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Nitric oxide releasing poly(vinylidene fluoride-co-hexafluoropropylene) films using a fluorinated nitric oxide donor to greatly decrease chemical leaching

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Abstract

Nitric oxide (NO) releasing polymers have been widely applied as biomaterials for a variety of biomedical implants and devices. However, the chemical leaching of NO donors and their byproduct species is almost always observed during the application of polymers doped with NO donors, unless the donor is covalently linked to the polymer. Herein, we report the first NO releasing poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) fluorinated copolymer prepared by incorporating a fluorinated *S*-nitrosothiol as the NO donor. Under physiological conditions, the resulting polymeric films can release NO for 16 days. Importantly, due to both fluorine-fluorine and electrostatic charge interactions between the fluorinated NO donor and the PVDF-HFP copolymer, the total chemical leaching of the fluorinated NO donor and its disulfide product after 9 day was only 0.6% (mol%) of the initial amount of NO donor loaded into the film. These new NO release PVDF-HFP films exhibit antimicrobial and anti-biofilm activities against both Gram positive *S. aureus* and Gram negative *P. aeruginosa* strains. The NO-releasing PVDF-HFP polymer can also be coated on Teflon tubing to release NO under physiological conditions for extended time periods. This NO-releasing PVDF-HFP copolymer with greatly reduced chemical leaching could help enhance the biocompatibility and antimicrobial activity of various biomedical devices.

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Author contributions

Mark E. Meyerhoff and Yang Zhou conceived this project and designed all the experiments. Yang Zhou synthesized the NO donors, prepared the NO releasing PVDF-HFP films and the surface coating of Teflon tubes, examined the NO release profiles from all the samples, analyzed the data and drafted the manuscript. Jinyi Tan fabricated SNAP doped PVDF-HFP films and carried out the leaching tests. Jianfeng Wu and Chuanwu Xi designed and conducted the antimicrobial and anti-biofilm studies. Qi Zhang characterized the NO releasing PVDF-HFP films by SEM spectroscopy. John Andre and Zhan Chen carried out tensile tests of pure PVDF-HFP and NO release PVDF-HFP films.

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Conflict of interest

The authors have no interest conflict to declare.

Graphic Abstract

Keywords

Nitric oxide releasing; Fluoropolymer; Poly(vinylidene fluoride-*co*-hexafluoropropylene); Chemical leaching; Antibacterial activity

1. Introduction

Fluoropolymers have a long history of application in creating various biomedical implants and devices [1, 2]. This is due to their unique properties, such as high chemical inertness, great thermal stability, high tensile strength, low coefficient of friction and improved biocompatibility [1, 2]. Specifically, expanded polytetrafluoroethylene (PTFE) has been widely applied in the construction of various vascular grafts, cardiovascular patches and stents, as well as other medical devices [3]. For the most part, such PTFE devices exhibit desirable biocompatibility in blood and vascular tissue contacting applications, including decreased inflammatory response, less thrombogenicity and rapid re-endothelialization [2, 3].

Poly(vinylidene fluoride-*co*-hexafluoropropylene) (PVDF-HFP) is a fluorinated copolymer formed with vinylidene fluoride and hexafluoropropylene. Due to having over 50% of its backbone carbons linked to fluorine atoms, PVDF-HFP also exhibits similar water resistance ($< 0.1\%$ (w/w)), chemical inertness and highly hydrophobic properties as PTFE. In addition, PVDF-HFP is a semi-crystalline polymer with a low glass transition temperature ($-29\text{ }^{\circ}\text{C}$) [4], providing this elastic material with greater elongation ability ($> 600\%$) as well as greater resistance to stress-cracking and fatigue compared to PTFE. Owing to the inherent anti-inflammatory and thromboresistant properties derived from fluorinated materials [5], PVDF-HFP also exhibits great biocompatibility when used in medical devices and tissue engineering [6–13]. For example, PVDF-HFP is employed as a top coating on the XIENCE V™ everolimus-eluting coronary stent and provides *in vivo* biocompatibility as well as outstanding chemical stability [11]. Importantly, the chemical properties of PVDF-HFP are not altered after the stent implantation for many years [14]. Although the PVDF-HFP copolymer provides promising properties to decrease surface induced thrombus formation and bacterial proliferation [12], blood clotting and significant bacterial adhesion are still observed during the applications of this material [4, 15–19]. For instance, bacterial adhesion

was significantly observed on the surface of PVDF-HFP during wound healing and antimicrobial studies [17–19]. PVDF-HFP also provoked a moderate curate inflammatory response during a subcutaneous implantation in mice [18]. Therefore, improvement in the antimicrobial activity and the biocompatibility of PVDF-HFP is required to further enhance its potential applications in preparing medical devices.

Nitric oxide (NO) releasing polymers have attracted increasing attention to improve the biocompatibility of medical devices [20–23], due to the potent antimicrobial and antithrombotic roles of NO. In such NO releasing polymers, NO precursor molecules are covalently attached or doped within the parent polymers. In contact with physiological fluids, surface NO release is expected to imitate the NO release that occurs at the surface of endothelial cells that line all the inner walls of all blood vessels in our bodies. Using this NO releasing approach, many groups have demonstrated the enhanced antimicrobial activity and biocompatibility of various polymers including Elast-eon E2As [24, 25], poly(lactic-co-glycolic acid) [26], polylactic acid [27], gelatin [28], silicone rubber [29–32], poly(ether-block-amide) [33], nylon [33], polyurethane [34, 35], Tygon [36, 37], and CarboSil polymer [38–41]. However, to the best of our knowledge, NO release fluoropolymers have not been well studied to date. Due to the high chemical inertness of fluoropolymers, fabrication of NO release fluoropolymers is very challenging. Thus far, only one example of a NO releasing fluoropolymer has been reported [42]. In that study, polyvinylidene fluoride (PVDF) tubing was doped with a newly synthesized fluorinated NO donor (*S*-nitroso-*N*-pentafluoropropionyl-penicillamine, C₂F₅-SNAP; see Fig. 1, below), which can release NO under physiological conditions for 11 d. As expected, this NO release PVDF tubing displayed significant antimicrobial properties [42].

