In vitro results of flexible light-emitting antimicrobial bandage designed for prevention of surgical site infections

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ABSTRACT

Surgical site infections (SSIs) are a leading cause of morbidity and mortality and a significant expense to the healthcare system and hospitals. The majority of these infections are preventable; however, increasing bacterial resistance, biofilm persistence, and human error contribute to the occurrence of these healthcare-associated infections. We present a flexible antimicrobial blue-light emitting bandage designed for use on postoperative incisions and wounds. The photonic device is designed to inactivate bacteria present on the skin and prevent bacterial colonization of the site, thus reducing the occurrence of SSIs. This antimicrobial light emitting bandage uses blue light's proven abilities to inactivate a wide range of clinical pathogens regardless of their resistance to antibiotics, inactivate bacteria without harming mammalian cells, improve wound healing, and inactivate bacteria in biofilms. The antimicrobial bandage consists of a thin 2"x2" silicone sheet with an array of 77 LEDs embedded in multiple layers of the material for thermal management. The 405 nm center wavelength LED array is designed to be a wearable device that integrates with standard hospital infection prevention protocols. The device was characterized for irradiance of 44.5 mW/cm^2. Methicillin-resistant *Staphylococcus aureus* seeded in a petri dish was used to evaluate bacterial inactivation *in vitro*. Starting with a concentration of 2.16 x 10^7 colony forming units (CFU)/mL, 45% of the bacteria was inactivated within 15 minutes, 65% had been inactivated by 30 minutes, 99% was inactivated by 60 minutes, and a 7 log reduction and complete sterilization was achieved within 120 minutes.

Keywords: blue light, antimicrobial, infectious diseases, infection prevention, surgical site infection

1. INTRODUCTION

The Centers for Disease Control praise the advances in infection control practices, including improved operating room ventilation, sterilization methods, barriers, surgical technique, and availability of antimicrobial prophylaxis [1]. Nevertheless, SSIs remain a substantial cause of morbidity, prolonged hospitalization, and death, since 2-3% of the 16 million surgical procedures in the U.S. result in a SSI. Additionally, these SSIs cost hospitals and the healthcare system over $10 billion annually [1-4]. The vast majority of these infections are preventable; however, increasing bacterial resistance, biofilm persistence, and human error contribute to the occurrence of these infections.

The increasing prevalence of antibiotic resistance in pathogenic bacteria has created a need for alternative antimicrobial therapies. Antibiotics are expensive, have significant side effects, and their overuse is a well-known contributor to an increasing antibiotic resistance. An increasing number of SSIs are attributable to antibiotic-resistant pathogens like methicillin-resistant *S. aureus* (MRSA) [5]. The most common source of pathogens responsible for SSIs originate from the patient's endogenous flora and exogenous sources such as the environment, instrumentation, or hospital staff. MRSA, *S. aureus,* coagulase-negative *staphylococci*, and *E. coli* are the most common organisms causing SSIs [5]. Pathogens present in the environment make their way to surgical sites through the air or through contact, and can proliferate, leading to an infection. Microorganisms protect themselves by growing in biofilms, which are prevalent in the environment because they are difficult to kill and typically resistant to antibiotic therapy [6].

The use of blue light in the wavelength rage of 400-470 nm is a potential solution to SSIs due to its proven antimicrobial effects, specifically for inactivating wound pathogens and improving wound healing [7-9]. Blue light is intrinsically antimicrobial, capable of photodynamic inactivation of a wide spectrum of bacteria (both gram-positive and negative) and fungi [8]. Additionally, blue light is equally effective against drug sensitive and resistant bacteria, blue light is less

