405 nm light technology for the inactivation of pathogens and its potential role for environmental disinfection and infection control

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Running Title: 405nm light environmental disinfection

Summary

Background: Although the germicidal properties of UV light have long been known it is only comparatively recently that the antimicrobial properties of visible violet-blue 405 nm light have been discovered and utilised for environmental disinfection and infection control applications.

Aim*:* To review the antimicrobial properties of 405 nm light and describe its application as an environmental decontamination technology with particular reference to disinfection of the hospital environment.

Methods*:* Extensive literature searches for relevant scientific papers and reports.

Findings*:* A large body of scientific evidence is now available that provides underpinning knowledge of the 405 nm light induced photodynamic inactivation process involved in the destruction of a wide range of prokaryotic and eukaryotic microbial species including resistant forms such as bacterial and fungal spores. For practical application, an environmental disinfection system (HINS-light EDS) has been developed and tested in hospital isolation rooms. The trial results have demonstrated that this 405 nm light system can provide continuous disinfection of air and exposed surfaces in occupied areas of the hospital, thereby substantially enhancing standard cleaning and infection control procedures.

Conclusions*:* Violet-blue light, particularly 405 nm light, has significant antimicrobial properties against a wide range of bacterial and fungal pathogens and, although germicidal efficacy is lower than UV-light, this limitation is offset by its facility for safe continuous use in occupied environments. Promising results on disinfection efficacy have been obtained in hospital trials but the full impact of this technology on reduction of HAI has yet to be determined.

Keywords:

Violet-blue 405 nm light; Hospital acquired infection; Infection control; Pathogens; Environment; Decontamination; Disinfection; Photodynamic inactivation; Air disinfection; Surface disinfection.

Introduction

Although intensive efforts over recent years are making an impact, healthcare-associated infections (HAI) still regularly occur and continue to pose a major challenge. In addition to the significant morbidity and financial costs, concern over contraction of a HAI is one of the greatest fears of patients being admitted to hospital.[1] Infection control procedures such as hand washing are of critical importance in addressing the HAI problem, however greater awareness of the hospital environment as a source of nosocomial pathogens has led to renewed focus on hospital cleaning and disinfection. Whilst effective physical cleaning remains essential for infection control and aesthetic reasons, there has been an upsurge of interest in the development of new cleaning and decontamination technologies.[2,3] A number of these employ novel methods of delivering antimicrobial chemicals, whereas others use the antimicrobial properties of light to enhance disinfection,[4,5,6] and it is this latter approach that forms the topic of this review.

The most germicidal wavelengths of light fall within the ultraviolet (UV) range and UVC (between 240-260 nm) irradiation has traditionally been used for disinfection, particularly for air and medical device decontamination applications.[7,8,9] More recently the antimicrobial properties of violet-blue visible light has emerged as an area of increasing research interest. Although less germicidal than UVC light, violet-blue light with wavelengths in the region of 405 nm, has proved effective for inactivation of a range of microbial species, and exploitation of these wavelengths may provide alternative methods of antimicrobial treatment for infection control applications. This paper will provide a brief background on the use of light for environmental decontamination applications within hospitals before presenting a detailed description of the broad spectrum antimicrobial effects of violet-blue light and how this knowledge has led to the development and clinical evaluation of a 405 nm light environmental disinfection system. In addition to environmental decontamination applications, other potential uses of violet-blue light for infection control proposes such as skin and wound treatment have been highlighted in recent literature but these topics are out with the scope of the current review.[10-17]

Inactivation of microorganisms by light in the hospital environment

Records of observations on the antibacterial effects of light go back to the latter part of the 19th century and these early historical observations have been documented by Kowalski.[18] The germicidal effects of light received further attention during the early part of the 20th century and the appreciation of the decontamination effect of light was translated into early hospital design features where natural ventilation and exposure to sunlight were regarded as beneficial.[19] The roles of sunlight and natural ventilation for controlling the transmission of infections within

healthcare settings has recently been reviewed by Hobday and Dancer, who provide a detailed record of the early – mid 20th century observations on the effects of natural sunlight on a wide range of nosocomial pathogens.[20] Whilst natural light and ventilation were originally considered beneficial, modern hospital design has tended to reduce these features. Recent interest in the application of 'artificial' lighting within hospitals has been with regard to energy reduction issues but also how lighting can affect the mood and circadian rhythm of patients.[21,22] Light from artificial sources with wavelength emission in the UV range, can have significant antimicrobial effects and new technologies for hospital decontamination have been developed around this concept.[6,23-25]

