In Vitro Bactericidal Effects of 625, 525, and 425 nm Wavelength (Red, Green, and Blue) Light-Emitting Diode Irradiation

SangWoo Kim, MS,^{1*} JiSun Kim, PhD,^{1*} WonBong Lim, PhD,¹ SangMi Jeon, BS,¹
OkSu Kim, DDS, PhD,² Jeong-Tae Koh, DDS, PhD,³ Chang-Su Kim, PhD,⁴
HongRan Choi, DDS, PhD,¹ and OkJoon Kim, DDS, PhD¹

Abstract

Objective: The purpose of this study was to evaluate the relationship of 625, 525, and 425 nm wavelengths, providing average power output and effects on three common pathogenic bacteria. **Background data:** Ultraviolet (UV) light kills bacteria, but the bactericidal effects of UV may not be unique, as 425 nm produces a similar effect. The bactericidal effects of light-emitting diode (LED) wavelengths such as 625 and 525 nm have not been described. Before conducting clinical trials, the appropriate wavelength with reasonable dose and exposure time should be established. **Materials and methods:** The bactericidal effects of 625, 525, and 425 nm wavelength LED irradiation were investigated *in vitro* for the anaerobic bacterium *Porphyromonas gingivalis* and two aerobes (*Staphylococcus aureus* and *Escherichia coli* DH5 α). Average power output was 6 mW/cm² for 1 h. The bacteria were exposed to LED irradiation for 1, 2, 4, and 8 h (21.6, 43.2, 86.4, and 172.8 J/cm², respectively). LED irradiation was performed during growth on agar and in broth. Control bacteria were incubated without LED irradiation. Bacterial growth was expressed in colony-forming units (CFU) and at an optical density at 600 nm in agar and broth. **Results:** The bactericidal effect of LED phototherapy depended upon wavelength, power density, bacterial viable number, and bacteria species. The bactericidal effect of 425 and 525 nm irradiation varied depending upon the bacterial inoculation, compared with unirradiated samples and samples irradiated with red light. Especially, *P. gingivalis* and *E. coli* DH5 α were killed by 425 nm, and *S. aureus* growth was inhibited by 525 nm. However, the wavelength of 625 nm was not bactericidal for *P. gingivalis*, *E. coli* DH5 α , or *S. aureus*. **Conclusions:** Irradiation at 625 nm light was not bactericidal to *S. aureus*, *E. coli*, and *P. gingivalis*, whereas wavelengths of 425 and 525 nm had bactericidal effects. *S. aureus* was also killed at 525 nm.

Introduction

THE INACTIVATION OF MICROORGANISMS BY ULTRAVIOLET (UV) light is a well-established phenomenon that is used in many applications.^{1–5} UV is used extensively for disinfection of drinking water, treatment of plants, and in clinical and industrial facilities to prevent bacterial infection.^{6,7} The sterilization efficiency of UV systems depends upon different parameters such as wavelength range and microbial concen-

tration.^{8,9} A wavelength ranging between 200 and 300 nm, which corresponds to the peak absorption of DNA, is effective. Absorption of UV light by DNA is lethal for microorganisms.¹⁰ Although UV is effective at retarding bacterial growth, it can be harmful to normal cells. UVB and UVA radiation are also responsible for a variety of skin disorders, including cancer.¹¹

The light emitting diode (LED) is a semiconductor device that emits noncoherent narrow-spectrum light when

¹Department of Oral Pathology, Medical Research Center for Biomineralization Disorders, School of Dentistry, Dental Science Research Institute, Chonnam National University, Bug-Gu, Gwangju, Republic of Korea.

²Department of Periodontology, Medical Research Center for Biomineralization Disorders, School of Dentistry, Dental Science Research Institute, Chonnam National University, Bug-Gu, Gwangju, Republic of Korea.

³Dental Science Research Institute, Medical Research Center for Biomineralization Disorders, School of Dentistry, Chonnam National University, Gwangju, Republic of Korea.

⁴Su Dental Clinical Research Center, Gangnam-gu, Seoul, Republic of Korea.

