Effects of Combined 405-nm and 880-nm Light on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in Vitro

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ABSTRACT

**Objective:** The aim of this study was to determine the effect of a combination of 405-nm blue light and 880-nm infrared light on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in vitro. **Background Data:** Reports indicate that certain wavelengths and treatment parameters of light promote the growth of bacteria, but our earlier study indicates that light at specific wavelengths and intensities are bactericidal for specific organisms (1). **Methods:** Two common aerobes, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were tested because of their frequent isolation from skin infections and wounds. Each organism was treated simultaneously with a combination of 405-nm and 880-nm light emitted by a cluster of Super Luminous Diodes (SLDs). Doses of 1, 3, 5, 10, and 20 J cm$^{-2}$ were used. Colony counts were performed and compared to untreated controls using Student *t* tests and one-way ANOVA with Tukey and Scheffe post hoc analyses. **Results:** The results revealed significant dose-dependent bactericidal effects of the combined blue and infrared light on *Staphylococcus aureus* ($F_{4,94} = 5.38, p = 0.001$) and *Pseudomonas aeruginosa* ($F_{4,95} = 21.35, p < 0.001$). With *P. aeruginosa*, the treatment reduced the number of bacteria colonies at all doses, achieving statistical significance at 1, 3, and 20 J cm$^{-2}$ doses and reducing bacterial colony by as much as 93.8%; the most effective dose being 20 J cm$^{-2}$. Irradiation of *S. aureus* resulted in statistically significant decreases in bacterial colonies at all dose levels; the most decrease, 72%, was also achieved with 20 J cm$^{-2}$. **Conclusion:** Appropriate doses of combined 405-nm and 880-nm phototherapy can kill *Staphylococcus aureus* and *Pseudomonas aeruginosa* in vitro, suggesting that a similar effect may be produced in clinical cases of bacterial infection.

INTRODUCTION

Light therapy has been suggested as a potentially effective treatment for a variety of human conditions. Suggested amenable conditions range from sleep disorders,2 photoaged facial skin,3 depression in the elderly,4 and treatment of acne vulgaris5 to a variety of neuromusculoskeletal conditions such as peripheral neuropathy,6 second degree ankle sprains,7 and osteoarthritis of the knee8 and cervical spine.9 Papageorgiou, Katsambas and Chu10 reported a significant improvement in the condition of patients suffering from acne vulgaris when treated with 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 effect (blue light). Blue light has been shown to kill bacteria in tissue.17 Studies11,12 have demonstrated bactericidal results using light therapy at 810 nm and at 630 nm. Phototherapy at 685 and 830 nm has recently been shown to increase collagen production and organization resulting in improved wound repair.13 Not all studies dealing with the application of light therapy have demonstrated a bactericidal effect. The results seem to be associated with both specific wavelengths and type of organism. Nussbaum et al.,14 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*.
Combined 405-nm and 880-nm Light in S. aureus and P. aeruginosa

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 for its bactericidal potential. As mentioned above, some studies\(^6,7,11,17\) have suggested that light therapy may retard 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*, whether the bactericidal effect we have already demonstrated\(^1\) for blue light (405 nm) would continue to result when this blue wavelength was combined with an infrared wavelength (880 nm) and irradiated upon two common organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) associated with skin conditions. We chose to examine this combination because recent literature\(^13,14,19,20\) has suggested that infrared wavelengths have the potential to promote wound healing. If promotion of wound healing can be achieved with certain wavelengths of light and bactericidal effect can be achieved simultaneously by combining such wavelengths with other specific wavelengths, then the potential for light as a treatment armamentarium for infected wounds would be immense.

**METHODS**

The strains of the organisms tested were *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853), both are aerobes. 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*, the suspensions were further diluted 1:1000. For *P. aeruginosa*, the suspension was diluted 1:2000. All dilutions were made immediately before the treatment.

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. Control counts were on plates receiving no light treatment. Counts were done at exposures to blue light of 1, 3, 5, 10, and 20 joules per square centimeter (Jcm\(^{-2}\)). The plates were incubated at 35°C under aerobic conditions and controlled humidity. Colony counts of *S. aureus* and *P. aeruginosa* were made after 18 hours of incubation, with a confirmation of the counts at 24–48 h to rule out a bacteriastic effect.

