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Antimicrobial blue light inactivation of pathogenic microbes: State of the art

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ABSTRACT

As an innovative non-antibiotic approach, antimicrobial blue light in the spectrum of 400–470 nm has demonstrated its intrinsic antimicrobial properties resulting from the presence of endogenous photosensitizing chromophores in pathogenic microbes and, subsequently, its promise as a counteracter of antibiotic resistance. Since we published our last review of antimicrobial blue light in 2012, there have been a substantial number of new studies reported in this area. Here we provide an updated overview of the findings from the new studies over the past 5 years, including the efficacy of antimicrobial blue light inactivation of different microbes, its mechanism of action, synergism of antimicrobial blue light with other angents, its effect on host cells and tissues, the potential development of resistance to antimicrobial blue light by microbes, and a novel interstitial delivery approach of antimicrobial blue light. The potential new applications of antimicrobial blue light are also discussed.

1. Introduction

Antibiotic resistance of pathogenic microbes is a quickly growing and extremely dangerous health threat. It is now indisputable that antibiotic resistance is life-threatening in the same sense as cancer, both in the number of cases and the likely outcome (Bush et al., 2011). The extensive use of antibiotics is the single most important factor leading to antibiotic resistance (Cowen et al., 2014; Hampton, 2013; Rice, 2003). There is consequently a critical need for the development of new approaches to tackle antibiotic resistance. Recently, Dr. Karen Bush and 29 other scientists who are experts in antibiotic resistance pointed out that:

> "*The investigation of novel non-antibiotic approaches for the prevention of and protection against infectious diseases should be encouraged, and such approaches must be high-priority research and development projects.*"
>
> Source: (Bush et al., 2011).

As an innovative non-antibiotic approach, antimicrobial blue light (aBL) in the spectrum of 400–470 nm has demonstrated its intrinsic antimicrobial properties resulting from the presence of endogenous photosensitizing chromophores in pathogenic microbes. It is envisioned that microbes are less able to develop resistance to aBL than to traditional antibiotics, because of the multi-target characteristics of aBL (Dai et al., 2012). In addition, it is well accepted that aBL is much less detrimental to host cells than UVC irradiation (Kleinpenning et al., 2010; Liebmann et al., 2010). Since we published our last review of aBL in 2012 (Dai et al., 2012), there have been a substantial number of new studies reported in this area. This review aims to update the findings from the new studies, including the efficacy of aBL inactivation of different microbes, its mechanism of action, synergism of aBL with other antimicrobials, its effect on host cells and tissues, the potential development of resistance to aBL by microbes, and a novel interstitial delivery approach of aBL. The potential new applications of aBL are also discussed.

2. Efficacy of antimicrobial blue light inactivation of pathogenic microbes

A wide range of microbial species was studied for aBL inactivation

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in the past five years, including Gram-positive bacteria, Gram-negative bacteria, mycobacteria, molds, yeasts and dermatophytes. The studies were carried out *in vitro* using planktonic cells or biofilms, *ex vivo*, and *in vivo* using animal models (pre-clinical) and in patients (clinical trials).

2.1. In vitro studies

2.1.1. Antimicrobial blue light inactivation of planktonic microbial cells

In vitro studies of planktonic microbial cells are usually performed in buffer suspensions or on agar plates.

2.1.1.1. Nosocomial bacterial pathogens. Healthcare-acquired infections (HAIs) are infections that patients acquire during the course of receiving treatment for other conditions in healthcare facilities (NIH, 2015). These infections are a leading cause of morbidity and mortality in the United States (Magill et al., 2014; McFee, 2009). According to recent studies, on any given day, about 1 in 25 patients has at least one HAI. There are an estimated 722,000 HAIs annually and about 75,000 patients with HAI die during their hospitalization (Magill et al., 2014). The total annual costs for HAI are as high as $10 billion in the United States (Zimlichman et al., 2013). The situation is exacerbated by the continued emergence of multidrug-resistant pathogenic microbes (Hidron et al., 2008; Sievert et al., 2013).

