Characterizing the Antimicrobial Properties of 405 nm Light and the Corning® Light-Diffusing Fiber Delivery System

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Background and Objectives: Hospital-acquired infections (HAIs) and multidrug resistant bacteria pose a significant threat to the U.S. healthcare system. With a dearth of new antibiotic approvals, novel antimicrobial strategies are required to help solve this problem. Violet-blue visible light (400–470 nm) has been shown to elicit strong antimicrobial effects toward many pathogens, including representatives of the ESKAPE bacterial pathogens, which have a high propensity to cause HAIs. However, phototherapeutic solutions to prevention or treating infections are currently limited by efficient and nonobtrusive light-delivery mechanisms.

Study Design/Materials and Methods: Here, we investigate the in vitro antimicrobial properties of flexible Corning® light-diffusing fiber (LDF) toward members of the ESKAPE pathogens in a variety of growth states and in the context of biological materials. Bacteria were grown on agar surfaces, in liquid culture and on abiotic surfaces. We also explored the effects of 405 nm light within the presence of lung surfactant, human serum, and on eukaryotic cells. Pathogens tested include *Enterococcus spp*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp*., *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Candida albicans*, and *Escherichia coli*.

Results: Overall, the LDF delivery of 405 nm violet-blue light exerted a significant degree of microbicidal activity against a wide range of pathogens under diverse experimental conditions.

Conclusions: The results exemplify the fiber's promise as a non-traditional approach for the prevention and/or therapeutic intervention of HAIs. Lasers Surg. Med. © 2019 The Authors. *Lasers in Surgery and Medicine* Published by Wiley Periodicals, Inc.

Key words: ESKAPE pathogens; 405 nm; antimicrobial

INTRODUCTION

Hospital-acquired infections (HAI) present a significant healthcare problem in the United States, resulting in high rates of morbidity and mortality, costing the U.S. healthcare system approximately $45 billion annually [1]. Concern has been further exacerbated by recent emergence of antibiotic-resistant bacterial pathogens that have rendered front-line antimicrobials largely ineffective. The so-called ESKAPE bacterial pathogens (*Enterococcus spp*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*.) are of particular concern, as these organisms have a high propensity to cause HAI and can "escape" antimicrobial therapy due to resistance [2].

Phototherapy is an emerging field that is gaining recognition as a promising approach for the prevention and/or treatment of bacterial infection, with visible, violet-blue light (400–470 nm) being both safe and effective (reviewed in Wang et al. [3]). For example, high intensity, narrow-band blue light exhibited potent antimicrobial activity toward *Propionibacterium acnes*, the causative agent of acne, in several clinical studies [4–9]. From a safety perspective, the Food and Drug Administration has approved 405 nm light delivery devices for the treatment of acne, illustrating its low toxicity against keratinized epithelium at therapeutic doses [10]. While the mechanism of blue light antimicrobial action is not fully understood, irradiation is hypothesized to excite endogenous photosensitizing chromophores produced within microbes, resulting in the production of cytotoxic reactive oxygen species that lead to lipid peroxidation, DNA and protein damage [11,12]. Given the multitude of potential cellular photooxidation-labile targets,

bacterial resistance to blue light has been hypothesized to be slow to develop, and adds to the attractiveness of developing phototherapeutic strategies for the prevention and/or treatment of HAI [3].

To that end, recent studies have revealed that light-emitting diode (LED) 405 nm blue light delivery of fluences ranging between 7.5 and 270 J/cm^2 display robust antibacterial activity toward ESKAPE and other high priority bacterial pathogens [5]. However, such studies have also made it clear that there are inherent challenges with effective 405 nm light delivery systems [3]. Chiefly among these, the light delivery device must be in direct proximity to—and deliver uniform illumination of—the offending pathogen, necessitating development of delivery devices capable of irradiating a variety of surfaces, some of which may be physically constrained and/or nonuniform.

Corning® light-diffusing fiber (LDF) is a multimode fused silica optical fiber that is extremely thin (115 μm core diameter), durable, and flexible, capable of withstanding a bend radius of 5 mm without breaking, that can be coupled to a high power multimode 405 nm laser diode and may overcome these light delivery limitations (reviewed in Tandon et al. [13]). Accordingly, the goal of this study was to characterize the *in vitro* antimicrobial properties of LDF delivered 405 nm light, based on the premise that its flexibility and durability may provide an attractive light delivery approach to luminal or nonuniform surfaces. Our results suggest that the LDF system effectively delivers 405 nm mediated antimicrobial properties toward the ESKAPE pathogens, the fungus *Candida albicans* and bacteria in disease-associated growth states. Consequently, it may serve as an effective option for the prevention and/or treatment of microbial pathogens under a variety of physiological and environmental conditions.

