



Evaluation of an Automated Dental Unit Water System's Contamination Control Protocol

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ABSTRACT

Background: This study addresses the efficacy of an automated decontamination protocol using the germicide 'tetra acetyl ethylene diamine (TAED) perborate' (Farmec SpA, Italy). The germicide TAED perborate protocol is used in the Castellini Dental Units fitted with an Autosteril unit (an automated device that can cycle 0.26% TAED perborate solution and sterile water for cleaning the water system between patients and overnight). Prior to testing the Autosteril and the 0.26% TAED perborate protocol on the Logos Jr Dental Unit (Castellini SpA, Italy), TAED perborate was used on a dental unit water system simulation device.

Methods: A dental unit water system simulation device equipped with four dental unit water systems and with naturally grown and mature biofilm contamination was used in this study (three treatment units and one control). One treatment group used a simulated 5 minutes contact with TAED perborate and sterile water for irrigation; the second used a simulated 5 minutes contact with TAED perborate and 2 ppm ClO₂ for irrigation; the third used a simulated 5 minutes contact with TAED perborate and municipal water for irrigation. The control group used municipal water for irrigation with no cleaning/disinfection protocols. This protocol was repeated for 30 cycles. Laser scanning confocal microscopy (LSCM) was used to study the effects on natural and mature biofilms, and R2A agar used to quantify heterotrophic plate counts in the effluent irrigant. Antimicrobial efficacy was evaluated by challenging TAED perborate with microbes and spores (*M. smegmatis* and *B. subtilis*). Deleterious effects of the germicide were evaluated on metal and nonmetal parts of dental unit water systems. Heterotrophic plate counts using R2A agar and LSCM of the lines were conducted to assess biofilm and microbial control.

Results: Baseline water samples showed mean contamination >5.6 log₁₀ cfu/ml. After initial cleaning, all three groups maintained mean contamination levels of less than 1.1 (SD <0.3) log₁₀ cfu/ml. LSCM of baseline samples was positive for live biofilm in all groups. At the end of the study, viable biofilm was only present in the control. In the microbial challenge test, all vegetative organisms were killed within 30 seconds of contact, while spores were killed within 5 minutes. Corrosion was seen in metals used in US-manufactured dental unit materials, while not observed in those used in the Castellini Logos Jr dental unit.

Conclusion: In this study, the TAED perborate protocol was effective in biofilm control and control of dental treatment water contamination. Use of sterile water or 2 ppm ClO₂ along with TAED treatment also controlled planktonic contamination effectively.

Clinical significance: Environmental biofilms contaminate dental unit water systems over time and affect the quality of dental treatment water. Contaminants include environmental biofilms, microbes, including gram-negative rods and endotoxins in high doses that are not of acceptable quality for treating patients. There are many germicidal protocols for treating this contamination and one such is the prescribed use of TAED perborate used in conjunction with sterile water for irrigation in the autosteril device, an integral component of the Castellini dental units for between patient decontamination of dental unit water systems. This study was conducted on an automated simulation dental unit water system to test the TAED perborate protocol's efficacy on naturally grown, mature environmental biofilms, its efficacy on microbes and spores and its effects on materials used in dental unit water systems. This translational research addresses both microbial control and material effects of TAED perborate in studying efficacy and possible deleterious effects and simulated use in dentistry. Currently, this antimicrobial use protocol is followed worldwide in the Castellini dental units that are used in day-to-day dental patient care.

Keywords: TAED perborate, Autosteril, Dental water system, Biofilms, Microbial contamination, Challenge test, Laser scanning confocal microscopy.

