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In Vitro Bactericidal Effects of 405-nm and 470-nm Blue Light

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ABSTRACT

Objective: **The aim of this study was to determine the bactericidal effect of 405- and 470-nm light on two bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa, in vitro*. *Background Data:* It is well-known that UV light kills bacteria, but the bactericidal effects of UV may not be unique since recent studies indicate that blue light produces a somewhat similar effect. The effects of blue light seem varied depending on wavelength, dose and the nature of the bacteria, hence this study. *Methods:* Two common aerobes, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and anaerobic *Propionibacterium acnes* were tested. Each organism was treated with Super Luminous Diode probes with peak emission at 405 and 470 nm. Treatment was timed to yield 1, 3, 5, 10, and 15 Jcm^{-2} doses. Colony counts were performed and compared to untreated controls. *Results:* The 405-nm light produced a dose dependent bactericidal effect on *Pseudomonas aeruginosa* and *Staphylococcus aureus* ($p < .05$)), achieving as much as 95.1% and nearly 90% kill rate for each, respectively. The 470-nm light effectively killed *Pseudomonas aeruginosa* at all dose levels, but only killed *Staphylococcus aureus* at 10 and 15 $J\ cm^{-2}$. With this wavelength, as much as 96.5% and 62% reduction of *Pseudomonas aeruginosa* and *Staphylococcus aureus* was achieved, respectively. Neither of the two wavelengths proved bactericidal with anaerobic *Propionibacterium acnes*. *Conclusion:* The results indicate that, *in vitro*, 405- and 470-nm blue light produce dose dependent bactericidal effects on *Pseudomonas aeruginosa* and *Staphylococcus aureus* but not *Propionibacterium acnes*.**

INTRODUCTION

LIGHT THERAPY has been suggested as a potentially effective treatment for a variety of human conditions. Suggested amenable conditions range from sleep disorders,[1] photoaged facial skin,[2] depression in the elderly,[3] and treatment of acne vulgaris[4] to a variety of neuromusculoskeletal conditions such as peripheral neuropathy,[5] second degree ankle sprains,[6] and osteoarthritis of the knee[7] and cervical spine.[8]

Papageorgiou et al.[9] reported a significant improvement in the condition of patients suffering from acne vulgaris resulting from exposure to a combination of red (660-nm) and blue (415-nm) light. They postulated that this combination provided both an anti-inflammatory benefit (red light) and an antibacterial benefit (blue light). Blue light has been shown to kill bacteria in the tissue.[16] Studies[10,11] have demonstrated bactericidal results using light therapy at 810 and 630 nm. Low-level light therapy at 685 and 830 nm has recently been shown to increase collagen production and organization resulting in improved wound repair.[12]

Not all studies dealing with the application of light therapy have demonstrated a bactericidal effect. The results seem to be associated with wavelength and type of organism. Nussbaum et al.,[13] while reporting a bactericidal effect at 630 nm for *Pseudomonas aeruginosa* and *E. coli*, also found that a wavelength of 810 nm facilitated growth of *E. coli.* These researchers also noted that growth of *Staphylococcus aureus* was facilitated by exposure to a wavelength of 905 nm.

With such an intriguing array of conditions that might respond to light therapy, we attempted to evaluate blue light therapy in terms of its bactericidal potential. As mentioned above, some studies[4,9–11,16] have suggested that light therapy may retard

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bacterial growth. The potential to positively impact various skin/wound conditions would be great if such a simple and relatively inexpensive treatment could be shown to significantly reduce bacterial growth.

The purpose of this study was to evaluate, *in vitro*, the bactericidal effect of blue light exposure (405 and 470 nm) on three common organisms (*Staphylococcus aureus, Pseudomonas aeruginosa*, and *Propionibacterium acnes*) associated with skin conditions.