MATERIALS AND METHODS

Microbial Species and Growth Conditions

All organisms used in these studies are listed in Table 1. When available, well-characterized laboratory strains were used, otherwise clinical isolates were acquired from the Strong Memorial Hospital Clinical Microbiology Laboratory, Rochester, NY. *S. aureus* strain UAMS-1112 is a stable small-colony variant of the common laboratory strain 8325-4 that harbors a *hemB* disruption [14]. Unless otherwise noted, bacteria were grown for 16 hours in Mueller–Hinton (MH) medium (Becton Dickinson, Franklin Lakes, NJ) at 37°C on a rotary shaker at 225 rotations per minutes, serially diluted in 0.9% saline solution, and then processed as described below. *C. albicans* cultures were grown overnight in yeast extract peptone dextrose (YPD) broth and processed.

LDF System

All assays were performed using 230 μm (outer) diameter 22 cm diffusion length LDFs (Corning Incorporated, Corning, NY). Ends of the LDF fiber were connected to 750 mW 405 nm lasers (World Star Technologies, Mark-

ham, ON, Canada) using Fiber Connector/Patch Connector (FC/PC) to FC/PC patch cable with 0.22 NA and 105 μm core size (Thorlabs, Newton, NJ). The fiber is composed of 115 μm high purity silica glass core with 0.05–0.3 μm diameter gas filled voids randomly distributed throughout the core and double clad with a 25-μm thick fluorine-doped (F-doped) silica and a 40 μm cycloaliphatic polymer containing ~0.1 μm diameter alumina particles, whereas the outer surface of the LDF is coated with ~70 μm fluorinated polymer (perfluoroalkoxy) to protect the fiber yet retain its flexibility. LDFs were placed in a 1 mm × 1 mm groove on a sheet of polytetrafuoroethylene for increased reflection and to stabilize the fiber's configuration across experiments [22]. Microorganisms within 96-well microtiter plates, colonizing agar or abiotic surfaces were positioned approximately 1 cm directly overtop the fiber's illumination path and irradiated at 405 nm to achieve the indicated light dose.

LDF Treatment of Bacteria Inoculated onto Agar Plate Surfaces

Bacteria were either dispensed as a spot (20 μl volume) or spread (100 μl volume) directly onto MH agar plates at final concentrations of 1×10^4 to 1×10^8 cells per plate. Once dry, plates were inverted and the surface was placed within 1 cm of the LDF light delivery path. Plates were treated at fluences ranging from 36 to 540 J/cm^2. Fluence rates were measured at the beginning of each test using an ILT 1400 Photometer and XSL340A detector (International Light Technologies, Peabody, MA). Fluence (radiant energy in J/cm^2) was calculated using the formula Fluence $= (X)(Y)(3.6)$ J/cm^2, where X = power density in mW/cm^2, and Y = exposure time in hours. Following treatment, plates were incubated for 16 h at 37°C to determine colony forming units (CFUs). Antimicrobial

TABLE 1. Organisms Used in These Studies

Species	Strain	Reference or source
Enterococcus faecium	824-05	[14]
Staphylococcus aureus	UAMS-1	[15]
	UAMS-1112	[14]
Staphylococcus epidermidis	1457	[16]
Klebsiella pneumoniae	cKP1	[17]
Acinetobacter baumannii	98-37-09	[18]
Pseudomonas aeruginosa	PA01	[19]
Enterobacter cloacae	PMD1001	[14]
Streptococcus pyogenes	Clinical isolate	URMC[a]
Candida albicans	SC5314	[20]
Escherichia coli	JM109	[21]

List of all pathogens used in this study, their strain, and source or reference.
[a]Strong Memorial Hospital Clinical Microbiology Laboratory, Rochester, NY.

effects were determined by comparing the cell viability of each treatment regimen to mock-treated (light-shielded) cells. All organisms were evaluated a total of three times on separate days.

LDF Treatment of Bacteria Cultured in Liquid Media

To approximate standard antimicrobial minimum inhibitory concentration (MIC) testing parameters, approximately 5×10^4 of the indicated bacterial species were inoculated (10 µl) in individual wells of a 96-well, round-bottom polystyrene microtiter plate (Corning, Inc., Corning NY) containing 190 µl MH medium, 100% human serum or mouse lung surfactant. Cell suspensions were then treated at 31.3, 62.7, 125.4, and 250.8 J/cm^2, or as otherwise indicated. Following treatment, an aliquot of cells was removed, serially diluted in 0.9% saline solution and plated to enumerate CFU. Antimicrobial effects were determined by comparing the number of CFU of each treatment to that of mock-treated (light-shielded) cells. All organisms were evaluated at least three times on separate days.

Cytotoxicity Testing

Human colon 38 cells were grown to 50% or 100% confluence in McCoy's 5A medium in a 24-well plate. Half the plate was shielded and the other half was treated at 72, 144, 360, and 720 J/cm^2. Following treatment, the cells were stained by adding 0.1 volume of Alamar blue solution (Invitrogen, Eugene, OR) and incubated at 37°C (protected from light) for 4 hours. To measure cell viability a 100 µl aliquot was transferred into a 96-well plate to be read by a plate reader at a fluorescence excitation wavelength of 540–570 nm (peak excitation is 570 nm) and fluorescence emission at 580–610 nm (peak emission is 585 nm). Each condition was tested in quadruplet.

