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Surface-Anchored Metal–Organic Framework–Cotton Material for Tunable Antibacterial Copper Delivery

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Abstract

In the present study, a new copper metal-organic framework (MOF)–cotton material was strategically fabricated to exploit its antibacterial properties for postsynthetic modification (PSM) to introduce a free amine to tune the physicochemical properties of the material. A modified methodology for carboxymethylation of natural cotton was utilized to enhance the number of nucleation sites for the MOF growth. Subsequently, MOF Cu₃(NH₂BTC)₂ was synthesized into a homogenous surface-supported film via a layer-by-layer dip-coating process. The resultant materials contained uniformly distributed 1 $\mu\text{m} \times 1 \mu\text{m}$ octahedral MOF crystals around each carboxymethylated fiber. Importantly, the accessible free amine of the MOF ligand allowed for the PSM of the MOF–cotton surface with valeric anhydride, yielding $23.5 \pm 2.2\%$ modified. The Cu²⁺ ion-releasing performance of the materials was probed under biological conditions per submersion in complex media at 37 °C. Indeed, PSM induces a change in the copper flux of the material over the first 6 h. The materials continue to slowly release Cu²⁺ ions beyond 24 h tested at a flux of $0.22 \pm 0.003 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ with the unmodified MOF–cotton and at $0.25 \pm 0.004 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$ with the modified MOF–cotton. The antibacterial activity of the material was explored using *Escherichia coli* by testing the planktonic and attached bacteria under a variety of conditions. MOF–cotton materials elicit antibacterial effects, yielding a 4-log reduction or greater, after 24 h of exposure. Additionally, the MOF–cotton materials inhibit the attachment of bacteria, under both dry and wet conditions. A material of this type would be ideal for clothing, bandages, and other

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Author Contributions

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.7b19455.

Optimization details of the carboxymethylation and MOF grown on cotton, additional SEM imaging, 3D drawings of the MOF (PDF)

Notes

The authors declare no competing financial interest.

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textile applications. As such, this work serves as a precedence toward developing uniform, tunable MOF–composite textile materials that can kill bacteria and prevent the attachment of bacteria to the surface.

Graphical Abstract:

Keywords

metal–organic frameworks; post-synthetic modification; tunability; cotton; antibacterial agent; antibacterial surface

INTRODUCTION

The development of antibacterial surfaces to prevent the spread and growth of bacteria remains a material necessity of high priority to combat a pressing global health concern.^{1,2} Bacteria may thrive for weeks to months upon common surfaces including bandages, clothing, and blankets.³ Once materials of this type are compromised with microbial attachment, it is likely for the bacteria to advance to biofilm formation, whereby microbial cells become increasingly impenetrable to traditional antibiotics, making treatment extremely difficult.⁴ Due to the emergence of “superbugs”, multidrug-resistant organisms, new antibacterial agents with decreased likelihood for resistance are needed.⁵ An optimal strategy is to develop efficient antimicrobial coatings, metals, and fabric materials which kill or prevent the attachment of bacteria altogether. Using small molecules which kill bacteria via multiple mechanisms is a relatively well-established route with excellent success rates to avoid bacterial resistance.^{6–8} On the other hand, it is easier to prevent than treat biofilms. Thus, materials with surfaces that prevent the attachment of bacteria altogether would minimize the spread of bacteria and in turn lessen the necessity for traditional antibiotics. Towards this goal, antimicrobial materials that kill or inhibit the growth of microorganisms have become a centralized focus for materials science.

Nano-antimicrobial materials offer the unique opportunity to fill the void associated with the current antibiotic treatment failure. While traditional antibiotics target inhibition of DNA replication, cell wall synthesis, or protein synthesis, all of which are primarily due to resultant secondary oxidative species. Materials containing nanoparticles, on the other hand, are suspected to be unaffected by most resistance mechanisms as they display multiple modes of action. This includes the direct interaction of the nanoparticles with the bacterial cell membranes to kill bacteria and prevent biofilm formation, induce metal-redox species

which causes an oxidative stress to damage the bacterial cell components, and disrupt critical permeability into or out of the cell.^{9,10} Nanomaterials also serve as excellent drug carriers, which would support and strengthen traditional antibiotic treatments.¹¹ Moreover, the release of small molecules or metal ions with antibacterial properties has shown promise for strategically developing antibacterial materials. Metal–organic frameworks (MOFs) are a unique subclass of nanomaterials consisting of tunable coordination networks. Manipulation at either the metal ion centers or organic linkers yields seemingly infinite possibilities to control the physicochemical properties of the materials. Minor adjustments to synthetic conditions permits synthetic regulation of the size and shape of the MOF crystals. Since a greater surface area is associated with increased antibacterial efficacy of nanoparticles, control over the particle size is valuable.¹² The unprecedented porosity achieved with MOFs gives these particular systems unique properties attributable to the increased surface area-to-mass ratio over other nanomaterials. This characteristic effectively increases MOF interactions with cells in biological mediums, adversely affecting the bacterial cell membranes. Additionally, the controlled release and decomposition kinetics of the MOF itself or the guest molecules preloaded into the pores yields effective synergistic therapeutic delivery systems. As such, MOFs are excellent candidates for incorporation into materials to achieve antibacterial activity.

