Evaluation of Ultrasonic Scaling Unit Waterline Contamination After Use of Chlorine Dioxide Mouthrinse Lavage

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Background: An infection control problem in dental operatories which is not fully controlled is waterline contamination by heterotrophic mesophilic bacteria. These bacteria are present in water supplies as a planktonic phase and adhere to the lumen of tubings as a biofilm comprised of their external cell surface glycocalyx and by production of extracellular carbohydrate polymers. The adherent film is most difficult to remove. The accumulated planktonic phase can be reduced significantly by flushing water from the lines before use in patient treatment, but will return when the equipment is idle through the accumulation of more planktonic phase and by slough of the biofilm surface-adsorbed phase not yet enmeshed in the carbohydrate matrix. Chlorine dioxide has antimicrobial activity against many bacteria, spores, and viruses. It is used in water supply treatment as a disinfectant and slime preventive and has an advantage over chlorine in that carcinogenic trihalomethanes are not generated.

Methods: This study compared use of phosphate buffer-stabilized chlorine dioxide (0.1%) mouthrinse as a lavage in ultrasonic dental scaler units with the use of tap water as a control. Sterile water flushed through the units onto heterotrophic plate count (HPC) sampler plates was cultured 7 days at room temperature and colonies were counted at 12×. One test and one control unit were used for biopsy of internal tubing and scanning electron microscopy imaging.

Results: The HPC counts, in colony forming units (CFU)/ml, were reduced 3- to 5-fold by flushing tap water through the units, but they returned after units were idle overnight. When phosphate-buffered chlorine dioxide mouthrinse was used as a lavage, CFU/ml were reduced 12- to 20-fold. Holding chlorine dioxide in waterlines overnight reduced recurrent buildup compared to water ($P < 0.05$). Scanning electron microscopy images indicated a significant reduction of biofilm coverage by chlorine dioxide as compared to water ($P < 0.001$).

Conclusions: Phosphate-buffered chlorine dioxide mouthrinse was effective in these short-term trials for control of waterline contamination in ultrasonic dental scaling units. It should prove as useful in dental professional waterline applications as it has in industrial uses for biofilm control. *J Periodontol 2001;72:401-410.*

KEY WORDS

Biofilms; dental equipment; infection control; risk factors; water pollution/prevention and control; wound healing.

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Infection control in dental offices remains a problem because of contamination of air and waterlines in the dental units. Recommendations to flush the lines before use, avoid aspirations into the lines, and disinfect with bleach do not always assure that the next patient seen will not be contaminated.[1,2]

Dental unit waterlines are a source of cross-contamination from aspirations and from biofilms that form in any aquatic environment. Aspirations can be dealt with by installation of check valves in units so that water retraction devices do not operate and suck contamination back into the handpiece and its lines at the end of a use in the mouth.[3]

Biofilms in the dental unit waterlines can be a particularly vexing problem.[4] Biofilms will form on solid surfaces in any aquatic environment. The surfaces collect films of low molecular weight hydrophobic molecules by adsorption. Bacteria in the water react with the films, at first reversibly, then irreversibly. The adsorbed bacteria in turn have many protruding molecules on their surface (a glycocalyx), which then cause adherence of other molecules and bacteria from the planktonic, or free-floating, phase. Thus the biofilm thickens by collection of additional bacteria, proliferation of microcolonies, and production of extracellular carbohydrate polymers. The microorganisms which are sessile in the biofilm are more active

in proliferation than those in the planktonic phase, which are living in near starvation in community water systems. These bacteria are called heterotrophs. While there may be an active ion exchange at the surface of the biofilm, those bacteria in its depths are protected (depending on their environment) from amoebae, white blood cells, bacteriophage, surfactants, antibiotics, antibodies, and disinfectants. Such biofilms are especially hard to eliminate.[5] There are many times between patients, overnight, and on weekends when the water flow is stagnant in dental units, and the community system water treatment of chlorine is insufficient to keep the biofilm in check. Even when there is flow, the greatest rate of flow is in mid-tubing, which is quite low near the biofilm surface.[4]

One community's drinking water sampled over a year using culture media specific for heterotrophs had counts ranging from <0.02 to 1×10^4 CFU/ml. Of those which were cultivable, the most numerous were pseudomonads (14%), *Actinomyces* (10.7%), *Aeromonas* (9.5%), *Acinetobacter* (5.5%), *Citrobacter freundii* (1.7%), *Enterobacter agglomerans* (1.2%), and *Escherichia coli* (0.3%).[6]

The persistence of coliforms in high-quality drinking water is perplexing. Their increased resistance to disinfection is thought to be due to adherence.[7] *Klebsiella pneumoniae* grown on glass slides in EPS broth was 150-fold more resistant to free chlorine than those grown on high-nutrient agar.[8] *Legionella pneumophila* grown as a biofilm in tap water is less sensitive to chlorine than when grown on agar.[9]

