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Metal–Organic Framework/Chitosan Hybrid Materials Promote Nitric Oxide Release from *S*-Nitrosoglutathione in Aqueous Solution

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Abstract

It has been previously demonstrated that copper-based metal–organic frameworks (MOFs) accelerate formation of the therapeutically active molecule nitric oxide (NO) from *S*-nitrosothiols (RSNOs). Because RSNOs are naturally present in blood, this function is hypothesized to permit the controlled production of NO through use of MOF-based blood-contacting materials. The practical implementation of MOFs in this application typically requires incorporation within a polymer support, yet this immobilization has been shown to impair the ability of the MOF to interact with the NO-forming RSNO substrate. Here, the water-stable, copper-based MOF $H_3[(Cu_4Cl)_3(BTTRI)_8]$ (H_3BTTRI = 1,3,5-tris(1*H*-1,2,3-triazol-5-yl)benzene), or Cu-BTTRI, was incorporated within the naturally derived polysaccharide chitosan to form membranes that were evaluated for their ability to enhance NO generation from the RSNO *S*-nitrosoglutathione (GSNO). This is the first report to evaluate MOF-induced NO release from GSNO, the most abundant small-molecule RSNO. At a 20 μ M initial GSNO concentration (pH 7.4 phosphate buffered saline, 37 °C), chitosan/Cu-BTTRI membranes induced the release of $97 \pm 3\%$ of theoretical NO within approximately 4 h, corresponding to a 65-fold increase over the baseline thermal decomposition of GSNO. Furthermore, incorporation of Cu-BTTRI within hydrophilic chitosan did not impair the activity of the MOF, unlike earlier efforts using hydrophobic polyurethane or poly(vinyl chloride). The reuse of the membranes continued to enhance NO production from GSNO in subsequent experiments, suggesting the potential for continued use.

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Author Contributions

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Supporting Information

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pXRD for chemical stability and biological testing under varying pH values, blood, acidic and basic conditions for chitosan/Cu-BTTRI membranes; TGA analysis for chitosan and chitosan/Cu-BTTRI membrane; cross-sectional SEM images for chitosan/Cu-BTTRI membranes with EDS overlays ([PDF](#))

Notes

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Additionally, the major organic product of Cu-BTtri-promoted GSNO decomposition was identified as oxidized glutathione via mass spectrometry, confirming prior hypotheses. Structural analysis by pXRD and assessment of copper leaching by ICP-AES indicated that Cu-BTtri retains crystallinity and exhibits no significant degradation following exposure to GSNO. Taken together, these findings provide insight into the function and utility of polymer/Cu-BTtri systems and may support the development of future MOF-based biomaterials.

Graphical Abstract

Keywords

chitosan; metal–organic frameworks; nitric oxide; S-nitrosothiols; biomaterials

1. INTRODUCTION

Metal–organic frameworks (MOFs) are crystalline structures consisting of organic linkers bound to metal centers, producing highly porous lattices that are frequently utilized in gas storage and heterogeneous catalysis, among other applications.¹ It has been previously reported by Harding et al. that copper-based MOFs may be capable of inducing the nitric oxide (NO)-forming decomposition of *S*-nitrosothiols (RSNOs), a class of NO-donating compounds that are unique in their biological occurrence.² NO itself is produced endogenously by a family of NO synthase enzymes, where it contributes to the immune response, neurotransmission, and regulation of vascular tone.³ Biological NO production is essential to the wound-healing process, and exogenous NO supplementation has been shown to promote accelerated wound closure.^{4–6} These properties have led to the exploitation of copper-based MOFs for their ability to induce the release of NO directly from RSNOs. Biological RSNOs are believed to function collectively as a physiological reservoir of NO, most commonly in the form of *S*-nitrosoglutathione (GSNO) and *S*-nitrosoalbumin, which exist in blood in the nM to μ M range.^{7,8} Biologically relevant GSNO and other RSNO species have been established to produce NO through thermal decomposition or photolysis that leads to the formation of disulfide, according to the equation $2\text{RSNO} \rightarrow 2\text{NO} + \text{RSSR}$.⁹ In addition to these reaction pathways, it has been shown that exposure to copper ions induces the catalytic, NO-forming decomposition of many RSNOs.¹⁰ This phenomenon has led to the development of copper-based materials intended to produce a localized supply of

NO through the controlled decomposition of bioavailable RSNOs, typically through inclusion of simple copper salts or complexes.^{11,12}

The ability of copper-based MOFs to promote NO release from RSNOs is exemplified by the decomposition of *S*-nitrosocysteine and *S*-nitrosocysteamine by copper benzene-1,3,5-tricarboxylate (HKUST-1) under nonaqueous conditions.^{2,13} It was later discovered that the triazolate-bridged framework (Figure 1a) derived from reaction of 1,3,5-tris(1*H*-1,2,3-triazol-5-yl)benzene (H3BTri) with CuCl₂·H₂O in DMF (H₃[(Cu₄Cl)₃-(BTri)₈], or Cu-BTTri) exhibits a similar ability to promote the decomposition of both *S*-nitrosocysteamine and *S*-nitroso-*N*-acetylpenicillamine (SNAP) under aqueous, physiological conditions and exhibits an exceptional degree of aqueous stability.^{14,15} For this reason, Cu-BTTri represents a useful candidate for the development of MOF-based biomaterials that are intended to trigger NO release from RSNOs in a physiological environment. However, the feasibility of utilizing copper-based MOFs for this application depends on their effective incorporation within a supporting polymeric matrix and the retention of useful activity thereafter. Consequently, the identification of polymers that allow the productive, NO-forming interaction between aqueous-phase RSNOs and Cu-BTTri is a prerequisite to biological implementation. Nevertheless, previous work has been confined to hydrophobic polymers that are unlikely to permit the rapid diffusion of polar (and potentially charged) RSNO substrates. For this reason, hydrophilic materials such as polysaccharides may represent a useful alternative.

Chitosan is a versatile, naturally derived polysaccharide consisting of β -(1,4)-linked glucosamine and *N*-acetyl glucosamine units (Figure 1b). Because chitosan is biodegradable, typically regarded as nontoxic, and exhibits important therapeutic properties, it has been widely utilized for biomedical applications ranging from drug delivery to tissue engineering.¹⁶ The conversion of chitosan to water-soluble polycationic salts or other easily processed derivatives permits the preparation of films, fibers, hydrogels, and other materials with useful characteristics.¹⁷ As a biomaterial, chitosan has been reported to exhibit inherent bacteriostatic/antimicrobial properties, in addition to well-characterized hemostatic properties that have resulted in its commercial adoption for the treatment of hemorrhagic injuries.¹⁸ Furthermore, numerous studies have shown that chitosan-based wound dressings can significantly accelerate the wound healing process.^{19,20} Notably, both the wound-healing and antimicrobial properties of chitosan overlap significantly with those of NO, suggesting the possibility for combined action. Due to the potential for NO and chitosan to exhibit synergistic effects as a therapeutic, multiple publications have reported the development of NO-releasing materials based on chitosan. NO-releasing chitosan derivatives have been proposed as potential candidate polymers for the development of wound-healing dressings, while NO-releasing chitosan oligosaccharides have been utilized as solution-phase antibacterial agents.^{21–23} These previous examples of NO-releasing chitosan derivatives have been universally prepared through the inclusion of unstable synthetic NO-donor species that release a finite quantity of NO through irreversible decomposition processes. In wound dressings, this may produce complications arising from both storage limitations and toxicity concerns.²⁴ For these reasons, an alternative method of producing localized NO release is desirable. In the case of potential blood-contacting materials, this goal may be achieved through the recruitment of natural RSNOs.^{25,26}