*The first two authors contributed equally to this work.

electrically biased in the forward direction. LED has become an effective alternative to laser systems for reasons including lower cost, availability in a variety of wavelengths ranging from ultraviolet to near-infrared region of the spectrum, narrow emission band ($\sim 5\text{--}10\text{ nm}$), and a light fluence rate that can achieve hundreds of mW/cm^2 . In addition, the arrays can be constructed in various sizes to accommodate large areas, and they do not emit any heat, which may cause additional tissue damage.¹²

Visible light is used clinically in the treatment of dermatitis, Alzheimer's disease, and muscle analgesia,¹³ and is effective at removing bacterial biofilms.¹⁴ Visible light phototherapy seems to be a promising alternative approach to eradicating bacteria with blue light.^{15,16} Guffey and Wilborn^{17,18} examined the *in vitro* effects of 405 and 470 nm light on two common aerobes, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and the anaerobe *Propionibacterium acnes*, and reported different bactericidal effects depending upon wavelength. The killing rate of 405 nm light was greater than 470 nm light (90% for *S. aureus* and 95.1% for *P. aeruginosa*). Hamblin et al.¹⁹ showed that high intensity visible light could kill *Helicobacter pylori* in the stomach of humans. High-intensity broad-spectrum polychromatic light with wavelengths in the range of 400–1000 nm killed bacteria in infected diabetic ulcers.²⁰

The effects of LED wavelength and optical density on bacteria growth and bactericidal effects are unclear. The bactericidal effects and bacteria growth of LED illumination are unclear, although the bactericidal effects of blue light are well known.²¹ Before conducting clinical trials, the wavelength, appropriate dose, and exposure time of the irradiation must be determined.

The acceleration or retardation of bacteria growth might be affected by the type and number of bacteria. The aim of this study was to evaluate the relationship of 625, 525, and 425 nm wavelengths, providing average power output and effects on three common pathogenic bacteria: *Porphyromonas gingivalis*, *Escherichia coli* DH5 α , and *S. aureus* *in vitro*. We hoped to replicate the bactericidal effects of UV using visible light to remove bacterial biofilms without posing risks to human, but with high efficiency and minimal side effects.

Materials and Methods

Reagents

Luria–Bertani agar (LBA), Luria–Bertani broth (LBB), Brain–Heart Infusion Agar (BHIA), Brain–Heart Infusion Broth (BHIB), Baird–Parker Agar (BPA), and Baird–Parker Broth (BPB) were purchased from Difco Laboratories (Detroit, MI).

Bacterial strains and growth media

P. gingivalis (KCTC 5352) is an anaerobic bacterium. *S. aureus* (KCTC 1916) and *E. coli* DH5 α (ATCC 25922) are aerobic bacteria. *E. coli* DH5 α was grown on LBA and LBB for 24 h at 37°C. *P. gingivalis* was grown on BHIA and BHIB for 24 h in a 37°C anaerobic chamber. *S. aureus* was grown on BPA and BPB for 24 h in a 37°C incubator. They were harvested by centrifugation and suspended in 0.85% saline. The final bacterial density in each 0.1 mL aliquot used in the ex-

periments was 2×10^8 colony-forming units (CFU)/mL or 1.6×10^9 CFU/mL.

