Evaluation of a hydrogen peroxide disinfectant for dental unit waterlines

JACKSON B. LINGER, D.M.D., M.S.; JOHN A. MOLINARI, Ph.D.; WILLIAM C. FORBES, D.D.S.; CRAIG F. FARTHING, B.S.; WILLIAM J. WINGET, B.S.

Background. The purpose of this study was to investigate the use of a hydrogen peroxide–based dental unit waterline, or DUWL, treatment to reduce the colonization and growth of heterotrophic bacteria.
Methods. Twenty-three dental units with self-contained water systems were randomly selected. Three of the units and tap water served as controls. Twenty-four water samples were taken at baseline and once a week for five weeks. They were serially diluted, spread-plated in duplicate onto R2A agar plates and incubated at 37 C for seven days.
Results. At baseline, the tap water control had a mean count of 0 colony-forming units/milliliter, or CFU/mL, the three control DUWLs had a median count of 8,440 CFU/mL and the 20 treated DUWLs had a median count of 9,760 CFU/mL. By week 1, 19 (95 percent) of the 20 treated DUWLs had counts of less than 200 CFU/mL, and by week 4, the median count for all of the treated DUWLs was 0 CFU/mL. The measurement at week 5 showed that the reduction to below 200 CFU/mL had been maintained. Scanning electron micrographs from processed DUWL tubing samples revealed a similar pattern of results, with biofilm accumulation more evident in the untreated control specimens.
Conclusions. Following the parameters of this study, the authors used a hydrogen peroxide–based disinfectant to achieve the ADA goal of no more than 200 CFU of heterotrophic, mesophilic bacteria per milliliter of unfiltered output water.
Clinical Implications. An easy-to-use hydrogen peroxide–based DUWL disinfectant demonstrated effectiveness in improving the quality of water used for intraoral procedures. Protocol compliance meets the ADA year 2000 goal.

Reports of microbial contamination of municipal water systems[1,2] as well as sporadic outbreaks of bacterial[3,4] and protozoan[5] disease have sparked a heightened concern about water quality. The existence of high concentrations of microbial accumulations in dental unit waterlines, or DUWLs, was first reported by Blake in 1963.[6] Subsequent reports have cited contamination levels ranging from 1,000 to 160 million colony-forming units/milliliter, or CFU/mL.[7-9] Microorganisms have been identified in water samples that are considered potentially pathogenic to humans. These include *Pseudomonas*, *Legionella* and nontuberculosis *Mycobacterium* species, although other contaminants ranging from fungi to aquatic nematodes have been identified less frequently.[10]

A hydrogen peroxide–based dental unit waterline disinfectant demonstrated effectiveness in improving the quality of water used for intraoral procedures.

Concern has been raised about the safety of dental health care workers, who may be at risk by virtue of their exposure to water spray and aerosols from the dental handpiece, air-water syringe, ultrasonic cleaning devices or other equipment that uses dental unit water. Patients also face a potential risk from these aqueous sources, but their exposure time is limited by the procedure. Exposure of both dental health care workers and patients is minimized by the use of universal precautions, antiretraction valves, high-volume suction and rubber dams. Although a potential risk to the general patient population exists, there have been no major outbreaks attributable to a DUWL that would suggest a quantifiable epidemiologic risk. Reports in the literature, however, do suggest that debilitated or immunocompromised patients are at greater

risk because they are less able to defend themselves against the challenge of opportunistic microorganisms.[11,12]

The Environmental Protection Agency's national primary drinking water regulations establish a mandated standard for potable water in community water systems of 500 CFU/mL or less.[13] Although not proposed as a standard of care, the American Dental Association set a goal of no more than 200 CFU of heterotrophic, mesophilic bacteria per milliliter of unfiltered output water as a benchmark for DUWLs.[14] Organized dentistry, industry and dental equipment manufacturers have made a concerted effort to meet this goal.