The most widespread applications of ultra-violet germicidal irradiation (UVGI) has been for air and water disinfection, as well as for decontamination of devices.[26-28] More recently, with the increased emphasis that has been directed towards enhanced decontamination of the hospital environment, novel technologies have been developed for the rapid delivery of UVC radiation to exposed surfaces in clinical areas. Several of these are automated or manually positioned robotic systems using either continuous or pulsed UV emission sources.[6,25] Detailed information on UVGI and other 'no-touch' automated room disinfection systems is provided in a recent review by Otter *et al*.[6]

Antimicrobial Effects of Violet-Blue Light

Until relatively recently light within the visible spectrum (400–700 nm) was considered to have little biocidal effect compared to UVC light due to the lower photon energy of these wavelengths. Wavelengths of violet-blue light, particularly around 405 nm, have however been shown to possess antimicrobial capabilities, and there is scope for exploiting these wavelengths for the control of problematic microorganisms in many areas of application including the disinfection of air and exposed surfaces in the clinical environment. The following section provides an overview of the antimicrobial inactivation mechanism, and the antimicrobial efficacy of high-intensity 405 nm violet-blue light.

Violet-Blue Light Inactivation Mechanism

Investigations into the mechanism of action of 405 nm violet-blue light indicate that photodynamic inactivation occurs as a result of the photo-excitation of intracellular porphyrin molecules within the exposed bacterial cells. Laboratory studies have shown that a range of violet-blue light wavelengths in the region 400-425 nm can be used for bacterial inactivation,[29-34] however, optimal antimicrobial activity has been found at 405 nm.[34,35] This peak in activity correlates with the absorption maximum of porphyrin molecules, termed the soret band, being

in this wavelength region.[36] Exposure to light of this wavelength induces an oxygen dependent photo-excitation reaction within exposed microorganisms, where excited porphyrins react with oxygen or cell components to produce reactive oxygen species (ROS) causing oxidative damage and microbial cell death.[29,37-41] Cell death has been accredited to oxidative damage to the cell membrane, with a recent study demonstrating disruption of the cytoplasmic content and cell walls of exposed *S. aureus*,[10] and it is likely that, due to the non-selective nature of ROS, multi-target damage will be induced in the microbial cells.

Antimicrobial Effects of Violet-Blue Light

Extensive laboratory studies have shown that 405 nm light, and the wider violet-blue light wavelengths, have a broad spectrum of activity, with successful inactivation demonstrated for a wide range of organisms, including antibiotic-resistant bacterial strains such as methicillin-resistant *Staphylococcus aureus* (MRSA).[30-32] Bacterial species which have demonstrated susceptibility include HAI-associated organisms, including *Staphylococcus aureus*, *Clostridium difficile*, *Acinetobacter baumanni*, *Escherichia coli, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes* and *Mycobacterium* species.[29-33,42,43] Bacterial sensitivity to violet-blue light inactivation tends to be species dependent, however the general trend suggests that Gram positive bacteria tend to be more susceptible to inactivation than Gram negative species.[32,44]

Two of the most significant pathogens associated with HAI are MRSA and *C. difficile*, and vegetative cells of these species both show susceptibility to violet-blue light inactivation. Vegetative cells of *C. difficile* are particularly sensitive to inactivation, and this is likely to be due to this organism being an obligate anaerobe, giving it increased sensitivity to oxidative damage.[33] *C. difficile* spores are a significant issue for infection control, particularly due to their prolonged survival in the environment, and their resilience to disinfection technologies is well documented.[45-47] *C. difficile* spores can be successfully inactivated by exposure to 405 nm light, however as expected, significantly higher doses (~50 times) are required for inactivation compared to vegetative cells.[33]

Laboratory studies have demonstrated the successful antimicrobial efficacy of violet-blue light for the inactivation of bacterial contamination in liquid,[10,11,29,32,34] artificially seeded on surfaces,[30,31,42,48] and most recently, in biofilms.[44] Within the clinical environment, biofilm formation is a major cross-contamination risk, with the presence of patient fluids such as saliva, blood and urine influencing biofilm adhesion and development on surfaces.[49] Indeed, a recent study attributed the presence of *Pseudomonas aeruginosa* biofilms on sinks to the acquisition of infections, with a 33% death rate.[50]

Although the germicidal efficacy of blue light is lower than that of ultraviolet light – UV inactivation typically required doses of the order of mili-joules rather than joules as is the case with violet-blue light [51,52] – significant bacterial inactivation can still be demonstrated, with up to 9-$\log_{10}$ orders of reduction being achieved in one study.[32] A major advantage of violet-blue light inactivation is that the susceptibility of strains isolated from the clinical environment is similar to their laboratory type strain counterparts i.e. clinical isolates do not show enhanced resistance and thus can be inactivated by 405 nm light with no inherent problems.[32] Also, it has recently been demonstrated that sublethally damaged bacterial cells are more susceptible to light inactivation[48], therefore, there is great potential for bacterial contamination that has been sub-lethally stressed by desiccation and disinfectants during routine cleaning of the hospital environment to be more susceptible to inactivation by exposure to violet-blue light.