The numbers of microorganisms in dental unit waterlines can be very numerous and varied, from 400 to one million CFU/ml, even in clinics where all water is drawn from the same drinking water source.[10,11] Drinking water with counts over 500 CFU/ml is considered unfit for human consumption. Microorganisms flushed from dental unit water lines have included *Streptococcus mitis, S. salivarius,* enterococci,[10,12] *Staphylococcus cohnii, Staph. warneri* A, *Klebsiella (Enterobacter) aerogenes, Bacillus subtilis, Pseudomonas* spp., *Streptococcus (Enterococcus) faecalis, Cloaca,*[13] *Legionella,*[14] *Alcaligenes faecalis,*[15] *Cladosporium, Cephalosporium, Aeromonas, Acinetobacter, Flavobacterium,* and *Moraxella.*[16] Meiller et al. recently reported isolates of *Burkholderia pickettii, B. cepacia, Psychrobacter phenylpyruvica,* staphylococci, *Morabella osloensis, Sphingomonas paucimobilis, Myroides odoratum, Brevindimonas vesicularis, Achromobacter* spp., and *Xanthomonas maltophilia.*[17]

Barbeau et al.[18] isolated *Sphingomonas paucimobilis, Acinetobacter calcoaceticus, Pseudomonas aeruginosa, P. maltophilia, P. putida, P. fluorescens, P. vesicularis,* and *P. acidovorans,* in addition to *Actinomyces* spp and *Bacillus* spp. Seen, but not identified, were yeasts and amoebae.[18] There are other bacteria in samples which may be dead, dormant, inhibited by residual chlorine, or not cultivable with the media used.[18] *Mycobacterium* spp. have been reported in drinking water supplies.[19]

Newly installed dental units had up to 2×10^5 CFU/ml counts within a week.[18] Tall et al. described the growth of biofilm in clean dental unit air-water syringe tubing from 0 to 120 days. The first week a few rods and spiral forms were seen with scanning electron microscopy (SEM), and by the end of the first month, there were many heterogeneous microcolonies. After 6 months there were multiple layers of different morphologies covering the lumen completely. The succession of species in order of appearance, as cultured, were *Pasturella pneumotropica, Pseudomonas paucimoblis, Pasturella multocida, Pseudomonas* spp., *Ochrobactrum anthropi, P. multiphilia, Pasturella haemolyticus, P. picketii, P. stutzeri, Pseudomonas acidovorans, Seromonas salmonicida, Acinetobacter calcoaceticus, P. vesicularis, Pasturella* spp., *P. cepacia, Moraxella phenylpyruvica, P. putrefaciens, Flavobacterium* spp., *Flavobacterium odoratum,* and *Moraxella urethalis.*[20]

Flushing the waterlines of a dental unit for 1 minute reduced the CFU/ml 97% and a 2-minute flush by 98.6%, according to Abel et al.[10] Barbeau et al.[18] found a 2-minute flush at the start of a day reduced counts by 96%. After a weekend of stagnant conditions, a 6- to 7-minute flush may be required.[21] A 6-minute flush of air-water syringe lines reduced heterotroph counts by 99.9% in the report of Mayo et al.,[22] but the residual mean counts were still 1.3×10^4. Flushing for 8 minutes was needed to get counts under 500 CFU/ml in another study.[18] Flushings only remove accumulated planktonic forms and perhaps a few of the biofilm surface-adsorbed microorganisms. SEM of flushed dental unit waterlines that were removed and split open showed patches and clumps of rod-shaped bacteria within fibrous strands.[17,22] Flushing will reduce counts, but not eliminate organisms and it might take over 7 minutes to flush 1 liter and get a mean CFU/ml of 0.5.[23] However, a subsequent stasis might result in counts that are higher than at the start.[11] After flushing for over 20 minutes to counts of zero, microorganisms reappeared in samples within 30 minutes from some dental units and all were positive again by 24 hours.[24] All of the flushing will be for naught, unless an autoclaved handpiece or syringe is then attached.[25]