LED light irradiation devices

A three-in-one mounted RGB LED package for light irradiation was a continuous wave LED (HELIO Optoelectronics, Taiwan) emitting three wavelengths: red LED peak at 625 nm (spectral width 620–630 nm), green LED peak at 525 nm (spectral width 520–530 nm), and blue LED peak at 425 nm (spectral width 420–430 nm), providing a power density of $10\text{ mW}/\text{cm}^2$. An average power output of $6\text{ mW}/\text{cm}^2/\text{h}$ was set, and individual potency was determined using a model AQ215OA handheld optical Multi meter (Ando Electric, Japan). The optical density of microorganisms was analyzed by spectrophotometry at a wavelength of 600 nm in a 0.1 mL cubic cell (GeneQuant Pro OD600; Biochrom, UK). The bacteria were irradiated with 21.6, 43.2, 86.4, and $172.8\text{ J}/\text{cm}^2$. To determine the effects of LED irradiation depending upon the cell density of *P. gingivalis*, 0.1 mL suspensions containing 2×10^8 or 1.6×10^9 CFU/mL were dispensed in wells of a 100 mm dish and irradiated with LED for 0, 8, 12, and 24 h. To deal with the generation of heat from the long-term use of each LED source, bacteria were cultured in an incubator attached to a cooling fan (KA1238HA2/H.T.R.130; KAKU, China). Also, LED lamps contained an attached heat sink. In agar plates, 0.1 mL suspensions containing 2×10^8 CFU/mL of *P. gingivalis*, *E. coli* DH5 α , or *S. aureus* were dispensed and LED irradiated for 1, 2, 4, and 8 h. Number of CFU was determined and compared with the untreated group. In broth culture, 0.1 mL of *P. gingivalis*, *E. coli* DH5 α , or *S. aureus* (2×10^8 CFU/mL) were inoculated to 50 mL of the medium and LED irradiated for 1, 2, 4, or 8 h. Bacterial growth was evaluated as the optical density at 600 nm (OD₆₀₀). Additionally, 0.1 mL of a suspension irradiated for 8 h was added to a well of a 100 mm dish. After 24 h, the number of CFU was measured. The distance between the LED and the Petri dish or Erlenmeyer flask was kept constant, to maintain the proper power density. The LED light was positioned 50 mm directly over the center of the Petri dish and Erlenmeyer flask (Fig. 1). The three-in-one mounted RGB LED package was powered by a DC power control system (0–9 A and 0–30 V). The current was set at $2 \pm 0.05\text{ A}$ at a voltage of $5 \pm 0.1\text{ V}$, giving an approximate irradiance of $6\text{ mW}/\text{cm}^2$ for 1 h at the surface of each plate or dish. Each sample exposure was calculated as $E = P \times t$, where E = energy density (dose) in J/cm^2 , P = power density (irradiance) in W/cm^2 , and t = time in seconds. The power supply system obtained from the Department of Oral Pathology, Chonnam National University School of Dentistry, was set in an incubator for the experiment (Fig. 2).

CFU

The results were provided by calculation of 0.1 mL of 2×10^8 CFU/mL and 0.1 mL of 1.6×10^9 CFU/mL at 24 h after incubation at 37°C. CFU/mL is the number of colonies multiplied by $10^{1 + \text{dilution factor}}$. It takes into account the need to multiply by an extra 10, because of the addition of 0.1 mL to the plate from the tube. The number of CFU/mL was calculated with and without irradiation. The experiment was repeated two times to ensure accurate results.

FIG. 1. Schematic diagram of set-up for the three-in-one approach mounting the RGB light-emitting diode (LED) package (a) agar plate and (b) broth setup. The LED was positioned 50 mm directly over the Petri dish and Erlenmeyer flask. The three-in-one approach mounting the RGB LED package for light irradiation and the power supply system was set in an incubator for the experiment (see Fig. 2). The electrical circuit system for experiment controlled time, color of RGB, current, and voltage.

FIG. 2. (a) Power supply system and (b) three-in-one approach mounting the RGB light-emitting diode (LED) package for light irradiation. Red LED peak at 625 nm presented spectral width 620 ~ 630 nm. Green LED peak at 525 nm presented spectral width 520 ~ 530 nm. Blue LED peak at 425 nm presented spectral width 420 ~ 430 nm.

Statistical analysis

Values obtained in CFU and OD₆₀₀ were expressed as mean and standard deviation. Statistical calculations were done using Graph Pad Prism version 5.01 (GraphPad Software, San Diego, CA) statistical data analysis software. The data are expressed as the mean±SEM and analyzed with two way analysis of variance (ANOVA). Bonferonni's test was used to perform multiple comparisons among different groups. Null hypotheses of no difference were rejected if *p* values were <0.05.