METHODS

The strains of the organisms tested were *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853), both aerobes, and *P. acnes* (ATCC 11827), an anaerobe. All organisms tested were from a 20-h-old culture from which a suspension equal to McFarland Standard 0.5 was prepared. Use of a 20-h-old culture is standard microbiological practice and serves to minimize the lag time for new growth. For *S. aureus* and *P. acnes*, the suspensions were further diluted 1:1000. For *P. aeruginosa* the suspension was diluted 1:2000. All dilutions were made immediately before the treatment with various wavelengths of light.

One microliter of dilutions of *S. aureus* and *P. aeruginosa* was inoculated on tryptic soy agar plates in a star-streak pattern for colony counts. The experimental treatment consisted of six subcomponents. Each organism was prepared (n = 10 plates) and treated at one wavelength (five separate doses, two plates each dose) on one day. The process was repeated a total of six times on separate days. Controls for a given day's subcomponent consisted of two plates handled under the same conditions of ambient light and temperature as the treated plates. Treated plates received exposures to blue light of 1, 3, 5, 10, and 15 Joules per square centimeter (Jcm^{-2}). Related quantitative data can be found in Tables 1–6.

The treated and control plates were incubated at 35°C under aerobic conditions for *S. aureus* and *P. aeruginosa*. Colony counts of *S. aureus* and *P. aeruginosa* were made after 18 h of incubation, with a confirmation of the counts at 24–48 h to rule out a bacteristatic effect. Aliquots of the dilution of *P. acnes* in a Petri dish were subjected to the same treatment and control methods as described above. Due to the nature of the organism, *P. acnes* was inoculated on sheep blood agar plates. The treated and control plates were placed in GasPak anaerobic pouches

TABLE 1. BACTERICIDAL EFFECT OF 405-NM LIGHT: *PSEUDOMONAS AERUGINOSA*

Dose (Jcm^{-2})	*Colonies* (*mean ± SD*)	*N*	*Percent change*	*t*	*p-value*
Control	81.0 ± 9.89	2			
1	25.5 ± 4.95	2	−68.5	15.857	0.04
3	11.5 ± 2.12	2	−85.8	12.636	0.05
5	7.5 ± 4.95	2	−90.7	21.000	0.03
10	4.0 ± 1.41	2	−95.1	12.833	0.05
15	8.5 ± 3.53	2	−89.5	16.111	0.04

TABLE 2. BACTERICIDAL EFFECT OF 405-NM LIGHT: *STAPHYLOCOCCUS AUREUS*

Dose (Jcm^{-2})	*Colonies* (*mean ± SD*)	*N*	*Percent change*	*t*	*p-value*
Control	152.7 ± 13.43	2			
1	281.5 ± 9.19	2	+84.3	−43.000	0.02[a]
3	58.5 ± 4.94	2	−61.7	15.667	0.04
5	67.0 ± 7.07	2	−56.1	19.000	0.03
10	36.5 ± 3.53	2	−76.1	16.571	0.04
15	18.5 ± 2.12	2	−87.9	16.750	0.04

[a]This dose level demonstrated a stimulatory effect.

TABLE 3. BACTERICIDAL EFFECT OF 405-NM LIGHT: *PROPIONIBACTERIUM ACNES*

Dose (Jcm^{-2})[a]	*Colonies* (*mean ± SD*)	*N*	*Percent change*	*t*	*p-value*
Control	5.5 ± 2.21	2			
1	14.5 ± 3.53	2	+163.6	−2.250	0.3
3	15.5 ± 4.95	2	+181.8	−5.000	0.1
5	32.0 ± 11.31	2	+481.8	−4.077	0.2
10	22.0 ± 4.24	2	+300.0	−11.000	0.06
15	29.0 ± 2.83	2	+427.3	−47.000	0.01

[a]Each dose level demonstrated a stimulatory effect.

