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Applications of visible blue light as a multipurpose disinfecting tool for medicine and public health: an overview

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APPLICATIONS OF VISIBLE BLUE LIGHT AS A MULTIPURPOSE DISINFECTING TOOL FOR MEDICINE AND PUBLIC HEALTH: AN OVERVIEW

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APPLICATIONS OF VISIBLE BLUE LIGHT AS A MULTIPURPOSE DISINFECTING TOOL FOR MEDICINE AND PUBLIC HEALTH: AN OVERVIEW

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ABSTRACT

Visible blue light wavelengths in the 400-470-nm range have been observed to have antimicrobial properties. The purpose of this review article is to delineate the mechanism of action, optimizing conditions, and public health and clinical applications of antimicrobial blue light (aBL) as characterized by the current literature. A widely accepted hypothesis for the mechanism of bacterial inactivation by aBL is that the light causes photoexcitation of endogenous photosensitizers, porphyrins and flavins, which leads to the release of reactive oxygen species that subsequently lead to cell toxicity. Factors that have been observed to be associated with enhanced antimicrobial action include increased duration of exposure, pre-treatment with quinine hydrochloride, and exposure of target bacteria to sub-lethal stress conditions. Studies examining the effect of repetitive exposure to sub-lethal levels of aBL on bacteria show no significant evidence of development of resistance. Additionally, aBL has exhibited the ability to inactivate multidrug-resistant (MDR) bacteria. While studies have also

observed aBL to have efficacy in inactivating fungal and viral pathogens, there is a need for further research to elucidate the mechanisms of photoinactivation of fungi and viruses by aBL. Multiple studies have shown that aBL is effective in causing significant CFU reduction in biofilms, an observation that supports the application of aBL for decontamination of surfaces and treatment of localized infections. aBL has demonstrated efficacy in eliminating foodborne pathogens found on food surfaces and exposed surfaces in the food processing environment. Studies applying aBL to decontamination of surfaces in the clinical environment have concluded that it is a viable decontamination practice that shows promise for helping to minimize the spread of healthcare-associated infections (HAIs). Existing literature provides evidence in favor of the application of aBL in clinical contexts such as the treatment of gonococcal infections, eye infections, and otitis media, and in the decontamination of stored platelets and plasma. Studies investigating these clinical applications have demonstrated the efficacy of aBL for inactivating the clinically relevant pathogens, as well as the preservation of normal human cells upon exposure to the doses of light that are lethal to the pathogens.

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LIST OF ABBREVIATIONS

aBL	Antimicrobial blue light
CFU	Colony-forming unit
FCV	Feline calicivirus
HAIs	Healthcare-associated infections
HINS-light EDS	High-intensity narrow-spectrum light environmental decontamination system
HIV	Human Immunodeficiency Virus
ICU	Intensive Care Unit
MDR	Multidrug-resistant
MRSA	Methicillin-resistant *S. aureus*
NADH	Nicotinamide Adenine Dinucleotide Hydride
PBS	Phosphate-buffered saline
SCID	Severe combined immunodeficient

INTRODUCTION

The presence of different forms of pathogens in environmental, clinical, and food processing settings presents a multitude of risks to health on the individual and population scales. For this reason, pathogen reduction technologies have applications in food safety, environmental health, and clinical contexts. Emerging technologies for pathogen reduction can also have far-reaching public health consequences, with implications for long-standing issues like antibiotic-resistant bacteria, and the incidence of hospital-acquired infections. One such emerging technology is the use of visible blue light, as it is increasingly being investigated for its antimicrobial properties against a variety of pathogens, including bacteria, fungi, viruses, and protozoa (Hessling et al. 2020; Jankowska et al. 2020; Maclean et al 2016; Zhang et al 2016). One major advantage of aBL over other pathogen reduction technologies currently in use, such as ultraviolet radiation, is that even at the irradiance levels required for bacterial inactivation, visible blue light is safer for human exposure (Maclean et al. 2016).

Potential clinical applications of pathogen reduction technologies include the treatment of localized infections, wound healing, and ensuring the safety of

stored blood products for transfusion (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019; Maclean et al. 2016; Maclean et al 2020; Zhang et al. 2016). Visible blue light also shows potential for facilitating a solution for the increasingly urgent public health concern of antibiotic resistance, as experiments involving repeated exposure to high-intensities of blue light have shown no significant evidence of bacterial resistance to photoinactivation by visible blue light (Amin et al. 2016; Tomb, Maclean, Coia, MacGregor, Anderson, 2017; Wang, Ferrer-Espada, Baglo, Goh et al. 2019).

Environmental decontamination applications of visible blue light include the disinfection of food, contact surfaces, and air. Foodborne pathogens are estimated to cause annually 9.4 million cases of illness in the United States (Scallan et al. 2011). Among the leading pathogens of these foodborne illnesses are norovirus and nontyphoidal *Salmonella* (Scallan et al. 2011). Microbial persistence and growth on surfaces are another major health concern, particularly in the hospital setting (Otter et al. 2014). Dry hospital surfaces have been observed to develop biofilms of microbes, which are then less susceptible to conventional disinfection methods (Otter et al. 2014).

The purpose of this literature review is to provide a comprehensive overview of the potential of visible blue light as an antimicrobial tool by examining its clinical and public health applications as characterized by experimental studies published in the last ten years. Additional areas of investigation within this literature review will include the mechanism of pathogen inactivation by visible blue light, conditions that optimize its antimicrobial activity, its efficacy on different forms of pathogens, and its effects on normal healthy non-pathogenic cells. This review will also endeavor to highlight areas within the study of visible blue light in which further investigation is necessary in order to ensure its viability for the proposed clinical and non-clinical applications. Experimental studies were collected with several searches for peer-reviewed experimental studies in the EMBASE and PubMed search engines, using search terms including "antimicrobial blue light" or "visible blue light" in conjunction with terms including "pathogen," "virus," "fungi," or "bacteria."

PUBLISHED STUDIES

Investigation of mechanism of pathogen inactivation by aBL

A widely accepted hypothesis regarding the mechanism of photoinactivation of organisms by blue light in the visible range is that the light causes photoexcitation of endogenous photosensitizers, which triggers the release of reactive oxygen species, which in turn leads to oxidative stress and cell toxicity (Ramakrishnan et al. 2016). A 2016 study by Ramakrishnan et al. sought to further elucidate the bacterial photoinactivation mechanism (Ramakrishnan et al. 2016). In experiments with *Staphylococcus epidermidis*, an overproduction of reactive oxygen species in cells treated with 405-nm light was observed with the use of a green fluorescent dye (Ramakrishnan et al. 2016). On application of sodium pyruvate, a reactive oxygen species scavenger which scavenges free H_2O_2, to light-treated *S. epidermidis* cells, it was observed that the extent of bacterial inactivation was substantially reduced when compared to light-treated *S. epidermidis* cells in the absence of sodium pyruvate (Ramakrishnan et al. 2016). The observation that sodium pyruvate was the single reactive oxygen species scavenger associated with the greatest reduction in bacterial inactivation by 405-nm light suggests that H_2O_2 plays a substantial role in the mechanism of

photoinactivation (Ramakrishnan et al. 2016). The greatest reduction in *S. epidermidis* inactivation after 405-nm light exposure was observed when a combination of three reactive oxygen species scavengers was administered to light-treated cells: sodium pyruvate, catalase, and dimethyl thiourea (Ramakrishnan et al. 2016). This finding was interpreted to mean that more than one reactive oxygen species takes part in the photoinactivation mechanism induced by 405-nm light (Ramakrishnan et al. 2016).

A 2020 study sought to investigate the reason for the known discrepancies in bacterial inactivation by different light wavelengths within the 400-470-nm range, working from the understanding that differences in the extent of microbial inactivation are typically explained by the presence of different photosensitizers within the targeted microbe (Hessling et al., 2020). Investigators studied the apparent contradiction in existing knowledge which indicates that 400-420-nm light is more efficient at bacterial inactivation than 450-470-nm light, even in enterococci which possess flavins but do not produce the porphyrin photosensitizers that would predispose them to be more vulnerable to 400-420-nm light (Hessling et al., 2020). On exposure of *Enterococcus moraviensis*, which does not produce porphyrins, to 405-nm and 450-nm light, it was found that 405-nm light led to a greater reduction in bacterial concentration, consistent with

findings in prior publications (Hessling et al., 2020). Fluorescent spectral analysis of *E. moraviensis* exposed to irradiation with 405-nm light showed decreasing flavin levels with increasing duration, as well as increasing levels of NADH and the fluorophore lumichrome (Hessling et al., 2020). The investigators proposed that the degradation of flavins into lumichrome upon irradiation with 405-nm light suggest that lumichrome could possibly have a role in the efficient inactivation of *E. moraviensis* by 405-nm light, as lumichrome is known to have high absorption at 405-nm and is an efficient photosensitizer (Hessling et al., 2020). However, investigators also note that both NADH and lumichrome were found in increasing concentrations with increasing duration of 405-nm light irradiation, and the data obtained in the present study do not provide evidence as to what role each plays in the photoinactivation of *E. moraviensis* (Hessling et al, 2020).