However, for most NO donor doped polymers, a significant amount of chemical leaching remains a problem for potential biomedical applications [21]. In many of these NO release materials, the NO donors are not covalently linked to the polymer chains. Indeed, leaching of the NO donors and/or their products formed after NO release can both decrease NO-release surface flux rates as well as NO release longevity and create the potentially toxic sources [21]. In recently reported *S*-nitroso-*N*-acetyl-D-penicillamine (SNAP, Fig. 1) doped/impregnated polymers, a significant amount of the NO donor SNAP, and its corresponding thiol and disulfide were found to leach into the surrounding aqueous environment [21]. Typically, in the study of CarboSil polymer doped with SNAP (at 10 wt%) without any topcoat, ~ 47% of the initial SNAP (> 200 nmol/mg) and SNAP by-products including thiol and disulfide (~ 40 nmol/mg, respectively) leached out in 22 days when the NO release CarboSil polymer was soaked continuously in PBS at 37 °C [38].

In our previous work with PVDF tubing doped with C₂F₅-SNAP, leaching of the C₂F₅-SNAP and its decomposition products was found to be somewhat decreased (~ 10%, total) [42], owing to specific fluorous-fluorous interactions present between the PVDF and the C₂F₅-SNAP NO donor. Comparing the chemical leaching results between NO release non-fluorinated polymers and NO release fluorinated polymers, fluorinated polymers offer the opportunity to decrease or prevent the undesirable chemical leaching. Indeed, it seems possible to solve the chemical leaching issue by taking advantage of fluorous-fluorous interactions between the given fluoropolymer and fluorinated NO donors.

In our previous studies, we reported the synthesis of a family of fluorinated *S*-nitrosothiols, in which DiCF₃Bn-SNAP was the most stable one, with the larger number of fluorine atoms (total of 6) [43]. In fact, the more fluorine atoms present in a fluorinated NO donor can potentially lead to stronger fluorine-fluorine interactions between the fluorinated NO donor and the fluoropolymer, presumably resulting in the lower chemical leaching of NO donor or its derivatives. Thus, DiCF₃Bn-SNAP was considered as the species with greatest potential to be applied in NO releasing fluoropolymer compositions in order to minimize chemical leaching. In this work, we now compare the NO-releasing behavior of PVDF-HFP doped with SNAP or a new fluorinated NO donor, DiCF₃Bn-SNAP (see Fig. 1). The SNAP or DiCF₃Bn-SNAP are doped into the PVDF-HFP polymer *via* a solvent casting method. The NO release profiles from SNAP or DiCF₃Bn-SNAP doped PVDF-HFP films are investigated under physiological conditions. The chemical leaching from the DiCF₃Bn-SNAP doped PVDF-HFP films are fully characterized by HPLC-HRMS measurements. In addition, the antimicrobial activity of DiCF₃Bn-SNAP doped PVDF-HFP films are examined. Finally, the NO release behavior from a Teflon tube coated with a DiCF₃ Bn-SNAP doped PVDF-HFP film is reported.

2. Materials and methods

2.1 General information

All chemicals in this work were purchased and used directly without any purification. Commercial PVDF-HFP pellets were obtained from Sigma-Aldrich (427187–100G). Teflon tubing was purchased from Allied Electronics, Inc. (28 AWG, TFT20028 NA005). A phosphate-buffered saline (PBS, 10.0 mM, pH 7.40, with 100 μM EDTA) solution was prepared using Na₂HPO₄, KH₂PO₄, NaCl, KCl and EDTA. The synthetic NO donors were characterized by ¹H and ¹⁹F NMR spectroscopy (Varian 500 MHz spectrometer).

2.2 Preparation of NO donors and NO-releasing PVDF-HFP films

SNAP, C₂F₅-SNAP and DiCF₃Bn-SNAP NO donors were synthesized according to the reported methods [42–44]. The synthetic SNAP was characterized by ¹H NMR spectroscopy. The pure C₂F₅-SNAP and DiCF₃Bn-SNAP target obtained were characterized by ¹H and ¹⁹F NMR spectroscopy. ¹H NMR data of SNAP (500 MHz, DMSO-*d*₆): δ 13.16 (s, 1 H), 8.50 (d, *J* = 9.5 Hz, 1 H), 5.16 (d, *J* = 9.5 Hz, 1 H), 1.97 (s, 3 H), 1.94 (s, 3 H), 1.87 (s, 3 H). ¹H NMR data for C₂F₅-SNAP (700 MHz, DMSO-*d*₆): δ 10.14 (d, *J* = 9.1 Hz, 1H), 5.33 (d, *J* = 9.1 Hz, 1 H), 2.05 (s, 3 H), 1.98 (s, 3 H). ¹⁹F NMR data for C₂F₅-SNAP (377 MHz, DMSO-*d*₆): δ −82.29 (s), −121.22 (d, *J* = 11.31 Hz). ¹H NMR data for DiCF₃Bn-SNAP (500 MHz, DMSO-*d*₆): δ 9.15 (t, *J* = 7.0 Hz, 1 H), 8.51 (d, *J* = 12.0 Hz, 1 H), 7.99 (s, 1 H), 7.93 (s, 2 H), 5.28 (d, *J* = 12.0 Hz, 1 H), 4.51 (dd, *J* = 20.0, 7.5 Hz, 1 H), 4.44 (dd, *J* = 20.0, 7.5 Hz, 1 H), 1.96 (s, 3 H), 1.94 (s, 3 H), 1.87 (s, 3 H). ¹⁹F NMR data for DiCF₃Bn-SNAP (377 MHz, DMSO-*d*₆): −61.48 (s).

