



**DioxiClear™ is the
Predecessor to the
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Evaluation of DioxiClear™ as a treatment for dental unit waterline biofilms & as a water contamination control agent

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Product

The product evaluated in this study was DioxiClear™, Frontier Pharmaceutical, Melville, New York, USA. DioxiClear™ is a Chlorine Dioxide (ClO₂) formulation that has detergents and produces 140 ppm of ClO₂. This product has specifically been developed for treating dental unit water systems as a periodic cleaner at 140 ppm. It has also been formulated for mixing with municipal water at 1-2 ppm, to control planktonic contamination and for short-term preservation of water quality in closed water systems. ClO₂ (DioxiClear) was evaluated on various aspects related to dental treatment water usage and common chemical effects over a three-year period.

Purpose

Items 1-4 were studied together in 1999 and item 5 in 2000

1. Study efficacy of DioxiClear™ (ClO₂) in controlling dental unit water system contamination including inorganic deposits and organic deposits such as microbial biofilms
2. Study efficacy of DioxiClear™ (ClO₂) in controlling dental treatment water contamination
3. Study effects of DioxiClear™ (ClO₂) on the metal components of the dental unit water system
4. Compare efficacy of DioxiClear™ (ClO₂) with that of NaOCl on biofilms and dental treatment water contamination control
5. Study effects of DioxiClear™ (ClO₂) on composite restorative material bonding to dentin

Background

Biofilms are routinely found in dental unit water and are formed when planktonic bacteria adhere to the inner luminal walls of water lines within dental treatment water delivery systems. Colonization and proliferation in dental unit water and dental unit waterlines of many and varied species of microorganisms has been well documented and observed under Scanning Electron Microscopy (SEM). Although many microorganisms found in this environment may be potentially pathogenic, three genera in particular are of concern in the dental office *Pseudomonas*, *Mycobacteria* and *Legionella* posing potential risks to both patient and clinic staff.

Materials & Methods

Two dental chairs (Virginia-Chayes with ADEC water system & controls) that had not been cleaned in the past 14 years were used in this study. Water from these two chairs was not used for patient care during the period of this study. One self-contained water reservoir (ADEC) was installed for each chair and a water pasteurizer (Health-Sonics) was used to provide microbe free dental treatment water during simulated patient care throughout this study.

Treatment 1: NaOCl 5000 ppm was used to treat Chair 1 (C1) for a 10-minute contact period in the water system followed by a flush with 700 mL of pasteurized water through all the lines to evacuate the bleach.

This was performed daily during the first four days and weekly for the remaining 4 weeks. 600 mL of pasteurized water was flushed intermittently (100 mL) to simulate patient care each day.

Treatment 2: DioxiClear™ (ClO₂) was used to treat Chair 2 (C2) at about 140 ppm at about 2 – 2.5 minutes of contact with the water system followed by a flush with 700 mL of pasteurized water. This was performed daily during the first four days and weekly for the remaining 4 weeks. 600 mL of pasteurized water was flushed intermittently (100 mL) to simulate patient care on a daily basis.