In addition to clinically relevant bacteria, the effectiveness of 405 nm light for microbial inactivation has also been demonstrated against bacterial species associated with foodborne infection including *Listeria*, *Campylobacter, Shigella* and *Salmonella* species;[32,34,53] pathogens *Helicobacter pylori, Chlamydia* and *Propionibacterium acnes*;[29,37,43] oral periodontal pathogens;[54,55] and fungal organisms including moulds and yeasts such as *Candida*.[56] To date the effect of violet-blue light on viruses has not been fully determined, however it is expected that due to the hypothesised involvement of porphyrins in the inactivation mechanism, it is unlikely that viruses will be highly susceptible to light exposure alone, and may require the addition of photosensitising material to enhance viricidal activity.[57]

Use of 405 nm Violet-Blue Light for Hospital Disinfection

The wide antimicrobial spectrum of activity combined with the ability to apply light intensities safe for human exposure make violet-blue light ideal for decontamination of occupied environments, and the development of a system which utilises high-intensity narrow spectrum (HINS) 405 nm light for environmental disinfection of the clinical environment has been recently described.[58-60] This new disinfection technology, termed the HINS-light Environmental Decontamination System (EDS) is a ceiling-mounted lighting system designed for the reduction of environmental contamination in hospital wards and other areas of the healthcare environment. The antimicrobial light from the system is generated from a matrix of light-emitting diodes (LEDs) which emit low irradiance violet-blue light with a narrow spectral profile centred on 405 nm.[58] The output of the antimicrobial light has been set to ensure, with reference to international guidelines,[61,62] that the light source does not pose a blue light hazard and is safe for use in occupied environments. Whilst biocidal, the 405 nm wavelengths is well below the blue light wavelengths which can impact on human health, particularly in the region

of 440 nm which is associated with photoretinitis, and 480 nm which is influences mood and circadian rhythm in humans, as shown in Figure 1. Whilst satisfying safety standards as an installed light source it is interesting to also note that, when comparing the susceptibility of mammalian cells and bacteria to 405 nm light, mammalian keratinocytes and osteoblasts were considerably more resistant and could be exposed to bactericidal levels of 405 nm light with no loss of cell viability.[10,11,63] The increased resistance of mammalian cells is likely due to the fact that these cells have much more advanced mechanisms for coping with oxidative damage compared to the more primitive microbial cells.

For practical application as an overhead light source, incorporation of white LEDs into the HINS-light EDS system ensures the illumination output is predominantly white, thus blending with the standard room lighting.[58] The system is designed to be operated continuously, providing on-going disinfection of the air and all exposed environmental surfaces within the treated area, with no disruption to day-to-day hospital procedures or patient care. Laboratory testing of the system confirms the efficacy for inactivation of a range of bacterial pathogens associated with HAI.[64] As mentioned, the low irradiance levels employed by the system were deliberately selected to enable continuous disinfection in occupied environments, and therefore require sufficient time to exert the antimicrobial effect. Significant inactivation of microbial contamination on simulated laboratory surfaces can be achieved by approximately 1-2 h light exposure,[64] however, inactivation kinetics are likely to be significantly enhanced in the 'real' clinical environment due to the stressed and dessicated state of the microorganisms.[48]

Clinical Assessment of 405nm Light for Environmental Disinfection

A number of published studies have presented results from clinical assessment of this 405 nm light system for continuous environmental decontamination of single-bed isolation rooms.[58-60] Evaluation of the technology has been carried out in isolation rooms within two main clinical areas: a Burns Unit and an Intensive Care Unit (ICU).

For evaluation, systems were installed within isolation rooms, and used as a complementary disinfection procedure, being operated continuously during daylight hours in occupied rooms, under conditions where normal clinical care and infection control measures were implemented. The effect of the system was assessed through contact-plate sampling of bacterial levels on a range of frequently touched contact surfaces (e.g. locker top, bed table, bed rails, bin lids, light switches & door handles) which are commonly associated with being 'high-risk' surfaces for cross-transmission of HAIs, as well as surfaces likely to have high contamination levels due to aerial deposition, such as ledges. Samples were typically collected (i) before use, (ii) during use, and (iii) a period after the HINS-light EDS units had been switched off, with the same contact

surfaces sampled throughout each study. Bacterial levels were assessed using 55 mm contact agar plates, with a surface area of 23.76 cm^2, which were inoculated by pressing the agar surface onto the environmental surface. Studies monitored the levels of staphylococcal bacteria (a good indicator of contamination of human origin),[58-60] and the total viable bacteria levels[60] in order to establish the effect of the system for reducing levels of bacterial contamination around the isolation room. For collection of staphylococcal organisms, Baird Parker with egg yolk telurite agar (BPA), a selective medium for the growth of staphylococcal-type organisms, contact plates were used. Tryptone soya agar contact plates (TSA), which use non-selective growth medium, were used to obtain total viable bacterial counts (TVC). Microbiological assessment, as colony forming unit (CFU) counts, was based upon growth on the contact agar plates after incubation at 37°C for 24 hours (TSA plates) or 48-hours (BPA plates).

