AD_____

Award Number: W81XWH-13-C-0048

TITLE: Durable and rechargeable antimicrobial textiles

PRINCIPAL INVESTIGATOR: Dr. Zhengbing Cao

CONTRACTING ORGANIZATION: Medetech Development Corp
LYNNWOOD, WA 98087-1532

REPORT DATE: December 2013

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE
December 2013

2. REPORT TYPE
Final

3. DATES COVERED
4 March 2013 - 4 September 2013

4. TITLE AND SUBTITLE
Durable and rechargeable antimicrobial textiles

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-C-0048

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Zhengbing Cao

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Medetech Development Corp
LYNNWOOD, WA 98087-1532

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

In accordance with the Phase I solicitation, Medetech Development Corporation proposed and investigated our novel water-soluble, self-curable, aqueous epoxy-based polymeric N-halamine antimicrobial textile finishing technology. The ultimate objective of this proposed project is to develop robust scalable architectures for antimicrobial textiles to provide antimicrobial uniforms and undergarments for soldiers to control body odor and reduce the risk of infection. The materials can also be used as antimicrobial medical textiles for medical shelters and military hospitals to control the transmission of pathogenic bacteria in the field. The main objective of phase I of this SBIR project was to demonstrate the feasibility of producing durable and rechargeable N-halamine-based antimicrobial textiles via a simple, practical, and eco-friendly approach. This goal has been achieved, Dr. Zhengbing Cao and his team's phase I work on the development of N-halamine-based fabrics in Medetech showed encouraging results, as described below. The Phase I results demonstrated that Medetech's new HalomineTM technology could reliably enable the creation of efficiacious functional coatings on cotton and polyester (see the sections below).

15. SUBJECT TERMS- none provided

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU		**19b. TELEPHONE NUMBER** *(include area code)*

1. PHASE I TECHNICAL RESULTS

In accordance with the Phase I solicitation, Medetech Development Corporation proposed and investigated our novel water-soluble, self-curable, aqueous epoxy-based polymeric N-halamine antimicrobial textile finishing technology. The ultimate objective of this proposed project is to develop robust scalable architectures for antimicrobial textiles to provide antimicrobial uniforms and undergarments for soldiers to control body odor and reduce the risk of infection. The materials can also be used as antimicrobial medical textiles for medical shelters and military hospitals to control the transmission of pathogenic bacteria in the field. The main objective of phase I of this SBIR project was to demonstrate the feasibility of producing durable and rechargeable N-halamine-based antimicrobial textiles via a simple, practical, and eco-friendly approach. This goal has been achieved, Dr. Zhengbing Cao and his team's phase I work on the development of N-halamine-based fabrics in Medetech showed encouraging results, as described below. The Phase I results demonstrated that Medetech's new Halomine™ technology could reliably enable the creation of efficiacious functional coatings on cotton and polyester (see the sections below).

1.1 Phase I tasks and results

Task 1. The technology results in covalent bonding of N-halamines onto fabrics with high density of crosslinking.

In the treatment of cotton fabrics, the effects of polymeric N-halamine precursor concentrations (0.1 wt%, 0.2 wt%, 0.5 wt%, 1.0 wt%, 2.0 wt and 5.0 wt%), wet pickups (80 wt%, 100 wt%, and 120 wt%), curing temperatures (120 ^{o}C, 150 ^{o}C, and 180 ^{o}C) and curing duration (1 min, 2 min, 5 min, and 30 min) on grafting yield and chlorine content were investigated. As shown in Figure 1, grafting yield rapidly increases from 0.04 to 3.12 wt% with the increase of polymeric N-halamine precursor concentration from 0.1 to 5 wt%. Correspondingly, the chlorine content markedly increases from 46 ppm to 1242 ppm. However, when the polymeric N-halamine precursor concentration is higher than 5 wt%, the textile finish slightly changes the hand of the treated fabrics. The effects of wet pickup are illustrated in Figure 2. Higher wet pickup leads to more chemical loading, higher grafting yield and higher chlorine content, but takes longer drying times. As shown in Figure 3, higher curing temperature significantly improves the grafting yield and chlorine content. However, extremely high temperature curing has the potential to damage the treated textile. Finally, longer curing time improves the grafting yield and chlorine content (Figure 4).

In the treatment of polyester fabrics, we tested the effects of polymeric N-halamine precursor concentrations (0.1 wt%, 0.2 wt%, 0.5 wt%, 1.0 wt%, 2.0 wt and 5.0 wt%) under 100 wt% wet pickups, 180 ^{o}C curing temperatures and 2 min curing. As can be seen in Figure 5, grafting yield rapidly increases from 0.02 to 1.35 % with the increase of polymeric N-halamine precursor concentration from 0.1 to 5%. Correspondingly, the chlorine binding content markedly increases initially from 23 ppm to 482 ppm.

These results suggest that our new polymeric N-halamine precursors can be used to introduce N-halamines into both cotton and polyester, two important military textiles, to achieve powerful antimicrobial effiacy (see the following tasks). Reaction conditions have significant effects on grafting yields and chlorine contents, which are fabric-specific; more work is needed to establish the optimal treatment conditions for the scaling up and commercialization of these and other related new products.

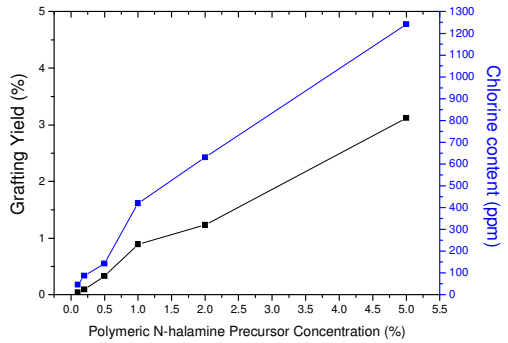

Figure 1. The effects of polymeric N-halamine precursor concentration on grafting yield and chlorine content.(100% wet pickup, 180 °C curing temperature, 2 min curing)