Herein, the concept of promoting accelerated NO release from naturally occurring GSNO using chitosan membranes containing water-stable Cu-BTtri was investigated. This work represents the first inclusion of a water-stable MOF within chitosan and expands the potential biological utility of Cu-BTtri blends to nonsynthetic polymers. Cu-BTtri was incorporated within a chitosan matrix and characterized by pXRD, ATR-FTIR, and SEM. The chitosan/Cu-BTtri membranes were subsequently exposed to GSNO at physiological temperature and pH, and chemiluminescence-based NO detection was utilized to confirm that the MOF retained its ability to accelerate the NO-forming decomposition of the RSNO substrate. Confinement of Cu-BTtri within the polysaccharide was not observed to significantly impact the enhanced NO generation observed when unbound Cu-BTtri particles were tested separately with GSNO. Additionally, mass spectrometry was used to qualitatively determine the major product of the MOF-promoted decomposition of GSNO as oxidized glutathione (GSSG). Although it is known that thermal or photolytic RSNO decomposition typically results in the formation of the corresponding disulfide dimer ($2\text{RSNO} \rightarrow 2\text{NO} + \text{RSSR}$), this is the first report to definitively identify the production of disulfide from an RSNO by Cu-BTtri.²⁷ These findings reveal that Cu-BTtri may be successfully immobilized within chitosan membranes without impairment of its ability to induce NO release from GSNO, providing further insight into the function of such MOF/polymer systems. The ability of Cu-BTtri to generate an enhanced level of NO release from GSNO complements the existing therapeutic uses of chitosan, particularly in the realm of future wound-healing applications. While previous reports have examined promotion of NO release from putatively endogenous *S*-nitrosocysteine, this structure primarily occurs in the form of *S*-nitrosated cysteine residues in biological macromolecules, which may limit the ability of a MOF to interact with the RSNO and induce NO release.²⁶ Similar efforts have been constrained to *S*-nitrosocysteamine and SNAP, which are not known to occur biologically. This work is the first to expand the substrate scope to include GSNO, a biologically available RSNO identified in human blood. Furthermore, the use of water-stable and potentially reusable Cu-BTtri and naturally derived chitosan represents a new and potentially more environmentally benign approach to achieving NO release from RSNOs compared to previous systems based on synthetic polymers.

2. METHODS

2.1. Materials.

1,3,5-Tribromobenzene (98%), trimethylsilyl-acetylene (98%), trimethylsilyl azide (94%), and diethylamine (99%) were purchased from Alfa Aesar (Ward Hill, MA, USA). Sodium nitrite (97%), phosphate buffered saline (PBS) tablets, and copper(II) chloride dihydrate were obtained from EMD Chemicals (Gibbstown, NJ, USA). Glutathione (98%) was purchased from AMRESCO (Solon, OH, USA). Low molecular weight chitosan (96% deacetylated) and copper(I) iodide (99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water (18.2 MΩ-cm) was obtained from a Millipore Direct-Q water purification system (EMD Millipore, Billerica, MA, USA). Ultrahigh purity nitrogen and oxygen gases were supplied by Airgas (Denver, CO, USA). Bis(triphenylphosphine)-palladium(II) dichloride (98%) was purchased from TCI America (Portland, OR, USA). All materials were used as received without any further purification.

2.2. Characterization Methods.

Powder X-ray diffraction (pXRD) measurements and ATR-FTIR were used to examine the incorporation of Cu-BTtri within chitosan. All pXRD measurements were made using a Bruker D-8 Discover DaVinci X-ray diffractometer (Cu K α X-ray source, line focus) (Bruker, Billerica, MA, USA). A 0.6 mm divergent slit was placed on the primary beam side and a high-resolution energy-dispersive LYNXEYE-XE-T detector on the diffracted beam side during the XRD studies. XRD measurements were performed with Soller slits on the primary and diffracted beam side (2.5° separation). The instrument alignment was tested using the NIST 1976b SRM. All measurements were performed at 4–50 θ with a step count of 1.0. ATR-FTIR spectra were obtained in the range of 600–4000 cm⁻¹ on a Nicolet 6700 FTIR spectrometer equipped with a Smart iTR ATR sampling module and ZnSe crystal plate (Thermo Electron Corporation, Madison, WI, USA). Images of chitosan/ Cu-BTtri membranes were acquired using a JEOL JSM-6500F scanning electron microscope with an accelerating voltage of 10.0 kV and a working distance of 10.0 mm (JEOL USA Inc., MA, USA). All samples were placed under vacuum and coated with 20 nm of gold prior to runs. Images were taken at magnifications of 250 $\times$, 1000 $\times$, 2000 $\times$, and 3000 $\times$. All data were processed using TEAM Software. Thermogravimetric analysis (TGA) was performed under nitrogen on a TA Instruments Q500 thermogravimetric analyzer at a rate of 5 °C/min from 25 to 500 °C (New Castle, DE, USA), and data were processed using TA Instruments Universal Analysis 2000.

2.3. Synthetic Methods.

Preparation of H₃[(Cu₄Cl)₃(BTtri)₈-(H₂O)₁₂]-72H₂O (Cu-BTtri-H₂O).—1,3,5-Tris(1*H*-1,2,3-triazol-5-yl)-benzene (H₃BTtri) was synthesized following the method reported by Demessence et al. and suspended (225 mg) in 10 mL of DMF.²⁸ This mixture was treated with 0.1 M hydrochloric acid to dissolve the triazole, and then copper(II) chloride dihydrate (383 mg) was added to the solution. After thorough agitation to ensure dissolution of all solids, the mixture was heated at 100 °C for 3 d to form Cu-BTtri-DMF as a dark violet precipitate. Cu-BTtri-DMF was isolated by centrifugation and washed with DMF and Millipore water, then heated in water at 80 °C for 3 d to produce Cu-BTtri-H₂O as a light purple solid. This solid was isolated by centrifugation, dried, and subsequently characterized by ATR-FTIR and pXRD.

Preparation of Chitosan Membranes.—Chitosan (2.5 g) was dissolved in 100 mL of 1% acetic acid, and the resulting water-soluble chitosan acetate was isolated by lyophilization. To prepare chitosan membranes, chitosan acetate (180 mg) was dissolved in 6 mL of Millipore water, followed by the addition of Cu-BTtri-H₂O (20 mg) to form a suspension. This mixture was allowed to air-dry within a PTFE mold, and the prepared film was treated twice with 100 mL of 250 mM pH 8.0 sodium phosphate buffer to produce a flexible hydrated sheet. This material was washed with 5 $\times$ 100 mL of Millipore water and subsequently punched into smaller 13 mm diameter membranes. Chitosan control membranes were prepared without Cu-BTtri-H₂O, following an otherwise identical procedure.