Halstead et al. (Halstead et al., 2016) recently assessed the effect of aBL at 400 nm from a light-emitting diode (LED) array on 34 bacterial strains commonly causing HAI, including *Acinetobacter baumannii, Enterobacter cloacae, Stenotrophomonas maltophilia, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Enterococcus faecium, Klebsiella pneumoniae*, and *Elizabethkingia meningoseptica*. All the bacteria in phosphate-buffered saline (PBS) suspensions were found to be susceptible to aBL inactivation, with the majority (71%) demonstrating a $> 5\text{-log}_{10}$ decrease in colony-forming units (CFU) after exposures of 54–108 J/cm^2 aBL.

The result of the above study is quantitatively in line with the observations in our group using aBL at 415 nm from LED arrays for inactivation of *P. aeruginosa, A. baumannii,* methicillin-resistant *Staphylococcus aureus* (MRSA), and *Candida albicans* in PBS suspensions. For *P. aeruginosa,* when 110 J/cm^2 aBL was delivered, an approximately 7.64-log_{10} CFU inactivation was achieved (Dai et al., 2013b). For *A. baumannii* and *C. albicans*, over 4-log_{10} CFU were inactivated after an exposure of 70 J/cm^2 aBL (Zhang et al., 2016; Zhang et al., 2014). The inactivation curves of the above pathogens approximately followed first-order kinetics (Xiong et al., 1999), a linear relation between the log-transformed cell survival fraction $\log_{10}$ and the aBL exposure. aBL inactivation of MRSA resulted in a survival kinetic with a shoulder or lag phase (Dai et al., 2013a). Only modest inactivation (0.17-log_{10}) was observed in the lag phase up until 56 J/cm^2 aBL was delivered. In the linear phase, approximately 4.75-log_{10} inactivation was achieved after 112 J/cm^2 more aBL was delivered.

Similar observations were also reported by Barneck et al. (Barneck et al., 2016). It was evidenced by the investigators that 405-nm aBL successfully inactivated *S. aureus*, *Streptococcus pneumoniae*, *E. coli*, and *P. aeruginosa* seeded on agar plates. At an exposure of 133 J/cm^2, reductions in $\log_{10}$ CFU were 6.27 for *E. coli*, 6.10 for *S. aureus*, 5.20 for *P. aeruginosa*, and 6.01 for *S. pneumoniae*. In another study from the same group of authors, the investigators found that β-lactam-resistant *E. coli*, which is resistant to penicillins, cephamycin, and carbapenems, is sensitive to aBL inactivation (Rhodes et al., 2016). Over 6-log_{10} CFU reduction of the β-lactam-resistant *E. coli* strain on agar plates was achieved after an exposure of 68 J/cm^2 aBL.

In another study, a panel of microbial isolates from cases of infected joint arthroplasty were tested (Gupta et al., 2015), including 39 isolates of Gram-positive bacteria (*S. aureus, S. epidermidis, E. faecalis, S. pneumoniae*, *Corynebacterium striatum*, Coagulase negative *Staphylococcus*, etc), 11 isolates of Gram-negative bacteria (*E. coli, K. pneumoniae, P. aeruginosa*, and *Serratia marcescens*) and one isolate of *C. albicans*. aBL was emitted from a high-intensity narrow-spectrum light at 405 nm. Complete inactivation ($> 4\text{-log}_{10}$ CFU) in suspensions was achieved in all of the isolates tested. The aBL exposures required for the complete inactivation of these pathogens ranged between 118 and 2214 J/cm^2, with *E. coli* showing the highest tolerance and *S. epidermidis* the lowest. In this study, the authors reported that Gram-positive bacteria were generally found to be more susceptible to aBL than Gram-negative bacteria.

