Journal of Applied Polymer Science

Antibacterial Activity Against *Escherichia coli* of Cu-BTC (MOF-199) Metal-Organic Framework Immobilized onto Cellulosic Fibers

Haendel S. Rodríguez,[1] Juan P. Hinestroza,[2] Cristian Ochoa-Puentes,[1] Cesar A. Sierra,[1] Carlos Y. Soto[1]

[1]Department of Chemistry, Faculty of Sciences, Universidad Nacional de Colombia, Carrera 30 # 45-03, Ciudad Universitaria, Bogotá, Colombia

[2]Department of Fiber Science and Apparel Design, Cornell University, Ithaca, New York 14853

Correspondence to: C. A. Sierra (E-mail: casierraa@unal.edu.co.)

ABSTRACT: A strong antimicrobial activity against *Escherichia coli* of Cu-BTC metal-organic frameworks immobilized over cellulosic fibers is hereby reported. The *in situ* synthesis of Cu-BTC metal-organic frameworks, aka MOF-199 or HKUST-1, onto cellulosic substrates was carried out by exposing carboxymethylated cellulosic substrates to $Cu(OAC)_2$, 1,3,5-benzenetricarboxylic acid and triethylamine solutions following a very specific order. Using an in vitro model, in accordance to ASTM E2149-13a, we observed that the cellulose-MOF system was able to completely eliminate the growth of *E. coli* on agar plates and liquid cultures. The antibacterial activity of the comprising components of MOF-199 and the cellulosic substrate was also evaluated and determined to be negligible. Since the method used to synthesize MOF-199 crystals provides a strong bond between the crystals and the cellulosic substrates, the crystals not detach from the anionic cellulosic fibers allowing the modified textile to be washed and reused hence opening a new avenue to fabricate antibacterial clinical fabrics. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40815.

KEYWORDS: cellulose and other wood products; nanoparticles; nanowires and nanocrystals; textiles

Received 4 February 2014; accepted 31 March 2014

DOI: 10.1002/app.40815

INTRODUCTION

Healthcare-associated infections in hospitals cause significant economic consequences on healthcare systems as ~1.7 million nosocomial infections are reported every year just in the USA.[1] These incidents represented financial losses ranging from $28 to $45 billion dollars.[2] Most of nosocomial infections are produced by drug-resistant or multi-drug resistant gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* as well as gram-positive bacteria such as *Staphylococcus aureus* and beta-hemolytic Streptococcus.[3] The design of new antibacterial materials has been one of the most important challenges in the development of new strategies for the control of healthcare-associated infections.

Bacterial adhesion, that is the interaction of cells with environmental surfaces mediated by van der Waals and electrostatic forces, involves multiple processes that culminate in a multicellular structure called biofilm.[4] Usually, bacteria contained in biofilms exhibit an increased tolerance against antimicrobial drugs[5,6]; thus, in addition to exhibiting biocidal characteristics, antimicrobial materials have to avoid the growth of bacteria and its adhesion to surfaces. Antibacterial strategies based on surface-immobilized polycations have been successful for the development of contact-killing surfaces. These materials often contain polymeric hydrophobic alkyl side chains that improve their cytotoxic and antimicrobial activity.[7] Another important issue is the lifetime of the active species, which can be improved by developing materials in which the active compound is covalently bonded to the surface hence offering very low to zero possibilities for leaching.[8]

The antibacterial activity of metallic surfaces, such copper, is well-known as Cu ions have been identified to induce damage to the bacterial envelope.[9,10] A synergistic effect between the presence of copper species and a very stable substrate is expected to largely improve the antibacterial effect of the modified surface. Hence, a cellulose-MOF-199 system (from now on "cellulose-MOF") could offer a unique solution, by merging the antibacterial behavior of MOF-199 with the immobilization of these compounds to cellulose fibers. The antibacterial activity of MOF-199 immobilized on fibers has not been thoroughly studied. Abbasi et al.[11] reported the first antimicrobial activity of MOF-199 coated on silk against *E. coli* via qualitative methods. As far as the authors are aware of, no further antimicrobial studies have been published in the scientific literature afterwards. This paper focuses on providing quantitative information of the

