Corrosive effect of disinfection solution containing hydroxyl radicals generated by photolysis of H₂O₂ on dental metals

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The purpose of the present study was to evaluate the corrosive effect of disinfection solution containing hydroxyl radicals generated by photolysis of H₂O₂ on dental metals. Static immersion test was performed on four different dental metals: Ti, Type 316L stainless steel, Ag-Pd-Cu-Au alloy, and Co-Cr alloy. Metal specimens were immersed in 1 M H₂O₂ (=3.4%) with or without light-emitting diode (LED) light irradiation (wavelength: 400 nm) for 1 week, and then the amounts of released ions were analyzed. Corrosive effect of the disinfection solution containing hydroxyl radicals on any dental metals tested in the present study never exceeded that of H₂O₂ alone. Therefore, disinfection systems based on the photolysis of H₂O₂ for the cleaning of dentures and treatment of oral infectious diseases would not cause problematic metal corrosion whenever the concentration of H₂O₂ does not exceed 3%, which is a concentration used as an oral disinfectant.

Keywords: Corrosion, Hydroxyl radical, Hydrogen peroxide, Dental alloys

INTRODUCTION

Oral infectious diseases—such as caries and periodontitis—are caused by bacteria found in dental plaque, a microbial biofilm. In our previous studies, we have developed a novel disinfection technique, based on hydroxyl radicals generated by photolysis of H₂O₂, which could effectively kill bacteria in biofilms within 3 min. Therefore, this new disinfection technique can be used as an adjunctive treatment for caries, periodontitis, and root canal infection by delivering light together with H₂O₂ to the lesion site.

In view of the importance of denture hygiene, this disinfection technique was also applied to a denture cleaning device to prevent microbiological problems derived from long-term use of removable dentures. Microorganisms in denture plaque were effectively reduced when removable dentures were immersed in H₂O₂ with light-emitting diode (LED) light irradiation at a wavelength of 405 nm for 20 min. On the other hand, there is a strong concern if the hydroxyl radical oxidizes dental metals. When the disinfection technique is used for denture cleaning, metal components in removable dentures would be corroded because of repeated exposure to hydroxyl radicals for daily cleaning. Since the hydroxyl radical has an unpaired electron, it might deprive dental metals of electrons resulting in metallic oxidation, also known as corrosion. Therefore, before the new disinfection technique could be applied clinically, the influence of long-term exposure of dental metals to hydroxyl radicals should be examined.

In general, dental metals are highly resistant to corrosion because the oral environment is highly aggressive and leads to corrosion. Nonetheless, the corrosion behavior of dental metals is susceptible to being altered by the environment. For example, a drop in the pH of the oral environment increases the risk of corrosion of dental metals. Similarly, a difference in electric potential between two or more dental metals in contact with each other in the corrosive oral environment can cause galvanic corrosion to occur.

Chemical disinfectants containing H₂O₂ probably corrode dental metals. Al-Salehi et al. showed that metal ion release from dental casting alloys increased with increase in H₂O₂ concentration. Similarly, electrochemical analysis in another study showed that corrosion current and corrosion rate increased with increase in H₂O₂ concentration. Therefore, H₂O₂ is a key contributing factor to the corrosion of dental metals.

The oxidizing power of hydroxyl radicals is higher than that of H₂O₂. However, little is known about the influence of hydroxyl radicals on the corrosion behavior of dental metals. In sufficient quantities, metal ions released from metallic dental restorations can induce inflammation of the adjacent periodontal tissues and oral mucosa, allergic reactions, and cytotoxic effects that may occur besides damage to the metallic restorations caused by corrosion. Therefore, it is crucial to examine if the corrosive effect of hydroxyl radicals generated by photolysis of H₂O₂ exceeds that of H₂O₂ alone.

The purpose of the present study was to evaluate...
the corrosive effect of a disinfection solution containing hydroxyl radicals generated by photolysis of H$_2$O$_2$ on various dental metals when they were immersed in the disinfection solution for 1 week.

**MATERIALS AND METHODS**

**Experimental LED device**

Hydroxyl radicals were generated by photolysis of 1 M H$_2$O$_2$ using an experimental LED device. The concentration of 1 M H$_2$O$_2$ is used in oral disinfectants and it corresponds to 3.4% H$_2$O$_2$ (wt/vol). In this study, 1 M H$_2$O$_2$ was prepared by diluting 31% H$_2$O$_2$ (Santoku Chemical Industries Co. Ltd., Tokyo, Japan) with pure water. The pH of 1 M H$_2$O$_2$ was 5.1±0.1, as measured using a pH meter (Twin pH meter, Horiba, Kyoto, Japan). Diluted H$_2$O$_2$ was used without further pH adjustment.