LDF Treatment of *S. aureus*, *A. baumannii*, and *P. aeruginosa* on Abiotic Surfaces

Approximately 1×10^6 to 1×10^8 CFU of the indicated organism in 20 µl 0.9% saline was inoculated onto approximately 1 cm^2 area of abiotic materials including fabric (Ameripride Services, Minnetonka, MN), silicone rubber, polystyrene, polypropylene (Nuc 96-well), and ceramic tile (Lowes, Mooresville, NC). Each material was placed in 24-well culture plates and allowed to dry for 24–48 hours at 37°C. Colonized surfaces were treated at 5, 10, or 25 mW/cm^2 for a total of 2, 4, or 6 hours. Bacteria were collected from treated surfaces by vigorous pipetting with 1 ml of 0.9% saline, serially diluted, and the recoverable CFU were enumerated by plating. The antimicrobial effects of each treatment regimen were determined by comparing the cell viability of each treatment to mock-treated cells. Additionally, two methods were used in parallel to ensure total viable bacterial recovery from each surface. First, 0.25 ml of 0.9% saline was added and the test surface was physically scraped using a spatula, transferred to a 96-well microtiter plate and the percent nonviable/viable organisms remaining was measured using a LIVE/DEAD stain at an excitation wavelength of 485 nm and emission wavelengths of 530 nm (SYTO 9; green) and 630 nm (propidium iodide; red) (Molecular Probes, Inc., Eugene, OR). Second, inoculated test surfaces were transferred to fresh culture medium after treatment, incubated for 16 hours at 37°C on an orbital shaker and optical density (OD600$_{nm}$) measured. All organisms were evaluated a total of three times on separate days for each abiotic material

RESULTS

LDF System

The experimental LDF system used in these studies is shown Figure 1A and was engineered to include cycloaliphatic polymer containing alumina particles (Materials and Methods) to increase light delivery. Integrated sphere testing measures of the total light scattering across emission wavelengths [23–25] revealed that the LDF light delivery efficiency was increased in comparison to a commercially available version of LDF (Corning® Fibrance® LDF) which was optimized for broad-band visible light, capable of >90% emission at wavelengths >400 nm (Fig. 1B). The increased light diffusion properties of LDF in comparison to a conventional delivery fiber suggested that it may be appropriate for antimicrobial 405 nm light delivery.

LDF 405 nm Light Delivery System Exerts Antimicrobial Activity Toward ESKAPE and Other Pathogens

LED-delivered 405 nm light (133 J/cm^2) has been shown to effectively reduce the growth of both *S. aureus* and *P. aeruginosa* seeded onto agar plates by approximately 6-log$_{10}$ and 5.2-log$_{10}$, respectively [26]. Thus, as an initial evaluation of whether an LDF system efficiently delivered 405 nm light approaching the antimicrobial effects of LED 405 nm exposure, *S. aureus* strain UAMS-1 or *P. aeruginosa* strain PA01 were spread onto the surface of MH agar plates. One half of the plate was shielded from light exposure, whereas the other half was exposed to 216 J/cm^2 and then incubated overnight to score bacterial growth. As shown in Figure 1C (left), LDF 405 nm light delivery effectively inhibited growth of irradiated *S. aureus* cells, whereas there were no discernable ablation of shielded cell growth. Similar antimicrobial effects were also observed in studies in which 10^6 *S. aureus* CFU were spot plated (20 µl) in the light exposure field (Fig. 1C; right), providing an efficient means of performing replicate light exposure tests on a single petri plate. Parallel studies of *P. aeruginosa* cells revealed the organism to be equally susceptible to the antimicrobial effects of LDF 405 nm light delivery (data not shown).