Synthesis of GSNO.—GSNO was prepared using a protocol adapted from a previously reported synthesis.²⁹ 1.53 g of glutathione was suspended in 8 mL of water at 0 °C, followed by the addition of 2.5 mL of 2 M hydrochloric acid and 0.345 g of sodium nitrite. This mixture was stirred at 0 °C for 40 min, and then the precipitated solid was isolated by filtration and washed with ice-cold water, acetone, and diethyl ether. The resulting light pink powder was placed under vacuum to remove residual solvent and stored at –20 °C in the absence of light. Subsequent to synthesis, an aqueous solution of GSNO was analyzed by ICP-AES, where no copper was detected. UV–vis: λ 335 ($\pi \rightarrow \pi^*$), 545 nm ($n_N \rightarrow \pi^*$).

2.4. Analytical Methods.

Membrane Swelling Properties.—Water swelling of chitosan and chitosan/Cu-BTtri membranes was determined gravimetrically by obtaining their dry masses (W_d), followed by immersion in Millipore water for 24 h. Samples were then withdrawn and reweighed (W_w) after the removal of excess water. The following calculation was then applied to determine the overall water uptake and swelling ratio of each sample.³⁰

$$\% \text{ swelling} = \frac{W_w - W_d}{W_d} \times 10$$

Measurement of NO Formation.—NO release measurements were obtained using Sievers chemiluminescence-based nitric oxide analyzers (NOA 280i, GE Analytical, Boulder, CO, USA). Calibration was performed with ultrahigh purity nitrogen (0 ppm of NO) and 45 ppm of NO/nitrogen. Prior to use, dry membranes were immersed in Millipore water overnight to produce 13 mm diameter hydrated membranes. In each experiment, a single chitosan/Cu-BTtri ($n = 3$) or chitosan control ($n = 3$) membrane was suspended in 5 mL of deoxygenated PBS (pH 7.4) in a custom analysis cell connected directly to an NO analyzer under a constant flow/purge of nitrogen gas and protected from exposure to light. Baseline was acquired for several minutes, and then an aqueous GSNO solution was injected into the cell to produce an initial concentration of 20 μ M. After GSNO injection, each experiment was allowed to continue until NO detection returned to the baseline level. As an additional control, the NO release profile from the decomposition of GSNO in the absence of membranes was also obtained. In all cases, gas phase NO concentration (ppb/ppm) was used to determine total NO release (mol) using an instrument-specific calibration constant obtained from the reduction of sodium nitrite. Following each experiment, the membrane was removed and the buffer solution was analyzed for copper content using ICP-AES. To evaluate reuse of the chitosan/Cu-BTtri membranes, each previously tested membrane was resuspended in Millipore water overnight and the previously described experimental procedure was repeated.

Mass Spectrometry Analysis.—The products formed following exposure of Cu-BTtri to GSNO were determined by suspension of approximately 20 mg of chitosan/Cu-BTtri membrane in a 1 mM solution of GSNO/Millipore water. The experiment was protected from light and maintained at 37 °C until disappearance of the characteristic red/pink color of GSNO. A control experiment was similarly conducted using chitosan membrane without

Cu-BTtri (intended to reflect thermal decomposition of GSNO), while a second control experiment involved exposure of GSNO to 365 nm UV light using a 100 W Blak-Ray B-100AP UV lamp (UVP, Upland, CA, USA). The solutions were subsequently analyzed using an Agilent 6224 LC-MS (Agilent Technologies, Palo Alto, CA). The instrument was equipped with an Agilent multimode ionization (MMI) source capable of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). For all the performed analyses, ESI in positive polarity was employed with the mass measurement range set at 70–3200 m/z . The sample solutions were introduced into the ion source using flow injection at a flow rate of 0.22 mL/min via the ESI nebulizer using a mobile phase consisting of 80/20 water/acetonitrile with 0.1% formic acid. The ion source conditions were as follows: capillary voltage, 3000 V; fragmentor voltage, 200 V; skimmer voltage, 60 V; drying gas temperature, 310 °C; drying gas flow-rate (nitrogen gas), 10 L/min; and nebulizer pressure, 45 psi. All collected data were analyzed with Agilent MassHunter Qualitative Analysis software (B.07.00).

Statistical Analysis.—Data are reported as the mean $\pm$ standard deviation of a minimum of triplicate measurements, unless otherwise noted.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of Cu-BTtri and Chitosan/Cu-BTtri Composite Materials.

Chitosan-based biomaterials have been shown to accelerate the wound healing process, resulting in significant overlap with the wound-healing properties of NO. Although previous reports have examined the inclusion of NO donor moieties within chitosan, this report is the first to evaluate an alternative approach involving the incorporation of Cu-BTtri, a copper-based MOF that has demonstrated the ability to promote release of NO from endogenous RSNOs. As previously described by Demessence et al., the cubic sodalite framework of Cu-BTtri consists of $[\text{Cu}_4\text{Cl}]^{7+}$ square planar units bound to BTtri^{3-} ligands. Within this structure, each triazolate ligand interacts with six distinct copper sites in $[\text{Cu}_4\text{Cl}]^{7+}$ units. This fundamental building unit is depicted in Figure 1a. Interactions between the triazolate ligands and copper produce an increase in metal–ligand bond strength, conferring greater water stability in comparison to many copper carboxylate based structures.

To prepare chitosan/Cu-BTtri membranes, water-soluble chitosan acetate was dissolved in Millipore water and Cu-BTtri was blended into the mixture to produce membranes containing 10% w/w MOF (Figure 2). The polysaccharide was deprotonated and rendered insoluble by treatment with 250 mM pH 8 phosphate buffer, resulting in 13 mm diameter circular chitosan membranes containing 295 ± 8 nmol of Cu mg^{-1} (11% w/w Cu-BTtri), as determined by dissolution and subsequent copper determination via ICP-AES. While aqueous sodium hydroxide is typically utilized to cure chitosan membranes, it was observed that treatment of Cu-BTtri with concentrated base had an adverse effect on the integrity of the MOF.³¹ Similarly, immersion of chitosan acetate/Cu-BTtri membranes in 10% sodium hydroxide produces a visual color change (dark violet to blue) and the loss of Cu-BTtri diffraction peaks when examined by pXRD. In order to ensure preservation of the MOF,

comparatively mild pH 8 phosphate buffer was used as an alternative method of neutralizing the membranes. To isolate the effect of Cu-BTtri in subsequent analyses, control membranes were prepared without addition of the MOF. Both chitosan/Cu-BTtri and chitosan control membranes were characterized by ATR-FTIR, pXRD, and SEM. IR absorptions characteristic of chitosan are present in the control membranes, including the broad hydroxyl O–H stretch feature occupying the region of 3650–3000 cm^{-1} (Figure 3a). This feature overlaps with the bands associated with the C2 primary amine, and both asymmetric and symmetric N–H stretching modes are identifiable at 3355 and 3278 cm^{-1} . Additional diagnostic bands are observed at 2849 (C–H stretch), 1636 (amide I), 1577 (amide II), 1056, and 1025 (C–O stretch). These typical features are accompanied by new absorptions at 2917 (acetate CH_3), 1542 (acetate salt), and 1258 cm^{-1} (phosphate), arising from preparation of the membrane. Incorporation of Cu-BTtri produces additional features characteristic of the MOF at 1617 (C=C stretch), 830, and 775 cm^{-1} (C–H out-of-plane bend). Furthermore, finished membranes exhibited the major pXRD diffraction peaks associated with Cu-BTtri (Figure 3b). The pXRD spectrum in conjunction with ATR-IR data indicates that the MOF is indeed present and has retained crystallinity after incorporation into the chitosan membrane.