Wavelength of LED light (NHH105UV, Lustrous Technology, Shiji, Taiwan) used in this study was 400±20 nm. Irradiance was 80 mW/cm$^2$, as measured using a portable irradiance gage (Delta OHM, Caselle di Selvazzano, Italy). The experimental device customized for this study was equipped with two LED light sources.

**Static immersion test**

Static immersion test was performed according to the guidelines of ISO 10271 “Dental metallic materials —Corrosion test methods (2001)”[1]. Dental metal/alloys used for investigation were commercially pure titanium (cpTi; Titanium sheet, Sanko Kozai Co. Ltd., Sagamihara, Japan), Type 316L stainless steel (Kousei Chemical Industries Co. Ltd., Tokyo, Japan), Ag-Pd-Cu-Au alloy (Castwell M.C., GC Corp., Tokyo, Japan), and Co-Cr alloy (Neorium Co., Ltd., Tsubame, Japan), Ag-Pd-Cu-Au alloy (Castwell M.C., GC Corp., Tokyo, Japan), and Co-Cr alloy (Neorium Co., Ltd., Tsubame, Japan). Compositions of the dental metal/alloys used are given in Table 1.

Ti and Type316L were cut into 15×10×1 mm pieces from a sheet. Ag-Pd-Cu-Au alloy and Co-Cr alloy were cast into 15×10×1 mm pieces according to the manufacturers’ instructions, followed by sandblasting with alumina. All specimens were polish-finished using #1200 silicon carbide papers to remove at least 0.1-mm layer from each surface. Precise measurement of the surface area of each specimen was done using a digital caliper. A nylon string was attached to each specimen to prepare for immersion. All specimens were ultrasonically cleaned in ethanol for 2 min before immersion and then randomly divided into two groups as follows:

- **Group 1:** Five specimens of each dental metal were immersed in 12 mL of 1 M H$_2$O$_2$. Irradiation was performed using the experimental LED device in a direction perpendicular to the specimens, for 1 week in an incubator maintained at 37°C.

- **Group 2:** Five specimens of each dental metal were immersed in 12 mL of 1 M H$_2$O$_2$ without LED light irradiation for 1 week in the incubator maintained at 37°C.

After 1-week immersion, specimens were removed and ions released into each immersion solution were qualitatively and quantitatively analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES; iCAP 6300 ICP spectrometer, Thermo Fisher Scientific Inc., Waltham, MA, USA). However, in the immersion solution of Ti in Group 2, a deposition mass was observed. As a result, the amount of released ions was evaluated by weighing the specimens before and after immersion test using an electronic analytical balance (GH-200, A&D, Tokyo, Japan). Amount of released ions was calculated as µg/cm$^2$/week.

Using Student’s t-test or Welch’s t-test following F-test, statistical analysis was performed on the total amounts of ions released by each dental metal in Groups 1 and 2. Statistical significance was set at $p<0.05$.

**Yield of hydroxyl radicals and time course changes of H$_2$O$_2$ concentration**

Apart from qualitative and quantitative determination of released ions, two other experiments were conducted in this study as follows:

- **Experiment 1:** To determine hydroxyl radical yield in the presence of different concentrations of H$_2$O$_2$ under LED light irradiation.

- **Experiment 2:** To monitor the time course changes of H$_2$O$_2$ concentration with and without LED light irradiation.

1. Determining yield of hydroxyl radicals

Electron spin resonance (ESR) and spin trapping technique using 5,5-dimethyl-1-pyrroline N-oxide (DMPO) were applied for the quantitative analysis of hydroxyl radicals in this study[14]. DMPO was the spin trapping agent. In a test glass vessel, 250 µL of H$_2$O$_2$ was mixed with 250 µL of DMPO (Labotec, Tokyo, Japan) to prepare 300 mM DMPO and H$_2$O$_2$ of 0–1000 mM concentrations. Immediately after mixing, glass vessel was placed in the experimental LED device and mixture was irradiated using LED for 1 min.