Disinfection of dental unit waterlines and reservoirs with chlorhexidine at 1:5,000 or 1:10,000 concentrations resulted in no growth after 24 hours,[13] but one has to deal with an objectionable taste for the patients. Various dilutions of bleach have generally reduced bacterial counts, but if not done repeatedly, the units soon return to shedding bacteria again.[10,14,26-37] Residual chlorine has objectionable smell and taste and there is

concern about development of trihalomethanes.[33] The biofilm matrix can remain even after strong bleach treatment.[28,37] Karpay et al. recently reported that weekly periodic high bleach (1:10 dilution, 10 minutes) and daily continuous low bleach (3 ppm) treatments of 10 dental units for 5 weeks reduced mean heterotroph counts to 0.74 CFU/ml and nearly eliminated biofilm, as seen on SEM in 6 of 10 units; yet the units still put out about 40 ppb of trihalomethanes.[38] An epidemiological study showed cancer risk from trihalomethanes and haloacetates.[39] Dental units treated weekly with bleach for 4 years were studied by SEM energy dispersive x-ray analysis of waterline samples and were found to have deposits of copper or iron, nickel, zinc, calcium, phosphorus, sulfur, and silicon, thought to be due to gradual corrosion of internal fittings.[40] Alcohols have reduced counts if left in lines long enough and repeatedly, but they do not remove the matrix of the biofilms, so there is recurrence.[15,17] Glutaraldehyde,[41,42] hydrogen peroxide,[16,43] cetylpyridinium chloride,[19] povidone-iodine,[44,45] desoxycholate, and Tween 80[16] have been recommended as flushing agents.

Mechanical methods using filters have short-lived benefits.[32,46] Some need daily changing,[46] and charcoal filters may remove the little chlorine present in the water supply.[33] Installation of separate, disinfectable water reservoirs for sterile water delivery do little if not maintained.[28,34,36] A simple method that needs to be tested further may be to purge the lines with compressed air and let them dry overnight and weekends.[15,34]

The same problems occurring in fixed dental units occur in portable accessory equipment such as ultrasonic scalers and polishing units. Typical ultrasonic scaling units might have over 16 feet of fine tubing, from the water connection to the end of the handpiece, in which to form a biofilm. Counts of viable bacteria from ultrasonic scaling units are variable and have ranged as high as 2.6 million CFU/ml.[26,47,48] *Pseudomonas, Alcalignes,* and *Legionella* have been reported.[48,49] Flushing ultrasonic units before use reduced counts up to 99%, but the residual counts were still high.[47,50] Intermittent weekly treatments with bleach reduced counts, but not to zero.[26,27] Use of 3.0 μm and 0.45 μm filters reduced counts to zero for only 48 to 52 hours.[51]

The purpose of this investigation was to test the effect of a phosphate buffer-stabilized chlorine dioxide (0.1%) mouthrinse on the counts of heterotrophic bacterial contamination in the waterlines of ultrasonic dental scaler units. Chlorine dioxide (ClO_2) has been used in the treatment of some American water supplies since 1944. It has 2.5 times the oxidizing power of chlorine (Cl_2).[52,53] ClO_2 has been shown to be more effective than Cl_2 on fecal strains of *E. coli,* having a 99% kill in 15 seconds.[54] Chlorine dioxide was found not only better than chlorine, but also faster acting on sewage effluent.[54,55] In vitro tests found ClO_2 able to disinfect raw sewage in 120 seconds at half the concentration of Cl_2.[56] ClO_2 efficiency increases with temperature and pH,[57] compared to chlorine. The enteric bacteria *Yersinia enterocolitica* and *Klebsiella pneumoniae* grown with limited nutrients in a chemostat were killed by ClO_2, but not as rapidly as those grown at more rapid rates.[58] Bacteria grown in "hardship" conditions tend to form biofilms, which increase their resistance to disinfectants. ClO_2 was found to significantly reduce *P. aeruginosa, Yersinia enterocolitica, S. pyogenes, Salmonella typhimurium,* and *Bacillus subtilis.*[59] Common water pathogens *Eberthela typhosa, Shigella dysenteriae, Salmonella paratyphi B, P. aeruginosa, Staph. aureus, E. coli,* and *Aerobacter aerogenes* were killed by ClO_2.[57] With no organic load, ClO_2 killed *E. coli* at a concentration of only 0.8 ppm.[60] ClO_2 has a better sporicidal activity than Cl_2.[61,62] It kills *Giardia, Cryptosporidium,*[63] *Enterovirus,*[64] *Rotavirus,*[65,66] and poliovirus 1.[55,56,67] Cultures of dental and oral pathogens *S. mutans, Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis,* and *Prevotella intermedia* were 99.9% to 100% killed by ClO_2 in 10 seconds.[68,69]

MATERIALS AND METHODS

Twelve magnetostrictive‡ and 3 piezoelectric§ ultrasonic units capable of 25,000 Hz were used. They were connected to a separate reservoir system‖ capable of supplying the ultrasonic unit with community drinking water, or any solution thought suitable in the mouth, such as sterile water, sterile saline, or mouthrinses. The reservoir system had 2 one pint bottles under pressure of filtered compressed air supplied by a dental unit. In this investigation, bottle A was filled with sterilized tap water, and bottle B with 0.1% chlorine dioxide/0.5% sodium phosphate mouthrinse.¶ The tap water connection was made between the dental unit and the reservoir system. The reservoir system lines were first treated by mixing sodium hypochlorite bleach with water in a 1:10 dilution and running a full load through bottle A, and then bottle B. The bottles were rinsed with sterile water, then a full load of sterile water was run through the lines from bottle A and then B. After the bottles were empty, compressed air was run through to purge the lines of any water and dry them.