Under controlled conditions, copper ions have proven to be nontoxic and highly effective at killing bacteria and preventing the formation of biofilms.¹³ Ancient civilizations used copper to sterilize wounds and drinking water.¹⁴ More recently, antimicrobial copper alloys have been approved by the United States Environmental Protection Agency and implemented as antibacterial surfaces for a multitude of common items from electronic cases, to handles, and countertops at healthcare facilities, community facilities, residential buildings, and mass transit facilities.¹⁵ Copper is suspected to elicit antibacterial action because of multiple mechanisms including inducing intracellular leakage, cell wall accumulation causing reduced permeability, disrupting the DNA structure, damaging critical enzymes, and causing cellular internalization.¹⁶ Copper-containing MOFs, therefore, may present a promising opportunity to develop useful and effective antibacterial materials.

Although release of Cu^{2+} ions has been observed with nonporous copper-containing materials, manipulation of ion release rates is limited to the surface area of the material or specialized coatings.^{17,18} Thus, few successful attempts to synthetically control the rate of the release of Cu^{2+} ions have been reported. Tunable ion release, however, is advantageous and necessary, namely, for various environmental and biomedical applications, as this would provide an opportunity to control the ion delivery rates. Cu-BTC is a well-known, commercially available copper-containing MOF, which when attached to surfaces, increases the antimicrobial activity at the interface.¹⁹ By utilizing a similar ligand scaffold that contains a functional handle for postsynthetic modification (PSM) of the material, a MOF system capable of releasing antibacterial agents would become amenable to fine tuning. This would offer an exclusive opportunity to manipulate the physicochemical properties of the materials, offering control over the stability to aqueous environments using late-stage synthetic functionalization. The low temperatures associated with MOF syntheses, as compared with other copper-containing synthetic processes, allow for MOF incorporation into more sensitive, flexible materials, like polymers and textiles. Such surfaces, including

bandages, curtains, and garments, all serve as proponents for bacteria to attach, proliferate, and spread. Thus, PSM of MOFs on commonly used textiles for such applications, like cotton, may yield new, useful antibacterial fabric materials with controllable Cu^{2+} ion release. This would have far-reaching consequences for textile applications including medical supplies, apparels, and common fabrics in shared facilities that will be able to prevent the attachment and therefore spread of deadly bacteria.

In the present study, we engineered a new MOF–cotton material for antibacterial applications that can undergo PSM. MOF $\text{Cu}_3(\text{NH}_2\text{BTC})_2$ was grown using a layer-by-layer process on the surface of a modified cotton fabric. The resultant MOF–cotton material was uniformly coated with distinct octahedral MOF crystals to yield a robust, flexible composite. PSM to the MOF grown on cotton changes the rate at which the material delivers Cu^{2+} ions when fully submerged in nutrient broth media at 37 °C over 24 h, representing extreme biological conditions. The antibacterial efficacy of the cotton swatches was demonstrated against *Escherichia coli* (*E. coli*) over 24 h to serve as a proof-of-concept for the intended application, such as a wound dressing. Importantly, not only did the release of Cu^{2+} from the modified materials kill the bacteria in solution, but the materials were also able to significantly reduce the attachment of bacteria to the surface as compared to plain cotton under both wet and dry conditions, demonstrating their usefulness to impede biofilm formation and act as antibacterial surfaces.

EXPERIMENTAL SECTION

Materials.

Absolute ethanol was obtained from Pharmco-Aaper. Anhydrous dimethylacetamide (DMA), anhydrous chloroform (CHCl_3), dimethylsulfoxide (DMSO), sodium hydroxide, and copper(II) nitrate were purchased through Sigma-Aldrich. Deuterium chloride (DCI) was purchased from Acros Organics. The anhydrides were purchased from TCI. Natural resized and bleached cotton was purchased through SDL Atlas. Sodium chloroacetate was purchased through Ark Pharm Inc. All materials were used as provided, without further purification. For experiments requiring water, a Millipore purification system set at 18 MΩ was used. Oxoid nutrient broth media (NBM, OXCM0001B), Oxoid nutrient agar (OXCM0003B), and sodium chloride were purchased from Fisher Scientific. *E. coli* (ATCC 25922) was purchased from the American Type Culture Collection.

Characterization.

An Agilent (Varian) 400 MR equipped with an automated tuning and a 7600 sample changer at room temperature was used for all NMR experiments. The attained free induction decay (FID) was processed using MestreNova NMR software. To quantify PSM, a small portion of the modified fiber was added to a vial with 0.8 mL DMSO and three drops of DCI. The solution was vortexed or shaken until the cotton fiber returned to its original white color, and the solution was transferred to an NMR tube for the analysis of the ligand component. The powder X-ray diffraction (PXRD) patterns were obtained to verify MOF crystallinity with a Bruker D8 DISCOVER DaVinci powder X-ray diffractometer with Cu K α radiation operated at 40 kV and 40 mA. A 0.6 mm divergent slit was placed on the primary beam side,

and a high-resolution energy-dispersive LYNXEYE-XE-T detector was placed on the diffracted beam side during the XRD studies. The instrument alignment was tested using the NIST 1976b SRM. The typical scan rate was 0.1 s/step, with the step size of 0.02°. Infrared (IR) data were acquired on a Nicolet 6700 Fourier transform IR spectrometer under a nitrogen flow. Diffuse reflectance UV–vis spectroscopy was performed using a Nicolet Evolution 300 UV–vis spectrophotometer equipped with a Praying Mantis diffuse reflectance accessory. To access the MOF coverage of the cotton fibers, scanning electron microscopy (SEM) imaging was performed using a JEOL JSM-6500F microscope. An accelerating voltage of 5.0 kV and a working distance of 10.0 mm were used. All samples were placed under vacuum and coated with 20 nm gold prior to imaging. Five to six representative images were obtained at three different magnifications for each sample. Cross-sectional transmission electron microscopy (TEM) imaging was performed to analyze the MOF crystals around individual cotton fibers using a JEOL JEM2100F microscope. For TEM imaging, the cross-sectioned samples were first embedded. In brief, the samples were heated under vacuo at 22 psi and 70 °C for 30 min, embedded in a mixture of 70% resin and 30% acetone for 40 min on the rotator, followed by 100% resin for 1 h on the rotator, and finally transferred to flat embedding molds and heated under vacuo at 22 psi and 70 °C for 20 h. The sections were then cut on a Reichert Ultracut E microtome to 70 nm thickness and imaged. additional TEM imaging was performed by isolating one cotton fiber. The fiber was drop-casted using ethanol between two copper grids prior to imaging. This imaging includes bright field TEM (BFTEM), high-angle annular dark-field scanning TEM (HAADF-STEM), and high-resolution TEM (HRTEM).

