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Calibration of optimal use parameters for an ultraviolet light-emitting diode in eliminating bacterial contamination on needleless connectors

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Abstract

Aims—Needleless connectors may develop bacterial contamination and cause central-line-associated bloodstream infections (CLABSI) despite rigorous application of best-practice. Ultraviolet (UV) light-emitting diodes (LED) are an emerging, increasingly affordable disinfection technology. We tested the hypothesis that a low-power UV LED could reliably eliminate bacteria on needleless central-line ports in a laboratory model of central-line contamination.

Methods and Results—Needleless central-line connectors were inoculated with *Staphylococcus aureus*. A 285 nm UV LED was used in calibrated fashion to expose contaminated connectors. Ports were directly applied to agar plates and flushed with sterile saline, allowing assessment of bacterial survival on the port surface and in simulated usage flow-through fluid. UV applied to needleless central-line connectors was highly lethal at 0.5 cm distance at all tested exposure times. At distances >1.5 cm both simulated flow-through and port surface cultures demonstrated significant bacterial growth following UV exposure. Logarithmic-phase *S. aureus* subcultures were highly susceptible to UV induction/maintenance dosing.

Conclusions—Low-power UV LED doses at fixed time and distance from needleless central-line connector ports reduced cultivable *S. aureus* from >10⁶ CFU to below detectable levels in this laboratory simulation of central-line port contamination.

Significance and Impact of the study—Low-power UV LEDs may represent a feasible alternative to current best-practice in connector decontamination.

Keywords

central venous catheter; central-line-associated bloodstream infections; disinfection; needleless connector; ultraviolet light-emitting diode

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Introduction

In the United States alone, there are estimated to be 41 000 cases of inpatient central-line-associated bloodstream infections (CLABSI) and 37 000 cases of outpatient CLABSI per year, with 30 million central line days per year (CDC 2011). These cases cause increased hospital stays and increase patient costs by tens of thousands of dollars per patient, resulting in an additional \$1.3 billion annual cost to the US health care system. Furthermore, CLABSI has an estimated 25% mortality rate at a cost of 20 000 patient lives lost annually (CDC 2011).

First introduced in the early 1990s, needleless connectors for central venous catheters (CVC) have become ubiquitous throughout the health care system, primarily because they reduce the risk of needle stick injury. Unfortunately, the rise in use of needleless connectors has also been associated with an increase in CLABSI (Jarvis *et al.* 2009; Hadaway 2012). The most common cause of CLABSI is direct contamination of the needleless connector, commonly called a port or hub, when handled by a healthcare provider (Crnich and Maki 2002b). Evidence suggests that the insertion site is the source of CLASBI related to short-term CVC (Mermel 2011). For CLASBI associated with CVC used for a prolonged period, improper aseptic technique using devices such as needleless connectors, tubing connectors and stopcocks additionally provide sources for contamination (Field *et al.* 2007; Rupp *et al.* 2007; Jarvis *et al.* 2009; Mermel 2011; Holroyd *et al.* 2014). The recommended practice to reduce contamination is cleaning the port interface for 5 s with an isopropyl alcohol wipe prior to use, often termed 'scrub the hub' (O'Grady *et al.* 2011). However, one study suggests that up to a third of healthcare providers do not disinfect ports before use and another suggests that over half of all nurses do not feel the need to disinfect ports before use (Karchmer *et al.* 2005; Hadaway 2012). Furthermore, even a 15 s 'scrub', while reducing contamination, does not completely sterilize a port (Simmons *et al.* 2011). Finally, ports can be accessed 10–30 times per day (Mahieu *et al.* 2001). This intervention can impose a hardship on health care workers, reducing their availability for other duties, and may be underperformed in emergencies.