A DiCF₃Bn-SNAP doped (15 wt%) PVDF-HFP polymer thin film was prepared by a solvent casting method. Eight pieces of PVDF-HFP pellets (~ 330 mg) were initially dissolved in anhydrous THF (2.0 mL) within a sample vial at 50 °C. After cooling to room temperature, DiCF₃Bn-SNAP dissolved in anhydrous THF (200 μL) was added into the PVDF-HFP

polymer solution. The resulting green mixture solution was continuously stirred for 10 min under dark conditions at room temperature to obtain a homogeneous polymer solution. Then, the green polymer solution was poured into a cast mold ($2\text{ cm} \times 2\text{ cm} \times 1\text{ cm}$) stabilized on a glass plate. After being covered by a second glass board on the top, the sample mixture was left in the hood to allow the THF solvent to evaporate overnight under dark conditions. The final green films ($2\text{ cm} \times 2\text{ cm} \times 0.50 \pm 0.02\text{ mm}$) were obtained after being dried under vacuum for another 4 h. To prepare a longer piece of the DiCF₃Bn-SNAP doped (15 wt%) PVDF-HFP thin film, a secondary cast mold ($5\text{ cm} \times 2\text{ cm} \times 1\text{ cm}$) was utilized with proportional increase of the mass of both PVDF-HFP pellets and DiCF₃Bn-SNAP donor used. SNAP doped (3/9/15 wt%) PVDF-HFP polymer thin films and DiCF₃Bn-SNAP doped (3/9 wt%) PVDF-HFP polymer thin films were prepared in a cast model ($2\text{ cm} \times 2\text{ cm} \times 1\text{ cm}$) using the same method described above in order to compare NO-releasing from both NO donors. The C₂F₅-SNAP doped PVDF-HFP film was prepared using the same solvent casting method under dark conditions. The molar amount of C₂F₅-SNAP doped in the PVDF-HFP film equals that of DiCF₃Bn-SNAP used in the DiCF₃Bn-SNAP (15%) doped PVDF-HFP film.

For the surface coating of Teflon tubes (28 AWG, ID: 0.330 mm, OD: $0.824 \pm 0.003\text{ mm}$), a solution of PVDF-HFP with the DiCF₃Bn-SNAP NO donor within was obtained using the same method described above. After the distal end of Teflon tube was sealed with silicone rubber, the Teflon tube was dipped in the green polymer THF solution for surface coating. After each dip coating, the wet Teflon tube was hung within a dark fume hood, to allow the THF to evaporate. The dip coating was repeated five times for each Teflon tube. Then the resulting coated tubes were kept in a dark fume hood overnight for drying. Finally, the coated Teflon tubes were dried under vacuum for another 1 h before the measurement of NO release rates. The OD of the coated Teflon tubes was measured to be $1.351 \pm 0.077\text{ mm}$. The thickness of surface layer of DiCF₃Bn-SNAP doped (15 wt%) PVDF-HFP polymer on Teflon was calculated to be $0.264 \pm 0.039\text{ mm}$.

2.3 Characterization of NO donor doped PVDF-HFP films

The surface morphologies of pure PVDF-HFP film and DiCF₃Bn-SNAP doped (15 wt%) PVDF-HFP polymer thin films were analyzed using scanningelectron microscope (SEM, JEOL-7800FLV FE). Mechanical pull testing of films was performed with an Instron 5544R mechanical testing system with a pull rate of 100 mm/min with a starting distance between grips on 10 mm (twice of each film ($5\text{ mm} \times 40\text{ mm} \times 0.5\text{ mm}$)). The Young's modulus was determined from the elastic region of the stress-strain curves from over the 0.05 mm to 0.10 mm range of the stress-strain curve. The NO release profiles for all films were measured in a solution of PBS under dark conditions at 37 °C using a Sievers 280i chemiluminescence Nitric Oxide Analyzer (NOA). The NO releasing films ($1.5\text{ cm} \times 0.5\text{ cm} \times 0.50\text{ mm}$) were stored in a fresh PBS (2.0 mL) at 37 °C under dark and unsealed conditions before and after each measurement. For the leaching tests, NO releasing films ($1.5\text{ cm} \times 0.5\text{ cm} \times 0.50\text{ mm}$) were placed in a fresh PBS solution (2.0 mL) each day. To determine the possible presence of DiCF₃Bn-SNAP donor and its corresponding thiol and disulfide in the soaking buffer, the daily soaking solutions were analyzed by an Agilent 6520 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) HPLC-HRMS equipped with a reversed-phase column (ZORBAX RRHD

Eclipse Plus C18 (2.1×50 mm, $1.8 \mu\text{m}$) in the electrospray positive-ion mode. A gradient method was used for the HPLC separations. The ratio between eluent A (water with 0.1% formic acid) and eluent B (acetonitrile) (A/B) was gradually changed from 95/5 to 0/100 over a 10 min period with a flow rate of 0.4 mL/min. The elution times of $\text{DiCF}_3\text{Bn-SNAP}$ and its disulfide were determined to be ~ 11.50 and ~ 13.70 min on the reversed-phase C18 column. The corresponding calibration curves for pure $\text{DiCF}_3\text{Bn-SNAP}$ NO donor and its disulfide were prepared to determine their concentrations in the daily soaking buffer.

2.4 Antimicrobial and anti-biofilm studies

The antimicrobial and anti-biofilm studies were performed using a CDC biofilm reactor (Biosurface Technologies, Bozeman, MT) as described previously [42]. All $\text{DiCF}_3\text{Bn-SNAP}$ doped (15 wt%) PVDF-HFP (3 pieces, $4.5 \text{ cm} \times 0.5 \text{ cm} \times 0.50 \text{ mm}$) and pure PVDF-HFP (3 pieces, $4.5 \text{ cm} \times 0.5 \text{ cm} \times 0.50 \text{ mm}$) thin films were sterilized first using 70% ethanol. After loading of the films on the sample holders in the CDC biofilm bioreactor, 1% of overnight grown *S. aureus*, or *P. aeruginosa* bacterial strains was inoculated into reactor and was allowed to incubate with the films under stagnant conditions for 1 hour. Then the same media composition, fresh without any bacteria present, was continuously supplemented at rate of 100 mL/h for 7 d. After this period, the films were removed from the bioreactor to count viable cells adhered on the surface using a plate count method and to observe biofilm formation *via* fluorescence imaging.

