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Authors

Kumaraswamy, Monika
Collignon, Sean
Do, Carter
et al.

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Decontaminating surfaces with atomized disinfectants generated by a novel thickness-mode lithium niobate device

Monika Kumaraswamy[1,2], **Sean Collignon**[3], **Carter Do**[4], **Janie Kim**[4], **Victor Nizet**[4,5], and **James Friend**[3]

[1]Division of Infectious Diseases, University of California San Diego, 9500 Gilman Drive, Mail Code 0711, La Jolla, CA 92093-0711, USA

[2]Infectious Diseases Section, VA San Diego Healthcare System, San Diego, CA 92161, USA

[3]Department of Mechanical and Aerospace Engineering, University of California San Diego, La Jolla, CA 92093, USA

[4]Department of Pediatrics, University of California San Diego, La Jolla, CA 92093, USA

[5]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, CA 92093, USA

Abstract

We evaluated the ability of a novel lithium niobate (LN) thickness-mode device to atomize disinfectants and reduce microbial burden on model surface materials. A small-scale plastic model housed the LN thickness-mode device and circular coupon surface materials including polycarbonate, polyethylene terephthalate, stainless steel, borosilicate glass, and natural rubber. Coupon surfaces were coated with methicillin-resistant *Staphylococcus aureus* (MRSA) or multidrug-resistant (MDR) strains of Gram-negative bacterial pathogens (*Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii*), atomized with disinfectant solutions of varying viscosity (including 10% bleach, 70% ethanol (EtOH), or 25% triethylene glycol (TEG)) using the LN thickness-mode device, and assessed for surviving bacteria. The LN thickness-mode device effectively atomized disinfectants ranging from low viscosity 10% bleach solution or 70% EtOH to highly viscous 25% TEG. Coupons harboring MDR bacteria and atomized with 10% bleach solution or 70% EtOH were effectively decontaminated with ~ 100% bacterial elimination. Atomized 25% TEG effectively eliminated 100% of *K. pneumoniae* (CRE) from contaminated coupon surfaces but not MRSA. The enclosed small-scale plastic model established proof-of-principle that the LN thickness-mode device could atomize disinfectants of varying viscosities and decontaminate coupon surface materials harboring MDR organisms. Future studies evaluating

scaled devices for patient rooms are warranted to determine their utility in hospital environmental decontamination.

Keywords

Lithium niobate thickness-mode device; Disinfection; Multidrug-resistant bacteria

Introduction

Multidrug-resistant organisms (MDROs) such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* sp. (VRE), carbapenem-resistant *Enterobacteriaceae* (CRE), multidrug-resistant (MDR) *Acinetobacter baumannii*, and extended spectrum β-lactamase (ESBL)-producing *Escherichia coli* frequently contaminate environmental surfaces and medical equipment (Huang et al. 2006). Items potentially contaminated by hospitalized patients include bed linens and rails, bedside tables and other furniture, floors, curtains, blood pressure cuffs, intravenous pumps and poles, nurse call buttons, television remotes, phones, light switches, door knobs, wheel chairs, toilet seats, faucet knobs, ventilators, respiratory tubing, foley tubing/bags, endoscopes, and laryngoscopes, to name but a few. Furthermore, these organisms can be unintentionally transferred to other sites outside the patient room, including computer keyboards, mouses, tablets, stethoscopes, desks, and chairs, and account for 20–40% of all healthcare-associated pathogens (Rutala and Weber 2013).

Cleaning and disinfection of environmental surfaces by housekeeping in healthcare facilities following patient discharge remains a critical infection prevention and control practice for reducing or eliminating the microbial burden on hospital surfaces, thereby preventing unintentional transmission of MDROs and consequent nosocomial infections (Dancer et al. 2009; Hayden et al. 2006). Common agents used in the manual disinfection of hospital environmental surfaces include sodium hypochlorite (bleach) solutions, sodium dichloroisocyanurate, demand release chlorine dioxide, chloramine-T, 70–90% ethyl or isopropyl alcohol, quaternary ammonium germicidal solutions, phenolic germicidal detergent solutions, iodophors, hydrogen peroxide, peracetic acid, glutaraldehyde, and formaldehyde (Rutala et al. 2008).

Nevertheless, cleaning of patient rooms by housekeeping upon discharge is often inadequate. Covert evaluations of terminal room cleaning by direct observation, swab or agar slide cultures, fluorescent markers, and ATP bioluminescence testing have revealed suboptimal cleaning of high touch surfaces with only 40–50% of objects being appropriately decontaminated (Carling et al. 2006; Eckstein et al. 2007). In institutions with poor cleaning and disinfection practices, environmental contamination of surfaces by patients harboring MDROs during hospitalization has been shown to increase the risk of MDRO transmission and healthcare-associated infections (HAIs) among subsequent room occupants (Drees et al. 2008; Huang et al. 2006).