A 2019 study sought to characterize the antimicrobial mechanism of visible blue light (400-470-nm) when applied to *Neisseria gonorrhoeae* (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). Upon examination of the fluorescence spectra and absorption spectra of endogenous photosensitizers derived from the *N. gonorrhoeae* cell lysates, there was evidence of the presence of both porphyrins and flavins (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). Spectroscopic and ultra-

performance liquid chromatography analysis showed that exposure to 405-nm light led to excitation of porphyrins and flavins and subsequent cell death (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). This study also examined the antimicrobial effect of 405-nm light under the hypoxic condition by deoxygenating the *N. gonorrhoeae* cell suspension with N_2 prior to the light exposure (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). It was found that the antimicrobial effect of 405-nm was substantially diminished in the hypoxic condition compared to the non-hypoxic control, with only a reduction of 1-$\log_{10}$ CFU in the hypoxic condition and complete eradication in the non-hypoxic control condition (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). The finding that there was still some level of bacterial reduction in the hypoxic condition led investigators to infer the involvement of reactive oxygen species besides $^1O_2$ in the photoinactivation mechanism, which in turn suggests that flavins have a role in the photosensitization reaction and subsequent photoinactivation of *N. gonorrhoeae* by 405-nm light (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019).

Conditions that optimize the antimicrobial activity of aBL

A common finding among studies examining photoinactivation of bacteria upon administration of aBL is that 405-nm light is the most effective at bacterial reduction, compared to other wavelengths within the 400-nm to 470-nm

range of blue light (Endarko et al. 2012; Hessling et al 2020; Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). A study comparing the efficacy of photoinactivation of *Listeria monocytogenes* by blue light wavelengths ranging from 400-nm to 450-nm in 10-nm increments observed 405 ± 5-nm to be the most efficacious (Endarko et al. 2012). As previously mentioned, it was found in the 2020 study by Hessling et al. that administration of 405-nm light led to a greater reduction in the bacterial concentration of *E. moraviensis* than did administration of 450-nm light (Hessling et al, 2020). Similarly, the 2019 Wang et al. study examining the role of wavelength on inactivation of *N. gonorrhoeae* found that 405-nm light led to greater reduction in bacterial concentration than did 470-nm light (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019).

A 2014 study by McKenzie et al. experimented with the use of 405-nm light in conjunction with different stress conditions to examine the stress conditions that most enhance the bactericidal activity of 405-nm light (McKenzie et al. 2014). *Escherichia coli* and *Listeria monocytogenes* were subjected to sub-lethal stress conditions including varying temperatures, pH levels, and salt concentrations (McKenzie et al. 2014). After being held in the sub-lethal stress conditions, the bacterial suspensions were exposed to 405-nm light at an irradiance of 70mW/cm^2 (McKenzie et al. 2014). Temperature stress was observed

to induce enhanced photoinactivation with 405-nm light in both species, with the populations grown in the 4˚C and 45˚C stress conditions exhibiting greater inactivation rates than those grown in the non-stress 22˚C condition (McKenzie et al. 2014). Acid stress was observed to be associated with the most substantial enhancement of photoinactivation (McKenzie et al. 2014). After being held in the pH 3 condition, the dose of 405-nm light required to achieve the same extent of inactivation as observed in the non-stressed population was reduced by 77% for *E. coli* and by 50% for *L. monocytogenes* (McKenzie et al. 2014). Increased salt concentration produced differing effects on the two species, with *E. coli* requiring a 50% smaller dose for inactivation, whereas *L. monocytogenes* became more tolerant to the light treatment and required a 50% greater dose for inactivation (McKenzie et al. 2014). Upon further examination, the apparent increase in 405-nm light tolerance in *L. monocytogenes* could be explained by the fact that the increased salt condition did not induce sub-lethal damage in *L. monocytogenes* as it did for *E. coli*, as *L. monocytogenes* is known to have a degree of osmotolerance (McKenzie et al. 2014).

In studies that compared the extent of pathogen inactivation at different durations of exposure to aBL, it was observed that increased duration of exposure is associated with an increased extent of pathogen inactivation (Bache

et al. 2017; Jankowska et al. 2020; Maclean et al. 2020). In the 2017 study by Bache et al. comparing the extent of bacterial reduction in a burns unit after different durations of aBL treatment, it was found that increased duration of exposure was associated with greater extent of bacterial reduction (Bache et al. 2017). Jankowska et al. observed that in platelets and plasma infected with *Trypanosoma cruzi* and subjected to 405-nm light treatments for durations of up to 5 hours, the plasma and platelet samples exposed to 5 hours of light treatment exhibited the greatest reduction in parasites (Jankowska et al. 2020). In a 2020 study, Maclean et al. observed that in *S. aureus*-seeded platelets treated with 405-nm light, the percentage inactivation and duration of exposure were positively correlated (Maclean et al. 2020).

aBL inactivation of bacteria

Drug-resistance in bacteria is an increasingly important issue that poses a substantial threat to health on the global scale, as existing antibiotic treatments will fail to be effective as MDR bacteria continue to surface (Choffnes et al. 2010; Wang et al. 2017). Because infectious disease is a major contributor to the global burden of disease and is associated with more than 25% of annual deaths worldwide, it is imperative to have an arsenal of effective antibacterial strategies to prevent and treat infectious disease (Fauci et al. 2010; Choffnes et al. 2010). The

development of drug resistance in bacteria limits the prevention and treatment options, and it is therefore of significant consequence to determine both the potential for bacteria to develop resistance to inactivation by aBL, and the efficacy with which aBL can inactivate drug-resistant bacteria. Multiple studies which sought to determine whether bacteria are likely to develop resistance to photoinactivation by aBL have subjected bacterial samples to repetitive exposures to sublethal light doses and observed no signs of the development of resistance (Amin et al. 2016; Leanse et al. 2018; Wang, Ferrer-Espada, Baglo, Goh et al. 2019; Tomb, Maclean, Coia, MacGregor, Anderson, 2017; Zhang et al. 2014). A 2019 study assessed whether *N. gonorrhoeae* cells could develop resistance to aBL of wavelength 405-nm by exposing the bacterial suspension to a subtherapeutic amount of light, growing the surviving cells in culture overnight, exposing the new suspension to 405-nm light, and repeating this cycle for 15 repetitions (Wang, Ferrer-Espada, Baglo, Goh et al. 2019). Analysis of the reduction in bacterial CFU after each successive round of 405-nm light exposure showed no statistically significant association between the number of exposures and extent of bacterial reduction, suggesting that *N. gonorrhoeae* is not inclined to develop resistance to 405-nm aBL (Wang, Ferrer-Espada, Baglo, Goh et al. 2019). A 2017 study reached a similar conclusion after examining the propensity of

Staphylococcus aureus to develop resistance to 405-nm light exposure (Tomb, Maclean, Coia, MacGregor, Anderson, 2017). The Tomb et al. study conducted one experiment in which *S. aureus* was cultured under low-level exposure to 405-nm light, at an irradiance level of 1 mW/cm^2 (Tomb, Maclean, Coia, MacGregor, Anderson, 2017). In the second experiment, *S. aureus* was subjected to 15 successive cycles of exposure to a sublethal dose of 108 J/cm^2 of high-intensity 405-nm light (Tomb, Maclean, Coia, MacGregor, Anderson, 2017). The *S. aureus* cultured under low-level light exposure was observed to require a greater dose of 405-nm light for complete inactivation (Tomb, Maclean, Coia, MacGregor, Anderson, 2017). Upon the subsequent observation of elevated H_2O_2 in the *S. aureus* cultured in low-level 405-nm light, it was suggested that the apparent tolerance of these cells to inactivation by 405-nm light may be attributable to the upregulation of protective enzymes induced by the stress of low-level 405-nm light exposure during culture (Tomb, Maclean, Coia, MacGregor, Anderson, 2017). This finding suggests that in order to avoid engendering bacterial tolerance to 405-nm light in clinical applications, 405-nm light should be administered at a bactericidal dose as opposed to a low-level exposure (Tomb, Maclean, Coia, MacGregor, Anderson, 2017). In the experiment subjecting *S. aureus* to 15 successive cycles of high-intensity 405-nm light at a sub-lethal dose,

there was no significant evidence of the development of tolerance to inactivation by 405-nm light (Tomb, Maclean, Coia, MacGregor, Anderson, 2017). The observation that bacteria do not appear to develop resistance to blue light after repetitive exposure was also supported by the findings of the 2014 study by Zhang et al. which subjected MDR *A. baumannii* to 10 successive cycles of sublethal exposure to 415-nm light and observed no development of resistance (Zhang et al. 2014). In a 2018 study, Leanse et al. concluded that there was no statistically significant correlation between the number of exposure cycles to 405-nm light and the susceptibility to photoinactivation *in vitro* for any of the three gram-negative bacteria examined, *P. aeruginosa, A. baumannii,* or *E. coli* (Leanse et al. 2018).