To further explore the antimicrobial potential of the LDF system, studies were expanded to to provide higher resolution measures of the system's performance toward *S. aureus*, *P. aeruginosa* and the remaining members of the ESKAPE pathogens, as well as other microbes of immediate healthcare concern including *Streptococcus pyogenes*, *Escherichia coli*, and the fungus *C. albicans*. To do so, various amounts of
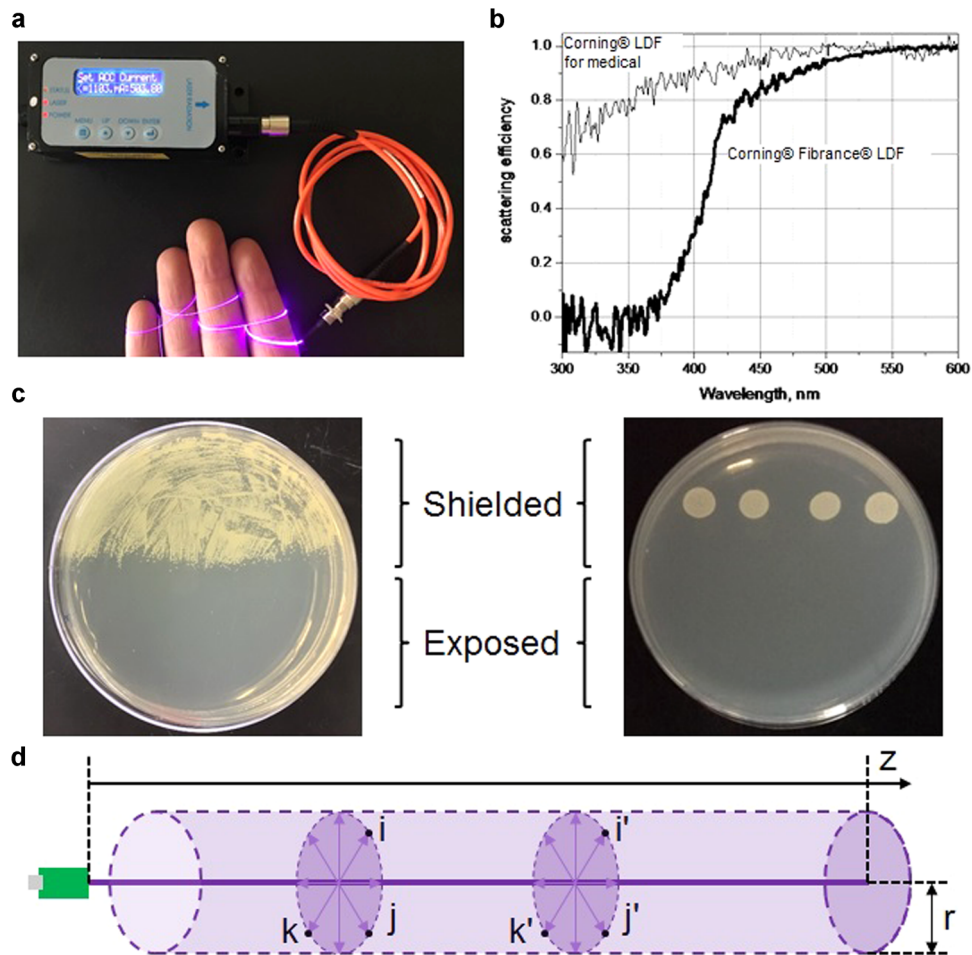
Fig. 1. (**a**) Photograph of 405 nm light-diffusing fiber (LDF) illumination system used in this study; clockwise from upper left: 405 nm 750 mW laser, orange transport fiber, Corning® LDF for medical applications wrapped around a hand. (**b**) Depicting the scattering efficiency differences over ultra violet–visible wavelength range (300–600 nm). As depicted in chart above, Corning® Fibrance® LDF has lower efficiency at 405 nm than does the Corning® LDF for medical fiber used in this study. (**c**) Two different antimicrobial efficacy assays on agar plates demonstrating the 6 Log and 4 Log reduction of *Staphylococcus aureus* (left-spread plate and right-spot plate). The top half of each agar plate was shielded with an opaque material, the bottom half was exposed to 405 nm light, delivered via the LDF system at 216 J/cm^2. (**d**) Figure depicting the diffusing properties and characteristics of LDF system. The solid purple line in the center depicts the LDF system fiber. Laser light is coming from the left to the right along the z axis with some length (z). The dashed lines represent the power of light at some distance away from the fiber, r. The fluence of the system depends on: laser power, efficiency of the fiber, the distance away from the fiber (r), the length of the fiber (z), and exposure time. Due to the radial symmetry of the fiber illumination, points i, j, and k all have the same fluence, as well as i′, j′, and k′ have an equal fluence. However, due to the exponential decay of the light scattering power along the length of the fiber, the fluence at point i does not equal i′; same with j and j′; and k and k′. Over short distances and when illuminated from both ends of the fiber, such as in this study, differences in illumination fluence can be averaged over the length of the fiber.

each organism were challenged with various light intensities (5, 10, or 25 mW/cm^2) and exposure times (2, 4, or 6 hours); each experiment was repeated at least three times, and the number of cells that were reproducibly and completely inhibited by each experimental condition was recorded (Table 2).

Results revealed that LDF delivery of 405 nm light elicited a dose-dependent antimicrobial effect toward each test organism. For *S. aureus,* 144 and 180 J/cm^2 treatment inhibited growth 10^4 CFU, whereas 216, 360, and 540 J/cm^2 treatments inhibited growth of 10^6 cells and 108 J/m^2 treatment reduced growth of <10^4 cells. *P. aeruginosa* displayed increased 405 nm light susceptibility; 36, 72, and 108 J/cm^2 treatment inhibited growth of 10^4, 10^6, and ≥10^6 *P. aeruginosa* cells, respectively. *E. coli* treatment at 180, 360, 540 J/cm^2 resulted in ≤10^4,