Efforts to reduce CLABSI should therefore include effective decontamination of needleless connector ports that is easily performed, perhaps automated, and does not interfere with patient care activities. Ultraviolet light (UV) has been recognized as an antimicrobial agent since the 1960s and is used today for everything from water treatment to disinfection of contact lenses (Berg 1973; Reed 2010; Gruber *et al.* 2013; Jindatha *et al.* 2014; Lonnen *et al.* 2014). The most widely used wavelength for bacterial decontamination is 254 nm; however, a range of wavelengths of UV and near UV have been successful at clearing bacterial contamination (Tyrrell and Peak 1978; Maclean *et al.* 2009; Zhang *et al.* 2014). Currently, the most common UV source for germicidal purpose is the mercury-vapour lamp, which suffers disadvantages including need for a high-frequency power source, shorter life relative to mature-technology light-emitting diodes, and breakable glass construction (Lui *et al.* 2014). However, recent availability of UV light-emitting diodes (LEDs) offers a practical alternative. LEDs negate many of the disadvantages of fluorescent lamps and, because of their small size and low-power requirement, could be incorporated in a medical device that could reduce port contamination. We hypothesized that a UV LED emitting a peak

wavelength of 285 nm was sufficient to eliminate both contamination on ports and flow-through contamination. We tested this hypothesis using simulated accidental port contamination and port usage, modelling real-life accidental bacterial contamination that could lead to CLABSI.

Materials and methods

Bacterial cultivation

Staphylococcus aureus (Non-MRSA clinical isolate, OHSU Hospital, Portland, OR, USA) was streaked for single colonies on a trypticase soy agar plate (TSA) and grown overnight at 37°C. Single colonies were selected and cultured on an orbital shaker to stationary phase in trypticase soy broth (TSB) at 37°C.

Ultraviolet light-emitting diode test device

We used a commercially available ultraviolet (UV) LED in this study (LED285W Thorlabs Inc. Newton, NJ, USA). This device is specified as having peak emission (centre wavelength) at 285 nm, full width, half maximum wavelength 12 nm, half viewing angle 60°, DC forward current (typical) 20 mA, forward voltage (at typical current) 5.8 V and optical power (typical) 0.8 mW (Thorlabs Inc. Newton, NJ, USA). The LED was fixed to the centre of a sliding carrier within a 2.5 cm opaque polyvinyl chloride tube (Fig. 1). The sliding carrier was calibrated such that the distance between the LED and the target port could be set and maintained with precision. A luer-lock connector, drilled to remove any light-obstructing plastic was placed at the centre of the end of the tube and allowed reproducible connection of standard needleless connection ports (MicroClave Clear, ICU Medical). The LED was powered for precise time intervals using a programmable DC power supply (Rigol DP1308a) set to 20 mA constant current. Unless otherwise indicated, the light source was positioned 0.5 cm from the immobilized port.

Simulation of port contamination, injection and quantification of bacterial growth

A 1 ml aliquot of *S. aureus* broth culture was diluted in sterile PBS (Hyclone Laboratories, Logan, UT, USA) such that the absorbance (A_{595}) approximated 1.0, which equated to $\sim 5 \times 10^8$ colony forming units per millilitre (CFU ml⁻¹). This sample was designated 'start culture'. To produce biofilm on ports, 5 μ l aliquots of start culture were inoculated on ports and dried for 1 h. Following exposure, ports were flushed with PBS. The ports were attached to a 10 ml Luer-lock syringe (BD) with the plunger removed. After placing them over a 14 ml snap cap tube (BD) for collection, 5 ml of sterile PBS was placed in the syringe, and the contents were pushed through the port using the plunger (Fig. 1). The contents of the collection tube (simulated usage flow-through) were mixed and plated on TSA in aliquots (10 μ l unexposed or 100 μ l UV exposed). Each condition was performed in triplicate. Additionally, immediately after flushing each port was pressed against a TSA plate for 5 s to create a bacterial stamp, providing a semi-quantitative representation of viable biofilm remaining on the port. To determine the concentration of the start culture, 5 μ l aliquots were diluted in 5 ml of PBS. 10 μ l aliquots from 1 : 10 dilutions were plated on TSA. All plates were incubated overnight at 37°C. CFU counts from each condition were compiled 24 h postinoculation. Bacterial stamp plates were documented using a digital camera. Colony

densities were quantified using IMAGEJ software (National Institutes of Health, Bethesda, MD, USA).

Ultraviolet dose-response

Ports were inoculated as described above and divided into groups of 3. The first group was exposed to UV for 10 s. Exposure time was increased in 10 s intervals up to 90 s. A control group was left unexposed. Bacteria were collected from a simulated usage flow-through fraction and stamp plated as above. All plates were incubated overnight at 37°C. CFU counts from each condition were compiled 24 h postinoculation. Bacterial stamp plates were documented and quantified as above.