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Conflict of interest: None declared

INTRODUCTION

Biofilms are routinely found in dental unit water (DUW) and formed when bacteria adhere to the luminal walls of

water lines within dental treatment water delivery systems.¹⁻⁸ In dental unit waterlines (DUWLs), macromolecules and other low-molecular-weight hydrophobic molecules or exopolysaccharide glycocalyx polymers may anchor to the surface forming conditioning films of 30 to 50 micron thickness, while planktonic or free-floating bacteria in the water adhere to these conditioning films laying the basis for a biofilm matrix in as little as 2 weeks.⁹⁻¹² Many potentially pathogenic and nonpathogenic species of microorganisms have been well documented contaminating the dental water system.¹³⁻²⁰ Amoebae species, such as *Naegleria*, *Acanthamoeba*, *Hartmanella*, *Vahlkampfia* and *vanella*, have been isolated from dental unit water systems 300 times more counts than in municipal drinking water samples.²¹ Microbes commonly found in DUWLs are *Pseudomonas*, *mycobacteria* and *Legionella*. *Pseudomonas cepacia* (gram-negative bacillus) commonly found in dental treatment water has, in the past, been associated with hospital infections through its presence and survival in aqueous disinfectants/germicides.²²⁻²⁶ In one study, investigators found *Exophiala mesophila*, a fungus, predominantly contaminating dental unit water systems that was using a stabilized ClO₂ irrigant formulation.²⁷ *Mycobacterium sps* have been isolated from hospital water supplies, some of which have been associated with hospital-related infections with *M. xenopi* implicated in 19 cases of pulmonary disease due to aerosols generated from a contaminated showerhead.²⁸⁻³¹ Water spray and aerosols common in the dental setting were associated with subclinical infection with *Legionella pneumophila* in a dental school environment.¹⁸ Fotos et al³² investigated exposure of students and employees at a dental clinic and found that of the 270 sera tested, 20% had significantly higher IgG antibody activity to the pooled *Legionella sp.* antigen as compared with controls. Reinthaler et al³³ also found a high prevalence of antibodies to *Legionella pneumophila* among dental personnel demonstrating the highest prevalence (50%) among dentists. Atlas et al³⁴ found that 68% of DUW samples collected from 28 dental facilities in six US states and 61% samples from institutional faucets and drinking water fountains showed presence of *Legionella spp.* High doses of bacterial endotoxins measured in one study showed that more than 100 endotoxin units per milliliter (EU/ml) were released in contaminated dental unit water systems during cleaning with 5,000 ppm bleach with municipal water containing more than 25 EU/ml.³⁵ Other studies have shown that the endotoxin levels could reach 6,200,000 EU/ml in untreated dental water systems and 3,295.0 EU/ml in treated water systems.³⁶ The types of organisms may range from amoebae, *Legionella* to *E. coli*,²¹ which may inherently be seen in dental units connected to

municipal water or be contaminated by the handlers of the water systems, if proper hygiene practices are not followed.³⁶ In summary, exposure of patients to microbial agents associated with respiratory, enteric diseases, conjunctivitis or other adverse health conditions may be plausible, if the dental treatment water quality is poor.³⁷

Considering the presence of potentially pathogenic bacteria, amoebae and endotoxins in extremely high quantities, control measures for cleaning and disinfecting the dental water system and preserving high quality of the irrigant/treatment water quality must be adopted. Biofilm contamination can be viewed as a dynamic process involving many contributing factors.^{35,38,39} Some of the main factors could be— (1) long periods of stagnation, (2) high surface to volume ratio, (3) nutritional content of water for microbial survival, (4) mineral content and hardness of water facilitating coating of the lumen, (5) fluid dynamics, such as laminar flow that does not facilitate physical purging of biofilms coating the lines, (6) low flow rate, (7) microbial quality (bacteria, fungi, protozoans and nematodes) of the water entering the system, and (8) failure of antiretraction valves leading to contamination from the oral cavity of patients and finally, (9) time/period of exposure to some of the above factors.