Light exposures were achieved using The Dynatron\(^\circledR\) Solaris\(^\text{TM}\) manufactured by Dyontronics 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, both simultaneously produced by a cluster of SLDs that emitted a band of light focused around the two (405 and 880 nm) primary wavelengths. The probe consisted of a 5-cm\(^2\) illuminating surface area. Additional specifics related to the light probe are as follows:

A. The probe was composed of both 405-nm and 880-nm SLDs.
B. The total power output of the probe was 450 milliwatts (mW).

**RESULTS**

As shown in Tables 1 and 2, the combined 405 and 880 nm light effectively killed both bacteria depending on the dose used. With *P. aeruginosa*, the treatment reduced the number of bacteria colonies at all doses, achieving statistical significance at 1, 3, and 20 J cm\(^{-2}\) doses but not at 5 and 10 J cm\(^{-2}\) (Table 1). The percent decrease in bacterial colony ranged from 3.6 to 93.8; the most effective dose being 20 J cm\(^{-2}\). One-way ANOVA revealed significant dose effect differences between groups \((F_{4,95} = 21.35, p < 0.001)\), with post-hoc analysis confirming that 20 J/cm\(^2\) was the most effective dose \((p < 0.001)\).

Irradiation of *S. aureus* resulted in statistically significant decreases in bacterial colonies at all dose levels. The corresponding percent decrease ranged from 39 to 72, again with 20 Jcm\(^{-2}\) being the most effective. Similarly, one-way ANOVA revealed significant dose effect differences between groups \((F_{4,84} = 5.38, p = 0.001)\), with post-hoc analysis confirming that 20 Jcm\(^{-2}\) was the most effective dose \((p = 0.022)\).

**DISCUSSION**

The degree to which various treatment techniques 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 it promotes wound healing\(^7,10,16-20\) and has potential bactericidal effect,\(^5,10,11,12,17\) we directed our efforts to evaluate the bactericidal effect *in vitro*, when a wavelength demonstrated to have bactericidal effects\(^7\) was combined with a wavelength thought to have wound healing properties.\(^18-20\) We undertook this effort for four reasons. First, Papageorgiou et al.\(^10\) 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 ourselves demonstrated that blue light has a bactericidal effect in vitro. Thirdly, others continue to report positive wound healing outcomes when light is used. Finally, the potential clinical value of combining wavelengths to kill bacteria and facilitate of tissue repair is significant. If one accepts for the moment that blue light is bactericidal and infrared light can promote healing, the question is whether combining the two significantly retards either outcome.

This combination of wavelength bands did produce a bactericidal effect on these aerobic organisms. The mechanisms for the bactericidal effect that we observed were 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, it may affect cytoplasmic membrane proteins and DNA, or it may have a direct effect on photolabile pigments in bacteria.

Dose and wavelength appear to be critical issues. *Pseudomonas aeruginosa* growth was negatively impacted at all doses, but the bactericidal effect peaked at 20 J cm\(^{-2}\) (Table 1). In our earlier work, where 405-nm light was applied alone, we found *Pseudomonas aeruginosa* growth to be much more significantly retarded at lower doses (1, 3, 5, and 10 J cm\(^{-2}\)). The combination of the 880 nm light, while not completely removing the bactericidal impact of 405 nm light on *Pseudomonas aeruginosa*, lessened the effect.

In our earlier work, *Staphylococcus aureus* was also negatively impacted by light irradiation, but required higher dosages for the type of bacterial kill we observed with *P. aeruginosa*. Using the 405-nm light, a dose of 15 J cm\(^{-2}\) was needed to approach the 90% kill rate seen with *Pseudomonas aeruginosa* achieved at 5 J cm\(^{-2}\). In the present study, the bactericidal effect seen on *Staphylococcus aureus* (Table 2) when the combination of 405 and 880 nm light was used more closely followed our original work. That is to say, the combination of wavelengths had a much less deleterious impact on the kill rate for *Staphylococcus aureus* than it did for *Pseudomonas aeruginosa*.

