Novel Denture-Cleaning System Based on Hydroxyl Radical Disinfection

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The purpose of this study was to evaluate a new denture-cleaning device using hydroxyl radicals generated from photolysis of hydrogen peroxide (H_2O_2). Electron spin resonance analysis demonstrated that the yield of hydroxyl radicals increased with the concentration of H_2O_2 and light irradiation time. *Staphylococcus aureus, Pseudomonas aeruginosa,* and methicillin-resistant *S aureus* were killed within 10 minutes with a > 5-log reduction when treated with photolysis of 500 mM H_2O_2; *Candida albicans* was killed within 30 minutes with a > 4-log reduction with photolysis of 1,000 mM H_2O_2. The clinical test demonstrated that the device could effectively reduce microorganisms in denture plaque by approximately 7-log order within 20 minutes. *Int J Prosthodont 2012;25:376–380.*

Mechanical and chemical denture-cleaning methods are available. Although toothpastes are the most popular mechanical denture cleansers, they are highly abrasive, so they can create microporosities on the denture surfaces that may harbor microorganisms.[1,2] The commercially available peroxide solution most widely used for chemical denture cleaning is insufficient because it can only kill bacteria with a 3-log reduction.[3] To boost the microbicidal effect of a low concentration of hydrogen peroxide (H_2O_2), the authors focused on photolysis of H_2O_2, which generates a highly reactive hydroxyl radical. In a previous study, it was demonstrated that hydroxyl radicals generated by photolysis of H_2O_2 could kill oral pathogenic bacteria effectively.[4] The purposes of this study were to develop a new denture-cleaning device using hydroxyl radical disinfection, to examine its microbicidal effect, and to clinically evaluate its cleaning efficacy.

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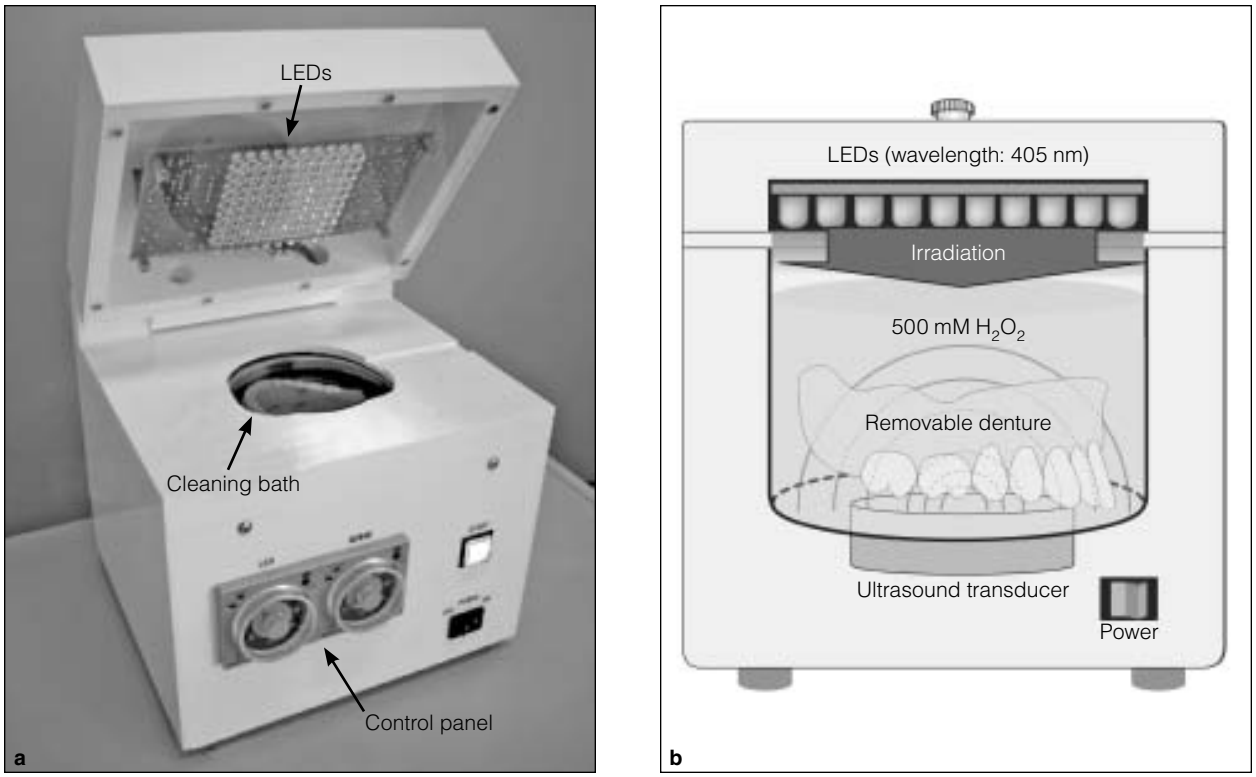
Materials and Methods

