Efficacy of Antimicrobial 405 nm Blue-light for Inactivation of Airborne Bacteria

Laura R. Dougall*[a], John G. Anderson[a], Igor V. Timoshkin[a], Scott J. MacGregor[a], Michelle Maclean[a, b]

[a] The Robertson Trust Laboratory for Electronic Sterilization Technologies, Department of Electronic & Electrical Engineering, University of Strathclyde, 204 George Street, Glasgow, UK, G1 1XW; [b] Department of Biomedical Engineering, University of Strathclyde, 106 Rottenrow, Glasgow, UK, G4 0NW.

ABSTRACT

Airborne transmission of infectious organisms is a considerable concern within the healthcare environment. A number of novel methods for 'whole room' decontamination, including antimicrobial 405 nm blue light, are being developed. To date, research has focused on its effects against surface-deposited contamination; however, it is important to also establish its efficacy against airborne bacteria. This study demonstrates evidence of the dose-response kinetics of airborne bacterial contamination when exposed to 405 nm light and compares bacterial susceptibility when exposed in three different media: air, liquid and surfaces. Bacterial aerosols of *Staphylococcus epidermidis*, generated using a 6-Jet Collison nebulizer, were introduced into an aerosol suspension chamber. Aerosolized bacteria were exposed to increasing doses of 405 nm light, and air samples were extracted from the chamber using a BioSampler liquid impinger, with viability analysed using pour-plate culture. Results have demonstrated successful aerosol inactivation, with a 99.1% reduction achieved with a 30 minute exposure to high irradiance (22 $mWcm^{-2}$) 405 nm light (P=0.001). Comparison to liquid and surface exposures proved bacteria to be 3-4 times more susceptible to 405 nm light inactivation when in aerosol form. Overall, results have provided fundamental evidence of the susceptibility of bacterial aerosols to antimicrobial 405 nm light treatment, which offers benefits in terms of increased safety for human exposure, and eradication of microbes regardless of antibiotic resistance. Such benefits provide advantages for a number of applications including 'whole room' environmental decontamination, in which reducing levels of airborne bacteria should reduce the number of infections arising from airborne contamination.

Keywords: 405 nm light, airborne transmission, air disinfection, environmental decontamination, antimicrobial, healthcare, violet-blue light, LED

1. INTRODUCTION

The global challenge of antibiotic resistance is well documented and ever-increasing. The more prominent likelihood of returning to a pre-antibiotic era has become a driving force for the need to develop novel methods of infection control to combat emerging microbial threats. In the healthcare environment, nosocomial infections are a critical concern, both in terms of patient wellbeing and economic burden[1]. Whilst traditional infection control methods such as good hand hygiene and manual disinfection should not be overlooked, it is estimated that up to 50% of high touch areas within patient settings are often missed, prompting the need for a different approach to disinfection technologies [2].

One such method is the novel light-based disinfection technology of visible light in the region of 400-420 nm, with optimal antimicrobial efficacy at 405 nm[3]. 405 nm light has wide antimicrobial properties against an extensive array of microorganisms at exposure levels that are non-detrimental to mammalian cells[4-8]. As a result, this violet-blue light

* Laura.dougall@strath.ac.uk; phone +44 141 548 2376

technology is being developed for clinical applications, including wound decontamination[9-11] and continuous environmental decontamination for use in patient-occupied environments[12-14].

With regards to environmental decontamination, in general, traditional cleaning focuses on reducing surface contamination, with little implemented to improve air quality, other than the use of HEPA filtration in critical areas. However, the airborne transmission of infectious microorganism is a major issue, with 10-33% of all hospital acquired infections estimated to spread via the air[15]. Airborne transmission is a route of many serious infectious organisms such as Influenza, clinically-relevant MRSA, and the highly contagious *Mycobacterium tuberculosis* and *Bacillus anthracis* (the respective causes of TB and anthrax). Microorganisms originating from the human respiratory tract can become airborne by coughing, sneezing or exhaling and can remain suspended in the air for prolonged periods of time, sometimes indefinitely[16, 17]. Infectious droplets have the ability to travel long distances on air currents, and thus can be easily dispersed throughout hospital buildings. Novel methods of whole-room environmental decontamination attempt to target both air and surfaces, and focus primarily on the use of gaseous disinfection (e.g. use of hydrogen peroxide vapor) and ultraviolet (UV) light. Although highly antimicrobial, safety restrictions of UV light and chemical disinfection limit their application to unoccupied environments, as a result of their mutagenic/toxic nature and ability to cause degradation of materials. 405 nm light presents a technique that can be employed for environmental decontamination in the presence of room occupants without the related safety issues associated with chemical and UV-based strategies.

There is a growing body of evidence available which demonstrates the efficacy of 405 nm light for decontamination of surface-deposited or liquid-suspended organisms, however efficacy against airborne contamination is yet to be fully established, with only circumstantial evidence reflecting its germicidal activity against airborne microorganisms. As a result, the aim of the present study was to establish the dose-response kinetics of laboratory-generated bacterial aerosols upon exposure to 405 nm light, and compare bacterial susceptibility when exposed in air or liquid and on surfaces.