A number of studies also characterised the staphylococcal isolates by subculturing selected isolates and then testing using Staphaurex Plus (Remel Europe Ltd, Dartford, UK) and PBP2 Latex Agglutination Test (Oxoid Ltd), to identify *S. aureus* and methicillin *S. aureus* isolates, respectively.

Inpatient Studies

An initial study evaluated use of the system for disinfection of an unoccupied isolation room, and results demonstrated a significant 90% reduction (P=0.000) in the staphylococcal contamination on surfaces around the room after 24-hour use.[58] Studies in burns isolation rooms occupied by MRSA positive patients, with treatment periods ranging from 2-7 days, demonstrated that staphylococcal contamination on surfaces around the rooms were significantly reduced by 56 to 86%, over and above the reductions achieved by cleaning alone. Levels of presumptive *S. aureus* and MRSA showed similar reductions.[58] Significantly, once use of the system ceased, recontamination of the room was observed, to levels similar to pre-treatment contamination levels.

An example of the data from one published study is shown in Figure 2, which demonstrates the mean reductions in the total staphylococcal counts and the presumptive *S. aureus* levels in an occupied burns unit isolation room, before, during and after 5-day use of HINS-light EDS. Samples (n=70) were collected twice during each of the three phases, and the results from all sampled surfaces have been pooled to demonstrate the overall decontamination effect the system had across the room. In this study, data demonstrated that a significant 62% decrease in total staphylococcal counts, and 50% decrease in presumptive *S. aureus* was achieved ($P<0.05$) after 5-days use of the system. 'After use' samples, collected during a 6-day period after the system had been turned off, showed that contamination around the room had significantly

risen, with a 126% and 98% increase in the total staphylococci and presumptive *S. aureus* counts, respectively ($P<0.05$), thus reinforcing the recontamination effect that occurs after removal of the light-treatment.[58] Extended use of the system also proved to further reduce the bacterial contamination around the room, supporting the continuous use of this system for maintaining low contamination levels around isolation rooms.[58] Importantly, studies were performed to show that the decontamination effect was not patient or room dependent.[59]

Studies carried out in an ICU isolation room also demonstrated system efficacy, with 60 to 70% reductions in both the staphylococcal and the total bacterial contamination across the entire sampled room environment.[60] In addition to demonstrating an overall reduction in contamination around the room, results demonstrated that exposed surfaces had reduced contamination levels as a result of use of the system, and an example of this is shown in Figure 3. Levels of bacteria on various surfaces around an occupied ICU isolation room were determined before use of the HINS-light EDS, and resampled after a 5-day exposure period. Results demonstrated that despite marked variation in the initial bacterial bioburden there was a marked decrease in levels of bacterial contamination at all tested sites.

In addition to these findings, a significant factor noted in the studies carried out in the ICU isolation room was that despite asymmetrical positioning of the EDS units within the room, results demonstrated that the special distribution of bacterial contamination was reduced almost uniformly across all the sampled contact surfaces. This suggested that disinfection of airborne bacteria contributes to the reductions in bacterial contamination levels, and the installation positions of the systems may not be critical.[60]

Outpatient studies

In addition to its use for disinfection of occupied inpatient isolation rooms, the HINS-light EDS has also proved effective when used in an outpatient clinic.[59] Communal use of outpatient clinic rooms provides a recognised risk of cross-contamination between subsequently treated patients, therefore it is important to maintain cleanliness in these areas throughout the day. Studies carried were carried out to evaluate the environmental bacterial levels at the start and end of 8-hour clinic sessions, with and without use of the EDS. Results demonstrated that a statistically significant 61% efficacy was achieved ($P=0.02$), and these successful results lead to the suggestion that use of this system would be beneficial in other similar communal patient rooms such as the bathroom or physiotherapy room, where decontamination of all surfaces is unachievable between each patient due to time limitations.[59]

Overall, results have been successful, showing evidence that use of 405 nm light achieves significant reductions in bacterial contamination levels around isolation room environments.[58-60] Results also demonstrated that when switched off, the decontamination effect ceases and bacterial contamination levels return to around pre-treatment levels, further confirming the effectiveness of the 405 nm light. It is important to note that these results were achieved under a range of clinical conditions within a busy city hospital environment, and that the bacterial disinfection results obtained were over and above those achieved by the hospital's normal stringent infection control procedures which remained fully in place throughout the study.[58-60] Further studies are still required to establish the effectiveness of 405 nm light for disinfection of larger communal environments.