Carboxymethylation of Cotton Swatch.

To increase the nucleation sites for the growth of the MOF, the appendant hydroxyl moieties of the cotton fabric were first modified to yield available carboxylates on the surface (Figure 1). In a typical modification procedure, a 15 wt % NaOH (15 g per swatch) solution was prepared with 100 mL of 2:1 absolute ethanol/deionized water per swatch. Precut cotton swatches with the dimensions of 4.5 cm × 2 cm were added and gently stirred for 30 min. The swatches were removed, and sodium chloroacetate was added to the original solution to attain a 1 M solution (11.6 g). After complete dissolution, the swatches were added back to the solution and gently stirred for 1 h. The resultant modified cotton was soaked for 30 min in Millipore water and rinsed with 100 mL Millipore water, followed by 100 mL ethanol. After drying in an open atmosphere, the modified swatches were dried in an oven for 1 h at 60 °C.

Layer-by-Layer Growth Process of $\text{Cu}_3(\text{NH}_2\text{BTC})_2$ on Modified Cotton.

A uniform growth of the MOF onto the cotton fibers was achieved via layer-by-layer dip-coating process using a mechanical dip coater (Figure 1). Each swatch was dipped into independently prepared copper- and ligand-containing solutions. In a typical procedure, 20 mL scintillation vials were first filled with 20 mL of the solution. Copper-containing solution A was prepared using 1:1:1 DMA, ethanol, Millipore water, and 1.90 g copper(II) nitrate. The ligand, 2-aminobenzene-1,3,5-tricarboxylic acid, was synthesized following our previously reported procedure.²⁰ The ligand-containing solution B was prepared using 2:1:1 DMA, ethanol, Millipore water, and 675 mg of the ligand. All solutions were vortexed,

shaken, and sonicated (10 min) to ensure all compounds were completely dissolved. The solutions were transferred to 20 mL beakers. The carboxymethylated cotton swatches trimmed to 1.7 cm × 3.8 cm were loaded to a mechanical dip coater and hydrated with absolute ethanol. The materials were then dipped into solution A for 17 min, a solution of ethanol for 5 s, solution B for 17 min, followed by ethanol for 5 s. The process was repeated for a total of 15 cycles (30 total layers, 15 of each solution A and B). To remove any residual nonbonded copper or ligand, the swatches were soaked in 20 mL of ethanol overnight. After this, each swatch was added to a new solution of ethanol, washed, and vortexed six times with a new solvent in a new vial each time.

Postsynthetic Modification of $\text{Cu}_3(\text{NH}_2\text{BTC})_2$ on Modified Cotton.

After the MOF–cotton composites were characterized, the swatches were subjected to PSM with valeric anhydride (designated AM-5-modified MOF, Figure 2). The modification of the MOF on cotton was optimized when a swatch was submerged in a 1.5 M solution of valeric anhydride in CHCl_3 for 24 h. The MOF–cotton composites were added to 20 mL scintillation vials followed by 12 mL CHCl_3 and 3.55 mL valeric anhydride and vortexed to mix. After 24 h of soaking, the cotton swatch was removed, rinsed several times with CHCl_3 , and soaked overnight. Each swatch was subsequently soaked in ethanol for 24 h, after which the solution was decanted, the swatches were rinsed with 100 mL ethanol, and then dried in an oven at 60 °C for 1 h.

Copper-Releasing Studies.

Soaking studies were performed to test the rate at which the materials released copper into the aqueous environments under biologically relevant conditions. The MOF–cotton composites were first cut to 0.7 cm × 0.7 cm individual pieces and placed into four dram vials. The NBM (1 mL) solution was added, and the materials were incubated at 37 °C for various time points. After the intended time, the solutions were pipetted into a new vial, diluted with 4 mL Millipore water, and analyzed by inductively coupled plasma optical emission spectrometry (ICP–OES). Each data point reported is representative of the three trials from the three independent swatches with the standard deviation.

Bacteria Studies.

The antibacterial efficacy of the MOF–cotton materials was evaluated against *E. coli* using the standard broth dilution agar plating method. The stock cultures of *E. coli* were obtained by first streaking onto agar plates and placing in a 37 °C incubator until the formation of colony-forming units (CFUs). A colony was removed from the agar plate, placed in NBM, and allowed to grow at 37 °C under shaking conditions until reaching an $\text{O.D.}_{600\text{nm}} \sim 1.0$. The aliquots of the culture were mixed with 30% (v/v) glycerol solution in a 1:1 ratio before being stored at –80 °C until use for future bacterial studies. To perform the antibacterial kill rate studies, the stock culture was thawed on benchtop and subsequently centrifuged at 4700 rpm for 10 min. The supernatant was discarded, and the remaining pellet was resuspended using 5 mL warmed NBM. An additional 45 mL NBM was added to the culture and placed under incubated and shaking conditions overnight, until reaching an $\text{O.D.}_{600\text{nm}} \sim 1.0$.