2. SYSTEM DESIGN & METHODOLOGY

2.1 Microbiological Preparation

The bacterial strain used throughout this study was *Staphylococcus epidermidis* LMG 10273 (Laboratorium voor Microbologie, Universiteit Gent, Belgium). For experimental use, *S. epidermidis* was cultured in 100 mL Tryptone Soya Broth (Oxoid Ltd, UK) at 37°C under rotary conditions (120 rpm) for 18-24 hours. Post incubation the bacterial suspension was centrifuged at 3939 × g for 10 minutes and the pellet re-suspended in 100 mL phosphate buffered saline (PBS; Oxoid Ltd, UK), and serially diluted to obtain the required cell density (colony forming units per millilitre, CFU mL^{-1}) for experimental use.

2.2 405 nm Light Source

The light source used in this study was an ENFIS PhotonStar Innovate Uno 24 405 nm light-emitting diode (LED) array (PhotonStar Technologies, UK), consisting of 24 LEDs with peak output at approximately 405 nm as shown in Figure 1. The LED array was bonded to a heat sink and fan for thermal management.

Irradiance was measured using a photodiode detector (Model 1Z02413; Ophir) and radiant optical power meter (Model-70260; Oriel Instruments) calibrated at 405 nm. Throughout the study, the irradiance of 405 nm light was set at approximately 22 mW cm^{-2}. To calculate the dose of light exposure (J cm^{-2}) using the measured irradiance and exposure times, the following calculation was used:

$$\text{Dose (J cm}^{-2}\text{)} = \text{Irradiance (W cm}^{-2}\text{)} \times \text{time (seconds)}$$

* Laura.dougall@strath.ac.uk; phone +44 141 548 2376

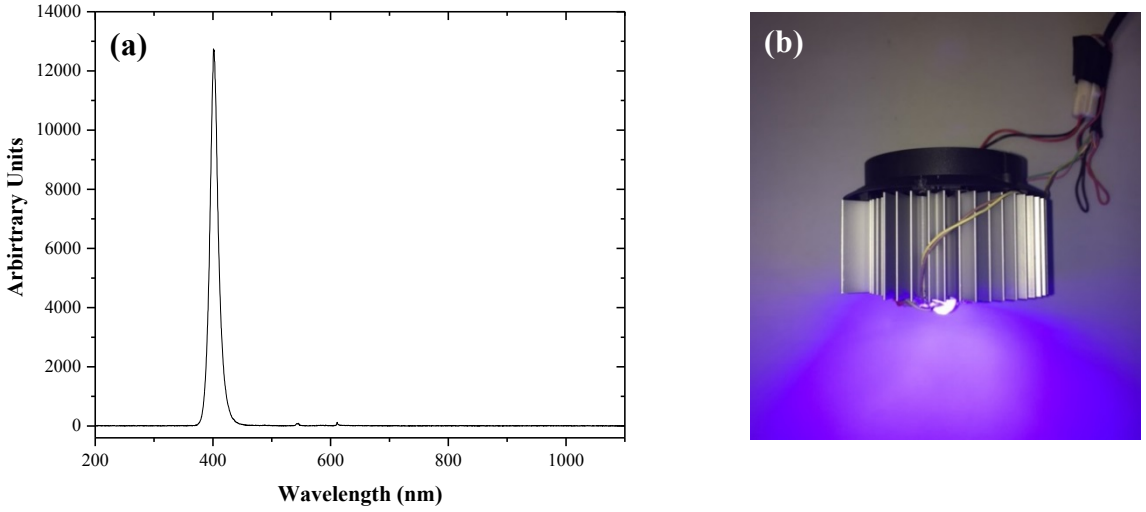


Figure 1. Optical emission spectrum of the 405 nm LED array, measured using a HR4000 spectrometer (Ocean Optics, Germany) and Spectra Suite Software (A) and 405 nm LED array attached to heatsink and fan (B).

2.3 405 nm Light Exposure of Bacteria in Air

The aerosol suspension chamber, shown in Figure 2, was a 15 L cylindrical acrylic tube with a diameter of 24 cm and height of 35 cm. The chamber lid contained a glass window with a 13 cm diameter, to permit light transmission from the top-mounted LED array. Four additional LED arrays were mounted onto the outer walls of the chamber. Irradiance was measured at multiple points inside the aerosol chamber, with the average irradiance calculated to be approximately 22 mW cm^{-2}. Aluminium panels were fitted to the chamber exterior to aid internal light reflection and to prevent stray light exposure through the chamber walls. An inlet for the nebulizer and outlet for the BioSampler were also present and were sealed with HEPA vents during exposure. A small fan was attached to the internal base of the chamber to aid aerosol circulation.

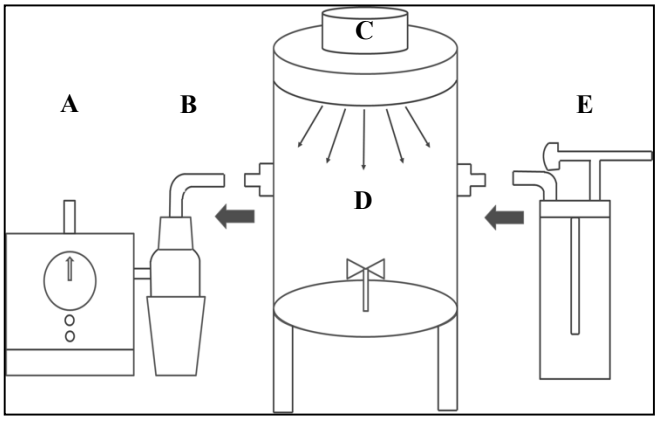


Figure 2. Schematic diagram of the aerosol chamber for exposure of airborne bacteria to 405 nm light. A= vacuum pump, B= BioSampler liquid impinger, C= top-mounted light source, D= aerosol chamber and E= nebulizer. During operation, the aerosol chamber (D) had 4 additional light sources mounted around the exterior, and was shielded with aluminium panels to aid internal light reflection.