As a result, several strategies have evolved to reduce bacterial colonization and growth, including use of waterline flushing, independent water reservoir systems, distilled or pasteurized water, ultrasonics, ultraviolet light, inline micropore filtration and periodic or continuous chemical disinfection.[14-16] These strategies vary in their approaches, either by reducing the number of bacteria introduced into the system or by directly attacking the biofilm. We conducted this study to investigate the use of a recently developed disinfectant formulation and a protocol to reduce the colonization and growth of heterotrophic bacteria in previously untreated DUWLs.

MATERIALS AND METHODS

Twenty-three dental units (A-dec preclinical simulators, A-dec Inc.) that used a self-contained water system were selected randomly for inclusion in this study from the 76 units available in the University of Detroit Mercy Simulation Lab. Three of these units were selected as controls in which no periodic disinfectant procedures were instituted. A tap water source also was used as a study control. Four investigators, two faculty members (J.L., W.F.) and two students (C.F., W.W.), coordinated the clinical procedures followed in this investigation. The students maintained all of the units that normally were assigned to them, and were given no specific instructions regarding DUWLs.

The investigators collected a total of 24 water samples for a baseline measure before the study began, and another 24 samples once a week for the next five weeks. The study was ongoing during the normal school workweek. Start-up was initiated by treating the DUWLs with an alkaline-based peroxide disinfectant (Sterilex Ultra, The Sterilex Corp.) for three consecutive nights. This initial "shock treatment" consisted of a 0.5 percent hydrogen peroxide solution freshly prepared each night by mixing 12.5 grams of disinfectant powder in 250 mL of hot water. Routine weekly treatment protocols then were implemented, as described below.

Waterlines were flushed for 30 seconds, with samples subsequently collected from the air-water syringe in sterile test tubes. Samples for microbial assay were taken at a worst-case point immediately before chemical treatment. Water reservoir bottles were emptied and an 8-ounce solution of freshly prepared disinfectant was placed into the bottles. The disinfecting solution is easy to mix and has the advantage of being pink, which enables the operator to easily see that the solution is in the water reservoir, that it is evident in the effluent water when the system is charged and that it has cleared the system when the unit is flushed. We then used the water syringe to flush the waterline until the pink-dyed hydrogen peroxide solution was observed to flow from the water syringe. The chemical solution then remained in the units overnight. Before class the next day, we emptied the units' reservoirs, refilled them with tap water and flushed the lines for 60 seconds.

All water samples collected for microbial culture were diluted serially in sterile distilled water in a 1:100 ratio. We spread-plated 1 mL of each of the diluted samples in duplicate onto agar plates containing R2A medium. Cultures were incubated at 37 C for seven days. We chose this temperature to simulate body temperature as a condition for growth of aqueous organisms. Microbial counts from the duplicate cultures then were averaged.

At the conclusion of the five-week study, we randomly selected 10 units for scanning electron microscopy, or SEM, to evaluate the biofilm. A one-inch piece of the waterline adjacent to the air-water syringe was sectioned and placed into a sterile test tube containing 3.2 percent glutaraldehyde. SEM was performed at the University of Louisville, Ky. Each tubing section was cut longitudinally and a 1-centimeter sample was processed according to a standard dehydration protocol, mounted with adhesive on aluminum studs, sputter-coated with 2 nanometers of gold/palladium in a 60:40 ratio and then examined with a scanning electron microscope. Photographs of representative sample areas were

TABLE

EVALUATION OF A HYDROGEN PEROXIDE DISINFECTANT FOR DENTAL UNIT WATERLINES.

DUWL*	MEAN CFU/mL†‡				
	Baseline	Week 1	Week 3	Week 4	Week 5
1	450	2,880	0	0	0
2	12,600	0	0	0	0
3 (Control)	220	4,800	856	2,115	5,000
4	> 50,000	0	0	0	0
5	9,320	0	0	0	0
6	7,160	0	0	0	0
7	560	0	0	0	0
8	10,200	0	0	0	0
9	9,240	0	0	0	0
10	10,400	0	0	0	0
11 (Control)	9,400	130	2,288	> 50,000	5,000
12	8,320	0	0	0	0
13	> 50,000	0	0	0	0
14	> 50,000	0	0	0	0
15	24,800	0	32	0	0
16	> 50,000	0	0	0	0
17	0	0	1	0	0
18 (Control)	8,440	8,160	1,880	800	5,000
19	6	0	1	0	0
20	> 50,000	0	0	0	0
21	> 50,000	0	520	0	0
22	0	0	0	0	0
23	7,560	0	0	0	0
24 (Tap Water)	0	0	81	0	0

* DUWL: Dental unit waterline.
† CFU/mL: Colony-forming units per milliliter.
‡ Data are unavailable for week 2 because the water samples were contaminated as a result of accidental exposure to high heat.

taken at ×250, ×500 and ×1,000 magnification.

