotics. In addition, the *S. marcescens* strain was also resistant to rifampin antibiotic. These results confirmed the

Table 1 Antibiotic susceptibility of antibiotic-resistant bacteria

Antibiotic	Zone of inhibition (mm) against *E. coli* (AR-0089)	Zone of inhibition (mm) against *S. marcescens* (AR-0099)
Ampicillin	Resistant (no zone)	Resistant (no zone)
Vancomycin	Resistant (no zone)	Resistant (no zone)
Gentamicin	10.33 ± 0.47	20.83 ± 0.62
Rifampin	9.83 ± 0.23	Resistant (no zone)
Cefotaxime	19.66 ± 0.47	31.83 ± 0.23

multi-drug resistance of these two bacteria strains to more than one standard antibiotic. All further bacteria studies were done using these two bacterial strains.

3.3.2 Determination of minimum inhibitory concentration and ability to prevent bacterial viability. The growing dilemma of bacterial resistance to gold-standard antibiotic drugs means that antibacterial drugs used in clinical settings must be carefully chosen. Clinicians use minimum inhibitory concentration (MIC) analysis to identify resistance and susceptibility patterns toward various drugs.[29] The MIC of a drug refers to the lowest concentration that will inhibit the visible growth of a particular bacteria strain; a lower MIC indicates that less of the drug is needed to prevent the growth of bacteria, which is advantageous over high, potentially cytotoxic dosages. Here, MIC stands for the MIC_{90} values calculated based on the drug concentration needed to reduce relative growth to below 10%.

To determine the drug concentration needed to eradicate the chosen resistant strains of bacteria, concentrations of SNAP, ampicillin, and SNAPicillin were evaluated against *E. coli* and *S. marcescens*. Bacteria at a starting concentration of approximately 1×10^8 CFU per mL was exposed to various drug concentrations for a 24 h period. CFU plating counting methods were used to determine the percent reduction when the relative growth of SNAPicillin-treated bacteria was below 0.1. The MIC assays revealed that SNAPicillin had the greatest antibacterial activity after 24 h for both *E. coli* and *S. marcescens* (Fig. 3A and B, respectively). The 24 h relative growth graph for *E. coli* shows that SNAPicillin impacted bacterial growth at concentrations as low as 250 μM, with no visible growth starting at 1 mM concentrations. In contrast, only 8 mM SNAP was effective, and only 4 mM was effective for ampicillin treatments. The drug with the lowest MIC against *E. coli* is SNAPicillin (SNAPicillin MIC *vs. E. coli* = 1 mM). Therefore, all 1 mM drug treatments were plated to quantify the relative reductions of the effective SNAPicillin drug treatments compared to untreated *E. coli* (Fig. 3C). The planktonic solutions from the well plates were spiral plated and counted *via* a colony counter. Compared to untreated bacteria, 1 mM SNAPicillin demonstrated a phenomenal 8.24-log reduction ($p < 0.0001$). SNAPicillin performed significantly better than ampicillin ($p < 0.0001$) even though these treatments resulted in *ca.* 2-log reduction. Lastly, SNAPicillin performed also significantly better than SNAP, as 1 mM SNAP yielded no significant difference compared to untreated *E. coli*.

The 24 h relative growth graph for *S. marcescens* shows that SNAPicillin began to affect bacterial growth at 125 μM substantially, and there was no visible growth observed starting at 2 mM. However, ampicillin was not effective at any concentration tested. This trend follows the earlier reported ZOI results (Table 1) and published standards from the Clinical Laboratory Standards Institute (CLSI) denoting *S. marcescens* as ampicillin resistant.[30] Given *S. marcescens* strong resistance characteristics, SNAP treatment was observed to have a similar trend. Only 8 mM of SNAP was found to be effective against the bacteria. Nevertheless, the drug with the lowest MIC against *S. marcescens* is SNAPicillin (SNAPicillin MIC *vs. S. marcescens* = 2 mM). Although the NO release studies demonstrate similar release levels observed for 1–2 mM concentration of SNAP and SNAPicillin (Fig. 2C and D), SNAPicillin-treated samples show a substantially more effective reduction in bacterial viability due to the dual-action of NO and ampicillin.