Many dental units today are equipped with anti-retraction valves to prevent suck-back of water through handpieces after operation.⁴⁰ Even reliable functioning of new and unused antiretraction valves have been questioned due to failure that could potentially lead to suck-back of saliva and microbes into the waterline system from the oral cavity.^{39,41} Flushing of DUWLs at the beginning and end of patient treatment has been previously advocated.⁴²⁻⁴⁵ One study concluded that a two-minute flushing cycle reduced counts of planktonic organisms, on average by one-third, but did not reduce counts to zero.⁶ Purely flushing the water for a few minutes prior to treatment was not effective in biofilm removal, while it may reduce protozoans and planktonic organisms for a short period.^{21,46}

There are many physical and chemical methods of improving dental treatment water quality. Investigators have tested methods, such as using inline microfilter devices,^{7,8} flushing water lines with various disinfectant solutions which include hydrogen peroxide,^{47,48} chlorhexidine gluconate,^{49,50} sodium hypochlorite,⁵¹⁻⁵⁴ povidone-iodine,²⁰ iodine⁵⁵ and mouthwash.⁵⁶ Each of these methods, though effective at controlling planktonic organisms and possibly biofilms to a certain extent does not eliminate biofilm formation due to the inherent contamination of source or city water supplies. Tap water was found 'not reliable' with respect to microbial contamination.⁵⁷ Studies have measured planktonic contamination levels of tap water and repeatedly

shown heterotrophic plate counts ranging from zero to at least a few hundred cfu/ml, exceeding even the contamination levels set per current CDCs recommendations for dental treatment water of 500 cfu/ml.^{7,8,35,48,50,53,55,58,59} Only cleaning/disinfecting the lines periodically does not ensure that tap water can meet the ADA's goal or the CDC's guidelines. The ADA's statement on dental unit waterlines implies that there should be a control over the quality of water used during 'boil water alerts' in the community.⁹ There are no data on commercially available distilled and bottled water being microbiologically reliable for dental use.

Filters (activated carbon casing fused to a high intensity UV light) have been used to improve source water.⁶⁰ Most available membrane filters are consistent in controlling microbes/planktonic microorganisms in dental treatment water, while membrane filters with the additional function of endotoxin retention are even more beneficial.⁸ When using filters, it may be pragmatic to periodically control the biofilm in the DUW systems to reduce the bacterial and endotoxin challenge to the filters. Furthermore, it is absolutely essential to change the filters based on the manufacturers' recommended optimal performance time.⁸ Chemical treatment or constantly present chemicals to control the microbes and biofilms in DUWLs are some of the options available to dentists. Examples are low concentrations of constantly present citric acid in the DUW system used as an irrigant,⁵⁴ chlorhexidine⁵⁵ and iodine.⁵² Germicides must be approved by the FDA and the EPA for use in the jurisdiction of the United States^{61,62} safe for patients, noncorrosive to the components of the DUW system and compatible with other materials used in the patient's mouth. Bleach can be damaging to the dental unit and produce high amounts of trihalomethanes when it reacts with organic matter, such as biofilms.⁶³ Low concentrations of NaOCl in the presence of organic matter also increased the total trihalomethane levels beyond levels set by the US Environmental Protection Agency. The use of NaOCl for the specified purpose of cleaning DUWLs has not been approved by the US FDA.

OBJECTIVES

In this investigation, we evaluated the effects of a periodic use germicide, 0.26% TAED perborate on—(a) naturally

grown biofilms in the dental unit water system, (b) planktonic organisms in the dental treatment water when used with various irrigants, (c) marker organisms for hospital infections, (d) spores, and (e) compatibility with metals in the conventional dental unit water systems manufactured in the United States (A-DEC, Newburg OR, USA), and a dental unit manufactured in Europe with an automated flushing feature (Autosteril, Logos Jr Dental Unit, Castellini SpA, Bologna, Italy).

