The effect of hydrogen peroxide/colloidal silver on reducing the colonization and growth of heterotrophic bacteria in dental unit waterlines*

Meral ÖZALP1,*, Ömer Engin BULUT2, Atilla Stephan ATAÇ3, MelikeEKİZÖGÜL1, Didem KART1, Hakan Hamdi ÇELİK1, İlkan TATAR4

1Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Hacettepe University, 06100, Sıhhiye, Ankara, Turkey
2Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Başkent University, 06490, Bahçelievler, Ankara, Turkey
3Department of Pediatric Dentistry, Faculty of Dentistry, Hacettepe University, 06100, Sıhhiye, Ankara, Turkey
4Department of Anatomy, Faculty of Medicine, Hacettepe University, 06100, Sıhhiye, Ankara, Turkey

Abstract: The objective of the present study was to investigate reduction in the colonization and growth of heterotrophic bacteria in dental unit waterlines by using hydrogen peroxide/colloidal silver as disinfectant. Twenty-seven dental units were included; 6 units that were more than 20 years old and 7 units that had been in use for 2 years comprised the old and new treatment groups, respectively. Fourteen units served as controls. The treatment groups were disinfected continuously and every 4 weeks shock doses were applied over a 20-week period. Water samples were taken before treatment, 1 and 2 weeks after treatment, and thereafter every 4 weeks; then they were inoculated onto R2A agar plates. While 1–16-week results for the old treatment group showed total heterotrophic bacterial counts of higher than 1 × 105 cfu/mL, at 20 weeks they were below 7.5 × 102 cfu/mL. Only 2 units were able to reach levels of ≤200 cfu/mL, which is the dental unit water quality standard. For the new treatment group it was achieved for all units after 1 week. Electron microscopic analysis also revealed that while biofilm formation was more evident in the old treatment group, after a longer treatment period biofilms were eliminated completely. The findings indicate that disinfection was effective in improving the output water quality using hydrogen peroxide/colloidal silver disinfectant.

Key words: Biofilm, aerobic heterotrophic bacterial count, dental unit waterlines, hydrogen peroxide/colloidal silver

1. Introduction
Water in dental units is used for cooling the handpieces, flushing, and irrigating the oral cavity during dental treatment. Water delivered from dental units is not sterile and has been shown to contain high numbers of bacteria (1–4). Several reasons can cause these high numbers, such as ambient temperature, source of water, type of tubing, and stagnation, which facilitates the formation of biofilms on the inner surface of the waterline tubing (1,2). Biofilms are well-organized communities of cooperating microorganisms that can include bacteria, protozoa, diatoms, and fungi (5,6). These microbes range from environmental-origin to opportunistic pathogens, such as Moraxella, Klebsiella, Pseudomonas aeruginosa, nontuberculosis Mycobacterium spp., Legionella pneumophila, and Candida spp. (6–8).

During almost every visit, the patient and the dental health care staff are exposed to the water from dental unit waterlines (DUWLs). We have limited knowledge about whether contaminated DUWLs pose a risk of infection for dental clinicians and patients. There are only a few reports linking exposure to contaminated DUWLs with infection (8–10). Even in these conditions, the infection control and prevention program does not take account of the risk of contaminated DUWLs (11,12).

The aim of the present study was to investigate reduction in the colonization and growth of aerobic heterotrophic bacteria to or below 200 colony-forming units per milliliter (cfu/mL) in previously untreated old and relatively new dental units by using hydrogen peroxide/colloidal silver disinfectant.

2. Materials and methods
2.1. Dental units
This study was performed in clinics belonging to dentistry faculties in 2 different universities: Hacettepe and Başkent. Twenty-seven dental units that directly used the municipal water system and had never been treated with any disinfectant were selected. Six units that were more than 20 years old and 7 units that had been in use for 2 years comprised the old and new treatment groups, respectively. Fourteen units served as controls. The older 6 units in Hacettepe University were

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** Correspondence: mozalp@hacettepe.edu.tr
classified as the old treatment group. These units have been in use for more than 20 years. In Başkent University, 7 units that were not more than 2 years old comprised the new treatment group. The DUWL was made of polyvinyl and the diameter was 2.5 mm. In order to see the difference between older and newer devices, the 2 treatment groups were formed with distinctive model year differences.