Consequently, all 2 mM drug treatments were plated to quantify the relative reductions of the effective SNAPicillin drug treatments compared to untreated *S. marcescens* (Fig. 3D). Here, 2 mM SNAPicillin demonstrated a 4.28-log reduction compared to untreated bacteria ($p < 0.0001$). For 2 mM SNAP and ampicillin, no significant difference was seen when compared to untreated *S. marcescens*. As anticipated, the lack of significant reduction in growth corresponds to the previously obtained curves and our knowledge of *S. marcescens* resistance to ampicillin. Typically, about 30 mM concentration of ampicillin is used for clinical lock solution application.[7] Compared to the very high concentration of ampicillin solution, a significantly lower concentration of SNAPicillin (1–2 mM) was found to be extremely effective against antibiotic-resistant bacteria in this study.

The NO release levels that are achievable from NO-releasing catheter lock solutions are dependent on the NO donor concentration utilized. The NO release levels obtained in these SNAPicillin studies were found to be lower compared to previously reported studies comprising of the less bulky and hydrophilic SNACET, GSNO, and GSNO-cyclodextrin-based NO-releasing lock solutions.[14,15,31] However, the high NO release levels seen from these previously reported studies can be attributed to the higher concentrations of solutions utilized (as high as 100 mM) compared to 10 mM concentration of SNAPicillin used in this study. Although the NO release levels from the SNAPicillin solution were observed to be lower, the antibacterial efficiency of SNAPicillin is noted to be the most efficient with 4.28 and 8.24 log reductions in *S. marcescens* and *E. coli* bacteria due to the synergy with the covalently bound ampicillin, respectively, when compared to previously reported NO-releasing lock solutions.

3.3.3 Extermination of established biofilm on polymer. Antibiotic resistance in bacterial species is one of the major reasons for the increase in chronic infections arising from medical devices. The development and propagation of multi-drug resistant strains have exacerbated the existing situation of antibiotic resistance around the world. The bacteria encapsulated in the biofilm matrix have additional resistance mecha-