TABLE 2. Antimicrobial Effect on a Semi-Solid Surface

Species	Viability reduction								Minimum fluence
	36 J/cm^2	72 J/cm^2	108 J/cm^2	144 J/cm^2	180 J/cm^2	216 J/cm^2	360 J/cm^2	540 J/cm^2	
Staphylococcus aureus	–	–	$\leq 10^4$	10^4	10^4	10^6	10^6	10^6	144
Pseudomonas aeruginosa	$\leq 10^4$	$\leq 10^4$	10^6	10^6	10^7	10^6	10^6	10^7	72
Enterococcus faecium	–	–	–	–	–	–	$\leq 10^4$	10^4	540
Staphylococcus epidermidis	–	–	–	10^5	10^4	10^7	10^4	10^7	144
Klebsiella pneumoniae	–	–	$\leq 10^4$	$\leq 10^4$	–	10^4	$\leq 10^4$	10^5	144
Acinetobacter baumannii	–	–	$\leq 10^4$	10^4	10^4	$\leq 10^4$	10^4	10^4	144
Enterobacter sp.	–	–	–	$\leq 10^4$	$\leq 10^4$	$\leq 10^4$	10^5	10^5	360
Streptococcus pyogenes	10^8	10^8	10^8	10^8	10^8	10^8	10^8	10^8	36
Candida albicans	–	$\leq 10^4$	$\leq 10^4$	$\leq 10^4$	$\leq 10^4$	10^6	10^6	$\leq 10^7$	216
Escherichia coli	–	–	$\leq 10^4$	$\leq 10^4$	$\leq 10^4$	10^5	10^5	10^6	216

List of antimicrobial efficacy of all organisms tested. Dosing is measured in J/cm^2. The right most column represents the minimum radiant energy density dose required to cause a 4-log reduction in viability. All antimicrobial effects reported are bactericidal. $\leq 10^X$ indicates >50% reduction in the indicated inoculum; (–) indicates no measurable reduction in inoculum.

10^5, and 10^6 reduction in CFU, respectively; 108 and 216 J/cm^2 dose resulted in a reduction of 10^5 and 10^4 CFU, respectively. Similar dose-dependent antimicrobial activity was observed for all other organisms evaluated, although the magnitude of the system's performance did vary for microbial species. *S. pyogenes* appeared to be the most susceptible, as all doses uniformly produced an approximately 8-log reduction in inoculum. Further, consistent with previously published reports, *E. faecium* appeared to be the most 405 nm-tolerant organism tested, displaying a 4-log decrease in cell viability but only at the highest dosing conditions (540 J/cm^2). However, there was no significant reduction in the organism's survival at lower light exposures [27].

To facilitate comparison of the antimicrobial efficacy of LDF to other technologies using 405 nm light reported in the literature, Table 2 also summarizes the radiant energy in J/cm^2 required to kill $\geq 4\text{-log}_{10}$ CFU of each test organism. As elaborated above, *S. pyogenes* appeared to be the most susceptible organism tested, demonstrating >4 log reduction in cell viability at 36 J/cm^2. Of the other species evaluated, *P. aeruginosa* was also highly susceptible to 405 nm light (72 J/cm^2), followed by *S. aureus*, *S. epidermidis*, *K. pneumoniae*, and *A. baumannii* (144 J/cm^2). *C. albicans*, *E. coli*, and *E. cloacae* appeared somewhat more blue light-tolerant (360 J/cm^2), whereas *E. faecium* was the least susceptible, requiring 540 J/m^2 irradiation to achieve at least a 4 log reduction in CFU.

Effects of LDF System-Delivered 405 nm Light Toward ESKAPE Pathogen-Colonized Abiotic Surfaces

Colonization of fomite material is well-recognized to serve as a source of bacterial transmission (reviewed in Otter et al. [28]). Thus, studies were expanded to assess the system's performance toward representatives of the ESKAPE pathogens when colonizing abiotic surfaces common to hospital environments, including polystyrene, fabric, silicone rubber, polypropylene, and ceramic tile. Based, in part, on their observed high degree of 405 nm susceptibility on semi-solid agar surfaces combined with their propensity to colonize abiotic surfaces, we chose three representative ESKAPE pathogens for these studies; *S. aureus* and *P. aeruginosa*, which are commonly acquired nosocomial pathogens prevalent in the hospital environment, and *A. baumannii*, an environmental organism that is notoriously tolerant to desiccation, and a prominent cause of wound infections [29–33]. Each abiotic surface was inoculated with various amounts of *S. aureus*, *P. aeruginosa* or *A. baumannii*, treated at 0, 5, 10, or 25 mW/cm^2 for 2, 4, or 6 hours and bacterial viability was enumerated.

The LDF system displayed modest decolonization properties toward *P. aeruginosa* and limited activity toward *S. aureus* adhered to each substrate (Table 3). More specifically, in comparison to mock-treated cells (shielded from light), *P. aeruginosa* exhibited a dose response dependent effect when adhered to cloth and polystyrene resulting in a maximum of 2-log_{10} and 5-log_{10} decrease in viability following 4 and 6 hours 25 mW/cm^2 treatment, respectively. *P. aeruginosa* exhibited a 2-log_{10} to 3-log_{10} decrease in recoverable cells when adhered to all other surfaces, with the exception of silicone rubber. For *S. aureus*, 6 hours irradiation at 25 mW/cm^2 (540 J/m^2) resulted in a maximum of 2 to 3-log_{10} reduction in viability to the organism adhered to cloth, ceramic tile, polypropylene, and polystyrene, whereas no antimicrobial activity was detected toward the organism when adhered to silicone rubber. Similarly, LDF delivered 405 nm light had no detectable antimicrobial effect toward *A. baumannii* on the tested colonized surfaces at any dose ($\leq 540 \text{ J/m}^2$).