TABLE 4, BACTERICIDAL EFFECT OF 470-NM LIGHT: *PSEUDOMONAS AERUGINOSA*

Dose (Jcm^{-2})	*Colonies* (*mean ± SD*)	*N*	*Percent change*	*t*	*p-value*
Control	114.5 ± 7.77	2			
1	45.5 ± 6.36	2	−60.3	69.000	0.01
3	62.0 ± 5.65	2	−45.9	5.526	0.10
5	40.0 ± 4.24	2	−96.5	29.800	0.02
10	58.5 ± 13.43	2	−48.9	14.000	0.05
15	69.5 ± 9.19	2	−39.3	45.000	0.01

TABLE 5. BACTERICIDAL EFFECT OF 470-NM LIGHT: *STAPHYLOCOCCUS AUREUS*

Dose (Jcm^{-2})	*Colonies* (*mean ± SD*)	*N*	*Percent change*	*t*	*p-value*
Control	93.5 ± 4.95	2			
1	113.5 ± 16.26	2	+21.4	−2.500	0.20
3	108.0 ± 5.66	2	+15.5	−29.000	0.02[a]
5	99.0 ± 46.66	2	+5.9	−0.186	0.90
10	71.5 ± 13.43	2	−23.5	3.667	0.17
15	35.5 ± 2.12	2	−62.0	29.000	0.02

[a]This dose level demonstrated a stimulatory effect.

TABLE 6. BACTERICIDAL EFFECT OF 470-NM LIGHT: *PROPIONIBACTERIUM ACNES*

Dose (Jcm^{-2})	*Colonies (mean ± SD)*	*N*	*Percent change*	*t*	*p-value*
Control	161.0 ± 29.69	2			
1	178.0 ± 16.97	2	+10.6	−1.889	0.31
3	252.0 ± 12.73	2	+56.5	−7.583	0.08
5	224.0 ± 8.48	2	+39.1	−4.200	0.15
10	179.0 ± 22.62	2	+11.2	−0.486	0.71
15	244.0 ± 19.79	2	+51.6	−12.000	0.05

(Becton Dickinson Microbiological Systems, Sparks, MD) with anaerobic indicators, and incubated at 35°C. Incubation was monitored for 3–5 days at 35°C, attainment of anaerobic conditions was verified, and, when colonies were well developed, counts were made.

Light exposures were achieved using The Dynatron® Solaris™ manufactured by Dynatronics Corp. (Salt Lake City, UT). This device is designed to accommodate a variety of light probes. For this experiment, we chose to illuminate the cultures using two different wavelength bands, each produced by a different SLD light probe that emitted a band of light focused around a primary wavelength. The two primary wavelengths used were 405 and 470 nm. The specifics of each light probe in terms of diode composition and power output are listed below. Each probe consisted of a 5-cm^2 illuminating surface area.

A. The 405-nm probe was composed of 32–405-nm SLDs and 4–470-nm SLDs with an average power output of 160 mW.
B. The 470 was composed of 32–470-nm and 4–660-nm SLDs producing 150 mW of power.

Dose was calculated in Jcm^{-2}. Since output for each probe was held constant, adjustment in time of irradiation provided the scale of doses (1, 3, 5, 10, 15 Jcm^{-2}). The Dynatron® Solaris™ automatically calculates time of irradiation when desired dosage is selected.

Statistical analysis

Data were analyzed using Student *t*-tests.

RESULTS

The 405 nm light produced a dose dependent bactericidal effect on the two aerobes, *Pseudomonas aeruginosa and Staphylococcus aureus* (Tables 1 and 2; Figs. 1 and 2). As much as 95.1% and nearly 90% kill rates were achieved with *Pseudomonas aeruginosa and Staphylococcus aureus*, respectively (Tables 1 and 2). The 470-nm light effectively killed *Pseudomonas aeruginosa* at all dose levels, but only killed *Staphylococcus aureus* at high dosages, 10 and 15 J cm^{-2}. As much as 96.5% and 62% reduction was achieved with *Pseudomonas aeruginosa and Staphylococcus aureus* respectively. Neither of the two wavelengths proved bactericidal when applied to the anaerobic *Propionibacterium acnes* (Tables 3 and 6; Figs. 3–6).