HAIs that may result from the transmission of organisms or spores from environmental surfaces include central line-associated bloodstream infections, catheter-associated urinary

tract infections, ventilator-associated pneumonia, surgical site infections, and *Clostridium difficile* infection. According to the Centers for Disease Control (CDC), approximately 1 in 25 hospitalized patients will develop a HAI (Magill et al. 2014). In 2011, there were an estimated 722,000 HAIs in US acute care hospitals resulting in 75,000 deaths (Magill et al. 2014). Additionally, HAIs cost healthcare facilities $9.8 billion annually and are no longer reimbursed by the Centers for Medicare and Medicaid Services (CMS) (Zimlichman et al. 2013). Cornerstones to preventing HAIs and MDRO transmission include hand hygiene, patient isolation, antimicrobial stewardship, and improved environmental cleaning and disinfection practices.

Factors contributing to suboptimal environmental decontamination include variations in the amount of time spent cleaning surfaces, demand for fast "turnaround times" upon patient discharge and re-occupancy, personnel shortages/turnover, confusion among housekeepers and nursing personnel about who is responsible for cleaning specific surfaces and equipment, inappropriate disinfectant use, inadequate disinfectant contact time, inappropriate overdilution of disinfectant solutions, contamination of disinfectant solutions (with organisms resistant to the disinfectant), and inadvertent transfer of pathogens between surfaces using a wipe with insufficient antimicrobial activity. Conventional cleaning is not always effective for eliminating pathogens and is prone to human error. Automated disinfection systems, new disinfectants, novel methods for disinfectant application, light-activated photosensitizers, and self-disinfecting surface technologies are emerging as alternatives to traditional decontamination strategies employed in healthcare facilities (Boyce 2016).

Acoustic wave microdevices (a.k.a. atomizers) can be utilized to aerosolize and disperse microfluidic solutions, cells, nanoparticles, microparticles, and even biomolecules. However, their potential use in disinfecting environmental surfaces has yet to be evaluated. Here, we describe a pilot evaluation of a novel lithium niobate (LN) thickness-mode portable atomizer, its ability to atomize disinfectants of varying viscosity and inactivate leading MDR pathogens on engineered surfaces typically found in the hospital environment.

Materials and methods

Device description

Our atomizer is a custom device employing the fundamental thickness mode resonance at 7 MHz in a 10×15 mm $\times$ 500 μm thick piezoelectric single-crystal LN element in a 127.68 Y-rotated cut. Planar electrodes serve to transmit a 850–2000 mW sinusoidal input electrical signal into the hysteresis-free piezoelectric element and generates vibration sufficient to atomize a fluid meniscus present upon its surface as formed from a wick carrying fluid to it from a separate reservoir due to the hydrophilicity of the LN (Fig. 1a and Video S1).

Device fabrication and characterization

The atomizer device employs single-crystal LN, a widely used piezoelectric material for telecommunications, with a 127.68 degree Y-rotated cut developed decades ago for surface acoustic wave devices, and again in the past few years for micro- and nano-scale fluidics

(Campbell 1998; Friend and Yeo 2011). Wafers of this material, optically polished on both sides, 500 μm thick, and 125 mm in diameter were obtained (Roditi, London, UK) and cleaned using isopropyl alcohol, acetone, and deionized water in succession and in a class 10,000 clean room (NANO3, UCSD) before being dried under dry nitrogen gas flow. Standard ultraviolet (UV) photolithography was used to pattern the semicircular regions for each of the devices where the fluid meniscus was formed directly upon the hydrophilic LN. The positive-tone photoresist AZ4562 (MicroChemicals GmbH, Ulm, Germany) was used with a standard mask for defining the pattern on one side of the wafer via UV light, including the semicircular regions and 1 mm of gap along each of the dicing lines later used to cut the individual devices from the wafer. After development, the photoresist remained in regions where we chose to prevent metal deposition. Plasma vapor deposition was used to coat both sides of the wafers, one side at a time, with 10 nm of Ti followed by 1 μm of Al. Immersion in acetone within an ultrasonic bath for 5 min released the remaining photoresist from the wafer and lifted off the thin metal layers upon it, leaving behind the metal in the remaining regions. Each device, 10×15 mm in size, was diced from the wafer using a standard diamond wafer saw (DAD-321, DISCO, Tokyo, Japan). Metal was removed from the dicing lines to facilitate easier post-sawing separation of the devices.

We recently discovered placing planar electrodes upon both faces of the material creates an effective atomizer via fundamental thickness-mode vibration at 7 MHz (Collignon et al. 2017). There are many choices of vibration modes and materials for these devices, and the thickness mode in combination with the single crystal hysteresis-free LN material provides superior atomization efficiency an order of magnitude greater than past devices employing surface acoustic waves. Furthermore, LN requires far lower power requirements than the nearly ubiquitous lead zirconate titanate (PZT) in traditional high-power ultrasound. Unlike PZT with elemental lead present in significant quantities within the polycrystalline structure, LN is conveniently nontoxic. Laser Doppler vibrometry (UHF-120, Polytec, Irvine, CA, USA) was used to verify the mode of vibration, the resonance frequency suitable for device operation, and the amplitude of the vibration phenomena. Past studies have indicated the relationship between the vibration amplitude and the onset of atomization for a variety of fluids (Collignon et al. 2017).