Antibacterial Activity under Wet Conditions.

After an overnight growth of the stock culture, the *E. coli* solution was diluted using NBM to a working concentration associated with an $\text{o.d.}_{600\text{nm}} \sim 0.35$. The cotton materials were placed in a 24-well plate before the addition of 1 mL of the diluted *E. coli* culture. The well plate was placed in a 37 °C incubator, and 100 μL aliquots were pulled from each well at 1, 6, and 24 h after exposure to the cotton materials. At each time point, the aliquots underwent 10-fold serial dilutions using 0.85% NaCl solution and subsequently plated on agar using 50 μL . At the end of the total exposure period of 24 h, all of the remaining solution was removed, the wells were washed once with warmed NBM, all swatches were moved to a new well, and 1 mL NBM was added. The plate was sonicated for 5 min (100 W power, 42 kHz frequency) to liberate any bacteria that were adhered to the cotton materials. The aliquots were then taken from the sonicated wells and plated on agar following the above protocol. The agar plates were placed in a 37 °C incubator, and the CFUs were counted the following day. The assessed CFUs were normalized by the dilution factor and volume by eq 1. All kill rates by the MOF–cotton swatches are relative to the clean natural cotton swatches without the presence of MOF.

$$\frac{\text{CFU}}{\text{mL}} = \frac{\text{no. of CFUs}}{(\text{dilution factor}) \times (\text{vol plated})} \quad (1)$$

Antibacterial Activity under Dry Conditions.

The overnight stock culture of *E. coli* ($\text{O.D.}_{600\text{nm}} \sim 1.0$) was used to assess bacterial attachment onto the cotton swatches under dry conditions. The three types of swatches were placed in a 24-well plate, and 10 μL aliquot of the concentrated bacterial solution was added to each swatch. The plate was placed in a 37 °C incubator for 5 min before being assessed for bacterial attachment. The plate was removed after the 5 min exposure period, 1 mL of warmed NBM was added, and the plate was sonicated for 5 min. The viable bacteria were plated for CFUs and assessed via eq 1.

RESULTS AND DISCUSSION

Development of MOF–Cotton Material.

MOF coatings on surfaces often result in nonuniform coverage with heterogenous crystal formation. The resultant polycrystallinity and/or inhomogeneity may interfere with the material's final performance, leading to substantial batch-to-batch inconsistencies. An alternative approach is to synthetically grow MOF crystals or films bound to the surface. This commonly includes using a layer-by-layer growth method or liquid-phase epitaxy. These techniques result in the formation of more consistent, homogenous surface-supported, highly oriented MOFs, known as SURMOFs.²¹ First, the material is synthetically manipulated to provide a nucleation site which mimics that of the ligands within the desired MOF. To this end, natural cotton was first functionalized with sodium chloroacetate under basic conditions to yield available carboxylate on the surface of the fabric. An initial immobilization of the MOF on cotton was attempted utilizing previously reported

methodology, whereby cotton carboxymethylation occurs in 100% water.²² Using 100% water, however, minimal surface coverage of the MOF $\text{Cu}_3(\text{NH}_2\text{BTC})_2$ was observed. Thus, a new protocol for carboxymethylation was employed to optimize surface coverage of the cotton fibers. Recent advances in the understanding of carboxymethylation suggest that an increased degree of substitution, yielding a greater percentage of carboxylate moieties on the surface of the cotton fibers, can be achieved by adding alcohol to the carboxymethylation solution. A 100% water carboxymethylation solution yields a degree of substitution of just 0.13, whereas a 2:1 ethanol to water solution has been shown to exhibit a degree of substitution of 0.39, three times that of using water alone.²³ Knowing this, the cotton swatches were carboxymethylated in a 2:1 ethanol to water solution to increase the available nucleation sites for subsequent MOF growth. To verify carboxymethylation was successful, IR spectroscopy was performed on both natural cotton and the carboxymethylated material (Figure 3). In the chemically modified cotton spectra, the appearance of a peak at 1630 cm^{-1} indicates the presence of the $\text{C}=\text{O}$ bond stretch associated with the carboxylate moiety. Additionally, a decrease in the intensity and peak shape of the OH stretch at 3380 cm^{-1} is observed, consistent with the change associated with the carboxylate as well.

To ensure consistent results across the batches of material, the carboxymethylated cotton swatches were attached to a mechanical robot and subjected to dip-coating to generate the MOF particles chemically bound to the cotton fibers. First, the swatches were introduced into a concentrated solution of copper nitrate for 17 min, after which the material exhibits a characteristic blue color associated with the copper coordination to the carboxymethylated cotton. The material was briefly rinsed with ethanol and then introduced into a concentrated solution containing the $\text{NH}_2\text{H}_3\text{BTC}$ ligand for 17 min. Immediately upon submerging the material, a distinct color shift to green is observed. This change in color is attributable to the interaction of the ligand (having an electron-donating amine) with the copper layer on the surface of the cotton fibers. After 15 total cycles, the material has a uniform green appearance, characteristic of the MOF $\text{Cu}_3(\text{NH}_2\text{BTC})_2$ (Figure 4). To remove any unattached MOF particles, the materials were thoroughly rinsed and agitated. The MOF–cotton material was then dried in an open atmosphere for several hours and then in an oven at $60\text{ }^\circ\text{C}$ to remove any remaining ethanol.

Characterization of MOF–Cotton Material.

Diffuse reflectance UV–vis and PXRD were performed to confirm the presence and orientation of the crystalline MOF on the surface of the carboxymethylated cotton.