TABLE 3. Antimicrobial Effect on a Semi-Solid Surface

	Species	Viability reduction							
		36 J/cm^2	72 J/cm^2	108 J/cm^2	144 J/cm^2	180 J/cm^2	216 J/cm^2	360 J/cm^2	540 J/cm^2
Polystyrene	*Staphylococcus aureus*	–	–	–	–	–	–	–	10^3
	Pseudomonas aeruginosa	–	–	–	–	–	10^2	10^2	10^5
	Acinetobacter baumannii	–	–	–	–	–	–	–	–
Polypropylene	*Staphylococcus aureus*	–	–	–	–	–	–	–	10^2
	Pseudomonas aeruginosa	–	–	–	–	–	–	10^2	10^3
	Acinetobacter baumannii	–	–	–	–	–	–	–	–
Ceramic Tile	*Staphylococcus aureus*	–	–	–	–	–	–	–	10^2
	Pseudomonas aeruginosa	–	–	–	–	–	–	–	10^3
	Acinetobacter baumannii	–	–	–	–	–	–	–	–
Rubber	*Staphylococcus aureus*	–	–	–	–	–	–	–	–
	Pseudomonas aeruginosa	–	–	–	–	–	–	–	–
	Acinetobacter baumannii	–	–	–	–	–	–	–	–
Cloth	*Staphylococcus aureus*	–	–	–	–	–	10^3	10^4	10^3
	Pseudomonas aeruginosa	–	–	–	–	–	–	–	10^3
	Acinetobacter baumannii	–	–	–	–	–	–	–	–

List of antimicrobial efficacy of all organisms tested on abiotic surfaces listed above. Gray indicates conditions that elicited ≥10^2 reduction in cell viability; (–) indicates no measurable effect.

Taken together, these results indicate that the LDF system may be amenable to decolonizing *P. aeruginosa* and, to a lesser extent, *S. aureus* colonizing many abiotic surfaces common to the hospital setting. Yet, effective decolonization would likely require significantly higher blue light doses than investigated here using either higher power and/or longer exposure times. Conversely, *A. baumannii* decolonization is not likely to be achievable, which is consistent with the organism's well-established hardiness and ability to tolerate disinfectants and long-term desiccation.

Comparison of LDF-Delivered 405 nm Light on Cultured Eukaryotic Cells and Bacterial Pathogens

We next explored the technology's potential as a therapeutic in the context of the host setting. In doing so, it was recognized that establishing blue light's therapeutic index (antimicrobial dose vs. human cell toxicity) is a requisite in characterizing whether the technology is likely to be applicable to treating infection without causing extensive, collateral host cell damage. Thus, human cell cytotoxicity measures were first used to establish a relative safe dose of blue light that displayed limited human cell cytotoxicity. Cells irradiated at 144 J/cm^2 cells displayed between 96% and 100% metabolic activity of mock-treated cells, whereas higher doses resulted in significant reduction in cellular metabolism expected of human cell cytotoxic response (not shown). Thus we considered 144 J/cm^2 irradiation to be the maximum blue light dose tolerated by representative host cells, and set out to evaluate whether blue light could deliver therapeutically relevant antimicrobial activity toward ESKAPE pathogens during planktonic growth at ≤144 J/cm^2.

Results of pathogens exposed to light while in a liquid culture revealed two distinct susceptibility phenotypes (detailed in Table 4). *S. aureus*, *S. epidermidis*, and *S. pyogenes* were all highly susceptible to 405 nm irradiation. More specifically, *S. pyogenes* exhibited a complete loss of cell viability at all doses evaluated (≥31 J/cm^2), whereas both *S. aureus* and *S. epidermidis* showed a dose-dependent antimicrobial effect with a maximum and complete loss of cell viability at 125.4 J/cm^2, respectively. Conversely, the other organisms tested displayed no significant susceptibility toward 405 nm light during growth in liquid culture conditions. Nonetheless, the two organisms that displayed the greatest therapeutic index, *S. pyogenes* and *S. aureus*, are predominant causes of bolus impetigo, indicating that a LDF 405 nm light delivery system may represent a safe and attractive strategy for the therapeutic intervention of this serious bacterial skin infection.

Characterization of the Effects of 405 nm Light Toward *S. aureus* Disease-Associated Growth States

In comparison to *S. pyogenes*, *S. aureus* has greater propensity to cause a multitude of diverse infections ranging in severity from skin to lung disease and sepsis.