The effect of aBL on drug-resistant strains of bacteria is also of significant consequence, as the growing issue of antibiotic-resistant bacteria presents a need for novel methods of controlling bacterial spread (Leanse et al. 2020). Multiple studies have shown aBL to have efficacy in inactivating MDR bacteria (Barneck et al. 2016; Bumah et al. 2015; dos Anjos et al. 2019; Leanse et al. 2020; Maclean et al. 2010). In a 2010 study that experimented with high-intensity narrow-spectrum light (HINS-light) with a peak wavelength of 405-nm on a hospital isolation room with high levels of *S. aureus* and methicillin-resistant *S. aureus* (MRSA), the

use of HINS-light was associated with a reduction from 0.84 CFU/cm^2 to 0.42 CFU/cm^2 in *S. aureus* count, and a reduction from 0.73 CFU/cm^2 to 0.26 CFU/cm^2 in MRSA count (Maclean et al. 2010). A 2016 study by Barneck et al. found 405-nm light to be effective in inactivating *E. coli* that had been conferred with ampicillin resistance, with an 82% mean reduction after 120 minutes of exposure at an irradiance of 2.89 mW/cm^2, and a 100% mean reduction after 120 minutes of exposure at an irradiance of 9.37 mW/cm^2 (Barneck et al. 2016). Bumah et al. observed aBL of wavelengths 405-nm and 470-nm to be effective in reducing bacterial CFU of MRSA (Bumah et al. 2015). A trend seen in this study was that greater fluences of light were required to achieve 100% growth suppression in the more dense (greater CFU/mL) bacterial colonies (Bumah et al. 2015). In an *in vitro* study, suspensions of MDR strains of *E. coli* were seen to be susceptible to photoinactivation by 410-nm light, with all strains exhibiting a greater than 3log$_{10}$ reduction after 180 minutes of light exposure (dos Anjos et al. 2019). The strains of *E. coli* used in this study were resistant to colistin, broad spectrum cephalosporin, or carbapenem, antibiotics that are considered the last resort for controlling MDR bacteria (dos Anjos et al. 2019). A study by Leanse et al. proposed that aBL could be used in conjunction with quinine hydrochloride to inactivate multi-drug resistant bacteria (Leanse et al. 2020). The use of quinine

hydrochloride in conjunction with 405-nm light irradiation was associated with a greater than 10^3-fold reduction in planktonic suspensions of MDR strains of *P. aeruginosa* and *A. baumanii* (Leanse et al. 2020). It has also been proposed that sublethal doses of aBL can be used in conjunction with traditional antibiotics to treat MDR bacteria (Wozniak et al. 2019). The findings of a 2019 study by Wozniak et al. showed extensively drug-resistant *A. baumannii* to have enhanced susceptibility to antibiotics after sublethal exposure to aBL (Wozniak et al. 2019).

There is consensus among a number of studies that there is a difference in susceptibility to 405-nm light between gram-positive and gram-negative bacteria, with gram-positive bacteria typically being the more vulnerable and gram-negative bacteria requiring relatively greater doses of 405-nm light for inactivation (Guffey et al. 2016; Maclean et al. 2016; McKenzie et al. 2014). In a 2016 study, it was seen that among plasma samples seeded with *Staphylococcus aureus*, *S. epidermidis*, and *E. coli*, samples seeded with *E. coli* required an approximately four times greater dose to achieve a similar extent of bacterial inactivation compared to the samples seeded with the staphylococci (Maclean et al. 2016). This phenomenon was even more pronounced in phosphate-buffered saline (PBS) samples seeded with the same bacteria, where it was seen that *E. coli*-seeded samples required a 7.5 times greater dose for a similar extent of

bacterial inactivation compared to the PBS samples seeded with the staphylococci (Maclean et al. 2016). The 2014 McKenzie et al. study also found that *L. monocytogenes,* a gram-positive bacterium, had greater susceptibility to inactivation by 405-nm light compared to *E. coli*, a gram-negative bacterium (McKenzie et al 2014). After culture in optimal growth conditions, complete inactivation of 5-$\log_{10}$ populations was achieved with a dose of 84 J/cm^2 405-nm light for *L. monocytogenes*, whereas a greater dose of 378 J/cm^2 was required to achieve the same extent of inactivation in the *E. coli* populations (McKenzie et al 2014). This observation of *E. coli* requiring a 4.5 times greater dose of 405-nm light than *L. monocytogenes* to exhibit the same extent of inactivation echoes the finding in the 2016 study by Maclean et al. in which the plasma samples seeded with gram negative *E. coli* required an approximate 4 times greater dose of 405-nm light to achieve the same level of inactivation as seen in the plasma samples seeded with gram-positive species (Maclean et al. 2016; McKenzie et al. 2014). A contrasting finding was observed in a 2016 study which examined the relative bactericidal effects of 405-nm light against four different clinically relevant bacteria and found gram-positive and gram-negative bacteria to have similar susceptibility to 405-nm light, with no statistically significant differences in susceptibility between the species tested (Barneck et al. 2016). This study found

ampicillin-resistant *E. coli* to be the most susceptible to photoinactivation by 405-nm light, followed by *S. pneumoniae*, *S. aureus*, and *P. aeruginosa* (Barneck et al. 2016). This departure from the more widely observed tendency of gram-positive bacteria to have greater susceptibility to 405-nm light than gram-negative bacteria was attributed to possible variability in species, strains, and stain family (Barneck et al. 2016).

aBL inactivation of fungi

aBL has had demonstrated efficacy across studies in inactivating fungal pathogens. Studies have shown aBL to be effective in inactivating *Candida albicans*, a clinically relevant fungal pathogen responsible for commonly seen fungal infections (Yan et al 2018; Zhang et al. 2016). It has been proposed that endogenous photosensitizers may be responsible for the phototoxicity effect of aBL on *C. albicans,* as fluorescence spectroscopy has indicated the presence of porphyrins and flavins in *C. albicans* (Zhang et al. 2016). One such study administered 405-nm light to *ex vivo* rabbit corneas with *C. albicans* keratitis and concluded that it was indeed an effective antifungal treatment and a viable option for treatment pending studies investigating its safety for the surrounding eye tissue (Yan et al. 2018). *In vitro* experiments conducted by Zhang et al. exposing both human keratinocytes and *C. albicans* to 415-nm light showed that

C. albicans was more susceptible than human keratinocytes to inactivation upon light exposure, and that there is a therapeutic window of exposure wherein *C. albicans* cells will be inactivated and human keratinocytes unharmed (Zhang et al. 2016). In the in vivo experiment by Zhang et al. administering 415-nm light to *C. albicans*-infected mouse burn wounds, the light exposure substantially reduced the fungal burden, although there was a noted recurrence of infection after light treatment (Zhang et al. 2016).

In a study using *Saccharomyces cerevisiae* as the model organism, blue light of wavelengths 405-nm and 450-nm were administered it was found that a $1\log_{10}$ reduction in CFU could be achieved with a 182 J/cm^2 dose of 405-nm light, and with a 526 J/cm^2 dose of 450-nm light (Hoenes et al. 2018). Subsequent analysis of trypan blue staining produced no evidence of damage to the cell membranes of the light-treated samples, suggesting that the aBL treatment did not impair the viability of the fungal cells but rather impacted their ability to be cultured (Hoenes et al. 2018). Analysis of the *S. cerevisiae* cell lysates indicated that the presence of the endogenous photosensitizer protoporphyrin IX may play a role in the sensitivity of the fungal cells to 405-nm light (Hoenes et al. 2018). The sensitivity of the fungal cells to 450-nm could conceivably be explained by the presence of riboflavin and other flavins (Hoenes et al. 2018).

An area for future investigation within the application of aBL to fungal pathogens is the impact of melanization (Zhang et al. 2016). Fungal pathogens like *C. albicans* have been known to produce melanin in the context of an infection, which could conceivably shield fungal cells from light exposure and thus impact the efficacy of fungal inactivation by aBL (Zhang et al. 2016). Future studies investigating the interaction between this phenomenon and aBL will be necessary in order to more fully ascertain whether aBL is a viable disinfecting strategy for fungal pathogens in public health and clinical applications.

aBL inactivation of viruses

Because virions are known not to contain endogenous porphyrins, there is relatively less understanding of the mechanism and efficacy of aBL in inactivation of viruses compared to bacteria and fungi (Tomb, Maclean, Coia, Graham et al 2017). There have been studies investigating the potential of aBL for inactivating human norovirus, which is a leading cause of acute gastroenteritis and can be transmitted in a variety of ways including contaminated food and surfaces (Tomb, Maclean, Coia, Graham et al 2017; Kingsley et al. 2018). A major limitation of studies that seek to investigate the inactivation of norovirus, an issue of substantial clinical and public health importance, is that there is currently no standardized protocol for propagation of human norovirus, and

experimental studies must be conducted using a surrogate virus (Tomb, Maclean, Coia, Graham et al 2017; Kingsley et al. 2018).