### 2.2. Modifications of DUWLs

The dental units were modified to facilitate the addition of a disinfectant to the water used in the DUWLs by fitting an externally mounted purge system. Using this system, the municipal water supply could be bypassed and the tank was used to add a disinfectant to the DUWLs throughout patient treatment sessions. Disinfectant solution was continuously used and every 4 weeks shock doses were applied, as recommended by the disinfectant’s manufacturer. For shock doses, all of the liquids were removed and then 250 mL of 5% hydrogen peroxide colloidal silver (Huwa-San Dent-6, Roamchemie, Belgium) with 750 mL of municipal water was applied to the DUWLs for 2 h. For continuous application 25 mL of 5% disinfectant was added to the 15-L tank supply, which was previously filled with municipal water (the final concentration of hydrogen peroxide colloidal silver was 83 µg/mL) and was left in the tank overnight.

### 2.3. Microbiological analysis of the water samples

Water samples from 27 dental units were taken before treatment (baseline), 1 and 2 weeks following the application of disinfectant, and every 4 weeks during the next 20 weeks (treatment groups). Control units were not subjected to waterline disinfection. After 30 s flushing, approximately 50 mL of water sample was collected into a sterile water bottle containing sodium thiosulfate [Fluka Chemie, Buchs, Switzerland (final concentration 0.1%)] in order to neutralize the residual disinfectant in water (13). Samples were delivered immediately to the microbiology laboratory in a cool box. The samples were maintained at 4 °C and processed within 3 h. Each sample was diluted and spread-plated onto triple R2A agar plates (Merck, Darmstadt, Germany) and then incubated at 28 °C for 7 days. Since it is particularly efficient at isolating aerobic heterotrophic bacteria from water, R2A agar was used to determine aerobic heterotrophic bacterial count (14). At the end of the incubation period, average bacterial counts were determined as cfu/mL.

### 2.4. Detection of biofilm

Five units from both groups were randomly selected for scanning electron microscopy to evaluate biofilm formation before the treatment and at the end of the 20-week period. The waterline tubing samples were fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer and then removed, washed in purified water, cut into sections, and split lengthwise to expose the interior surface. After fixation, the specimens were dehydrated in a series of ethyl alcohol, dried with 100% hexamethyldisilazane to minimize shrinkage due to drying, as described by Wirthlin et al. (15), and then examined by scanning electron microscope (Zeiss EVO, Germany) at an operation voltage of 5 KeV.

### 2.5. Statistical analysis

To compare pre- and postsed disinfection total bacterial count values for both old and new dental units, the Wilcoxon test was used. The Mann–Whitney U test was subsequently used to compare the old and new units with their control groups separately (SPSS 12.0.1 for Windows; SPSS, Chicago, IL, USA). In order to test statistical differences between all groups, a Kruskal–Wallis test was used. Statistical significance was defined as P < 0.05.

### 3. Results and discussion

Bacterial densities of old and new dental units' output water before and after disinfection using hydrogen peroxide/colloidal silver are given in Tables 1 and 2. Samples of the old dental units control group had a minimum count of 1.4 × 10³ cfu/mL and these high values were maintained during the 20 weeks (Table 1). For control groups of both new and old dental units, there were no significant differences between the baseline and other weeks in terms of output water heterotrophic bacteria count (P > 0.05 for Wilcoxon test). For the old dental units, statistically significant differences in median reduction (P = 0.035 for Mann–Whitney U test) in heterotrophic bacteria count were found between the treatment and control groups in week 20, while they existed for all weeks in the new dental units (P = 0.002 for Mann–Whitney U test).

In the comparison of the treatment groups in old and new dental units, statistically significant differences were found between before and after treatment in the new units (P = 0.018 for Wilcoxon test) (Table 2), while there were no significant differences in values for the old units during the 16-week period (data not given) (P > 0.05 for Wilcoxon test) (Table 1).

Differences between before and after 20 weeks of treatment were significant for the treatment group of old dental units (P = 0.028 for Wilcoxon test). Furthermore, 2 units in this group reached levels of ≤200 cfu/mL at the end of 20 weeks (Table 1).

Further statistical tests showed that the reduction in heterotrophic bacteria to the levels below 200 cfu/mL recommended by the American Dental Association (ADA) in the treatment group of new dental units was significantly more successful than in all of the old dental units (P < 0.05 for Kruskal–Wallis).