RESULTS

As the table shows, the CFU counts varied considerably at baseline, with the vast majority far exceeding the 200 CFU/mL goal. After one week, however, the count for only one of the 20 study units was above 200 CFU/mL, and by week 4, all units had counts well below 200 CFU/mL. Control units continued to yield high concentrations of environmental organisms throughout the five weeks of the study. Using the Mann-Whitney U test at the .05 level of significance, we found no statistical evidence of a difference in findings between the control DUWLs; however, we found statistical evidence of a difference in findings between the disinfectant-treated waterlines over the five weeks of the study.

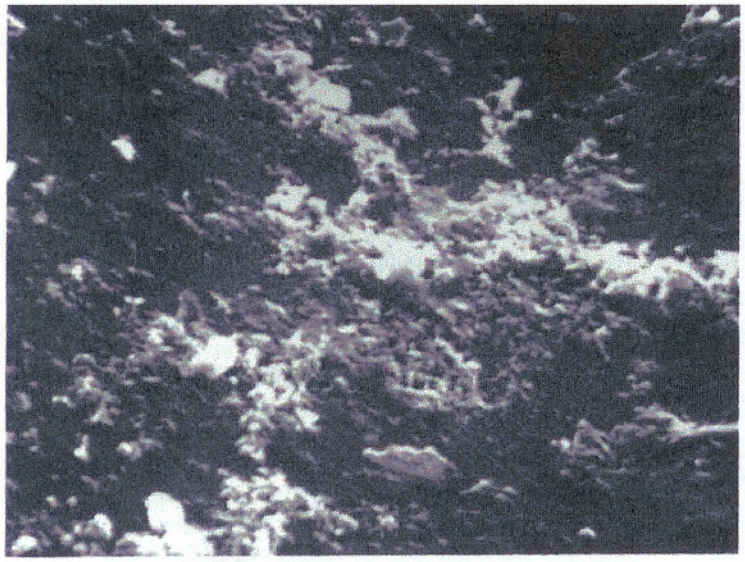

Figure 1. Scanning electron micrograph of baseline biofilm in a section of the air-water syringe line. Evidence of extensive microbial colonization with extensive organic matrix material is discernible (×1,000).

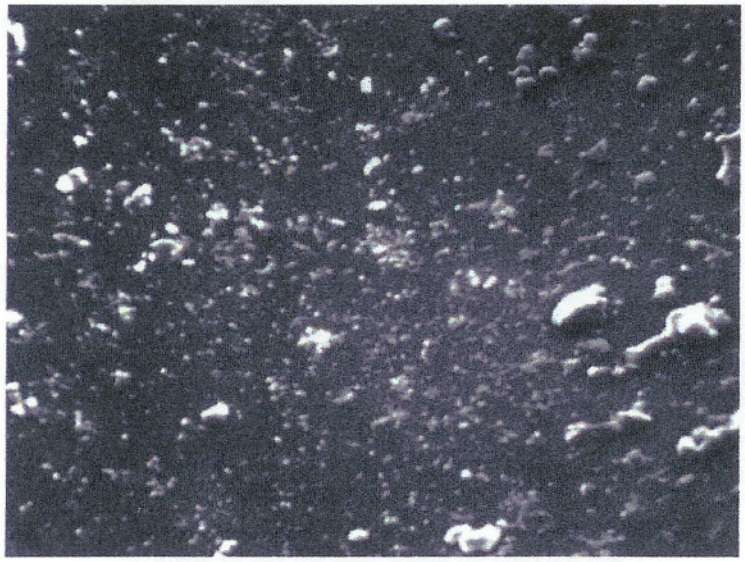

Figure 2. Scanning electron micrograph of a representative section of the air-water syringe line after five weeks of periodic disinfectant treatment. Presence of irregular material on the surface was observed with few, if any, microbial forms evident (×250).