2.5 Statistical Methods

In addition to the specific explanation, all data with standard errors was obtained with the measurements of three independent samples. Statistical significance between the pure PVDF-HFP films and fluorinated NO donor doped PVDF-HFP films was determined by a Student's *t*-test. Vales less than 0.05 were regarded as statistically significant.

3. Results

3.1 NO release from SNAP doped PVDF-HFP films

SNAP doped PVDF-HFP polymer films ($2 \text{ cm} \times 2 \text{ cm} \times 0.50 \text{ mm}$) were prepared *via* a solvent casting method as described in Section 2.2. The corresponding NO release on the first day from these SNAP (15 wt%) doped PVDF-HFP films was measured by the NOA in PBS solution at 37°C (see Fig. 2). Interestingly, the surface NO release flux ($\sim 2 \times 10^{-10} \text{ mol/cm}^2\text{-min}$) from this film was only observed on the first day. After switching into a fresh soaking solution on the second day, the NO release was no longer detected by chemiluminescence. The 3 and 9 wt% SNAP doped PVDF-HFP polymer films also showed a similar NO release behavior, with NO release only observed on the first day (see Fig. S1). With the doping of 3 wt% of SNAP in the PVDF-HFP polymer, NO release was observed to be $\sim 1 \times 10^{-10} \text{ mol/cm}^2\text{-min}$. When the doping amount of SNAP was increased to 9%, a clear increase of NO release rate was obtained ($\sim 2 \times 10^{-10} \text{ mol/cm}^2\text{-min}$). However, the NO release flux was not significantly enhanced when the doping amount was increased up to 15 wt% SNAP (see Fig. 2).

3.2 Surface Characterization of DiCF₃Bn-SNAP doped PVDF-HFP films

The new DiCF₃Bn-SNAP species was used to prepare a NO release PVDF-HFP thin film (15 wt%, thickness 0.50 ± 0.02 mm). The resulting PVDF-HFP film has the characteristic green color of this NO donor (see Fig. 3). The SEM images of pure PVDF-HFP and DiCF₃Bn-SNAP doped PVDF-HFP polymer films are shown in Fig. 4. The Pure PVDF-HFP polymer film has a somewhat roughened surface (see Fig. 4a and 4b). Interestingly, with the incorporated DiCF₃Bn-SNAP species, the corresponding surface of the thin film becomes slightly less rough (see Fig. 4c and 4d). To gain more information about the surface chemical composition, an SEM image (see Fig. S4a) was recorded after the DiCF₃Bn-SNAP (15 wt%) doped PVDF-HFP thin film was washed with CH₂Cl₂. Clearly, NO releasing films after washed by CH₂Cl₂ shows the same surface morphology to the one (Fig. 4d) without washing.

3.3 NO release from DiCF₃Bn-SNAP doped PVDF-HFP films

The corresponding NO release data for the DiCF₃Bn-SNAP (15 wt%) doped PVDF-HFP film were measured in PBS solution at 37 °C using an NOA instrument (see Fig. 5). NO release is observed to last for 16 days when sample films were stored at these physiological conditions. This NO release time far exceeds that observed for the PVDF-HFP films containing SNAP (only one day). NO release flux numbers were observed to ca. 5×10^{-10} mol/cm²-min on the first day, which then significantly decreased on the second day ($\sim 0.52 \times 10^{-10}$ mol/cm²-min). Thereafter, the NO release flux remained almost constant between 0.2 and 0.4 ($\times 10^{-10}$ mol/cm²-min) over the following days. When using less DiCF₃Bn-SNAP NO donor (3 and 9 wt%) within the PVDF-HFP polymer film, the observed NO release time is much shorter (4 days for 3 wt% and 7 days for 9 wt%) compared to that observed for the 15 wt% doped PVDF-HFP film. Thus, as expected, increasing the level of NO donor within the polymer leads to an elongated NO release period.

3.4 Chemical leaching from DiCF₃Bn-SNAP doped PVDF-HFP films

The DiCF₃Bn-SNAP (15 wt%) doped PVDF-HFP films were soaked in fresh solutions of PBS each day and the amount of donor and byproducts was measured daily *via* HPLC-HRMS. Interestingly, the free thiol of DiCF₃Bn-SNAP was not detected during these leaching tests. Based on the corresponding calibration curves obtained for DiCF₃Bn-SNAP and its disulfide (see Fig. S7), the chemical leaching results for PVDF-HFP containing this fluorinated SNAP derivative are summarized in Fig. 6. Fig. 6a shows the cumulative leaching of the DiCF₃Bn-SNAP species (~ 1.86 nmol/mg) after 9 days of soaking. In Fig. 6b, the disulfide derived from DiCF₃Bn-SNAP donor was found to be ~ 0.15 nmol/mg after 9 days of soaking. Therefore, the total leaching of chemicals (DiCF₃Bn-SNAP and its disulfide) was 0.6% (mol%) of the total initial amount of the DiCF₃Bn-SNAP doped within the film.

3.5 Antimicrobial and anti-biofilm studies

The antimicrobial activity of the PVDF-HFP films doped with DiCF₃Bn-SNAP (15 wt%) was examined toward *S. aureus* and *P. aeruginosa* bacterial strains over a 7 day test period. The results of these studies are shown in Fig. 7. For *S. aureus*, the NO-releasing PVDF-HFP

film displayed more than 1 order of magnitude less viable bacterial counts than the control group with pure PVDF-HFP film (see Fig. 7a). Fig. 7b shows that the pure PVDF-HFP polymer has a more significant *P. aeruginosa* bacterial adhesion after 7 d. However, in the presence of the NO-release, a greater effect (> 1.5 order of magnitude) for the reduction of *P. aeruginosa* adhesion was observed in comparison to the control PVDF-HFP film.