A 2017 study sought to investigate the potential of aBL as a preventative for norovirus outbreak in the hospital setting, using Feline Calicivirus (FCV) as a surrogate for human norovirus (Tomb, Maclean, Coia, Graham et al 2017). Female embryonic cells were inoculated with FCV and incubated, and after 90% of the cell monolayer was destroyed, the virus-containing supernatant was collected and stored (Tomb, Maclean, Coia, Graham et al 2017). The media in which the virus-containing supernatant was suspended during exposure to the 405-nm light treatment appeared to affect the efficiency and extent of virus inactivation, with minimal media requiring the greatest dose, presumably because of the absence of porphyrins both in the virus and in the medium (Tomb, Maclean, Coia, Graham et al 2017).

Table 1. Extent of FCV (Feline Calicivirus) inactivation relative to the dose of 405-nm light exposure, for each experimental media composition. Data from Tomb, Maclean, Coia, Graham et al 2017.

Media	**Dose of 405-nm light (J/cm^2)**	**Inactivation of FCV ($log_{10}PFU\ mL^{-1}$)**
Minimal media	2.8×10^3	3.9
Minimal media + riboflavin	421	1.3
Organically rich media – Dulbecco's modified eagle's medium + 10% fetal bovine serum	421	4.8
Biologically relevant organically rich media – artificial feces	1.4×10^3	4.5
Biologically relevant organically rich media – blood plasma	561	4.8

Biologically relevant organically rich media – artificial saliva	421	5.1

It was seen that FCV suspended in organically rich media or biologically relevant organically rich media showed the greatest reductions in FCV and necessitated relatively lower doses of 405-nm light (Table 1; Tomb, Maclean, Coia, Graham et al 2017). These findings support the idea that the presence of porphyrins enhances inactivation by 405-nm light, as the organically rich and biologically relevant media contained porphyrins, whereas minimal media did not contain porphyrins and showed relatively lower efficiency in FCV inactivation (Tomb, Maclean, Coia, Graham et al 2017). The greater susceptibility of FCV when in the presence of biologically relevant substances including plasma and artificial saliva suggest that 405-nm light could potentially be a promising antimicrobial tool in the context of hospital environments, where virus contaminants are likely to be in the presence of similar biological substances containing endogenous photosensitizers.

Another study which used Tulane virus as a surrogate for human norovirus in the context of food safety and the decontamination of produce also

found that viral inactivation by 405-nm light was limited in the absence of some form of enhancer (Kingsley et al. 2017). Although the singlet oxygen enhancers riboflavin and rose bengal did appear to enhance viral inactivation by 405-nm light, the overall extent of inactivation was limited, and there is a need for further research into food-grade compounds that can more substantially enhance the production of reactive oxygen species and subsequently inactivate viruses (Kingsley et al. 2017).

aBL inactivation of biofilms

Biofilms are aggregations of microorganisms that are understood to be an adaptive mechanism to improve survival under conditions of stress (de la Fuente-Núñez et al. 2013). Biofilms have been observed to form on both living and non-living surfaces in clinical settings, and their increased resistance to antimicrobial treatments and host immune response present a considerable risk for the spread of infectious disease (de la Fuente-Núñez et al. 2013; Wang et al. 2016). The increased resistance to stress conditions observed in bacterial biofilms is thought to be attributable to the formation of an extracellular polymeric substance (EPS) that envelops bacteria (de la Fuente-Núñez et al. 2013; Ferrer-Espada et al. 2020; Liu et al. 2020). Polymicrobial biofilms, biofilms composed of more than one species of microorganism, are thought to have increased

antimicrobial resistance attributable to the transfer of antimicrobial resistance genes between microorganisms (Ferrer-Espada et al. 2019). Studies have investigated the use of aBL as an antimicrobial strategy targeting both monomicrobial and polymicrobial biofilms.

aBL wavelengths have been observed to inactivate bacterial biofilms, as seen in a 2020 study which concluded that 400-nm light effectively inactivated *Pseudomonas fluorescens* and *S. epidermidis* biofilms (Angarano et al. 2020). A 2020 study by Ferrer-Espada et al. concluded that 405-nm light exhibits efficacy in inactivating monomicrobial biofilms, with the *A. baumannii, S. aureus, N. gonorrhoeae,* and *P. aeruginosa* biofilms being the most susceptible to inactivation (Ferrer-Espada et al. 2020). It has also been shown that the monomicrobial biofilms composed of MDR strains of *A. baumanni* and *P. aeruginosa* could also be inactivated with the 405-nm light treatment (Ferrer-Espada et al. 2020). In a study that applied 405-nm aBL to monomicrobial and polymicrobial biofilms, significant reductions in bacterial CFU were observed after light treatment (Ferrer-Espada et al. 2019). Monomicrobial biofilms of *P. aeruginosa*, *C. albicans*, and MRSA were subjected to 405-nm light at a dose of 500 J/cm^2, and the resultant inactivation in CFU were 6.30log$_{10}$, 2.33log$_{10}$, and 3.48log$_{10}$, respectively (Ferrer-Espada et al. 2019). The polymicrobial biofilms composed of *P. aeruginosa*

and *C. albicans* exhibited a 6.34$\log_{10}$ CFU inactivation in *P. aeruginosa* and a 3.11$\log_{10}$ CFU inactivation in *C. albicans* after exposure to the same 500 J/cm^2 dose of 405-nm light (Ferrer-Espada et al. 2019). In the polymicrobial biofilms composed of *P. aeruginosa* and MRSA, the same 500 J/cm^2 dose of 405-nm light led to a 3.40$\log_{10}$ CFU inactivation of *P. aeruginosa* and a 2.37$\log_{10}$ CFU inactivation of MRSA (Ferrer-Espada et al. 2019). This study demonstrated the efficacy of 405-nm blue light against polymicrobial biofilms, and suggests the importance of future *in vivo* studies to determine whether this could be applied to the clinical setting in the context of wound healing (Ferrer-Espada et al. 2019).

A 2020 study by Liu et al. applied 405-nm light to biofilms of *Moraxella catarrhalis*, a common causative pathogen for otitis media seen in children which is also known to form biofilms in the middle ear (Liu et al. 2020). Upon exposure to 405-nm light at a dose of 216 J/cm^2, a reduction in biofilms of greater than 3$\log_{10}$ CFU was observed (Liu et al. 2020). Scanning electron microscopy analysis of the light-treated *M. catarrhalis* biofilms showed evidence of damage to the cell walls and loss of the EPS which provides structure to the biofilms (Liu et al. 2020). The findings of this study suggest a potential for 405-nm light to be used as a treatment for *M. catarrhalis* otitis media, pending further investigation to

determine a suitable mode of delivery of 405-nm light to the middle ear in the clinical setting (Liu et al. 2020).

A 2020 study investigated the effect of aBL on *Pseudomonas aeruginosa* biofilms, which pose a significant risk for the spread of HAIs in the clinical environment (Martegani et al. 2020). It was observed that 410-nm light at a low dose of 75 J/cm^2 effectively prevented the formation of the biofilm matrix structure, and the higher doses of 410-nm light at 225 J/cm^2 and 450 J/cm^2 were observed to cause damage to the bacterial cells themselves and impair further cell growth (Martegani et al. 2020). The elimination of existing biofilms by 410-nm light was observed to be dose-dependent, with greater doses of light being associated with greater efficacy in decreasing the biofilm matrix, detaching the bacterial cells, and inactivating the bacterial cells (Martegani et al. 2020).

aBL in food safety

Foodborne illness is a major public health concern in the United States, and proper prevention of widespread foodborne illness necessitates the use of effective disinfection techniques to ensure the safety of the food supply (Guffey et al., 2016). Existing decontamination methods including thermal control, ultraviolet light, and chemical sanitizers have raised concerns about reduced nutritional value and carcinogenicity of the food product (Guffey et al., 2016).

Ultraviolet light has been demonstrated to be efficacious for decontamination in the food industry in contexts such as juice pasteurization and meat treatment, although its viability for widespread use in the food industry is limited by factors including safety concerns, limited ability to penetrate through packaging material, and potential for degradation of plastics (Koutchma 2008; McKenzie et al. 2014). A 2016 study examined the viability of aBL as a food disinfection technique by administering different doses of aBL to processed meat inoculated with *E. coli* and to cucumbers inoculated with *Salmonella typhimurium* (Guffey et al., 2016). In both treatment conditions, there was an observed increase in inactivation rate of the bacteria with increasing fluence, with complete inactivation of the *S. typhimurium* on cucumber peels with administration of 464-nm light at 18 J/cm^2, and 96.3% inactivation of *E. coli* on processed meat with administration of 405-nm light at 100 J/cm^2 (Guffey et al., 2016). While this study establishes that aBL is effective against foods inoculated with *E. coli* and *S. typhimurium*, it remains to be seen whether the aBL has any other effects on the quality and safety of the light-treated food items themselves (Guffey et al., 2016).