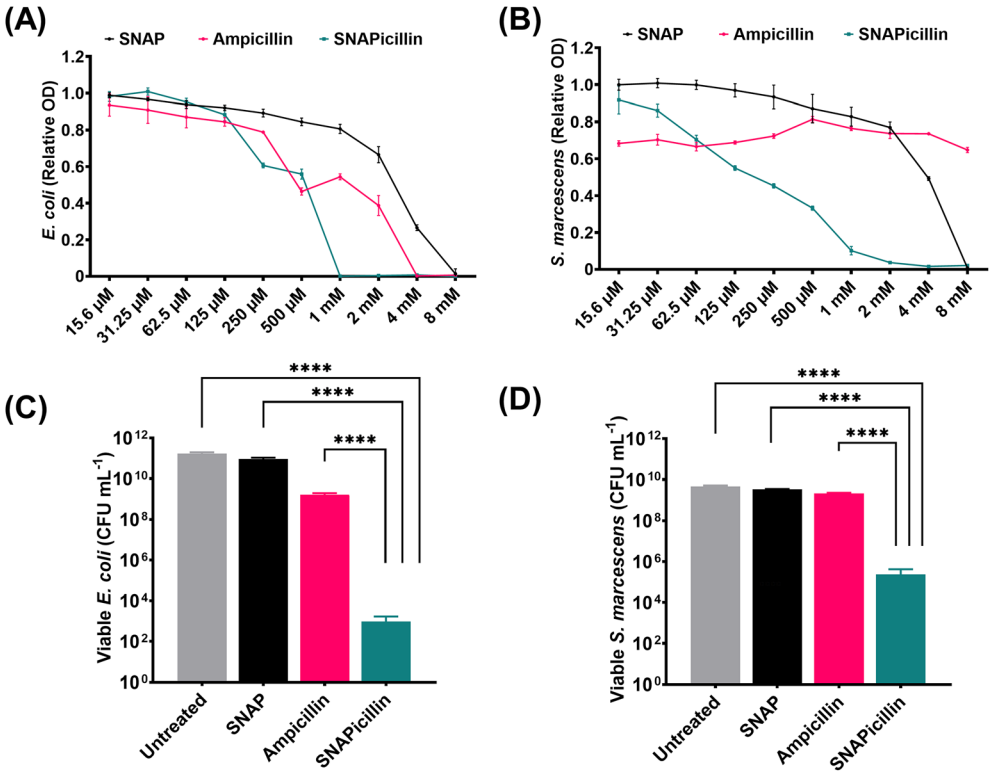

Fig. 3 Drug susceptibility curves for (A) *E. coli* and (B) *S. marcescens* (n = 3) exposed to SNAP, ampicillin, and SNAPicillin at various incremental concentrations. Viable colony forming units (CFU) of (C) *E. coli* after 24 h of exposure to 1 mM SNAPicillin lock solution (n = 3), and (D) *S. marcescens* after 24 h of exposure to 2 mM SNAPicillin lock solution (n = 3) (F) (**** = $p < 0.0001$). All data are presented as mean ± standard deviation (n = 3).

nisms as opposed to the free-floating planktonic bacteria which inhibits the action of antibiotics and other antibacterial agents. However, the gaseous nature of NO and its promising antibacterial and antibiofilm properties allow it to penetrate the EPS matrix and disperse the biofilm.[32] *E. coli* and *S. marcescens* are two prominent bacterial strains that are most commonly reported across all hospital-acquired infections (HAIs) and medical devices such as CVC's, urinary catheters, prosthetic devices, *etc.*[13,33] In this study, a promising alternative to combat antibacterial resistance in bacteria and their emergence in the form of lock solution is presented where the conjugate of NO donor SNAP and ampicillin antibiotic (SNAPicillin) possesses the superior ability to eradicate the bacteria on polymeric medical devices. The biofilm eradication potential of SNAPicillin solution was evaluated against *E. coli* and *S. marscences* biofilms on PDMS polymer films to mimic mature infection of a catheter device (Fig. 4). The biofilm on these surfaces was grown for 48 h in the presence of nutrient-rich media, followed by treatment of biofilm with the solution of ampicillin and SNAPicillin (10 mM) and PBS control for 24 h. Results from the study revealed that SNAPicillin-treated samples led to 99.06 and 99.75% reduction in viable *E. coli* bacteria on the polymer surface compared to ampicillin-treated and control samples, respectively ($p < 0.05$) (Fig. 4A). Similar results were observed for *S. marcescens* bacteria where SNAPicillin-treated samples resulted in 99.94 and 99.96% reduction in viable bacteria on polymer surface compared to ampicillin-treated and untreated control samples, respectively ($p < 0.05$) (Fig. 4B).

In both bacteria studies, ampicillin-treated samples showed no reduction in bacterial viability compared to untreated control samples. On the other hand, SNAPicillin-treated samples demonstrated a >99% reduction in viability. These results clearly demonstrate the limitation of antibiotics in treating biofilms comprising of antibiotic-resistant bacteria and are in agreement with the resistance profiles listed by the CDC for *E. coli* (AR-0089) and *S. marcescens* (AR-0099) which confirmed the resistance of the bacteria to at least ten standard antibiotics such as ampicillin, cefazolin, cefoxitin, *etc*. *via* ACRF, aph(3″)-Ib, aph(3′)-Ia, aph(6)-Id, CMY-2, sul2, tet(B) and aac(6′)-Ic, SMDA, SMDB, SME-4, SRT-2, SSME mechanism, respectively.