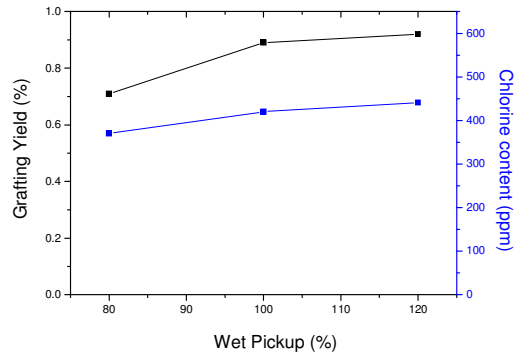

Figure 2. The effects of wet pickup on grafting yield and chlorine content. (1% polymer concentration, 180 °C curing temperature, 2 min curing)

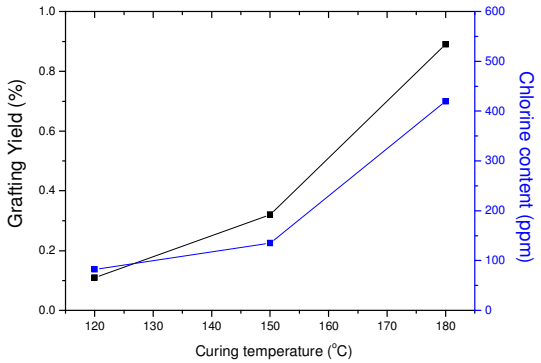

Figure 3. The effects of curing temperature on grafting yield and chlorine content. (1% polymer concentration, 100% wet pickup, 2 min curing)

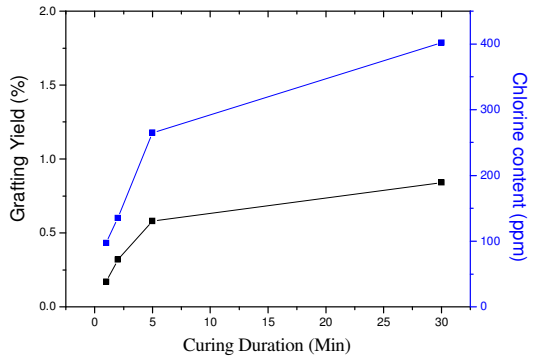

Figure 4. The effects of curing duration on grafting yield and chlorine content. (1% polymer concentration, 100% wet pickup, 150 °C curing temperature)

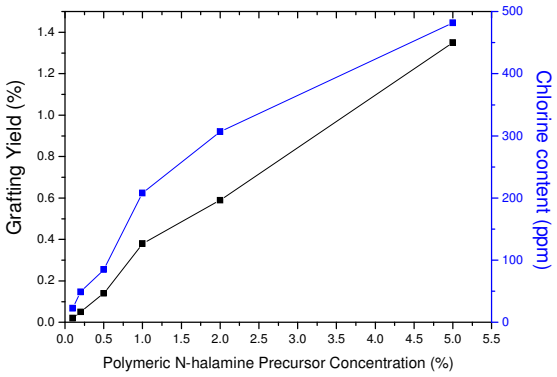

Figure 5. The effects of Polymeric N-halamine precursor concentration on grafting yield and chlorine content for polyester fabrics.(100% wet pickup, 180 °C curing temperature, 2 min curing).

Task 2. Characterize the physical/mechanical and other properties.

Water vapor permeability was used to simulate the body moisture and sweat during uniform wearing conditions. The water vapor permeability of the samples was measured using the cup method, according to the ASTM E96 standard. As shown in Table 1, the antimicrobial finishing process did not significantly alter the water vapor permeability of the resulting fabrics.

Table 1. Comparison of water vapor permeability of treated and untreated cotton samples

Water Vapor Permeability Index (%)			
Treated Cotton Sample		Untreated Cotton Sample	
Sample with 46 ppm chlorine	428 ± 8.4	Control 1	431 ± 10.3
Sample with 420 ppm chlorine	442 ± 6.1	Control 2	428 ± 6.9
Sample with 1242 ppm chlorine	431 ± 9.2	Control 3	435 ± 7.6

Abrasion resistance, tear resistance and bursting strength were determined according to ASTM D4966, ASTM, and ASTM D3786, respectively. As can be seen from Tables 2-3, Medetech's antimicrobial textile finishing did not significantly change the mechanical properties of the resulting fabrics.

Table 2. Comparison of mechanical properties of treated and untreated cotton samples

Mechanical Properties	Control		Sample	
Abrasion resistance(rubs)	19,543 ± 2,068		18,653± 1,5101	
Bursting strength (lbf)	29.1 ± 2.3		27.2 ± 3.9	
Tear resistance (lbs)	Warp Direction	Fill Direction	Warp Direction	Fill Direction
	1.425 ± 0.102	1.208 ± 0.045	1.470 ± 0.038	1.266 ± 0.077

No obvious difference between control and sample. (Less than 10%)

Table 3. Comparison of fabric hand of treated and untreated cotton samples

Untreated Cotton Fabrics-Control			Treated Cotton Fabrics- Sample		
Bending	Shearing	Surface	Bending	Shearing	Surface
Warp Direction	Springy	Pliable	Warp Direction	Springy	Pliable
Fill Direction	Springy	Pliable	Fill Direction	Springy	Pliable

No obvious difference between control and sample.

Medetech's novel antimicrobial finishing process did not significantly alter the physical/ mechanical properties of the resulting fabrics.

Task 3. Evaluate in vitro antimicrobial performance of the treated fabrics.