To further confirm the antimicrobial activity of the films containing the DiCF₃Bn-SNAP species (at 15 wt%), the representative fluorescence images of the biofilms on the surfaces of test films are also provided (see Fig. 8). For *S. aureus*, a very significant difference in these fluorescence images are clearly observed between the control and the NO-releasing PVDF-HFP film (see Fig. 8a and 8b). In addition, a significant variation between image 8c and 8d also proves the role of NO release helping to prevent *P. aeruginosa* biofilm growth on the polymer surface.

3.6 Surface coating application on Teflon tube

We coated the surface of a Teflon tube (28 AWG) with the PVDF-HFP polymer containing the DiCF₃Bn-SNAP (15 wt%) species (see Fig. S9). The NO releasing PVDF-HFP surface layer on the Teflon tube displayed a pale greenish color. The surface layer of the film on Teflon tube was very thin (0.264 ± 0.039 mm). The NO release profile of the NO-releasing PVDF-HFP polymer coating on Teflon tubing is shown Fig. 9. The NO flux number was observed to be $\sim 0.27 \times 10^{-10}$ mol/cm²-min on the first day. Then, NO flux number continuously decreased in the following days. With this very thin layer of polymer, a measurable NO release was still observed for 12 days under physiological conditions.

4. Discussion

The SNAP doped PVDF-HFP thin films can only have surface NO release on the first day, but they still displayed a greenish color on the second day, which is the characteristic color of SNAP. Therefore, we can conclude that a significant fraction of the SNAP crystals were “locked” within the PVDF-HFP polymer, and cannot further decompose to generate NO within the polymer phase. Indeed, the PVDF-HFP polymer is highly water resistant ($< 0.1\%$ (w/w)) [4]. Without the presence of significant water molecules, it seems that the SNAP molecules within PVDF-HFP polymer are quite stable. The NO release observed on the first day from SNAP doped PVDF-HFP films likely results from SNAP on or very near the outer surface dissolving into the soaking solution. In contrast, NO release in SNAP doped non-fluorinated polymers can last for several more months [33]. Hence, the NO release time period from SNAP doped PVDF-HFP polymer is totally different with those observed for SNAP doped non-fluorinated polymers. In our previous report, SNAP exhibited a zero-order ($100 \mu\text{M}$, $k = (1.586 \pm 0.012) \times 10^{-5} \mu\text{M}/\text{min}$, $t_{1/2} = 56.22$ h) thermal decomposition reaction in a mixture of PBS and DMSO (50:50, v/v) at 37°C [43]. However, under the same conditions, DiCF₃Bn-SNAP showed a much faster decomposition reaction, with a half-life of 6.49 h ($100 \mu\text{M}$, $k = (8.089 \pm 0.062) \times 10^{-5} \mu\text{M}/\text{min}$) [43]. Therefore, SNAP is much more (8.6 times) stable than DiCF₃Bn-SNAP in aqueous solutions. In addition, the stability of NO donors in the PVDF-HFP fluoropolymer can also be observed by the color change of NO donors doped films over time. The SNAP-doped PVDF-HFP film exhibits a

greenish characteristic color after soaking in the solution for 2 weeks. However, the green color of DiCF₃Bn-SNAP doped PVDF-HFP film gradually disappears over the same period, and finally turns into a pale yellow color over one week soaking period in the PBS solution at 37 °C. This result demonstrates that SNAP is much more stable in the PVDF-HFP fluoropolymer phase. To further simulate the decomposition of SNAP in the PVDF-HFP film, we characterized the films by ¹H NMR spectroscopy (see Fig. S2). The disulfide product derived from the decomposition of SNAP was observed based on ¹H NMR spectra after films soaked in the PBS solution at 37 °C over 1 day and several days after dissolving the films in THF-*d*₈. These results infer that SNAP still slowly decomposes in the PVDF-HFP film, consistent with the fact that *S*-nitrosothiols are thermally unstable. However, after continuous soaking over 8 d, very low NO release (~ 0.1 flux) was observed. This data also indicate that SNAP is thermal unstable in the polymer phase. In addition, after a long time of soaking in PBS, more moisture can incorporate into the thin film, which might also slightly accelerate the decomposition of SNAP. However, in fact, the NO flux number is observed to be too low for any specific application. Therefore, we conclude that SNAP is not a suitable NO donor for fabricating useful NO releasing fluorinated polymers.

The C₂F₅-SNAP doped PVDF-HFP film was also prepared for the purpose of comparison. Surprisingly, the resulting C₂F₅-SNAP doped film had the same color as pure PVDF-HFP film (see Fig. S3). The appearance of C₂F₅-SNAP doped in PVDF-HFP film clearly indicates that the C₂F₅-SNAP donor is almost completely decomposed during the preparation. Compared to the green color obtained in DiCF₃Bn-SNAP (15%) doped PVDF-HFP films (Fig. 3), the C₂F₅-SNAP donor demonstrates a much poorer stability in PVDF-HFP. This result is consistent with the storage stability of donors we have previously reported [42]. The C₂F₅-SNAP donor can decompose at room temperature and even decompose under -20 °C [42], while the DiCF₃Bn-SNAP species is stable at room temperature and under -20 °C [43]. Specifically, the C₂F₅-SNAP doped PVDF-HFP film can only release NO gas ($1.99 \pm 0.55 \times 10^{-10}$ mol cm⁻²min⁻¹) on the first day. This result concluded that C₂F₅-SNAP NO donor was quite unstable in the PVDF-HFP polymer. The short NO release time limits the further application of C₂F₅-SNAP doped in PVDF-HFP film.