The potential clinical application of these results is promising. The delivery of the light energy used in this investigation is a simple process. The probe construction allows for a handheld 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 3–20 J cm\(^{-2}\) does appear to be sufficient for significant bacterial kill when the organism is *Pseudomonas aeruginosa*. These results strongly support the higher end of the range for this organism. When *Staphylococcus aureus* is present, doses of 1, 3, 10, and 20 J cm\(^{-2}\) all have the potential to give over a 50% kill rate. While in our initial work *Staphylococcus aureus* proved slightly more resistant to light energy at 405 nm compared to *Pseudomonas aeruginosa*, the kill rate for *Staphylococcus aureus* in this study using the combination of 405 and 880 nm was not lessened to any great degree. This was not the case when the combination was used on *Pseudomonas aeruginosa*.

Our initial work strongly supported the use of 405-nm light to produce a bactericidal effect on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This work examining the combination of 405- and 880-nm light continues to support the bactericidal benefits of light. However, there is little doubt that these results do indicate some loss of bactericidal impact when the 880-nm light is combined (specifically in relation to *Pseudomonas aeruginosa*). The question that is not addressed by our research is whether the loss of bactericidal impact is offset by a significant increase in tissue repair. This question requires further investigation. The combination of these wavelengths may well produce excellent clinical outcomes. It is also possible that treatment should be individually designed. It may be that

### Table 1. Bactericidal Effect of Combination 405-nm and 880-nm Light: *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>Dose (J cm(^{-2}))</th>
<th>Treated (mean ± SD)</th>
<th>Control (mean ± SD)</th>
<th>Percent change</th>
<th>N</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>352.00 ± 93.1</td>
<td>400 ± 17.32</td>
<td>-12.0</td>
<td>30</td>
<td>0.009</td>
</tr>
<tr>
<td>3</td>
<td>337.00 ± 71.6</td>
<td>400 ± 17.32</td>
<td>-15.8</td>
<td>30</td>
<td>0.000</td>
</tr>
<tr>
<td>5</td>
<td>384.00 ± 81.9</td>
<td>400 ± 17.32</td>
<td>-4.0</td>
<td>30</td>
<td>0.337</td>
</tr>
<tr>
<td>10</td>
<td>268.00 ± 22.3</td>
<td>278 ± 43.31</td>
<td>-3.6</td>
<td>5</td>
<td>0.742</td>
</tr>
<tr>
<td>20</td>
<td>17.80 ± 5.7</td>
<td>286.4 ± 63.93</td>
<td>-93.8</td>
<td>5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### Table 2. Bactericidal Effect of Combination 405-nm and 880-nm Light: *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>Dose (J cm(^{-2}))</th>
<th>Treated (mean ± SD)</th>
<th>Control (mean ± SD)</th>
<th>Percent change</th>
<th>N</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95.20 ± 51.1</td>
<td>195 ± 27.48</td>
<td>-51.2</td>
<td>30</td>
<td>0.000</td>
</tr>
<tr>
<td>5</td>
<td>54.00 ± 28.7</td>
<td>195 ± 27.48</td>
<td>-72.3</td>
<td>30</td>
<td>0.000</td>
</tr>
<tr>
<td>10</td>
<td>12.20 ± 8.1</td>
<td>20 ± 6.92</td>
<td>-39.0</td>
<td>30</td>
<td>0.000</td>
</tr>
<tr>
<td>20</td>
<td>45.40 ± 13.3</td>
<td>116 ± 30.22</td>
<td>-60.9</td>
<td>5</td>
<td>0.006</td>
</tr>
<tr>
<td>20</td>
<td>32.20 ± 14.7</td>
<td>115.8 ± 42.69</td>
<td>-72.2</td>
<td>5</td>
<td>0.022</td>
</tr>
</tbody>
</table>
infected wounds should be briefly treated with blue light. Once control of the infecting organisms is gained, infrared light might be best given alone to promote repair.

**CONCLUSION**

Within the limits of our experimental paradigm, our findings mandate the following conclusions:

1. Combined blue and infrared light (405 nm and 880 nm) produce bactericidal effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
2. In vitro, the optimal bactericidal dose of blue light, when combined with 880 nm light, for the treatment of *Pseudomonas aeruginosa* is 20 Jcm$^{-2}$. Lower doses give statistically significant kill rates, but 20 Jcm$^{-2}$ was found to be most effective.
3. *Staphylococcus aureus* responds to the 405 and 880 nm combination at doses of 1, 3, 10, and 20 Jcm$^{-2}$.

We recommend that future research be directed toward the mechanisms involved and how combinations of blue and infrared light affect healing when compared to infrared alone or combined red and infrared light in laboratory and clinical situations.

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**REFERENCES**


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