MATERIALS AND METHODS

Testing on the Automated Dental Unit Water System Simulator

An automated dental unit water system simulatorⁱ (Fig. 1) was used in this evaluation to study the effects of TAED perborate on the biofilms. The waterlines retrofitted to the device were at least 10 years old, obtained from dental units that had not been cleaned. Presence of mature and viable biofilms was confirmed using laser scanning confocal microscopy (LSCM; SP2, Leica, Heidelberg, Germany) after staining with BacLight[®] Live/Dead Stain (Molecular Probes, Eugene, OR, USA) and scanning electron microscopy (JEOL, Peabody, MA, USA) after the lines were retrofitted. Four water systems were used to study the effects

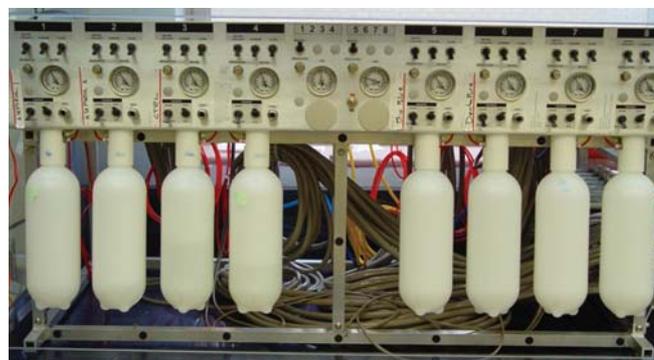


Fig. 1: Automated dental unit water system simulator was developed to grow natural biofilms and conduct standardized *in vitro* experiments in the control of biofilms and treatment of water contamination prior to use in dental units. The simulator is equipped with 8 ADEC (Newburg, OR, USA) dental unit water systems built to scale, an Allen Bradley logic controller and RSLogix automation software (Rockwell Automation, Milwaukee, WI, USA), and specifically developed algorithms to simulate treatment water use in general dental practice

ⁱOriginally designed by Dr Puttaiah, Dr Mills and Mr Gambal at the Dental Investigations Service, Brooks AFB in 1994, modified and automated by Drs Puttaiah, Zawada, and Siebert in 1998 to evaluate the effects of the periodic use to grow natural biofilms and conduct *in vitro* methods of control of both biofilms and water contamination prior to use in dental units treating patients. The simulator is equipped with 8 ADEC (Newburg, OR, USA) dental unit water systems built to scale, an Allen Bradley logic controller and RSLogix automation software (Rockwell Automation, Milwaukee, WI, USA), and specifically developed algorithms to simulate treatment water use in general dental practice.

of the germicides on biofilms and planktonic organisms on the simulator.

LSCM procedures included waterlines (1 cm length) from the four water systems were harvested, split lengthwise exposing the lumen and immediately treated with the BacLight® Live/Dead stain. Presence of mature and viable biofilms was confirmed using LSCM at 400× and 1600×. Live organisms stained green, while dead organisms stained red. In LSCM images, where the organisms picked up both dyes they appear yellow and were dead. A 40× water dipping lens was used to obtain z-series stacks. The image stacks were projected into a single image to obtain the biofilm on the curved water lines (Fig. 2).

Heterotrophic plate counts (HPC) for assessing treatment water contamination were carried out by taking a 10 ml sample (less than the inherent volume of that unit's waterlines) in a sterile test tube. Water samples for HPC were neutralized with 3% polysorbate 80, 0.1% L-histidine, 0.3% lecithin, 0.3% sodium thiosulphate in phosphate buffer 0.25 N. 1 ml of this neutralized water was then plated on R2A agar and incubated for a period of 7 days at 22 to 24° C (room temperature) before counts made.

The periodic-use germicide evaluated on the simulator was TAED perborate (Farmec SpA, Italy) used per manufacturer's recommendation for the three 'treatment groups' using different irrigants, while the fourth group was the control, with municipal water as irrigant and for between-patient flush. The flush in this unit comprised purging lines with municipal water for 30 seconds between simulated patient care.

Methods for the three treatment groups were as follows:

Group 1: Simulated 5 minutes between-patient flush with TAED perborate and sterile water as irrigant.

Group 2: Simulated 5 minutes between-patients flush with TAED perborate and ClO₂ (BioCleans™, Frontier Pharmaceuticals, Melville, NY, USA) diluted to 2 ppm municipal water as the irrigant.