Results

LED irradiation of *P. gingivalis*

To determine the effects of LED irradiation on *P. gingivalis*, 2×10^8 and 1.6×10^9 CFU/mL were irradiated at 625, 525, and 425 nm for 0, 8, 12, and 24 h. For the samples containing 1.6×10^9 CFU/mL, OD₆₀₀ was decreased by 425 nm light by 60% at 8 h, but not decreased as precipitously by 525 and 625 nm light (Fig. 3A). However, bacterial growth recovered to the control level within 24 h following irradiation at all three wavelengths. For the suspensions containing 2×10^8 CFU/mL, the OD₆₀₀ was decreased at 425 nm by 90~100% at all time points (Fig. 3B). After 24 h, irradiation at 425 nm was completely bactericidal. In contrast, bacterial growth peaked in samples irradiated at 625 nm for 8 h as much as 2.5-fold over control values.

Time dependence of LED irradiation on *P. gingivalis* on agar and in broth

To examine the effects of LED irradiation on *P. gingivalis*, bacteria (2×10^8 CFU/mL) were irradiated with LED wavelengths of 625, 525, and 425 nm at 6 mW/cm²/h. *P. gingivalis* viability was decreased by irradiation at 425 nm (40~60% reduction) and 525 nm (10~20% reduction) both in agar (Fig. 4A) and broth (Fig. 4C). In contrast, *P. gingivalis* viability was only slightly reduced by irradiation at 625 nm for 4 h. To confirm the growth and bactericidal effects on *P. gingivalis*, bacteria were LED irradiated in broth, and viability was

analyzed by OD₆₀₀ and CFU. Survival of *P. gingivalis* was markedly reduced by 425 nm (90~100% reduction) and 525 nm (40~70% reduction) after 8 h (Fig. 4C and D). In contrast, *P. gingivalis* viability increased during an 8 h irradiation at 625 nm compared with control.

Time dependence of LED irradiation of *E. coli* DH5 α in solid and liquid media

To examine the effects of LED irradiation on *E. coli* DH5 α , cells (2×10^8 CFU/mL) were exposed to LED wavelengths of 625, 525, and 425 nm (density of 6 mW/cm²/h) when growing on agar and in broth. Viability was decreased during irradiation at 425 nm (90~100% reduction) and 525 nm (~40% reduction) in agar (Fig. 5A). Viability was not affected at 625 nm. Survival was markedly reduced by irradiation at 425 nm (80~100% reduction) and 525 nm (40~50% reduction) for 8 h (Fig. 5C and D). In contrast, viability was unaffected by irradiation at 625 nm.

Time dependence of LED irradiation of *S. aureus* in solid and liquid media

To examine the effects of LED irradiation on *S. aureus*, bacteria (2×10^8 CFU/mL) were LED irradiated at 625, 525, and 425 nm (density of 6 mW/cm²); for 1 h during growth on agar and in broth. As shown in Fig. 6A, *S. aureus* viability decreased during an 8 h irradiation at 525 nm (10~60% reduction) on agar. The bactericidal effects of *S. aureus* were increased by 8 h irradiation at 425 nm (40% reduction) and 525 nm (10~60% reduction) in broth (Fig. 6C). In contrast, *S. aureus* viability was unaffected at 625 nm (Fig. 6A). To confirm the growth and bactericidal effects on *S. aureus*, *S. aureus* exposed to LED irradiation in broth was analyzed by OD₆₀₀ and CFU. Survival of *S. aureus* was reduced by 30~90% at 525 nm irradiation, both for 8 h (Fig. 6C and D). Viability was unaffected by irradiation at 625 nm.

Discussion

Light therapy has begun to be considered for various treatments. Bactericidal effects of LED irradiation have been

FIG. 3. Bacterial survival by LED irradiation on *Porphyromonas gingivalis*. **(A)** Bacterial survival is shown in optical density (OD₆₀₀) of 0.1 mL of 1.6×10^9 colony-forming units (CFU)/mL. The X-axis represents irradiated time, and OD₆₀₀ is shown on the Y-axis. **(B)** Bacterial survival is shown in OD₆₀₀ of 0.1 mL of 2×10^8 CFU/mL. The X-axis represents irradiated time, and OD₆₀₀ is shown on the Y-axis. The data are expressed as the mean±SEM and analyzed by using the two way analysis of variance (ANOVA). **p*<0.05, ***p*<0.01, ****p*<0.001.