Comparison of 405 nm Light with Other Environmental Decontamination Systems

The increased awareness of the importance of the hospital environment as a source of nosocomial pathogens has focused attention not only on improving the efficiency of conventional cleaning and disinfection procedures, but has led to the development of a range of novel technologies for enhanced decontamination of whole room environments, including new UV systems (as discussed earlier), steam cleaning, hydrogen peroxide vapour and super-oxidised water fogging.[7,65-67] Although these systems are effective for widespread disinfection of the room environment, they require, for safety reasons, experienced operator supervision and their use is restricted to unoccupied, sealed rooms, thereby resulting in rooms being out-of-commission for periods of time – a consequence which can be costly and undesirable in busy ward areas. Additionally, whilst these systems provide effective decontamination, studies have found that once treatment has finished, there is rapid and widespread recontamination of the room.[68] In addition to human safety considerations, another problem associated with UV-light and chemically-based technologies is the potential for long-term material degradation of furniture and equipment within the treated room if these are repeatedly exposed.[69,70] Therefore these methods are best-suited for terminal- and deep-cleaning procedures, but are ineffective for maintaining low levels of contamination.

Whilst UV irradiation and 405 nm light technology share some similar features they are, in many respects quite distinct technologies both in their modes of action and methods of application (Table I and Fig. 1). Whilst UV light is strongly germicidal it is dangerous to humans and the different UV waveband regions corresponding to UVC, UVB and UVA can cause a wide range of detrimental effects on the human eye and skin.[70] Violet-blue within the visible spectrum can also cause harmful effects at high irradiance levels but these are particularly at

440 nm which can cause photoretinitis,[61,62] and 480 nm which is the peak sensitivity of mammalian photosensitive retinal ganglion cells (pRGCs) which modulate diverse physiological responses to light, including circadian physiology and pupil constriction.[76] A comparison of the biological effects of radiation extending from the UV and into the visible light regions is presented in Figure 1. Whilst 405 nm light is germicidal it falls within a relatively benign wavelength region and if operated at appropriate irradiance levels it is safe for human exposure.[61,62]

The above features explain why the 405 nm light environmental disinfection technology, in comparison with other whole-room decontamination systems including UV technology, can be operated continuously in the presence of patients and staff, thus facilitating a background decontamination effect which maintains low levels of contamination.[58-60] Continuous operation of the 405 nm light system ensures that there is a level of disinfection concurrently being applied even during periods of high activity, such as visiting hours, and bed and bandage changing.[77,78] Whilst disinfectant cleaning and hand hygiene are critical for maintaining a clean environment and minimising the spread of potential pathogens, compliance with hand-washing tends to be low after direct contact with a patient, and significantly, healthcare workers are even less likely to wash their hands after being in contact with the environmental surfaces around the patients, even though these surfaces can be reservoirs of potential pathogens.[79] Use of the 405 nm light technology can strategically augment this by enhancing the low levels of contamination achieved with intermittent cleaning, and also provide decontamination of surfaces within rooms, such as walls and high ledges, as well as delicate equipment, which may not be routinely cleaned using disinfectants. Moreover the system can be automatically operated with no user training required, and consequently problems with staff and patient compliance do not apply.[58-60]

As with all methods of cleaning and disinfection there are inherent disadvantages with any procedure. A limitation of the 405 nm light technology is that, to ensure that patient friendly room illumination conditions are used, relatively low irradiance levels are applied and this impacts on microbial inactivation rates which are inevitably lower than can be achieved with other decontamination technologies albeit only in short term comparisons. The high doses of 405 nm light required for inactivation of endospores means it is unlikely that 405 nm light alone could be realistically applicable for the specific environmental decontamination of *C. difficile* spores, however enhancement of the inactivation may be achieved when combined with other decontamination methods such as oxidative biocides, due to the similar oxidative damage that is exerted on the bacteria by both treatments.[33] In addition to the resilience of spores, the antiviral efficacy of violet-blue light has not been fully established, therefore further research in

this area is required. Also, similar to UVC technology, 405 nm light effectively treats hospital air, but only surfaces that are directly or reflectively exposed to the light are treated, and the effects on occluded or darkly shadowed areas are limited. It is also the case that whilst all of the new technologies including 405 nm light can claim to have demonstrated enhanced disinfection of the hospital environment translation of this potential benefit into a significant reduction in infection rates will be required to ensure the widespread uptake of these new disinfection technologies.