Fig. 2: Laser scanning confocal micrograph of a naturally occurring biofilm in a dental unit waterline showing formation on the line surface. Clumps of bacteria adhere together as well as stick to the line surface with the help of an extracellular polymer matrix produced by the microbes

Group 3: Simulated 5 minutes between-patient flush with TAED perborate and municipal water as the irrigant.

The three treatment groups included initial overnight contact with TAED perborate followed by a 60 seconds flush with the respective irrigants, thereafter, between simulated patient care system was purged for 30 seconds with institutional air, initial loading and contact with TAED perborate for 60 seconds, a second flush of TAED perborate for 30 seconds, a final contact pause with the germicide for 30 seconds and rinse with the respective irrigants for 60 seconds to purge the germicide from the lines. Before beginning the germicide protocols, all four units were programmed to simulate care for eight patients per day and have an overnight inactive period. For the first 3 days, the system was operated with no germicide treatment. For each group, baseline waterline samples harvested for LSCM and SEM to study biofilms and five random water samples for HPC plated per day. From day 4 through day 9, the simulator was operated with respective treatments and irrigants (simulated treatment of eight patients per day with between-patient germicide protocols for the treatment groups and between-patient flush for the control). Five water samples were taken per day for heterotrophic plate counts. Absolute HPCs were converted to Log₁₀ values to normalize data. On day 10, water line sections were prepared for LSCM and SEM from each of the four units to study presence or absence of biofilms.

Antimicrobial Efficacy Tests

Efficacy of use dilution TAED perborate on controlling hospital organisms and spores was tested at an independent laboratory.ⁱⁱ *P. aeruginosa* (ATCC No. 9027), *E. coli* (ATCC No. 8739), *S. aureus* (ATCC No. 6538), *M. smegmatis* (ATCC No. 14468) all at concentration > 10⁷ and *C. albicans* (ATCC No. 10231) at >10⁶ were exposed to use dilution of TAED perborate with time ranging from 30 seconds to 1 hour. *M. smegmatis* at >10¹⁰ was also exposed to TAED perborate. *B. subtilis* var niger 2.5 × 10⁶ and *B. stearothermophilus* 2.5 × 10⁵ were also exposed to TAED perborate. After each time period of exposure, they were neutralized and incubated in the media and at temperatures according to standard microbiological methods, all samples were checked for positive or negative growth after 72 hours except *M. smegmatis* which had to be incubated for a longer period of time (AOAC test—method 965.12).

ⁱⁱMicroconsult Inc. 3218 Commander Dr Suite 100, Carrollton, Texas 75006. <http://www.microconsultinc.com/>

Compatibility of TAED Perborate with Metals

TAED perborate use concentration for between-patient periodic cleaning and other germicides at use concentration for irrigation were tested for compatibility with metals used in conventional water systems of dental units manufactured in the United States as well as the metals in the autosteril and the Logos Jr dental unit. Brass, aluminum and steel metal parts found in the water systems were placed individually in nonreactive glass containers with two different chemicals TAED perborate (use dilution) and water with 2 ppm ClO₂. These chemicals with the metal samples were kept in an incubator at 56°C for a period of 60 days. Dissimilar metals were also placed together in the chemicals to study, if corrosion and galvanic effect either tarnished or corrode the metals. Twice daily, solutions were changed with metal samples being rinsed with deionized water. The samples were examined for visible tarnish or corrosion at 12× using a dissecting microscope. Digital images before and after exposure to the chemicals were made for comparison.

To study the effects of the chemicals and irrigants, 100 ml water samples were collected in nonreactive containers with nitric acid (as a preservative) for elemental metal analysis, from the following tap water (chairside faucet), tap water effluent from the dental unit water system, sterile water effluent from the dental unit water system, TAED perborate 5-day contact at 56°C with steel and aluminum, TAED perborate overnight contact with lines, TAED perborate 5 minutes contact with lines, TAED perborate 2 minutes contact with lines, ClO₂ 2 ppm with tap water (freshly mixed), ClO₂ 2 ppm effluent from the lines. These samples were analyzed using the EPA 6000/7000 series method at a registered environmental testing laboratory.