The surface morphology of biomaterials plays a very important role in their roughness and performance. With the doping of fluorinated NO donors, the surface film is smoother (see Fig. 4c and 4d). The DiCF₃Bn-SNAP has a good solubility in CH₂Cl₂. Hence, any excess of DiCF₃Bn-SNAP present on the surface of PVDF-HFP film can be readily removed during this washing step. Based on the texture comparison between Fig. 4d and S4a, the fluorinated NO donor doped PVDF-HFP film has the same morphology before and after being washed by CH₂Cl₂. Thus, we presume that the fluorinated NO donor is homogeneously mixed within the polymer phase rather than physically deposited primarily on the surface of fluoropolymer during the drying process. Moreover, no gradient color was observed when we took an edge-on view of the film (after cutting) under a microscope, indicating the homogeneous dispersion of the DiCF₃Bn-SNAP within the PVDF-HFP polymer film. The DiCF₃Bn-SNAP doped (15%) PVDF-HFP film was further characterized by Auger Electron Spectroscopy. The corresponding element mapping images of nitrogen, oxygen and sulfur for DiCF₃Bn-SNAP doped (15%) PVDF-HFP film are shown in Fig. S4b within the

Supporting Information. All nitrogen, oxygen and sulfur elements were found to be uniformly distributed within the DiCF₃Bn-SNAP doped (15%) PVDF-HFP film. Since nitrogen, oxygen and sulfur elements are only present in the DiCF₃Bn-SNAP NO donor and not in the PVDF-HFP polymer, these results further confirm the homogeneous distribution of the NO donor throughout the film.

We also studied the mechanical properties of the NO release PVDF-HFP film. The corresponding stress-strain curves and the Young's modulus fit curves are shown in Fig. S5 within the supporting information. Interestingly, the ultimate breaking strength for the DiCF₃Bn-SNAP doped (15%) PVDF-HFP film (38.7 ± 0.4 N) was slightly higher compared to that of PVDF-HFP film (34.8 ± 1.8 N). An increase by about 20 mm in the maximum elongation was also observed for the NO release PVDF-HFP film when compared to the pure PVDF-HFP film. Furthermore, the Young's modulus of pure PVDF-HFP film was calculated to be 4.7 ± 0.5 MPa, while the NO release PVDF-HFP film had a Young's modulus of 3.5 ± 0.2 MPa. These results indicate that the DiCF₃Bn-SNAP (15%) doped PVDF-HFP films are more elastic than undoped PVDF-HFP. Thus, the mechanical properties have actually been improved by doping the polymer with the fluorinated DiCF₃Bn-SNAP NO donor. In fact, the increase of strength, elongation and elasticity by doping PVDF-HFP polymer with the fluorinated DiCF₃Bn-SNAP NO donor species could also be attractive for the potential biomedical applications of this new NO release PVDF-HFP film.

In our previous studies, we found that fluorinated DiCF₃Bn-SNAP has much lower thermal stability than SNAP under the same conditions [43]. SNAP impregnated in the PVDF-HFP polymer cannot yield any detectable NO gas *via* thermal decomposition at 37 °C after the very first day. However, due to the lower thermal stability of DiCF₃Bn-SNAP, the NO release from the fluorinated DiCF₃Bn-SNAP (15 wt%) doped PVDF-HFP polymer thin film can be observed for 16 days. This result also suggests that NO donor stability within the polymer plays a significant role in the observed NO release behavior. NO release from the *S*-nitrosothiols can be triggered *via* metal ion catalysis, photolysis or thermolysis. Due to the lack of a metal ion species and irradiation during the NO release measurements, we assume that the NO release from the PVDF-HFP polymer occurs *via* a thermal decomposition mechanism, especially in the presence of some trace moisture coming from the soaking solution. In the absence of water, the NO release ability from the NO donor within the polymer is completely dependent on the thermal stability of the NO donor. Based on our results, a less stable NO donor seems to be more suitable for use within fluoropolymers since such polymers are highly water resistant. In addition, the DiCF₃Bn-SNAP (15 wt%) doped PVDF-HFP film is stable under long-term freezer storage (-20 °C), and still showed the characteristic green color after more than 6 months of storage.

The chemical leaching greatly decreased (totally, $\sim 10\%$) for the NO release from PVDF tubing [42], due to the presence of fluorine and fluorine interactions. However, the PVDF-HFP copolymer has a greater percentage of fluorine atoms compared to pure PVDF. Accordingly, we also expected that leaching of the DiCF₃Bn-SNAP NO donor and its byproducts would be even lower from these PVDF-HFP films. Interestingly, the free thiol of DiCF₃Bn-SNAP was not formed, which is quite different than results found when SNAP was impregnated in non-fluorinated polymers [38]. However, this result is consistent with

the decomposition product formation from DiCF₃Bn-SNAP found in the solution phase, where no free thiol product was observed [43].

Compared to the leaching results of our previous fluorinated NO donor in PVDF tubing (totally, ~ 10% observed over 11 days) [42] and SNAP doped in CarboSil films (totally, ~ 47% of SNAP observed over 22 days) [38], the new PVDF-HFP film doped with DiCF₃Bn-SNAP has significantly reduced chemical leaching (totally, ~ 0.6% (mol%)). In addition to the fluorine-fluorine interaction between DiCF₃Bn-SNAP and the PVDF-HFP polymer [45–47], a secondary factor to cause such low chemical leaching might be the interaction between the aromatic ring of DiCF₃Bn-SNAP and the fluorine atoms within the PVDF-HFP polymer [46, 47]. Due to the presence of strong electron withdrawing group (two CF₃ groups) on the phenyl ring, the aromatic ring of DiCF₃Bn-SNAP becomes electron deficient and will have a slight positive charge [46, 47]. In contrast, the fluorine atoms in the PVDF-HFP polymer are electron rich and thus have a slight negative charge. Hence, due to both the existence of fluorine-fluorine and positive-negative charge interactions [46, 47], the total chemical leaching from the PVDF-HFP film doped with DiCF₃Bn-SNAP was found to be greatly reduced to < 1% after 9 days. It should be noted that concentration of leached disulfide (0.15 nmol/mg) was observed to be much lower compared to the intact NO donor DiCF₃Bn-SNAP (~ 1.86 nmol/mg) after 9 days of soaking. This data is reasonable based on the number of fluorine atoms in DiCF₃Bn-SNAP and its corresponding disulfide species. Indeed, the disulfide has double the number of fluorine atoms and two positively charged aromatic rings leading to an enhanced attraction with the PVDF-HFP polymer.