In the antimicrobial efficacy tests, all the microbial species were provided by the American Type Culture Collection (ATCC). *Staphylococcus epidermidis* (*S. epidermidis*, ATCC 35984, gram-positive) and *Escherichia coli* (*E. coli*, ATCC 15597, gram-negative) were used as representative examples of clinically skin-related non-resistant bacteria. *Candida albicans* (*C. albicans*, ATCC 10231, fungi), a diploid fungus, was used as a representative example of fungi. As shown in

Table 4-6, Treated fabrics were challenged with 10^7-10^8 CFU/mL of the bacteria and yeast. The treated fabrics with low chlorine contents demonstrated obvious antimicrobial effect against the bacteria: after 30 min of contact, provided around 90% reduction of *S. epidermidis* and *E. coli*. However, we achieved more powerful antimicrobial efficacy when we prolonged the contact duration. Usually, it's powerful enough for textile-related odor control. Medical antimicrobial textiles products for infection control, usually, 99.9% reduction of bacteria in 30 min will be the optimum killing power which is the goal we are trying to achieve for this project. As shown in Table 4-6, higher chlorine contents significantly improved the antimicrobial activity. The new fabrics with higher than 404 ppm of chlorine provided more than 99.9% reduction of the test microorganisms within 30 min, meeting the EPA standard for antimicrobial materials for medical products. [1]

Table 4. Antibacterial activities of chlorinated fabrics with various active chlorine contents against *Staphylococcus epidermidis*, ATCC 35984

Active chlorine content (ppm)			Reduction of the microbes after contacting with the treated fabrics for different periods of time** (%)			
			15 min	30 min	60 min	120 min
Cotton	Sample 1	40	21.78	51.32	75.44	79.33
	Sample 2	82	60.36	80.32	87.25	91.21
	Sample 3	130	67.82	81.45	92.12	99.61
	Sample 4	404	99.91	99.99	99.99	99.99
	Sample 5	620	99.91	99.99	99.99	99.99
	Sample 6	1201	99.99	99.99	99.99	99.99
Polyester	Sample 1	81	80.89	89.32	95.76	99.49
	Sample 2	200	85.46	99.38	99.45	99.94
	Sample 3	310	99.28	99.93	99.99	99.99
	Sample 4	455	99.92	99.99	99.99	99.99

Table 5. Antibacterial activities of chlorinated fabrics with various active chlorine contents against *Escherichia coli*, ATCC 15597

Active chlorine content (ppm)			Reduction of the microbes after contacting with the treated fabrics for different periods of time** (%)			
			15 min	30 min	60 min	120 min
Cotton	Sample 1	40	50.65	70.38	87.98	91.36
	Sample 2	82	67.46	86.43	94.65	99.43
	Sample 3	130	91.43	91.69	99.31	99.92
	Sample 4	404	99.23	99.91	99.99	99.99
	Sample 5	620	99.99	99.99	99.99	99.99
	Sample 6	1201	99.99	99.99	99.99	99.99
Polyester	Sample 1	81	80.89	89.32	95.76	99.49
	Sample 2	200	85.46	99.38	99.45	99.94
	Sample 3	310	99.28	99.91	99.97	99.99
	Sample 4	455	99.92	99.99	99.99	99.99

Table 6. Antibacterial activities of chlorinated fabrics with various active chlorine contents against *Candida albicans, ATCC 10231*

Active chlorine content (ppm)			Reduction of the microbes after contacting with the treated fabrics for different periods of time** (%)			
			15 min	30 min	60 min	120 min
Cotton	Sample 1	40	11.91	33.48	45.44	69.33
	Sample 2	82	45.32	70.76	73.25	90.43
	Sample 3	130	59.82	90.21	90.65	99.15
	Sample 4	404	99.16	99.93	99.98	99.99
	Sample 5	620	99.90	99.99	99.99	99.99
	Sample 6	1201	99.99	99.99	99.99	99.99
Polyester	Sample 1	81	30.42	45.76	58.94	89.17
	Sample 2	200	80.32	90.32	96.32	99.91
	Sample 3	310	99.26	99.94	99.99	99.99
	Sample 4	455	99.91	99.99	99.99	99.99

*Calculate % reduction to formula 1) 100 (B-A)/B=R,

*Explanation: the higher the % value the more organism were destroyed; negative values equal an increase in bacterial numbers

Furthermore, the new N-halamine-based fabrics demonstrated a powerful ability to control bacterially-generated ammonia for cotton and polyester (Figure 6, 7). In the Drager R tube tests, with higher than 82 ppm of chlorine on the N-halamine fabrics, at the end of the six-hour incubation, no ammonia could be detected. Under the same conditions, the control samples (pure cotton and pure polyester) generated respectively more than 45 and 34 ppm of ammonium, with a strong odor to human nose.

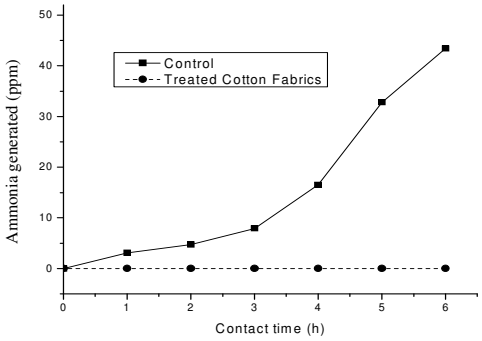

Figure 6. Prevention of ammonia formation on treated cotton fabrics. The treated cotton fabrics contained 82 ppm of covalently bound chlorine.

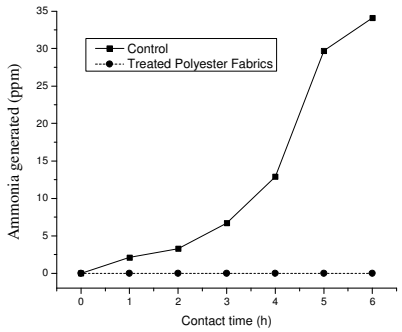

Figure 7. Prevention of ammonia formation on treated polyester fabrics. The treated polyester fabrics contained 78 ppm of covalently bound chlorine

Task 4. Evaluate washing durability, rechargeability and shelf life.

In the leaching tests, a 3 × 3 cm N-halamine treated cotton fabric with 620 ppm chlorine was put in 10 ml water. After 24 h agitation, KI was used to test the free chlorine/N-halamine in the water, and no observable leaching of active chlorine/N-halamine was detected. This non-leaching test demonstration is available on youtube: http://www.youtube.com/watch?v=qZaOCwL51EM. In washing durability tests as shown in Table 7, after 50 cycles of simulated home laundering and recharging, the antimicrobial functions of the new N-halamine fabrics were essentially unchanged.