RESULTS

LSCM showed that all baseline samples demonstrated mature biofilm. The cells dyed red were dead cells within the biofilm, while the green color indicated live or viable cells. When the two cell types colocalized, the recorded color was yellow, indicating that the cells were dead. After 6 days treatment regimen (poststudy) with TAED perborate, all treatment groups had no biofilm (Figs 3A and 3B), while the control group still had a mature biofilm.

Heterotrophic plate control studies supported the LSCM results in that all base line control samples had high cfu/ml (>100,000 in automated water system simulator), whereas the samples taken after treatment were <10 cfu/ml in

all treatment groups. In contrast, control samples continued to have high cfu/ml counts not different from the baseline (Fig. 4).

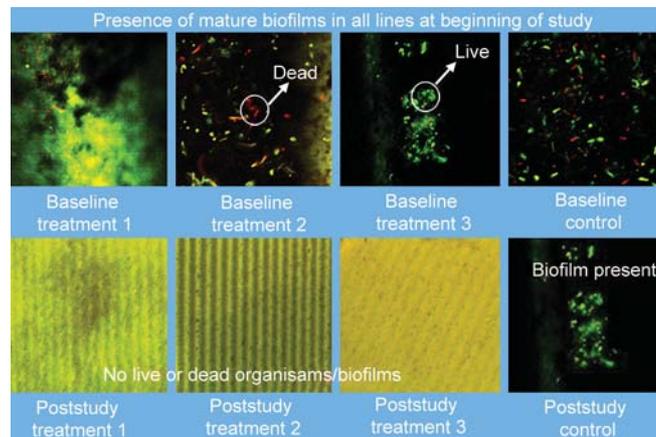


Fig. 3A: All baseline samples at baseline demonstrated mature biofilm. The cells dyed in red color are dead cells within the biofilm while the green cells indicate live or viable cells. Poststudy samples of treatment groups 1 to 3 showed no biofilm while the control group showed presence of mature biofilm [laser confocal microscopy (1600×)]

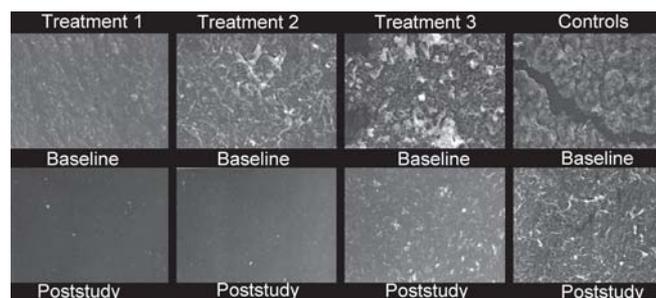


Fig. 3B: All baseline samples at baseline demonstrated mature biofilm. Poststudy samples of treatment groups 1 and 2 showed no biofilm. Treatment group 3 showed disruption of the biofilm matrix demonstrating residual individual cells or clumps of cells, while the control group showed presence of mature biofilm [scanning electron microscopy (1500×)]

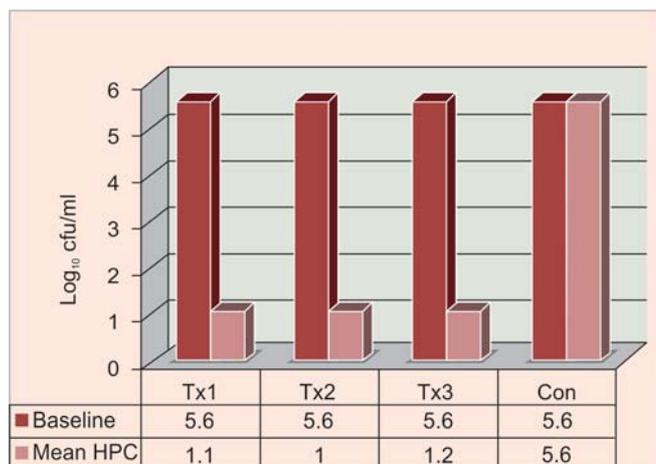


Fig. 4: At baseline, all groups showed contamination levels of >40,000 cfu/ml. Mean contamination level of the effluent water in all treatment groups was <500 cfu/ml. The control group was significantly more contaminated than treatment groups (p < 0.05). Mean heterotrophic plate counts of effluent water