We also repeated the NO release measurements of DiCF₃Bn-SNAP doped (15%) PVDF-HFP film in a bovine serum medium over first 7 d. The corresponding NO release profile is summarized in Fig. S6. Interestingly, NO release results under serum conditions were similar to those obtained when the films are soaked in PBS (within experimental error). Thus, we can conclude that serum proteins are not likely to influence the NO release behavior for the DiCF₃Bn-SNAP doped PVDF-HFP film. In fact, any greater chemical leaching of the NO donor into the serum medium would likely lead to a decrease in the rate of NO release. However, the same NO release data was obtained when the films are soaked in serum and PBS. Therefore, the presence of the serum medium does not significantly influence the chemical leaching from the DiCF₃Bn-SNAP doped PVDF-HFP film. Otherwise, a totally different NO release profile would be obtained when soaked in serum if any further leaching of the NO donor occurred.

In fact, detecting the color change of film would not be an accurate method to determine the NO donor amount remaining in the polymer phase. To further characterize the level of DiCF₃Bn-SNAP NO donor present in the PVDF-HFP, ¹H NMR spectroscopy was utilized to analyze the amount of DiCF₃Bn-SNAP NO donor after dissolving the NO release film in THF-*d*₈ (see Fig. S8). After the DiCF₃Bn-SNAP (15%) doped PVDF-HFP film was soaked under physiological conditions over 13 d, the amount of DiCF₃Bn-SNAP remained in the film was measured to be ~ 13 % of the original loaded level based on the ¹H NMR results. Based on the cumulative calculation of NO release in Fig. 5, a total of 44.81 nmol/mg surface NO (only 13% of donor initially doped) was found during the 16 days of chemiluminescence testing. However, these NO-releasing PVDF-HFP films (15 wt%) were

initially doped with 336.55 nmol/mg of the DiCF₃Bn-SNAP NO donor. And since these NO releasing films exhibit extremely low leaching of chemicals (totally 2.16 nmol/mg, 0.6% of NO donor initially doped, see Fig. 6), we can presume that the majority of the NO was lost during the film preparation process, possibly resulting from the low stability of the DiCF₃Bn-SNAP NO donor. Hence, developing methods to prevent loss of the NO during the formulation process should be helpful to increase the levels of NO that can be emitted from the PVDF-HFP films (e.g., faster evaporation of the THF, etc.).

With respect to antimicrobial activity, several studies have shown that the pure PVDF-HFP copolymer does not have any intrinsic antimicrobial activity [17–19]. NO-releasing polymers are widely reported to have very significant antimicrobial activity to inhibit the risk of infection associated with various medical devices (e.g., intravascular catheters/cannula, etc.) [21]. For our NO-releasing PVDF-HFP films (15 wt%), it should be noted that given the low fluxes of NO emitted by these films after day 1, the observed antimicrobial activity, while not ideal, is still significant. In the application of these NO-releasing polymers for biomedical implants and devices, the amount of surface NO release is an important factor in preventing clotting and infection [21, 48]. However, although the average NO flux numbers from day 2 to day 16 were very low ($\sim 0.32 \times 10^{-10}$ mol/cm²-min), we still observed significant antimicrobial activity (see Figs. 7 and 8) toward both *S. aureus* and *P. aeruginosa* bacterial strains. These results are also in agreement with previous antimicrobial activity of NO release polymers toward *S. aureus*, *P. aeruginosa* and *S. epidermidis*, that had relatively low NO flux values ($\sim 0.3 \times 10^{-10}$ mol/cm²-min) [33, 48]. Furthermore, our group recently demonstrated that even lower NO fluxes of $< 0.3 \times 10^{-10}$ mol/cm²-min can successfully suppress subcutaneous tissue inflammatory response in animal studies [33]. Thus, even with the relatively low NO flux levels observed for these new formulations, it is expected that the PVDF-HFP films doped with DiCF₃Bn-SNAP can also prevent the inflammatory response often observed for subcutaneously implanted biomedical applications (e.g., insulin infusion cannula, etc.).

For device applications, the PDVF-HFP polymer can be easily engineered into different shapes (film or tubing) or simply coated on the surface of existing materials, such as stainless steel, Teflon, etc. We successfully demonstrated a more practical application of the NO releasing PVDF-HFP films by coating them on Teflon tubes (28 AWG). To date, the release of NO from Teflon material has been extremely difficult to achieve due to its chemical inertness. Only surface coating or fabrication of composites have provided a successful approach for achieving NO release from Teflon materials. For example, a diazeniumdiolate-based polymer coating on expanded PTFE vascular grafts was reported by the Ameer group [49], which exhibited sustained NO release for 2 days. In another study, a composite disc (1 mm) containing NO-loading Zinc zeolite and PTFE polymer (50%/50%) was fabricated by Fox et al. [50], which released NO for < 1 h. Compared to these Teflon-based NO release materials, our NO-releasing PDVF-HFP polymer coating on Teflon exhibits significantly longer term NO release behavior. The NO flux number was low, mainly due to the decomposition of NO donor during the coating and drying processes.