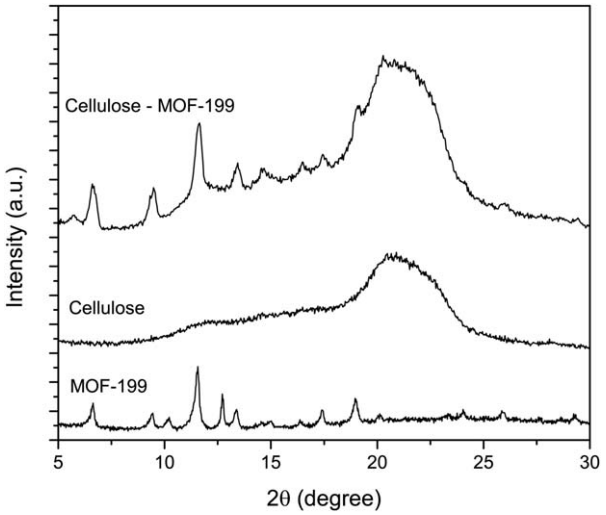

Figure 1. XRD patterns of MOF-199, anionic cellulose and anionic cellulose after *in situ* synthesis of MOF-199.

antimicrobial activity of MOF-199 and its individual components and it also explores the antimicrobial activity of cotton-based substrates conformally coated with MOF-199 crystals.

EXPERIMENTAL

Materials

Copper (II) acetate, 1,3,5-benzene-tricarboxylic acid, methanol, *N,N*-dimethylformamide (DMF), sodium hydroxide, sodium chloroacetate and ethanol were purchased from Sigma-Aldrich (St. Louis, MO), while cellulose fabrics (TIC/400R standard woven cotton fabrics) were obtained from SD Atlas (Greenville, NC). All chemicals and reagents were analytical grade and used as received.

Instrumentation

X-ray diffraction and FT-IR analyses were performed in a BRUKER D8-ADVANCE diffractometer and a Nicolet Magna 760 FTIR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA) in single attenuated total reflectance (ATR) mode. For UV-Vis measurements, an ELISA Reader from Bio-Rad was used.

Anionic Cellulose Synthesis

In order to create a suitable substrate for the synthesis of a cellulose-MOF system, carboxymethylation of cellulose fabrics was necessary. Carboxymethylation was achieved by reacting cellulose and chloroacetate salt under the presence of sodium hydroxide as a catalyst.[12]

Cellulose-MOF System Synthesis

The growth of MOF-199 to the anionic cellulose was performed according to our previously reported method.[13] Cellulose-MOF fabrics were washed with water, DMF and methanol, for 5 h in each solvent to remove MOF-199 crystals that were not chemically attached to the anionic cellulose.

Antibacterial Assays

The antibacterial activity of the cellulose-MOF system was determined on *E. coli* BL21 cells (Stratagene, USA) according to ASTM E2149-13a,[14] with minor modifications explained as follows. Bacterial cells were cultured in LB broth at 37°C with agitation (83 rpm) until achieving a stationary phase of growth (18 h). Then, bacteria were diluted in 0.3 m*M* KH_2PO_4, pH 7.2, until an $OD_{470} = 0.28 \pm 0.02$ to obtain a working solution corresponding to 1.6×10^4 colonies forming units (CFU) $\times$ mL^{-1}, approximately.

In one test, under sterile conditions and by triplicate, 0.25 ± 0.1 g of the cellulose-MOF system (2×2 cm^2 size fabric swatches) were placed into 50 mL polycarbonate tubes containing 12.5 mL of the working solution. As a control, in a separate tube containing an identical volume of the working solution, a similarly sized fabric swatch of anionic cellulosic was placed. The two specimens were incubated at 37°C with agitation for 1 h. Subsequently, serial dilutions of the incubated samples (supernatant) were plated on LB-agar. The inoculated plates were incubated at 37°C for 24 h and surviving CFU were counted.

On the other hand, The minimal inhibitory concentration (MIC) of unattached MOF-199 on *E. coli* cells was determined using a microplate colorimetric-based test with resazurin as a bacterial growth indicator.[15] The growth of bacteria was indicated by a change in the color of broth from blue to rose, and the MIC was defined as the lowest MOF-199 concentration preventing this color change.

In order to know if the substances involved in the synthesis of the cellulose-MOF system were individually responsible for the antimicrobial activity, a standard zone of inhibition test under static conditions was also performed. In this method, 100 µL of 1,3,5-benzenetricarboxylic acid (24.0 m*M*), copper(II) acetate (35.3 m*M*) and DMF:ethanol:water (1 : 1 : 1) solutions were placed in holes (1×1 cm^2) bored into LB plates that were previously seeded with a confluent layer of *E. coli* as shown in the top of Figure 2.[16]

To evaluate bactericidal efficiency under contact, a standard zone of inhibition test was performed. Swatch pieces of 2×2 cm^2 of cellulose-MOF system and anionic cellulose (control) were placed on the surface of LB plates containing confluent bacteria as shown in the bottom of Figure 2. The plates were incubated at 37°C for 24 h, and the inhibition of bacterial growth was visually evaluated after removing the fabric swatches.