Imaging of the membranes by SEM showed clear incorporation of Cu-BTtri within the chitosan matrix (Figure 4c–f) with distinct crystals observed at the membrane surface. In Figure 4b, the distribution of Cu-BTtri at the surface of the membrane is shown. At greater magnification, the octahedral morphology of Cu-BTtri was evident, and the crystals displayed an apparent trimodal particle size distribution with an overall range of 1–15 μm (Figure 4e,f). The materials were further evaluated for overall copper distribution by SEM-EDX using a copper analysis probe. Figure 4e and Figure 4f depict the copper overlay on the SEM image of the chitosan/Cu-BTtri membrane. As can be observed, the overall distribution of copper is concentrated in regions that contain Cu-BTtri. Cross-sectional SEM images of nonhydrated chitosan/Cu-BTtri membrane indicated a thickness of 150 μm (Figure 4 g). Additionally, SEM-EDS was performed on the cross-section to assess Cu-BTtri particle distribution throughout the bulk of the membrane (Figure 4g,h). This analysis showed copper distributed within the membrane, with greater concentration in regions of noticeable particle agglomeration (Figure S1 in Supporting Information). Taken together, these findings suggest dispersion of Cu-BTtri throughout the entire membrane, with certain areas of higher concentration indicative of Cu-BTtri agglomeration following incorporation within the polymer matrix.³²

Additionally, the water swelling ratio of each material was determined gravimetrically after 24 h of immersion to assess if incorporation of Cu-BTtri into the chitosan membrane produced changes to the ability of the polymer to uptake water. Chitosan/Cu-BTtri membranes exhibited $244 \pm 26\%$ swelling, while chitosan control membranes swelled by $240 \pm 26\%$. These results suggest that incorporation of Cu-BTtri does not have a significant impact on the ultimate water uptake of the chitosan materials. The stability of both the chitosan membrane and incorporated Cu-BTtri at pH 8 was established by characterization carried out subsequent to a preparatory step performed in pH 8 phosphate buffer (Figure 3b). Behavior of the membrane under other pH conditions was assessed by immersing the materials in Millipore water and adjusting the pH with dilute hydrochloric acid or sodium

hydroxide. The stability of the membrane when directly exposed to either 1 M hydrochloric acid or sodium hydroxide was also examined. Immersion in 1 M hydrochloric acid produced rapid decolorization and deterioration of the membrane, resulting from both the dissolution of chitosan and digestion of the MOF. At pH 4–5, the membrane exhibited swelling and a change in appearance consistent with the early stages of dissolution. This outcome was anticipated from the known solubility of chitosan following protonation in hydrochloric acid. Despite the onset of polymer dissolution, pXRD of the membrane indicated that Cu-BTTri remained intact under mildly acidic conditions (Figure S2e,f). At pH values of 6 and 9, the membrane retained a normal appearance and did not exhibit signs of swelling or deterioration. Subsequent pXRD characterization supported the survival of the MOF within this pH range, as well (Figure S2g,h). Immersion of the membrane in 1 M NaOH resulted in a pronounced color change from dark violet (imparted by Cu-BTTri) to blue and associated loss of the MOF diffraction pattern (Figure S2i). To examine the ability of the chitosan/Cu-BTTri membrane to tolerate biological media, samples of the material were immersed in both whole blood and platelet-rich plasma for 2 h, followed by rinsing with water. The stability of the MOF was then evaluated by pXRD, which showed the retention of all major diffraction peaks (Figure S2c,d). Analysis of chitosan/Cu-BTTri membrane by TGA yielded an onset decomposition temperature of 232 ± 0.12 °C, a value that was consistent with the decomposition of chitosan alone (Figure S3). In general, Cu-BTTri membrane appeared to demonstrate appropriate chemical and thermal stability for potential use in biomedical applications, where extreme pH or temperature conditions are not anticipated.

3.2. Evaluation of Enhanced NO Generation from GSNO with Chitosan/Cu-BTTri Membrane.

As previously noted, NO exhibits numerous beneficial properties, including the ability to accelerate healing when delivered to the site of an injury.^{4–6} It was reasoned that the ability of Cu-BTTri to significantly enhance the rate of NO release from biologically relevant RSNOs could potentially permit the conscription of these endogenous species as useful sources of NO. The retention of this function when incorporated within a chitosan membrane may allow for the future development of blood-contacting materials exhibiting both the desirable antimicrobial and wound-healing effects of chitosan, as well as the ability to generate enhanced levels of therapeutic NO from endogenous sources. To assess the ability of chitosan/Cu-BTTri membranes to generate NO from physiologically relevant GSNO, membranes were swelled in Millipore water (18.2 MΩ·cm) and subsequently immersed in pH 7.4 10 mM phosphate buffered saline (PBS) at 37 °C inside custom gas-flow analysis cells connected to Sievers chemiluminescence-based NO analyzers (NOA 280i). The effect of chitosan/Cu-BTTri membranes on NO formation from GSNO was evaluated through the addition of 100 nmol of GSNO to the analysis cell (sufficient to produce a 20 μM initial concentration), and the resulting gas-phase NO concentration (ppb) was recorded under nitrogen atmosphere until reaching baseline levels ($n = 3$) (Figure 5). Subsequently, a predetermined NO calibration constant (mol of NO ppb⁻¹ s⁻¹) was used to determine the overall NO release (mol). Control experiments ($n = 3$) were performed using GSNO alone, chitosan membranes without incorporated Cu-BTTri, and Cu-BTTri in free particulate form (equivalent to the mass incorporated within chitosan/Cu-BTTri membranes). In all cases, experiments were protected from light to avoid photolytic

decomposition of GSNO. At the end of experiments involving chitosan/Cu-BTtri membranes, the reaction medium was exposed to light to test for the presence of residual, nondecomposed GSNO.

When GSNO was added to analysis cells containing chitosan/Cu-BTtri membranes, a noticeable increase in NO generation was observed in comparison to the behavior of GSNO alone (Figure 6a). In the presence of chitosan/ Cu-BTtri membranes, NO generation was found to continuously increase over the duration of the experiment until depletion of the available GSNO after approximately 4 h (Figure 6a). In contrast, the decomposition and corresponding NO release of GSNO alone remained steady over the same interval, indicating the clear role of chitosan/Cu-BTtri membranes in promoting NO formation from the RSNO substrate. Chitosan/Cu-BTtri membranes induce a mean release of 97 ± 3 nmol of NO (mean $\pm$ SD) over 4.3 ± 0.3 h, resulting in 97% recovery of total theoretical NO (Figure 6b, Table 1). Comparatively, GSNO decomposition in the absence of chitosan/Cu-BTtri membranes resulted in the recovery of 1.5 ± 0.7 nmol of NO (1.5% of theoretical) over the same mean interval (Figure 6b, Table 1). In order to provide support that membrane-enhanced NO generation was primarily attributable to the inclusion of Cu-BTtri, chitosan membranes without incorporated MOF were assessed, and a moderate increase in GSNO decomposition (9.8 ± 0.3 nmol of NO, 9.8% of theoretical) was observed (Figure 6b, Table 1). This elevation was constant and did not result in the distinctive NO release profile produced by Cu-BTtri/membranes and may be attributable to the presence of trace metal ions chelated by the chitosan matrix (e.g., Fe^{2+}), which have been previously demonstrated to enhance NO generation.^{33,34} Overall, it was observed that chitosan/Cu-BTtri membranes are capable of producing a 65-fold increase in NO generation over the baseline thermal decomposition of GSNO, with a significantly smaller 7-fold elevation observed for chitosan control membranes.