Distance evaluation

Ports were inoculated as described above and divided into groups of 3. A control group was left unexposed. Remaining ports were exposed to UV for 40 s with the distance between the port and the LED starting at 0.5 cm, increasing in 1.0 cm intervals up to 3.5 cm. Bacteria were collected from a simulated usage flow-through fraction and stamp plated as above. All plates were incubated overnight at 37°C. CFU counts from each condition were compiled 24 h postinoculation. Bacterial stamp plates were documented and quantified as above.

Maintenance regime exposure and cell recovery

To simulate new bacterial growth on a freshly contaminated port, a log-phase culture was prepared by diluting a stationary phase culture 1 : 50 into fresh TSB and incubating on a shaker at 37°C to an A_{595} of 0.5–0.6. Twenty-seven ports were inoculated with 5 μ l aliquots and dried for 1 h. Ports were divided into three groups of 9. In the first group (designated T₀), three ports were left untreated, three ports were exposed to UV for 10 s, and three ports were exposed for 60 s. For the second (T₈) and third groups (T₂₄), ports were initially exposed to UV for 10 s (as the first step in the maintenance regime), covered and incubated at 37°C. Eight hours after the initial UV exposure, the T₈ set was exposed to UV, as above, with three ports left untreated to exemplify cell recovery at 8 h. Additionally, three ports were exposed to UV again for 10 s (maintenance), and three ports were exposed for 60 s. For the 24 h maintenance test (T₂₄), three ports were left unexposed to UV after the initial 10 s treatment (24 h recovery). Three ports received a 10 s maintenance UV exposure 8 and 21 h after the initial 10 s exposure, and three ports were exposed for 60 s, 24 h after the initial 10 s exposure. Each group of nine ports was flushed with PBS, plate stamped, and streaked for CFU counts, as described above, upon completion of its UV exposure regime. A CFU count of the inoculum was performed as described above. All plates were incubated overnight at 37°C. CFU counts from each condition were compiled 24 h postinoculation. Bacterial stamp plates were documented using a digital camera. Colony densities were quantified using IMAGEJ software.

Statistical analysis

Differences between groups in multiple-group experiments were assessed with analysis of variance (ANOVA), with Tukey posttesting, performed using Prism 6.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was inferred from $P < 0.05$.

Results

Exposure time

UV light delivered by a 285 nm peak emission light-emitting diode was highly lethal to *Staphylococcus aureus* cultures at a distance of 0.5 cm, as evidenced by the significant decline in the CFU counts between unsterilized ports and all UV exposure time points examined ($P < 0.0001$) when simulated usage flow-through aliquots were cultivated and quantified (Fig. 2a). Small increments in time-exposure greatly enhanced killing, as there was a significant difference between 10 and 20 s UV exposure ($P < 0.01$) and a highly significant difference between 10 and 30 s or beyond ($P < 0.0001$). By 70 s zero-growth on TSA plates suggested the ports were free of cultivable bacteria recovered using either the simulated usage flow-through or plate stamp methodology. As is illustrated in Fig. 2b,c, there was a noticeable decline in bacterial colonies recovered from the port surface with each increase in UV exposure time. Similar to the CFU counts, no bacteria were present at or beyond a 70 s exposure. We concluded that UV light delivered by LED can eliminate both simulated usage flow-through and port surface bacteria in this model; where even brief UV exposure times significantly reduced bacterial viability.