Atomization flow rate

The atomizer operates via destabilization of the fluid meniscus to form capillary waves that break up to form droplets ejected from the surface at approximately 1 m per second (m/s) (Fig. 1b, c). Assessment of the atomizer's ability to deliver mists of disinfectants appropriate for this study was made by measuring the volumetric flow rate of the fluid from the reservoir. Ten microliters of fluid was pipetted into the reservoir, and the time taken to atomize this volume of fluid was determined through high-speed video (1000 fps, UX100 greyscale camera, Photron, San Diego, CA) mated to a K-series long working-distance microscope (Infinity USA, Boulder, CO) with four repeats. The typical power input for these fluids was between 850 mW and 2 W, strongly dependent upon the viscosity and density of the fluid as defined via the *atomization Reynolds number* $\mathrm{Re}_A = \rho u_v L/\mu$, where ρ, L, μ, and u_v are the fluid density; a linear measure, here the typical size of the meniscus at about 1 mm, the fluid viscosity; and the vibration velocity (or particle velocity) induced in the fluid

by the atomizer, itself dependent upon the input power, P, into the piezoelectric substrate such that $u_v \propto P$ (Collignon et al. 2017).

Bacterial strains

Bacterial strains utilized in the study included methicillin-sensitive *Staphylococcus aureus* (MSSA), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), *Klebsiella pneumoniae*—a carbapenem-resistant *Enterobacteriaceae* (CRE), extended spectrum β-lactamase (ESBL)-producing *Escherichia coli*, MDR *Pseudomonas aeruginosa*, and MDR *Acinetobacter baumannii*. The strains of MSSA (ATCC 29213), MRSA (ATCC BAA-1720), VRE (ATCC 51299), and CRE *K. pneumoniae* (ATCC BAA-1898) were obtained from the American Type Culture Collection. The ESBL *E. coli*, MDR *P. aeruginosa* (strain P4), and MDR *A. baumannii* were clinical isolates obtained from UC San Diego Healthcare System, a tertiary academic hospital in the New York metropolitan area and Walter Reed Medical Center respectively (Fair et al. 2012; Zurawski et al. 2012).

Minimum inhibitory concentration (MIC) testing and time-kill assays

Broth microdilution disinfectant susceptibility testing with triethylene glycol (TEG) was performed against MSSA, MRSA, VRE, ESBL-producing *E. coli*, CRE *K. pneumoniae*, MDR *P. aeruginosa*, and MDR *A. baumannii*, using cation-adjusted (Ca^{2+} 20–25 mg/L and Mg^{2+} 10–12.5 mg/L) Mueller-Hinton Broth (CA-MHB) (Spectrum Chemicals), in accordance with CLSI guidelines. The MIC endpoint was defined as the lowest TEG concentration required to inhibit bacterial growth by 90% vs. disinfectant-free positive control at 24 h. Bactericidal vs. bacteriostatic activities of TEG (25 or 50%) compared to other disinfectants, namely 10% bleach, 70% ethanol (EtOH), or 0.23% peroxide + 0.23% peracetic acid (HP + PA) in CA-MHB, were determined via time-kill assays (10, 30, and 60 min) performed in CA-MHB using 1×10^7 cfu/ml of bacteria. Bactericidal activity was defined as a reduction in viable bacteria by 3 $\log_{10}$ cfu/ml, and bacteriostatic activity a reduction in viable bacteria by < 3 $\log_{10}$ cfu/ml at 24 h, compared to the starting inoculum of 1×10^7 cfu/ml.

Device testing

A comprehensive analysis of the LN thickness-mode device's ability to atomize disinfectants and reduce the microbial burden on common environmental surfaces contaminated with MDROs was performed using an enclosed small-scale plastic model to establish proof-of-principle. The enclosed plastic container (4 in × 4 in × 7 in) housed the LN thickness-mode device and circular coupon surface materials (polycarbonate (PC), polyethylene terephthalate (PT), stainless steel (SS), borosilicate glass (BG), and natural rubber (NR)) (Biosurface Technologies) contaminated with MDR bacteria (Fig. 1d). The dimension and surface area of each circular coupon was 12.7 mm dia. × 3.8 mm and 1.26cm^2 (one side), respectively. Three sets of each coupon surface material were coated with 50 μL of stationary phase bacteria: MRSA, CRE *K. pneumoniae*, ESBL-producing *E. coli*, or MDR *A. baumannii*. Coupons were air-dried for 1 to 2 h to achieve a recoverable and viable inoculum of 7 $\log_{10}$ CFU per coupon prior to placement in the enclosed plastic container and initiation of disinfectant atomization with one of two disinfectants: 10%