Further commentary regarding the application of 405 nm light for hospital disinfection

Regarding the deployment of the HINS-light system within hospitals, although important issues such as disinfection efficacy and patient safety have been addressed, other questions relating to the use of such a novel light source in clinical settings must also be considered. Undoubtedly enrichment of room lighting with additional violet-blue light will alter the normal lighting effect. This could have some impact on patient and staff comfort levels, and possible effects on medical procedures that involve colour perception must also be considered. In the hospital trials already conducted with the HINS-light EDS no such issues have been problematic (unpublished observations) but monitoring for such effects must remain during uptake of this technology. Further hospital-based studies, funded by the Scottish Infection Research Network and the Chief Scientist Office, are currently being initiated to investigate the acceptability of the technology, and to ensure the technology is optimised with staff and patient comfort fully taken into account. There may conceivably also be implications for colours employed in hospital furnishings and fabrics as these may serve to amplify or supress the reflection or absorption of violet-blue light.

As already discussed, a benefit of 405 nm light over UV-light for disinfection purposes is that, unlike UV-light, 405 nm light, because of its lower photon energy, does not cause photo-degradation of photosensitive materials such as rubbers and plastics used in the hospital environment and equipment.[69] However strong visible light can cause photochemical changes in light-sensitive solutions, and this aspect requires consideration if such solutions were to be exposed for long periods. At the relatively low 405 nm light intensities used[58-60], and considering the fact that light intensity reduces upon transmission through materials e.g. plastic tubing or IV bag material, then this issue is not anticipated to be problematic but nevertheless must remain a consideration if highly light-sensitive pharmaceuticals were introduced.

The HINS-light system utilises LED-based technology and as such it benefits from the well-established characteristics of LED lighting, namely reduced energy requirements, long

operational (lifetime) use, and low maintenance characteristics. In the hospital trials already conducted, the HINS-light EDS unit is designed to be easily retrofitted into the ceiling in place of a ceiling tile. Installed units have remained maintenance-free and fully-operational over the trial period which now extends to several years. From a lighting technology perspective, it is interesting that the introduction of this LED-based disinfection system is concurrent with major potential changes taking place in general lighting technology. Considerable debate is underway regarding the advantages and disadvantages of replacing conventional fluorescent lighting with LED sources, a discussion that is mainly being driven by potential energy efficiency gains associated with LED lighting. Another potential advantage of LED technology is the capacity to blend different colours to 'fine tune' the colour spectrum to suit different environments and applications. In this context it is interesting that it is now appreciated, and as previously discussed in this review, that the nature of the light spectrum can affect circadian rhythmicity, sleep and mood and that this is associated with photosensitive retinal ganglion cells in the eye.[76] Such effects are not only important in the home and workplace but also for patients in the hospital environment, where it has been suggested that more research is required to better understand how lighting in the hospital environment can influence sleep, mood and pain in medical inpatients.[22] Future development of the HINS-light EDS system will undoubtedly be influenced by the various considerations outlined above.

Conclusions

Although the germicidal effects of sunlight and UV-light have been known for well over a century it is only comparatively recently that the antimicrobial properties of visible light in the violet-blue region of the spectrum have been recognised and studied in a number of laboratories. Given the severity of current and anticipated future microbiological problems faced by society, the development of any new antimicrobial weapon is to be welcomed. Violet-blue light, with particular efficacy at 405 nm, has been shown to possess broad spectrum photodynamic antimicrobial activity, and as such its use has been suggested for a range of potential clinical and medical applications.

One such application is the use of 405 nm light for environmental disinfection. The increased safety of 405 nm light wavelengths compared to UV-light, has facilitated development of this light technology for safe continuous disinfection of occupied environments, and results have shown the successful application of this system for environmental disinfection of hospital isolation rooms. This technology termed, the HINS-light EDS, has demonstrated a significant capability for reducing environmental bacterial contamination in clinical patient areas, over and above reductions achieved using the conventional cleaning and infection control strategies

alone. In common with the aspirations of other novel whole room disinfection systems, it is intended that this intervention technology, when used in conjunction with conventional infection control procedures, can help reduce levels of pathogens in the environment, thereby limiting the likelihood of pathogen transmission from the environment to patients, and thus contribute to reducing levels of HAIs.

Whilst violet-blue 405 nm light irradiation represents a new antimicrobial approach, the physical nature of this light source and the limitations of its antimicrobial effects must be understood. Inevitably microbial inactivation rates using 405 nm light are slower than can be achieved with the typical application of many other physical and chemical disinfection and sterilisation treatments. This limitation is however mitigated by its operational facility for continuous application to disinfect air and all illuminated surfaces in occupied environments and by the biochemical mechanism of 405 nm light inactivation. The photodynamic inactivation process induced by 405 nm light exposure involves a multi-targeted intracellular killing effect resulting from the generation of reactive oxygen species, a killing mechanism that is not conducive to microbial resistance development. Given these unique features, it is evident that 405 nm violet-blue light technology represents a novel antimicrobial approach that hopefully can make some contribution to tackling the challenge posed by ubiquitous environmental contamination, and to the ongoing health and resource problems associated with healthcare-associated infections (HAI).