Samples of sterile water passed through the ultrasonic units were evaluated with culture plates especially designed for waterline bacteria.# These culture plates had a clear outer plastic case which contained an inner paddle on which heterotrophic plate count

‡ Cavitron Bobcat ultrasonic scaler, Dentsply, York, PA.
§ Suprasson P5 Booster, Satelec, Merignac Cedex, France.
‖ Dual-Select Dispensing System, Dentsply.
¶ CloSYS *II,* formerly called Retardex, Rowpar Pharmaceutical, Scottsdale, AZ.
# Millipore HPC Sampler, Millipore Corp., Bedford, MA.

(HPC) medium was spread and covered with a 0.45 μm perforated membrane filter. There was enough medium so that, when wet, it would absorb 1 ml through the membrane. Samples were collected in the plastic case, the paddle inserted by means of an exterior handle for 30 seconds until the medium was completely wet, then the excess water sample was poured out of the case, the excess shaken from the paddle, and the paddle reinserted into the case. Samples were incubated at room temperature in the dark for 1 week. After 1 week, the colonies on the paddle were examined under a dissecting microscope at 12×, with side illumination to see the tiniest of colonies. Each sample was counted twice and the average count recorded as CFU/ml. Each batch of sterile water was sampled directly from the flask as a control of sterility. Test samples were taken in triplicate. Identification of microorganisms was not done.

The test procedure for the 15 ultrasonic scaler units located in a dental school clinic, which had been idle for one day or more, was to connect them to bottle A of the reservoir system and flush them with sterile tap water for 2 minutes without a handpiece insert. The water volume control of the ultrasonic unit was set at that which would provide the proper spray, if an insert was in use. A 1.8 ml sample was taken and diluted to 18 ml with sterile water (1:10). The sterile water was flushed through the ultrasonic unit handpiece for 1 more minute, and another 1.8 ml sample was taken and diluted 1:10. After sterile water was flushed through the ultrasonic unit for another minute, a third sample was taken without dilution.

The ultrasonic unit was then connected to bottle B, containing the stabilized ClO_2 mouthrinse, and 16 oz (473 ml) were run through without an insert. This process took 4 to 5 minutes to empty the reservoir. The mouthrinse in the lines was allowed to sit undisturbed for 30 minutes, then flushed out with sterile water for 30 seconds, and the lines were purged dry with compressed air.

Flushing of sterile water for HPC samples through the ultrasonic unit was repeated as outlined above. Following the collection of 3 more samples, the ultrasonic unit waterlines were purged with compressed air and the unit returned to service in the clinic. The mean and standard deviation of each sample stage were determined and the initial sample compared to the final sample using the Fisher-Behrens *t* test for variances with significant differences.

The next tests were done to simulate the flushing effect of a hygienist's work day on the ultrasonic scaling units. The 12 magnetostrictive type units were assigned to either test or control alternatively from their alphanumeric order (odd numbers to control, even numbers to test; or A to control, B to test, C to control, D to test, etc.). The unit was first sampled after a 2-minute flush with sterile water, then used in 8 successive runs of 15 minutes with a sterilized insert in the handpiece, followed by a rest. Before each succeeding run, a 1-minute flush was done with the insert removed. The rests were 15 minutes each, except between runs 4 and 5, which was 45 minutes to simulate a lunch break (Table 1). Control runs used 473 ml tap water in 6 units, and test runs used 473 ml of the stabilized phosphate-buffered ClO_2 in 6 other units. At the completion of a test with a unit, it was flushed with sterile water, its water line purged with air to dry it, and it was returned to service.

One ultrasonic unit using tap water lavage as a control and one unit using ClO_2 lavage as a test were selected to biopsy a 5 mm section of the plastic waterline tubing from the interior of the unit. The samples were processed for SEM. They were fixed in 3% glutaraldehyde in a 0.2 M sodium cacodylate buffer and stored in the fixative for several weeks. The tubing was removed from the fixing solution, washed in purified water, cut into sections, and split lengthwise to expose the interior wall surface. After fixation, the specimens were dehydrated, using a graded series of ethyl alcohol with a minimum of 1 hour at each step (50, 70, 80, 90, 95, and 100%, and 100% repeated) and dried using 100% hexamethyldisilazane to minimize shrinkage due to drying, following the method of Perdigao et al.[70]

Table 1.