In the UV–vis spectra, a characteristic absorbance peak around 700 nm is observed, indicating the MOF grown on cotton exhibits nearly the same diffuse reflectance of dispersed light inside the crystalline lattice as the MOF powder (Figure 5A). This suggests the MOF is indeed present on the surface of the carboxymethylated cotton and would be expected to behave electronically like the MOF powder itself. Furthermore, the diffractogram of the unmodified MOF–cotton composite supports that the crystallinity of the carboxymethylated cotton is retained and the MOF particles on cotton have the same morphology and orientation as the MOF powder materials. This is evidenced by the sharp Bragg reflections at relevant 2θ (Figure 5B) with matching relative peak intensities. Finally,

IR spectroscopy shows a clear difference between the carboxymethylated cotton and the MOF grown on cotton (Figure 5C). The unmodified MOF cotton contains several characteristic features of the MOF, as observed by the comparison with the MOF powder. With the MOF present, the distinct features at 3400 cm^{-1} show the presence of an amine; a new feature at 1640 cm^{-1} is representative of the $\text{C}=\text{O}$ stretch associated with the MOF, and distinct features within the thumbprint region between 750 and 850 cm^{-1} are also present. Taken together, these data indicate that cotton contains crystalline MOF crystals uniformly on the surface.

The surface coverage of the MOF onto the cotton fibers was investigated and analyzed using SEM and TEM imaging. The initial experiments demonstrated a significant increase in the MOF growth on the cotton fibers that were carboxymethylated in 2:1 ethanol/water rather than previous protocols with 100% water (Supporting Information Figure S2). As a result, these materials were carried on for further optimization. The coverage of the cotton surface was optimized by varying the number of layers made and the deposition time allowed. A complete and consistent coverage of the cotton fibers was accomplished with 17 min in each solution and with at least 30 total layers (15 times in each copper or ligand solution). As previously mentioned, the use of a mechanical dip coater ensured uniformity among the individual MOF–cotton swatches. As shown in Figure 6, the MOF crystals individually appear to be approximately $1\text{ }\mu\text{m}$ in diameter, with little twinning of the crystallites. In conclusion, SEM imaging reveals that the resultant materials contain consistent full coverage of the surface of the cotton swatches with abutting MOF crystals of uniform size and morphology.

To analyze the MOF coverage around the entire cotton fiber, the material was cross-sectioned and analyzed with SEM and TEM imaging (Figure 7). SEM imaging coupled with EDS mapping of a cross section of the MOF–cotton material shows that copper is present around the entire surface of the individual cotton fibers (Figure 7A). TEM imaging of the cross-sectioned material reveals that the optimized conditions generate a single uniform layer of adjoining crystallites around each cotton fiber of roughly $1\text{ }\mu\text{m} \times 1\text{ }\mu\text{m}$ in size (Figure 7B). This indicates that the detailed growth parameters maximize the surface area and size of each individual MOF crystal attached, as opposed to increasing the number of MOF crystals on the surface. Upon further magnification, the individual MOF crystals displayed distinct ligand and copper layers as light- and dark-layered regions of the BFTEM images (Figure 7C). TEM imaging allows for detailed monitoring of individual MOF components, a critical spectroscopic technique for characterizing MOF interactions including future studies with biological systems or MOF loading. Such studies with MOFs are limited because of the instability of MOFs to the high-energy electrons associated with using TEM.²⁴ Using HAADF-STEM, however, the MOF–cotton materials were successfully imaged (Figure 7D–F). Figure 7D shows a single fiber of the material, whereby the surface appears entirely covered with MOF crystals. Higher magnification further supports this conclusion, showing two crystals attached to the cotton surface (Figure 7E). The crystals appear to show copper striations attributed to the layers of copper in the materials (Figure 7F). To further assess the crystallinity and orientation of the crystals, free particles liberated during the isolation of a single MOF–cotton fiber were imaged using HRTEM (Figure 7G). Magnification of the framed regions is shown with the insets of the associated Fourier

transforms. Several crystal orientations are shown depicting clear lattice fringes throughout the crystals. Taken together, the imaging data suggest that detailed methodology is advantageous for ensuring that natural cotton is sufficiently carboxymethylated to yield increased crystalline MOF growth around the entire surface of individual modified cotton fibers. Additionally, this method results in the uniform growth of MOF $\text{Cu}_3(\text{NH}_2\text{BTC})_2$ octahedral crystals on the surface of each individual cotton fiber.

PSM of the MOF–Cotton Material.