Antimicrobial Leaching Assay

To establish potential leaching of the antimicrobial (i.e. MOF) compound during the experiments, similarly sized samples (2×2 cm^2) of cellulose-MOF system and anionic cellulose fibers (0.25 ± 0.1 g) were placed in 50 mL of buffer 0.3 m*M* KH_2PO_4, pH 7.2, and incubated for 1 h. Then, 100 µL of these solutions were separately inoculated in the borehole of LB plates previously seeded with a confluent layer of *E. coli*. The plates were incubated at 37°C for 24 h, and the presence or absence of inhibition halos was used to assess potential leaching of the antimicrobial substances.

RESULTS AND DISCUSSION

MOF-199 Is Strongly Immobilized onto Cellulosic Fibers

Cellulose-MOF fabrics were washed in three different solvents (water, DMF, and methanol) until no blue turquoise color, characteristic color of MOF-199 crystals, was observed in the

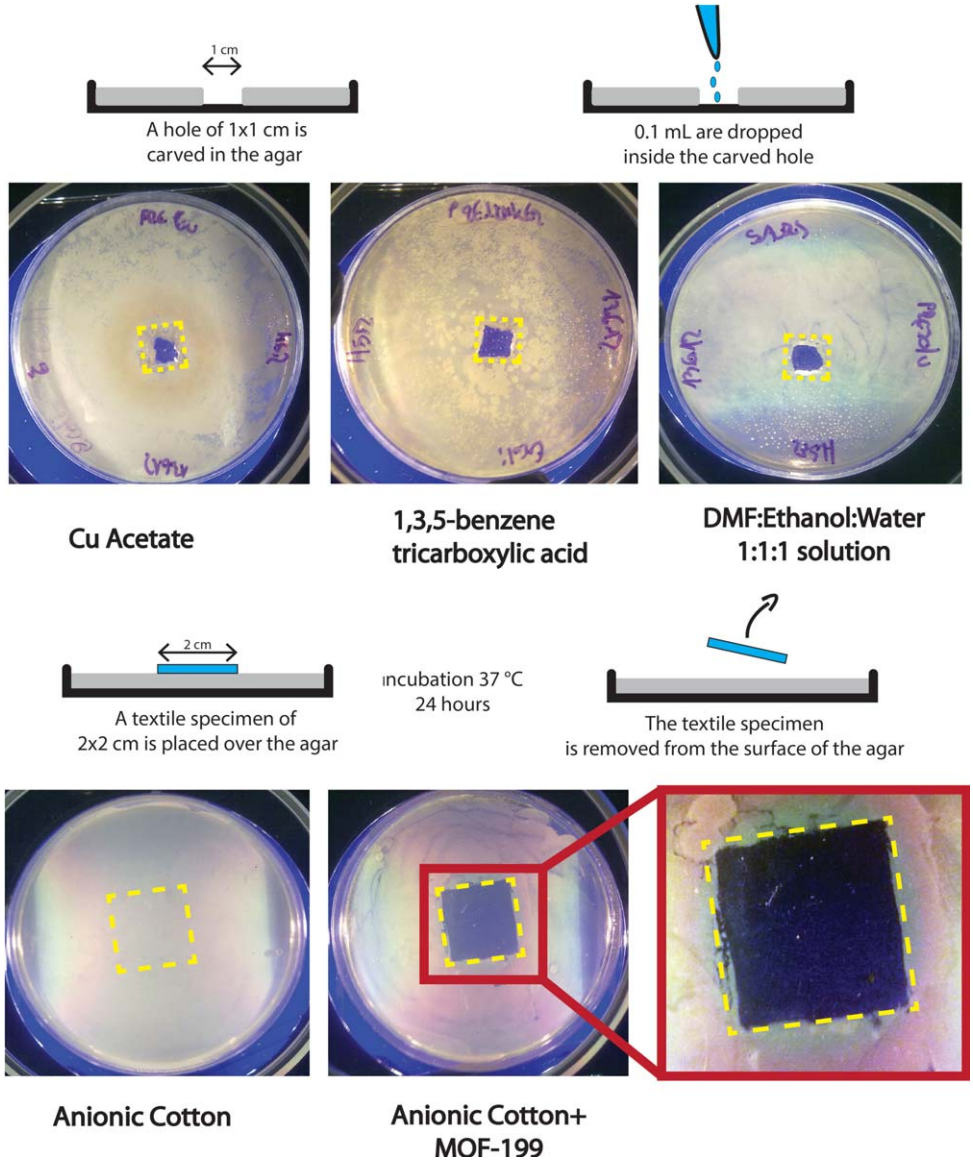

Figure 2. Antibacterial activity results according to ASTM E2149-13a. Bottom: The cellulose-MOF fabric (anionic cotton+MOF-199) shows complete inhibition of bacterial growth on the contact area. The absence of halo indicates lack of diffusion of the antimicrobial compound around the contact area. In sharp contrast, no inhibition zone was formed when only anionic cellulose (anionic cotton) was placed on the LB plates seeded with confluent *E. coli*. Top: No inhibition halos were observed when cells were grown in contact with solutions of copper acetate, 1,3,5-benzenetricarboxylic and DMF:ethanol:water, indicating that substances involved in the MOF-199 synthesis are not individually able to kill *E. coli* at the reported experimental conditions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

filtrates.[17] The thoroughly washed specimens were weighted and it was determined that they contained a loading of 42.5 mg of MOF-199 per gram of fabric.