detrimental to mammalian cells than UV radiation, and bacteria do not develop a resistance to blue light therapy [10]. The mechanism of blue light's antimicrobial effect is similar to photodynamic therapy (PDT), however, no exogenous photosensitizer is required for blue light therapy [8]. Blue light bacterial inactivation occurs due to the photo-excitation of intracellular porphyrins, resulting in production of cytotoxic reactive oxygen species (ROS), such as oxygen radicals, singlet oxygen and peroxides [10, 11]. When the endogenous photosensitizer is transformed to an excited state it will undergo a type I or type II photochemical reaction forming hydroxyl radicals or singlet oxygen, respectively [12]. The excitation of endogenous porphyrins generates an abundance of ROS which overwhelm the bacterial cell's antioxidant capacity causing damage to proteins, enzymes, lipids, and DNA, resulting in bacterial cell death and inactivation of virulence factors [11-13]. Blue light is an ideal technology for use on the human body for infection prevention because it can excite endogenous porphyrins in bacterial cells, such as MRSA, that are not present in host cells, allowing for bacteria to be specifically inactivated while host tissue cells are preserved, preventing harm to mammalian host cells [11, 14].

We present preliminary results with a device utilizing blue light LEDs centered at 405 nm (outside the range of UV light). The device is designed to be a flexible antimicrobial bandage to prevent bacterial contamination of postoperative incisions and wound sites, thus reducing the occurrence of SSIs. This antimicrobial light emitting bandage uses blue light's proven abilities to inactivate a wide range of clinical pathogens regardless of their resistance to antibiotics, inactivate bacteria without developing resistance or harming mammalian cells, improve wound healing, and inactivate bacteria in the biofilm state [8-10]. The thin transparent multilayer thermally-managed LED sheet delivers antimicrobial blue light therapy using a compact wearable device that can be easily incorporated into infection prevention protocols.

2. CLINICAL PROBLEMS

An ideal clinical device will solve its intended problem without creating new ones. Therefore, proper design necessitates close consideration of the clinical problems that contribute to healthcare associated infections (HAI) as well as an attempt to anticipate difficulties in implementing a new tool for HAI prevention.

In the current state, HAI prevention in the hospital is best-defined as a "culture." Healthcare workers are continually-encouraged to wash their hands through written reminders, email campaigns, slogans, and the threat of being caught by an "undercover" auditor who may see them on the wards neglecting proper hand hygiene [17-19]. Outside the surgical suites, physical barriers often denote the "no-pass" zone, where staff and visitors must change into clean scrubs and cover their shoes and hair prior to entering the halls outside the operating rooms. Surgeons scrub their hands for two (or more) minutes with chlorhexidine soap and a nail pick, and then apply (sometimes two layers of) sterile gloves after donning a disposable sterile gown [19].

Each floor on the hospital contains a well-stocked supply room, full of sterile gloves, soaps, bandages, gauze, scissors, tape, and surgical drains. Wounds are meticulously-checked, dressings changed, and signs of infection documented by the nursing team in mandatory check-boxes on the electronic medical record. Wounds are re-checked by the surgeons. Patients are monitored for signs of fever, dropping blood pressure, or elevated heartrate, and blood is drawn to monitor white blood cell concentration, all early signs of infection. Patients are frequently subjected to CT scans to search for a possible abscess or "hidden infection" lying deep in a wound. The specter of HAI pervades every department in a hospital, from executives to physicians and nurses to the custodial staff who clean beds and rooms with specialty antimicrobial equipment.

Since nurses are tasked with multiple time-sensitive duties for multiple patients, any additional task or inconvenience to them comes at a premium and introduces more opportunity for errors to occur. Minimizing the complexity and time requirement for a new infection-control procedure is crucial. In addition, any new infection-prevention tool should integrate with the current standard of care. For instance, new devices which are incompatible with surgical soap will never become effective because they will never be accepted by hospitals. A "disruptive" technology should not disrupt nursing protocols, hospital supply chains, and surgeon's preferences (at least at first) if it hopes to be welcomed in the hospital.

The challenge of developing a successful tool for preventing HAI's becomes thus: "How can a new tool lower the risk of developing an HAI from inadvertent exposure to pathogens without creating new challenges for healthcare staff?" HAI's, including surgical site infections, are the result of a pathogen exposure from the patient's endogenous flora and/or a "lapse" in proper hygiene as briefly discussed above, exposures which may happen at any phase of a patient's care [19]. A tool which eliminates or reduces pathogen entry into a wound at all phases of a patient's care should therefore eliminate or greatly reduce surgical site infections. Additionally, a device which is intuitive, facile to use, and does not interfere in hospital workflows is most likely to achieve optimal user compliance and acceptance in the hospital system.