The PSM of surfaces allows access to tunable and biocompatible surfaces with far-reaching applications including wound dressings, nanomedicine, and antifouling surfaces.²⁵ Previously reported PSM to the free amine moiety of the powdered MOF achieves a controllable change in the stability to water of the material.²⁰ This premise suggests that functionalizing the MOF–cotton composite may also yield control of the stability of the material to biologically relevant NBM. Ultimately, this would enable the unprecedented tunable release of Cu^{2+} ions into aqueous environments. This would be of great value for various applications including as an antibacterial agent. Thus, the materials were postsynthetically modified with valeric anhydride to determine the resultant impact on the stability of the composite material to NBM before and after modification. IR spectroscopy of the AM-5-modified MOF on cotton is consistent with the modified MOF powder. An increase in sp^3 C–H at 3005 cm^{-1} further verifies the presence of the valeric anhydride carbon chain. A small section of the modified swatches was cut and digested in acidic $\text{DMSO}-d_6$ and examined by ^1H NMR to quantify the percentage of modification achieved. The average observed percent modification with valeric anhydride was $23.5 \pm 2.2\%$ ($n = 3$). After modification, the total amount of copper per cm^2 section of the material measured $6.65 \pm 0.30\text{ }\mu\text{mol}\cdot\text{cm}^{-2}$, whereas the unmodified material contained $9.56 \pm 0.66\text{ }\mu\text{mol}\cdot\text{cm}^{-2}$. This suggests that modification under these conditions does lead to a slight depreciation of the MOF on the surface. These results are consistent with those previously observed using anhydrides to modify $\text{Cu}_3(\text{NH}_2\text{BTC})_2$, whereby a loss in copper is attributed to the by-product carboxylate released during modification.²⁰ For these studies, PSM was maximized relative to the total amount of MOF to ensure maximum modification possible; however, to retain more SURMOF, a less concentrated solution may be used. To analyze any changes the MOF may have undergone during the modification process, PXRD and SEM imaging were performed following modification. The retention of the diffraction pattern after PSM suggests that the crystallinity of the MOF does not change upon modification (Figure 5B). The SEM images indicate there is no visual damage to the structural integrity of the MOF crystals, morphology, or to the overall size (Supporting Information Figure S4). In all, the PSM procedure employed slightly reduces the amount of MOF remaining on cotton; however, it does not cause any detectable change to the morphology or size of the MOF crystals.

Copper Delivery Performance.

Although obtaining surfaces which prevent the growth of biofilms is extremely critical and the central goal of this study, we also recognize the advantages associated with a material that may slowly release Cu^{2+} ions into solution. A slow and controlled release of Cu^{2+} ions would be of significance for biomedical applications, agricultural applications, and to shape

the future for nano-antibacterial materials. As such, using PSM to tune the copper release rate of the material was investigated. Toward this goal, modified and unmodified MOF–cotton materials were subjected to extreme biological conditions, whereby the materials were submerged in complex media at elevated temperatures. $\text{Cu}_3(\text{NH}_2\text{BTC})_2$ on cotton is resistant to detectable damage from sonication and washing with organic solvents including ethanol, methanol, and chloroform. When submerged in aqueous environments, however, the MOF may undergo a slow structural collapse and release Cu^{2+} ions.²⁰ The rate of copper release from the materials was carefully monitored over various time points. The modified materials were found to release less copper into the solution within the first 6 h than the unmodified materials (Table 1). The unmodified MOF–cotton initially releases Cu^{2+} ions into the solution most quickly over the first 6 h, after which time the copper release remains slow and consistent. If the material is postsynthetically modified, initially a slower release of Cu^{2+} ions is observed compared to the unmodified material. After 6 h, the modified material releases Cu^{2+} ions at a faster rate than the unmodified material. Clearly, the modification of the MOF yields interesting changes to the releasing capabilities of the material. Importantly, both modified and unmodified materials continue to slowly release Cu^{2+} ions beyond the 24 h tested. At 24 h, the modified material released ~90% of the total copper content, while the unmodified material released just ~54% of the total copper content. These results suggest that these materials may be advantageous for the slow and controlled release of Cu^{2+} ions.

To better understand the copper-releasing performance of these materials, the copper flux of each was determined and plotted for the first 6 h (Figure 8). The rate of copper flux of the unmodified material at 30 min is significantly greater than that of the modified MOF. At 1 h, the MOF–cotton material still exhibits a greater flux than the unmodified material. After 2.5 h, the materials begin to behave similarly and slowly release Cu^{2+} ions. After 6 h, the copper ions are continually released for both materials at a flux less than or equal to $0.9 \mu\text{mol}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$. On the basis of previous reports of PSM yielding more stable materials, we anticipated the modified materials would release copper ions slower than the unmodified materials. Conversely, the results of the PSM to the materials lead to an increase in the copper flux. Although the results with valeric anhydride are not what were hypothesized, they excitingly suggest that this strategy does indeed yield tunable materials. As such, these findings serve as a necessary precedence to encourage further exploration using the PSM of MOFs in textile materials for controllable ion release.

PXRD of the MOF–cotton materials after 1 h of being submerged in NBM at room temperature revealed that while these materials release copper, the MOF retains crystallinity (Supporting Information, Figure S5). These results are particularly interesting when compared to previous reports investigating the biocompatibility of MOF coatings on solid Au surfaces and the stability to water and complex media of the materials.²⁶ Notably, a similar copper-SURMOF (Cu-BTC) releases Cu^{2+} ions because of the dissolution of the MOF, most rapidly in cell culture media. A complete structural collapse is observed within 30 min of Cu-BTC submersion in water. Herein, the materials retain crystallinity after 1 h of submersion in NBM. In addition, the materials continue to release Cu^{2+} ions beyond the 24 h tested. The retention of crystallinity may directly enhance the overall efficacy of using a MOF for antibacterial applications. The direct interaction of a marine bacterium with the copper-MOF surfaces has been reported to lead to the decomposition of SURMOF

crystallinity and release of Cu^{2+} ions.²⁷ Taken together, the data suggest that the particular solid support to which the MOF is bound, the MOF growth process, and PSM may all contribute to the stability and overall copper-releasing activity observed with the materials and therefore should be carefully considered for the desired application. As the Cu^{2+} ions are reportedly toxic to microorganisms including bacteria, the $\text{Cu}_3(\text{NH}_2\text{BTC})_2$ -cotton materials warrant further investigation of antimicrobial efficacy.

Bacteria Studies.