Scanning electron micrographs from processed DUWL tubing samples revealed similar patterns of microbiological study results, with biofilm accumulation more evident in
the control groups both at baseline and at the end of the study (Figure 1a, 1b). Moreover, biofilm formation existed in the treatment groups before the application (Figure 1c, 1d). However, continuous treatment with hydrogen peroxide/colloidal silver disinfectant was able to remove the biofilm attached to the inner surfaces of the DUWL tubing in the treatment groups (Figure 1e, 1f). In contrast to the treatment groups, at the end of the study biofilm accumulation was still more evident in the control groups (Figure 1b). Biofilm was almost absent from the inner surface of the waterline tubing from both new and old dental units at the end of the study (Figure 1e, 1f).

Table 1. Bacterial density of old dental units output water before and after hydrogen peroxide/colloidal silver disinfection.

<table>
<thead>
<tr>
<th>Units</th>
<th>Control units</th>
<th>Treatment units</th>
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<tbody>
<tr>
<td></td>
<td>Baseline*</td>
<td>Week 1</td>
</tr>
<tr>
<td>1</td>
<td>1.4 × 10³</td>
<td>tntc</td>
</tr>
<tr>
<td>2</td>
<td>3.2 × 10⁴</td>
<td>2.7 × 10⁴</td>
</tr>
<tr>
<td>3</td>
<td>1.1 × 10⁵</td>
<td>7.6 × 10⁴</td>
</tr>
<tr>
<td>4</td>
<td>8.0 × 10⁴</td>
<td>6.1 × 10⁴</td>
</tr>
<tr>
<td>5</td>
<td>tntc</td>
<td>4.8 × 10⁴</td>
</tr>
<tr>
<td>6</td>
<td>tntc</td>
<td>6.8 × 10⁴</td>
</tr>
<tr>
<td>7</td>
<td>1.4 × 10⁴</td>
<td>1.1 × 10⁴</td>
</tr>
</tbody>
</table>

Abbreviations: cfu, colony forming unit; tntc, too numerous to count
* P > 0.05 for Wilcoxon test
** P = 0.035 for Mann–Whitney U test at 20 weeks
†P = 0.028 for Wilcoxon test
‡ P < 0.05 for Kruskal–Wallis test

Table 2. Bacterial density of new dental units output water before and after hydrogen peroxide/colloidal silver disinfection.

<table>
<thead>
<tr>
<th>Units</th>
<th>Control units</th>
<th>Treatment units</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline*</td>
<td>Week 1</td>
</tr>
<tr>
<td>1</td>
<td>1.2 × 10⁴</td>
<td>2.0 × 10³</td>
</tr>
<tr>
<td>2</td>
<td>2 × 10⁵</td>
<td>1.8 × 10⁴</td>
</tr>
<tr>
<td>3</td>
<td>1.8 × 10⁴</td>
<td>4.0 × 10³</td>
</tr>
<tr>
<td>4</td>
<td>2.8 × 10³</td>
<td>6.5 × 10³</td>
</tr>
<tr>
<td>5</td>
<td>2.2 × 10²</td>
<td>3.0 × 10⁴</td>
</tr>
<tr>
<td>6</td>
<td>3.9 × 10²</td>
<td>2.3 × 10³</td>
</tr>
<tr>
<td>7</td>
<td>9.8 × 10²</td>
<td>1.0 × 10³</td>
</tr>
</tbody>
</table>

* P > 0.05 for Wilcoxon test
** P = 0.002 for Mann–Whitney U test for all weeks
† P = 0.018 for Wilcoxon test
‡ P < 0.05 for Kruskal–Wallis test

Since old dental units have thicker and stronger biofilms, it is very important to remove them completely...
from old units. The results of the present study showed that the treatment of old dental units with hydrogen peroxide/silver ions disinfectant was efficient in both reducing the heterotrophic bacteria in output water and eradicating the biofilm in a longer treatment period compared with the new ones.

Figure 1. Scanning electron micrographs of dental unit waterline’s internal surfaces from a control unit for baseline (a) and after treatment units (b), treatment groups before the application from old units (c) and new units (d), treatment groups after 20 weeks’ disinfection from old units (e) and new units (f).
It was interesting to find that even though both the old and new dental units' water sources have similar bacterial density (data not shown), which are less than 200 cfu/mL, they do not necessarily meet the ADA's recommendation for dental unit output water. It was revealed that although the dental unit's output water quality was directly influenced by the source water quality (1,16), a significant reduction in biofilm inside the DUWLs was the main factor improving output water quality in the dental units.