Table 7. Washing durability testing

Testing Results: As is	Results: CFU/Sample		
	Zeo Contact Time	24hr Contact Time	Percent Reduction*
Staphylococcus auresus ATCC 6538	1.50E + 05	9.99E + 02	99.33%
Klebsiella pneumonia ATCC 4352	1.80E + 05	5.15E + 03	97.14%

Testing Results: 50X	Results: CFU/Sample		
	Zeo Contact Time	24hr Contact Time	Percent Reduction
Staphylococcus auresus ATCC 6538	1.50E + 05	9.99E + 02	99.33%
Klebsiella pneumonia ATCC 4352	1.80E + 05	9.99E + 02	99.45%

*Calculate % reduction to formula 1) 100 (B-A)/B=R, section 11.2

*Explanation: the higher the % value the more organism were destroyed; negative values equal an increase in bacterial numbers

To determine storage durability, the treated fabrics were stored under normal warehouse conditions. The chlorine content and antimicrobial potency of each sample was tested monthly over 6 months. The N-halamine fabrics retained more than 90% of their original chlorines as shown in Table 8.

Table 8. Textile stability study

Duration		Month 0	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Chlorine content		ppm	ppm	ppm	ppm	ppm	ppm	ppm
Chlorinated Cotton	Sample 1	46	44	41	45	40	40	36
	Sample 2	87	88	80	81	82	78	71
	Sample 3	143	133	135	132	130	125	120
	Sample 4	420	416	419	400	404	401	395
	Sample 5	631	641	630	622	620	610	588
	Sample 6	1242	1230	1220	1210	1201	1182	1162
Chlorinated Polyester	Sample 1	85	88	80	82	81	78	74
	Sample 2	208	211	199	205	200	195	191
	Sample 3	307	317	316	300	310	298	289
	Sample 4	482	471	473	453	455	451	451

Weather resistance as evaluated with UV light and moisture exposure following AATCC Test Method 186: a laboratory artificial weathering exposure apparatus employing fluorescent UV lamps as a light source and using water spray for wetting will be used to simulate real in-use conditions. Chlorine contents changes in exposed test specimens were evaluated by titration test method. Antimicrobial function changes in exposed test specimens were evaluated by antimicrobial test method. After either exposure to UV irradiation from 1h，2h, 4h, 8h, the fabric samples were titrated immediately and also after rechlorination.

As shown in Table 9, part of the chlorine in treated cotton lost after high intense UV irradiation and the lost chlorine could be regenerated upon rechlorination. After 1 h irradiation, some of the chlorine on coated cotton was lost, although after equal 8 h under UV light irradiation, of the N–Cl bond in coated cotton had decomposed. After equal exposure to high intense UV light for 8 h and rechlorination, more than 90% chlorine in coated cotton could be recovered. The UV light stability provides the treated fabrics for soldiers to control body odor and reduce the risk of infection, and to provide medical textiles with antimicrobial functions to help prevent the transmission of pathogenic bacteria under field conditions.

Table 9. UV light stability of chlorinated cotton fabrics (Cl^+ content, ppm)

Time (h)	Sample 4		Sample 6	
	UV exposure	Rechlorination	UV exposure	Rechlorination
0	400	395	1210	1206
1	391	393	1196	1201
2	385	390	1147	1197
4	373	379	980	1176
8	342	362	891	1092

Task 5. Evaluate the biocompatibility of the treated fabrics.

The biocompatibility (cytotoxicity) data of the treated fabrics was shown in Figure 8,9. As demonstrated by trypan blue assay, the viability of the balb/c mouse 3T3 fibroblast cells was not significantly affected by the presence of up to 1210 ppm of covalently bound chlorine after up to 3 days of contact. Of all the balb/c mouse 3T3 fibroblast cells exposed to the new treated fabrics, only a few had trypan blue-stained nuclei (indicating cell growth expiration), which was very similar to the culture-only controls and untreated cotton control, suggesting good biocompatibility.

In our preliminary cost analysis, we found that the total cost of the antimicrobial textile finishing treatment of 1 kg dry textile was around $0.346, including $0.096/kg for the antimicrobial finishing agent and $0.25/kg for the textile finishing process.

To sum up, our Phase I results demonstrated the feasibility of using the new technology for antimicrobial finishing of both natural and synthetic fabrics. Unique features of the Halomine™ technology include powerful antimicrobial activity (more than 99.9% reduction in less than 30 min), long antimicrobial duration (6 months), rechargeability of the antimicrobial function, good physical/mechanical properties, good biocompatibility, and low cost. Building on these Phase I results, a Phase II project will be need to further develop and scale up the new approach for the manufacturing and commercialization of this new generation of antimicrobial products, as described below.

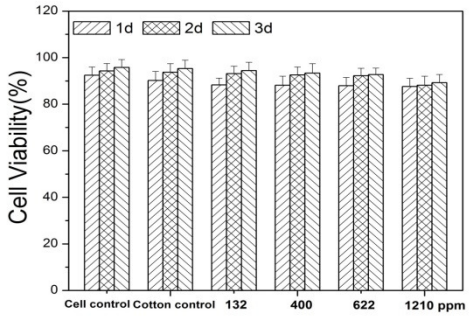

Figure 8. Cell viabilities of the treated cotton fabrics.

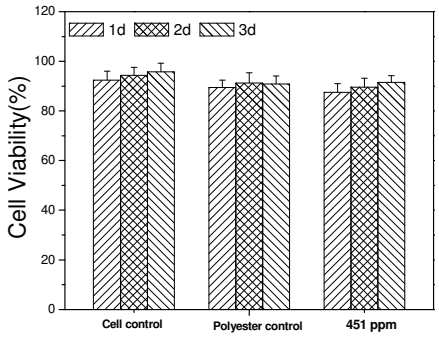

Figure 9. Cell viabilities of the treated polyester fabrics.

Our Phase I results already demonstrated the feasibility of the new technology, suggesting that Medetech's Halomine^{TM} antimicrobial textile finishing technology provides a solution to the problems associated with current antimicrobial textile approaches, by combining the power of the best antimicrobial agents, N-halamines (N-Cl), with the ease of production of commercially available low-potency products such as Microban, Aegis, etc. The resulting powerful and durable antimicrobial textiles have the potential to significantly improve the quality of infection control with soldiers and military healthcare personnel through management of odor issues and textile related infections. The treated fabrics will provide long-lasting and rechargeable protection against bacteria, fungi and viruses for both military applications and a broad range of civilian applications. A Phase II study will be needed to further develop the technology for real application and commercialization.