Yield of Hydroxyl Radicals

An experimental denture-cleaning device equipped with 100 light-emitting diodes (LEDs) (OSSV5111A, OptoSupply), which radiate light at a wavelength of 405 ± 20 nm and an energy density of 5 mW/cm^2, and an ultrasound transducer (40 kHz, 30 W) was made (Figs 1a and 1b). The yield of hydroxyl radicals was analyzed using the electron spin resonance (ESR) technique according to a previous study.[5]

Bactericidal and Fungicidal Testing

It has been suggested that gram-positive *Staphylococcus aureus* as well as *Candida albicans* are frequently detected in the oral cavity and sometimes cause serious infectious diseases such as pneumonia, toxic shock syndrome, and septicemia, especially in elderly patients.[6,7] In addition,



Figs 1a and 1b Experimental denture-cleaning device. A removable dental prosthesis is immersed in the cleaning bath containing 150 mL of 500 mM H_2O_2 and is then irradiated using the LED light and ultrasound.

gram-negative *Pseudomonas aeruginosa* in the oral cavity is implicated in the development of a range of systemic diseases, including atherothrombotic disorders.[8] Therefore, these microorganisms were used as in vitro assays. *S aureus* ATCC 25923 (American Type Culture Collection), *P aeruginosa* ATCC 27853 (American Type Culture Collection), *C albicans* IFO 1269 (Institute of Fermentation), and a clinically isolated strain of methicillin-resistant *S aureus* (MRSA) from a patient at Showa University Fujigaoka Hospital (Yokohama, Japan) were obtained. An aliquot (400 µL) of each microbial suspension in sterile physiologic saline was mixed with 400 µL of given concentrations of H_2O_2, placed in the cleaning device, and irradiated with the LED light. After irradiation, 50 µL of the sample was mixed with an equal volume of 5,000 U/mL catalase solution (Wako) to terminate the effect of any remaining H_2O_2, and the culture study was conducted to determine the colony-forming units (CFUs)/mL. The experimental conditions were photolysis of H_2O_2 (expressed as LED(+)H_2O_2(+)), LED light irradiation alone (LED(+)H_2O_2(–)), H_2O_2 alone (LED(–)H_2O_2(+)), and no treatment (LED(–)H_2O_2(–)).

Clinical Testing

Complete and removable partial dental prostheses worn by patients who visited Tohoku University Dental Hospital, Sendai, Japan, were recruited. This clinical research was approved by the ethical committee of Tohoku University Graduate School of Dentistry. Denture bases were washed with tap water for 5 to 10 seconds and inspected visually to determine the sites for sampling of the denture plaque. Two sites per denture were selected. Sterile sticky labels with a hole of 6 mm in diameter were attached at the sites to indicate the sampling area. Each site was assigned randomly to analysis before or after cleaning. The sites assigned to analysis before cleaning were swabbed using a sterile cotton swab that was subsequently immersed in 10 mL of phosphate-buffered saline. Then, the dentures were randomly divided into three groups: LED(+)H_2O_2(+), LED(–)H_2O_2(+), and LED(–)H_2O_2(–). For the condition of H_2O_2(+), 500 mM H_2O_2 was used. Irrespective of the groups, all dentures were irradiated by ultrasound during cleaning. For the sites assigned to analysis after cleaning, they were