Mixture was then transferred to a quartz cell, and its ESR spectrum was recorded using an X-band ESR spectrometer (JES-FA-100, Jeol, Tokyo, Japan). Measurement conditions for ESR were as follows —field sweep: 330.50–340.50 mT; field modulation frequency: 100 kHz; field modulation width: 0.1 mT; amplitude: 80; sweep time: 2 min; time constant: 0.03 s; microwave frequency: 9.420 GHz; microwave power: 4 mW. To calculate the concentration of DMPO-OH (a spin adduct of hydroxyl radical), 20 µM 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxide (TEMPOL; Sigma-Aldrich, St. Louis, MO, USA), a spin label, was used as a standard. The concentration of DMPO-OH was determined using Digital Data Processing (Jeol, Tokyo, Japan).

A calibration curve was constructed with the yield of DMPO-OH plotted against the different concentrations of H$_2$O$_2$ to calculate the generation rate of hydroxyl radicals (µM/min). The yield of hydroxyl radicals each day was derived from the generation rate, and the total
The amount of hydroxyl radicals generated in a week was thus estimated.

## Monitoring time course changes of H$_2$O$_2$ concentration

Time course changes of H$_2$O$_2$ concentration were monitored in this study, as it was expected that H$_2$O$_2$ concentration would decrease with LED irradiation time. Twelve milliliters of 1 M H$_2$O$_2$ was either irradiated with LED light or kept without LED light irradiation in the incubator at 37°C for 1 week. Quantitative analysis of H$_2$O$_2$ was performed every 24 h using a spectrophotometric assay for iodide oxidation by H$_2$O$_2$.

In brief, 100 µL of 1 M sodium iodide, 20 µL of 100 µM horseradish peroxidase, 1830 µL of 0.2 M acetate buffer (pH 3.8), and 50 µL of 20-fold dilution of the sample were mixed. Final concentrations of the reagents were 0.05 M for sodium iodide, 1 µM for horseradish peroxidase, and an 800-fold dilution for the sample. Absorption peak of periodide at 353 nm was read using a spectrophotometer (NanoVue Plus, GE Healthcare, Buckinghamshire, UK).

### RESULTS

#### Metal ion release

Table 2 lists the detection limits of ICP-OES for the elements, and the amounts of ions released from the four dental metals in Group 1 and Group 2 immersion solutions are summarized in Fig. 1.

Ti severely corroded in H$_2$O$_2$ (Fig. 1a), such that a yellow-colored deposited mass was formed in H$_2$O$_2$ alone without LED light irradiation (i.e., Group 2). With Type 316L stainless steel, slight amounts of Fe and Cr ions were detected in both Groups 1 and 2 (Fig. 1b). This indicated that Type 316L was resistant to H$_2$O$_2$ alone as well as the disinfection solution containing hydroxyl radicals generated by photolysis of H$_2$O$_2$. Ag-Pd-Cu-Au alloy also corroded in both Groups 1 and 2, resulting in elution of Ag and Cu ions while Au and Pd ions were hardly detected (Fig. 1c). With Co-Cr alloy, slight corrosion occurred in both Groups 1 and 2 (Fig. 1d). Of the three metal elements, Co ion was eluted the most in both groups.

Figure 2 shows the total amounts of ions released from each dental metal in Groups 1 and 2. Corrosive effect of disinfection solution containing hydroxyl radicals (Group 1) on Ti and Co-Cr alloy was significantly lower (p<0.01) than that of 1 M H$_2$O$_2$ alone (Group 2). For Type 316L and Ag-Pd-Cu-Au alloy, there were no significant differences in corrosive effect between Groups 1 and 2.

#### Yield of hydroxyl radicals

Generation of hydroxyl radicals increased in proportion to the concentration of H$_2$O$_2$ (Fig. 3). Equation for the linear relationship between the concentrations of DMPO-OH and H$_2$O$_2$ was $y=0.05x+9.07$, with a high correlation coefficient of $r=0.991$. Time course changes of H$_2$O$_2$ concentration

Concentration of H$_2$O$_2$ decreased in a time-dependent manner when irradiated with LED light, while that without LED irradiation remained almost unchanged (Fig. 4). After 1 week of irradiation, 1000 mM H$_2$O$_2$ was degraded to approximately 200 mM H$_2$O$_2$. Spectrophotometric assay for iodide oxidation by H$_2$O$_2$ showed that the average concentrations of H$_2$O$_2$ at 0, 24, 48, 72, 96, 120, 144, and 168 h were 1000, 775, 590, 487, 406, 321, 267, and 216 mM respectively.