3. DEVICE DESIGN

The photonic device is intended for automated antimicrobial light therapy while worn as a bandage over a surgical site to create a barrier to bacteria migrating from the patient, his or her surroundings, or healthcare workers' hands and thereby prevent spread into the deep tissues of the wound. The prototype bandage (Fig. 1) consists of a thin 2" x 2" silicone sheet with an array of 77 LEDs embedded in multiple layers, allowing for thermal insulation and protection of the patient from excess heat. The LEDs (Vishay VLMU 3500-405-060) have a center wavelength of 405 nm with a full-width half maximum bandwidth of 16 nm. The device is 2.8 mm in height, and the LEDs within the prototype are spaced accordingly to result in even distribution of light on the treatment surface. This prototype bandage, assembled in conjunction with LiteSheet, offers a low-cost system with reusable components, allowing for a novel approach to infection prevention.

Figure 1. LED array embedded in 2" x 2" silicone sheet provides a flexible surface capable of delivering 50 mW/cm^2 of 405 nm light to a treatment surface.

The bandage itself integrates with standard infection protocols (scrubs, dressing changes) rather than obviating them, allowing hospitals to continue their current standard of care. Flexible, thermally-managed materials result in a bandage that is comfortable for patients and allows contouring to a patient's wound surface.

For prototype testing, we ensured that the device was powered properly for output capability of 50 mW/cm^2 at the surface of the treatment area. 50 mW/cm^2 was chosen as an ideal irradiance because it is the maximum irradiance currently approved by the FDA for use on human skin for the treatment of acne by reduction of bacterial colonization from blue light's antimicrobial effects. The device is capable of delivering an irradiance greater than 250 mW/cm^2, however, for this experiment we focused on maintaining constant output of 50 mW/cm^2 at the treatment surface. To control power to the device, a DROK DC-DC numerical control step down voltage converter was used to supply constant current and constant voltage to the device. The low drive current supplied to the high power LEDs in combination with the multi-layer silicone design and a small CPU fan (8 mm x 10 mm x 1.4 mm) placed on top of the device provided thermal management to maintain treatment surface temperatures below 43 °C, which is below the burn threshold for skin and does not contribute to bacterial death. [21]. The LED array and fan were placed inside a custom housing for integration with experimental design and use with the petri dishes. This housing was used on enclosed petri dishes and allowed for blue light treatment without drying or heating of medium.

4. MATERIALS AND METHODS

4.1 Device testing

The flexible light emitting bandage is designed to emit blue light at 405 nm to achieve antimicrobial effects without damage to patient skin. The variability of the irradiance and thermal output of the device were first characterized to confirm that

design specifications would be maintained over time. To verify the LED's constant irradiance, the device was placed in a calibrated optical imaging setup using a Thorlabs PM100D optical power and energy meter and a Thorlabs S120C sensor, and tested for 10 minutes. Irradiance measurements were taken at one minute intervals, while maintaining constant current at a voltage of 3.7 V. The device surface temperature was also measured at the same time intervals using a 2 K-type thermocouple sensor probe.

4.2 Bacterial experiments

In order to test the functionality and antimicrobial effects of the flexible light emitting bandage, we tested its light treatment on MRSA strain Xen36 (PerkinElmer) through previously reported methods of blue light inactivation of bacteria [15]. The bacteria were prepared to be in the exponential growth phase during exposure to the blue light. The MRSA Xen36 inoculum was grown in Luria Bertani (LB) broth with shaking at 220 rpm at 37 °C overnight. The O.D. of the overnight culture was measured at 600 nm and adjusted to achieve a count of 10^7 CFU/ mL. The petri dish was seeded with 10^7 CFU/mL MRSA Xen36 in 10 mL Dulbecco's Modified Eagle Medium (DMEM) +5% fetal calf serum (FCS). This concentration of bacteria is used to simulate the amount of bacteria present in an infection. A magnetic stirring bar was placed in the solution to provide continuous stirring during exposure, as previously reported [14, 15]. The enclosed petri dish was treated with the flexible light emitting bandage within the custom housing (Fig. 2), and an experimental control without light exposure was also used. A 100 µL aliquot was withdrawn and diluted in phosphate-buffered saline (PBS) to make 10-fold dilutions and plated onto LB agar plates (20 µL spots in triplicate) to enumerate CFU at 0, 15, 30, 60 and 120 min after exposure to blue light. At each time-point, light intensity from the device was recorded to ensure constant irradiance on the sample. Percent survival was calculated for each time point.