2. PHASE II TECHNICAL OBJECTIVES AND APPROACHES

The Phase I results already demonstrated the feasibility and efficacy of Medetech's antimicrobial finishing technology for producing durable and rechargeable antimicrobial textiles for military applications via a simple, practical, and cost-effective approach. The immediate objective of this Phase II study is to establish the optimal conditions for the application of this new technology so as to enable scale-up and commercialization of new products for military and a broad range of related civilian applications. The scope of work will be expanded to not only cotton, polyester, but also nylon and polyaramid, which are all important military-related textiles. The planned approaches are to: (1) Establish optimal antimicrobial finishing conditions and extend antimicrobial performance evaluation for military textiles. In addition to cotton and polyester, a broad range of synthetic fabrics are widely used in military textiles. The Phase I results demonstrated the feasibility of the technology for cotton and polyester fabrics, and suggested that different textile materials need different treatment parameters to achieve optimal antimicrobial effects. In order to commercialize the technology to real world antimicrobial textile products in this SBIR Phase II project, we will extend our studies to include cotton, polyester, and nylon and polyaramid textiles in this proposed study. The storage stability, cytotoxicity, and antimicrobial efficacy of those treated materials against bacteria (including drug-resistant species), fungi and viruses will be investigated. (2) Evaluate the efficacy of the new antimicrobial textiles in mammalian cell-bacteria co-culture systems. To simulate real in-use conditions, the performance of the new antimicrobial textiles in mammalian cell-bacteria co-culture systems will be evaluated for bandage/wound dressing related military applications. (3) Evaluate the acute dermal toxicity

of the new antimicrobial textile materials. The safety of the treated fabrics will be further evaluated for its dermal toxicity and skin irritancy. (4) Design prototypes, scale up the finishing process, and prepare for EPA "antimicrobial" claim approval.

2.1 Phase II - Approaches

Approach 1- Establish optimal antimicrobial finishing conditions and extend antimicrobial performance evaluation for military textiles

Both natural fibers (such as cotton) and synthetic fibers (such as polyester, nylon, and polyaramids) are used for military as well as civilian applications. In this Phase II project, we will extend our studies to include cotton, polyester, and nylon and polyaramid textiles. On the completion of approach 1, we will create a series of N-halamine treated fabrics with a broad range of grafting yields and chlorine contents. The next approaches will fully evaluate these fabrics to identify the treatment conditions to achieve the best overall performance for each fabric type.

It has been well documented that Gram-positive and Gram-negative bacteria on the skin are the major cause of skin infections; fungi/yeasts can also contribute to infections. Thus, in this project, we will use three Gram-positive bacteria (*Staphylococcus epidermidis*, ATCC 35984, *Staphylococcus aureus*, ATCC 6538, and *Streptococcus pyogenes,* ATCC 19563,), two Gram-negative bacteria (*Klebsiella pneumoniae*, ATCC 13886, *Escherichia coli*, ATCC 15597), and one yeast *Candida albicans* (*C. albicans,* ATCC 10231) as the model test microorganisms. All these species have been reported to be responsible for skin problems.[2-4] Multidrug-resistant species, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE), have spread out of healthcare facilities, and MRSA infections have been reported in public sites, posing a growing risk for the general public. Multidrug-resistant pathogens caused huge numbers of deaths and billions of dollars in excess healthcare cost in the United States each year, and can cause serious military-related infections. In this Phase II project, Methicillin-resistant *S. aureus* (MRSA, ATCC BAA-811) and Vancomycin-resistant *E. faecium* (VRE, ATCC 700221) will be selected to represent drug-resistant strains because these species have caused serious healthcare-associated infections (HAIs) and community-acquired infections. It has been known for some time that clothing and textiles are able to transfer viruses as well as other pathogens, and hospital viral infections are a very real problem. *E. coli* bacteriophage MS2 (ATCC 15597-B1) virus will be used to represent viral species for antiviral functions evaluation. The microorganisms will be purchased from the American Type Culture Collection (ATCC). All the microbial studies will be performed following the guidelines provided by the CDC to ensure safety. The principal investigator and co-investigators have extensive experience in handling the test organisms and following accepted procedures and protocols.

To determine storage durability, the treated fabrics will be stored under normal warehouse conditions up to 12 months. The chlorine content and antimicrobial potency of each sample will be tested monthly over the 12 months of the storage and each weather resistance test period.

Approach 2. Evaluate the efficacy of the new antimicrobial textiles in mammalian cell-bacteria co-culture systems

In this Phase II project, cell biocompatibility and antimicrobial efficacy of the treated fabrics will be further evaluated in a mammalian cell-bacteria co-culture system consisting of *S. aureus* and mouse skin fibroblast cells to simulate military-relevant in-use conditions (e.g., antimicrobial textiles on skins with cuts, bandages, wound dressing materials, etc.) [5-7]

The mouse skin cell CRL-1213TM and the bacteria *S. aureus* (ATCC 6538) will be cultured and harvested as described in the sections above. A growth medium consisting of 98% of the tissue growth medium (DMEM+FBS) and 2% of the bacteria growth medium (TSB) will be used as the co-culture medium to ensure that both the mammalian cells and the bacterial cells would grow. [43-45] Prior to cell and/or bacteria seeding, all fabric swatches ($0.2 \pm 0.01 cm^2$ per swatch) will be sterilized with UV treatment for 24 h.

The co-culture experiments will be carried out in duplicate over a 14-day period. In each test, 10 µL of the bacteria suspension with 10^4-10^5 CFU/mL of *S. aureus* in PBS and 15 µL of the mouse skin cell suspension containing 20,000 cells in the co-culture medium will be placed concurrently on each fabric swatch in individual wells of a 96-well polystyrene plate for 1 h. Afterwards, 175 µL of the co-culture medium will be added in each well. One hundred (100) µL of the medium will be changed with the fresh co-culture medium every 2 h during daytime. The swatch samples will be taken out of the medium after 1, 3, 7, and 14 days of incubation at 37 °C in a humidified 5% CO_2 atmosphere, fixed, dehydrated, sputter coated with gold, and prepared for SEM observation, as described in the sections above.