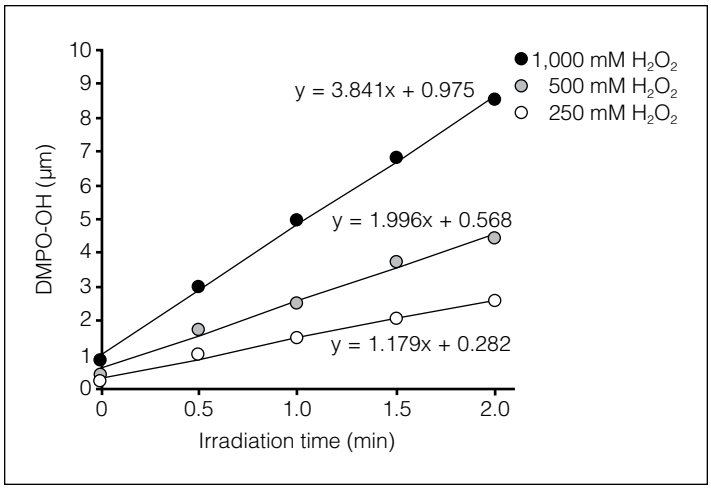


Fig 2 Yield of DMPO-OH in relation to the concentration of H_2O_2 and irradiation time. When H_2O_2 was irradiated with LED light in the device, DMPO-OH increased in a time-dependent manner. The generation rate of DMPO-OH, which corresponds to the slope value of the linear relationship, increased with the concentration of H_2O_2. Each value represents the mean of duplicate measurements.

swabbed in the same manner as described for those before cleaning. The specimen sample was plated on Petrifilm (Petrifilm Aerobic Count Plate, 3M ESPE) and incubated for 48 hours. If the CFU/area in the sample before cleaning was less than 10^5, the denture was regarded as relatively clean and excluded. Since a sterility assurance level of 10^{-6} is the recommended probability of survival for organisms on a sterilized device,[9] 10^5 CFU was set as an exclusion criterion. Of the removable dental prostheses recruited, 40 dental prostheses were subjected to the clinical test so that 10 prostheses were allocated to each treatment.

Results

Yield of Hydroxyl Radicals

When the H_2O_2 was irradiated using LED light, 5,5-dimethyl-1-pyrroline N-oxide ([DMPO]-OH), a spin adduct of DMPO and the hydroxyl radical, increased with the irradiation time and the concentration of H_2O_2 (Fig 2). The generation rates of hydroxyl radicals from 250, 500, and 1,000 mM H_2O_2 were 1.2, 2.0, and 3.8 μM/min, respectively.

Bactericidal and Fungicidal Testing

As shown in Figs 3a to 3d, *S aureus* in 250 mM H_2O_2 and MRSA in 500 mM H_2O_2 were killed in a time-dependent manner irrespective of LED light irradiation. LED(+)H_2O_2(+) killed the bacteria more effectively than LED(–)H_2O_2(+). *P aeruginosa* was completely killed by 250 mM H_2O_2 alone. Hydroxyl radicals generated under LED(+)H_2O_2(+) with 1,000 mM H_2O_2 could kill *C albicans* with a > 4-log reduction in 30 minutes. In all cases, LED light irradiation alone (LED(+)H_2O_2(–)) did not exert any bactericidal or fungicidal effect.

