A. COVER PAGE

Project Title: Rechargeable Antimicrobial Textiles to Reduce Occupational Risk of Healthcare Personnel	
Grant Number: 5R21OH011406-02	**Project/Grant Period:** 08/01/2019 - 07/31/2021
Reporting Period: 08/01/2020 - 07/31/2021	**Requested Budget Period:** 08/01/2020 - 07/31/2021
Report Term Frequency: Final	**Date Submitted:** 10/12/2022
Program Director/Principal Investigator Information: YUYU SUN , PHD **Phone Number:** 978-934-3637 **Email:** yuyu_sun@uml.edu	**Recipient Organization:** UNIVERSITY OF MASSACHUSETTS LOWELL UNIVERSITY OF MASSACHUSETTS LOWELL 600 Suffolk Street, Suite 415 LOWELL, MA 018543643 **DUNS:** 956072490 **UEI:** LTNVSTJ3R6D5 **EIN:** 1043167352E5 **RECIPIENT ID:**
Change of Contact PD/PI: No	
Administrative Official: JILL GUTIERREZ 600 Suffolk Street, Suite 212 Lowell, MA 018543692 **Phone number:** 978-934-3247 **Email:** jill_gutierrez@uml.edu	**Signing Official:** JACKIE BLACK 600 Suffolk Street - Suite 212 Suite 212 Lowell, MA 018543692 **Phone number:** 978-934-4739 **Email:** Awards@uml.edu
Human Subjects: NA	**Vertebrate Animals:** NA
hESC: No	**Inventions/Patents:** Yes **If yes, previously reported:** No

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Our long-term goal is to use a monitorable and rechargeable antimicrobial technology to control microbial contamination on healthcare service textiles to reduce the occupational burden of exposure to infectious agents, as a significant component of infection prevention. Our objectives here are to establish the feasibility of applying the new technology to materials that are currently used in healthcare facilities, including cotton, nylon, polyester and their blends, and to evaluate the antimicrobial performance of the new textiles under simulated in-use conditions (various soils, dry, repeated exposure, and multiple pathogenic species with normal skin flora). To accomplish these objectives, the specific aims (goals) of the proposed research are to:

Aim 1. Covalently bind N-halamines onto healthcare service textile materials and characterize the physical/mechanical properties of the new materials.
Aim 2. Evaluate in vitro the antimicrobial performance under simulated in-use conditions, the cytotoxicity of the new materials and the risk of microbial resistance to the materials.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Not Applicable

B.2 (B-2.pdf)

B.2 What was accomplished under these goals?

1) Major Activities: Our long-term goal is to use a monitorable and rechargeable antimicrobial technology to control microbial contamination on healthcare service textiles to reduce the occupational burden of exposure to infectious agents, as a significant component of infection prevention. Our objectives here are to establish the feasibility of applying the new technology to materials that are currently used in healthcare facilities, and to evaluate the antimicrobial performance of the new textiles under simulated in-use conditions (various soils, multiple pathogenic species with normal skin flora, etc.). To accomplish these objectives, the specific aims of the proposed research are to: (1) covalently bind N-halamines onto healthcare service textile materials and characterize the physical/mechanical properties of the new materials; and (2) evaluate in vitro the antimicrobial performance under simulated in-use conditions, the cytotoxicity of the new materials and the risk of microbial resistance to the materials. In this reporting period, we have made significant progress in both aims.

2) Specific Objectives:

2-1) Aim 1: In this aim, we continued to develop the pad-dry-cure grafting methods and evaluated the physical/mechanical properties of the grafted fabrics. In the grafting reactions, we designed and synthesized water-soluble copolymers, poly(methacrylamide-co-acrylic acid) (MAA-co-AA; MAA/AA ratio of 7/3, 8/2, and 9/1), which could be easily grafted/crosslinked onto various fabrics via epoxy curing reactions with poly(ethylene glycol) diglycidyl ether (PEGDGE, as the crosslinker). MAA moieties in the grafted copolymers were then transformed into N-halamines by rinsing with diluted chlorine bleach. The contents of the covalently bonded chlorine in each resulting textile material were determined with iodometric titration. The washing durability and rechargeability of the covalently bound chlorine were determined, the monitorability of the bound chlorine through color change was confirmed, and the effects of grafting/bleaching on physical properties were evaluated.