The 2014 McKenzie et al. study subjected *E. coli* and *L. monocytogenes* to sub-lethal stress conditions that mimic the conditions that bacterial contaminants would likely be exposed to in the food services environment, including hot and

cold temperatures, acidic environments, and high salt concentration environments (McKenzie et al. 2014). This study found that in both *E. coli* and *L. monocytogenes*, bacterial populations that experienced sublethal damage were more susceptible to inactivation by 405-nm light compared to bacterial populations grown in optimal conditions (McKenzie et al. 2014). Investigators propose that the observed enhancement in the bactericidal effects of 405-nm light when applied in conjunction with sub-lethal stress suggests a potential application of 405-nm light for decontamination of the food processing environment and thus helping to reduce the spread of foodborne pathogens (McKenzie et al. 2014).

Human norovirus is another major cause of foodborne illness, and a 2017 study sought to evaluate 405-nm light as a potential preventative by examining its effects on Tulane virus (a human norovirus surrogate) present on the surface of blueberries (Kingsley et al., 2017). Because viruses do not contain porphyrin, a major photosensitizer involved in the inactivation of bacteria and fungi by aBL, investigators in this study added food-grade singlet oxygen enhancers to the blueberries to enhance the production of the reactive oxygen species and thereby enhance the inactivating effect of the 405-nm light (Kingsley et al., 2017). Blueberries were inoculated with the Tulane virus, treated with one of two

singlet oxygen enhancers, and subjected to 30 minutes of 405-nm light exposure (Kingsley et al., 2017). In blueberries prepared with the singlet oxygen enhancer riboflavin, the 405-nm light treatment group exhibited greater inactivation of Tulane virus, with a statistically significant difference between the treated and untreated group (Kingsley et al. 2017). Among blueberries prepared with the singlet oxygen enhancer rose bengal, the 405-nm light-treated group showed greater inactivation of the virus, although the difference between the treated and untreated group was not statistically significant (Kingsley et al. 2017). Investigators concluded that there is a need for further research of singlet oxygen enhancers that can be used in conjunction with 405-nm light to more substantially inactivate a virus like human norovirus and help ensure the decontamination of produce (Kingsley et al. 2017).

aBL for decontaminating surfaces/environment

Healthcare-associated infections (HAI) are a cause for increased morbidity and mortality of hospital patients, and their prevention necessitates safe and effective methods of environmental decontamination within the hospital (Bache et al. 2011; Kollef et al. 2021). HAIs are also associated with prolonged hospital stay and increased healthcare costs, and new technologies for the decontamination of surfaces and equipment could potentially help to reduce the

burden of disease attributable to HAIs (Kollef et al. 2021). aBL has been investigated as a tool for environmental decontamination, as exemplified by the high-intensity narrow-spectrum light environmental decontamination system (HINS-light EDS) which uses a peak wavelength of 405-nm and has been shown across studies to have efficacy with inactivating environmental pathogens (Bache et al. 2011; Maclean et al. 2010; Maclean et al. 2013).

Burns patients are particularly susceptible to infection due to the immunosuppressed state and impaired integrity of the skin, and the complications can include sepsis and organ failure (Bache et al. 2011; Bache et al. 2017; Ramakrishnan et al. 2006). Burns patients are also likely to disperse bacteria into the surrounding environment, which makes infection control practices in burns units vitally important (Bache et al 2011; Bache et al. 2017). It has been observed that infection control practices in burn units have helped reduce fatality rates for burns patients (Church et al. 2006). A 2010 study by Maclean et al. examined the efficacy of HINS-light for disinfecting a hospital isolation room in the burns unit (Maclean et al. 2010). Two HINS-light sources were fitted in the ceiling of the hospital isolation room, and bacterial growth was examined in an unoccupied room, in an occupied room with intermittent HINS-light application, in an occupied room with constant HINS-light application, and in an occupied

room without HINS-light (Maclean et al 2010). Bacteria were sampled from frequently touched surfaces in the isolation room and examined after a 48-hour incubation period (Maclean et al. 2010). Results showed that the HINS-light effected a statistically significant reduction in bacterial counts in each experimental condition (Maclean et al. 2010). It was also noted that bacterial counts remained low for a period after discontinuation of HINS-light application in the unoccupied room, in contrast to the occupied room which exhibited an increase in bacterial contamination up to pre-treatment levels within two days of the discontinuation of HINS-light treatment (Maclean et al. 2010). As this study administered the HINS-light treatment concurrently with existing hospital decontamination practices, it was demonstrated that the 405-nm light treatment successfully achieves substantial bacterial decontamination that exceeds those achieved by currently used hospital decontamination practices alone, and thus provides strong evidence in support of the application of 405-nm light as a supplementary hospital decontamination method in the setting of a burn unit hospital environment (Maclean et al. 2010). Another study applied HINS-light EDS to an inpatient burn isolation room occupied by a single burns patient and to an outpatient burn clinic room which saw between seven to twelve burns patients (Bache et al. 2011). As with the 2010 Maclean et al. study, samples were

collected from several frequently-touched surfaces and examined after a 48-hour incubation period (Bache et al. 2011). With 14-hour treatment with HINS-light for two consecutive days, a 27% to 75% reduction in bacterial CFU was observed from samples collected at 08:00 hours from the inpatient burn isolation rooms (Bache et al. 2011). In the study conducted on the outpatient burn clinic rooms in which multiple patients were treated for burn wounds, the 8-hour application of HINS-light was associated with a lesser increase in bacterial contamination after clinic – the outpatient room without HINS-light exhibited a mean increase of 14.1 CFU/plate during clinic, whereas the outpatient room with 8-hour HINS-light treatment exhibited a mean increase of only 5.5 CFU/plate during clinic (Bache et al. 2011). Although complete bacterial inactivation was not achieved, this study provides further evidence of the efficacy of violet blue light in its potential application as a supplementary environmental decontamination tool in the inpatient and outpatient burn treatment settings to help prevent further spread of HAIs (Bache et al. 2011). The results of the outpatient clinic experiment indicated that the 405-nm light treatment may be a promising decontamination tool for communal environments where cross-contamination by multiple individuals may be a concern (Bache et al. 2011). A 2017 study by Bache et al. also applied HINS-light treatment to a burns unit at different irradiance levels

and for different durations of exposure (Bache et al. 2017). While no correlation was observed between irradiance level and bacterial reduction, it was observed that increased duration of exposure was correlated with increased reduction in bacteria (Bache et al. 2017). As in the 2010 study by Maclean et al. and the 2011 study by Bache et al, the 2017 study saw a decrease in bacterial counts upon exposure to HINS-light treatment, and an increase in bacterial counts following the discontinuation of HINS-light treatment (Bache et al. 2017).

A 2013 study by Maclean et al. ventured to test the 405-nm HINS-light technology on a different clinical environment in which HAIs are a major concern, namely the intensive care unit (ICU) (Maclean et al. 2013). An ICU room occupied by a patient since nine days prior to the start of the study exhibited a 67% mean percentage reduction in staphylococcal counts after HINS-light treatment (Maclean et al. 2013). A second experiment with a patient who had arrived to the ICU room just twelve hours prior to the start of the study did not exhibit a statistically significant mean percentage reduction in staphylococcal counts after HINS-light exposure, and this was thought to be because of a lower starting level of bacterial contamination due to the room having been cleaned more recently than in the previous experiment (Maclean et al 2013). Samples collected 24 hours following the discontinuation of HINS-light exposure

exhibited a 357% increase in staphylococcal count, which supports the hypothesis that the 405-nm light has a substantial decontaminating effect (Maclean et al. 2013). In a third experiment, the effect of direct versus indirect exposure to the HINS-light was examined (Maclean et al. 2013). The sampled surfaces under direct exposure were observed to have a 63% mean reduction in bacterial counts associated with HINS-light treatment, and a 94% mean increase after discontinuation of the HINS-light (Maclean et al. 2013). The sampled surfaces under indirect exposure to HINS-light were observed to have a 48% mean reduction in bacterial counts, and a 71% mean increase after discontinuation of HINS-light (Maclean et al. 2013). These results showed that both direct and indirect exposure to 405-nm light is associated with a significant reduction in bacterial contamination, although direct exposure does lead to a greater extent of bacterial inactivation (Maclean et al. 2013).

A 2017 study applied visible blue light to the issue of indoor air pollution, and specifically aimed to degrade gaseous formaldehyde which can be injurious to health (Laciste et al., 2017). Investigators first synthesized a titanium dioxide photocatalyst doped with the elements W, Ag, N, and F such that it would have photocatalytic activity in the visible light wavelengths, then combined the photocatalyst with formaldehyde in test tube reactors which were then exposed

to visible blue light (Laciste et al., 2017). Under this experimental setup, it was observed that the combined multi-element doped titanium dioxide was the most effective catalyst with 88.1% photocatalytic degradation of gaseous formaldehyde (Laciste et al., 2017). The findings of this study suggest a potentially promising application of visible blue light in conjunction with photocatalysts for the purification of air (Laciste et al., 2017).