The ability of NO to provoke biofilm dispersal across various bacterial species is well understood. The antibacterial property and ability to disperse biofilms on surfaces have made NO an important therapeutic strategy for biofilm-related infections. Bacterial cells are susceptible to the reactive NO gas that can easily permeate the bacterial membrane and disrupt structural proteins and enzymes. Unlike several target-specific antibiotics that can be impacted by modifications in the microbial cell (decreased penetration, expelling of antibiotic, inactivation of antibiotic, or alterations to target site), NO

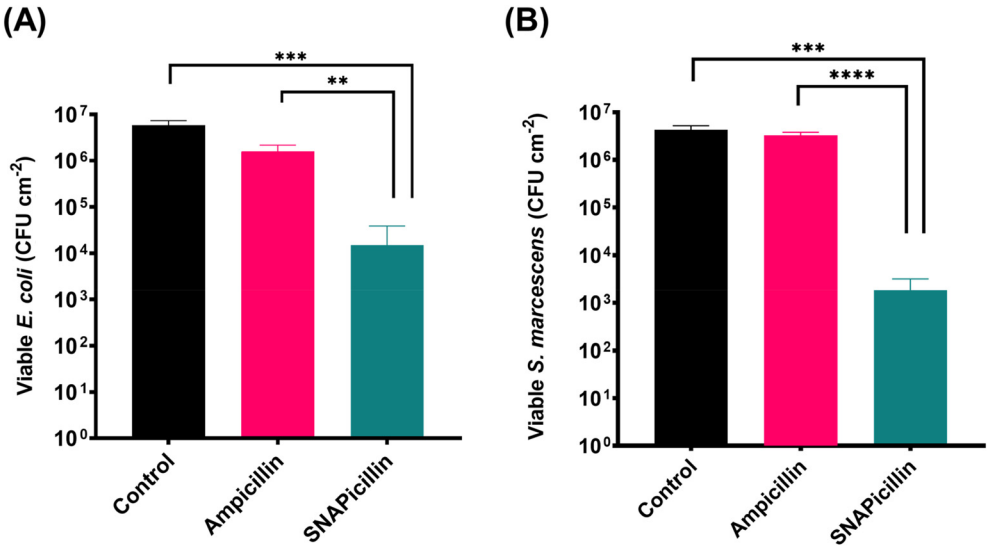

Fig. 4 Antibiofilm activity of the SNAPicillin solution calculated as a log of colony forming units (CFU) per cm^2 of surface area against (A) *E. coli*; ** represents $p \leq 0.01$ and *** represents $p \leq 0.001$, calculated for SNAPicillin *vs.* ampicillin and control and (B) *S. marcescens*; *** represents $p \leq 0.001$ and **** represents $p \leq 0.0001$, calculated for SNAPicillin *vs.* ampicillin and control. All data are represented as mean ± SD ($n \geq 3$).

demonstrates antibacterial properties through various nitrosative and oxidative pathways that lead to DNA deamination and membrane destruction.[34–37] Previous reports have shown that low amounts of NO release can prompt the shift of bacteria in biofilm from a sessile state to a planktonic state.[38] This can be attributed to the modulation of secondary cellular signaling molecules like cyclic di-GMP by NO that inhibits the motility and attachment of bacteria, therefore, preventing biofilm development and causing the dispersal of established biofilms.[39] Moreover, the reactive nitrogen and oxygen species triggered by NO and the superoxide ions diminish the generation of EPS matrix which is a critical element for the adhesion of bacteria on a surface. Unlike many antibiotics that target either Gram-positive or Gram-negative bacteria, the broad-spectrum capacity of NO in promoting biofilm dispersal is preserved across a variety of microorganisms.[40] Overall results obtained from the antibacterial ability of the engineered SNAPicillin molecule underscore the potential of enhancing the activity of less active antibiotics such as ampicillin by conjugating the NO donor to the antibiotic. The conjugated SNAPicillin has exhibited heightened antibacterial action against multi-drug resistant strains of *E. coli* and *S. marcescens*.