Marker organisms, such as *P. aeruginosa*, *E. coli*, *S. aureus*, *C. albicans* and *M. smegmatis*, were killed within 30 seconds of exposure (Table 1). A 10 log reduction of *M. smegmatis* was achieved with a 30 seconds exposure to TAED perborate (Table 2). Use dilution of TAED perborate showed a 5 log reduction of *B. stearothermophilus* and 6 log reduction of *B. subtilis* spores within 5 minutes as shown in Table 3.

TAED perborate did not corrode stainless steel, anodized aluminum and brass adversely even when exposed to extended periods of time and at higher temperatures (Fig. 5). TAED perborate removed patina (tarnish) on brass components of US-made system showing a potential for corrosion. Elemental metal analyses also showed minimal effects of TAED perborate on anodized aluminum and no effect on stainless steel used in the autosteril system (Fig. 6). TAED perborate, chlorine dioxide, sterile water and municipal water (tap water) reacted with metals and showed leaching of copper, nickel and zinc, when exposed to brass components of US manufactured dental equipment.

DISCUSSION

Many factors need to be considered in contamination control of dental unit water systems and treatment water, including

long periods of stagnation, high surface to volume ratio, fluid dynamics, such as laminar flow, low-flow rate and failure of antiretraction valves. On the other hand, nutritional and mineral content of water for patient care and microbial quality of the water entering the system can be controlled in addition to periodic cleaning or disinfection of the water system.

Biofilms in water systems should be controlled or removed periodically, and treated water or a low-grade germicide in water should be used regularly as an irrigant. In the absence of treatment of the water or using a low-grade germicide, one could use microfilters or the latter with endotoxin retention capabilities. These microfilters must be changed either daily or weekly based on the quantity of water filtered or based on the manufacturer's recommended use instructions. If the filters are not used properly, the possibility of clogging and breaching the filter could occur contaminating water entering the patient's mouth.^{8,9} Just cleaning or disinfecting the lines periodically and using municipal water do not provide a microbiologically reliable irrigant as in many instances, the inherent contamination of the municipal water exceeds the 2003 CDC's guidelines for dental treatment water of <500 cfu/ml.

Table 1: Kill rate of microorganisms exposed to TAED perborate using the AOAC (Association of Official Analytical Chemists) test. All microorganisms (marker organisms for hospital infections) and *M. smegmatis* were killed in 30 seconds of exposure to TAED perborate

Test microorganism	Challenge	Time of exposure	Outcome
<i>P. aeruginosa</i>	1.04×10^7	30 seconds	No growth
<i>E. coli</i>	3.04×10^7	30 seconds	No growth
<i>S. aureus</i>	3.32×10^7	30 seconds	No growth
<i>C. albicans</i>	1.15×10^6	30 seconds	No growth
<i>M. smegmatis</i>	4.81×10^7	30 seconds	No growth

Table 2: AOAC test for TB Kill time showed a 10 log reduction achieved in 30 seconds of exposure to TAED perborate time of exposure to TAED perborate

Test microorganism	Challenge	Time of exposure	Outcome
<i>M. smegmatis</i>	1.43×10^{10}	30 seconds	No growth