bleach solution or 70% EtOH. Additionally, testing with the highly viscous disinfectant, 25% TEG, was also performed but only using coupon surface materials coated with MRSA or CRE *K. pneumoniae*. Atomization was generated intermittently for 30 min with cycling 2 min on and 2 min off. Coupons were ultimately retrieved 30 min after atomization, vortexed in a Falcon tube containing 1 mL of phosphate-buffered saline (PBS) for 5 min, serially diluted, and plated on Luria-Bertani (LB) agar plates for bacterial enumeration. The percentage of surviving bacteria was calculated compared to the initial inoculum.

Statistics

Statistical analyses were performed using GraphPad Prism 6.0f (GraphPad Software). One-way ANOVA or two-way ANOVA were utilized where appropriate. *P* values < 0.05 were considered statistically significant.

Results

Working principle of thickness-mode LN device atomization

A defined fluid meniscus size of < 1 mm helps to avoid large (> 20 μm) droplet formation during atomization. A semicircular region of 2 mm in diameter was defined on the LN substrate for reliable formation of the meniscus. The characteristics of this interaction are described in a prior work (Qi et al. 2010). Fluid was drawn onto the substrate via capillary action from a wick placed against the side of the atomizer device. Thickness-mode vibration drives the face of the LN substrate into motion, and atomization occurs at 750 mW and up when using water. Fluids of greater viscosity require a greater input power to achieve atomization, and in our device fluids of viscosity up to approximately 100 cP can be atomized (Collignon et al. 2017). For the wick, we employed quantitative filter paper (Whatman, GE Healthcare Life Sciences, Sigma-Aldrich, St. Louis, MO, USA); grade 540 (8 μm) works well for most fluids of viscosity < 5 cP while grade 541 (25 μm) is adequate for the more viscous fluids used in this study. The wicks were cut to 2-cm length, and 1 cm was introduced into a polypropylene snap-cap microcentrifuge tube (1.5 mL Eppendorf 022363204, Fisher Scientific, San Diego, CA, USA), the fluid to be atomized was introduced into the tube to wet the wick, and the cap was closed shut upon the wick. The meniscus formed in most cases in about 30 s after the device was assembled in the exposure chamber.

The atomizer was operated using a continuous sinusoidal signal from a custom signal generator circuit operating at 7 MHz and with a defined voltage of approximately 1 VRMS passed through a 2-W RF amplifier (ZHL-2+, Mini-Circuits, Brooklyn, NY, USA) connected to the atomizer device via a twisted wire pair. Energy input resulted in destabilization of the fluid meniscus, capillary wave formation, capillary wave breakup, and then droplet ejection at 1 m/s (Fig. 1b, c). The droplets of the mist rapidly decelerate, drift within the chamber, and gradually settle upon surfaces. The droplets have been found to be approximately 1–10 μm in diameter, depending principally on their fluid characteristics (Collignon et al. 2017).

Thickness-mode LN device can atomize fluids of varying viscosities and densities

Table 1 provides the maximum continuous flow rate measured during atomization for sample fluids possessing a broad range of viscosities and densities, quantified by the atomization Reynolds number: Re_A. Generally, in this study, the maximum atomization flow rate was between 0.2 and 2 mL/min during continuous operation of the atomizer, found to be linearly dependent upon Re_A, and required 1–2 W of power during operation. Fluids of greater viscosity typically required greater power input.

Activity of viscous TEG in solution and atomized by the thickness-mode LN device against various MDROs

Activity of TEG against MSSA, MRSA, VRE, ESBL *E. coli*, CRE *K. pneumoniae*, MDR *P. aeruginosa*, and MDR *A. baumannii* was confirmed using CLSI broth microdilution methodology with MIC_{90} values ranging from 5 to 10% TEG (Table S1). Kinetic killing activity of 25 and 50% TEG against MRSA, ESBL *E. coli*, *K. pneumoniae* (CRE), and MDR *A. baumanni* was comparable to the common hospital disinfectants 10% bleach, 70% EtOH, and HP + PA. A solution of 25% TEG yielded a 100% reduction in MRSA and *K. pneumoniae* by 30 and 60 min, respectively (Fig. 2). Additionally, 25% TEG produced a 99.99% reduction in ESBL *E. coli* and MDR *A. baumanni* by 60 min (Fig. 2).

An attractive feature of the thickness-mode LN device is its ability to atomize viscous solutions. In our small-scale plastic model, the LN thickness-mode device effectively atomized 25% TEG and completely decontaminated coupon surface materials (PC, PT, SS, BS, NR) contaminated with CRE *K. pneumoniae* (Fig. 3). In contrast, MRSA persisted on the majority of coupon surfaces in face of 25% TEG atomization although a 42% bacterial reduction was observed for the MRSA contaminated BS coupon.