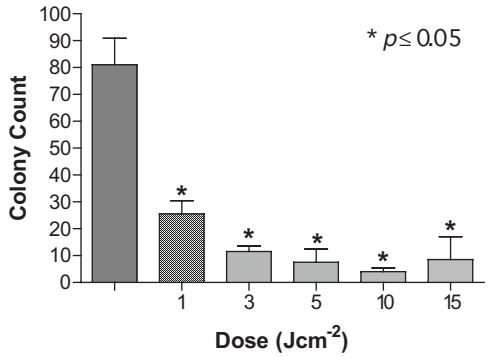

FIG. 1. Bactericidal effects of 405-nm light: *Pseudomonas aerugilosa.*

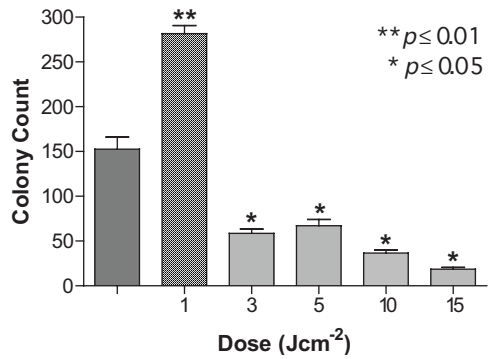

FIG. 2. Bactericidal effects of 405-nm light: *Staphylococcus aureus.*

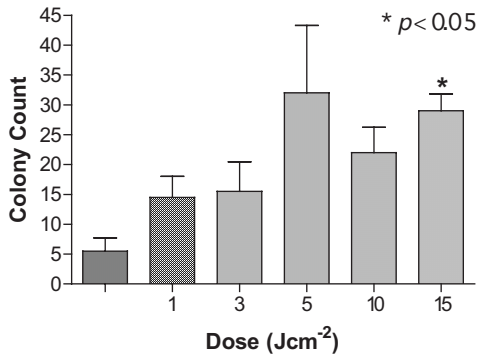

FIG. 3. Bactericidal effects of 405-nm light: *Propionibacterium acnes.*

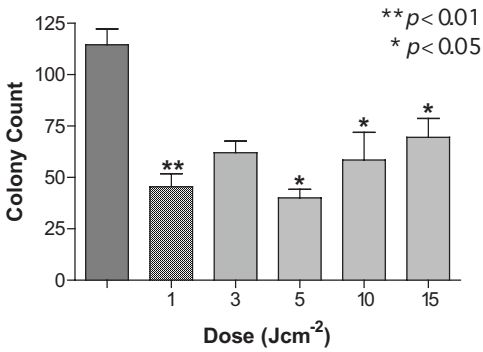

FIG. 4. Bactericidal effects of 470-nm light: *Pseudomonas aeruginosa.*

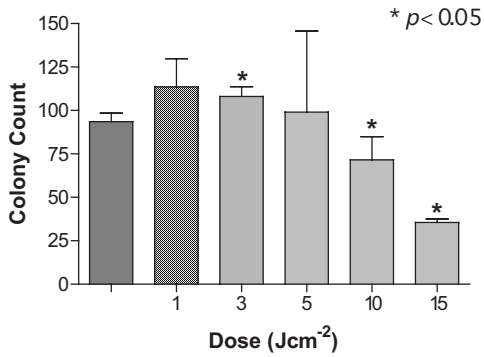

FIG. 5. Bactericidal effects of 470-nm light: *Staphylococcus aureus.*

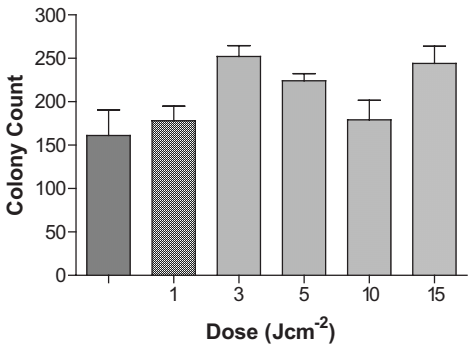

FIG. 6. Bactericidal effects of 470-nm light: *Proprionibacterium acnes.*

DISCUSSION

Determining the degree to which any treatment technique might be effective is an important obligation of all researchers and clinicians. Light therapy has, as discussed above, begun to be associated with various treatment possibilities. Because the facilitation of wound healing[12] is one of the applications that has been suggested, and because others have reported a bactericidal effect,[4,9–11,16] we directed our efforts to evaluate the bactericidal effect of two wavelengths of light *in vitro*. We chose the "blue" band of the visible light spectrum for two reasons. First, Papageorgiou et al.[9] set out a possible explanation for how light exposure might be bactericidal. Their model identified near ultraviolet and blue light as the important wavelengths. Secondly, we intend to continue examining various wavelengths in terms of retardation of bacterial growth and felt it logical to begin at the lower end of the visible spectrum and work toward the infrared range.

Both wavelengths produced a bactericidal effect on the aerobic organisms. The mechanism(s) for the bactericidal effect that we observed was not addressed by our design, but others have offered possible explanations. Light may be absorbed by porphyrins produced by bacteria or result in increased free radicals,[14] which may affect cytoplasmic membrane proteins and DNA,[15] or have a direct effect on photolabile pigments in bacteria.[17] These potentials will need to be examined by future studies.

It is interesting that we found no bactericidal effect when these wavelengths were used to irradiate *Propionibacterium acnes* (Tables 3 and 6). Others[9] have at least suggested such an effect associated with "blue" light when used to treat the same organism. We have no explanation for this finding, but can report that the processes of culturing and growing this organism for testing *in vitro* are tedious. Papageorgiou et al.[9] treated *in vivo*. We do not believe this complication had any impact on our results, but future investigations may yield differing results.