Examination of SEM micrographs of processed DUWL tubing samples revealed a similar pattern of results. Tubing samples from air-water syringe lines of untreated control units exhibited a variety of biofilm formations, ranging from relatively early stages to well-established organic matrixes containing numerous colonizing microbial forms (Figure 1). In contrast, we found few bacteria on the surfaces of tubing taken from units that had been treated for five weeks with the peroxide-based disinfectant (Figure 2). However, a residual matrix was evident on these treated samples.

DISCUSSION

We conducted this study in our preclinic simulation laboratory. A previous evaluation revealed that students' compliance with the DUWL maintenance protocol was only about 50 percent, which motivated us to explore options to improve effluent water quality. These units are not used for patient care, which eliminates the possibility of fluid retraction from a patient and creates an environment for minimum contamination of the air-water syringe. The tap water used in this study was routinely well below 200 CFU/mL. These conditions notwithstanding, the baseline water samples showed that several dental units had counts of greater than 50,000 CFU/mL.

Data for week 2 are not available because the water samples were contaminated as a result of exposure to high heat from a faulty heating, ventilation and air conditioning unit in the microbiology laboratory storage area. However, the data trends from week 1 to week 3 do not suggest a variance that would be identified by the missing data from week 2.

Differences in water sample CFU counts from the control units, as well as in CFU counts in general, can be explained by the variability inherent in the water sample itself. The water sample can vary as a result of differences in compliance with the maintenance protocol, the amount of dislodged biofilm present in the water sample, or the portion of the diluted water sample that is agar-plated. We can assume that CFU counts found in this study are conservatively low in comparison with those that would be found in a clinical setting or those that would occur with longer incubation times. However, the trends in CFU counts over time are more indicative of the success of treatment than are the absolute numbers of CFU counts.

Clinicians need to consider many factors when selecting a DUWL disinfectant. This study focused on CFU counts. Corrosion, disinfectant byproducts and a decrease in enamel and dentin bond strengths for adhesive restorative dental materials[17] have been reported for other disinfec-

tants, but we did not evaluate these factors in our study. In addition, any residual disinfectant trapped within the biofilm matrix then might be released over time in the effluent water. The ability of bacteria to adapt and change to survive suggests that resistant strains may develop within the biofilm.[18] The specific biocides and doses used to control a planktonic population are significantly different from the specific biocides and doses used to eliminate a particular biofilm matrix. Care must be used in the selection of any chemical disinfectant introduced into the system, particularly in light of the probability that the biofilm matrix will be left intact.

Success with any strategy will be measured in no small part by the degree of compliance achieved with the suggested protocol. Treatments that are easier to use, decrease the time allocated for disinfection procedures, and increase the cost effectiveness are more likely to achieve the desired compliance.

CONCLUSION

Clinicians need to consider many factors when selecting a DUWL disinfectant. Following the parameters of this study, we found that a hydrogen peroxide–based disinfectant achieved the ADA goal of no more than 200 CFU of heterotrophic, mesophilic bacteria per milliliter of unfiltered output water. ■

Dr. Linger is an assistant professor, Department of Restorative Dentistry, University of Detroit Mercy, School of Dentistry, 8200 W. Outer Drive, Detroit, Mich. 48219-0900, e-mail "lingerjb@udmercy.edu". Address reprint requests to Dr. Linger.

Dr. Molinari is a professor and chairman, Department of Biomedical Sciences, University of Detroit Mercy, School of Dentistry.

Dr. Forbes is an assistant professor, Department of Biomedical Sciences, University of Detroit Mercy, School of Dentistry.

Mr. Farthing is a third-year dental student, University of Detroit Mercy, School of Dentistry.

Mr. Winget is a third-year dental student, University of Detroit Mercy, School of Dentistry.

The Sterilex Corp., Owings Mills, Md., supplied the Sterilex Ultra disinfectant powder and provided scanning electron micrographs of the dental unit waterlines.

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