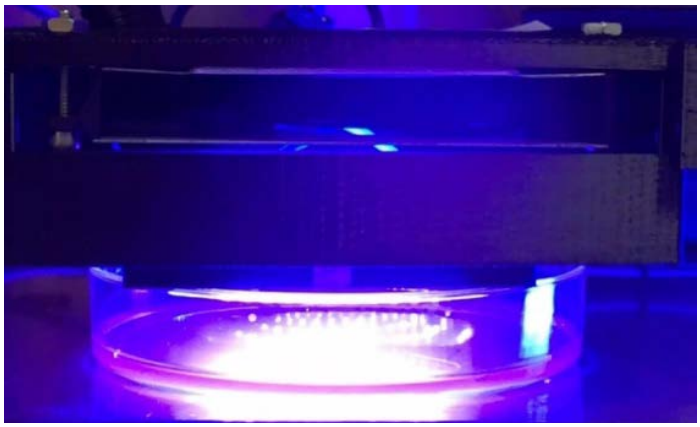

Figure 2. Custom housing for flexible light emitting bandage and fan positioned over a bacterial culture plate during light exposure.

5. RESULTS

5.1 Device performance

Results for the testing of the flexible light emitting bandage for stability in irradiance and thermal management are shown in Fig. 3. The device surface temperature was maintained at 35.4 +/- 0.3 °C, and the irradiance stayed constant at 50.2 +/- 0.2 mW/cm^2. The device was verified to produce light output adequate for blue light antimicrobial effects, while maintaining a constant temperature below the thermal burn threshold for skin tissue.

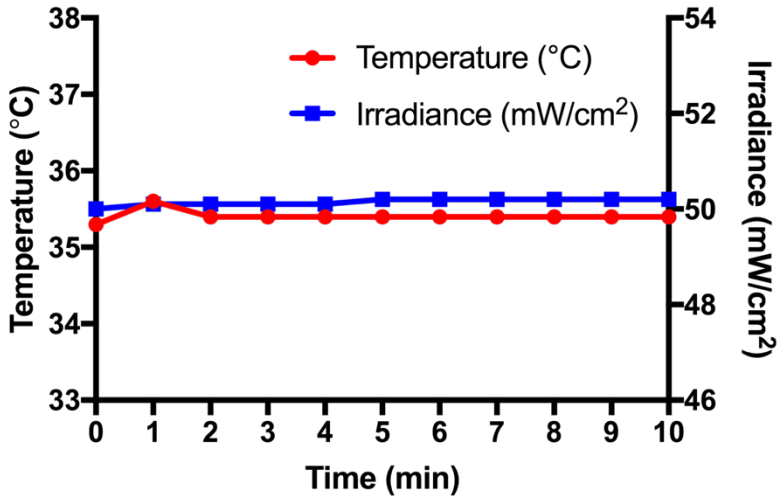

Figure 3. Flexible light emitting bandage maintained surface temperature of 35.4 °C and irradiance of 50.2 mW/cm^2 at a constant current.

5.2 Inactivation of MRSA with light emitting bandage

Results for testing the flexible light emitting bandage on an initial population of MRSA Xen36 at 2.167 x 10^7 CFU/mL are shown in Fig. 4, 5, 6, and Table 1. The device's irradiance and temperature met performance requirements. The measured irradiance at the surface of the broth was 44.5 +/- 0.7 mW/cm^2. The temperature inside the petri dish did not exceed 30.7 °C throughout the experiments. The sample obtained at 120 minutes did not have any viable bacteria present; therefore, complete sterilization occurred. By 30 minutes a 0.45 log reduction was achieved (65% reduction) with an 80.1 J/cm^2 dose. By 60 minutes a 1.9 log reduction was achieved (99% reduction) after a dose of 160 J/cm^2. Within 120 minutes, a 7 log reduction was achieved (100% reduction) from the initial 2.167 x 10^7 CFU/mL, reaching complete sterilization within 320 J/cm^2.