* Laura.dougall@strath.ac.uk; phone +44 141 548 2376

Bacterial aerosols were generated using a 6-Jet Collison Nebulizer (BGI Inc., USA). 60 mL of the bacterial suspension at 10^6 CFU mL^{-1} starting population was added to the nebulizer and operated at 20 psi for 1 minute to introduce airborne bacteria into the aerosol chamber. The chamber inlet was then sealed with HEPA vents and the light sources were switched on for a specified time period. Post-exposure, air samples were extracted from the test chamber using a BioSampler liquid impinger (SKC, UK). A vacuum pump connected to a glass collection vessel was operated at 12.5 L min^{-1} for 5 minutes to remove particle-laden air from the chamber and collect it into 20 mL of PBS inside the collection vessel. Samples were serially diluted and plated in triplicate onto Trytone Soya Agar (TSA; Oxoid Ltd, UK) using the Pour Plate Method and incubated at 37°C for 18-24 hours. Non-exposed control samples were subject to identical conditions, but non-light exposed. Post-incubation, samples were enumerated and results reported as CFU mL^{-1}. Experiments were repeated in triplicate.

2.4 405 nm Light Exposure of Bacteria in Liquid

For liquid exposures, the bacterial suspension was serially diluted to a starting population of approximately 10^6 CFU mL^{-1}. A 3 mL volume of the bacterial suspension was transferred to a single well of a 12-well plate. The well was positioned 11.5 cm below the LED array giving an irradiance of approximately 22 mW cm^{-2}. Bacterial suspensions were exposed to the light source for increasing exposure times, resulting in increasing doses of light. Control samples were subject to identical conditions, but exposed to normal laboratory lighting conditions only. Exposed and control samples were plated onto TSA using an automated spiral plater (Don Whitley Scientific, UK). Samples were incubated at 37°C for 18-24 hours and then enumerated, and viable bacterial counts recorded as CFU mL^{-1}. Experiments were repeated in triplicate.

2.5 405 nm Light Exposure of Bacteria on Surfaces

For surface exposures, the bacterial suspension was serially diluted to a starting population of approximately 10^7 CFU mL^{-1}. A 100 µL volume of the bacterial suspension was pipetted onto the surface of a 90 mm diameter TSA plate, and evenly distributed using an L-shaped spreader to provide a starting bacterial concentration of approximately 10^6 CFU/plate. The agar plate was positioned directly under the LED array, at a distance of 11.5 cm, giving an approximate irradiance of 22 mWcm^{-2} at the centre of the plate. Surface-seeded bacteria were exposed to the light source for increasing exposure times. Exposed and non-exposed control plates were then incubated at 37°C for 18-24 hours. Post incubation, plates were photographed for qualitative analysis and then enumerated, where possible, for quantitative analysis, with viable bacterial counts recorded as CFU/plate. Experiments were repeated in triplicate.

2.6 Data & Statistical Analysis

Experimental data points are presented as percentage surviving bacterial population, compared to the equivalent control population and are an average of a minimum of triplicate independent experimental results, measured in duplicate ($n \geq 6$) or triplicate ($n \geq 9$). Error bars represent the calculated standard deviation (SD) of the relevant data-set. Data were analysed using two sample T-tests using Minitab Statistical Software Version 17, with significant differences identified at the 95% confidence interval, $P \leq 0.05$.

* Laura.dougall@strath.ac.uk; phone +44 141 548 2376

3. EXPERIMENTAL RESULTS

3.1 405 nm Light Exposure of Bacteria in Air

Results in Figure 3 demonstrate the susceptibility of aerosolized *S. epidermidis* to 405 nm light exposure. Significant bacterial inactivation was achieved after an initial dose of 6.6 J cm^{-2}, equating to an exposure time of 5 minutes. At this dose, a 36.5% reduction was achieved, when compared to the equivalent non-exposed control sample ($P<0.001$). After a dose of 39.5 J cm^{-2} (30 minute exposure), a significant 99.1% bacterial reduction was observed, with less than 1% of the starting bacterial population surviving at this dose ($P=0.001$).