Based on the calibration curve, the yield of hydroxyl radicals each day was calculated. For example, H$_2$O$_2$ concentration decreased from 1000 mM to 775 mM during the first day, rendering the average H$_2$O$_2$ concentration to be 888 mM. Therefore, the yield of hydroxyl radicals generated by photolysis of 888 mM H$_2$O$_2$ per minute in the first day was calculated to be 53 µM ($=0.05\times888+9.07$). In 24 h, the yield of hydroxyl radicals was calculated to be 77 mM ($=0.053\times60\times24$). Accordingly, the yield of hydroxyl radicals each day was calculated to be 77, 62, 52, 45, 39, 34, and 30 mM respectively. After 1 week, the total amount of hydroxyl radicals generated was estimated to be 340 mM.

### Table 1 Compositions of the dental metal/alloys used in this study (wt%)

<table>
<thead>
<tr>
<th>Ti</th>
<th>Fe</th>
<th>Cr</th>
<th>Mo</th>
<th>Ni</th>
<th>Ag</th>
<th>Pd</th>
<th>Au</th>
<th>Cu</th>
<th>Co</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
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<td>99.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.2</td>
</tr>
<tr>
<td>Type 316L</td>
<td>–</td>
<td>balance</td>
<td>18</td>
<td>2.5</td>
<td>12</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>&lt;3.1</td>
</tr>
<tr>
<td>Ag-Pd-Au-Cu</td>
<td>–</td>
<td>–</td>
<td>28</td>
<td>5.7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>64.7</td>
</tr>
<tr>
<td>Co-Cr</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>28</td>
<td>5.7</td>
<td>–</td>
<td>64.7</td>
</tr>
</tbody>
</table>

### Table 2 Detection limits of ICP-OES for the elements

<table>
<thead>
<tr>
<th>Ti</th>
<th>Fe</th>
<th>Cr</th>
<th>Mo</th>
<th>Ni</th>
<th>Ag</th>
<th>Pd</th>
<th>Au</th>
<th>Cu</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>ppm</td>
<td>0.0147</td>
<td>0.0014</td>
<td>0.0377</td>
<td>0.0011</td>
<td>0.0003</td>
<td>0.0320</td>
<td>0.0023</td>
<td>0.0016</td>
<td>0.0034</td>
</tr>
<tr>
<td>µg/cm$^2$</td>
<td>0.0503</td>
<td>0.0048</td>
<td>0.1293</td>
<td>0.0037</td>
<td>0.0009</td>
<td>0.1098</td>
<td>0.0079</td>
<td>0.0054</td>
<td>0.0118</td>
</tr>
</tbody>
</table>
Fig. 2 Comparison of total amounts of ions released from each dental metal. Specimens in Group 1 were immersed in 1 M H₂O₂ and irradiated with LED light, while those in Group 2 were immersed in 1 M H₂O₂ without LED light irradiation. Each value represents the mean of quintuplicate assays with standard deviation. ∗: p<0.01.

DISCUSSION

Ti, Type 316L stainless steel, Ag-Pd-Cu-Au alloy, and Co-Cr alloy were selected as representative dental metals for investigation in this study because they are widely used in the dental field. Ti is applied for dental implants and metal components of dentures; Type 316L stainless steel is applied for dental instruments as well as orthodontic wires and brackets; Ag-Pd-Cu-Au alloy is applied for metal components of dentures and metallic restorations such as inlays, crowns, and bridges in Japan; and Co-Cr alloy is applied for metal components of dentures and frameworks of metal-ceramic restorations.

Static immersion test in this study was performed according to the guidelines of ISO 10271 to examine the corrosive effect of hydroxyl radicals on dental metals. Hydroxyl radicals probably oxidize dental metals, and the corrosive effect may intensify with repeated and prolonged exposure to disinfection solutions containing hydroxyl radicals. To date, there is no information on the clinically acceptable level of ion release from dental metals. Hence, the corrosive effect of disinfection solution containing hydroxyl radicals generated by photolysis of H₂O₂ was examined by comparing against that of H₂O₂ alone—which is used as a disinfectant in
Fig. 3 Calibration curve between the yield of DMPO-OH and the concentration of H$_2$O$_2$. Linear relationship showed a high correlation coefficient. Each value represents the mean of triplicate assays.

Fig. 4 Changes in concentration of H$_2$O$_2$ with and without LED irradiation. Concentration of H$_2$O$_2$ was determined by spectrophotometric analysis of oxidation of NaI. Each value represents the mean of triplicate assays.

clinical dentistry.