Thickness-mode LN device can atomize disinfectants and decontaminate surfaces harboring MDROs

The thickness-mode LN device effectively atomized disinfectants including 10% bleach and 70% EtOH intermittently for 30 min (cycled 2 min on and 2 min off). All coupon surface materials (PC, PT, SS, BS, NR) reflecting common hospital environmental surfaces and contaminated with MDROs (MRSA, ESBL *E. coli*, CRE *K. pneumoniae*, or MDR *A. baumannii*) were successfully decontaminated with the disinfectants atomized by the thickness-mode LN device and allowing 60-min total contact time. The most effective atomized disinfectant was 10% bleach, eliminating 99–100% of bacteria tested on all contaminated surfaces (Fig. 4). Additionally, 70% EtOH completely eliminated all of the Gram-negative MDROs tested and reduced MRSA levels to 2–15% of the untreated control, with the greatest bacterial survival noted solely on stainless steel (Fig. 4).

Discussion

Contaminated hospital surfaces, a persistent reservoir of nosocomial and MDR pathogens, are an important component in the inadvertent transmission and acquisition of bacteria involved in HAIs. MDROs can persist on environmental surfaces for prolonged periods and be directly transferred to patients or acquired via the cross-contaminated hands of healthcare

personnel (Kramer et al. 2006). Environmental cleaning and disinfection remains a critical mainstay in reducing the microbial burden of contaminated high touch surface areas in patient rooms, and is touted by various guidelines including the Centers for Disease Control and Prevention, the Society for Healthcare Epidemiology in America, and the British National Health Service as a critical measure in reducing the prevalence of nosocomial pathogens (Health 2003; Muto et al. 2003; Rutala et al. 2008). Unfortunately, conventional cleaning methods for decontaminating hospital surfaces can be lacking, wrought with human error, and requires constant supervision and education of environmental services staff to be effective.

No touch automated technologies such as hydrogen peroxide vapor systems, ultraviolet light devices, pulsed xenon light devices, high-intensity narrow-spectrum light, saturated steam systems, gaseous ozone, and paracetic acid/hydrogen peroxide fogging have emerged and garnered significant attention as alternatives to conventional healthcare disinfection practices (Boyce 2016). These technologies were developed as a means to reduce dependence on environmental services personnel. However, they remain expensive, require removal of healthcare workers and the patient thereby increasing room turnover time, and alone cannot replace standard manual cleaning and disinfection.

Here, we evaluated a novel low-cost LN thickness-mode device's ability to atomize disinfectants and reduce the microbial burden on multiple coupon surface materials using a proof-of-principle enclosed small-scale plastic model. The LN thickness-mode device effectively atomized 10% bleach solution and 70% EtOH to decontaminate several common hospital environmental surface materials with a high burden of several MDROs important in human medicine. Our concentration of 7 $\log_{10}$ CFU bacteria per disk was selected as a maximal challenge scenario and is likely considerably higher than bacterial concentrations encountered in the hospital environment. Atomized 10% bleach solution eliminated nearly 100% of all strains tested, while 70% EtOH eliminated nearly 100% of ESBL *E. coli*, CRE *K. pneumoniae*, MDR *A. baumannii*, and 85–98% of MRSA on contaminated surfaces (Fig. 4). The activity of atomized 10% bleach and 70% EtOH against MDROs was comparable to the activity observed with direct immersion in each disinfectant via time-kill assays.

Additionally, the LN thickness-mode device effectively atomized viscous 25% TEG to eliminate 100% of *K. pneumoniae* (CRE) from contaminated coupon surface materials but not MRSA. Unlike *K. pneumoniae* (CRE), the activity of atomized 25% TEG against MRSA was not comparable to the activity observed with direct immersion assessed via time-kill assays. MRSA may have persisted on coupon surface materials atomized with 25% TEG due to inadequate contact time or surface exposure to 25% TEG. TEG, a colorless odorless viscous fluid approved by the Environmental Protection Agency for use as an air disinfectant in hospitals, was first registered for use in 1947. Its antimicrobial activity is broad with studies demonstrating inactivation of bacteria, viruses, fungi, and spores with its application (USEPA 2003). However, its activity against MDROs was previously uncharacterized, and we showed that MRSA, VRE, ESBL *E. coli*, CRE *K, pneumoniae*, MDR *P. aeruginosa*, and MDR *A. baumannii* were exquisitely sensitive to TEG (MIC$_{90}$ 5–10% TEG) with comparable kinetic killing of 25% TEG vs. 10% bleach, 70% EtOH, or HP + PA.

