Utilization of two concentrations of ClO2 Cleaner in controlling dental waterline contamination

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ABSTRACT

Purpose: The purpose of this study was to conduct a preliminary evaluation of two concentrations of chlorine dioxide (ClO2)[1] in controlling biofilms and planktonic microbial control in dental unit water systems.

Methods: Two dental clinics each with 3 chairs (2 treatment and 1 control in each clinic) were used in this study. One clinic had old dental units with established biofilms and the second clinic was equipped with dental units that were less than a month old. A concentration of about 140 ppm ClO2 was used at baseline and once a month to purge the lines (2.5 minute contact). A concentration of 2-3 ppm ClO2 was used as a continuous irrigant (municipal water mixed with ClO2) during the 12 weeks of this study in the treatment units. The control units were not treated with any cleaner. Baseline and post study waterline samples were examined for biofilm using an SEM. Baseline and weekly water samples (20 mL per unit) were analyzed for contamination using HPC water samplers (Millipore). Municipal water samples were treated for 10 minutes with ClO2 resulting in a concentration of 2-3ppm of ClO2. After 10 minutes exposure of municipal water to ClO2, this mixture was neutralized with sodium thiosulphate and plated using HPC water samplers.

Results: Biofilm was seen in all old units at baseline and only in the control units at the end of the study. At baseline the heterotrophic plate counts of all units showed contamination ranging from >4000 to >400,000 colony forming units (cfu/mL). All treated units showed a reduction in contamination <30 cfu/mL by the end of the third week and remained so for the rest of the study. Municipal water contamination ranged from 10 cfu/mL to >4000 cfu/mL. When this water was mixed with ClO2 resulting in a 2-3 ppm solution utilized for irrigation during dental treatment, contamination levels dropped to <10 cfu/mL.

Conclusion: In this study, 140 ppm Chlorine Dioxide when used as a 2.5-minute purge and 2-3 ppm as an irrigant for dental care was found to control biofilms and provide safe dental treatment water. Further studies should be conducted to study effects on dental equipment.

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[1] DioxiClear is a chlorine dioxide product manufactured by Frontier Pharmaceutical, Melville, New York, USA

DioxiClear is the former name for BioClenz™

<div align="center">**PURPOSE**</div>

- To study the effects of ClO2 on planktonic microorganisms in providing microbiologically safe dental treatment water
- To study the effects of ClO2 when used periodically on naturally occurring mature biofilms in the dental waterlines in dental units with previously established biofilm, and in new biofilm-free dental units

<div align="center">**MATERIALS AND METHODS**</div>

Study Sample

Two dental practices were used in this study

- *Treatment 1:* A 15 year old dental practice with untreated dental unit water systems
  - Three dental units retrofitted with self-contained water systems
    - Two treatment units with presence of mature biofilms
    - One control unit with presence of mature biofilms
- *Treatment 2:* A 1 month old dental practice with new dental unit water systems
  - Three dental units retrofitted with self-contained water systems
    - Two treatment units with no mature biofilms
    - One control unit with no mature biofilms

Definition of Treatment and Control Groups

- Treatment Groups
  - Chairs/units treated three times (2.5 minutes each time) on the day of commencement of the study with 140 ppm ClO2 & post flush (750 mL of microbe free water that had been previously treated with chlorine dioxide resulting in 2-3 ppm of available ClO2), followed by monthly cleaning
  - The dental treatment water/irrigant was municipal mixed with chlorine dioxide resulting in treatment water/irrigant with 2-3 ppm of available ClO2 the previous day. Residual ClO2 was less that 1 ppm during patient care.
- Control Group
  - No treatment or flushing with any chemical, and municipal water used as an irrigant

Study period

- 60 working days

Scanning Electron Microscopy

Baseline and end of study line samples were harvested for Scanning Electron Microscopy from each of the dental units water system. The samples were fixed with 3% glutaraldehyde and treated with increasing concentrations of alcohol (60% through 95%) and desiccated overnight. The desiccated samples were mounted and coated with gold palladium (about 9 nanometers). The samples were viewed

and photographed at 1500 X. The surface morphology of the lumen were categorized using the following criteria—