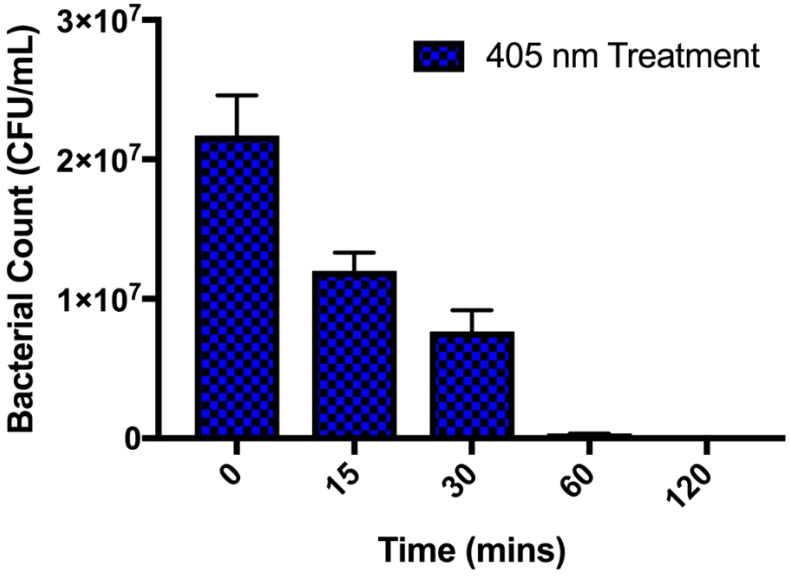

Figure 4. Bacterial count in CFU/mL of MRSA Xen36 after treatment with 405 nm flexible bandage emitting 44.5 mW/cm^2 of 405 nm light at the surface of the medium.

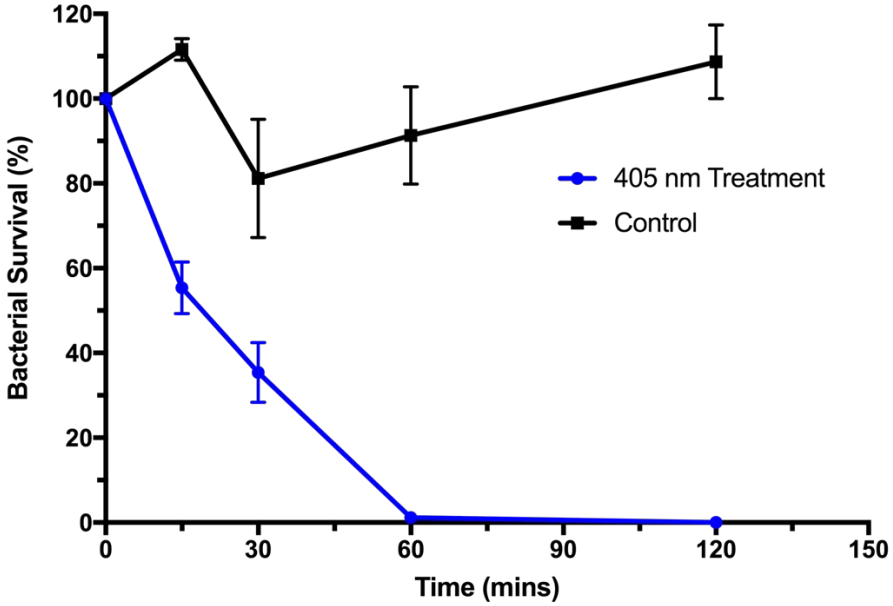

Figure 5. Percent survival of MRSA Xen36 after treatment with 405nm flexible light emitting bandage.