In addition, the harvested bacteria *S. aureus* will be labeled with FITC as described in the literature. [8] A co-culture of the FITC-labeled bacteria (50 µL suspension with 10^7-10^8 CFU/mL of *S. aureus* in PBS) and mouse skin fibroblasts (100 µL suspension with 400,000 cells/mL in the co-culture medium) will be seeded on each fabric swatch in individual wells of a 96-well polystyrene plate. An additional 50 µL of the co-culture medium will then be added to each well. The samples will be collected after 1, 3, and 12 h of incubation at 37 °C in a humidified 5% CO_2 atmosphere for fluorescence microscope observation. Live fibroblast cells on each sample will be stained with 0.5 U/mL of rhodamine-conjugated phalloidin using previously described methods. [9] The distribution of the fluorescently labeled *S. aureus* and fibroblast cells on the fibrous materials will be observed with an Axiovert 200 fluorescence microscope (Zeiss).

Approach 3. Evaluate the acute dermal toxicity of the new antimicrobial textile materials.

The safety of the treated fabrics will be further evaluated for their dermal toxicity potential and relative skin irritancy. Single samples, moistened with 1 mL of deionized water/g test substance, at a level of 5050 mg/kg, will be applied to the intact skin of albino rabbits. The objective of this study will be to assess the systemic toxicity potential and relative skin irritancy of the treated fabrics when administered to rabbits in accordance with US EPA OCSPP 870.1200, which is intended to meet testing requirements of FIFR 7 USC 136, *et seq*, and TSCA 15 USC 2601. All procedures in this study will be done in compliance with Animal Welfare Act Regulations.

For each fabric class, there will be no mortality expected in this study; the estimated acute dermal LD_{50} will be greater than 5050 mg/kg body weight. All animals will appear normal for the duration of the study. We expect no sign of dermal irritation at any observation point during this study. At necropsy at termination of the study we expect to see no observable abnormalities. These studies will determine the "chlorine content-acute dermal toxicity" relationship for each fabric class.). Our Phase I studies have demonstrated the high biocompatibility of the treated fabrics. Thus it is highly likely that the treated materials will not show dermal irritation.

Approach 4. Design prototypes, scale up the finishing process, and prepare for EPA "antimicrobial" claim approval.

In the second year of this Phase II project, Medetech will pursue scaling up and demonstration production of two prototypes for military applications: antimicrobial underwear for soldiers, and antimicrobial linens for military hospitals. Medetech will consult with experts in the regulatory

approval process to plan its strategy for starting an antimicrobial claim registration for Halomine™.

Medetech has already made progress in establishing commercial partners and licensing arrangements. Medetech has identified and confirmed the working OEM relationship with Precision Fabrics Group (PFG) in Greensboro of North Carolina. Precision Fabrics manufactures, markets and sells value-added products and services to selected, highly specified markets. Their high-performance products play a key role in several diverse markets, which demand engineered, finished fabrics. The common thread among these markets is the technical nature of their requirements. Precision Fabrics was the first ISO-qualified textile supplier in the USA. ISO continues to provide the discipline and framework for effective and efficient product development, customer service, and manufacturing. Precision Fabrics has been ISO-registered to 9001 since 1993 and upgraded to 9001-2008 in October 2009. Medetech anticipates working with PFG for prototype designing and scaling-up for this Phase II project. Medetech is already in ongoing discussions with Clorox and Chemetall Group to partner on the sale and distribution of other products related to the technology being deployed in this SBIR project. These partners have extensive experience in textiles products, and have been advising Medetech as to how to establish procedures and standards to support future manufacturing in-house.

Quality control standards and testing procedures will be evaluated and established in this Phase II project. Based on data generated to date in Phase I, and from results targeted in the plan of work in Phase II, we will be in a position to define the Raw Material (RM) specifications for implementation of the coating technology at commercial scale. We will work with suppliers who will be obliged to meet our requirements in order to be qualified, and will provide appropriate Certificates of Analysis (C of A) for incoming RM. At Medetech, all materials will undergo three forms of quality testing during the scaling up: grafting yield, active chlorine content and physical/mechanical properties. Grafting yield, indicating the amount of covalently bound polymeric N-halamine precursor on the treated fabrics, can be easily determined with a laboratory balance. The presence of active chlorine in the fabrics can be determined with potassium iodine (KI) titration. With the bound antimicrobial Cl technology, however, a simple KI test with a testing strip can be performed in a matter of minutes at the mill to verify proper treatment of the fabric or other surface. This is a very important part of a quality assurance (QA) program that gives the manufacturer confidence that a feature, normally invisible to the senses, can be seen and is actually on the product providing the protection. Physical/mechanical properties are checked on a random basis to ensure that manufacturers adhere to the quality standards. Maximum 10% deviation from expected will be set as the criterion.

In the second year of the Phase II effort, we plan to begin final regulatory testing, in preparation for EPA antimicrobial claim-related regulatory clearance. In Western economies where product claims that truly impact public health and disease risks are subject to rigorous regulatory agency scrutiny, there are no valid claims yet allowed for any of the antimicrobial product examples cited above—none whatsoever. The US EPA, the controlling agency for non-therapeutic antimicrobial claims in the USA, does not prevent manufacturers from incorporating certain technologies into end user products (provided these do no harm), but they do stop companies making claims that are not true about their benefits. As of today, it remains the case that US EPA has not approved any hard or soft surface claims for health benefits derived from use of antimicrobial consumer products; it has granted only limited claims on one, rather expensive institutional hard surface product entry (based on copper).