Schedule for Trial of Effect of Flushing Tap Water or ClO_2 Through Ultrasonic Units in a "Typical" Day From 8 AM to 5 PM*

Time	Minutes/Flush	Run/Rest
8:00	2†	15 min run-15 min rest
9:00	1	15 min run-15 min rest
10:00	1	15 min run-15 min rest
11:00	1	15 min run-45 min rest
1:00	1	15 min run-15 min rest
2:00	1	15 min run-15 min rest
3:00	1	15 min run-15 min rest
4:00	1	15 min run-15 min rest
	2‡*	
	2§*	

* Each 15-minute run used 1 pint of tap water or mouthwash. Triplicate HPC samples were taken following a 2-minute sterile water flush before, after, and the following morning.
† Samples taken.
‡ Samples taken; overnight rest.
§ Samples taken; next day.

The tubing samples were mounted on stubs, a 10-nm gold layer was sputtered on the samples, and they were observed in the SEM** at 15 KeV accelerating voltage. Selected images of deposits and microorganisms were made at magnifications of 300× to 5,000×. In addition, 1,000× images were taken at approximately equal intervals along the length of each sample to compare the relative quantities of deposits for the mouthwash-treated and the tap water control lavage ultrasonic waterlines. The resulting 25 SEM images were ranked and tested using a Mann-Whitney rank order sum test to determine any significant difference in surface coverage of the 2 samples.

RESULTS

The original and the repeat counts of colonies on the HPC samplers were compared and a correlation coefficient of r = 0.9914 was found. However, there was a large variation in counts from unit to unit, and from sample to sample with any one unit. The 15 ultrasonic units which had been idle for a day or more had, after a 2-minute flush with sterile water, a mean CFU/ml of 582 ± 451 (SD), with a range of 110 to 1,870 CFU/ml (Table 2). After 2 succeeding 1-minute flushes, the mean count was reduced 3.5-fold to 162 ± 123 CFU/ml ($P<0.05$). Ten of the 15 units (66%) had a final mean count of less than 200 CFU/ml, as recommended by the American Dental Association (ADA).[71]

After a single treatment with the pint of phosphate-buffered ClO_2 mouthrinse, the mean count following a 2-minute flush with sterile water was 709 CFU/ml ± 1,396, with a range of 0 to 5,290 CFU/ml. Four of the units had exceptionally high counts, creating a large standard deviation, but the mean did not differ from the initial counts after use of tap water lavage. Following 2 more 1-minute flushes, the mean HPC sampler count was reduced about 20-fold to 35 ± 36 CFU/ml, but this was not significantly different than after the first 2-minute flush due to the large variance of that first count. However, the final count after the ClO_2 mouthrinse use was about 5-fold less than the count after use of tap water ($P<0.05$), and all 15 units (100%) had a final count under the recommended 200 CFU/ml.

We compared HPC sampler counts of 6 magnetostrictive ultrasonic scaler units to be run for a simulated day with tap water lavage with 6 other units to be run for a simulated day with ClO_2 mouthrinse lavage (Table 1). The initial counts after a 2-minute flush with sterile water in the tap water group were 2,489 ± 2,831.8 CFU/ml and in the ClO_2 group, 2,266 ± 2,039.2 CFU/ml (Table 3). Both simulated day counts had large variance and there was not a significant difference. At the end of the simulated day, the mean count was reduced by the repeated flushing of tap water lavage about 5-fold to 458 ± 506.9 CFU/ml, and the mean count in the ClO_2 group was reduced about 12-fold to 186 ± 248 CFU/ml. Although the result using ClO_2 was about 2.5 times smaller, these counts were not significantly different due to large variances. The mean count in the ClO_2 group was under the ADA recommended[71] 200 CFU/ml. Following an overnight rest with the tap water or ClO_2 lavage remaining in the lines, the mean count of tap water controls was 3,168 ± 1,764.7 CFU/ml and for the ClO_2 group, 717 ± 566.8 CFU/ml, a significantly smaller difference despite large variance ($P<0.05$).

The sequential images along the length of the interior tubing sample from an ultrasonic unit treated with

Table 2.

Heterotroph Plate Counts of Ultrasonic Units Before and After Use of ClO_2 Mouthrinse

	Before ClO_2			After ClO_2		
Unit	1	2	3	4	5	6
1	490	410	68	2100	110	35
2	330	550	211	1330	930	9
3	290	60	88	200	170	32
4	490	330	149	5290	80	29
5	430	230	62	15	45	24
6	110	400	51	110	180	53
A	430	120	26	340	30	qns*
B	440	330	140	30	10	2
C	1330	715	361	220	140	128
D	700	490	294	120	40	6
F	230	260	125	610	240	14
EMS	430	150	72	0	10	1
P5a	1870	900	372	240	100	100
P5b	500	100	68	0	0	7
200	665	350	349	30	80	47
$\bar{X}$	582	359	162	709	144	35
S.D.	451	233	123	1396	228	36
	†1 > 3 $P<0.05$			‡4 versus 6 $P>0.10$		
	†3 > 6 $P<0.05$					

* Quantity not sufficient (for a full sample).
† Analysis using Fisher-Behrens t test for variances with significant differences.