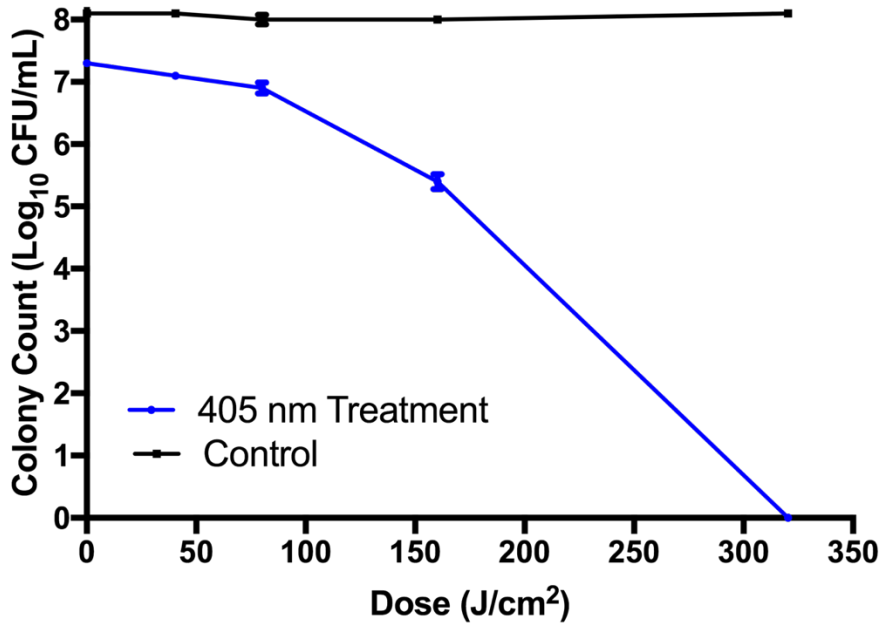

Figure 6. Results of 405nm light inactivation of MRSA Xen36 with 405 nm light emitting bandage

Table 1. Summary of Experimental Results

Time (mins)	Log Reduction	Percent Reduction	Dose (J/cm^2)
0	0	0%	0
15	0.26	45%	40.5
30	0.45	65%	80.1
60	1.9	99%	160.2
120	7	100%	320.4

6. CONCLUSION

This study successfully demonstrates the capabilities of the flexible light emitting bandage to inactivate MRSA, and shows the ability of the device and its form factor to perform in a manner that could be easily incorporated into the clinical setting. The study results are consistent with previous antimicrobial blue light experiments performed in a similar manner, showing that MRSA bacterial log reduction is based on both time and irradiance of 405-415 nm blue light [14, 15]. The inactivation curves in our study are consistent with previous blue light bacterial inactivation studies [14-16]. Our results, along with previous studies, indicate that when MRSA is exposed to blue light there is an approximate 50% reduction in bacterial population by 30 minutes, and when exposure is longer than 30 minutes the bacteria continue to die off at an accelerated rate of multiple orders of magnitude. Our device reduced MRSA population to less than 1% by 60 minutes of blue light exposure, and achieved a 7 log reduction (sterilization) within 120 minutes of blue light exposure. With different strains of bacteria, different bacterial starting concentrations, and variable light irradiance the precise log reduction effect varies; however, these experiments all confirm blue light's ability to inactivate bacteria. Though these results are limited to in vitro MRSA populations, previous experiments provide evidence that blue light exposure results in excellent in vivo disinfection as well [7, 9, 14].

Although it is known from an ever-increasing body of literature that 405nm blue light results in bacterial inactivation, further work should be performed with reduced variations in experimental design to analyze the trends between these blue light bacterial inactivation experiments. To apply this antibacterial treatment clinically for infection prevention a device in form and function was needed that could be implemented within clinical practices. The device presented here accomplishes this in both device performance and antibacterial effects. The next step to implement an infection prevention solution utilizing blue light is to test this device *in vivo* to confirm the appropriate irradiance of light and necessary exposure time to adequately reduce MRSA populations, as well as other wound pathogens such as *Staphylococcal* species, *Streptococcus*, *Escherichia*, *Enterococcus, Pseudomonas aeruginosa, and Acinetobacter baumannii*.

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