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Killing Bacterial Spores with Blue Light: When Innate Resistance Meets the Power of Light

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Abstract

This article is a highlight of the study by Maclean *et al.* in this issue of *Photochemistry and Photobiology* describing the sporicidal effects 405 nm visible light alone on endospores of the *Clostridium* and *Bacillus* genera. 1.73 kJ cm^{-2} was capable of reducing endospore colony-forming units by up to 4-log_{10}. These findings have never been previously demonstrated and may be incorporated into decontamination methods that span medical, military and food preparatory applications.

INTRODUCTION

Since the discovery of bacterial endospores by Tyndall, Cohn and Koch in the late 1800s, spores have been heralded as the hardiest biological entities on earth (1). Microbial life first appeared on earth over 3.5 billion years ago, and microbes therefore evolved in extremely harsh environmental conditions. One subsection of Gram-positive Firmicutes, the low (G + C) Gram-positive bacteria, evolved the ability to endosporulate so as to lie dormant until environmental conditions favored germination and subsequent replication (2).

Microbial endospores are capable of withstanding excessive physical insults including heat, ionizing (ultraviolet and gamma) radiation, osmotic pressure and desiccation. The extreme resistance conferred by spores protects them from chemical and biological disinfectants such as iodine, peroxides, alkylating agents, formaldehyde and glutaraldehyde (1). Although this is biologically fascinating, endospore-forming bacteria are a formidable threat in medicine. A list of the most dangerous spore forming bacteria and their respective threats to human health—all of which are found in the *Clostridium* or *Bacillus* genera, low (G + C) Gram-positive bacilli—is found in Table 1. Note that these species are notoriously resistant to conventional antibiotic modalities in addition to antiseptic techniques.

The extreme resistance of endospores is illustrated by the historical story of Gruinard Island, a remote island off the west coast of Scotland (3). Gruinard was the site of a biological warfare test by British military scientists from Porton Down in 1942, during the Second World War. It was recognized that the tests would cause widespread and long-lasting contamination of the immediate area by anthrax spores. To limit contamination, a remote and uninhabited island was required. Gruinard was surveyed, deemed suitable and

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compulsorily purchased from its owners by the British Government. Military scientists exploded a series of anthrax-spore laden bombs, testing their killing efficiency using sheep (4). Initial efforts to decontaminate the island after the biological warfare trials had ended, failed due to the high durability of anthrax spores. For many years it was judged too hazardous and expensive to decontaminate the island sufficiently to allow public access. Gruinard Island was quarantined indefinitely as a result. Visits to the island were prohibited, except for periodic checks by Porton Down personnel to determine the level of contamination. Starting in 1986 (44 years later) a determined effort was made to decontaminate the island, with 280 tonnes of formaldehyde diluted in seawater being sprayed over all 196 hectares of the island and the worst-contaminated topsoil around the dispersal site being physically removed (5). A flock of sheep was then placed on the island and remained healthy (6).

The intentional attacks by anthrax-spore laden mailed envelopes in the US in the fall of 2001 led to a large international effort to investigate methods of decontaminating bacillus spores (7). *In situ* generated chlorine dioxide gas was determined to be the best method for buildings and inanimate surfaces (8).

In a 2005 landmark publication by Demidova and Hamblin (9), photodynamic therapy, previously shown to be ineffective in the removal of *Bacillus* spores, effectively and efficiently reduced *Bacillus* spore colony-forming units by 5 to 6-$\log_{10}$ provided phenothiazinium photosensitizers (50 μM toluidine blue O incubated for 3 h and followed by 20 J cm^{-2} red light) were used. This finding demonstrated that, despite the high resistance to chemical stressors, *Bacillus* spores are susceptible to destruction by reactive oxygen species (ROS) including partially reduced oxygen species such as the hydroxyl radical or superoxide radical anion and the nonradical species, excited singlet state oxygen.