To confirm the presence of MOF-199 onto the cellulose, X-ray diffraction (XRD) analyses were performed as shown in Figure 1. The comparison between the cellulose-MOF system and the XRD patterns of MOF-199 crystals, synthesized according to Tranchemontagne et al.,[18] clearly indicates the superposition of some of the characteristic peaks of the MOF-199 along with patterns of amorphous cellulose.[13]

Table I. Quantification of Antibacterial Activity Against *E. coli*

Sample	Type of culture	Initial CFU (mL)	Final CFU (mL)	Bacterial reduction (%)
Cellulose-MOF	Liquid	16,000 ± 3.7	0	100
Anionic cellulosic fiber	Liquid	16,000 ± 3.7	5,700 ± 6.3	64

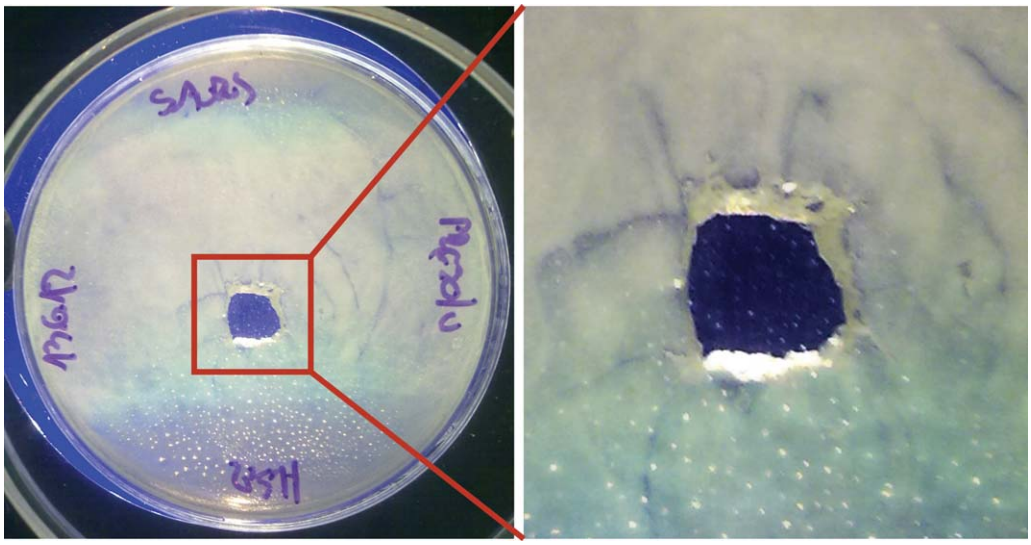

Figure 3. Leaching test. Inhibition of *E. coli* growth was not detected when cells were grown in contact with aliquots of a working solution previously exposed to a cellulose-MOF. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

MOF-199 Is Responsible for the Antimicrobial Activity Against E. coli

The standard zone of inhibition test, a qualitative test performed under static conditions (ASTM E2149-13a), indicated that bacterial growth below the contact area between the *E. coli* and the 2 × 2 cm^2 specimen of cellulose-MOF fabric was completely inhibited as shown in the lower section of Figure 2. That is to say that no growth was detected on or beneath the fabric, and no inhibition halo was observed around the contact area. These results indicate that the MOF-199 crystals display antibacterial activity against *E. coli* and that MOF-199 did not diffusing off the fabric to the nearby bacterial film as shown in the magnified image in lower section of Figure 2. On the other hand, inhibition of *E. coli* growth was not observed when the cells were cultured in contact with a piece of untreated anionic cellulose.

