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Major article

Biofilms on environmental surfaces: Evaluation of the disinfection efficacy of a novel steam vapor system

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Background: Environmental surfaces in health care settings are often contaminated by microorganisms, and biofilms can develop on the surfaces in these settings. Steam vapor technology is of potential use for disinfection of biofilms on the environmental surfaces.

Methods: We tested the disinfection efficacy of a thermal-accelerated nanocrystal sanitation (TANCS)-equipped steam vapor technology against biofilms through disinfecting biofilms developed by 4 bacterial strains—*Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*—on an identical test surface (ie, polycarbonate) and biofilms developed by *E coli* on 4 different test surfaces: polycarbonate, rubber, stainless steel, and ceramics.

Results: Our data show that a 3-second steam treatment rapidly killed each biofilm tested (>99.95 % killing efficiency). For biofilms developed on different surfaces, 3-second steam treatment achieved 99.95% killing of *E coli* biofilms developed on different surfaces. Compared with chemical disinfection, steam treatment for <1 second a similar level of biofilm disinfection as provided by incubation with 10-ppm sodium hypochlorite (bleach) for 10-20 minutes of contact time.

Conclusions: Our data suggest that the TANCS-equipped steam vapor disinfection is an emerging and potentially useful technology for disinfecting biofilms on environmental surfaces.

Environmental surfaces in health care, educational, and food service settings are often contaminated by microorganisms. Contaminated surfaces are commonly linked to disease transmission,[1-4] and environmental cleaning is important for controlling hospital-acquired infections.[5-7] Bacteria grow in the hospital environment mainly attached to surfaces as biofilms,[8] aggregates of active cells embedded within a polymeric matrix and attached to a biotic or abiotic surface. Biofilms can act as reservoirs of pathogens in the hospital environment and offer favorable environments to enable pathogens to persist for extended periods. The biofilm structure also protects embedded pathogens against many antimicrobial agents, such as antibiotics and biocides,[9-11] making it very difficult to eradicate these pathogens with commonly used decontamination techniques.[12] Biofilms in the hospital environment not only increase patients' likelihood of exposure of pathogens, but also provide a higher infectious dose due to the concentrated cells in biofilm aggregates. Bacterial biofilms are responsible for approximately 65% of nosocomial infections[13]; thus, developing effective procedures to combat biofilms in the hospital environment to control hospital-acquired infections is of critical importance.

Liquid chemical disinfectants, such as sodium hypochlorite, quaternary ammoniums,[14] phenolic disinfectants,[15] hydrogen peroxide, and silver ions,[2] are usually used to decontaminate environmental surfaces, and the use of these compounds is periodically reviewed by the Hospital Infection Control Practices Advisory Committee.[16,17] These compounds are often toxic to humans and inefficient in eradicating biofilms, or require long contact times for disinfection, especially in the presence of high organic matter loads. Thermal disinfection with steam autoclaves has been used for decades to sterilize critical medical devices. The advent of mobile, portable steam generators has made steam disinfection of environmental surfaces much more practical.

A novel steam disinfection system developed by Advanced Vapor Technologies (Seattle, WA) equipped with proprietary thermal-accelerated nanocrystal sanitation (TANCS) technology has demonstrated excellent disinfection efficacy against different

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types of microorganisms, reducing the risk of surface-mediated infections.[18] The TANCS technology uses the naturally occurring minerals in tap water to form crystals. As these crystals pass through the boiler, they gain energy from the heat. When the water transforms into superheated low-moisture steam (dry steam), these energized crystals are accelerated along with the steam.[19] This process helps disrupt the cell membrane, allowing lethal temperatures to quickly destroy bacterial cells. This novel steam vapor disinfection technology is considered to have great potential for eliminating biofilms on environmental surfaces in the aforementioned settings; however, a detailed study on the efficacy of this device against biofilms has not yet been reported.

Escherichia coli, *Acinetobacter baumannii*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* are important epidemiologic pathogens commonly detected on environmental surfaces.[20-23] We tested the disinfection efficacy of the TANCS technology against the biofilms developed by these bacterial strains. Given that various surfaces influence the initial adhesion and colonization of bacteria and consequently affect biofilm development and structure,[24] we also evaluated the disinfection efficiency against biofilms developed by *E coli* on 4 different test surfaces commonly found in the hospital environment.