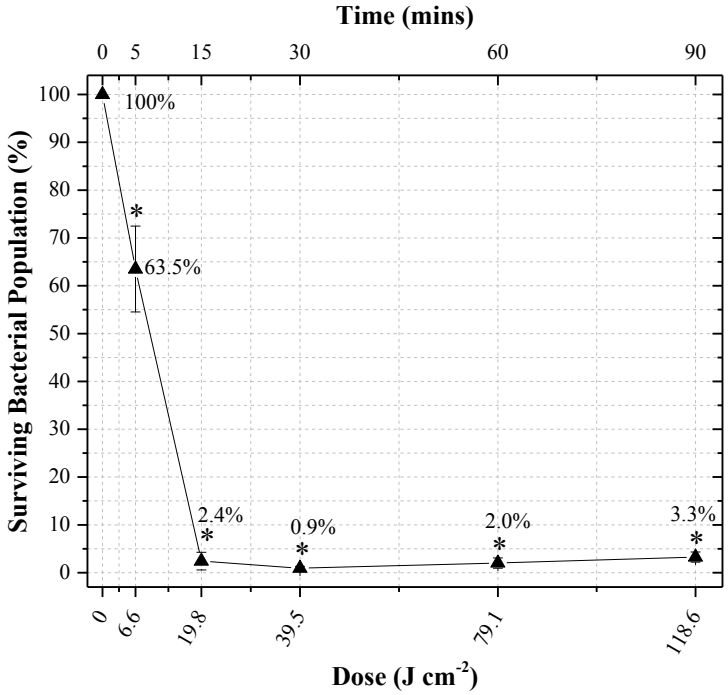


Figure 3. Inactivation of aerosolized *S. epidermidis* by exposure to 405 nm light at an irradiance of 22 $mWcm^{-2}$. Surviving bacterial populations are calculated as a percentage of the equivalent non-exposed control population. * represent significant bacterial inactivation when compared to the associated non-exposed control samples ($P<0.05$). Each data point is a mean value ± SD (n=9).

Tailing was observed after exposure times of greater than 30 minutes. It can be seen in Figure 3 that percentage reduction started to decrease slightly at longer exposure times, displaying a slight upward trend. This was a result of the natural decay of the suspended bacterial aerosol over extended time periods in both exposed and non-exposed test scenarios, however, this reduction was significantly less than the reduction achieved in the presence of 405 nm light exposure.

3.2 405 nm Light Exposure of Bacteria in Liquid

The inactivation kinetics of bacteria exposed to 405 nm light when suspended in liquid were also investigated. A significant bacterial reduction of 31.2% was achieved after a dose of 19.8 J cm^{-2}, an exposure time of 15 minutes ($P<0.001$), as shown in Figure 4.

* Laura.dougall@strath.ac.uk; phone +44 141 548 2376

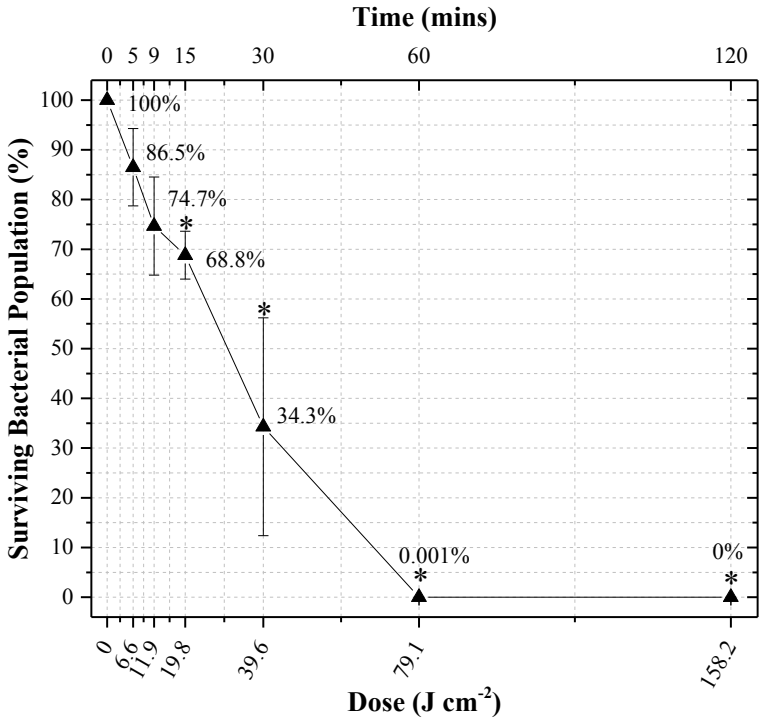
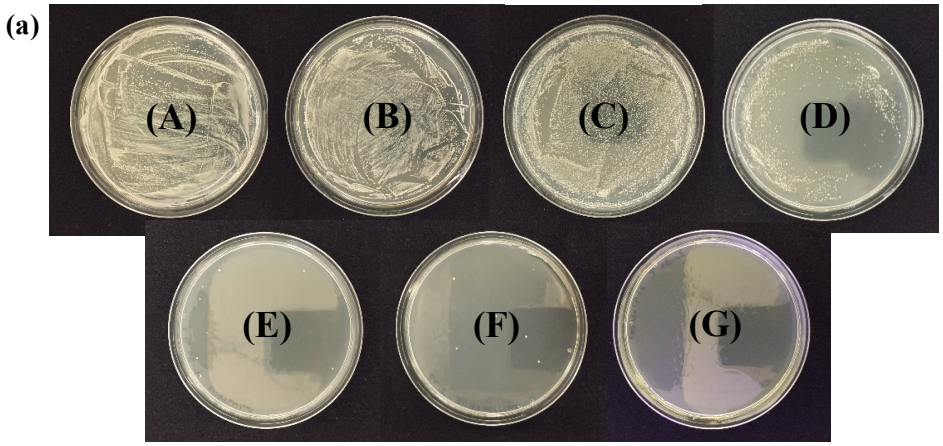


Figure 4. Inactivation of liquid-suspended *S. epidermidis* by exposure to 405 nm light at an irradiance of 22 $mWcm^{-2}$. Surviving bacterial populations are calculated as a percentage of the equivalent non-exposed control population. * represent significant bacterial inactivation when compared to the associated non-exposed control samples ($P<0.05$). Each data point is a mean value ± SD (n=6).