To assess if the substances involved in the MOF-199 synthesis exhibited antimicrobial activity, *E. coli* cells grown in LB plates were exposed to each one of the reagents conforming the MOF using a standard zone of inhibition test.[14,16] In this experiment holes were bored in the middle of agar plates where confluent *E. coli* was previously seeded, and solutions of the substances were separately placed in these holes. As it can be seen in the top section of Figure 2, when cells were grown in contact with solutions of copper (II) acetate, 1,3,5-benzenetricarboxylic acid and DMF:ethanol:water (1 : 1 : 1), no zone of inhibition was observed around the holes indicating that substances involved in the MOF-199 synthesis were not individually able to kill bacteria. Also, an invasion of *E. coli* around the holes was noted as the initial square area of 1 cm was reduced after incubation. An independent experiment, Minimal Inhibitory Concentration (MIC) test, was performed to evaluate the antimicrobial activity of unattached MOF-199. The MIC of the unattached MOF-199 crystals was determined to be 25 $\mu g \times mL^{-1}$ highlighting the deleterious effect of MOF-199 on bacterial cells. These results indicate that the MOF-199 structure is responsible for the antimicrobial effect, instead of its individual constituents.

To quantify the antimicrobial activity of the cellulose-MOF system, another procedure outlined in ASTM E2149-13a was performed under dynamic conditions. In this test, bacterial suspensions of *E. coli* in buffer (working solution) were separately incubated in presence of cellulose-MOF and anionic cellulosic swatches. The bacterial reduction was estimated using the supernatant of culture of samples. We found that the viability of *E. coli* was lower (5700 ± 6.3 CFU $\times$ mL^{-1}) when the cells were incubated with the anionic cellulose swatch in comparison to the case when the cells were incubated without the presence of any fabric specimens (16000 ± 3.5 CFU $\times$ mL^{-1}). More importantly, CFU were not observable when *E. coli* cells were incubated in the presence of the cellulose-MOF-199 system. These results are presented in Table I. At first glance, this quantitative test may attribute antibacterial activity of the anionic cellulose specimens under dynamic conditions, which contrast with the results from the static tests shown in Figure 2. However, it is important to note that the reduction of CFU could be the result of mechanical stress or adhesion of cells to the fabric during the immersion process rather than the actual antibacterial characteristics of anionic cellulose. Discrepancies between static and dynamic tests for immobilized antimicrobial agents continue to be an open debate.[19]

It is known that copper's antimicrobial mechanisms are complex and diverse, and we speculate that the observed behavior is probably caused by the copper (II) interaction with the cell membrane via oxidation of membrane proteins and fatty acids or transmembrane potential alteration leading to cell lysis.[20] We hypothesize that the oxidized state of copper in the MOF-199 structure combined with the abundance of open metal sides could cause the rupture of the membrane. This special situation in the MOF-199 structure could enhance the antimicrobial potential of copper.

No MOF-199 Leaching

The possible antimicrobial activity of leachates from the cellulose-MOF system was also evaluated using a standard zone

of inhibition test as shown in Figure 3. In this case, aliquots (100 μL) of working solution after incubation with both types of fabrics (anionic cellulosic and cellulose-MOF fabrics) were inoculated in a hole carved in LB plates previously seeded with confluent *E. coli* cells. No zone of inhibition around the holes was detected after cell incubation at 37°C during 24 h. These results indicate the negligible presence of antimicrobial substances in the leachate solution suggesting that MOF-199 crystals do not leach from the fabric upon exposure to the reported experimental conditions.[13]

CONCLUSIONS

In this study, a cellulose-MOF system was successfully synthesized and its antibacterial properties against *E. coli* were evaluated and quantified. The results indicated that the cellulose-MOF system exhibits a strong antibacterial activity giving a complete inhibition of microorganism growth on solid and liquid cultures. Experimental results also indicate that MOF-199 crystals and not its individual components were responsible of this enhanced antibacterial activity. It can be suggested that the usage of the cellulose-MOF system for the fabrication of antibacterial clinical fabrics has a big potential since this system showed to be useful for bacteria control and the nondetachment of MOF crystals could improve the fabric's lifetime and help to solve environmental problems related with antibacterial compounds release.