Exposure distance

Bacterial counts from simulated usage flow-through samples in trials testing UV exposure at variable distance produced a nonsignificant difference in average CFU counts from 0.5 to 1.5 cm, a significant difference between 1.5 and 2.5 cm ($P < 0.01$), and no significant difference between 2.5 and 3.5 cm (Fig. 3a). A transition point occurs between 1.5 and 2.5 cm after which the most effective clearance is lost. These results are quantified in Fig. 3a, where a three-fold increase in average CFU counts between these two distances was observed. While change in distance from 0.5 to 1.5 cm increased bacterial survival, it was not before a distance of 2.5 cm that a significant increase in bacterial viability was observed ($P < 0.0001$). With respect to this particular LED, there was little difference in the germicidal capacity from the 0 to 1.5 cm distance from the port surface. Beyond the 1.5 cm distance, increase in exposure time would be necessary to assure reduced bacterial viability. Parallel results were reflected in CFUs recovered from port surfaces (Fig. 3b,c). Interestingly, although the bacterial growth in flow-through was significantly greater at 2.5 cm over 1.5 cm, the growth of retained bacteria on the port was similar following exposure to UV at these distances. While there are only a few colonies present from the 0.5 cm stamps, there is little distinguishable difference between the stamps for the other distances (Fig. 3b). When stamps were varied by UV-exposure time (Fig. 1b), the first apparent drop in visible colonies compared to unsterilized ports was at 20 s. Yet mean CFU count between unsterilized ports and 20 s UV-exposed ports went from 202 to 2.56 CFU, a highly significant change ($P < 0.0001$). The mean CFU count at 1.5 cm is 20.6 CFU, which may be high enough to obscure any difference between the bacterial stamps even there was a significant difference between the mean CFU counts at the 2.5 cm ($P < 0.01$) and 3.5 cm ($P < 0.001$) distances.

Maintenance regime

We tested a regimen of repeated UV dosing to determine whether needleless ports could be kept near-sterile for prolonged periods. Three sets of experiments were performed where each exposure was set at a fixed 0.5 cm distance (Fig. 4a). The initial exposure (T_0) served as an internal control and examined bacterial cell viability after either 10 or 60 s UV exposure times. An 8 h maintenance regimen (T_8) included an initial nonlethal 10 s UV exposure followed by an 8 h recovery period. After 8 h, ports were either left untreated or exposed to UV for an additional 10 or 60 s. A 24 h maintenance regimen (T_{24}) included an initial nonlethal 10 s UV exposure followed by a 24 h recovery period after which control samples were cultivated on media. One set of ports was treated with 10 s UV exposures 8 and 21 h after the initial treatment and cultivated at the 24 h time-point. A third set of ports received the initial nonlethal 10 s UV exposure and was left to recover for 24 h after which they were UV-exposed for 60 s. Simulated usage flow-through was collected and cultivated and each port was stamped on agar plates to determine viability.

As expected, at T_0 there was a significant difference between the mean CFU recovered from the nonexposed ports in comparison to the 10 and 60 s UV exposure ports ($P < 0.0001$) (Fig. 4b). There was also a significant difference between the 10 and 60 s UV exposures at T_0 ($P < 0.01$). Eight hours after the initial 10 s UV exposure, simulated usage flow-through from untreated ports and those treated a second time for 10 s recovered $<10\%$ of mean CFUs that were observed in the initial 10 s UV exposure observed with the 10 s treatment at T_0 (Fig. 4b,c). Although the mean CFU counts observed from the ports exposed for the additional 10 s was half that of the untreated ports, the difference between the two was not statistically significant. There was a significant difference in mean CFU between 8 h-recovered ports when untreated and the 60 s UV-exposed ports ($P < 0.01$) were compared (Fig. 4c). After 24 h, simulated usage flow-through from ports that received no further treatment yielded twice the mean CFUs as those recovered for 8 h (Fig. 4c,d). However, counts were only 10% of the CFU recovered after the initial 10 s UV exposure at T_0 (Fig. 4b). No CFUs were observed from ports that had received either a series of 10 s UV exposures over the 24 h span or a single 60 s treatment at the 24 h endpoint. The results were significant in both cases ($P < 0.01$), suggesting that after an incidental contamination event, a series of short maintenance UV exposures is as effective at clearing the bacterial contamination as a single extended exposure. The bacterial stamps on TSA plates further illustrate this point (Fig. 4e–h).

Discussion

CLABSI are a serious health concern worldwide. They are responsible for increased health care costs per patient, extended hospital stays, and patient mortality. Needleless connectors are thought to be a source of CLABSI. The standard protocol to prevent port derived CLABSI is to ‘scrub the hub’ with an isopropyl alcohol impregnated wipe. However, studies suggest that this may not be performed consistently and that the practice may be less effective than other methods of reducing contamination of needleless ports (Karchmer *et al.* 2005; Hadaway 2012). Furthermore, because ports may be accessed nearly three dozen times per day, the alcohol-wipe practice imposes demand on healthcare worker’s time.