FIG. 4. Bacterial survival of light-emitting diode (LED) irradiation by $6 \text{ mW}/\text{cm}^2$; for 1 h irradiation in *Porphyromonas gingivalis*. **(A)** Bacterial survival is shown in colony forming units (CFU). The X-axis represents irradiated time, and CFU are shown on the Y-axis. **(B)** Representative photographs showing colonies of *P. gingivalis* irradiated at $48 \text{ mW}/\text{cm}^2$ for 8 h in agar plates. **(a)** Control, **(b)** 625 nm red, **(c)** 525 nm green, and **(d)** 425 nm blue. **(C)** Bacterial survival is shown in optical density (OD_{600}). The X-axis represents irradiated time, and OD_{600} is shown on the Y-axis. **(D)** After *P. gingivalis* was irradiated at $48 \text{ mW}/\text{cm}^2$ for 8 h in broth, representative photographs showing colonies were shown in CFU. **(a)** Control, **(b)** 625 nm red, **(c)** 525 nm green, and **(d)** 425 nm blue. The data are expressed as the mean $\pm$ SEM and analyzed by using the two way analysis of variance (ANOVA). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

reported, with results differing by wavelength.^{17,22} Wavelengths of red, green, and blue light are commonly used in dentistry for wound healing,²³ periodontitis,²³ and photopolymerization of restorative materials.

In the present study, two gram-negative bacteria (*P. gingivalis* and *E. coli* DH5 α) and a gram-positive species (*S. aureus*) were chosen. *E. coli* DH5 α is not pathogenic and was used as a model organism for the experimental procedures. *P. gingivalis* is associated with periodontal bone loss.²⁴ *S. aureus* is one of the most common bacteria present at infected soft tissue lesions.²⁵ Increasing evidence suggests that *S. aureus* may be an important pathogen in the initiation of peri-implantitis.²⁶

Light irradiation has been used as a bactericidal agent against oral disease.²⁷ Presently, the lethal exposure dose is

dependent upon bacterial species and bacterial density. As is shown in Fig. 3, the growth or inhibition at each wavelength seemed to be dependent upon the inoculum quantity of *P. gingivalis*. When 1.6×10^9 CFU/mL was inoculated, bacterial growth recovered by blue light irradiation in 24 h, whereas red and green light had no effect. However, with a smaller number (2×10^8 CFU/mL) of *P. gingivalis* as the inoculum, blue light irradiation inhibited bacteria growth over time. Neither red nor green light irradiation was bactericidal. The collective results indicate that bacterial growth was dependent upon the viable numbers of the inoculated bacteria. Light therapy is a proper measure for prevention and/or reduction, not for an absolute treatment and/or elimination of bacteria.

Bactericidal effects were also dependent upon wavelength. Blue light irradiation was bactericidal against

FIG. 5. Bacterial survival of light-emitting diode (LED) irradiation at 6 mW/cm² for 1 h irradiation in DH5α. **(A)** Bacterial survival is shown in colony forming units (CFU). The X-axis represents irradiated time, and CFU are shown on the Y-axis. **(B)** Representative photographs showing colonies of DH5α irradiated at 48 mW/cm² for 8 h in agar. **(a)** Control, **(b)** 625 nm red, **(c)** 525 nm green, **(d)** 425 nm blue. **(C)** Bacterial survival is shown in optical density (OD₆₀₀). The X-axis represents irradiated time, and OD₆₀₀ is shown on the Y-axis. **(D)** After DH5α irradiated at 48 mW/cm² for 8 h in broth, representative photographs showing colonies were shown in CFU. **(a)** Control, **(b)** 625 nm red, **(c)** 525 nm green, and **(d)** 425 nm blue. The data are expressed as the mean ± SEM and analyzed by using the two way analysis of variance (ANOVA). **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

P. gingivalis, *E. coli* DH5α, and *S. aureus*, whereas red light irradiation was not. However, *P. gingivalis* was increased only at 8 h irradiation, because of the energy dose. Blue light might induce bactericidal effects by the generation of reactive oxygen species (ROS).¹⁵ Lipovsky et al.²⁸ reported that visible light (400 ~ 800 nm) at high intensity killed bacteria frequently found in infected wounds, whereas low power white light enhanced bacterial proliferation. ROS production following blue light (400 ~ 500 nm) illumination was higher than that of red light (500 ~ 800 nm).²⁸ Feuerstein et al.²⁹ showed that blue light (400 ~ 500 nm) is phototoxic to *P. gingivalis* and *Fusobacterium nucleatum*.