ACKNOWLEDGMENTS

This work was supported by Colciencias, and the "División de Investigación Bogotá, DIB", Vicerrectoría de Investigación, Universidad Nacional de Colombia, grants 16060 and 16071. This work made use of the Cornell Center for Materials Research Shared Facilities, which are supported through the NSF MRSEC program (DMR-1120296).

REFERENCES

1. Klevens, R. M.; Edwards, J. R.; Horan, T. C.; Gaynes, R. P.; Pollack, D. A.; Cardo, D. M. *Public Health Rep.* **2007**, *122*, 160.
2. Scott, R. D. The direct medical costs of healthcare-associated infections in U.S. hospitals and the benefits of prevention, 2009. Available at: http://www.cdc.gov/hai/pdfs/hai/scott_costpaper.pdf (accessed June 2, **2013**).
3. Jean, S. S.; Hsueh, P. R.; Lee, W. S.; Chang, H. T.; Chou, M. Y.; Chen, I. S.; Wang, J. H.; Lin, C. F.; Shyr, J. M.; Ko, W. C.; Wu, J. J.; Liu, Y. C.; Huang, W. K.; Teng, L. J.; Liu, C. Y. *Eur. J. Clin. Microbiol. Infect. Dis.* **2010**, *29*, 471.
4. Vuong, C.; Otto, M. *Microbes. Infect.* **2002**, *4*, 481.
5. Roosjen, A.; Busscher, H. J.; Norde, W.; Van der Mei, H. C. *Microbiology* **2006**, *152*, 2673.
6. Ojha, A. K.; Baughn, A. D.; Sambandan, D.; Hsu, T.; Trivelli, X.; Guerardel, Y.; Alahari, A.; Kremer, L.; Jacobs, W. R.; Hatfull, G. F. *Mol. Microbiol.* **2008**, *69*, 164.
7. Lichter, J.; Van Vliet, K.; Rubner, M. *Macromolecules* **2009**, *42*, 8573.
8. Murata, H.; Koepsel, R. R.; Matyjaszewski, K.; Russell, A. J. *Biomaterials* **2007**, *28*, 4870.
9. Espirito Santo, C.; Quaranta, D.; Grass, G. *Microbioly Open* **2012**, *1*, 46.
10. Casey, A. L.; Adams, D.; Karpanen, T. J.; Lambert, P. A.; Cookson, B. D.; Nightingale, P.; MIruszenko, L.; Shillam, R.; Christian, P.; Elliot, T. S. *J. Hosp. Infect.* **2010**, *74*, 72.
11. Abbasi, A.; Akhbari, K.; Morsali, A. *Ultrason. Sonochem.* **2012**, *19*, 846.
12. Wang, Z.; Hauser, P. J.; Laine, J.; Rojas, O. J. *J. Adhes. Sci. Technol.* **2010**, *25*, 643.
13. Da Silva, M.; Sierra, C. A.; Hinestroza, J. P. *Cellulose* **2012**, *19*, 1771.
14. ASTM E2149-13a. Standard test method for determining the antimicrobial activity of antimicrobial agents under dynamic contact conditions. West Conshohocken, PA: American Society for Testing and Materials; **2013**.
15. Santos, P.; Gordillo, A.; Osses, L.; Salazar, L.; Soto, C. Y. *Peptides* **2012**, *36*, 121.
16. Martin, T.; Kooi, S.; Chang, S.; Sedransk, K.; Gleason, K. *Biomaterial* **2007**, *28*, 909.
17. Bordiga, S.; Regli, L.; Bonino, F.; Groppo, E.; Lamberti, C.; Xiao, B.; Wheatley, P. S.; Morris, R. E.; Zecchina, A. *Phys. Chem. Chem. Phys.* **2007**, *9*, 2676.
18. Tranchemontagne, D. J.; Hunt, J. R.; Yaghi, O. M. *Tetrahedron* **2008**, *64*, 8553.
19. Green, J. D.; Fulghum, T., Nordhaus, M. A. *Biointerphases* **2011**, *6*, MR13.
20. Hong, R., Kang, T. Y., Michels, C. A., Gadura, N. *Appl. Environ. Microbiol.* **2012**, *78*, 1776.