Antimicrobial textiles have huge potential for minimizing body odor and infection. A number of textiles products on the market claim antimicrobial functions, but all of these products have significant shortcomings, including weak antimicrobial activity, poor durability, and difficulty in extending antimicrobial function. The potency and durability do not come close to meeting EPA

registration requirements for public health claims as antimicrobial textiles (e.g., control microorganisms infectious for man in any area of the inanimate environment where these microorganisms may present a hazard to human health), EPA requires 99.9% reduction of test microorganisms on the test surface over an exposure period identical to the use pattern for which the product is intended; the length of time the residual activity can be expected to exist under the expected use conditions must be documented.[1] Not one of these commercial available products can come close to meeting the EPA requirements for antimicrobial textiles with public health claim.

Medetech has an experienced team in the development and commercialization of antimicrobial technologies with a long track record of collaborative work. Based on our Phase I results and our extensive experience with N-halamine-based antimicrobial technology, we are confident that this goal is achievable. The proposed technology will produce the first truly antimicrobial textiles products with EPA registration to provide potent, long-lasting and rechargeable protection against bacteria and fungi with low risk of inducing microbial resistance. Medetech has identified and secured the services of attorney Luke Conyac who has the unique EPA and regulatory experience required to assist the company in pursuing the appropriate claims and registrations. Much work has been done in this area by other companies in N-halamine-based chemistry. We believe that, in view of the preceding work done, we will be able to essentially fast-track our products with the EPA. Medetech also will pursue regulatory clearance from the FDA to market our products for hospital, wound infection control, etc.

Medetech's surface treatment technology portfolio, built around variations on a central, novel family of polymer coating structures, brings unprecedented functional power, and none of the baggage of prior art. The new durable coating chemistries are eminently practical, relying on water-based application methods. They have binding properties that allow for value-enhancing modification of a wide range of soft and hard surfaces, conferring unprecedented, fast-acting germ-killing power. All of which paves the way towards realization of long-sought dreams: legally defensible, EPA-allowable claims of true public health significance and benefit. These prospects are now within our reach, and represent an exciting and rewarding business opportunity.

3. RELATED WORK

Medetech has assembled a strong interdisciplinary team for the proposed study. The principal investigator (PI), Dr. Zhengbing Cao, has 8 years of experience in the development of antimicrobial materials, such as silver, zinc, quaternary ammonium and N-halamines. His major research interest is the application of N-halamine technology in textiles, paint, coatings, and water disinfection, etc. Dr. Cao is currently serving as the PI in this Phase I project. The PI is well experienced in commercializing N-halamine technologies. He was serving as a co-investigator in an NIH STTR grant (Antibacterial, Antifungal, Antiviral, and Antispore Paints**,** R41 NR 011545-01A1). As the key technologies inventor of Permara LLC, a South Dakota-based company, the PI was responsible for technological support during the commercialization of socks for persons with diabetes, as well as other R&D activities. The PI is in close contact with other researchers exploring the N-halamine technique for surface coatings, including antimicrobial textile finishing and water disinfection.[10-17]

4. RELATIONSHIP WITH FUTURE RESEARCH OR RESEARCH AND DEVELOPMENT

Anticipated Results of Phase I

The successful result of the Phase I effort is the viability of the proposed durable and rechargeable N-halamine-based antimicrobial textiles. The innovative simple, practical, and eco-friendly approach provides a durable, scalable, and highly robust antimicrobial textiles to control textile-related odor and infection for military. The Phase II prototype effort described will ultimately demonstrate its effectiveness.

Phase I as Foundation for Phase II

Phase I demonstrated the feasibility of producing durable and rechargeable N-halamine-based antimicrobial textiles via a simple, practical, and eco-friendly approach, suggesting that Medetech's HalomineTM antimicrobial textile finishing technology provides a solution to the problems associated with current antimicrobial textile approaches, by combining the power of the best antimicrobial agents, N-halamines (N-Cl), with the ease of production of commercially available low-potency products such as Microban, Aegis, etc. Encouraged by our Phase I results, in order to commercialize our novel technology to real world products for defense as well as civilian applications, Phase I acts as the foundation for our Phase II, we will further develop the technology for real application and commercialization in Phase II study. The Phase II research and development is designed to pursue advanced development of antimicrobial textiles using Medetech's patent pending technology and the efficacy of these materials.

The Phase II studies will fully evaluate Medetech's Halomine^{TM} antimicrobial textile finishing technology as a candidate for commercial process optimization and characterization. The investigations will allow for expansion of our Phase I findings to a full range of textile compositions, including cotton, polyester, and nylon and polyaramid. Antimicrobial activity will be fully determined for each fabric class. The treated textile properties will be fully investigated based on simulation of actual use conditions encountered by soldiers and military hospitals/shelters. If necessary, modifications or improvements will be made to improve the performance and practicality of the technology on the full range of textiles, under these challenge circumstances.

Phase II as Foundation for Military Textile Products

This Phase II approaches are intended to establish a firm the basis for commercialization of the technology, with a goal of finalizing simple, practical, and cost-effective approaches for Phase III production and distribution of an entirely new range of truly antimicrobial textiles.

5. LETTERS OF INTENT

Louis Stokes
Cleveland Department of
Veterans Affairs Medical Center
10701 East Boulevard
Cleveland, OH 44106

August 31, 2013

Dr. Zhengbing Cao, Ph.D
Medetech Development Corp.
12527 Mukilteo Speedway
Suite 103
Lynnwood, WA 98087-1532

Dear Dr. Cao,

Louis Stokes Cleveland VA Medical Center and Case Western Reserve University are very interested in the antimicrobial technology developed by Medetech. We would like to offer support to your effort to develop and commercialize a durable, rechargeable antimicrobial chemistry for use on woven textiles, and specifically for the use by the Department of Defense

I am an Infectious Diseases physician and researcher at the Louis Stokes Cleveland VA Medical Center and Case Western Reserve University. My research focuses primarily on the role of the environment, including skin and clothing, in transmission of healthcare-associated pathogens such as *Clostridium difficile* and methicillin-resistant *Staphylococcus aureus* (MRSA). We are particularly interested in developing new and improved strategies for environmental and skin/clothing disinfection. Our work is funded by the Department of Veterans Affairs, the Centers for Disease Control and Prevention, and the Agency for Healthcare Research and Quality (AHRQ).