1. Baseline waterline samples were taken at the beginning of the study for Scanning Electron Microscopy (SEM) analysis of biofilm in the lumen of the lines before any treatment regimen was implemented and again at the end of Week 1 and Week 5 (end of study). Fixed contaminants in the water lines and water system could be inorganic salts and organic material such as microbial biofilms. Therefore, to study the effects of chlorine dioxide, we used scanning electron microscopy. Line samples were harvested aseptically and sectioned to longitudinally expose the lumen. The lines were then fixed using a 3% glutaraldehyde for a period of 1.5 hours. The lines were exposed serially to increasing concentrations of ethyl alcohol to remove water from the biofilms and desiccated overnight. Scanning Electron Micrographs were done of the waterline samples using a JEOL Scanning Electron Microscope (Tokyo, Japan) at a magnification of X1500.
2. Since the contaminants in the dental unit water could be inorganic materials and organic materials, we used quantification of heterotrophic mesophilic organisms (one of the contaminants) as a marker for organic contamination of the water. Heterotrophic Plate Counts of the source and effluent water contamination was carried out using HPC water samplers (Millipore, Inc.). During the first week (five working days), baseline and daily water samples from the tap (municipal water), pasteurizer, source water bottle and effluent from the AWS syringe lines were collected and assayed for heterotrophic plate counts to study the contamination levels. For the remaining 4 weeks, water samples from the mentioned sources were plated twice a week.
3. Treatment chemical and water samples were collected on the Day 3 of the first week for metal analysis (resident volume in the lines with 10 minute contact, next five consecutive flush of 30mL each with pasteurized water for the NaOCl group; resident volume in the lines of the 2-4 minute contact, next 5 consecutive flush of 30 mL each for the ClO₂ group). These samples were shipped on ice to an independent laboratory and preserved using 0.1 mL high purity nitric acid until the time of test. Metal analysis was conducted on these samples to study the corrosive effects of 5000 ppm NaOCl and 140 ppm ClO₂ by the quantitative analysis of elemental zinc, copper and nickel removal from the dental unit system's metal (brass) components. The second set of water samples for metal analysis was collected in the second week of phase two and at the end of week five. The samples were analyzed on a Perkin-Elmer's Atomic Absorption Model 3030B Spectrophotometer according to standard methods (EPA 502.2).
4. To test the efficacy of low-grade presence of DioxiClear™ (<2 ppm) in municipal water, 700 mL of tap water was collected and placed in a reservoir bottle along with 2mL of part A and 2mL of part B solutions. After 20 minutes, a water sample was collected and plated on buffered R2A agar. Heterotrophic counts of the tap water samples were compared to their matched 20-minute contact water samples. A total of 26 samples of each were done over a period of 3 months.
5. Non-carious third molars were invested in die stone and sectioned. Fifteen teeth were randomly allocated to be treated with DioxiClear™ and Dallas municipal water. The teeth were acid etched, rinsed with an irrigant dependent on treatment group, conditioned with a bonding agent, and bonded to a composite button that was held in the perimeter with a steel washer standardized for the internal diameter with minimal standard deviation. The specimens were thermally cycled at hot and cold temperature using comparative methods as per previous studies to simulate 2 years of use. Each composite button was mechanically loaded until debonding occurred. Debonded surfaces were classified according to failure mode. The irrigants were tested for differences in mean failure loads using one-way ANOVA.

Results

1. **Scanning Electron Microscopy (SEM)** of the baseline in both groups showed that lines were excessively contaminated by biofilms with extreme colonization of rod shaped organisms and presence of a glycocalyx matrix throughout the samples. SEM at the end of week 1 showed disruption and removal of the biofilm with presence of occasional microorganisms on the lumen in both groups. The NaOCl group showed deposition of crystalline or amorphous material on the lumen while ClO₂ showed removal of inorganic and organic contaminants. By the end of the week 5, both groups showed no biofilm, but the NaOCl group had the same crystalline deposition seen at the end of week 1. The DioxiClear™ (ClO₂) group had no such deposition and had clean lines.