Conflict of Interest Statement

The intellectual property rights of the HINS-light EDS belong to the University of Strathclyde. The University has made all systems for research purposes only and no commercial company manufactures this technology.

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663 **Table I.** *Comparison of the properties of ultraviolet C (UVC) and 405 nm light for environmental*
664 *disinfection applications.*

	UVC LIGHT	**405nm LIGHT**
Typical/Potential use	Terminal clean of air and light exposed surfaces	Continuous disinfection of air and light exposed surfaces
Safety	Significant safety hazards associated with human exposure; can cause DNA mutations, erythema [70]	Can be utilised safely in the presence of people at recommended irradiation levels [58-60]
Mechanism of Action	DNA damage kills cells. Sub-lethally damaged cells can recover using photoreactivation mechanism to repair DNA [71,72]	Photoexcitation of intracellular molecules induces oxidation of microbial cells. No known repair mechanism [73,74]
Antimicrobial Activity	Broad spectrum action against a range of microorganisms including spores and viruses [51,52]	Effective against bacteria, fungi, yeasts and spores; antiviral activity not yet fully established [32,33,56]
Antimicrobial Efficacy	Rapid inactivation rate within treatment zone [6,24]	Comparably slower inactivation rate within treatment zone [58-60]
Materials Compatibility	UV light associated polymer damage [69]	Lower energy 405 nm wavelengths more materials compatible [69]
Ease of Use for Environmental Disinfection	Rooms/wards need to be vacated during use; operator training required [6,24]	Can be safely used during room occupation; no operator safety training required [58-60]
Microbial Mutagenic Potential	Powerful mutagen that may encourage resistance development	Multi-target oxidative action mitigates against resistance development [75]
Penetrability	Does not penetrate through plastics and glass and weakly penetrates into water and fabrics	Can penetrate through plastics and glass and penetrates into water and fabrics [44]

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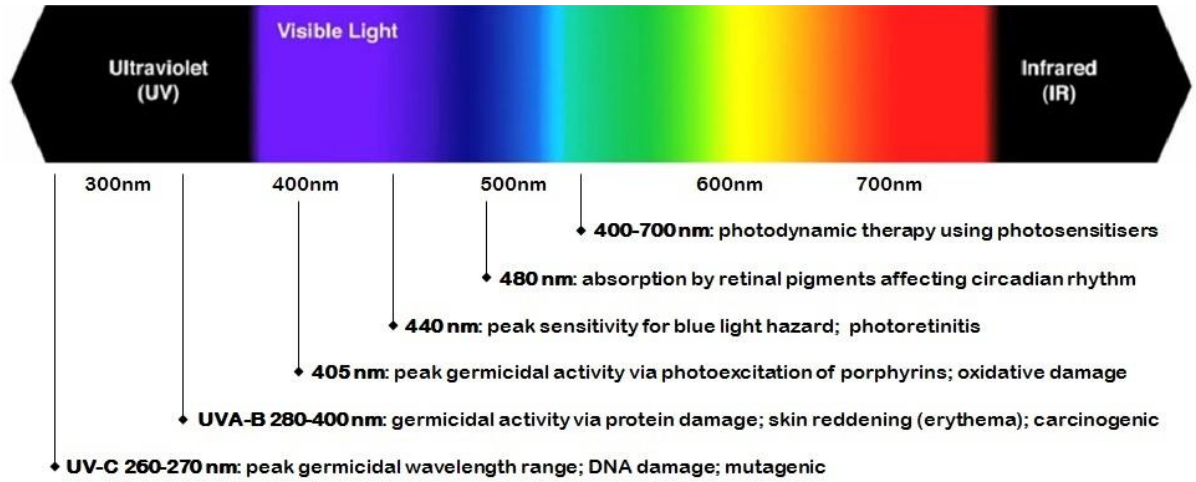
Figure Legends

Figure 1. *UV, visible light and infrared regions of the electromagnetic spectrum. Highlighted are key UV and violet/blue wavelengths with detail of their germicidal action and safety aspects.*

Figure 2. *Mean reductions in the total staphylococcal counts and the presumptive S. aureus levels across an occupied burns unit isolation room, before, during and after 5-day use of HINS-light EDS. Contact plate samples (n=70) were collected twice during each phase and the results pooled to assess the overall decontamination effect. A significant 62% decrease in total staphylococcal counts, and 50% decrease in presumptive S. aureus was achieved (P<0.05). 'After use' samples, showed that contamination around the room had significantly risen over the 6-days after the system was switched off: 126% and 98% increase in the total staphylococci and presumptive S. aureus counts, respectively (P<0.05). (Data adapted from [53]).*