The minimal inhibitory dose for *P. gingivalis* and *F. nucleatum* was reported to be 16 ~ 62 J/cm².²⁹ Near infrared diode laser irradiation did not affect any of the bacteria tested.²⁹ The results of the latter study suggest that visible light sources without exogenous photosensitizers have a phototoxic effect, mainly on gram-negative periodontal pathogens.²⁹ In contrast to their findings, bacteria exposed to 625 nm light in the present study was not affected (Fig. 4).

Green light was especially effective against *S. aureus* (Fig. 6). These results suggest that bacteria species is an important factor in the bactericidal effects of light therapy,

FIG. 6. Bacterial survival of LED irradiation at $6 \text{ mW}/\text{cm}^2$ for 1 h in *Staphylococcus aureus*. **(A)** Bacterial survival is shown in colony forming units (CFU). The X-axis represents irradiated time and CFU are shown on the Y-axis. **(B)** Representative photographs showing colonies of *S. aureus* irradiated at $48 \text{ mW}/\text{cm}^2$ for 8 h in agar. **(a)** Control, **(b)** 625 nm red, **(c)** 525 nm green, and **(d)** 425 nm blue. **(C)** Bacterial survival is shown in optical density (OD_{600}). The X-axis represents irradiated time and OD_{600} is shown on the Y-axis. **(D)** After *S. aureus* was irradiated at $48 \text{ mW}/\text{cm}^2$ for 8 h in broth, representative photographs showing colonies were shown in CFU. **(a)** Control, **(b)** 625 nm red, **(c)** 525 nm green, and **(d)** 425 nm blue. The data are expressed as the mean $\pm$ SEM and analyzed by using the two way analysis of variance (ANOVA). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

because of differences of bacteria structure, such as membranes. Dadras et al.³⁰ proposed that an Ar-ion laser at 514 nm be used to determine the effect of various energy densities of green light on these bacteria. All energy densities of Ar-ion laser had a proliferative effect on *P. aeruginosa* and an inhibitory effect on *S. aureus*. Similarly, second harmonic generation (SHG) Nd:YAG (532 nm) and He-Ne (633 nm) lasers with given energy densities increased proliferation of *P. aeruginosa*, but inhibited *S. aureus*.³⁰ Application of 525 nm green light in our study would be expected to have a bactericidal effect on other gram-positive microbes such as *S. aureus*. Additionally, it can be expected to have bactericidal effects when dental bleaching is treated with green light.

Regarding the power density for bactericidal effects, high light power ($6 \text{ mW}/\text{cm}^2$) was more effective for bactericidal effects than low power ($3 \text{ mW}/\text{cm}^2$) (data not shown). The reason may be a high concentration of light-induced ROS, which are lethal to the cell, although low levels stimulate cell growth.³¹

Finally, these results suggest that 625 nm irradiation has potential applications for oral periodontal disease, as *P. gingivalis* grew after irradiation. This might be expected to have additional merits for dental bleaching and resin curing in dental clinical practice. Irradiation at 625 nm irradiation can reduce inflammatory reactions and induce cell proliferation³² in periodontal disease, which involves the interaction of bacteria and oral cells. However, the absence

of a bactericidal effect at 625 nm and the lack of bacterial proliferation were noted in the present study. We can infer that 625 nm irradiation has potential applications for periodontal disease.

Conclusions

The effects of LED phototherapy are dependent upon wavelength, power density, quantity (or number) of bacteria, and species. At 625 nm, the growth of *S. aureus*, *E. coli*, and *P. gingivalis* increased, whereas 425 and 525 nm were bactericidal. Especially, *S. aureus* was killed only by 525 nm.