Scanning Electron Microscopy

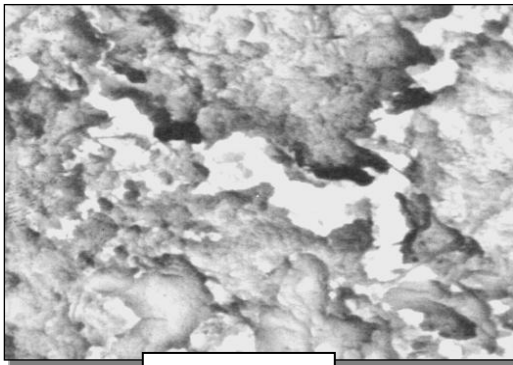


Figure 1

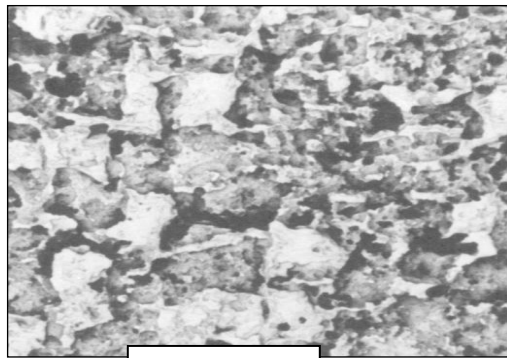


Figure 2

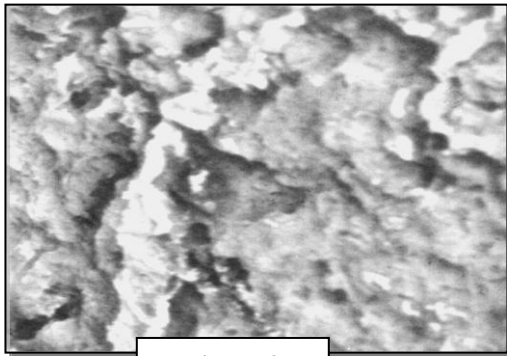


Figure 3

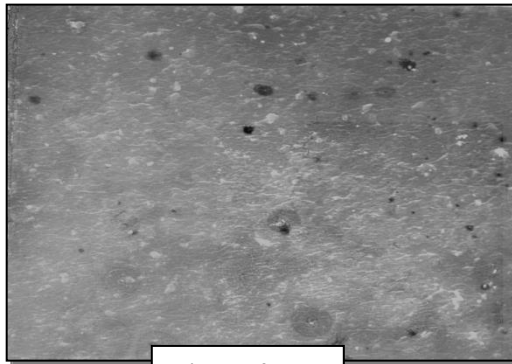


Figure 4

Legend: Figures 1 and 3 represent baseline SEM of the NaOCl and ClO₂ waterlines respectively, demonstrating presence of mature biofilm matrix and microorganisms. Figure 2 represents end of study SEM of the NaOCl group waterline with the absence of biofilms and microorganisms but with the presence of amorphous/crystalline deposits. Figure 4 represents end of study SEM of the ClO₂ group waterline demonstrating the removal of biofilm and absence of amorphous or crystalline deposits.

2. Heterotrophic Plate Counts are listed in the table below--

Table 1. HPC counts (CFU/mL)* of Tap, Pasteurized, Source and Effluent Waters

Time	Tap	Pasteurized	Source C1 NaOCl Chair 1	Effluent C1	Source C2 DioxiClear™ Chair 2	Effluent C2
Baseline	42	0	(>4,000)	>40,000	>4,000	>40,000
Day 1	71	0	(>4,000)	>4,000	97	>4,000
Day 2	88	0	0	0	>400	>400
Day 3	4	0	1	0	1	3
Day 4	94	0	0	0	0	>400
Week 1a	154	0	1	0	20	24
Week1b	37	1	4	89	3	210
Week 2a	82	0	0	139	0	220
Week 2b	71	0	0	0	12	31
Week 3a	7	0	0	0	3	104
Week 3b	0	0	0	0	0	0
Week 4a	140	0	0	0	0	0
Week 4b	92	0	0	4	0	0

CFU/mL = colony forming units per mL of water (1 CFU/mL = 1 microorganism per mL)

Table 1 demonstrates that the tap water in this study was well below the 200 CFU/mL (ADA's goal) but had organisms ranging from 0 to 154 CFU/mL. Pasteurization of tap water showed 0 cfu/ml in all but one sample. The bottle systems (Source Bottles of the Self-Contained Water System) in both groups showed levels that were less than 200 CFU/mL after day 3 of treatment. When the effluent water was measured for contamination, cleaning with 5000-ppm bleach was effective within 2 days of using pasteurized water. Cleaning with DioxiClear™ (140 ppm) was found to be effective by the end of day 4 (potable water) and thus met the ADA's goal in week 2 (dental treatment water <200 CFU/mL) when used in conjunction with pasteurized water as an irrigant.

Although a significant difference was noted between the 5000ppm NaOCl and 140ppm ClO₂ groups, both groups achieved the needed results by 2 weeks. 5000ppm of NaOCl was in contact with the dental unit water system for 10 minutes, while 140ppm of ClO₂ for 2 minutes. 5000ppm NaOCl is very concentrated and corrosive to metals while 140ppm ClO₂ is friendlier to metals.