The antibacterial efficacy of the unmodified and modified MOF-cotton swatches was tested against *E. coli* both under wet conditions (over a 24 h period) and dry conditions (5 min exposure period). Bacteria strains that are naturally present on skin (including *E. coli*) can readily attach onto textile surfaces, leading to potentially lethal bloodstream infections.²⁸ To determine the extent of cellular viability after exposure to the materials, a broth dilution, agar plating method was employed. This allows for extremely sensitive measurements to be attained, with a LOD of 1 CFU/mL.^{29,30} This agar plating method works not only for planktonic bacteria (where the aliquots can be removed at the desired time points), but also allows for the quantification of the viable bacteria remaining on the swatches after the exposure period. A 5 min sonication period of the cotton swatches liberates the bacteria that were previously attached to the surface without causing any loss of cellular viability in the process. Once the sonication period is complete, the aliquots can be removed and plated in an identical fashion as the planktonic bacteria.³¹

Antibacterial Activity under Wet Conditions.

Given the application of cotton swatches acting as wound dressings, it is useful to determine the remaining cellular viability after exposure to cotton at varying time points as it has been shown that the relative amount of bacteria present at a wound site is directly attributable to the potential to alter the normal wound-healing process.^{32,33} Therefore, the remaining viable *E. coli* cells were assessed after 1, 6, and 24 h exposure to the cotton swatches. Indeed, 6 h is considered the ideal time necessary for the bacteria to colonize a wound; however, it is also necessary to test the cellular viability after 24 h to ensure no regrowth of the bacteria has occurred.³⁴ These are considered “wet” experimental conditions, as the materials are fully submerged in the bacterial solution for the entire challenge period. Additionally, both the planktonic (in solution) bacteria and the viable bacteria that attached to the cotton swatches were assessed after the 24 h exposure period. This gives insight into both the antibacterial efficacy of the Cu^{2+} ions being released from the MOF-cotton swatches and the ability of the MOF-cotton swatches to impede bacterial attachment and act as an antibacterial surface.

Figure 9 shows the results from the *E. coli* kill rate study over the entire 24 h challenge period. The results show that the cotton swatches alone have no effect on the cellular viability of *E. coli* at any tested point. Indeed, the *E. coli* cells assessed in the presence of both cotton and the carboxymethylated cotton swatches were not statistically different when compared to *E. coli* without any cotton present. As an additional control, the cellular viability of *E. coli* in the presence of the ligand was assessed (at equal amounts that are present on the MOF-cotton swatches), which demonstrated very little antibacterial effect (10^7 CFUs/mL after 24 h exposure). After the initial hours of exposure to the MOF-cotton

swatches, there does not appear to be any significant decrease in viability. However, after 6 h of exposure to both the unmodified and modified MOF–cotton swatches, a decrease in planktonic viability is apparent. Both MOF-grown surfaces result in a 3-log reduction in cellular viability when compared to the cotton swatch alone. This is a substantial reduction to achieve, given the current industry standard is considered a 3-log reduction (equivalent to 99.9% reduction in viable cells). After 24 h of exposure, the reduction in viability is even greater, resulting in a 4-log for the modified MOF and 5-log for the unmodified MOF. This assessment after 24 h is critical as reports have shown significant regrowth of bacteria after the initial efficacy was determined.^{35,36}

In addition to the planktonic kill rate achieved using both the modified and unmodified MOF surfaces, the bacterial attachment onto cotton was depleted in the presence of the MOF–cotton swatches when compared with cotton on its own. Figure 9 shows the results of this study, where there is a massive 3-log reduction in viable bacteria attached to the MOF surfaces compared to the cotton surface. Bacterial attachment is the first step in biofilm formation and thus becomes the critical step to ensuring this detrimental effect does not occur.^{37,38} The observed reduction in attachment after 24 h indicates that these materials could indeed act as antibacterial surfaces, particularly through additional tuning of Cu²⁺ ion release to increase the reduction in attachment. It is also interesting to note that there is no statistical difference between the reduction in attachment between the modified and unmodified MOF, given their unique behavior against the planktonic bacteria. This would suggest that the remaining MOF (and subsequent copper) that is still present on both types of MOF–swatches is within the threshold to elicit these impressive effects.

Antibacterial Activity under Dry Conditions.

To understand how these materials behave as antibacterial surfaces under circumstances more similar to a pure copper surface application, dry conditions were also employed. This examines the efficacy of the materials without a direct contribution to the metal ions releasing into solutions and killing planktonic bacteria. This method is also more representative of the wound-healing model, where there is no continuous solution of bacteria surrounding the materials. A 5 min exposure period was chosen based on previous bacteria studies using metal surfaces.^{39,40} The results from this study (and the wet attachment study) are shown in Figure 10, where there is a further enhanced reduction in attachment onto the unmodified swatches under dry conditions than that was observed under wet conditions (4-log vs 3-log), and a similar reduction in attachment was observed for the modified swatch. This is a remarkable reduction in attachment to achieve using a minimal 5 min exposure period. This indicates that the inability for the *E. coli* cells to begin the biofilm process is near immediate, representing an attractive option for biofilm impediment. It is also fascinating that the total amount of attached cells onto natural cotton is the same, regardless of the exposure time or wet versus dry conditions.