Acknowledgments

This study was supported by the National Research Foundation of Korea (NRF) grants funded by the Korea government (MSIP) (No. 2011-0030759).

Author Disclosure Statement

No competing financial interests exist.

References

- Zemke, V., Podgorsek, L., and Schoenen, D. (1990). Ultraviolet disinfection of drinking water. 1. Communication: Inactivation of *E. coli* and coliform bacteria. *Zentralbl. Hyg. Umweltmed.* 190, 51–61.
- Rames, J., Chaloupecky, V., Sojkova, N., and Bencko, V. (1997). An attempt to demonstrate the increased resistance of selected bacterial strains during repeated exposure to UV radiation at 254 nm. *Cent. Eur. J. Public Health* 5, 30–31.
- Warriner, K., Kolstad, J., Rumsby, P., and Waites, W.M. (2002). Carton sterilization by uv-C excimer laser light: recovery of *Bacillus subtilis* spores on vegetable extracts and food simulation matrices. *J. Appl. Microbiol.* 92, 1051–1057.
- Warriner, K., Rysstad, G., Murden, A., Rumsby, P., Thomas, D., and Waites, W.M. (2000). Inactivation of *Bacillus subtilis* spores on aluminum and polyethylene preformed cartons by UV-excimer laser irradiation. *J. Food Prot.* 63, 753–757.
- Favier, G.I., Escudero, M.E., and de Guzman, A.M.S. (2001). Effect of chlorine, sodium chloride, trisodium phosphate, and ultraviolet radiation on the reduction of *Yersinia enterocolitica* and mesophilic aerobic bacteria from eggshell surface. *J. Food. Prot.* 64, 1621–1623.
- Wallbank, A.M., Lebtog, I., Meyer, W., and Boehm, P. (1985). Inactivation of bacteria and viruses in water: passage of germicidal ultraviolet light through Teflon. *Lab. Anim.* 19, 273–274.
- Bourrouet, A., Garcia, J., Mujeriego, R., and Penuelas, G. (2001). Faecal bacteria and bacteriophage inactivation in a full-scale UV disinfection system used for wastewater reclamation. *Water Sci. Technol.* 43, 187–194.
- Ishida, H., Nahara, Y., Tamamoto, M., and Hamada, T. (1991). The fungicidal effect of ultraviolet light on impression materials. *J. Prosthet. Dent.* 65, 532–535.
- Wilson, M. (1994). Bactericidal effect of laser light and its potential use in the treatment of plaque-related diseases. *Int. Dent. J.* 44, 181–189.
- Decho, A.W. (2000). Microbial biofilms in intertidal systems: an overview. *Cont. Shelf Res.* 20, 1257–1273.
- Afaq, F., and Mukhtar, H. (2006). Botanical antioxidants in the prevention of photocarcinogenesis and photoaging. *Exp. Dermatol.* 15, 678–684.
- Lim, W., Lee, S., Kim, I., Chung, M., Kim, M., Lim, H., et al., (2007). The anti-inflammatory mechanism of 635 nm light-emitting-diode irradiation compared with existing COX inhibitors. *Lasers Surg. Med.* 39, 614–621.
- Desmet, K.D., Paz, D.A., Corry, J.J., Eells, J.T., Wong-Riley, M.T., Henry, M.M., et al., (2006). Clinical and experimental applications of NIR-LED photobiomodulation. *Photomed. Laser Surg.* 24, 121–128.
- Nandakumar, K., Keeler, W., Schraft, H., and Leung, K.T. (2006). Visible laser and UV-A radiation impact on a PNP degrading *Moraxella* strain and its *rpoS* mutant. *Biotechnol. Bioeng.* 94, 793–802.
- Papageorgiou, P., Katsambas, A., and Chu, A. (2000). Phototherapy with blue (415 nm) and red (660 nm) light in the treatment of acne vulgaris. *Br. J. Dermatol.* 142, 973–978.
- Kawada, A., Aragane, Y., Kameyama, H., Sangen, Y., and Tezuka, T. (2002). Acne phototherapy with a high-intensity, enhanced, narrow-band, blue light source: an open study and in vitro investigation. *J. Dermatol. Sci.* 30, 129–135.
- Guffey, J.S., and Wilborn, J. (2006). In vitro bactericidal effects of 405 nm and 470 nm Blue Light. *Photomed. Laser Surg.* 24, 684–688.
- Guffey, J.S., and Wilborn, J. (2006). Effects of combined 405-nm and 880-nm light on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in vitro. *Photomed. Laser Surg.* 24, 680–683.
- Hamblin, M.R., Viveiros, J., Yang, C., Ahmadi, A., Ganz, R.A., and Tolkoff, M.J. (2005). *Helicobacter pylori* accumulates photoactive porphyrins and is killed by visible light. *Antimicrob. Agents Chemother.* 49, 2822–2827.
- Lipovsky, A., Nitzan, Y., and Lubart, R. (2008). A possible mechanism for visible light-induced wound healing. *Lasers Surg. Med.* 40, 509–514.
- Ashkenazi, H., Malik, Z., Harth, Y., and Nitzan, Y. (2003). Eradication of *Propionibacterium acnes* by its endogenic porphyrins after illumination with high intensity blue light. *FEMS Immunol. Med. Microbiol.* 35, 17–24.
- Wilson, M. (2004). Lethal photosensitization of oral bacteria and its potential in the photodynamic therapy of oral infections. *Photochem. Photobiol. Sci.* 3, 412–418.
- Carlson, N.E., and Roach, R.B. (2002). Platelet-rich plasma - Clinical applications in dentistry. *J. Am. Dent. Assoc.* 133, 1383–1386.
- O'Brien-Simpson, N.M., Pathirana, R.D., Paolini, R.A., Chen, Y.Y., Veith, P.D., and Tam, V., et al., (2005). An immune response directed to proteinase and adhesin functional epitopes protects against *Porphyromonas gingivalis*-induced periodontal bone loss. *J. Immunol.* 175, 3980–3989.
- Konttinen, S., and Rinne, E. (1987). Bacteria isolated from skin and soft tissue lesions. *Eur. J. Clin. Microbiol.* 6, 420–422.
- Heitz-Mayfield, L.J., and Lang, N.P. (2010). Comparative biology of chronic and aggressive periodontitis vs. peri-implantitis. *Periodontol.* 2000 53, 167–181.
- Wilson, M. (1993). Photolysis of oral bacteria and its potential use in the treatment of caries and periodontal disease. *J. Appl. Bacteriol.* 75, 299–306.