Overall, the results show a downward trend with a 6.4-log_{10} reduction achieved after 158.2 J cm^{-2} of 405 nm light exposure ($P<0.001$).

3.3 405 nm Light Exposure of Bacteria on Surfaces

In order to fully characterize the susceptibly of airborne bacteria to 405 nm light inactivation, a further comparison of inactivation kinetics was conducted on surfaces.



* Laura.dougall@strath.ac.uk; phone +44 141 548 2376

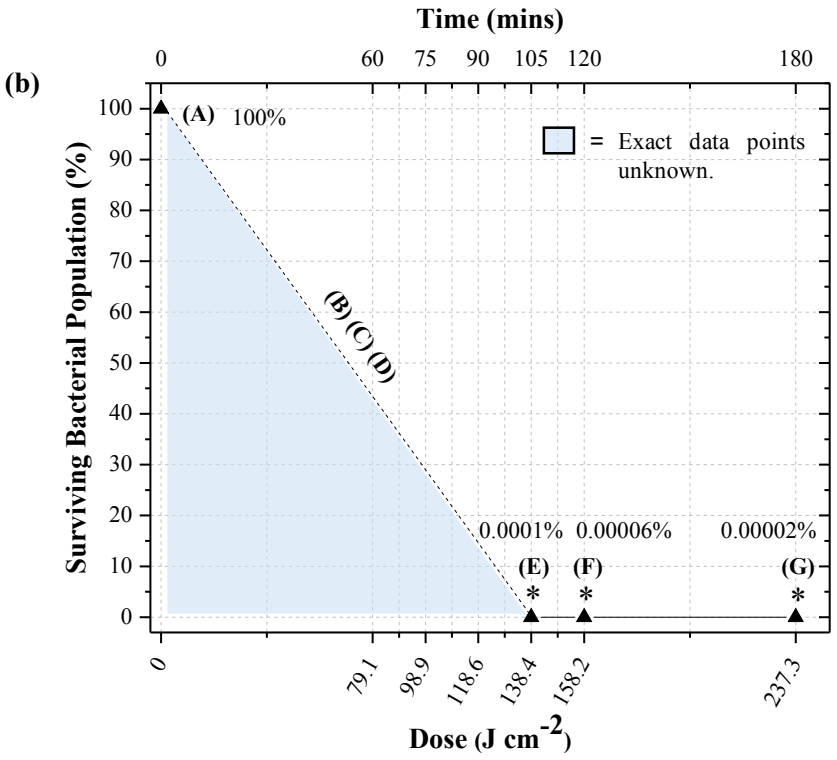


Figure 5. Inactivation of surface-seeded *S. epidermidis* exposed to 405 nm light at an irradiance of 22 mWcm^{-2}. (A) Qualitative results, showing agar plates seeded with bacteria and exposed to increasing doses of light treatment: A = 0 J cm^{-2}; B = 79.1 J cm^{-2}; C = 98.9 J cm^{-2}; D = 118.6 J cm^{-2}; E = 138.4 J cm^{-2}; F = 158.2 J cm^{-2}; G = 237.3 J cm^{-2}. (B) Quantitative results showing surviving bacterial populations, which were calculated as a percentage of the equivalent non-exposed control population. * represent significant bacterial inactivation when compared to associated non-exposed control samples. Each data point is a mean value ± SD (n=3).

Due to the high starting populations used in the experiments, both qualitative and quantitative data were necessary for evaluation of the inactivation efficacy on surfaces. Figure 5(A) shows the decrease in bacterial survival upon increasing light dose (A = 0 J cm^{-2} (starting population of 10^6 CFU/plate); B = 79.1 J cm^{-2}; C = 98.9 J cm^{-2}; D =118.6 J cm^{-2}; E = 138.4 J cm^{-2}; F = 158.2 J cm^{-2}; G = 237.3 J cm^{-2}), with near complete inactivation achieved (<10 CFU remaining) after exposure to 138.4 Jcm^{-2}. Figure 5(B) presents quantitative detail of the inactivation of *S. epidermidis* exposed to 405 nm light on agar plate surfaces, with only 0.0001% of the starting population surviving after 138.4 J cm^{-2} ($P<0.001$). Subsequent doses achieved similar levels of inactivation with a 6.6-log$_{10}$ reduction achieved after the maximum dose of 237.3 J cm^{-2} ($P<0.001$). Due to the high population density used, quantitative data was only available for doses of 138.4 J cm^{-2} and above (the starting population was calculated by serial dilution of the prepared bacterial suspension), therefore for graphical purposes, the shaded area in Figure 5(B) represents the fact that at doses of 79.1 J cm^{-2}, 98.9 J cm^{-2} and 118.6 J cm^{-2}, bacterial colonies on the agar surface were too numerous to count (as shown in Fig 5A), and the trend line is just an estimate of the downward trend. It is likely that a statistically significant degree of inactivation was achieved at point (D), 118.6 J cm^{-2}, however although visibly reduced, the surviving population was still too concentrated to obtain an accurate count.