After the chitosan/Cu-BTtri membranes were demonstrated to successfully enhance NO generation from GSNO, they were reused to produce NO from subsequent additions of GSNO (up to 3 additional times). Following initial experiments, the membranes were removed from the reaction medium and suspended in Millipore water for 1 d. The NO generation experiment was then repeated as described previously (3 times), with a consistent quantitative NO recovery (Table 2). This result suggests that the chitosan/Cu-BTtri membranes possess the capability for reuse, potentially indicating the ability to continuously generate NO from endogenous GSNO. This property may reduce concerns related to the finite loading currently associated with many NO-releasing polymeric materials.

3.3. Impact of Cu-BTtri Immobilization on Enhancement of NO Generation.

To evaluate the effect of Cu-BTtri immobilization within chitosan on the activity of the MOF, the ability of free Cu-BTtri particles to decompose GSNO was examined for comparison. Cu-BTtri particles were found to promote the release of 97 ± 6 nmol of NO (97% of theoretical) over 5.0 ± 1.6 h, indicating the absence of a statistical difference between the performance of chitosan/Cu-BTtri membranes and free Cu-BTtri particles (Figure 6a and Figure 6b, Table 1). In contrast, it was previously shown that incorporation of Cu-BTtri within a biomedical grade polyurethane resulted in an 8-fold reduction in the

activity of Cu-BTTRI.¹⁴ Additionally, Cu-BTTRI particles incorporated into poly(vinyl chloride) (PVC) were shown to require 10 h to reach a steady rate of NO release in the presence of SNAP.¹⁵ These observed differences in NO generation may be attributable to the restricted ability of RSNO to diffuse through hydrophobic polymer matrices, inhibiting interaction with incorporated Cu-BTTRI.^{15,35} This hypothesis is supported by the calculated swelling ratio of 244% for chitosan/ Cu-BTTRI membranes, indicating significant uptake of water, while PVC and polyurethane compositions exhibit weight increases from water uptake ranging from 0.02% to 20%.^{36–38} Since hydrophilic chitosan membranes readily swell in the presence of water, this process may encourage uptake and diffusion of GSNO, permitting more rapid interaction with the MOF. It was also observed that Cu-BTTRI particles showed wider variability in the overall time required to fully decompose GSNO, which ranged from 3 to 7 h. This variability may be due to random particle agglomeration resulting in decreased access to Cu-BTTRI active sites, while immobilization of the MOF within the chitosan membrane limits this outcome. Because incorporation of Cu-BTTRI within chitosan reduces variability in reaction time, it allows for increased control of NO generation from GSNO. For this reason, further understanding of these types of MOF/polymer composite systems may provide valuable insight into the tunability of such materials for future applications.

3.4. Evaluation of Structural Integrity and Copper Leaching.

Following NO release experiments with GSNO, the chitosan/Cu-BTTRI membranes were analyzed by pXRD to ensure that they remained structurally intact following NO generation. The observed pXRD pattern retained all major diffraction peaks corresponding to Cu-BTTRI, supporting that the MOF remains intact over the course of the experiment (Figure 6c). Furthermore, the buffer solutions remaining at the end of each experiment were subjected to copper content determination by ICP-AES after the removal of the chitosan/ Cu-BTTRI material. This analysis tests for the presence of copper that may have been released by deterioration of the MOF or detachment of fine MOF particles. Results indicated that $(1.5 \pm 1.1) \times 10^{-9}$ mol of copper was present in solution following exposure to GSNO during NO release experiments, corresponding to $0.10 \pm 0.07\%$ of theoretical (based on the mass of incorporated Cu-BTTRI) and resulting in a mean copper concentration of approximately 3×10^{-7} M. This lack of significant copper leaching was particularly encouraging due to the fact that solution-phase glutathione (the parent thiol of GSNO) is an established copper chelator.³⁹

GSNO decomposition experiments performed in the presence of copper(II) chloride at a concentration of 3×10^{-7} M did not result in a greater rate of NO production, as evaluated by chemiluminescence-based detection. This outcome reduces the likelihood that dissolved copper ions contribute to the acceleration of the NO-forming decomposition of GSNO and is consistent with reports from Williams et al., where higher copper concentrations of 10^{-6} M were needed to initiate decomposition.⁹ Over a total of three subsequent cycles (each performed in quadruplicate), a mean copper quantity of $(5.2 \pm 3.5) \times 10^{-9}$ mol of copper was found in solution (corresponding to $0.3 \pm 0.2\%$ of theoretical), with individual cycles varying from $(1.9 \pm 0.3) \times 10^{-9}$ to $(8.8 \pm 9.6) \times 10^{-9}$ mol. These values were not found to statistically differ from the amount of copper determined in the original experiment and

generally indicate that loss of copper from the membranes remains low. To contrast potential MOF-related copper leaching from chitosan/Cu-BTtri membranes with that from copper(II) ions directly chelated to chitosan, 13 mm chitosan membranes were exposed to copper(II) chloride to prepare additional control materials containing an average of 300 nmol of Cu mg⁻¹. These membranes were exposed to GSNO as described in previous experiments, and the buffer solution was subsequently tested for copper content. Notably, the NO-forming reaction completed within 0.68 ± 0.08 h and resulted in the recovery of 86 ± 6 of theoretical NO, indicating a significantly faster rate of GSNO decomposition. More importantly, ICP-AES was used to determine a solution-phase copper content equivalent to $3.6 \pm 0.8\%$ of theoretical, indicating up to 36-fold greater leaching than in the case of chitosan/Cu-BTtri membranes.