28. Lipovsky, A., Nitzan, Y., Gedanken, A., and Lubart, R. (2010). Visible light-induced killing of bacteria as a function of wavelength: Implication for wound healing. *Lasers Surg. Med.* 42, 467–472.
29. Feuerstein, O., Persman, N., and Weiss, E.I. (2004). Phototoxic effect of visible light on *Porphyromonas gingivalis* and *Fusobacterium nucleatum*: An in vitro study. *Photochem. Photobiol.* 80, 412–415.
30. Dadras, S., Mohajerani, E., Eftekhari, F., and Hosseini, M. (2006). Different photoresponses of *Staphylococcus aureus* and *Pseudomonas aeruginosa* to 514, 532, and 633 nm low level lasers in vitro. *Curr. Microbiol.* 53, 282–286.
31. Lavi, R., Sinyakov, M., Samuni, A., Shatz, S., Friedmann, H., Shainberg, A., et al., (2004). ESR detection of $1O_2$ reveals enhanced redox activity in illuminated cell cultures. *Free Radic. Res.* 38, 893–902.
32. Komerik, N., Nakanishi, H., MacRobert, A.J., Henderson, B., Speight, P., and Wilson, M. (2003). In vivo killing of *Porphyromonas gingivalis* by toluidine blue-mediated photosensitization in an animal model. *Antimicrob. Agents Chemother.* 47, 932–940.

Address correspondence to:

OkJoon Kim

Department of Oral Pathology

Medical Research Center for Biomineralization Disorders

School of Dentistry

Dental Science Research Institute

Chonnam National University

Bug-Gu, Gwangju, 500-757

Republic of Korea

E-mail: js3894@chonnam.ac.kr