While there have been preliminary works reporting copper-based MOFs as antibacterial agents, the results achieved in this work represent the most promising to date toward utilizing MOFs as antibacterial surfaces. Abbasi et al. use Cu-BTC grown on silk fibers to test the growth inhibition of *E. coli* and *Staphylococcus aureus*, but do not attempt to

quantify the antibacterial efficacy of their MOF materials.¹⁹ Rodríguez et al. expose *E. coli* cells to Cu-BTC grown on cellulose fibers, but the quantification method (resazurin) lacks the sensitivity to achieve the reductions seen in this work.⁴¹ Most important, however, is the determination of the cotton swatches to act as an antibacterial surface, a property that has not been tested in any of the mentioned literature. It is critical to test the surviving bacteria not only in solution (where it is known to be most susceptible to antibacterial agents) but also in its ability to attach to a surface.³⁸ The ability to impede bacterial attachment is indeed the most promising avenue for novel biomaterials, making this work particularly powerful in its ability to inhibit *E. coli* attachment under both wet and dry conditions.

CONCLUSIONS

This work demonstrates the potential to use surface-mounted MOF-containing materials to control the copper flux from materials and exploit the antibacterial properties of the metal ions in MOFs for antibacterial effects. The carboxymethylation process implemented establishes a new standard for increasing the amount of modification to cotton to improve the number of nucleation sites on the cotton fibers for MOF growth. The layer-by-layer growth process utilized herein leads to a highly uniform and evenly dispersed MOF around the entire cotton fibers. Importantly, this MOF permits PSM to the free amine to tune = physicochemical properties of the material. By manipulating the stability of the material to complex media at biologically relevant conditions, the overall flux of the material may be changed over the first 6 h. This serves as a critical precedent to enable synthetic control, demonstrating the potential power of PSM to tune the antibacterial textile surfaces.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic of the carboxymethylation process and growth of the MOF $\text{Cu}_3(\text{NH}_2\text{BTC})_2$ using a layer-by-layer process on the surface of the cotton fabric. First, the cotton swatches were modified under basic conditions with sodium chloroacetate. The resultant nucleation sites enabled the immobilization of the MOF chemically bound to the cotton fibers via a layer-by-layer growth process using repetitive dipping into the solutions of copper nitrate or ligand $\text{NH}_2\text{H}_3\text{BTC}$.

Figure 2.
PSM of the MOF $\text{Cu}_3(\text{NH}_2\text{BTC})_2$ with valeric anhydride to yield an AM-5-modified MOF.

Figure 3.

IR spectra of carboxymethylated cotton (red) and natural cotton (black). Verification of carboxymethylation highlighted at 3380 cm^{-1} indicates the functionalization of free alcohol to carboxylate ion as well as the appearance of a new $\text{C}=\text{O}$ shift at 1630 cm^{-1} .

Figure 4. Images of natural cotton used (left) and the uniformly grown MOF on the cotton material (right).

Figure 5.

(A) Diffuse reflectance UV-vis of the MOF-powder (red) and MOF-cotton (blue) materials, showing the presence of the MOF on cotton. (B) PXRD data of all relevant samples indicating the crystalline MOF on the surface of the carboxymethylated cotton. Postsynthetically modifying the materials does not impact the structural integrity or morphology of the MOF crystals. (C) IR spectroscopy of the MOF-powder and MOF-cotton samples with relevant features noted.

Figure 6. SEM images obtained at 5 kV of the MOF–cotton materials, showing excellent surface coverage of the MOF fibers. (A–C) are images of natural cotton. (D–F) are images of the MOF immobilized onto the cotton fibers under optimized conditions. All the cotton swatches were imaged over at least three different sites (not shown).

Figure 7.

(A) SEM image of a cross-sectioned MOF-cotton material and EDS mapping of copper. (B) TEM images of an embedded and cross-sectioned MOF-cotton material showing coverage around the entire cotton fiber. (C) BFTEM image of a MOF crystal anchored on the cotton material. (D) HAADF-STEM image of MOF crystals on the surface of a single fiber of the MOF-cotton material. (E) HAADF-STEM image of MOF crystals on the MOF-cotton material. (F) HAADF-STEM image of a MOF crystal on the MOF-cotton material; the lines of copper are visible. (G) HRTEM image of MOF crystals from MOF-cotton material liberated during isolation of a single fiber of material. Framed sections 1, 2, and 3 are magnified and shown with associated Fourier transform.

Figure 8. Copper ion-releasing flux data of the MOF-cotton materials soaked in NBM solution for 6 h at 37 °C. The modified MOF cotton materials exhibit a different copper-releasing flux, which is most substantial over the first 6 h. Data presented as the average with the associated standard deviation, $n = 3$.

Figure 9. Bacterial kill rate of *E. coli* in the presence of the three cotton swatches, with viable cells vs time displayed. The graph shows the average and standard deviation, $n = 6$.

Figure 10.

Attachment of *E. coli* onto the three cotton swatches under wet and dry conditions, with viable bacteria vs the type of swatch displayed. Wet conditions indicate the films were submerged in bacteria solution for 24 h before the assessment of viability; dry conditions indicate an aliquot of bacteria solution was added to each swatch for 5 min before the assessment of viability. Data show the average standard deviation, $n = 6$.

Table 1.

Copper in Solution After Soaking 0.49 cm² Swatch in 1 mL NBM at 37 °C, Average and Standard Deviation Displayed ($n = 3$)

time (h)	MOF-cotton ($\mu\text{mol.cm}^{-2}$)	AM-5 modified cotton ($\mu\text{mol.cm}^{-2}$)
0.5	3.21 $\pm$ 0.21	2.55 $\pm$ 0.08
1	3.70 $\pm$ 0.42	3.07 $\pm$ 0.10
2.5	4.10 $\pm$ 0.11	3.85 $\pm$ 0.20
6	5.28 $\pm$ 0.36	5.16 $\pm$ 0.22
12	5.01 $\pm$ 0.11	5.76 $\pm$ 0.17
24	5.18 $\pm$ 0.07	6.15 $\pm$ 0.10