1. Presence of a mature biofilm lattice and presence of microorganisms
2. Presence of biofilm and microorganisms but no lattice/matrix
3. Presence of microorganisms without the presence of biofilm lattice/matrix
4. No biofilm or microorganisms present

Heterotrophic Plate Counts of Planktonic Organisms

Baseline and weekly water samples were collected from the municipal water source, irrigant/dental treatment water from the self-contained water system, and the effluent water pooled from the air/water syringe and high-speed handpiece lines from each unit. The maximum volume of each water sample was 20 mL. This sample was neutralized using sodium thiosulphate buffer, vortexed and plated at 1:10 using HPC water samplers (Millipore Corporation).   The samples were incubated at $22^{\circ}C$ for 7 days and colony counts enumerated. Absolute colony forming units were converted to $\log_{10}$.

RESULTS

Table 1. Results of Scanning Electron Microscopy

|	1 Mature Biofilm with matrix organisms	2 Biofilm with organisms, no matrix	3 No Biofilm, but organisms only	4 No Biofilm and no organisms
Units with biofilm at baseline (Clinic 1)				
Baseline unit 1 (Treatment)	X			
Baseline unit 2 (Treatment)	X			
Baseline unit 3 (Control)	X			
Post Study unit 1 (Treatment)				X
Post Study unit 2 (Treatment)				X
Post Study unit 3 (Control)	X			
Units with no biofilm at baseline (Clinic 2)				
Baseline unit 1 (Treatment)				X
Baseline unit 2 (Treatment)				X
Baseline unit 3 (Control)				X
Post Study unit 1 (Treatment)				X
Post Study unit 2 (Treatment)				X
Post Study unit 3 (Control)	X			

Scanning Electron Microscopy:

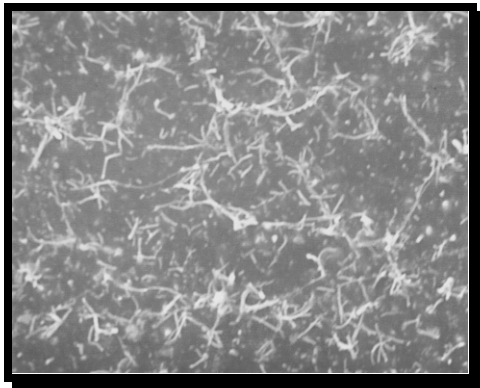### Figure 1: Baseline, New TX Unit
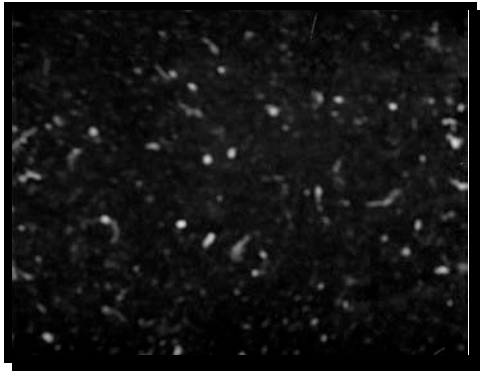
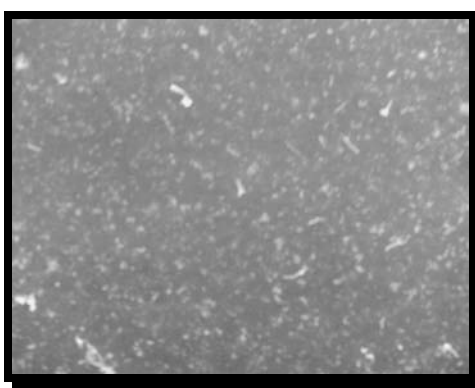1500 X No Biofilm