2-2) Aim 2: In this aim, we continued to evaluate the antimicrobial effects of the new textiles under simulated in-use conditions. Test organisms included: *Acinetobacter baumannii* (ATCC 19606), *Candida albicans* (ATCC 24433), *Escherichia coli* (ATCC 29214), vancomycin-resistant *Enterococcus faecalis* (ATCC 51575), methicillin-resistant *Staphylococcus aureus* (ATCC 43300), methicillin-susceptible *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 10145), and MS-2 virus (ATCC 15597-B). Representative normal skin flora included: *Acinetobacter lwoffii* (ATCC 15309), *Corynebacterium striatum* (BAA1293), *Micrococcus luteus* (ATCC 49732), and *Staphylococcus epidermidis* (ATCC 14900). We continued to use artificial soils including sterile PBS (the control soil), sterile artificial sweat, and sterile 5% serum in artificial sweat. We evaluated the antimicrobial activity of N-halamine fabrics with a single organism and mixed organisms in the presence of various soils.

Cytotoxicity of the fabric was evaluated with the XTT (sodium 3´-[1- (phenylaminocarbonyl)- 3,4- tetrazolium]-bis (4-methoxy6-nitro) benzene sulfonic acid hydrate) assay on L929 mouse fibroblasts (ATCC CCL-1) as specified by ISO 10993-5:2009. Triton X-100 (1wt%) was used as the positive control. Extracts in culture media from the N-halamine-containing fabrics were added to L929 cell cultures and cell viability was compared with the controls.

To address a logical concern about the antimicrobial fabrics, we also studied whether bacteria would develop resistance with several rounds of subculture in the presence of the N-halamine fabrics.

3) Significant Results:

3-1): New grafting methods in the preparation of N-halamine fabrics: By controlling reaction conditions, a wide range of N-halamine contents (from less than 100 ppm to more than 10,000 ppm) could be readily achieved. The treatment did not negatively affect the tearing strength and significantly improved the abrasion resistance of the resulting fabrics. The covalently bound chlorines in the N-halamines were both durable and rechargeable. For example, on N-halamine containing fabrics, at 20 °C and 60% relative humidity, the level of active chlorine on the fabrics was not changed after six months of storage. Even after 12 months of storage, more than 60% of the original chlorines were maintained. In laundering tests, washing gradually reduced the active chlorine contents. Nevertheless, even after 50 cycles of laundering, the chlorinated fabrics still contained residual chlorines. Further, after 50 washing cycles, the fabrics were re-chlorinated, and around 90% of the original chlorine was regenerated.

B.2 (B-2.pdf)

3-2): Monitorability of the covalently bound N-halamines: The presence of covalently bonded N-halamines in the fabrics could be easily detected with KI/starch test strips. When contacted with the untreated original fabrics, the test strip did not show any color change; however, when contacted with the N-halamine-based fabric containing 500 ppm of covalently bonded chorine, the test strip generated a light purple color; and when contacted with the N-halamine fabric containing 1500 ppm or 4000 ppm of chlorine, the strip showed much darker color after 2 min of contact.

3-3): Antimicrobial effects of the N-halamine fabrics: Antimicrobial effects were initially evaluated following the specifications of the AATCC (American Association of Textile Chemists and Colorists) test method 100, and the N-halamine fabrics we developed provided 99.9983% kill of seven common bacteria (including drug-resistant bacteria) and fungi in 15 min, and 100% kill of MS2 virus in 30 min.

To better model challenging in-use conditions (presence of various soils and multiple microbial species mixed with skin flora), we developed a novel comprehensive antimicrobial efficacy evaluation method through testing standardized mixtures of pathogens and normal skin microorganisms in the presence of artificial soils including artificial sweat and 5% human serum. This method contains four main steps: 1) freezing individual organisms at a consistent absorbance, 2) identifying the concentration of organisms in frozen aliquots, 3) mixing specific quantities of each to obtain the desired concentration, and 4) preparing frozen aliquots of the final mixture. The soils were found to have no impact on microbial growth. The maximum number of mixed organisms attempted was five. Colony morphologies were easily distinguishable. Frozen aliquot preparation generated the desired volume of broth containing approximately equivalent CFU/mL of each representative organism. Freezing aliquots of mixtures successfully allowed for thawing and utilization of the mixtures in subsequent experiments. Organism growth between freeze-thaw cycles was consistent between runs and reliably generated plates with countable colonies of representative organisms.

Microbial mixtures containing representative normal flora organisms with and without an additional pathogen prepared as above were tested against N-halamine fabrics with a 15-minute contact time. There was a statistically significant reduction in all organisms in the mixtures on the N-halamine fabric in the presence of all soils (P <0.05). All organisms were reduced by 4.00 $\log_{10}$ or greater, and the overall average $\log_{10}$ reduction for all mixtures was 4.86. When separated by soil type, the overall average $\log_{10}$ reductions were 4.86 for PBS, 5.03 for artificial sweat, and 4.86 for 5% serum. No statistical significance was found between soil types (P >0.05).

3-4): No cytotoxicity and microbial resistance: Compared to blank media, L929 cell viability was not significantly affected by any of the N-halamine extracts, suggesting excellent cytocompatibility of the N-halamine finished fabrics. Furthermore, no microbial resistance was found after 10 cycles of repeated exposure of the bacteria toward the N-halamine fabrics.