Makdoumi et al. compared the antimicrobial efficacy of 412 and 450 nm against MRSA planktonic cells (Makdoumi et al., 2017). Fifteen (15) or 40 μL MRSA suspensions were placed on microscope slides, creating fluid layers with 0.40 mm thick. At an exposure of 5.4 J/cm^2, the reduction of MRSA CFU was minimal for both wavelengths. At an exposure of 28.5 J/cm^2, the CFU reductions were 72% with 412 nm and 81% with 450 nm. The investigators also showed that the addition of riboflavin increased the antimicrobial efficacy of both aBL wavelengths.

de Sousa analyzed the influence of aBL emitted from a blue laser on the bacterial growth of *S. aureus, P. aeruginosa,* and *E. coli* (de Sousa et al., 2015b). Bacterial suspensions were exposed to a single exposure of a 450-nm blue laser at varying radiant exposures from 0 to 24 J/cm^2. It was shown that the blue laser exhibited bacterial inhibitory effect against *S. aureus* and *P. aeruginosa* at the exposures > 6 J/cm^2, and against *E. coli* > 3 J/cm^2.

Fang et al. employed 470-nm aBL emitted from a LED-array to inactivate *P. aeruginosa* (Fang et al., 2015). Suspensions of *P. aeruginosa* were exposed to aBL at the irradiance of 100 mW/cm^2. After 80-min aBL exposure, 92.4% inactivation of *P. aeruginosa* was produced. The less effective inactivation of *P. aeruginosa* using 470-nm aBL is possibly due to the lack of sufficient amount of flavins in the bacterial strain used.

2.1.1.2. Periodontal bacterial pathogens. Periodontal diseases include infections that affect the periodontium. Bacterial plaque, a biofilm or mass of bacteria that develops over the surface of teeth, is the most common cause of periodontal disease. Left untreated, periodontal diseases can eventually result in tooth loss, and may have effects on general health. For example, periodontal disease has been associated with an increased risk of stroke, heart attack, and other health problems (Bouchard et al., 2017; Mira et al., 2017).

In a recent study, two anaerobic periodontal pathogens, *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, were found to be susceptible to aBL with the spectrum of 400–520 nm (Song et al., 2013). Results exhibited that complete inactivation (6-log_{10} CFU) of bacteria on agar plates was achieved at 30 J/cm^2 for *F. nucleatum* and 7.5 J/cm^2 for *P. gingivalis*. In another study, Yoshida et al. showed that *P. gingivalis* in suspensions was effectively inactivated with 460-nm LED, and an exposure of 100 J/cm^2 produced approximately 4-log_{10} CFU inactivation of *P. gingivalis* (Yoshida et al., 2017).

Cieplik et al. treated another periodontal pathogen, *Aggregatibacter actinomycetemcomitans*, with aBL at 460 nm derived from a LED (Cieplik et al., 2014). The investigators observed a $\geq 5\text{-log}_{10}$ reduction in CFU of *A. actinomycetemcomitans* in suspensions after an exposure of 150 J/cm^2 aBL, whereas no effect of aBL was found against *E. coli* at the same aBL exposure. Spectrally resolved measurements of singlet oxygen luminescence showed clearly that a singlet oxygen signal was generated from lysed *A. actinomycetemcomitans* upon excitation at 460 nm.

Chui et al. investigated whether exposure to aBL kills *P. gingivalis* or only inhibits its growth (Chui et al., 2012) under anaerobic conditions. *P. gingivalis* suspensions were exposed to aBL (425–500 nm) at 135 J/cm^2 anaerobically. The investigators observed delayed growth of *P. gingivalis* after the aBL exposure. The RNA integrity number value indicated no RNA degradation in the aBL-treated cultures. The expression levels of stress-related genes remained either constant or increased 15 min after the aBL exposure compared to that before the aBL exposure, thus suggesting that aBL may not kill *P. gingivalis* cells