Until all organic matter has been removed, 5000ppm of Bleach can potentiate liberation of very toxic levels of trihalomethanes (total THMs in excess of 80 times the limit set by the EPA, as shown by Dr. Puttaiah in a previous study). ClO₂ does not generate THMs and is friendlier than bleach to the user/employee and the environment.

As a work practice, it is easier to flush a solution through the water system and do an immediate post flush as with the use of ClO₂, as opposed to loading the solution and waiting for 10 minutes before doing a post flush as in bleach protocols. A ClO₂ based solution at 140ppm is a better choice as a cleaner.

In most studies conducted by the author, tap water contamination ranged between 0 to a few thousand colony-forming units and did not consistently meet the ADA's goal for the year 2000. Furthermore, if the environmental conditions are right, the microorganism will undergo mitosis (replication) every 20 minutes. Therefore, it is necessary to treat the tap water and keep it free of viable microorganisms.

3. **Metal Analysis** of the effluent water is listed below in Table 2—

Table 2. Metal Analyses of Samples (Zinc in ppm) for the first set of samples

Sample	Contact	Flush 1	Flush 2	Flush 3	Flush 4	Flush5
NaOCl 10 minute	13.6	1.8	1.7	NT	NT	NT
ClO2 2-4 minute	NT	0.99	NT	2.32	NT	NT

1-minute contact between Flush 1, Flush 2, Flush 3, Flush 4 and Flush 5 for both NaOCl and ClO2
 NT = Non-traceable

The second and third set of samples that were collected mirrored the results in Table 2, except that the zinc concentration was <1.0 ppm in the initial solution. Flush 1 showed some zinc in both treatment groups while Flush 2 through Flush 5 were non-traceable for zinc. The copper concentration was detectable (1-2 ppm) for both groups. Trace elements of nickel were seen in both groups, while hardly any was seen in the second and third water samples.

4. **Control of municipal water contamination** using 2-2.5 ppm of Chlorine dioxide is listed in table 3--

Table 3. Efficacy of 2-2.5ppm of DioxiClear™ (ClO2) in Controlling Planktonic Water Contamination (Heterotrophic plate counts in CFU/mL)

Sample	Municipal Water	<2ppm DioxiClear™ (ClO2) in Municipal water post 20 minute contact
1	260	0
2	70	0
3	10	0
4	210	0
5	170	0
6	50	0
7	>400 (TNTC)	0
8	200	0
9	80	0
10	40	0
11	>400 (TNTC)	0
12	50	0
13	130	0
14	60	10
15	90	0
16	50	0
17	>400 (TNTC)	0
18	240	0
19	80	0
20	320	0
21	>400 (TNTC)	0
22	130	0
23	180	0
24	100	0
25	>400 (TNTC)	0
26	90	0

CFU/mL = colony forming units per mL of water (1 CFU/mL = 1 microorganism per mL)

Table 3 shows that tap water taken at another location in the same facility was contaminated and in many instances exceeded the 200 CFU/mL. When the water samples were treated with DioxiClear™ with a resulting solution of <2 ppm, the water in all instances but one was 0 CFU/mL. This concentration of ClO₂ is the same level added to drinking water, a safer alternative than using any other additives.

If the dental treatment water is prepared in the bottle overnight (<2ppm of ClO₂) and the bottle not totally tightened, there will be dissipation of gases and viable microbe free treatment water for the next day. If the water has to be replaced during patient care, the dentist may have to wait up to 20 minutes for the disinfection of the water to occur before treating the patient. At <2 ppm a very faint odor may be noticed, while post 1 hour of mixing, odor was absent.

This experiment demonstrates that tap water of reasonably poor microbial quality may be adequately treated to meet the ADA's goal of <200 CFU/mL.

5. Comparison of dentin bond strength between municipal water and chlorine dioxide is listed below--

Table 4. Failure modes and mean failure loads for dentin bonding specimens prepared with Dallas Municipal Water and DioxiClear™ tested by shear loading.