3.4 Biocompatibility screening

3.4.1 Hemolytic activity assessment. Due to the design of the indwelling catheter with an open-ended tip, lock solution leakages and mixing with blood at the tip are inevitable. The amount of lock solution that typically leaks from the catheter usually has a low risk of toxicity due to minimal leakage from low blood flow rates and the small inner diameter of the catheter.[41–43] Clinically, some catheters may be locked with as much as 10 mL of lock solution.[41] Therefore, to simulate varying severities of leakages, a range of leaked lock solutions volumes were tested. As a human body typically contains approximately 5 L of blood,[44] 3 mL, 4.5 mL, 10 mL, and 25 mL of leaked lock solution correspond with 0.06%, 0.09%, 0.2%, and 0.5% dilutions to mimic potential leakage events. The NAMSA ASTM F756 protocol considers a hemolytic index of >5% to be hemolytic. The results indicate that all lock solutions at all blood/lock solution ratios were not hemolytic (Table 2) and would not cause major complications if leaked into the bloodstream.

3.4.2 Cytotoxicity assay. The biocompatibility of the SNAPicillin and related antibiotic lock solutions was evaluated in a human fibroblasts model to investigate the potential for any cytotoxic effect from lock solution leakage. Based on preliminary studies of NO release, lock solutions of SNAP, ampicillin, and SNAPicillin, at concentrations of 10 mM were screened at different dilution ratios corresponding to solution leakage. Generally, no cytotoxic effects were observed after 24 h incubation (Fig. 5). These findings align with prior studies of lock solutions using *S*-nitrosoglutathione, suggesting strong biocompatibility of NO-releasing lock solutions.[15] Although previous studies have tested the biocompat-

Table 2 Percent hemolysis of all blood/lock solution ratios

Sample type	0.06%	0.09%	0.2%	0.5%
Control	1.53 ± 0.33	1.70 ± 0.74	2.27 ± 0.20	2.57 ± 0.33
Ampicillin (10 mM)	1.48 ± 0.20	2.09 ± 0.94	2.27 ± 0.30	2.75 ± 1.07
SNAP (10 mM)	1.57 ± 0.45	2.40 ± 0.59	2.23 ± 0.00	2.05 ± 0.66
SNAP + Ampicillin (10 mM)	1.61 ± 0.15	1.27 ± 0.50	2.40 ± 0.53	1.96 ± 0.82
SNAPicillin (10 mM)	1.44 ± 0.45	3.27 ± 1.59	2.49 ± 0.23	2.05 ± 0.50

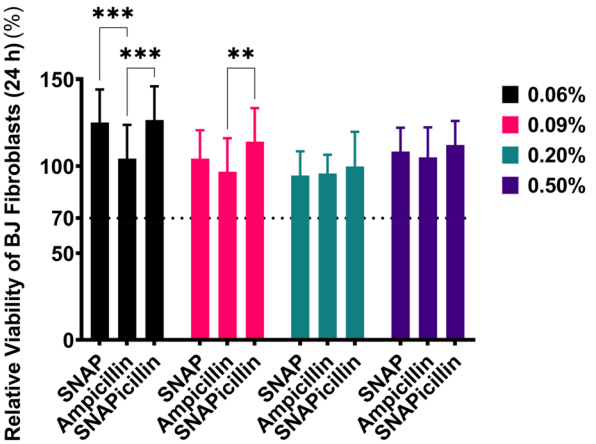

Fig. 5 Screening of antibiotic lock solutions against human fibroblasts in a model for lock solution leakage demonstrated no significant induction of cytotoxic effect with any of the antibiotic formulations after 24 h. Data are shown as mean cellular viability ± standard deviation (N = 3 independent preparations). Statistical significance is expressed as * (P < 0.05), ** (P < 0.01), and **** (P < 0.0001).