** ISI Modified SX-40A, Topcon Instruments, Pleasanton, CA.

the phosphate-buffered ClO_2 mouthrinse appeared to have few biofilm deposits. There were irregular thin lines in the background thought to be the texture of the tubing wall, and perhaps affected by the processing of the SEM image (Fig. 1A). In contrast, the samples of tubing from the unit using tap water had a well-developed coating and numerous patches of deposits common along the length of the sample (Fig. 1B). The statistical comparison of the ranking of images taken at intervals along the length of the tubing samples showed there was a significantly higher coverage of the surface with biofilm for the water lavage than for the mouthwash treatment ($P<0.001$).

A wide variety of different structures and microorganisms were observed in these samples. Usually these were in isolated small patches in the mouthwash-treated samples, and were more abundant in the water lavage samples. Several examples are seen in Figure 2, with the morphology of cocci, rods, and small rods with very long flagellae, possibly *Caulobacter*. Also seen are the differences in the amounts of granular to amorphous polysaccharide background material of the biofilm.

Table 3.

Comparison of Heterotroph Plate Counts of Ultrasonic Units in a Simulated Day

	Tap Water Lavage				ClO_2 Mouthrinse Lavage		
Unit	Before	After	Next Day	Unit	Before	After	Next Day
1	386	26	1811	2	1666	6	591
3	8088	1177	5005	4	1544	630	1397
9	2478	265	3827	6	5623	0	323
A	1589	223	1877	B	453	275	609
C	1050	39	1215	D	524	210	1386
F	1287	1018	5275	200	3789	0	0
*$\bar{X}$	2489	458	3168		2266	186	717
S.D.	2832	507	1765		2039	248	567

* No significant difference between means, except tap water next day > ClO_2 next day, $P<0.05$.

DISCUSSION

The significance of dental unit and ultrasonic scaler waterline contamination with heterotrophs or aspirated microorganisms lies in the reports of potential pathogens such as alpha-hemolytic streptococci,[12] enterococci,[12] *P. aeruginosa, Legionella*,[32,48] and other Gram-negative rods.[72] Cross-infections between patients; chronic infection of dental personnel with long-term exposure to oral fluids, splatter, and aerosols; and direct infections of open surgical wounds should concern any therapist. The microorganisms capable of forming biofilms on surfaces of dental unit waterlines might also form biofilms on heart valves, creating endocarditis.[73,74] Waterline bacteria might cause disease in immunocompromised persons. Hospitalized patients are at risk of nosocomial infections from pseudomonads, *Acinetobacter*, and other waterline bacteria.[75,76] Martin[77] reported 2