* Laura.dougall@strath.ac.uk; phone +44 141 548 2376

3.4 Comparison of Inactivation Kinetics in Different Media

The inactivation kinetics of *S. epidermidis* in air, liquid suspension and on surfaces are compared in Figure 6.

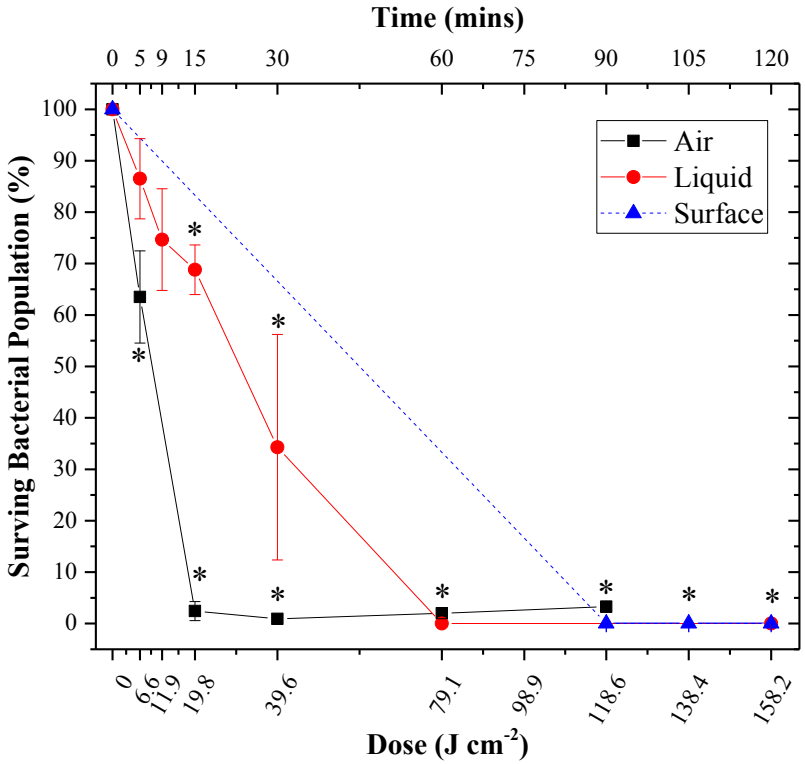


Figure 6. Comparison of the bacterial inactivation kinetics of *S. epidermidis* when exposed to 405 nm light (at an approximate irradiance of 22 mW cm^{-2}) in air, suspended in liquid and seeded onto surfaces.

In each case, a significant downward trend in surviving bacterial population was achieved, however the inactivation kinetics in response to 405 nm light exposure varied between each medium. When suspended as an aerosol, *S. epidermidis* was found to be most susceptible to inactivation, with approximately 4 times less dose required for a similar reduction compared to when in liquid; >97% reduction after 19.8 J cm^{-2} in air compared to >99% reduction after 79.1 J cm^{-2} when in liquid.

4. DISCUSSION AND CONCLUSIONS

This study has successfully demonstrated, for the first time, the efficacy of 405 nm violet-blue light technology for inactivation of airborne bacteria, and has additionally highlighted the significantly increased susceptibility when aerosolized, in comparison to when suspended in liquid or when exposed on surfaces.

Liquid-suspended bacteria required a dose 3-4 times greater than that of airborne bacteria to achieve an initial significant degree of inactivation. It is also worth indicating that after 15 minutes of light exposure, only 2.4% of airborne bacteria remained. In contrast, 68.8% of liquid-suspended bacteria survived at this dose, suggesting that bacteria are more susceptible to 405 nm light inactivation when suspended in the air. Aerosol research is challenging due to the variable

* Laura.dougall@strath.ac.uk; phone +44 141 548 2376

nature of experimental results, and the natural decay of artificially suspended organisms. In order to ensure data reliability, independent air experiments were carried out a minimum of three times, with triplicate samples plated for each, providing a sample size of 9 for each data point. The data obtained represents the percentage decrease between non-exposed control samples and exposed test samples. As a result, the efficient inactivation of airborne microorganisms achieved in this study can be accurately and confidently attributable to 405 nm light exposure alone.

As mentioned, natural decay of the suspended aerosolized bacteria was observed, and it is likely that this is unavoidable due to gravitational settling of airborne particles. Other potential causes include desiccation, impaction with the chamber walls and undesirable temperature and humidity conditions. An aerosol study by King *et al* (2011), experienced a 10% loss of viable bacteria inside their test chamber after only 170-330 seconds[18]. Microbiological particle aging studies have been conducted inside rotating aerosol chambers, with prolonged particle suspension achieved by altering the environmental conditions inside a rotating aerosol chamber[19]. This may provide a direction for potential future work.