ibility of lock solutions at as high as 60 mM concentration, SNAPicillin solution was only tested up to 15 mM due to the solubility limit of hydrophobic SNAPicillin molecule in aqueous solvents. Moreover, it is anticipated that the viability of cells is preserved at higher concentrations of RSNOs, however, higher concentrations of antibiotics are known to exhibit a cytotoxic response.[45] Previously, we have observed that both SNAP and GSNO exhibit proliferative effects against human fibroblasts at low doses (*e.g.*, <250 μM),[46,47] with similar effects observed herein for both SNAP and SNAPicillin-based lock solutions at dilutions of 0.06, 0.09, 0.20, 0.50% v/v, corresponding to 6, 9, 20, and 50 μM solutions. At both 0.06 and 0.09% v/v dilutions, both SNAP and SNAPicillin were able to significantly enhance viability compared to the ampicillin lock solution (p < 0.05). Overall, these findings support the general biocompatibility and suitability of these antibiotic-based lock solutions for further antimicrobial screening.

4. Conclusions

Bacteria and biofilm development often challenge the lifetime of catheters. This issue is further intensified by the rise in antibiotic resistance that negatively influences the current clinical regimens to prevent and treat catheter infections. In this study, a novel conjugate of ampicillin antibiotic with the ability to release nitric oxide (NO) for the use of antimicrobial lock therapy is reported. This was done by covalent attachment of the NO donor *S*-nitroso-*N*-acetyl-penicillamine (SNAP) to the antibiotic ampicillin, resulting in a dual-active antimicrobial SNAPicillin molecule. SNAPicillin was characterized by NMR and FTIR which confirmed the successful synthesis of the molecule. The storage stability of SNAPicillin powder was analyzed over 28 d at four different conditions (−20, 4, RT, and 37 °C) using spectrometric analysis. SNAPicillin exhibited higher stability at −20 and 4 °C with >90% of NO remaining after 28 d of storage. Then, to generate a catheter lock solution, SNAPicillin was dissolved in PBS (10 mM, pH 7.4) containing 100 μM EDTA, injected into SR tubing, and tested for NO release using a chemiluminescence NOA. The SNAPicillin lock solution revealed tunable NO release properties with the change in the concentration of SNAPicillin solution ranging from 1–10 mM resulting in physiological levels of NO. The antibacterial activity of the SNAPicillin solution was evaluated against antibiotic-resistant strains provided by CDC (resistance confirmed by ZOI assay against conventional antibiotics). The minimum inhibitory concentration of SNAPicillin lock solution was found to be 1 and 2 mM against the *E. coli* (AR-0089) and *S. marcescens* (AR-0099) strains of bacteria, respectively. The SNAPicillin lock solution at these MIC values exhibited the highest antibacterial activity after 24 h for both *E. coli* and *S. marcescens* compared to controls, with 8.24- and 4.28-log reduction in bacterial load, respectively. The ability of the SNAPicillin lock solution to exterminate an established biofilm infection was also determined. The SNAPicillin solution eradicated >99% of viable *E. coli* and *S. marcescens* bacteria compared to untreated control and ampicillin-treated bacteria which demonstrates the ability of this new lock solution to help prevent and treat catheter infections. Additionally, hemolysis and cell compatibility assays confirmed the biocompatibility of the SNAPicillin lock solution. The encouraging findings presented in this study highlight the capacity of dual-functional SNAPicillin for use as a versatile catheter lock solution that can be utilized with virtually any catheter currently used in the clinical setting in order to prevent and treat biofilm infections on indwelling catheters and other medical device interfaces.

Author contributions

Manjyot Kaur Chug: conceptualization, methodology, visualization, writing – original draft preparation, writing – reviewing & editing, and project administration. Lauren Griffin: methodology, writing – original draft preparation, writing – reviewing & editing. Mark Garren: methodology, writing – original draft preparation, writing – reviewing & editing. Emma Tharp: methodology, writing – reviewing & editing. Grace H. Nguyen: methodology, writing – original draft preparation, writing – reviewing & editing. Hitesh Handa: investigation, supervision, validation, funding acquisition, writing – reviewing & editing. Elizabeth Brisbois: conceptualization, data curation, visualization, investigation, supervision, validation, funding acquisition, writing – reviewing & editing, and project administration.

Conflicts of interest

There are no conflicts to declare.

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