Dose appears to be a critical issue. *Pseudomonas aeruginosa* growth was negatively impacted at all doses, but the bactericidal effect peaked at 10 Jcm^{-2} for the 405-nm light and at 5 Jcm^{-2} for the 470-nm light (Tables 1 and 4). *Staphylococcus aureus*, while also negatively impacted by blue light irradiation, required a higher dose for a similar rate of bacterial kill. Using the 405-nm light, a dose of 15 Jcm^{-2} was needed to approach the 90% kill rate seen with *Pseudomonas aeruginosa* achieved at 5 Jcm^{-2}. When 470 nm was applied to *Staphylococcus aureus*, we observed a linearly progressive kill rate. However, even at 15 Jcm^{-2} only a 62% kill rate could be achieved (Table 5). Based on the results shown in Table 5, one could expect that a higher dose might have produced the higher (87.9%) kill rate obtained with 405-nm light.

The potential clinical application of these results is high. The delivery of the light energy used in this investigation is a simple process. The probe construction allows for a hand held application to a circumscribed site. This arrangement would nicely adapt to an open wound such as is commonly seen secondary to pressure or trauma. While two of the organisms we tested are common to most open wounds, these results suggest that the identification of the particular organism resident in the wound may be critical. A dose of 5–15 Jcm^{-2} appear to be sufficient for significant bacterial kill when the organism is *Pseudomonas aeruginosa*. When *Staphylococcus aureus* is present, a higher dose appears to be indicated. *Staphylococcus aureus* appears to be more resistant to being killed by light energy in these wavelengths. Some organisms do not appear to respond to light therapy in the wavelengths we have tested; indeed these wavelengths seem to stimulate their growth as evidenced by our results with *Propionibacterium acnes*. Other wavelengths might be more effective; supporting the argument that knowledge of the organism, associated dose, and wavelength are critical.

CONCLUSION

The following conclusions were drawn from the data collected in this study:

1. Irradiation with blue light (405 and 470 nm) resulted in a bactericidal effect on both *Staphylococcus aureus* and *Pseudomonas aeruginosa.*
2. These wavelengths of blue light had no bactericidal effect on *Propionibacterium acnes.*
3. The results from this study indicated that the optimal dose of blue light for the treatment of *Pseudomonas aeruginosa* was 5–15 Jcm^{-2}. *Staphylococcus aureus* did respond to the 405-nm light at these doses and when 470-nm light was used a stronger dose, 10–15 J cm^{-2} was needed.

We recommend that future studies be directed toward the mechanisms involved in the observed bactericidal effect.

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