TABLE 4. Antimicrobial Effect Planktonic Growth Conditions

Organism	Viability reduction[1] (J/cm^2)			
	31.3 ± 8.8	62.7 ± 17.7	125.4 ± 35.4	250.8 ± 70.8
Staphylococcus aureus	–	10^2	10^4	10^4
Human serum	–	10^1	10^2	10^4
Lung surfactant	–	–	–	10^4
Pseudomonas aeruginosa	10^1	–	–	–
Enterococcus faecium	—	—	—	—
Staphylococcus epidermidis	–	10^1	10^4	10^4
Klebsiella pneumoniae	10^1	–	–	–
Acinetobacter baumannii	10^2	10^1	–	–
Enterobacter sp.	–	–	–	–
Streptococcus pyogenes	10^4	10^4	10^4	10^4
Candida albicans	–	–	–	–
Escherichia coli	–	–	–	–

List and results of all bacteria tested in planktonic growth conditions. Gray indicates conditions that elicited $\geq 10^2$ reduction in cell viability; (–) indicates no measurable effect.

Thus as an entrée toward assessing the antimicrobial properties of LDF 405 nm light delivery in conditions that approximate *S. aureus* disease settings, we assessed whether the system was capable of delivering antimicrobial activity toward the organism during growth in either human serum or mouse lung surfactant.

Accordingly, *S. aureus* were suspended in either 100% human serum or lung surfactant, and exposed to ≤144 J/m² 405 nm light. During growth in human serum, 405 nm light delivered via an LDF system resulted in a dose-dependent reduction in *S. aureus* viability, resulting in a 1.65 and 2 log reduction in recoverable CFU when irradiated at 62.7 and 125 J/cm², respectively. During growth in mouse lung surfactant, *S. aureus* appeared to be recalcitrant to low-dose exposures ≤125 J/cm², but exhibited complete loss of viability at 250 J/cm² although this dose is expected to be detrimental to host cells (Table 4). Taken together, these results suggest that future 405 nm light delivery strategies may serve as an effective and safe approach to reducing *S. aureus* burden in the context of systemic infections, but the approach may not be effective/safe in treating lung infections.

DISCUSSION

Light-diffusing fiber provides radially uniform illumination (described in Fig. 1D), that requires electricity at the light source opposed to along its length. Accordingly, the goal of the current work was to provide a preliminary survey of the antimicrobial properties of the LDF system for delivering 405 nm light toward bacterial and fungal species of immediate healthcare concern. In doing so, we had two overarching objectives. The first was to explore the system's potential as a means to decolonize semi-solid and abiotic surfaces. The second was to begin to characterize the system's performance in the context of biologically relevant conditions.

Most previously reported 405 nm surface associated antimicrobial studies have been performed by assessing the effects of LED irradiation toward bacterial species plated on agar plate surfaces [4–10]. Accordingly, as an entrée toward evaluating the potential of the LDF 405 nm light delivery system, we first performed analogous studies to establish whether the system performed with equipotency to LED systems. Our studies focused on the ESKAPE pathogens, because they cause the majority of nosocomial bacterial infections and can "escape" the antibacterial effects of current antibiotics due to resistance. We also included other pathogens of emerging healthcare concern both to explore the spectrum of activity of the approach and to compare results from LDF-delivered 405 nm light with previously reported results from 405 nm LED studies [4–10]. All organisms responded to 405 nm light delivered via an LDF system in a dose-dependent manner and magnitude that paralleled the results of LED irradiation. *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, and *A. baumannii* were all found to be highly susceptible to 405 nm light resulting in ≥ 4-$\log_{10}$ reduction in cell viability at ≤144 J/cm², which mimics the results of LED delivered 405 nm light [34–37]. *Enterococcus* was found to be recalcitrant to 405 nm light, which is also consistent to previous findings [38]. Interestingly, *S. pyogenes* appeared to be the most susceptible organism to 405 nm light that we tested, which to our knowledge, has not been previously reported. From these perspectives the LDF system appears to represent a novel, flexible antimicrobial light delivery agent with pronounced activity toward *S. pyogenes* and a subset of the ESKAPE pathogens.

Building from this, we recognized that the LDF system uniquely allows flexible 405 nm light delivery that, in turn, may be exploited for decolonizing abiotic surfaces common to the hospital setting that may not be easily accessible by traditional LED devices. While our pilot

study described above is limited in scope based on the laser power available, it allowed an early measure of the system's ability to decolonize polystyrene, polypropylene, silicone rubber, ceramic tile, and cloth surfaces. In general, the degree of bacterial killing on abiotic surfaces at a given fluence was reduced compared with organisms plated on nutrient medium permitting growth that were treated at the same fluence. This reduced efficacy may reflect a diminished level of available target molecules that may be activated by 405 nm light to produce reactive oxygen species in dried, static cells compared to growing cells. Of the test bacterial species, 405 nm light was ineffective in reducing the viability of *A. baumannii* when colonizing abiotic surfaces, which is consistent with the organism's tolerance of desiccation and disinfectants. Yet, this appeared to be an organism-specific phenomenon, as another Gram-negative pathogen, *P. aeruginosa*, displayed the greatest susceptibility as measured by reduction in cell viability of the bacterial species tested. Similarly, *S. aureus* exhibited appreciable 405 nm decolonization properties suggesting that the technology may be applicable to decolonizing hospital associated surfaces and warrant future studies aimed at comprehensively understanding the spectrum of susceptible species, dosing powers, and the recently recognized synergistic potential of 405 nm light in combination with chemical disinfectants [39].