Clinical applications of aBL

Effects of aBL on normal human cells

Multiple studies examining the viability of aBL for pathogen inactivation in clinical contexts have observed that photoinactivation of clinically relevant pathogens can be achieved at lower dosages of aBL relative to the dosages required to cause damage to human cells (Liu et al. 2020; Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019; Wang, Ferrer-Espada, Baglo, Goh et al. 2019; Zhang et al. 2016). A 2019 study by Wang et al. observed a selectivity of 405-nm aBL for *N. gonorrhoeae* cells over human vaginal epithelial cells, which supports its potential application as a treatment for gonococcal infections (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). Another study found that although human vaginal epithelial infected with *N. gonorrhoeae* were more susceptible to photoinactivation by 405-nm light than uninfected human vaginal epithelial cells, the *N. gonorrhoeae* cells

were substantially more susceptible to photoinactivation than even the infected human vaginal epithelial cells (Wang, Ferrer-Espada, Baglo, Goh et al. 2019). This finding suggests that 405-nm light treatment can substantially reduce bacterial load without significantly harming the infected vaginal epithelial cells (Wang, Ferrer-Espada, Baglo, Goh et al. 2019). A 2016 study investigating the potential of 415-nm light as a treatment for *C. albicans* keratitis exposed *C. albicans* and human keratinocytes to light treatment and found *C. albicans* to be substantially more sensitive to photoinactivation than the human keratinocytes, further reinforcing the idea of a therapeutic window in which aBL can eliminate pathogens without harming normal human cells (Zhang et al. 2016). A 2020 study by Liu et al. similarly observed *M. catarrhalis* in planktonic suspensions and in biofilms to have far greater susceptibility to photoinactivation by 405-nm light compared to human keratinocytes (Liu et al. 2020). At an exposure dose of 216 J/cm^2, there was a reduction of greater than 4log$_{10}$ CFU in planktonic suspensions of *M. catarrhalis*, a reduction of greater than 3log$_{10}$ CFU in *M. catarrhalis* biofilms, and no significant reduction in the viability of human keratinocytes (Liu et al. 2020). This observation is corroborated by findings in the 2019 study by Ferrer-Espada et al., where it was observed that human keratinocytes exhibited no significant reduction in viability after exposure to 405-

nm light at a dose of 216 J/cm^2, whereas the same dosage of light caused significant reduction of viability in the polymicrobial biofilms (Ferrer-Espada et al. 2019). A greater dosage of 405-nm light, 500 J/cm^2 was observed to cause cytotoxicity in the human keratinocyte cells (Ferrer-Espada et al. 2019). A study using 415-nm blue light also observed MDR *A. baumannii* to be substantially more susceptible to photoinactivation compared to human keratinocytes (Zhang et al. 2014).

aBL for the treatment of gonococcal infections

The previously referenced 2019 study by Wang et al. performed analysis of fluorescence emission spectra and ultra-performance liquid chromatography of normal human vaginal epithelial cells (VK2/E6E7) after exposure to 405-nm aBL (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). The co-cultures of *N. gonorrhoeae* cells and vaginal epithelial cells exposed to 108 J/cm^2 405-nm light showed a substantial increase in nonviable *N. gonorrhoeae* cells (from 32.85% ± 7.70% before light exposure to 78.15% ± 6.52% after light exposure), with no notable increase in the percentage of nonviable vaginal epithelial cells (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). Fluorescence emission spectra showed a small peak at 460-nm, indicating the presence of flavins, and otherwise minimal absorbance between 350-nm and 700-nm, indicating minimal presence of

porphyrins (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). The ultra-performance liquid chromatography analysis showed that VK2/E6E7 vaginal epithelial cells had substantially lower concentrations of endogenous flavins FAD, FMN, and riboflavin compared to ATCC 700825 *N. gonorrhoeae* cells with total flavin concentrations of 28.12 nmol/g and 363.63 nmol/g respectively (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). Thus, it appears that the selective inactivation of *N. gonorrhoeae* cells over normal human vaginal epithelial cells upon exposure to 405-nm light can be explained by the increased presence of endogenous photosensitizers in the bacterial cells (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). Investigators proposed administration of the 405-nm light via a laser with an optical fiber diffuser directed to the vaginal canal, similar to the present mode of administration of photodynamic therapy for treatment of condyloma acuminata in the vagina (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019).

A separate 2019 study by Wang et al. further investigated the potential use of 405-nm aBL for the treatment of gonococcal infections by examining the efficacy of 405-nm light at inactivating *N. gonorrhoeae* that has invaded human vaginal epithelial cells (VK2/E6E7), as well as the susceptibility of the human vaginal epithelial cells themselves to the aBL (Wang, Ferrer-Espada, Baglo, Goh et al. 2019). It was observed that vaginal epithelial cells that had been infected by

N. gonorrhoeae cells were more vulnerable to the 405-nm light exposure than noninfected vaginal epithelial cells, showing a reduction of 0.05-$\log_{10}$ CFU at the lower exposure of 54 J/cm^2 and a reduction of 0.12-$\log_{10}$ CFU at the higher exposure of 108 J/cm^2 (Wang, Ferrer-Espada, Baglo, Goh et al. 2019). It was also of note that the 405-nm light led to a more substantial inactivation of the *N. gonorrhoeae* cells that had adhered to or invaded the vaginal epithelial cells, with a 4.18-$\log_{10}$ reduction in bacterial CFU at the lower exposure of 54 J/cm^2, and a 5.07-$\log_{10}$ reduction in bacterial CFU at the higher exposure of 108 J/cm^2 (Wang, Ferrer-Espada, Baglo, Goh et al. 2019). Investigators concluded that although infected vaginal epithelial cells were more vulnerable to 405-nm light than non-infected vaginal epithelial cells, the substantially greater susceptibility of *N. gonorrhoeae* cells to 405-nm light indicated a possible therapeutic window of 405-nm light exposure whereby *N. gonorrhoeae* would be selectively inactivated and the infected vaginal epithelial cells would be largely unharmed (Wang, Ferrer-Espada, Baglo, Goh et al. 2019).

aBL in the prevention and treatment of eye infections

Recent studies have explored the potential application of aBL for prevention and treatment of infection relating to the eyes (Zhu et al. 2017; Hoenes et al. 2020; Yan et al. 2018). A 2017 study by Zhu et al. tested the efficacy

of 415-nm aBL for the treatment of keratitis in an *ex vivo* rabbit model and an *in vivo* mouse model, where the causative agent was a bioluminescent strain of *P. aeruginosa* (Zhu et al. 2017). In the experiment with *ex vivo* rabbit corneas, it was seen that antimicrobial light treatment effected the greatest extent of reduction of bacterial luminescence when administered 6 hours after inoculation, compared to 24 hours after inoculation (Zhu et al. 2017). There was no recurrence of infection observed in the rabbit corneas after antimicrobial light treatment (Zhu et al. 2017). A similar *ex vivo* study of keratitis induced in rabbit corneas with a bioluminescent strain of *C. albicans* found that 405-nm aBL was effective in substantially reducing the fungal infection (Yan et al. 2018). In the *in vivo* mouse study, complete bacterial luminescence eradication was achieved with treatment both 6 hours and 24 hours after inoculation, although recurrence of infection was observed in both groups with the 24-hour post inoculation treatment group exhibiting the more severe recurrence of infection (Zhu et al. 2017). The observation that infection recurred in the *in vivo* experiment with mice and not in the *ex vivo* experiment with rabbit corneas suggests that the eyelids of mice in the *in vivo* experiments may have covered parts of the cornea during the 415-nm light exposure, thus preventing all of the bacteria from being exposed to the light (Zhu et al. 2017). The findings from this study provide evidence of the potential

of 415-nm light as a treatment for *P. aeruginosa* keratitis, although further studies are needed to establish sound methods of adequately irradiating all of the cornea in the *in vivo* context and mitigating the recurrence of infection after light treatment.