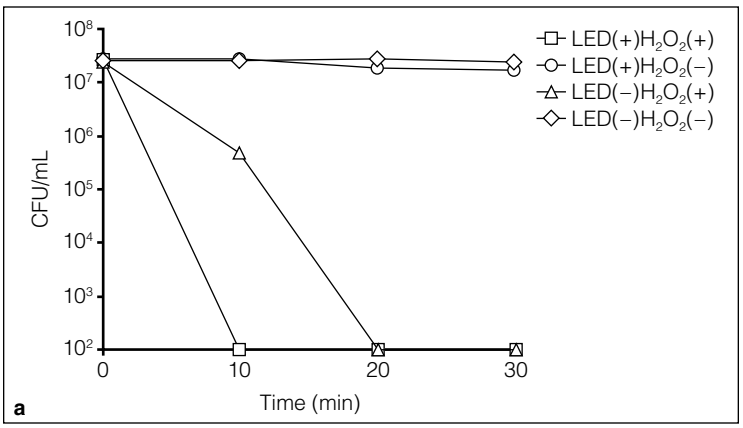
Clinical Testing

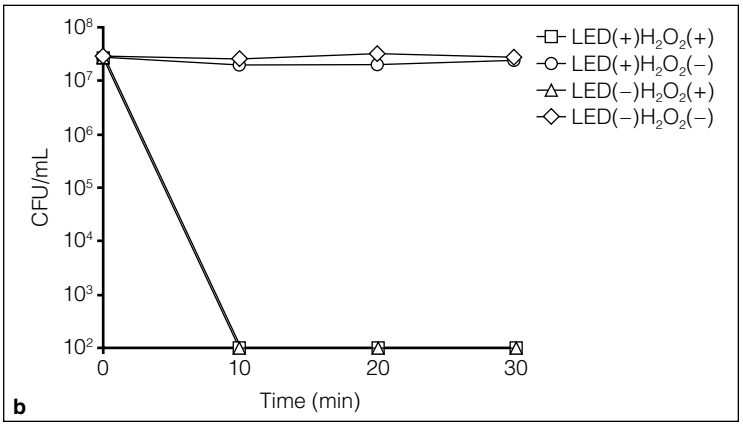
Before cleaning, the average numwber of bacteria in denture plaque was approximately 10^8 CFU/area. Since all dentures used in this study were irradiated by ultrasound regardless of the cleansing conditions, the number of bacteria decreased with time even under the condition LED(–)H_2O_2(–) (Fig 4). LED(+)H_2O_2(+) showed the highest reduction of CFU/area, with approximately 7-log order in 20 minutes, while LED(–)H_2O_2(+) and LED(–)H_2O_2(–) showed similar reduction of CFU/area of 3- to 4-log order within 20 minutes.

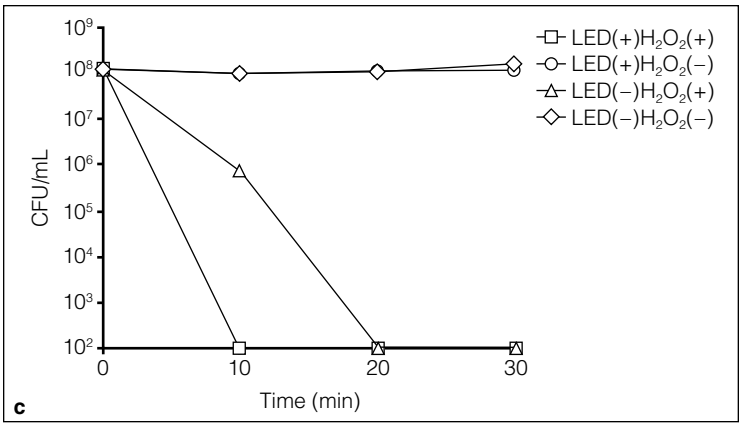
Discussion

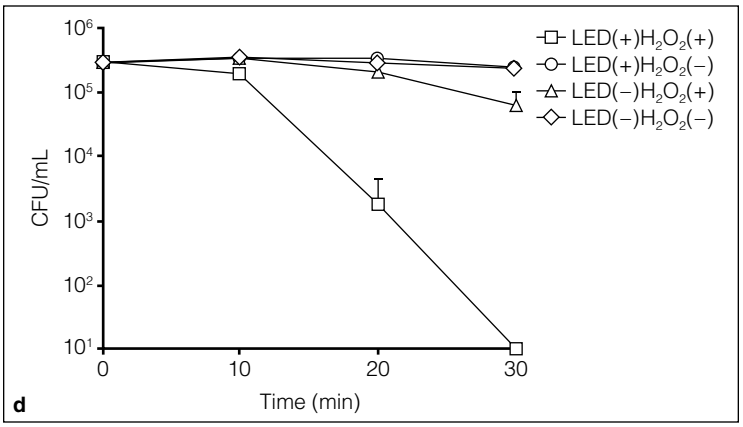
LED light irradiation with 500 mM H_2O_2 could enhance the bactericidal effect of 500 mM H_2O_2 alone, resulting in a reduction of > 5-log order within 10 minutes. Similarly, LED light irradiation with 1,000 mM H_2O_2 could kill *C albicans* within 30 minutes. In the former case, the amount of hydroxyl radical generated was calculated to be 20 μM, and in the latter, it was 114 μM. These enhanced microbicidal effects by the hydroxyl radical were well reflected in the clinical test, demonstrating that treatment with the device resulted in a significant reduction of microorganisms on the surface of the dentures within 20 minutes. Further studies are required to evaluate the effectiveness of the cleaning device on bacteria harboring in porosities.