Previous publications have described the incorporation of copper nanoparticles or copper complexes within polymers to produce materials capable of promoting NO release from RSNOs. These approaches often rely upon the deliberate generation of copper ions or use of labile complexes that readily release copper ions into solution.^{40,41} In one significant report, Hwang et al. covalently attached copper(II)–cyclen complexes to biomedical polymers and evaluated these materials for their ability to induce NO-forming RSNO decomposition.⁴² In this example, copper leaching as high as 25% was observed, which may limit applicability due to toxicity concerns. In comparison, the substantially lower copper leaching observed for chitosan/ CuBTtri membrane suggests greater retention of copper attributable to the stability of the MOF. In the case of potential biological applications, this lower level of copper leaching may reduce concerns related to copper toxicity.⁴³

3.5. Determination of Reaction Products.

To complement data supporting the preservation of Cu-BTtri following NO release experiments with chitosan/Cu-BTtri membranes, an additional experiment was performed in which chitosan/ Cu-BTtri membrane was exposed to a higher 1 mM concentration of GSNO in Millipore water at 37 °C. This solution was subsequently analyzed via mass spectrometry to screen for evidence of Cu-BTtri degradation, which was anticipated to regenerate the triazole ligand H₃BTtri. This compound was not detected in solution following the experiment, supporting the lack of MOF degradation. The thermal decomposition of GSNO in the presence of chitosan membrane (without incorporated Cu-BTtri) and the UV-initiated decomposition of GSNO in the absence of any polysaccharide were carried out for comparative purposes. In all cases, GSSG (C₂₀H₃₂N₆O₁₂S₂) was identified as the major organic decomposition product of GSNO (Table 3, Scheme 1). In comparative experiments performed using chitosan membrane at 37 °C or GSNO alone in the presence of UV light, the formation of GSSG was anticipated by the equation $2\text{RSNO} \rightarrow 2\text{NO} + \text{RSSR}$, which describes the decomposition of RSNO to form the corresponding disulfide dimer.⁹ In the case of chitosan/Cu-BTtri membranes, the identification of GSSG provides new evidence that the end products of the MOF-accelerated reaction are consistent with the decomposition under thermal or UV-mediated conditions. In general, the mass spectra resulting from analysis of GSNO decomposition solutions containing either chitosan/ Cu-BTtri or chitosan membranes were consistent with one another, while the GSNO solution exposed to UV light (without the presence of any material) predictably did not

exhibit the peaks associated with the polysaccharide (e.g., monomeric glucosamine or *N*-acetylglucosamine).

In the case of thermal or UV-induced decomposition, NO-forming homolytic cleavage of the S–N bond is believed to be followed by the combination of thiyl radicals to form disulfide.⁹ It has been observed that dissolved copper ions are capable of catalyzing NO release from RSNOs, with five- and six-membered copper chelates described as potential mechanistic intermediates.⁴⁴ Previous reports examining the use of copper-based MOFs for the generation of NO from RSNOs have suggested that the MOF behaves similarly to copper ions by reduction of the copper(II) sites to copper(I), producing both NO and disulfide (Scheme 1).^{9,27,44} However, it has not been previously verified that Cu-BTtri (or any MOF) forms products consistent with those generated in other known decomposition pathways. In this study, it is shown that the formation of GSSG accompanies the accelerated NO release from GSNO in the presence of chitosan/Cu-BTtri membranes, indicating that the ultimate fate of GSNO remains the same when decomposed by the MOF. While the overall mechanism remains unknown, it has been argued that steric considerations prevent the formation of the ring intermediates often invoked in the case of dissolved copper ions. For certain systems, it has been proposed that MOF copper sites may act as Lewis acids that facilitate the formation of NO from RSNOs.⁴⁵ A computational study carried out by Li et al. suggests that free copper ions interact with an initial RSNO molecule in solution to form a Cu–S bond, accompanied by the release of NO. This process is repeated with a second RSNO molecule to form a $\text{CH}_3\text{S–Cu–SCH}_3^+$ species, which subsequently leads to disulfide formation and regeneration of the copper species. Comparison of this solution-phase scenario to the MOF Cu-BTC indicated that the approach of the initial RSNO molecule was nearly isoenergetic with free copper, but the addition of a subsequent RSNO molecule to the same copper site or adjacent copper sites displayed a higher energy barrier. This suggests that this particular reaction would only be plausible for a flexible MOF that could allow for distortion around the copper center to accommodate the additional RSNO molecule.⁴⁶ Regardless of actual mechanism, the identification of GSSG is a crucial step toward understanding the interaction of copper-based MOFs with RSNOs.

4. CONCLUSIONS

These findings demonstrate the ability of chitosan/Cu-BTtri membranes to generate an enhanced level of NO release from GSNO, a compound that occurs naturally in blood as a hypothesized NO storage and transport agent. Preservation of Cu-BTtri following incorporation within chitosan was verified by pXRD and ATR-FTIR, and SEM revealed the clear distribution of octahedral Cu-BTtri crystals on the surface of the material. In the presence of 20 μM (100 nmol total) GSNO at physiological temperature and pH, chitosan/Cu-BTtri membranes produced 97 ± 3 nmol over 4.3 ± 0.3 h, representing a 65-fold increase in NO generation relative to the baseline thermal decomposition of GSNO. Under otherwise identical conditions, Cu-BTtri powder (without immobilization in chitosan) produced 97 ± 6 nmol NO over 5.0 ± 1.6 h, indicating no significant difference in performance compared to chitosan/Cu-BTtri membranes. While Cu-BTtri has been previously shown to induce the accelerated decomposition of synthetic RSNOs such as *S*-nitrosocysteamine and SNAP, this is the first extension of that function to GSNO.

Furthermore, inclusion of Cu-BTtri within chitosan membranes did not result in a significant impairment of function, contrasting with earlier polyurethane and PVC-based systems, where polymeric incorporation diminished the activity of the MOF. Following initial experiments, chitosan/Cu-BTtri membranes were capable of promoting accelerated NO release for at least three additional cycles. Characterization by pXRD and SEM subsequent to GSNO exposure supported the lack of extensive Cu-BTtri decomposition, and this outcome was corroborated by the low level of copper leaching as determined by ICP-AES. Moreover, mass spectrometry was used to identify GSSG as the major product of GSNO decomposition in the presence of chitosan/Cu-BTtri, substantiating the hypothesis that disulfides are formed from RSNOs by copper-based MOFs. Taken together, this work indicates that chitosan-based materials incorporating Cu-BTtri may be capable of inducing NO formation from endogenous GSNO in the blood. Examining the assembly and properties of these and similar materials facilitates the use of MOFs for localized NO release, further expanding their potential applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- (1). Furukawa H; Cordova KE; O’Keeffe M; Yaghi OM The Chemistry and Applications of Metal-Organic Frameworks. *Science* 2013, 341, 974–986.
- (2). Harding JL; Reynolds MM Metal Organic Frameworks as Nitric Oxide Catalysts. *J. Am. Chem. Soc* 2012, 134, 3330–3333. [PubMed: 22263610]
- (3). Ignarro LJ, Ed. Nitric Oxide: Biology and Pathobiology, 2nd ed; Elsevier: Amsterdam, 2010.
- (4). Witte MB; Barbul A Role of Nitric Oxide in Wound Repair. *Am. J. Surg* 2002, 183, 406–412. [PubMed: 11975928]
- (5). Shabani M; Pulfer SK; Bulgrin JP; Smith DJ Enhancement of Wound Repair with a Topically Applied Nitric Oxide-Releasing Polymer. *Wound Repair Regen.* 1996, 4, 353–362. [PubMed: 17177732]
- (6). Lowe A; Bills J; Verma R; Lavery L; Davis K; Balkus KJ, Jr. Electrospun Nitric Oxide Releasing Bandage with Enhanced Wound Healing. *Acta Biomater* 2015, 13, 121–130. [PubMed: 25463501]
- (7). Stamler JS S-Nitrosothiols in the Blood: Roles, Amounts, and Methods of Analysis. *Circ. Res* 2004, 94, 414–417. [PubMed: 15001539]
- (8). Giustarini D; Milzani A; Dalle-Donne I; Rossi R Detection of S-Nitrosothiols in Biological Fluids: A Comparison Among the Most Widely Applied Methodologies. *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci* 2007, 851, 124–139.
- (9). Williams DLH The Mechanism of Nitric Oxide Formation from S-Nitrosothiols (Thionitrites). *Chem. Commun* 1996, 1085–1091.