Studies have focused on many techniques to achieve the ADA's dental treatment water quality standards of ≤200 cfu/mL, namely antiretraction valves, waterline flushing, independent water reservoir systems, distilled water, ultraviolet light, ultrasonics, drying of DUWLs, inline micropore filtration, and autoclavable systems. Flushing the waterlines before patients were treated and dehydrating the units when they were not in use were ineffective and inconsistent in reducing heterotrophic bacteria to acceptable levels or removing biofilm (15,17). Filters placed inline did not prevent the formation of biofilm (5). Kettering (18) reported that even in a closed-circuit water system, sterile distilled water alone could not reduce microbial contamination of dental output water from DUWLs to the ADA stated goal of 200 cfu/mL. The intermittent and/or continuous application of disinfectant solutions through the DUWLs has also been proposed (5,6,19). Currently there are no microbial quality standards for DUWL output water in this country. In the present study, the combination of hydrogen peroxide colloidal silver ion-containing disinfectant, Huwa-san Dent-6, was used to achieve the ADA quality standard. This combination has been one of the most widely used chemical methods for DUWL disinfection because of its rapid and broad spectrum biocidal activity, biodegradability, and lack of corrosion damage to dental instruments. A number of DUWL treatment products that have been shown to be effective at controlling DUWL biofilm essentially have a residual effect within the DUWL. As the disinfectant was used continuously in the present study, the residual effect could not be examined. By their very nature, these waterline-cleaning products are claimed not to be detrimental to human health. Silver ions destabilize the biofilm matrix by binding to electron donor groups of biological molecules, leading to reductions in the number of binding sites for hydrogen bonds and electrostatic and hydrophobic interactions. The antimicrobial activity of hydrogen peroxide has oxidizing properties, and thus disrupts membrane lipids, DNA, and essential cell components (20).

In a series of trials, treatment of DUWLs with isopropanol (6), chlorhexidine (3), sodium hypochlorite (21), and glutaraldehyde (6) was investigated. These agents reduced microorganisms in effluent water but did not destroy the biofilm matrix in the DUWLs, even with periodic treatments (6). Similar results were obtained for some mouth rinses (Listerine, Bio 2000, Rembrandt) and 0.5% sodium fluoride (22). On the other hand, a study that tested the efficacy of reactive chlorine dioxide concluded that intermittent treatment with 50 ppm resulted in a temporary decrease in bacterial counts, but it did not maintain a long-term reduction (23).

Many studies have also evaluated that efficacy of hydrogen peroxide-based disinfectants (3,4,16,24–26). Linger et al. (24) studied 0.5% hydrogen peroxide disinfectant for DUWLs in 23 dental units over 5 weeks. That short-term study revealed that an easy-to-use hydrogen peroxide-based disinfectant was effective in improving the quality of water used for intraoral procedures and protocol compliance met the ADA goal. Szymanska (25) investigated the effect of 0.4% hydrogen peroxide/silver ions disinfectant on the bacterial microflora in DUWLs. The application of this agent caused a significant decrease in the number of total bacteria. Schel et al. (3) compared the abilities of 8 different disinfectants to improve DUWL quality for 134 units in 7 European countries. Their clinical trial showed that continuous treatment with hydrogen peroxide silver ions disinfectants caused a significant difference between the baseline and treatment values in bacterial count and 91% of the treated water samples had values of ≤200 cfu/mL.

In addition to a novel automated waterline cleaning system, O'Donnell (20) et al. also investigated the effectiveness of 2 different hydrogen peroxide/silver ions disinfectants, Planosil and Planosil Forte. They found that the automated waterline cleaning system provided a reduction in aerobic heterotrophic bacteria and removed biofilm sufficiently when used with Planosil, especially Planosil Forte. The same results have been obtained for Sanosil [1% (v/v) hydrogen peroxide, 0.001% (w/v) silver] and Sterilex Ultra [5% (w/v)] (4). However, another study stated that the selection of disinfectant-tolerant bacterial species is one of the reasons for long-term waterline disinfection failure. In order to prevent those difficulties, the periodic use of a different disinfectant would be reasonable (27).

In conclusion, according to the results of the present study, when continuously used with shock doses, hydrogen peroxide/silver ions disinfectant was particularly efficient at reducing the heterotrophic bacteria in output water and eradicating the biofilm in DUWLs.
References