We also measured the LDF system's performance toward planktonic bacteria. In doing so it was recognized that antimicrobial measures of 405 nm light toward bacteria in liquid format is typically performed by irradiation of buffered bacterial suspensions to allow optimal light diffusion/delivery, and approximate certain therapeutic applications, such as aquaculture. Such studies have revealed that many of the organisms evaluated here, including the ESKAPE pathogens *A. baumannii*, *E. cloacae*, *P. aeruginosa*, *S. aureus*, *E faecium*, and *K. pneumoniae* all display $\geq$5-$\log_{10}$ decrease in viability at $\leq$108 J/cm^2 exposure [27]. Nonetheless, conventional antimicrobial MIC testing and development is conventionally performed in culture medium instead of aqueous buffer for a multitude of reasons. Thus, we set out to measure the antimicrobial performance of LDF system delivery of 405 nm light toward each test organism in liquid culture conditions, both to allow comparison to the performance of conventional antibiotics and to simultaneously establish 405 nm light's base-line performance toward planktonic bacteria that could, in turn, be referenced for studies of bacteria in alternative liquid culture conditions recognized to be representative of the host environment.

As a prerequisite to liquid culture testing, we first determined the light dosage that was reproducibly well tolerated by cultured human cells based on the premise that host-associated light exposure would be best performed at a fluence levels that are not detrimental to human cells. Our work revealed that 144 J/cm^2 was the maximum tolerated dose by human colon 38 cells, but it should be noted that parallel tests of human primary fibroblasts displayed considerable toxicity at much lower

light doses (data not shown), consistent with earlier studies of 405 nm fibroblast cytotoxicity [40]. In a clinical scenario, risk-benefit pay-off must be considered based on any potential negative effects of 405 nm light exposure to host tissue versus the damage caused by a potential antibiotic-resistant infection. The results reported here suggest that the relatively low human cytotoxicity threshold necessitates careful consideration of the potential collateral damage associated with use of 405 nm light in the context of the host environment. Nonetheless, survey of the antimicrobial properties of LDF system-delivered 405 nm light toward bacteria in MH liquid medium at sub-144 J/cm^2 doses revealed that, similar to semi-solid agar testing, *S. pyogenes* was found to be the most susceptible organism evaluated. More specifically, the organism was eradicated at all doses tested, which prompted us to evaluate whether the high degree of 405 nm light sensitivity was strain-specific. Testing of five additional genetically divergent clinical *S. pyogenes* isolates confirmed the phenotype was conserved across the test panel, with each strain demonstrating at least a 4 log reduction at <50 J/cm^2 (data not shown). Killing of *S. pyogenes* by 405 nm light in the absence of exogenous photosensitizers has not been reported previously. Although the mechanistic basis of exquisite sensitivity of *S. pyogenes* to 405 nm light has not been defined, it may be due to elevated levels of endogenous photosensitizers, a reduced capacity to produce antioxidant compounds capable of neutralizing reactive oxygen species, or both in combination. Similarly, other Gram-positive species were also susceptible to 405 nm light irradiation delivered by an LDF system. Both *S. aureus* and *S. epidermidis* demonstrated dose-dependent antimicrobial effects and complete loss of cell viability at higher test doses tested. Conversely, *E. faecium* and the Gram-negative species evaluated did not exhibit appreciable 405 nm susceptibility in liquid culture conditions. It is possible that adjusting the treatment exposure time may improve efficacy. To that end, Biener et al. [41] have recently found that two 405 nm applications are more effective than a single application with equivalent light exposure. Regardless, the finding that the technology exhibited pronounced antimicrobial effects toward *S. pyogenes* and *S. aureus*, which are the two most predominant causes of bacterial impetigo, suggests a clinical application.

Taken together, the studies provide proof-of-principle of the utility of 405 nm light delivered by an LDF system as an antimicrobial system for the antisepsis of infections and disinfection of abiotic surfaces. Additionally, these results suggested that 405 nm light delivered via an LDF system is capable of producing an effective antimicrobial dose that approximates the antimicrobial performance of LED directed light exposure.

CONCLUSION

High-intensity blue-violet light (405–470 nm) has been demonstrated in the literature as an effective antimicrobial agent. Most of these studies use LEDs or other flood

illumination sources. Here we examined the antimicrobial capabilities of 405 nm light delivered with a laser source and Corning® LDF. Since LDF delivers light radially along its length in a flexible and thin format, its geometric characteristics may be advantageous for clinical applications. The LDF system and 405 nm light displayed significant antimicrobial activity toward the ESKAPE bacterial pathogens, as well as *S. epidermidis*, *S. pyogenes* and the fungal pathogen *C. albicans*.

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