- (10). McAninly J; Williams DLH; Askew SC; Butler AR; Russell C Metal Ion Catalysis in Nitrosothiol (RSNO) Decomposition. *J. Chem. Soc., Chem. Commun* 1993, 1758–1759.
- (11). Oh BK; Meyerhoff ME Spontaneous Catalytic Generation of Nitric Oxide from S-Nitrosothiols at the Surface of Polymer Films Doped with Lipophilic Copper(II) Complex. *J. Am. Chem. Soc* 2003, 125, 9552–9553. [PubMed: 12903997]
- (12). Puiu SC; Zhou Z; White CC; Neubauer LJ; Zhang Z; Lange LE; Mansfield JA; Meyerhoff ME; Reynolds MM Metal Ion-Mediated Nitric Oxide Generation from Polyurethanes via Covalently Linked Copper(II)-Cyclen Moieties. *J. Biomed. Mater. Res., Part B* 2009, 91B, 203–212.
- (13). Neufeld MJ; Harding JL; Reynolds MM Immobilization of Metal-Organic Framework Copper(II) Benzene-1,3,5-tricarboxylate (CuBTC) onto Cotton Fabric as a Nitric Oxide Release Catalyst. *ACS Appl. Mater. Interfaces* 2015, 7, 26742–26750. [PubMed: 26595600]
- (14). Harding JL; Metz JM; Reynolds MM A Tunable, Stable, and Bioactive MOF Catalyst for Generating a Localized Therapeutic from Endogenous Sources. *Adv. Funct. Mater* 2014, 24, 7503–7509.
- (15). Neufeld MJ; Ware BR; Lutzke A; Khetani SR; Reynolds MM Water-Stable Metal–Organic Framework/Polymer Composites Compatible with Human Hepatocytes. *ACS Appl. Mater. Interfaces* 2016, 8, 19343–19352. [PubMed: 27447022]
- (16). Kumar MNVR A Review of Chitin and Chitosan Applications. *React. Funct. Polym* 2000, 46, 1–27.
- (17). Rinaudo M Chitin and Chitosan: Properties and Applications. *Prog. Polym. Sci* 2006, 31, 603–632.
- (18). Croisier F; Jérôme C Chitosan-based Biomaterials for Tissue Engineering. *Eur. Polym. J* 2013, 49, 780–792.
- (19). Dai T; Tanaka M; Huang Y-Y; Hamblin MR Chitosan Preparations for Wounds and Burns: Antimicrobial and Wound-Healing Effects. *Expert Rev. Anti-Infect. Ther* 2011, 9, 857–879. [PubMed: 21810057]
- (20). Azad AK; Sermisintham N; Chandkrachang S; Stevens WF Chitosan Membrane as a Wound-Healing Dressing: Characterization and Clinical Application. *J. Biomed. Mater. Res* 2004, 69B, 216–222.
- (21). Lu Y; Slomberg DL; Schoenfish MH Nitric Oxide-releasing Chitosan Oligosaccharides as Antibacterial Agents. *Bio-materials* 2014, 35, 1716–1724.
- (22). Lu Y; Shah A; Hunter RA; Soto RJ; Schoenfish MH S-Nitrosothiol-modified Nitric Oxide-releasing Chitosan Oligosaccharides as Antibacterial Agents. *Acta Biomater* 2015, 12, 62–69. [PubMed: 25449913]
- (23). Lutzke A; Pegalajar-Jurado A; Neufeld BH; Reynolds MM Nitric Oxide-releasing S-Nitrosated Derivatives of Chitin and Chitosan for Biomedical Applications. *J. Mater. Chem. B* 2014, 2, 7449–7458.
- (24). Keefer KL Fifty Years of Diazeniumdiolate Research. From Laboratory Curiosity to Broad-Spectrum Biomedical Advances. *ACS Chem. Biol* 2011, 6 (11), 1147–1155. [PubMed: 21932836]
- (25). Giustarini D; Milzani A; Colombo R; Dalle-Donne I; Rossi R Nitric Oxide and S-Nitrosothiols in Human Blood. *Clin. Chim. Acta* 2003, 330, 85–98. [PubMed: 12636927]
- (26). Stamler JS; Jaraki O; Osborne J; Simon DI; Keaney J; Vita J; Singel D; Valeri CR; Loscalzo J Nitric Oxide Circulates in Mammalian Plasma Primarily as an S-Nitroso Adduct of Serum Albumin. *Proc. Natl. Acad. Sci. U. S. A* 1992, 89, 7674–7677. [PubMed: 1502182]
- (27). Singh RJ; Hogg N; Joseph J; Kalyanaraman B Mechanism of Nitric Oxide Release from S-Nitrosothiols. *J. Biol. Chem* 1996, 271, 18596–18603. [PubMed: 8702510]
- (28). Demessence A; D'Alessandro DM; Foo ML; Long JR Strong CO₂ Binding in a Water-Stable, Triazolate-Bridged Metal-Organic Framework Functionalized with Ethylenediamine. *J. Am. Chem. Soc* 2009, 131, 8784–8786. [PubMed: 19505094]
- (29). Hart TW Some Observations Concerning the S-Nitroso and S-Phenylsulphonyl Derivatives of L-Cysteine and Glutathione. *Tetrahedron Lett* 1985, 26, 2013–2016.
- (30). Liu M; Zheng H; Chen J; Li S; Huang J; Zhou C Chitosan-chitin Nanocrystal Composite Scaffolds for Tissue Engineering. *Carbohydr. Polym* 2016, 152, 832–840. [PubMed: 27516335]