Figure 3. *Reductions in the mean levels of environmental bacteria on a range of surfaces in an ICU isolation room before and after 5-day use of the HINS-light EDS. Tryptone soya agar contact plate samples were collected from each surface and results pooled to show the mean reduction in contamination on the sampled surface (data adapted from [55]).*


Ultraviolet (UV)
Visible Light
Infrared (IR)
300nm
400nm
500nm
600nm
700nm
400-700 nm: photodynamic therapy using photosensitisers
480 nm: absorption by retinal pigments affecting circadian rhythm
440 nm: peak sensitivity for blue light hazard; photoretinitis
405 nm: peak germicidal activity via photoexcitation of porphyrins; oxidative damage
UVA-B 280-400 nm: germicidal activity via protein damage; skin reddening (erythema); carcinogenic
UV-C 260-270 nm: peak germicidal wavelength range; DNA damage; mutagenic

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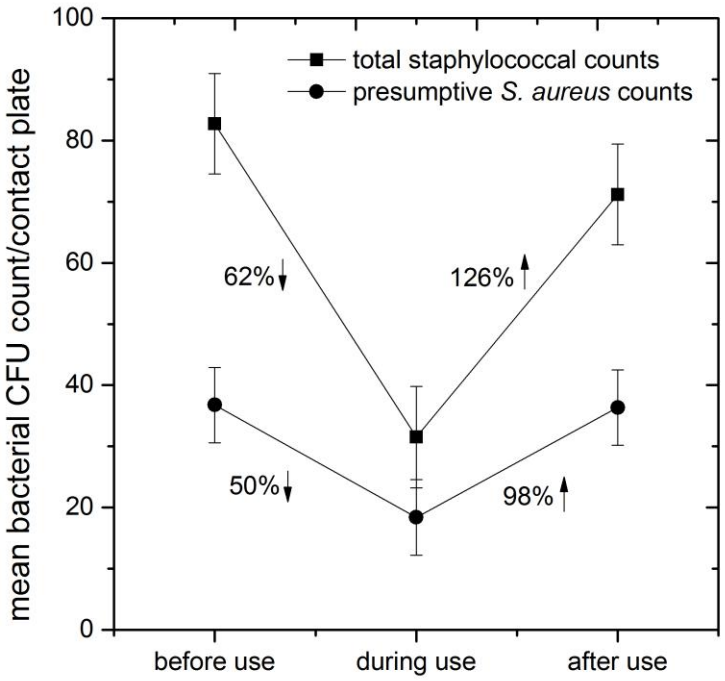

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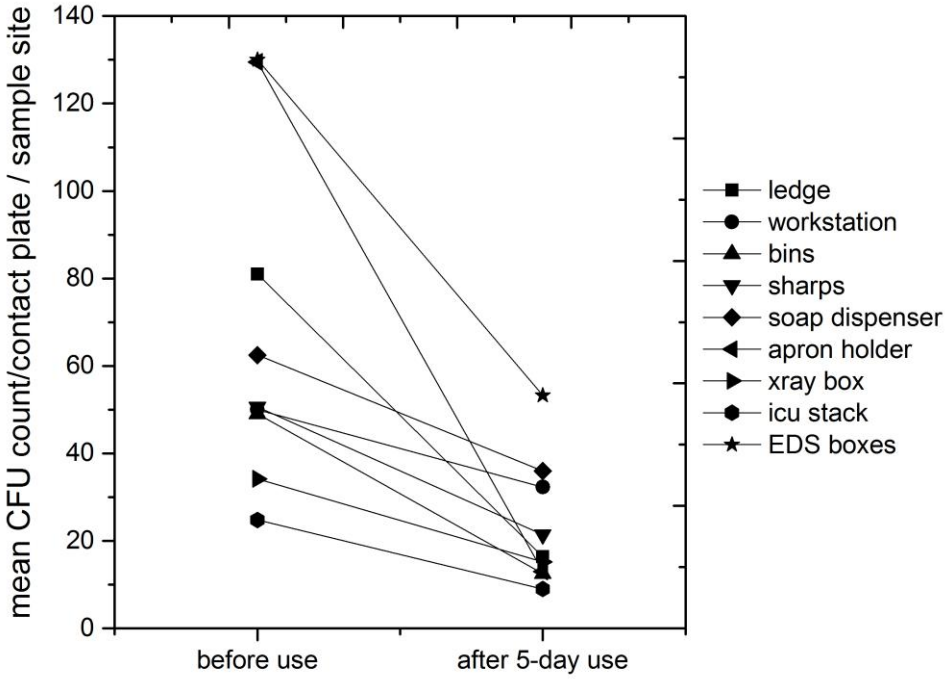

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