Figure 2 End of Study New TX
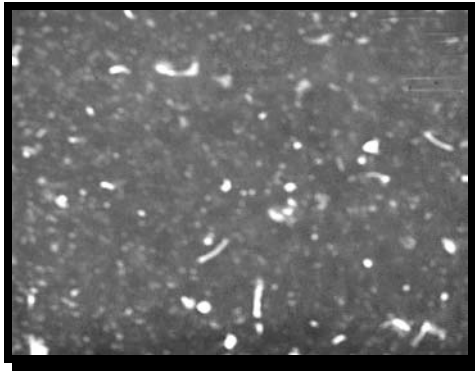
1500 X No Biofilm

Figure 3: Baseline Old Unit TX

1500 X Biofilm Present

Figure 4. End of Study Old Unit TX


1500 X No Biofilm

Heterotrophic Plate Counts:

Table 2. Clinic with New Dental Units (cfu/ml)

Sample	TX unit 1	TX unit 2	Control Unit	Tap water	Bottle (reservoir)
BL	>4000	>4000	>4000	260	>4000
Week 1	10	0	>4000	70	0
Week 2	0	0	>4000	10	10
Week 3	0	30	>4000	210	0
Week 4	20	0	>4000	170	0
Week 5	0	0	>4000	50	0
Week 6	30	0	>4000	>400	0
Week 7	10	0	>4000	200	0
Week 8	0	10	>4000	80	0
Week 9	0	0	>4000	40	0
Week 10	30	0	>4000	>400	0
Week 11	10	0	>4000	50	0
Week 12	10	0	>4000	130	0

Table 3. Clinic with Old Dental Units (cfu/ml)

Sample	TX unit 1	TX unit 2	Control Unit	Tap water	Bottle (reservoir)
BL	>40000	>40000	>40000	60	80
Week 1	60	80	>40000	90	10
Week 2	440	0	>40000	50	0
Week 3	90	0	>40000	>400	0
Week 4	0	10	>40000	240	10
Week 5	0	0	>40000	80	0
Week 6	0	0	>40000	320	0
Week 7	0	0	>40000	>400	0
Week 8	0	0	>40000	130	0
Week 9	10	0	>40000	180	0
Week 10	10	0	>40000	100	0
Week 11	0	0	>40000	>400	0
Week 12	20	0	>40000	90	0

Figure 1. Heterotrophic plate counts of dental units with biofilm
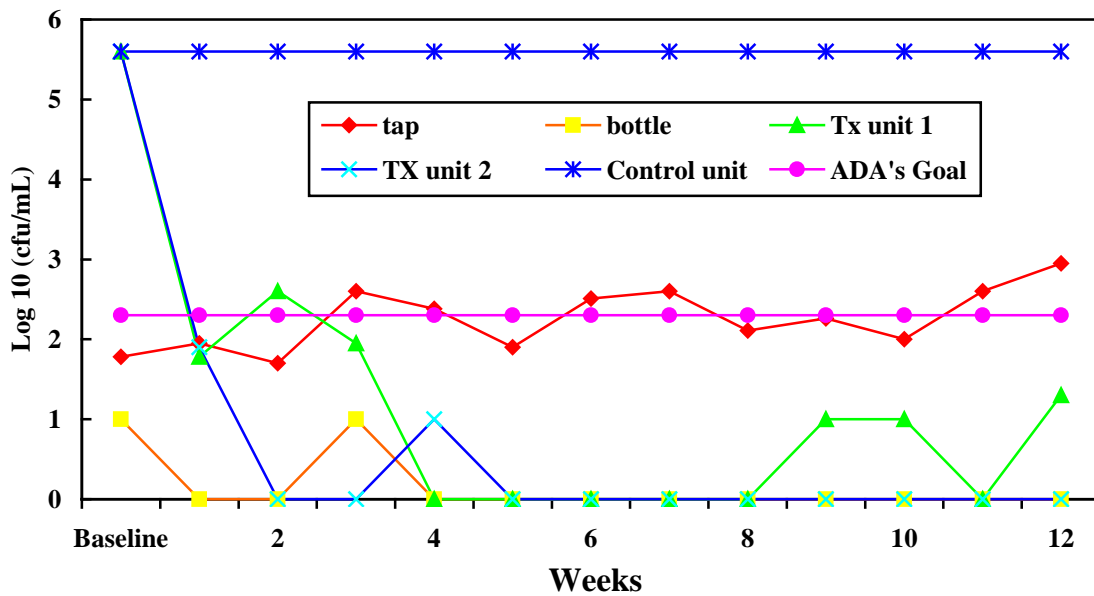
All Control effluent samples remained over 400,000 cfu/mL. All Treatment effluent samples reached below 200 cfu/mL immediately after the first week. All bottle water samples treated with 2-3 ppm ClO_2 remained <10 cfu/mL. Tap water did not meet the ADA's goal consistently.