- (31). Takara EA; Marchese J; Ochoa NA NaOH Treatment of Chitosan Films: Impact on Macromolecular Structure and Film Properties. *Carbohydr. Polym* 2015, 132, 25–30. [PubMed: 26256320]
- (32). Zhang Y; Feng X; Yuan S; Zhou J; Wang B Challenges and Recent Advances in MOF-polymer Composite Membranes for Gas Separation. *Inorg. Chem. Front* 2016, 3, 896–909.
- (33). Vanin AF; Malenkova IV; Serezhenkov VA Iron Catalyzes both Decomposition and Synthesis of S-Nitrosothiols: Optical and Electron Paramagnetic Resonance Studies. *Nitric Oxide* 1997, 1, 191–203. [PubMed: 9704580]
- (34). McAninly J; Williams DLH; Askew SC; Butler AR; Russell C Metal Ion Catalysis in Nitrosothiol (RSNO) Decomposition. *J. Chem. Soc., Chem. Commun* 1993, 1758–1759.
- (35). Harding JL; Reynolds MM Composite Materials with Embedded Metal Organic Framework Catalysts for Nitric Oxide Release from Bioavailable S-Nitrosothiols. *J. Mater. Chem. B* 2014, 2, 2530–2536.
- (36). Pethrick RA, Eds. *Physical Properties, Synthesis and Applications of Thermoplastic Polymers Polymer Yearbook*, 5; Harwood Academic Publishers GmbH, 1989; p 6.
- (37). Chan ADC; Li X; Harrison DJ Evidence for Water-Rich Surface Region in Poly(vinylchloride)-Based Ion-Selective Electrode Membranes. *Anal. Chem* 1992, 64, 2512–2517.
- (38). Brisbois EJ; Bayliss J; Wu J; Major TC; Xi C; Wang SC; Bartlett RH; Handa H; Meyerhoff ME Optimized Polymeric Film-Based Nitric Oxide Delivery Inhibits Bacterial Growth in a Mouse Burn Wound Model. *Acta Biomater* 2014, 10, 4136–4142. [PubMed: 24980058]
- (39). Noble DR; Swift HR; Williams DLH Nitric Oxide Release from S-Nitrosoglutathione. *Chem. Commun* 1999, 2317–2318.
- (40). Oh BK; Meyerhoff ME Catalytic Generation of Nitric Oxide from Nitrite at the Interface of Polymeric Films Doped with Lipophilic Cu(II)-complex: A Potential Route to the Preparation of Thromboresistant Coatings. *Biomaterials* 2004, 25, 283–293. [PubMed: 14585716]
- (41). Major TC; Brant DO; Burney CP; Amoako KA; Annich GA; Meyerhoff ME; Handa H; Bartlett RH The Hemocompatibility of a Nitric Oxide Generating Polymer that Catalyzes S-Nitrosothiol Decomposition in an Extracorporeal Circulation Model. *Biomaterials* 2011, 32, 5957–5969. [PubMed: 21696821]
- (42). Hwang S; Meyerhoff ME Polyurethane with Tethered Copper (II)-Cyclen Complex: Preparation, Characterization and Catalytic Generation of Nitric Oxide from S-Nitrosothiols. *Biomaterials* 2008, 29, 2443–2452. [PubMed: 18314189]
- (43). Luza SC; Speisky HC Liver Copper Storage and Transport During Development: Implications for Cytotoxicity. *Am. J. Clin. Nutr* 1996, 63, 812–820.
- (44). Dicks AP; Swift HR; Williams DLH; Butler AR; Al-Sa'doni HH; Cox BG Identification of Cu⁺ as the Effective Reagent in Nitric Oxide Formation from S-Nitrosothiols. *J. Chem. Soc., Perkin Trans. 2* 1996, 2, 481–487.
- (45). Baciú C; Cho KB; Gauld JW Influence of Cu⁺ on the RSNO Bond Dissociation Energy of S-Nitrosothiols. *J. Phys. Chem. B* 2005, 109, 1334–1336. [PubMed: 16851099]
- (46). Li T; Taylor-Edinbyrd K; Kumar R A Computation Study of the Effect of the Metal Organic Framework Environment on the Release of Chemically Stored Nitric Oxide. *Phys. Chem. Chem. Phys* 2015, 17, 23403–23412. [PubMed: 26292051]

Figure 1.

(a) Fundamental building unit of Cu-BTtri. Copper (red), chlorine (green), carbon (black), and nitrogen (blue) atoms are depicted. Hydrogen atoms have been omitted for clarity. (b) Structure of the glucosamine (x) and *N*-acetyl glucosamine (y) units of chitosan.

Figure 2. Images of (a) chitosan membrane and (b) chitosan/Cu-BTtri membrane, depicting the dispersion of Cu-BTtri throughout the material. Images were taken after the materials had been hydrated by immersion in deionized water.

Figure 3.

(a) ATR-FTIR spectrum for (i) Cu-BTtri, (ii) chitosan/ Cu-BTtri membrane, and (iii) chitosan. In (a), the weak C=C stretch and more prominent C-H out-of-plane bend from Cu-BTtri (i) are conserved in the spectrum of chitosan/Cu-BTtri (ii). (b) pXRD diffraction patterns of (i) Cu-BTtri, (ii) chitosan/Cu-BTtri membrane, and (iii) chitosan. Inset: 4–25 2θ .

Figure 4.

(a) 250× magnification of chitosan membrane; (b) 250× (c) 2000×, and (d) 3000× magnification of chitosan/Cu-BTtri membrane; (e) 1000× magnification of chitosan/Cu-BTtri membrane and (f) 1000× magnification with EDX copper analysis overlaid; (g) 500× magnification of cross-sectional of chitosan/Cu-BTtri membrane; (h) EDX copper analysis overlay of cross-section.

Figure 5. Schematic illustrating the NO analyzer cell, where chitosan/ Cu-BTtri membrane is suspended in pH 7.4 PBS at 37 °C, followed by injection of GSNO and subsequent chemiluminescence-based monitoring of NO formation.

Figure 6.

(a) Representative real-time NO release from (i) Cu-BTtri + GSNO, (ii) chitosan/Cu-BTtri membranes + GSNO, (iii) chitosan + GSNO, and (iv) GSNO assessed under physiological conditions (PBS, pH 7.4, 37 °C). (b) Cumulative NO release from (i) Cu-BTtri + GSNO (ii) chitosan/Cu-BTtri membranes + GSNO (iii) chitosan + GSNO, and (iv) GSNO all under physiological conditions (PBS, pH7.4, 37 °C). For (i) and (ii) data collection ended after the depletion of GSNO. (c) (i) pXRD diffraction pattern of chitosan/Cu-BTtri materials taken after NO analyzer experiment and (ii) prior to the NO analyzer runs; pXRD clearly shows the retention of all diffraction peaks. Inset: 4–25 θ .

Scheme 1.

Proposed Overall Reaction for GSNO in the Presence of Solution-Phase Copper Ions,
Resulting in the Subsequent Formation of NO and Oxidized Glutathione

Table 1.Total Average NO Release from GSNO^a

substrate	total NO release (nmol)	total recovery (%)
GSNO	1.5 ± 0.7	1.5 ± 0.7
chitosan + GSNO	9.8 ± 0.3	9.8 ± 0.3
chitosan/Cu-BTtri + GSNO	97 ± 3	97 ± 3
Cu-BTtri + GSNO	97 ± 6	97 ± 6

^aData reported as the mean ± SD (*n* > 3).

Table 2.Total Average NO Release for Chitosan/Cu-BT[†]ri Membranes after Reuse^a

chitosan/Cu-BT [†] ri membrane + GSNO	total NO release (nmol)
cycle 1	97 ± 3
cycle 2	107 ± 7
cycle 3	98 ± 3

^aData reported as the mean ± SD (*n* = 3).

Table 3.

Mass Spectrometry Data

name	formula	exact mass (m/z), H-adduct	measured mass (m/z), H-adduct	mass error (ppm)	polarity
oxidized glutathione	$C_{20}H_{32}N_6O_{15}S_2$	613.1592	613.1598	1.02	+
glucosamine monomers	$C_6H_{11}NO_4$	162.0761	162.0767	3.62	+
N-acetylglucosamine monomers	$C_8H_{13}NO_5$	204.0866	204.0874	3.36	+