4) Key outcomes or other achievements

Successfully binding N-halamines onto healthcare fabrics using continuous methods: We have established methods for covalently binding N-halamines onto healthcare textiles. The continuous binding treatments were much simpler and more practical than the batch methods, and we have submitted a patent application to protect this technology. The treatments did not negatively affect the physical properties of the resulting fabrics. The N-halamines on the fabrics were monitorable, durable, and rechargeable.

New antimicrobial testing methods: We successfully created the first method to simulate in-use conditions with mixed organisms in the presence of various soils. The new method provides a more rigorous performance evaluation by including commonly encountered pathogens in the presence of normal flora and soils. This is also the first reproducible method for mixing organisms at relatively equal concentrations.

Attractive antimicrobial performance of N-halamine fabrics: The N-halamine fabrics have potent antibacterial, antifungal, and antiviral effects, in the presence of various soils. The antimicrobial activity did not show variability between soils, indicating that sweat and proteinaceous serum do not impede the action of the N-halamine at the concentrations tested. The N-halamine fabrics had no cytotoxicity and did not induce microbial resistance under our testing conditions, pointing to the great potential of the fabrics as new infection-control strategies in healthcare settings.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
N/A: Not NIH Funded	Nicoloro JM, Wen J, Queiroz S, Sun Y, Goodyear N. A novel comprehensive efficacy test for textiles intended for use in the healthcare setting. Journal of microbiological methods. 2020 June;173:105937. PubMed PMID: 32387116; PubMed Central PMCID: PMC9453850; DOI: 10.1016/j.mimet.2020.105937.
N/A: Not NIH Funded	Wen J, Khan AD, Sartorelli JB, Goodyear N, Sun Y. Aqueous-based continuous antimicrobial finishing of polyester fabrics to achieve durable and rechargeable antibacterial, antifungal, and antiviral functions. Journal of Industrial and Engineering Chemistry. 2022 March 25;107:249-259.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? Yes

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
YUYUSUN	Y	SUN, YUYU	PHD	PD/PI	2.5	0.0	0.0			NA
GOODYEAR4427	Y	Goodyear, Nancy	PHD	PD/PI	0.0	0.0	1.5			NA
ADORRAHKHAN	N	Khan, Adorrah-Le		Graduate Student (research assistant)	0.0	9.0	3.0			NA
JIANCHUAN_WEN	N	Wen, Jianchuan		Staff scientist (Doctoral level)	4.0	0.0	0.0			NA

Glossary of acronyms:
S/K - Senior/Key
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RS - Reentry Supplement
DS - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Not Applicable

D.2.b New Senior/Key Personnel

Not Applicable

D.2.c Changes in Other Support

Not Applicable

D.2.d New Other Significant Contributors

Not Applicable

D.2.e Multi-PI (MPI) Leadership Plan

Not Applicable

E. IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

G. SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Not Applicable

G.4.b Inclusion Enrollment Data

NOTHING TO REPORT

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

NOT APPLICABLE

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT
No foreign component
G.10 ESTIMATED UNOBLIGATED BALANCE
Not Applicable
G.11 PROGRAM INCOME
Not Applicable
G.12 F&A COSTS
Not Applicable

I. OUTCOMES

I.1 What were the outcomes of the award?

Healthcare personnel (HCP) have a high occupational burden of exposures to infectious agents. Service textiles are high-touch surfaces in the healthcare environment and they play an important role in the acquisition and transmission of pathogenic microorganisms. Extensive studies have shown that in general practice, "clean" healthcare service textiles are rapidly and heavily contaminated/re-contaminated with pathogenic bacteria, including multi-drug resistant organisms that can survive for weeks on soft fabrics and easily transfer onto HCP's hands and other clean surfaces.

In this project, we developed a pad-dry-cure continuous treatment technique to covalently bind (not coat or impregnate) N-halamines onto service textiles. Our main findings include:

(1) The N-halamine fabrics provided 99.9983% kill of seven common bacteria (including drug-resistant bacteria) and fungi in 15 min, and 100% kill of MS2 virus in 30 min. The presence of normal skin flora, artificial sweat, and/or human serum did not affect antimicrobial potency. In comparison, a commercial silver-based antimicrobial scrub achieved only a 26.09% reduction of the bacteria.
(2) The antimicrobial activity was maintained for 12 months and could be easily monitored with potassium iodide (KI) test strips through color change (i.e., N-halamine reacts with KI to produce iodine with a yellow/brown color).
(3) If the test showed that the function was reduced, the fabric could be fully recharged by rinsing with diluted bleach. The recharging could be repeated through the entire service life of the fabric.
(4) Further, the N-halamine fabrics showed excellent in vitro cytocompatibility toward mammalian cells.