Potential advantages of the LN thickness-mode device for environmental decontamination of nosocomial and MDR pathogens include its low cost ($100), portability, energy savings (requires only 1 W), and ability to atomize non-viscous and viscous disinfectants. Additionally, the device can serve as a modality for airborne disinfection, thoroughly coat environmental hard surfaces, and be automated to help remove elements of human error. However, further investigation is required utilizing a scaled device for patient rooms and extending analysis of atomized disinfectants against other pathogen types (viruses, fungi, amoeba, spores, etc.). With the scaled LN thickness-mode device, testing can be extended to a full array of porous and nonporous surfaces to determine the optimal atomized disinfectant contact time to inactivate microbes (including heterogenous contamination) in the hospital environment. In summary, our proof-of-principle study provides an important first step and basis in the evidentiary hierarchy needed to determine the efficacy of the LN thickness-mode device in disinfectant atomization and environmental decontamination. However, future studies comparing and contrasting environmental decontamination by conventional manual disinfection and cleaning practices to the LN thickness-mode device disinfectant atomization alone and in combination, and their effect on patient colonization and HAI rates will be warranted to determine the device's true utility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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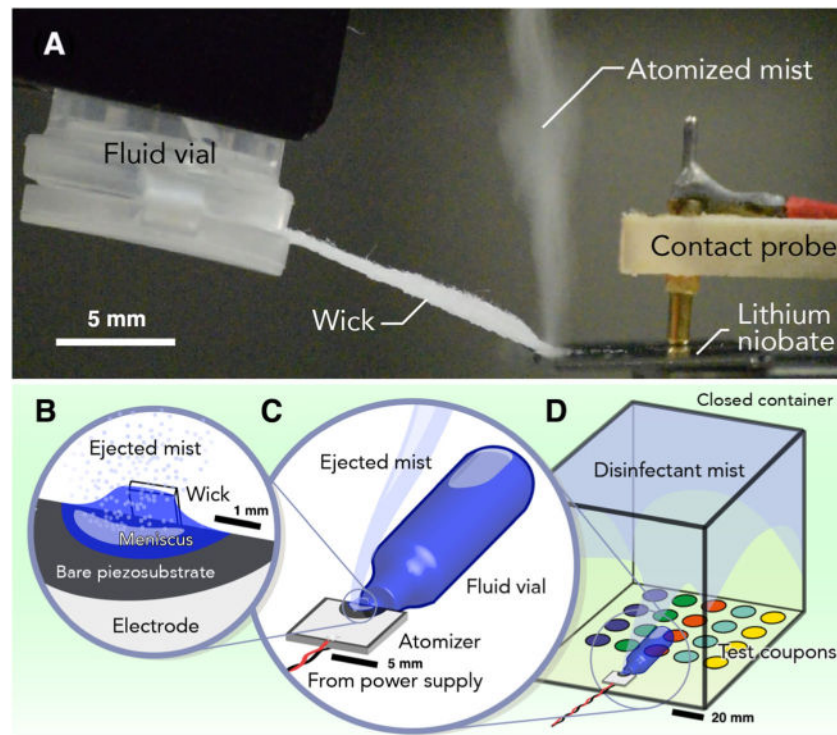
Fig. 1.
Atomizing disinfectants using a novel thickness-mode lithium niobate (LN) device. a Fluid delivered to the LN substrate via a wick and its associated reservoir forms a meniscus on the surface while electric input into the piezoelectric single crystal LN element generates thickness-mode vibration and drives fluid atomization. b, c Atomizer schematic depicting the piezoelectric LN material, delivery of fluid to LN substrate from wick/fluid vial, destabilization of fluid meniscus, capillary wave formation, and surface droplet ejection due to thickness-mode vibration and electric input. d Schematic of small-scale plastic model housing LN thickness-mode device and circular coupon materials used to establish proof-of-principle, study disinfectant atomization, and surface decontamination
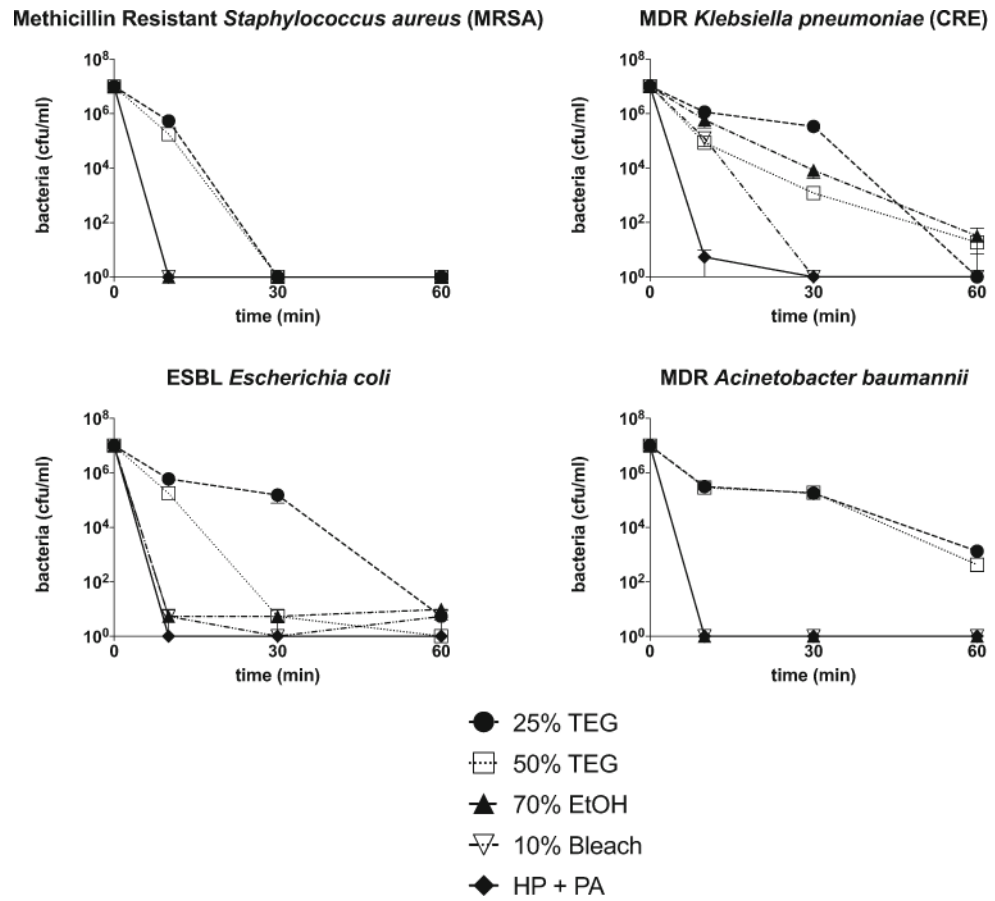
Fig. 2.
Time-kill curves comparing the activity of 25 and 50% tri-ethylene glycol (TEG) to other common disinfectants (10% bleach, 70% ethanol (EtOH), 7.35% hydrogen peroxide + 0.23% peracetic acid (HP + PA)). Data are plotted as mean ± SEM and represent the combination of three experiments performed in triplicate