The aerosol state is considered a harsh environment for microorganisms, and this may explain the high degree of susceptibility of airborne *S. epidermidis* to 405 nm light. At a dose of 19.8 J cm^{-2}, a 97.6% and 31.2% reduction in bacterial population was achieved in air and liquid, respectively. Results with the chamber used in the present study found the optimum antimicrobial dose to be 39.5 J cm^{-2}, equivalent to 30 minutes of exposure. At this dose, a 99.1% reduction was observed. All subsequent doses resulted in a slight decrease in germicidal efficiency, however this is an artefact of the natural decay experienced with both the control and test aerosol populations affecting the calculated percentage reductions. Complete inactivation was not observed in this study, and a possible explanation for this is bacterial clustering. If numerous bacteria clump together, those in the centre of the cluster will be shielded from light exposure, and thus, appear to be resistant. Kesevan and Sagripanti (2012) stated that if one bacterium was shielded by two others, then only 37% of the irradiated light would reach that third bacterium[20]. Although the authors' findings relate to UV-irradiated bacteria, it is highly likely that a similar phenomenon would occur for visible light exposure.

Although 405 nm light has previously been shown to be effective against a wide array of microorganism suspended in liquid, it was important to establish independent results in this study, and in order to provide a direct comparison between air and liquid inactivation kinetics, the same bacterial strain and irradiance level of light were used. It is generally considered that bacterial inactivation kinetics in liquid do not predict that in air, and this was also evidenced in the present study, where in order to achieve similar percentage reduction, liquid suspended *S. epidermidis* required 3-4 times the dose of airborne *S. epidermidis*.

Surface exposures were also conducted for comparison however, in order to utilize similar population densities to those used in the aerosol and liquid suspension tests, both qualitative and quantitative data was required. Figure 5(A) provides a visual representation of the inactivation of surface-seeded *S. epidermidis* upon exposure to increasing doses of 405 nm light, and utilizing the first quantifiable result, it can be seen that approximately 6-times the dose was required to achieve similar, near complete, inactivation when compared to suspension in air. The reduced rate of inactivation on surfaces compared to air may be a result of shielding, with the 10^6 CFU population on the plate surface likely to be in multiple layers therefore meaning some cells will have a degree of shielding from the light photons. It is also the case that bacteria located on a moist agar surface or suspended in liquid are protected from rapid desiccation which can occur when suspended in air.

Much research has been conducted to assess the antimicrobial efficacy of 405 nm light, with the majority of work using liquid suspensions or seeded surfaces, therefore the data of this study has expanded this by providing quantitative evidence of its efficacy for inactivation of airborne microbes. It was important to draw a comparison between air and surface exposures of 405 nm light, due to its developing use as a method of environmental decontamination. This technology has been shown to successfully reduce levels of environmental contamination in various clinical environments, with studies in a Burns Unit isolation room achieving bacterial reductions of up to 86%, over and above standard cleaning and infection control procedures[12]. The results of these previous clinical environmental decontamination studies are based on decreases in the levels of surface bioburden, with up till now, only circumstantial

* Laura.dougall@strath.ac.uk; phone +44 141 548 2376

evidence suggesting its efficacy for air decontamination. The results of the present study now provide the first fundamental evidence of this[21].

Overall, the results of this study provide evidence that airborne bacteria are readily inactivated by exposure to 405 nm light. It has also demonstrated the increased level of susceptibility of bacteria when in the airborne state, compared to other exposure media. These findings enhance the value of 405 nm light technology for environmental decontamination, as they demonstrate its potential to inactivate aerial contamination before it settles onto surfaces. Further work is required to fully characterize the dynamics of airborne microorganisms, and to establish the efficacy of 405nm light against natural airborne contamination within the healthcare setting. Such data is required to more fully understand the contribution that this technology can make to reducing the number of healthcare associated infections arising from both air-borne and surface contamination sources, and thereby positively impacting on patient care within the international healthcare sector.

AKNOWLEDGEMENTS

LRD wishes to thank the Engineering and Physical Sciences Research Council (EPSRC) for their support through a Doctoral Training Grant (Reference: EP/M508159/1). All data are provided in full in the Results section of this article. The authors would like to thank Andy Carlin and Sean Doak for their technical expertise for construction of the experimental aerosol chamber. All authors wish to thank The Robertson Trust for their support.

REFERENCES

[1] National Audit Office, "The management and control of Hospital Acquired Infection in Acute NHS Trusts in England", 17 February 2000, https://www.nao.org.uk/report/the-management-and-control-of-hospital-acquired-infection-in-acute-nhs-trusts-in-england/ (11 December 2017).

[2] Carling, P. C., Briggs, J. L., Perkins, J., and Highlander D., "Improved cleaning of patient rooms using a new targeted method", Clin. Infect. Dis. 42(3), 385-388 (2006).

[3] Maclean, M., MacGregor, S. J., Anderson, J. G., and Woolsey, G., "High-intensity narrow-spectrum light inactivation and wavelength sensitivity of *Staphylococcus aureus*", FEMS Microbiol. Lett. 285(2), 227-232 (2008).

[4] Maclean, M., MacGregor, S. J., Anderson, J. G., and Woolsey, G., "Inactivation of bacterial pathogens following exposure to light from a 405-nanometer light-emitting diode array", Appl. Environ. Microbiol. 75(7), 1932-1937 (2009).

[5] Maclean, M., McKenzie, K., Anderson, J. G., Woolsey, G., and MacGregor, S. J., "405 nm light technology for the inactivation of pathogens and its potential role for environmental disinfection and infection control", J. Hosp. Infect. 88(1), 1-11 (2014).