Figs 3a to 3d Bactericidal and fungicidal effect of the hydroxyl radical disinfection system applied for the denture-cleaning device. The microorganisms tended to be killed dependent on the concentration of H_2O_2 and LED irradiation time. Each value represents the mean of triplicate measurements with standard deviations. **(a)** *S aureus* with 500 mM H_2O_2; **(b)** *P aeruginosa* with 250 mM H_2O_2; **(c)** MRSA with 500 mM H_2O_2; **(d)** *C albicans* with 1,000 mM H_2O_2.









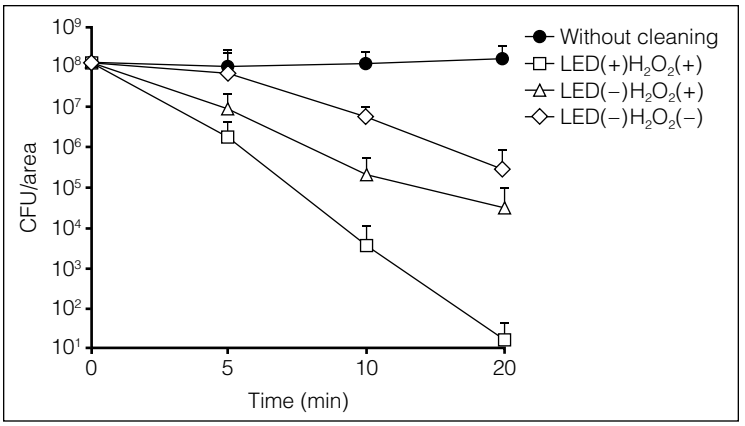


Fig 4 Denture-cleaning effect of the device in clinical use. Even in the case of LED(–)H_2O_2(–), the targets were irradiated by ultrasound, so the bacteria likely detached from the removable dental prosthesis and were somewhat killed by the increased temperature of the solutions. Hydroxyl radicals generated in LED(+)H_2O_2(+) in combination with H_2O_2 could most effectively decrease the number of bacteria with an approximate 7-log reduction. Each value represents the mean of 10 specimens with standard deviations.

Conclusion

This novel denture-cleaning system utilizing hydroxy radicals generated by photolysis of H_2O_2 was proven to be effective against microorganisms in denture plaque.

Acknowledgment

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Literature Abstract

Coffee intake and oral/esophageal cancer: Follow-up of 389,624 Norwegian men and women 40–45 years

This prospective study was conducted to investigate the effect of coffee on oral/esophageal cancer. Participants (n = 389,624) in the Norway national survey program were followed up with regarding their cancer status for 15 years. Participants' daily coffee intakes were categorized as 0 or < 1 cup, 1 to 4 cups, 5 to 8 cups, or 9+ cups. Participants' cancer statuses were obtained from the Cancer Registry of Norway. Squamous cell carcinoma in the buccal cavity or esophagus was considered the end point of this study. Cox proportional hazard regressions were used to investigate the hazard ratio of coffee intake for oral/esophageal cancer. Covariates included smoking, Body Mass Index, and alcohol consumption. The adjusted hazard ratio for squamous esophageal cancer in the four categories of coffee intake ranged from 0.96 to 1.16. No trend could be detected. The authors concluded that coffee intake has no inverse correlation with the incidence of oral/esophageal cancer. However, the possibility of a weak inverse relationship could not be excluded.

Tverdal A, Hjellvik V, Selmer R. *Br J Cancer* 2011;105:157–161. **References:** 16. **Reprints:** Dr V. Hjellvik, Department of Pharmacoepidemiology, Norwegian Institute of Public Health, PO Box 4404, Nydalen, Oslo NO-0403, Norway—*H.D. Khoo, Singapore*