Methicillin Resistant *Staphylococcus aureus* (MRSA)

MDR *Klebsiella pneumoniae* (CRE)

- ☐ Polycarbonate (PC)
- ☐ Polyethylene terephthalate (PT)
- ☐ Stainless Steel (SS)
- ☐ Borosilicate (BS)
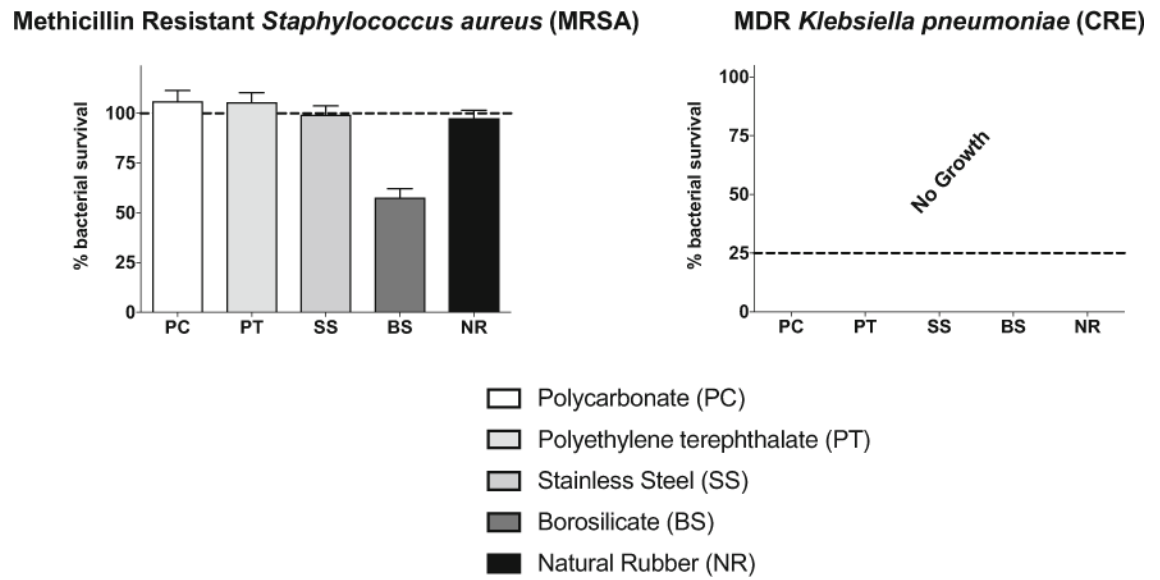- ■ Natural Rubber (NR)