[6] Murdoch, L. E., McKenzie, K., Maclean, M., MacGregor, S. J., and Anderson, J. G., "Lethal effects of high-intensity violet 405-nm light on *Saccharomyces cerevisiae*, *Candida albicans*, and on dormant and germinating spores of *Aspergillus niger*", Fungal Biol. 117(7-8), 519-527 (2013).

[7] Moorhead, S., Maclean, M., Coia, J. E., MacGregor, S. J., and Anderson, J. G., "Synergistic efficacy of 405 nm light and chlorinated disinfectants for the enhanced decontamination of *Clostridium difficile* spores", Anaerobe. 37(1), 72-77 (2016).

[8] Tomb, R. M., Maclean, M., Coia, J. E., Graham, E., McDonald, M., Atreya, C. D., MacGregor, S. J., and Anderson, J. E., "New proof-of-concept in viral inactivation: virucidal efficacy of 405 nm light against feline calicivirus as a model for norovirus decontamination", Food. Environ. Virol. 9(2), 159-167 (2017).

* Laura.dougall@strath.ac.uk; phone +44 141 548 2376

[9] Dai, T., Gupta, A., Huang, Y. Y., Sherwood, M. E., Murray, C. K., Vrahas, M. S., Kialian, T., and Hamblin, M. R., "Blue light eliminates community-acquired Methicillin-resistant *Staphylococcus aureus* in infected mouse skin abrasions", Photomed Laser Surg. 31(11), 531-538 (2013).

[10] Dai, T., Gupta, A., Huang, Yin, R., Murray, C. K., Vrahas, M. S., Sherwood, M. E., Tegos, G. P., and Hamblin, M. R., "Blue light rescues mice from potentially fatal *Pseudomonas aeruginosa* burn infection: efficacy, safety and mechanism of action", Antimicrob. Agents Chemother. 57(3), 1238-1245 (2013).

[11] Zhang, Y., Zhu, Y., Gupta, A., Huang, Y., Murray, C. K., Vrahas, M. S., Sherwood, M. E., Baer, D. G., Hamblin, M. R., and Dai, T., "Anti-microbial blue light therapy for multidrug-resistant *Acinetobacter baumannii* infection in a mouse burn model: implications for prophylaxis and treatment of combat-related wound infections" J. Infect. Dis. 209(12), 1963-1971 (2014).

[12] Maclean, M., MacGregor, S. J., Anderson, J. G., Woolsey, G., Coia, J. E., Hamilton, K., Taggart, I., Watson, S. B., Thakker, B., and Gettinby, G., "Environmental decontamination of a hospital isolation room using high-intensity narrow-spectrum light", J. Hosp. Infect. 76(30, 247-251 (2010).

[13] Bache, S. E., Maclean, M., MacGregor, S. J., Anderson, J. G., Gettinby, G., Coia, J. E., and Taggart, I., "Clinical studies of the High-intensity Narrow-spectrum light Environmental Decontamination System (HINS-light EDS), for continuous disinfection in the burn unit inpatient and outpatient settings" Burns. 38(1), 69-76 (2012).

[14] Maclean, M., Booth, M. G., Anderson, J. G., MacGregor, S. J., Woolsey, G. A., Coia, J. E., Hamilton, K., and Gettinby, G., "Continuous decontamination of an intensive care isolation room during patient occupancy using 405 nm light technology", J. Infect. Prev. 14(5), 176-181 (2013).

[15] House of Commons Public Accounts Committee, "Reducing healthcare-associated infections in hospitals in England", Fifty-second report of session 2008-9, http://www.publications.parliament.uk/pa/cm200809/cmselect/cmpubacc/812/812.pdf. (15 July 2017).

[16] Fitzgerald, D., and Haas, D.W., "*Mycobacterium tuberculosis*". In: Mandell, G. L., Bennett, J. E. and Dolin, R. Principles and Practice of Infectious diseases. 6th edition. Churchill Livinstone, Philidephia, 2852-2886 (2005).

[17] Beggs, C. B., "Indoor and Built The Airborne Transmission of Infection in Hospital Buildings : Fact or Fiction ?" Indoor. Built. Environ. 12(1-2), 9-18 (2003).

[18] King, B., Kesavan, J., and Sagripanti, J. L., "UV sensitivity of bacteria in aerosols and on contaminated surfaces", Aerosol. Sci. Technol. 45(5), 645-653 (2011).

[19] Verreault, D., Duchaine, C., Marcoux-Voiselle, M., Turgeon, N., and Roy, C. J., "Design of an environmentally controlled rotating chamber for bioaerosol aging studies" Inhal. Toxicol. 26(9), 554-558 (2014).

[20] Kesavan, J., and Sagripanti, J. L., "Disinfection of airborne organisms by ultraviolet C radiation and sunlight", in Aerosol Science: Technology and Applications, I. Colbeck, M. Lazaridis, 1st Ed., West Sussex: John Wiley and Sons Ltd, 417-439 (2012).

[21] Bache, S. E., Maclean, M., Gettinby, G., Anderson, J. G., MacGregor, S. J., and Taggart, I., "Universal decontamination of hospital surfaces in an occupied inpatient room with a continuous 405 nm light source" J. Hosp. Infect. (2017), https://doi.org/10.1016/j.jhin.2017.07.010.

* Laura.dougall@strath.ac.uk; phone +44 141 548 2376