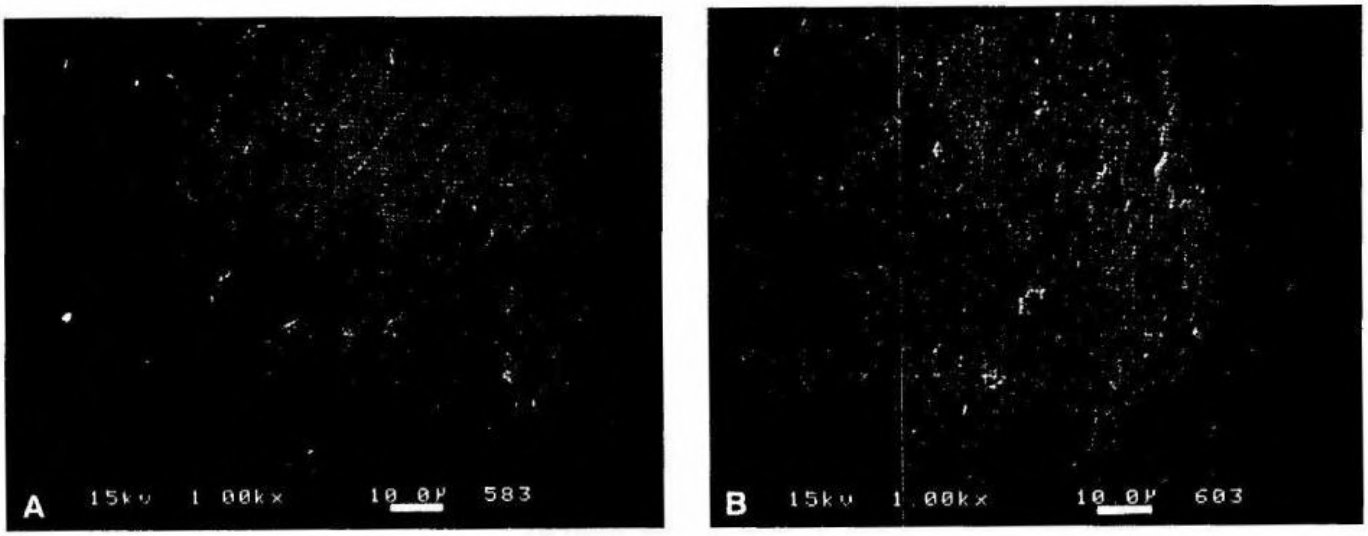

Figure 1.
SEM images of areas taken at intervals along the tubing length. **A.** *Typical area of mouthwash-treated tubing revealed a few small areas with deposits, and a network of fine lines probably related to the wall texture of the tubing.* **B.** *An area with a heavy deposit on the tubing used with tap water. Such dense accumulations and deposits were common on the tubing from the unit using water lavage (original magnification 1.0 kx).*

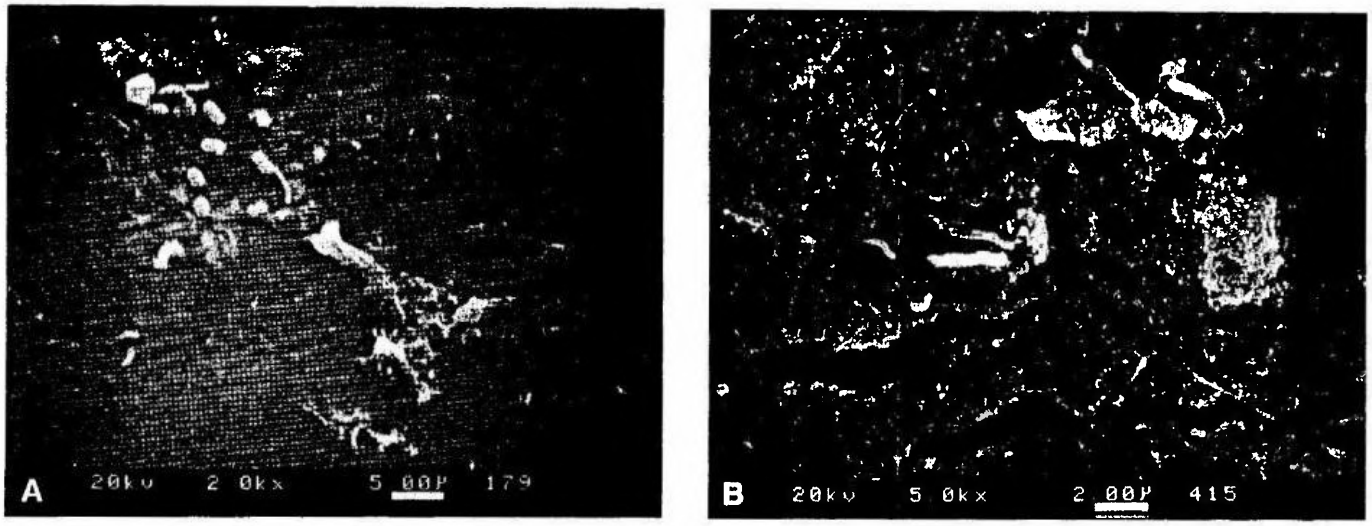

Figure 2.

Higher magnification of deposits seen in SEM images of ultrasonic waterline tubing. ***A.*** *Cocci and a sparse amount of film after use of chlorine dioxide mouthwash (original magnification 2.0 kx).* ***B.*** *Cocci, rods, and forms with very long flagellae among heavier deposits of biofilm in units used with tap water (original magnification 5.0 kx).*

cases of dental abscesses following operative dentistry procedures which upon culture grew out *P. aeruginosa.* In his prospective study of 78 dental patients, he tested the dorsum of their tongues for the presence of *P. aeruginosa* before and after they had been treated at a contaminated dental unit. None had *P. aeruginosa* before treatment; all had it afterward for up to 5 weeks later.[77]

Slots et al.[78,79] have reported on over 3,000 cases of samples of subgingival bacteria cultured from patients considered refractory to periodontal treatment because of progressive pocket deepening. Many of these samples grew out enteric rod bacteria Enterobacteriaceae such as *Enterobacter cloacae, E. agglomerans,* or *Klebsiella pneumoniae, K. oxytoca, Citrobacter freundii, Acinetobacter* spp., *P. aeruginosa,* and *Proteus mirabilis.* One might wonder if these microorganisms were inoculated from dental unit waterline contamination during their previous unsuccessful treatment.