Fig. 3.
Percentage bacterial survival observed following LN thickness mode device atomization of 25% tri-ethylene glycol (TEG) onto coupon surfaces (polycarbonate (PC), polyethylene terephthalate (PT), stainless steel (SS), borosilicate (BS), natural rubber (NR)) initially inoculated with 7 $\log_{10}$ CFU bacteria (methicillin-resistant *Staphylococcus aureus* (MRSA) or MDR *Klebsiella pneumoniae* (CRE)) using a small-scale model. Data are plotted as mean ± SEM and represent the combination of three experiments performed in triplicate
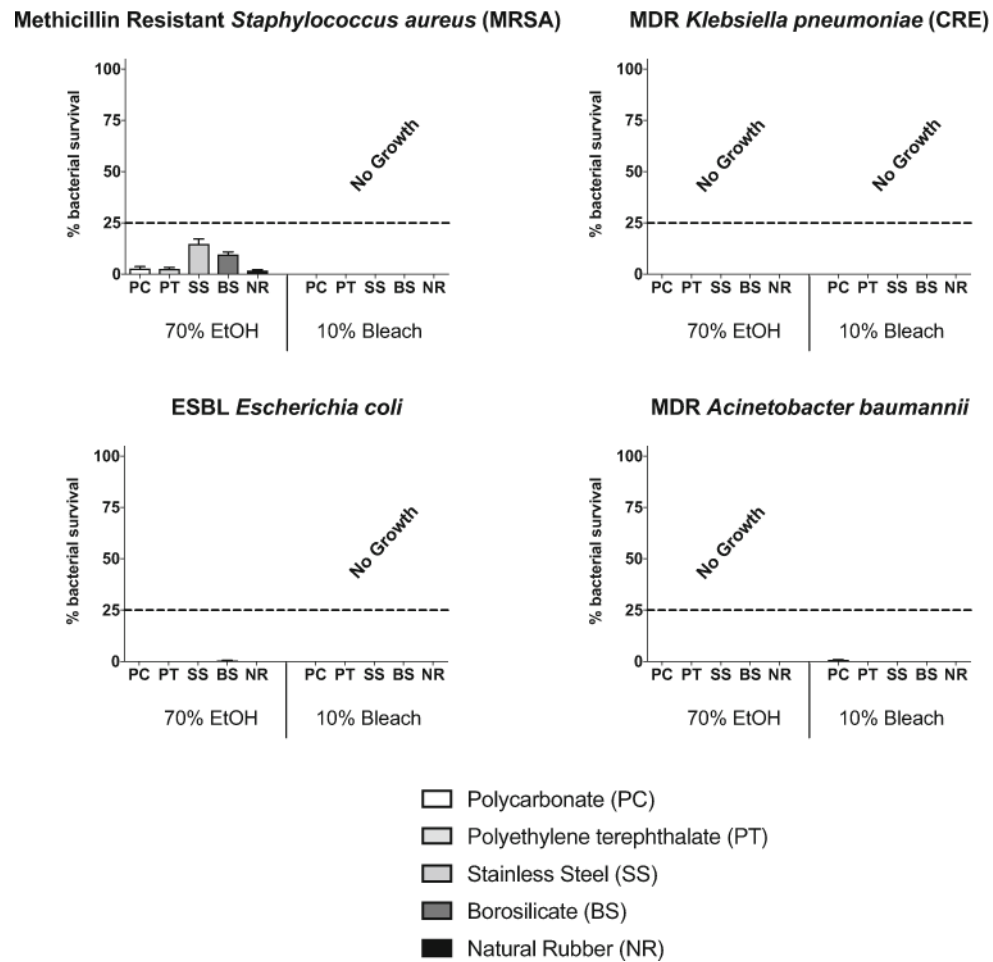
Fig. 4.

Percentage bacterial survival observed following LN thickness-mode device atomization of 70% ethanol (EtOH) or 10% bleach onto coupon surfaces (polycarbonate (PC), polyethylene terephthalate (PT), stainless steel (SS), borosilicate (BS), natural rubber (NR)) initially inoculated with 7 $\log_{10}$ CFU bacteria (methicillin-resistant *Staphylococcus aureus* (MRSA), MDR *Klebsiella pneumoniae* (CRE), ESBL *Escherichia coli*, or MDR *Acinetobacter baumannii*) using a small-scale model. Data are plotted as mean ± SEM and represent the combination of three experiments performed in triplicate

Table 1

Atomization of fluid samples with variable viscosities and densities (from least viscous and dense (methanol) to most viscous and dense (50% triethylene glycol) was measured using the volumetric flow rate (mL/min) of the fluid, typical power input for these fluids and the atomization Reynolds number (Re_A – a dimensionless quantity in fluid mechanics used to depict the transition from laminar to turbulent flow)

Fluid	Atomization Reynolds Number (Re_A)	Maximum Flow Rate (mL/min)	Power Input (Watts)
Methanol	7120.00	2.40	1.04
Acetone	3818.00	1.95	0.85
Isopropyl alcohol	1644.00	0.32	1.21
Water	1684.00	0.90	1.77
25% Triethylene Glycol	550.10	0.36	1.90
50% Triethylene Glycol	223.60	0.19	2.00