405-nm light – based pathogen inactivation of stored human platelets and plasma

The ability to safely administer blood and blood products is of great importance in the clinical setting, where a blood transfusion is often necessary as a life-saving measure. Paramount to the safe administration of blood products is the ability to ensure that stored blood products are free of pathogens, so as to avoid the often life-threatening consequences of blood-borne pathogens (Levy et al. 2018). In the 1970s, administration of inadequately decontaminated blood products to patients with hemophilia led to infection of many of these patients with Human Immunodeficiency Virus (HIV) (Seltsam 2017). With the emergence of viruses like HIV, Dengue virus, Chikungunya virus, and West Nile virus, elucidating techniques for the isolation and elimination of pathogens in blood products became exceedingly important to minimize the transmission of disease via blood transfusion (Schlenke 2014). Models suggest that a new infectious agent transmissible by blood emerges approximately every five years, and this

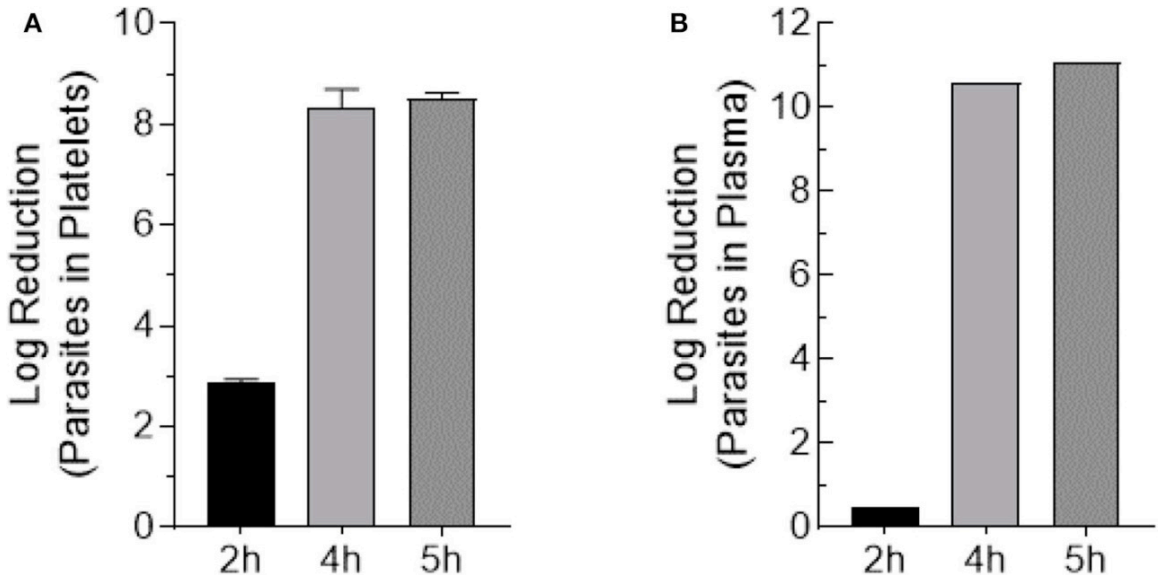
underscores the need for exploration of novel methods of pathogen inactivation in stored blood (Schlenke 2014).

The first study to investigate the use of aBL for the decontamination of plasma was a 2016 study by Maclean et al. Investigators examined the bactericidal effects of 405-nm light when applied to human plasma and rabbit plasma seeded with *S. aureus*, *S. epidermidis*, and *E. coli* (Maclean et al. 2016). Seeded rabbit and human plasma samples were prepared at different volumes and subjected to 405-nm light exposure at different irradiance levels (Maclean et al. 2016). Bacterial inactivation was observed in all test conditions, with *E. coli*-seeded samples generally requiring higher doses of 405-nm light than the *S. aureus*- and *S. epidermidis*-seeded samples for comparable extent of inactivation (Maclean et al. 2016). In bagged plasma seeded with *S. aureus*, light-treated bags were observed to have near complete bacterial inactivation after an exposure dose of 144 J/cm^2, in contrast to the untreated control plasma bags which exhibited a 0.5log$_{10}$ increase in bacterial contamination (Maclean et al. 2016). The findings of this study suggest a strong potential for the use of 405-nm light as a disinfecting tool for blood plasma given its efficacy for different bacterial species at different volumes, its efficacy even when administered through transparent packaging, and its safety for human exposure (Maclean et al. 2016).

A 2020 study by Jankowska et al. investigated whether 405-nm light could also be used to decontaminate plasma and platelets infected with *Trypanosoma cruzi,* a blood-borne protozoan parasite which causes Chagas disease in the host, which is associated with cardiac tissue damage and chronic heart disease (Jankowska et al. 2020). In the United States, the most likely mode of transmission of Chagas disease is via blood transfusion from an infected donor, and it is therefore of great public health importance to establish a safe and effective method of inactivating *T. cruzi* in stored blood products (Jankowska et al. 2020). Human plasma and platelet concentrates infected with *T. cruzi* were subjected to 405-nm light at an irradiance of 15mW/cm^2 for durations up to 5 hours (Jankowska et al. 2020). While light microscopy showed that the trypomastigotes were morphologically intact after 5 hours of 405-nm light exposure, they appeared to have lost the ability to infect or grow in host cells *in vitro*, as observations over a 28-day period showed (Jankowska et al. 2020). Log reduction in parasites treated with 405-nm light exposure in the plasma experiments and platelet experiments are displayed in Figure 1 below. As seen in Figure 1, each fixed duration of exposure to 405-nm light at an irradiance of 15mW/cm^2 led to a reduction in parasites in both the platelets and plasma

experiments, with the 5-hour exposure time leading to the greatest extent of parasite reduction (Figure 1, Jankowska et al. 2020).

Figure 1. Log reduction of parasites after fixed durations of 405-nm light exposure. (A) Log reduction of active *T. cruzi* trypomastigote after infected platelet samples were exposed to 405-nm light at irradiance 15 mW/cm² at the 2-hour, 4-hour, and 5-hour time points. (B) Log reduction of active *T. cruzi* trypomastigote after infected plasma samples were exposed to 405-nm light at irradiance 15 mW/cm² at the 2-hour, 4-hour, and 5-hour time points.



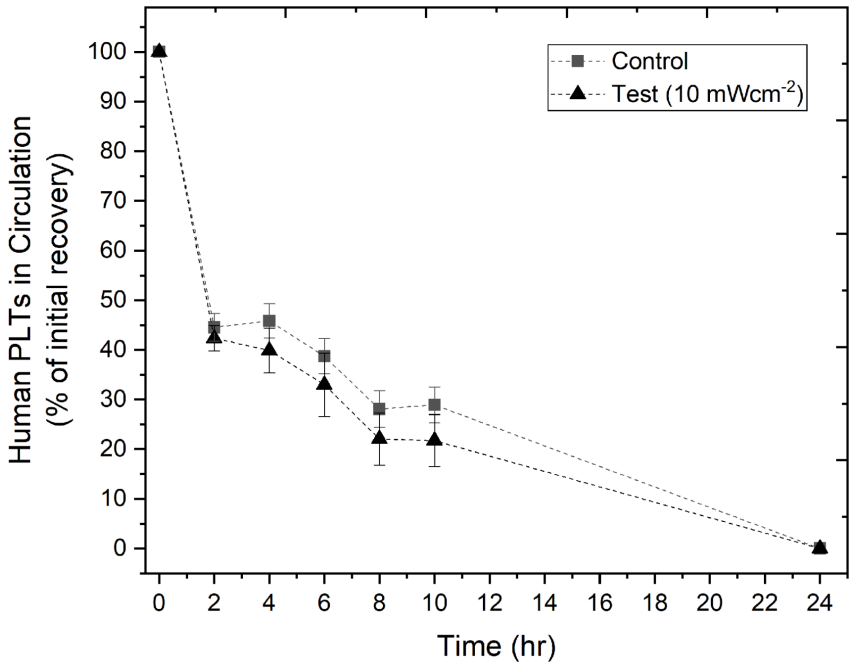
The *in vitro* findings were confirmed in an *in vivo* test wherein Swiss Webster mice were injected with 405-nm light-treated trypomastigotes in plasma,

and the mice showed no evidence of parasitemia even after mice were treated with induced immunosuppression (Jankowska et al. 2020).

Violet-blue 405-nm light is increasingly being investigated for its potential for use as an antimicrobial agent for platelets and plasma. Violet-blue light has been seen to be efficacious at eliminating bacteria in plasma, and a 2020 study sought to investigate whether it could be applicable to platelets as well (Maclean et al. 2020). Human platelets bags were seeded with *S. aureus* at a concentration of 10^2 colony-forming units per milliliter, exposed to 405-nm light at four fixed irradiance levels for up to 8 hours duration, and then plated and incubated for enumeration after 24 hours (Maclean et al., 2020). In addition, severe combined immunodeficient (SCID) mice were infused with light-treated platelets and subsequently assessed at increasing time intervals for the recovery of platelets (Maclean et al. 2020). In the *S. aureus*-seeded platelets experiment, it was seen in general that the percentage inactivation increased with increasing duration of light exposure, and with the increasing level of irradiance with 405-nm light (Maclean et al. 2020). Substantial bacterial inactivation was observed at all irradiation levels, with at least 92.5% inactivation at 6 hours exposure, and at least 99% inactivation at 8 hours exposure (Maclean et al. 2020). As seen in Figure 2 below, the recovery of light-treated platelets from the SCID mice following

platelet infusion closely mirrors the recovery of untreated platelets over the 24-hour period, suggesting that treatment with 405-nm light did not adversely affect the normal functioning of the platelets in vivo (Maclean et al. 2020).

Figure 2. Recovery of human platelets from SCID mice. Hourly percentage recovery of human platelets from SCID mice following infusion with untreated human platelets (control) and light-treated human platelets irradiated with 10mWcm^{-2} of 405-nm light (test). Figure obtained from Maclean et al. 2020.