Figure 2. Heterotrophic plate counts of dental units without biofilm
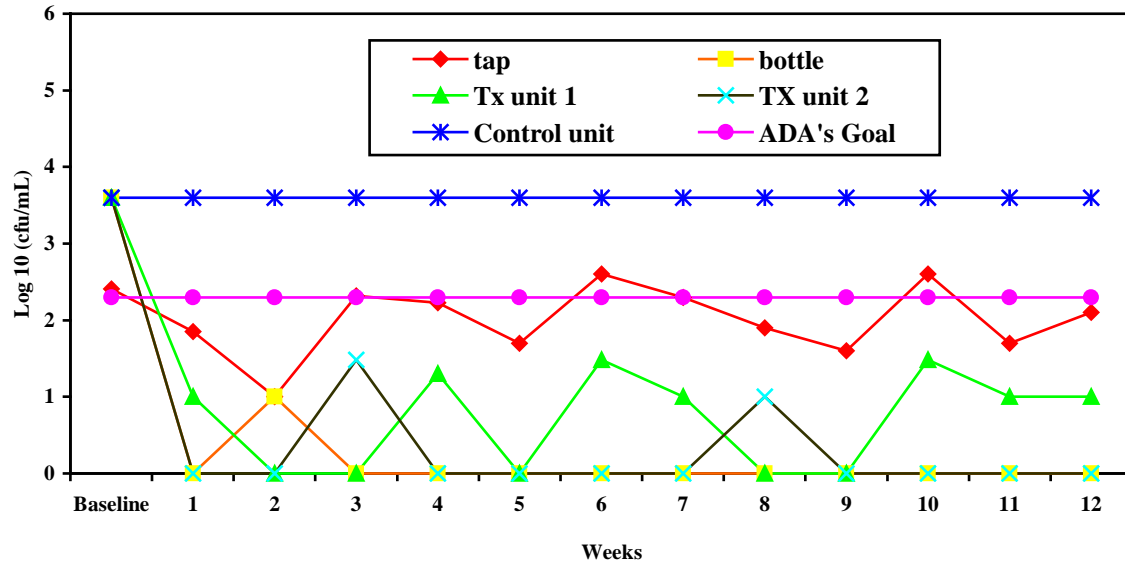
All Control unit effluent samples remained over 4,000 cfu/mL. Tap Water samples were not consistently below 200 cfu/mL. All bottle water samples (treated with 2-3 ppm ClO2 were below 200 cfu/mL. All Treatment unit effluent samples reached below 200 cfu/mL within the first week.

CONCLUSIONS
- Water from municipal sources in this study did not meet the ADA's goal of <200 cfu/ml consistently
- A post treatment with 2-3 ppm of ClO2 was required to keep contamination of the municipal water lower than 200 cfu/ml
- Lines with mature biofilms at baseline when flushed weekly with 140 ppm ClO2 (2-3 minute contact) disrupted and removed biofilms
- Lines that were biofilm free to begin with remained biofilm-free throughout the study
- More evaluation on lower concentration of ClO2 needs to be attempted with respect to microbial control and compatibility with dental unit water system materials