Based on the literature cited within the introduction to this paper, fluoropolymers have a long history of application in creating various biomedical implants and devices. However,

improvement in their antimicrobial activity and biocompatibility is required to further enhance their potential applications in preparing medical devices. Many NO releasing non-fluorinated materials have been reported to date. But NO releasing fluoropolymers have not been explored, and still remain quite challenging due their extremely hydrophobicity and high chemical inertness. So far, there is only one example from our group reported on NO releasing PVDF tubing [42]. In addition, a significant amount of NO donors and their byproduct species almost always leach into the surrounding aqueous environment from polymeric phase doped with NO donors. Indeed, leaching of the NO donors and/or their products formed after NO release can both decrease NO-release surface flux rates as well as NO release longevity, and further create the potential for toxicity. Therefore, studies on new approaches to decrease the chemical leaching is of great interest, not only for researchers in the academic community, but also those in the industrial area of biomedical materials. Furthermore, ref. [44] only reported the organic synthetic method for a family of fluorinated NO donors, rather than the NO release from fluorinated polymers of these completely new compounds, and observed antimicrobial activity. Hence, the new results of NO release from a PDVF-HFP polymer, the chemical leaching data, and the antimicrobial activity reported here are novel.

5. Conclusions

In summary, a NO-releasing PVDF-HFP polymer has been prepared by incorporating SNAP or a fluorinated SNAP derivative (DiCF₃Bn-SNAP) within films of this material. The NO-releasing PVDF-HFP films were fabricated *via* a solvent casting method. Nitric oxide release from SNAP doped PVDF-HFP films can only be observed on the first day. In contrast, the DiCF₃Bn-SNAP doped PVDF-HFP films can release NO for at least 16 days under physiological conditions. Using DiCF₃Bn-SNAP as a fluorinated NO donor, the chemical leaching from the PVDF-HFP films was greatly decreased. Data from HPLC-HRMS analysis indicated that only 0.6% (mol%) of initially doped DiCF₃Bn-SNAP species leached into the solution phase over a 9 days soaking period. This extremely low chemical leaching likely results from the presence of both fluorine-fluorine and charge-charge interactions between the DiCF₃Bn-SNAP molecule and the PVDF-HFP copolymer. The NO release exhibited from the new DiCF₃Bn-SNAP doped PVDF-HFP films can decrease growth of both *S. aureus* and *P. aeruginosa* on the surface of the polymer films (compared to control films without NO release). Further the NO-releasing PVDF-HFP copolymer can be coated on the outer and inner surfaces of Teflon tubing often used as cannula devices in medicine, to further improve the biocompatibility and antimicrobial activity of such devices. Even higher levels of NO release should be possible from these PVDF-HFP films containing the DiCF₃Bn-SNAP molecules, if decomposition of this NO donor during the casting/drying process can be greatly reduced, either by faster evaporation of the solvent, or using a different solvent system in which the NO donor is more stable. Efforts in this direction are currently in progress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of Significance

Fluoropolymers have been widely used in creating various biomedical implants and devices. However, nitric oxide (NO) release fluoropolymers have not been well studied to date. Additionally, in the application of biomaterials doped with NO donors, a significant amount of NO donors and their byproducts almost always leach into aqueous environment.

We now report an NO releasing poly(vinylidene fluoride-*co*-hexafluoropropylene) (PVDF-HFP) fluoropolymer by incorporating a new fluorinated *S*-nitrosothiol. The NO release can last for 16 days under physiological conditions. The total chemical leaching was determined to be only 0.6 mol% of the initial *S*-nitrosothiol loaded. As expected, significant antimicrobial/anti-biofilm activities of the NO release PVDF-HFP film were observed against Gram positive *S. aureus* and Gram negative *P. aeruginosa* bacterial strains.

Fig. 1.
Chemical structures of C₂F₅-SNAP, SNAP, DiCF₃Bn-SNAP NO donors and PVDF-HFP polymer.

Fig. 2. NO release profile on the first day from PVDF-HFP doped with SNAP (15 wt%) in PBS (10 mM, pH 7.4, 100 μ M EDTA) solution at 37 $^{\circ}$ C in the dark.

Fig. 3. Films of pure PVDF-HFP (left) and PVDF-HFP doped with DiCF₃Bn-SNAP NO donor (right).

Fig. 4. SEM images of pure PVDF-HFP (a and b) and DiCF₃Bn-SNAP (15 wt%) doped PVDF-HFP polymer (c and d) films.

Fig. 5.
NO flux for the PDVF-HFP film incorporated with DiCF₃Bn-SNAP under physiological conditions (PBS with 100 μ M EDTA at 37 $^{\circ}$ C) under dark conditions.

(a)

(b)

Fig. 6. Cumulative leaching of DiCF₃Bn-SNAP NO donor (a) and its corresponding disulfide species (b) into a PBS soaking solution (2.0 mL) from the DiCF₃Bn-SNAP (15 wt%) doped PDVF-HFP film at 37 °C in the dark.

Fig. 7. Results of antimicrobial studies for 7 days. (a) Plate count number of viable *S. aureus* on pure PVDF-HFP and DiCF₃Bn-SNAP doped PVDF-HFP films. (b) Plate count number of viable *P. aeruginosa* on pure PVDF-HFP and DiCF₃Bn-SNAP doped PVDF-HFP films.

Fig. 8. Anti-biofilm studies with *S. aureus* and *P. aeruginosa* bacterial strains for 7 d. (a) and (b) Representative fluorescence images of the biofilms on the film surfaces with *S. aureus* ((a): Control of PVDF-HFP film, (b): DiCF₃Bn-SNAP doped PVDF-HFP film). (c) and (d) Representative fluorescence images of the biofilms on the film surfaces with *P. aeruginosa* ((c): Control of PVDF-HFP film, (d): DiCF₃Bn-SNAP doped PVDF-HFP film).

Fig. 9. NO flux for the NO-releasing PDVF-HFP polymer coating on Teflon tubing under physiological conditions (PBS with 100 μM EDTA at 37 $^{\circ}\text{C}$) in the dark (Data was obtained from the NO release flux number of six samples (length: 1 cm) over 5 h on each day).