MATERIALS AND METHODS

Bacterial strains

Four bacterial strains were tested in this study. *E coli* K-12 MG16653, *A baumannii* (ATCC 17978), and *S aureus* (ATCC 45330) were purchased from American Type Culture Collection (Manassas, VA). *P aeruginosa* PAO300, a *mucA22* derivative of *P aeruginosa* PAO1 that constitutively overproduces alginate,[25] was obtained from the University of Copenhagen. Luria Bertani (LB) medium (broth and agar) was obtained from Fisher Scientific (Pittsburgh, PA).

Chemical disinfectant

Bleach (5% sodium hypochlorite) was purchased from Fisher Scientific. Chemical disinfection was performed according to the manufacturer's instructions.

Biofilm reactor

A 1-L Centers for Disease Control and Prevention (CDC) biofilm reactor with a coupon holder and 10-mm-diameter coupons of different materials (polycarbonate, rubber, stainless steel, and ceramic) were purchased from BioSurface Technologies (Bozeman, MT). The CDC biofilm reactor was developed to provide consistent biofilm samples and growth conditions for evaluation of antimicrobial agents, surface treatments, and materials as described in ASTM 2562-07, Standard Test Method for Quantification of *Pseudomonas aeruginosa* Grown with High Shear and Continuous Flow Using a CDC Biofilm Reactor.

Steam vapor device

The steam vapor device (MondoVap 2400 commercial model equipped with TANCS component SV-C/T) was provided by Advanced Vapor Technologies (Seattle, WA). For the tests described herein, the unit was outfitted with a hose connected to a 14 × 14 × 14 cm triangular cleaning head. A cotton terry towel that had been machine-washed with ordinary detergent was affixed to the cleaning head in accordance with the manufacturer's instructions. Before the experiments, the unit was filled with ordinary tap water, activated, and allowed to reach the functional operating boiler pressure of 66 psi. The steam delivery output was set to 12-15 psi for all experiments.

Table 1
Disinfection efficacy (expressed as log reduction, with coefficient of variation in parentheses) of dry steam against different biofilms developed on the polycarbonate surface

Biofilm	Treatment time, seconds	Disinfection efficacy
E coli	0	0.0 (0.49)
	1	−3.8 (0.27)
	3	−5.0 (1.41)
	10	−6.87
P aeruginosa 300	0	0.0 (0.12)
	1	−3.0 (0.20)
	3	−4.2 (0.14)
	5	−5.3 (0.38)
S aureus	0	0.0 (0.29)
	1	−2.9 (0.39)
	3	−4.7 (0.26)
	5	−5.3 (0.43)
A baumannii	0	0.0 (0.18)
	1	−2.6 (0.38)
	3	−4.1 (0.17)
	5	−4.9 (0.27)

Biofilm development

All biofilms for the study were developed in the CDC biofilm reactor. For this, 10% LB medium was pumped into the CDC reactor continuously at a flow rate of 120 mL/h using a mini pump (Fisher Scientific). The reactor has a residual volume of 400 mL and a hydraulic retention time of 3 hours, 20 minutes. The stirring rate was maintained at 50 rpm for all experiments. Test bacteria were grown in LB medium at 37 °C overnight, after which 4 mL of the overnight culture was injected into the CDC reactor for inoculation. Biofilms developed over 4 days after inoculation for *E coli* and over 5 days after inoculation for *A baumannii*, *S aureus*, and *P aeruginosa* were used for the disinfection experiments.

Biofilms composed of the individual test organisms of *E coli*, *A baumannii*, *S aureus*, and *P aeruginosa* grown on polycarbonate coupons were used to evaluate the disinfection efficacy of the TANCS-equipped steam vapor device (against different biofilms) and bleach (against *E coli* biofilm). *E coli* biofilms developed on ceramic, stainless steel, rubber, and polycarbonate coupons were used to evaluate the disinfection efficacy of the TANCS-equipped steam vapor device against biofilms developed by the same strain on different surfaces. *E coli* was chosen for this test because it is often used as an indicator strain for water and environmental contaminations.

Disinfection

The coupons with well-developed biofilms were placed in a 60 × 15 mm sterile polystyrene dish and disinfected on both sides with the steam vapor device. After 1 second, 3 seconds, and 5 seconds of steam vapor disinfection, the treated coupons were transferred into a Corning tube containing 2 mL of 1× PBS buffer. The biofilm biomass was released from the coupons by vortexing for 10 seconds, followed by homogenization. The homogenized mixtures were serially diluted and plated onto LB agar plates. Viable cells were determined by counting colonies formed on the agar plates. The disinfection efficiency was expressed as log reduction ($\log_{10} N/N_0$, where N is the number of viable cells after disinfection and N_0 is the number of viable cells in biofilms on coupons used as controls without disinfection).