The discussions we have had to date with The University of Akron Research Foundation about Medetech's Department of Defense SBIR project titled "Durable and Rechargeable Antimicrobial Textiles" align very well with the capabilities of our research group and our core strengths involving evaluation of new disinfection technologies. We have conducted preliminary studies with your product with C. difficile and MRSA and the initial results are very promising. We would like to work with Medetech to evaluate opportunities to develop and manufacture products using your technology for this project with the DoD.

We look forward to working with you on this opportunity. Please feel free to contact me should you have any questions or if we can be of service.
Sincerely,

Curtis Donskey M.D.

Curtis Donskey, M.D.

Infectious Diseases Section, Cleveland Veterans Affairs Medical Center
Associate Professor of Medicine, Case Western Reserve University
Phone: 216-791-3800 ext. 4788 or 6153; email: Curtis.Donskey@va.gov

PRECISION FABRICS GROUP INC.

301 North Elm Street
Greensboro, NC 27401
(336) 510 - 8011

September 13, 2013

Dr. Zhengbing Cao, Ph.D
Medetech Development Corp.
12527 Mukilteo Speedway
Suite 103
Lynnwood, WA 98087-1532

Dear Dr. Cao,

Precision Fabrics Group, Inc. is very interested in the antimicrobial technology developed by Medetech. We would like to offer support to your effort to develop and commercialize a durable, rechargeable antimicrobial chemistry for use on woven textiles, and specifically for the use by the Department of Defense.

PFG develops, manufactures and markets fine denier polyester and nylon fabrics for a number of technical and highly specified markets. Our high-performance fabrics play a key role in products for the aerospace, industrial, medical, and home furnishings markets and we are current suppliers to the Department of Defense. PFG was the first ISO qualified textile company in the U.S. and we are regularly audited by our customers for compliance with their specifications. We operate plants in Virginia, North Carolina and Tennessee and our corporate headquarters is located in Greensboro NC.

The discussions we have had to date about your Department of Defense SBIR project titled "Durable and Rechargeable Antimicrobial Textiles" appears to be right in line with the capabilities of our company and our core strengths. We would like to work with Medetech to evaluate opportunities to develop and manufacture products using your technology for this project with the DoD.

We look forward to working with you on this opportunity. Please feel free to contact me should you have any questions or we can be of service.

Best regards,

Mark P. Painley

Business Director

REFERENCES

[1] Questions and Discussion on Treated Articles.
http://www.epa.gov/scipoly/sap/meetings/1997/september/1097trtd.htm Accessed on Aug 2, 2013.
[2] Whitman TJ, Herlihy RK, Schlett CD, Murray PR, Grandits GA, Ganesan A, et al. Chlorhexidine-impregnated cloths to prevent skin and soft-tissue infection in Marine recruits: a cluster-randomized, double-blind, controlled effectiveness trial. Infection Control and Hospital Epidemiology : The Official Journal of the Society of Hospital Epidemiologists of America. 2010; 31: 1207-15.
[3] Stillman RM, Bella FJ, Seligman SJ. Skin wound closure. The effect of various wound closure methods on susceptibility to infection. Archives of Surgery. 1980; 115: 674-675.
[4] Saymen DG, Nathan P, Holder IA, Hill EO, Macmillan BG. Control of surface wound infection: skin versus synthetic grafts. Applied Microbiology. 1973; 25: 921-934.
[5] Subbiahdoss, G.; Kuijer, R.; Grijpma, D.W.; van der Mei, H.C.; Busscher, H.J. Acta Biomaterialia. 2009, 5, 1399–1404.
[6] Lee, J.H.; Wang, H.J.; Kaplan, J.B.; Lee, W.Y. Acta Biomaterialia. 2010, 6, 4422–4429.
[7] Gristina, A.G.; Hobgood, C.D.; Barth, E. In Pathogenesis and clinical significance of coagulase-negative staphylococci. Pischer Verlag: Stuttgart, 1987, 143–157.
[8] Astarie-Dequeker, C.; Carreno, S.; Cougoule, C.; Maridonneau-Parini, I. Journal of Cell Science. 2002, 115, 81–89.
[9] Khalil, H.; Williams, R.J.; Stenbeck, G.; Henderson, B.; Meghji, S.; Nair, S.P. Microbes and Infection. 2007, 9, 460–465.
[10] Sun J, Sun YY. Acyclic N-halamine-based fibrous materials: Preparation, characterization, and biocidal functions. Journal of Polymer Science Part A, Polymer Chemistry. 2006; 44: 3588-3600.
[11] Sun Y, Cao Z. Antimicrobial polymers and coatings. The University of South Dakota, USA . 2009. WO2009158285A2.
[12] Braun M, Sun YY. Antimicrobial polymers containing melamine derivatives. I. Preparation and characterization of chloromelamine-based cellulose. Journal of Polymer Science Part A, Polymer Chemistry. 2004; 42: 3818-3827.
[13] Porteous N, Schoolfield J, Luo J, Sun Y. The biofilm-controlling functions of rechargeable antimicrobial N-halamine dental unit waterline tubing. The Journal of Clinical Dentistry. 2011; 22: 163-170.
[14] Luo J, Chen Z, Sun Y. Controlling biofilm formation with an N-halamine-based polymeric additive. Journal of Biomedical Materials Research Part A, Polymer Chemistry. 2006; 77:823-831.
[15] Porteous N, Luo J, Hererra M, Schoolfield J, Sun Y. Growth and identification of bacteria in N-halamine dental unit waterline tubing using an ultrapure water source. International Journal of Microbiology. 2011; 2011: 1-6.
[16] Luo SH, Xiao W, Wei XJ, Jia WT, Zhang CQ, Huang WH, et al. In vitro evaluation of cytotoxicity of silver-containing borate bioactive glass. Journal of Biomedical Materials Research Part B, Applied Biomaterials. 2010; 95: 441-448.
[17] Sun YY, Chen ZB, Braun M. Preparation and physical and antimicrobial properties of a cellulose-supported chloromelamine derivative. Industrial & Engineering Chemistry Research. 2005; 44: 7916-7920.