Up until now, all physical methods of destroying endospores (*e.g.* heat, radiation, etc.) have been relatively ineffective, and the application of electromagnetic radiation in the destruction of endospores was, as documented by Demidova and Hamblin, was absolutely dependent on the addition of an exogenous photosensitizer. In this issue of *Photochemistry and Photobiology*, work by Maclean *et al.* of the University of Strathclyde in Glasgow, Scotland has demonstrated (10) that high-intensity, nonionizing blue light is capable of destroying *Bacillus and Clostridium* endospores with an impressive reduction of 4 $\log_{10}$ colony-forming units. Testing 1.73 kJ cm^{-2} of 405 nm light, Maclean's team was able to inactivate *B. cereus*, *B. megaterium*, *B. subtilis* and *C. difficile* endospores. It is important to note that Demidova and Hamblin's photoinactivation procedures were incapable of killing *B. megaterium*. Maclean also verified that their high-intensity light technique does not encourage endospore germination. The efficacy of this 405 nm therapy may be explained by the presence of endogenous porphyrins such as coproporphyrin with Soret bands in the 400–420 nm regions of the visible spectrum in *Bacillus* and *Clostridium* bacteria (11–13). Thus, the sporicidal effect of blue light is probably an oxygen-dependent process akin to the light-mediated destruction of *Helicobacter pylori* and *Propionobacterium acnes*—a photodynamic inactivation relying on pathogen's biosynthesis of endogenous photosensitizers (14, 15). The mechanisms of blue light spore inactivation and photodynamic therapy (PDT) spore inactivation is probably fairly similar, especially when the PDT is mediated by porphyrins and other tetrapyrroles that mainly produce singlet oxygen. In case of other photosensitizers that produce hydroxyl radicals, the mechanisms may have some differences.

Blue light has recently attracted much attention as an alternative antimicrobial approach (16) due to its intrinsic antimicrobial properties without the involvement of added exogenous photosensitizers (17). Blue light can be sensed by numerous microorganisms and can induce physiological responses elicited by blue light receptors (18). As a result of this, blue light

can regulate bacterial motility, suppress biofilm formation and potentiate light inactivation of bacteria. On the other hand, the presence of blue light may also up-regulate bacterial virulence factors (19).

Studies have found that blue light inactivation of bacteria is oxygen dependent (20, 21), suggesting that the antimicrobial effect of blue light is associated with the photoexcitation of intracellular chromophores and the subsequent generation of cytotoxic ROS such as singlet oxygen. Intracellular photosensitizing porphyrins were found in *P. acnes* (22), *H. pylori* (23) and some oral bacteria (24). Different porphyrin patterns were observed in different bacteria.

Blue light therapy is now an accepted protocol for acne vulgaris (25–28), and additional clinical trials have been carried out using blue light for *H. pylori* gastritis showing promising outcomes (14, 29). Blue light may also be effective in oral infections by killing dental anaerobic bacterial species.

In comparison to PDT, blue light inactivates bacteria without the involvement of exogenous photosensitizer. As a result, the use of blue light inactivation is technically easier to carry out. Delivery of photosensitizers to the target microbes embedded deep within tissue has been somewhat challenging when PDT is used for infectious diseases. One question that will have to be addressed is “Can microbial cells develop resistance to blue light inactivation?” To our knowledge, this question has not yet been experimentally addressed. The possible development of microbial resistance to photodynamic inactivation has been studied. After repeated cycles of partial inactivation followed by regrowth, different bacterial species failed to develop resistance to the photodynamic process after 10 (30) or even 20 cycles (31). At the very least, it will be necessary to repeatedly deliver suberadication doses of blue light to susceptible cultures with regrowth between cycles to investigate whether resistant clones can be selected, or mutants with increased blue light damage repairing enzymes can be produced.

Although the amount of light energy needed to kill endospores in Maclean’s study was 10-fold the Joule output needed to kill vegetative *B. cereus* and *C. difficile*, and is also considered too intense for therapeutic purposes, the light-mediated killing of endospores is of considerable antiseptic and decontamination interest. High-intensity 405 nm light may have application in the medical, military and agricultural fields to combat *B. anthracis* spore exposure. *B. anthracis* is known to have endospores of comparable robustness to *B. cereus* and *B. subtilis*; noting the findings of this study, 405 nm light may find application in the removal of *B. anthracis* from medical and military devices (32–34). Simple speculation suggests several applications of sporicidal high-intensity 405 nm light: hospital rooms may be decontaminated of *C. difficile* and *C. perfringens* spores, agricultural products and tools may be cleaned of *C. tetani* and *C. botulinum* and food preparatory devices may be disinfected of *B. cereus*.

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Table 1

Medically relevant endospore-forming bacteria, and their respective abilities to cause diseases harmful to human health.

Endospore-forming bacterium	Human health threat	References
Bacillus anthracis	Anthrax *via* the anthrax toxin	(35)
Bacillus cereus	Foodborne illness	(36)
Clostridium botulinum	Botulism *via* botulinum toxin	(37)
Clostridium difficile	Pseudomembranous colitis, toxic megacolon	(38)
Clostridium perfringens	Bacteremia, clostridial myonecrosis (gas gangrene), foodborne illness all *via* the *C. perfringens* alpha toxin	(39)
Clostridium tetani	Tetanus *via* tetanospasmin	(40)