Serological tests of dental personnel for various *Legionella* species found a prevalence of 50% in dentists, 38% in their assistants, 20% in their lab technicians, but only 5% in other employees (controls).[80] The number of dental personnel serologically positive increased with their time in the profession.[81] A fatal case of legionellosis in a dentist was found to contain the same DNA typing as that of contamination in his dental unit.[82] In a gene probe method for detection of *Legionella* in 268 dental unit waterlines from 28 clinics in 6 states, 68% were positive and 8% had *Legionella pneumophila.* Only 2% of the comparable domestic water samples had *L. pneumophila.* Samples from 30 ultrasonic scalers were 85% positive for *Legionella* spp.[83]

Reinhardt et al.[84] reported that ultrasonic scaling with non-sterile tap water resulted in positive blood cultures in 53% of their subjects, whereas sterile water incidence was 50%, a non-significant difference. They do not report the significance of differences they found in Gram-negative anaerobic rods: 18.8% with tap water and 13.3% with sterile water; nor Gram-positive anaerobic rods: 18.1% with tap water versus 0 with sterile water. One wonders if those were heterotrophs. The review by Miller[85] summarizes the risks of the waterline contamination problems.

The bactericidal action of ClO_2 is thought to be due to cell wall disruption and the halting of protein synthesis.[86] Irreversible oxidation of sulphydryl groups to disulphide or sulphones is possible.[87] In experimental inactivation of heterotrophic bacteria and *E. coli* in a deionized water system, hypochlorous acid, chlorine dioxide, hypochlorite, and monochloramine were used. The unattached heterotrophs were generally more resistant than the *E. coli,* and the relative effectiveness was $HOCl>ClO_2>>OCl^- >>NH_2Cl$. Yet, low-nutrient-grown heterotrophs were slightly more sensitive to ClO_2 than *E. coli.* The attached bacteria, in contrast, were more effectively killed by monochloramine, and next by ClO_2, than by hypochlorous acid.[88]

The HPC samplers were easy to use and have been considered more appropriate for growth and enumeration of heterotrophic mesophilic bacteria in waterlines than either high-nutrient blood agars or heart-brain infusion media.[89,90] It was found that the counts had to be made under a dissecting microscope at 12× power, as many colonies were too small to be seen with the naked eye or even a hand lens. The inked

ruled cross-hatch squares greatly helped with counts; yet some colonies were so small they had a diameter of about one-fourth the width of the inked lines. Diluted samples were necessary, as some counts of undiluted samples were very high and it was difficult to discern individual colonies. Despite these difficulties, the correlation of original and repeat counts of over 100 plates was r = 0.9914.

In this study, samples were taken directly from the ultrasonic handpiece into the HPC samplers, turning the flow on and off with the foot pedal switch common to these ultrasonic scaler units. It is thought that some of the large variance of repeated samples might be due to a "shock" caused by the abrupt opening and closing of the units' electrical solenoid water valve, and subsequent surge of pressure through the waterlines that might have a disrupting effect on microorganisms lightly attached to the surface of waterline biofilm. Another possible effect was the breaking up of the biofilm itself by the antimicrobial activity of ClO_2. Table 2 shows that 4 of the ultrasonic units had higher initial counts after use of ClO_2 than they did after use of tap water. In several of the undiluted samples taken after use of ClO_2, there were thin, irregular flakes up to 2 mm in size seen in the samples, even after the preceeding 2-minute flush with sterile water. Those large counts might have been a result of disruption of flakes from the biofilm on the lumen of the tubing after the use of ClO_2 mouthwash. We recommend that future trials take larger samples of about 100 ml into clean sterile glassware, then inoculate the HPC samplers indirectly to avoid possible shock effects.

While long-term use of ClO_2 lavage in the ultrasonic scaler units has not been studied, we speculate that it would have a definitive action in reducing biofilm in such units and in meeting the ADA recommendations. All the ultrasonic scaling units used in this study had been used in the school clinic for 5 or more years with community tap water lavage. It is most likely that the phosphate-buffered ClO_2-treated sample seen in Figure 1A looked much like that in Figure 1B before these tests. Chlorine dioxide is reported to reduce slimes and also inorganic deposits in pulp paper processing,[91] biofouling of water treatment tanks,[92] drip irrigation systems,[93] chilling tanks in canned vegetable processing,[94] hospital potable water supply,[95] and indwelling atrial catheters.[96] Very recently, initial reports of the effectiveness of phosphate-buffered ClO_2 mouthrinse on fixed dental unit waterline contamination were published.[97,98]

Use of ClO_2 as a lavage for ultrasonic scaling would be a type of "continuous chemical treatment" as suggested by the ADA Council on Scientific Affairs.[99] The HPC samplers could be a reasonable approach for monitoring. There was no discernable deleterious effect of the use of this mouthrinse on the materials or operation of the ultrasonic scaler units. There was no objectionable odor nor taste from its use as a lavage. It is not known what effect the ClO_2 lavage combined with ultrasonic scaling might have on the oral biofilm, dental bacterial plaque. That is currently under investigation in our clinic.

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