Evaluation of the Effects of Hydrogen Peroxide and LED Irradiation on Biofilms of Clinical Isolates from Orthopedic Infections

Hiroto Kono[1], Hyonmin Choe[1], Kimi Ishikawa[1], Hiroyuki Ike[1], Akira Morita[1], Yuta Hieda[1], Hironori Yamane[1], Akira Ihara[1], Takaaki Kitano[1], Yutaka Inaba[1]
[1]Department of Orthopaedic Surgery, Yokohama City University, Yokohama, Japan
Email: kono.hir.uh@yokohama-cu.ac.jp

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INTRODUCTION:
Orthopedic implant-associated infections often become intractable due to the formation of biofilms composed of polysaccharides and proteins secreted by bacteria. These biofilms confer strong resistance to antimicrobial agents. Light of near-ultraviolet wavelengths has been reported to generate reactive oxygen species (ROS) when irradiated in the presence of hydrogen peroxide (H_2O_2), thereby exhibiting bactericidal effects independent of antibiotic susceptibility. While the efficacy of this approach against biofilms of various species has been described, its effects on clinical isolates derived from patients with orthopedic implant infections remain unclear. The aim of this study was to evaluate the biofilm-disrupting effects of H_2O_2 combined with LED (Light Emitting Diode) irradiation on clinically relevant isolates commonly encountered in orthopedic infections.

METHODS:
Clinical isolates of *Methicillin-Susceptible Staphylococcus aureus* (MSSA), *Methicillin-Resistant Staphylococcus epidermidis* (MRSE), *Methicillin-Resistant Staphylococcus aureus* (MRSA), *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* were used. Bacterial suspensions were adjusted to 1×10^6 CFU/ml, and titanium discs (pure titanium, φ5 mm, 2 mm thick) were immersed and incubated overnight at 37 °C with shaking to allow biofilm formation. On the following day, after washing with PBS, discs (n=3 per group) were treated for 1 min under one of five conditions: control, 365nm LED irradiation alone, 3%H_2O_2 alone, 365nm LED+3%H_2O_2, or 405nm LED+3%H_2O_2. LED intensity was set at 1000mW/cm². Following treatment, discs were washed and incubated in WST solution, and metabolic activity was assessed by measuring absorbance at 450nm after the optimal incubation period for each species (5h for MSSA, MRSE, MRSA, and *E. coli*, 7h for *C. albicans*, and 16h for *P. aeruginosa*).

RESULTS:
Relative to the untreated control (100%), mean metabolic activity (n=3 per group, except C. albicans with n=2) was reduced, in the order of LED, H_2O_2, LED+H_2O_2, and 405LED+H_2O_2, to 44%*, 4.8%*, 3.9%*, and 3.8%* for MSSA; 30.3%*, 7.2%*, 5.4%*, and 5.3%* for MRSE; 92.4%, 21.6%$^{*\#}$, 4.5%$^{*\#}$, and 3.6%$^{*\#}$ for MRSA; 34.1%, 6.6%*, 6.6%*, and 6.6%* for E. coli; 90.1%, 6.6%$^{*\#}$, 5.8%$^{*\#}$, and 5.9%$^{*\#}$ for P. aeruginosa; and 14.0%*, 19.7%*, 12.2%*, and 10.9%* for C. albicans (* indicates significant difference compared with control, $^\#$ indicates significant difference compared with LED irradiation alone, respectively).Statistical analysis was performed using ANOVA with multiple comparisons. EZR (Easy R), a graphical user interface for R, was employed for all analyses.

DISCUSSION:
Both H_2O_2 alone and LED+H_2O_2 significantly reduced metabolic activity across all tested clinical isolates compared with untreated controls. In MRSA, combining LED with H_2O_2 further enhanced the inhibitory effect compared with H_2O_2 alone. In other species, additional synergy was not observed, likely due to the strong bactericidal action of H_2O_2 itself. The mechanism is thought to involve ROS-mediated oxidative damage to bacterial cell membranes, independent of antibiotic susceptibility. These findings support previous reports demonstrating that this approach is effective against resistant strains such as MRSA and MRSE. Notably, in staphylococcal species, 365nm irradiation tended to yield lower metabolic activity than 405nm, reflecting wavelength-dependent energy differences and ROS generation. 365nm irradiation lies in the ultraviolet range and has higher bactericidal potential but lower safety, whereas 405nm irradiation belongs to the visible spectrum and is considered safer. As both wavelengths achieved comparable antibacterial effects in this study, 405nm may represent a safer alternative for clinical application. This study is limited by the small sample size (n=3 per condition), the use of isolates from a single institution, and the restricted in vitro model, which may not fully represent clinical implant-associated infections.

SIGNIFICANCE/CLINICAL RELEVANCE:
H_2O_2 combined with LED irradiation demonstrated significant antibiofilm activity against clinical isolates from orthopedic infections. 405nm irradiation was shown to have bactericidal effects comparable to those of 365 nm, with relatively higher safety.

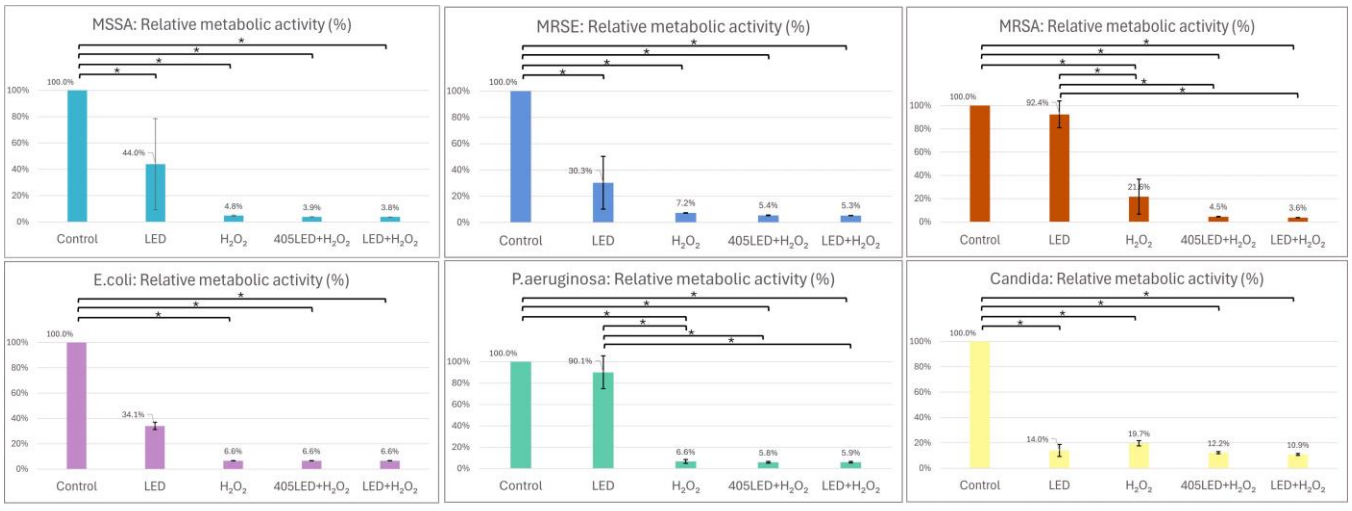

Figure.1 Metabolic activity of biofilms formed by clinical isolates under different treatment conditions. *p < 0.05