We therefore hypothesized that automated delivery of UV using a low-power LED could effectively reduce port contamination. The effectiveness of UV is well known and it is widely used for sterilization (Maclean *et al.* 2009). However, ultraviolet-emitting LEDs represent relatively new technology and have not been tested in this context. In our study, UV delivered by a low-power LED greatly reduced bacterial contamination on needleless port surfaces and in simulated usage flow-through, both avenues for CLABSI initiation. We chose *Staphylococcus aureus* as a representative model not only for its nosocomial infection potential, but also importantly for its Gram-positive cell wall architecture which is more rigid and less permeable to environmental stimuli than other groups of bacteria (Bukhari *et al.* 2014). Coagulase-negative *Staphylococcus* is the most frequent organism associated with CLABSI, followed by *S. aureus* and gram-negative bacteria. All of these organisms have similar susceptibility to UV (Kowalski 2009), suggesting that the proposed use of UV LEDs may be applicable to the broad category of CLABSI-causative organisms.

Under the experimental conditions examined, not only was a 60 s UV exposure effective in significantly reducing bacterial contamination, but additionally, a series of short 10 s UV exposures were equally as effective over extended times (Fig. 4d). Furthermore, any bacteria remaining viable after an initial 10 s UV exposure would be severely compromised, as indicated by the low CFU averages on ports left untreated after both 8 and 24 h of recovery (Fig. 4c,d). This is exemplified by a minimal expansion of bacteria on the port surfaces even after incubation at elevated temperature (37°C) during the recovery period in these experiments.

There have been a number of technologies designed to prevent CLABSI, with the majority focusing on the CVC itself (Crnich and Maki 2002a,b). Recent innovations include several directed at contamination of ports (Edmiston and Markina 2010; Maki 2010). In this study, we examined the bactericidal capacity of a low-power UV LED. A current limitation to the use of UV LEDs is that they are expensive, with the cost increasing as the wavelength decreases. While a 260 nm diode might be considered ideal from a bactericidal standpoint, it also costs 35–45% more than longer wavelength UV LEDs. Any medical device must meet stringent cost limitations so that it is practical as well as effective. To this end, we sought a balance between cost and bactericidal action. We elected to use a 285 nm LED in a laboratory simulation of an automated UV delivery device designed to clear bacterial contamination from ports. Our work suggests that low-power UV LEDs represent a viable option for disinfection of ports from an effective distance ranging 0.5–1.5 cm from the port surface. We estimate that total power requirement would be within the range of existing power sources (i.e. batteries) creating an effective and viable option for maintenance of port surfaces.

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Figure 1.

Experimental setup. (a) Images of the device fabricated for the study. Dimensions are depicted in white. Range of exposure distance between light-emitting diodes (LED) and port interface is shown in hatched box. (b) Cross-sectional diagram of the device connected to port. (c) Diagram of the simulated usage flow-through and plate stamp cultivation methods used in the study.

Figure 2.

Survival vs ultraviolet (UV) exposure. (a) Average CFU count per ml recovered from ports after timed UV exposure. (b) Plate stamp cultivation of port surface after simulated usage flow-through. (c) Semi-quantitative representation of viable biofilm remaining on the port surface. *Depicts port that did not transfer material to plate but contained bacteria in the simulated usage flow-through. (**) denotes $P < 0.01$, (****) denotes $P < 0.0001$.

Figure 3. Survival vs Distance. (a) Average CFU count per ml recovered from ports after 40 s ultraviolet (UV) exposure at varying distances between port surface and UV light-emitting diodes (LED). (b) Plate stamp cultivation of port surface after simulated usage flow-through. (c) Semi-quantitative representation of viable biofilm remaining on the port surface. (**) denotes $P < 0.01$, (****) denotes $P < 0.0001$.

Figure 4.

Maintenance protocol. (a) Diagrams of three experimental groups (T₀, T₈, T₂₄) outlining ultraviolet (UV) exposure (white bars) vs recovery time (black bars). Samples were processed for simulated usage flow-through and plate stamp cultivation at endpoints indicated with (S). (b) Average CFU count per ml recovered from ports under T₀ conditions, (c) T₈ conditions, (d) T₂₄ conditions. (e–g) Plate stamp cultivation of port surface after simulated usage flow-through. (h) Semi-quantitative representation of viable biofilm remaining on the port surface. (**) denotes $P < 0.01$, (****) denotes $P < 0.0001$, ns = not significant.