Irrigant	Failure Load		Failure Mode		
	N		Adhesive	Mixed	Cohesive
Dallas municipal water	173	(89) ^a	10 (83%)	2 (17%)	0 (0%)
DioxiClear™	165	(86) ^a	8 (67%)	4 (33%)	0 (0%)

^{*} mean (standard deviation), ^{**} number observed (proportion)

^a not significantly different according to one-way ANOVA ($\alpha = 0.05$)

Adhesive = Adhesive failure

Mixed = Failure/fracture extending into both the dentin and the composite

Cohesive = Cohesive failure

Table 4 details the mean loads required for debonding the composite from dentin surfaces recorded as 173 ± 89 N (Dallas municipal water) and 165 ± 86 N (DioxiClear™).

One-way ANOVA showed no significant difference in mean failure load between irrigants ($p = 0.07$).

There was no significant difference between the bond strengths of the Dallas municipal water treated surfaces and the DioxiClear™ surfaces.

Examples of failure when shear force was applied

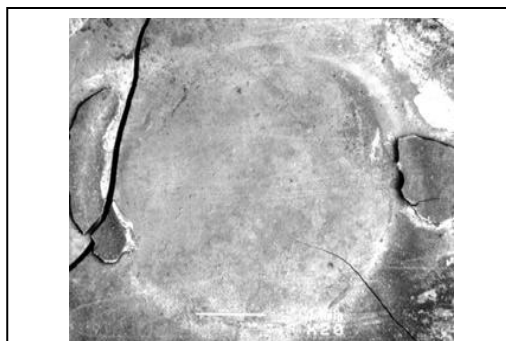


Figure 5. Representative adhesive failure on the surface of dentin after composite debonded under shear loading (23 N).

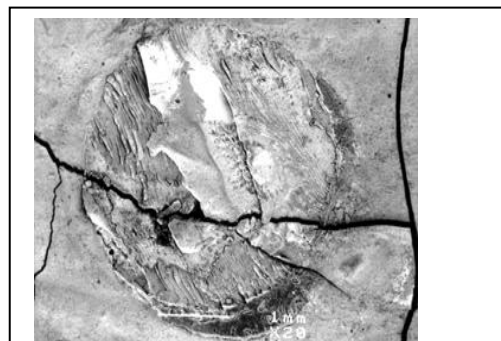


Figure 6. Representative cohesive failure on the surface of dentin after composite debonded under shear loading (301 N).



Figure 7. Representative mixed adhesive-cohesive failure on the surface of dentin after composite is debonded under shear loading (237 N).

Conclusions

DioxiClear™ (Chlorine dioxide) was efficacious in controlling the biofilms in dental unit waterlines and was comparable to using 5000 ppm of NaOCl, with the notable exception that DioxiClear™ does not form trihalomethanes (THMs are formed when NaOCl is used on biofilms). DioxiClear™ (ClO₂) when used to treat dental unit waterlines for a contact of less than 4 minutes on a weekly basis can control contamination in dental unit water systems and meet the American Dental Association's Goals for the Year 2000 AD of <200 CFU/mL. Periodic waterline treatment with 140 ppm of DioxiClear™ and use of pasteurized water as an irrigant was efficacious in meeting the ADA's goal of <200cfu/mL. Tap water was decontaminated when DioxiClear™ (ClO₂) was added to bring about a concentration of 2-2.5 ppm. DioxiClear™ could be used as an irrigant and meet the ADA's goal of introducing water of low microbial count. As an alternative to pasteurization, 2ppm of ClO₂ in municipal water may be evaluated for efficacy of contamination control in future clinical studies. DioxiClear™ as used in this study (less than 4 minutes contact time of 140 ppm for periodic cleaning and 2-2.5 ppm as a constantly present irrigant) did not corrode metals in the dental unit water system or leach out metals unacceptable for patient care. There was no significant difference in mean bond strength between Dallas Municipal Water and DioxiClear with regards to dentin bonding. DioxiClear™ did not affect dentin bonding when used as a waterline irrigant.

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