Ti and Cr-containing alloys usually have a thin layer of oxide—called the passive film—formed on their surfaces. These stable and insulating passive films, which contain TiO$_2$ or Cr$_2$O$_3$, act as a barrier to protect their underlying metal substrates from corrosion$^{16,17}$. Type 316L stainless steel and Co-Cr alloy contain Cr in their compositions. Therefore, they exhibited relatively high corrosion resistance to 1 M H$_2$O$_2$ with only a small amount of ions released after 1 week of immersion (<20 µg/cm$^2$/week). Another reason which accounted for their high corrosion resistance was the absence of chloride ions in the immersion solutions used in the present study. Chloride ions penetrate the passive film and initiate pitting corrosion, especially in the case of stainless steel$^{18,19}$. On this note, the effect of chloride ion of chloride-containing disinfection systems on metal corrosion should be examined in future studies.

Ti was severely corroded by H$_2$O$_2$ in this study, a finding consistent with previous studies$^{20-22}$. Although Ti has high corrosion resistance generally, this property was reportedly impaired in the presence of H$_2$O$_2$, and that the presence of some metal chlorides caused accelerated corrosion$^{20}$. On the mechanism of Ti corrosion, Tengvall et al. proposed that TiO$_2$ is hydrated to form Ti(IV)-H$_2$O$_2$ complex, followed by formation of TiOOH adduct which is a yellow-colored deposition$^{20}$. In other words, TiO$_2$ passive film is more vulnerable towards H$_2$O$_2$ than Cr$_2$O$_3$. Takemoto et al. also showed that Ti-H$_2$O$_2$ complexes were formed on the surface of Ti treated with H$_2$O$_2$ via Ti-peroxide radical species$^{24}$.

Ag-Pd-Cu-Au alloy is one of the dental metals admitted by Japan's public medical insurance system for metal restorations. This alloy contains 20% Pd and 12% Au (Table 1). According to the classifications set by the American Dental Association Council on Dental Materials, Ag-Pd-Cu-Au alloy is classified as a noble alloy as its noble metal content exceeds 25%. Although this alloy is known to have high corrosion resistance$^{25}$, the present study showed that the corrosive environments used in the present study induced the elution of Ag and Cu ions.

For all the dental metals tested in this study, their total amounts of ions released when immersed in the disinfection solution containing hydroxyl radicals never exceeded those eluted in 1 M H$_2$O$_2$ without LED light irradiation. This finding suggested that hydroxyl radicals generated by photolysis of H$_2$O$_2$ did not have a more severe corrosive effect than H$_2$O$_2$ alone.

Quantitative analysis of H$_2$O$_2$ revealed that with LED light irradiation, there was an approximately 20% decline in the concentration of H$_2$O$_2$ per day such that 1000 mM H$_2$O$_2$ degraded to become 216 mM after 1 week. Under the same experimental conditions, an estimated total amount of 340 mM hydroxyl radical was generated by photolysis of H$_2$O$_2$ at the end of 1 week. It must be highlighted that the concentration of hydroxyl radicals does not cumulatively increase in a time-dependent manner because the radicals are very reactive and have very short lifetimes (less than 10 ns)$^{26,27}$. This meant that the concentration of hydroxyl radicals remained constantly low at all times while the concentration of H$_2$O$_2$ continuously decreased with LED light irradiation. For this reason, H$_2$O$_2$ without LED light irradiation caused more severe corrosion of the dental metals than the disinfection solution containing hydroxyl radicals.

The disinfection system based on the photolysis of H$_2$O$_2$ could be applied to denture cleaning system as well as the treatment of oral infectious diseases in terms of metal corrosion as far as the concentration of H$_2$O$_2$ is 3% or less, the former of which is a concentration used in the oral cavity as a disinfectant. Since H$_2$O$_2$ generated
in peroxides solution has been clinically used as a denture cleanser without serious problems\(^{28}\), it is also considered that the disinfection system by photolysis of \(\text{H}_2\text{O}_2\) can be applied to denture cleaning device without problematic metal corrosion unless the dentures contain Ti components. Concerning the application of the disinfection technique to the treatment of oral infectious diseases, the metals would be exposed to hydroxyl radicals only for much shorter time than the case of denture cleaning. For instance, \(\text{H}_2\text{O}_2\) is clinically used for the treatment of peri-implantitis to clean the implant from the Ministry of Economy, Trade and Industry.

\[ \text{H}_2\text{O}_2 \]

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ACKNOWLEDGMENTS

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