This study provides strong evidence for the viability of 405-nm light as a bactericidal treatment for stored platelets in the clinical context, as seen by the efficient inactivation of *S. aureus* seeded in bagged platelets, as well as the

approximately normal recovery of light-treated platelets in the murine model (Maclean et al. 2020).

DISCUSSION

This study has presented an overview of the existing understanding of aBL with regard to its mechanism of antimicrobial action, efficacy with inactivating different pathogens and degrading contaminants, applications in clinical and public health contexts, and safety for host or human exposure. This study has also provided an assessment of the viability of potentially implementing aBL as a disinfecting protocol in settings such as environmental decontamination, food safety, treatment of localized infections, and decontamination of stored plasma and platelets.

Bacteria are the most thoroughly investigated and well-understood pathogen target of aBL. A widely accepted theory of the mechanism of inactivation of bacteria is that the blue light causes the photoexcitation of endogenous photosensitizers, namely porphyrins and flavins, which then precipitate the release of reactive oxygen species leading to toxicity and cell death (Hessling et al. 2020; Maclean et al. 2016; Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). There also appears to be consensus in the field that the sensitivity of a bacterium to a given wavelength of aBL may be affected by the type and

prevalence of endogenous photosensitizers within the bacterium, and the wavelength of peak absorbance specific to the endogenous photosensitizers that are present (Hessling et al. 2020; Maclean et al. 2016; Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). Porphyrins have been noted to have peak absorbance around a wavelength of 405-nm, and flavins around 470-nm, and the relative abundance of these two endogenous photosensitizers may contribute to the sensitivity of the bacterium to photoinactivation by certain wavelength of aBL (Hessling et al. 2020; Maclean et al. 2016; Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). While porphyrins and flavins seem to be the most ubiquitous photosensitizers, there has also been evidence of the possible involvement of other photosensitizers in the photoinactivation of bacteria by aBL (Hessling et al. 2020). An observed characteristic of bacterial inactivation by 405-nm aBL across studies is the greater susceptibility of gram-positive bacteria compared to gram-negative bacteria, with gram-negative bacteria generally requiring greater doses of light for inactivation (Guffey et al. 2016; Maclean et al. 2016). aBL has also shown promise for addressing the issue of bacterial resistance, as studies using *N. gonorrhoeae* and *S. aureus* have shown no significant change in bacterial reduction in response to 405-nm light even after repetitive exposure to successive cycles of high-intensity light (Wang, Ferrer-Espada, Baglo, Goh et al. 2019; Tomb, Maclean,

Coia, MacGregor, Anderson, 2017). The finding that *S. aureus* exposed to 405-nm light at a low-level during culture exhibited a level of tolerance to inactivation by high-intensity 405-nm light suggests that bactericidal levels of light should be used in practice, and also indicates a need for further investigation of the mechanism through which this tolerance is developed (Tomb, Maclean, Coia, MacGregor, Anderson, 2017).

Studies with fungi have shown aBL to have significant efficacy with reducing fungal burden (Hoenes et al. 2018; Yan et al 2018; Zhang et al. 2016). The presence of endogenous photosensitizers including porphyrins and flavins in *C. albicans* has been observed with fluorescence spectroscopy, which suggests the possibility that these photosensitizers play a role in the photoinactivation of *C. albicans*, as has been theorized to be the mechanism of photoinactivation in bacteria (Zhang et al. 2016). Studies using aBL of wavelength 405-nm and 415-nm have observed *C. albicans*' susceptibility at both wavelengths (Yan et al 2018; Zhang et al. 2016). There is a need for further investigation of fungal inactivation by aBL to characterize the effects of melanization by fungi in the context of infection, as this could potentially limit the efficacy of aBL (Zhang et al. 2016). While there is evidence of fungal susceptibility to aBL, further investigation will

be necessary in order to determine whether aBL is a viable antifungal treatment in practice.

Studies of viral inactivation by aBL also show some degree of antiviral efficacy. Because virions, unlike bacteria and fungi, do not contain endogenous porphyrins, there is relatively more ambiguity surrounding the mechanism and efficacy of photoinactivation (Kingsley et al. 2018). It has been observed that photoinactivation of viruses by aBL in the absence of other factors is limited, but is enhanced in the presence of singlet oxygen enhancers or nutrient-rich media containing photosensitive compounds (Tomb, Maclean, Coia, Graham et al 2017; Kingsley et al. 2018).

aBL has also been investigated as a tool for eliminating biofilms, which are of significant concern in contexts including environmental decontamination and treatment of localized infections due to their characteristic durability and decreased sensitivity to conventional antimicrobials (de la Fuente-Núñez et al. 2013; Liu et al. 2020; Wang et al. 2016). Studies show that treatment with aBL results in significant reduction in bacterial CFU when applied to monomicrobial and polymicrobial biofilms (Ferrer-Espada et al. 2019; Ferrer-Espada et al. 2020; Liu et al. 2020; Martegani et al. 2020).

Several studies have also shown that aBL could be a promising tool for environmental decontamination and non-clinical applications. Studies in food safety have shown aBL to be effective in reducing the bacterial or viral burden on the surface of food products, although there is a need for more research to optimize the antimicrobial effects and understand potential effects on the quality of the food products themselves (Guffey et al. 2016; Kingsley et al. 2018). aBL has been shown in multiple studies to be effective for reducing the bacterial burden within the clinical environment, as seen in studies of hospital isolation rooms, inpatient and outpatient burn treatment settings, and the hospital intensive care unit (Bache et al. 2011; Bache et al. 2017; Maclean et al. 2010; Maclean et al. 2013). There appears to be agreement amongst studies that aBL is a viable tool that could potentially supplement existing hospital decontamination practices and help reduce the spread of HAIs (Bache et al. 2011; Bache et al. 2017; Maclean et al. 2010; Maclean et al. 2013). In addition, visible blue light has been shown to be effective when used in conjunction with a novel photocatalyst to degrade gaseous formaldehyde, a major contributor to indoor air pollution that is injurious to health (Laciste et al. 2017).

Numerous studies have assessed the potential of aBL for use in clinical applications, including treatment of gonococcal infections, localized infections,

wound healing, and decontamination of stored platelets and plasma. A common finding among these studies is that pathogens appear to have greater susceptibility to aBL than normal host cells, such that there is a therapeutic window of exposure within which the pathogen would be selectively inactivated, and the host cells preserved (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019; Wang, Ferrer-Espada, Baglo, Goh et al. 2019; Zhang et al. 2016). These findings suggest that aBL could indeed be a viable treatment for infection in the clinical setting. The observation of recurrence of infection after light treatment seen in more than one *in vivo* study is of concern, and suggests that further investigation is needed to more effectively quantitate the prevalence of pathogen in the target area and more effectively administer the light to the entirety of the infected area (Zhang et al. 2016; Zhu et al. 2017). Additionally, aBL exposure has been shown to be efficacious at eliminating pathogens in stored platelets and plasma (Jankowska et al. 2020; Maclean et al. 2016; Maclean et al. 2020). It has also been seen that 405-nm light-treated platelets exhibit approximately normal recovery *in vivo* compared to untreated platelets in the murine model, which suggests that the visible light treatment does not substantially affect platelet function (Maclean et al. 2020).

The existing literature supports the efficacy of aBL as an antimicrobial tool in a number of applications including environmental decontamination and treatment of clinically relevant pathogens. It has been widely observed that although human cells are vulnerable to aBL at high doses, the doses that are lethal to many clinically relevant pathogens do not appear to cause damage to human cells (Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019; Wang, Ferrer-Espada, Baglo, Goh et al. 2019; Zhang et al. 2016). Although further studies will be needed to determine with more certainty the impact on normal human tissue and disease progression, current evidence points toward the viability of aBL for treatment of infections including otitis media, keratitis, gonococcal infections, and wound infections, as well as the decontamination of stored plasma and platelets for transfusion. At this time, bacteria are the most widely investigated target of 405-nm light treatment, and evidence across studies supports the hypothesized mechanism of bacterial photoinactivation involving the photoexcitation of endogenous photosensitizers and subsequent release of ROS and cytotoxicity (Hessling et al. 2020; Maclean et al. 2016; Wang, Ferrer-Espada, Baglo, Gu, Dai, 2019). Multiple studies have observed aBL to have efficacy in inactivating MDR bacteria, suggesting that the use of aBL can potentially help to slow the spread of antibiotic resistance and untreatable infections (Bumah et al.

2015; dos Anjos et al. 2019; Leanse et al. 2020; Wozniak et al. 2019). Further research is necessary to adequately understand the mechanism of inactivation for fungal and viral pathogens in order to better inform the manner in which aBL can be optimized to eliminate these pathogens in contexts such as the localized treatment of infections and decontamination of food and food processing environments. With regard to the application of aBL to disinfection of produce and other food products, further investigation will be needed to determine any adverse impacts on the nutritional content and shelf life of the light-treated foods. Another direction for future research in the use of aBL as an antimicrobial tool is to further identify new factors that can be used in conjunction with aBL to potentiate its antimicrobial effects.

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