Table 3: Exposure of spore population to TAED perborate

Time of exposure to TAED perborate	Growth outcomes					
	<i>B. subtilis</i> *			<i>B. stearothermophilus</i> **		
Samples	1	2	3	1	2	3
1 minute	+ve	+ve	+ve	+ve	+ve	+ve
5 minutes	-ve	-ve	-ve	-ve	-ve	-ve
30 minutes	-ve	-ve	-ve	-ve	-ve	-ve
60 minutes	-ve	-ve	-ve	-ve	-ve	-ve
120 minutes	-ve	-ve	-ve	-ve	-ve	-ve
180 minutes	-ve	-ve	-ve	-ve	-ve	-ve
240 minutes	-ve	-ve	-ve	-ve	-ve	-ve

- * *B. subtilis* var niger 2.5×10^6 incubated at 37°C 72 hours
- ** *B. stearothermophilus* 2.5×10^5 incubated at 56°C 72 hours growth was positive only on the 1 minute exposure to TAED perborate, while all other times of exposure showed no growth

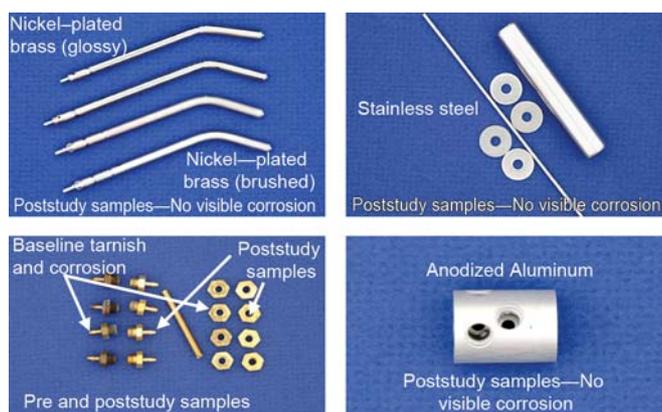


Fig. 5: No corrosion was noted in nickel-plated brass, stainless steel and anodized aluminum components of the dental unit water system after 60 days of exposure to daily prepared TAED perborate solution. Brass components that had tarnish showed removal of tarnish. TAED perborate in this accelerated compatibility test was found to be compatible with the Castellini Logos dental unit components (accelerated corrosion tests for 2 months in TAED perborate at 56°C)

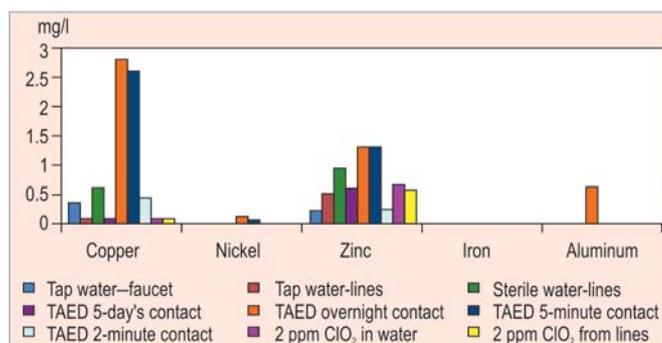


Fig. 6: This experiment shows effects on metals when used in conventional dental unit water system other than the Castellini Autosteril system. Copper, zinc and aluminum showed varying levels of corrosion or leaching of the metals when exposed to TAED perborate, chlorine dioxide, sterile water and tap water. All components of the Autosteril system do not show leaching of metals when exposed to chemicals as they are specifically formulated for use in the Castellini dental unit water system where the components are anodized aluminum and stainless steel (metal analysis: EPA 6000/7000 series method)

Alternatively, using only sterile, boiled or distilled water, or a low-grade antimicrobial in water toward controlling the planktonic microbes will not ensure cleaning or removal of the biofilm nor will it provide microbiologically reliable irrigant that meets the ADA's or the CDC's recommendation for dental treatment water. This is more important when some dental units are used less frequently than others within the same premises, where frequent replacement of low-grade antimicrobial does not occur in the lower-use units. Most manufacturers do not expect existing biofilm contamination to be removed prior to introducing the constantly present low-grade antimicrobial; therefore, the biofilm disruption or removal does not occur and a higher level of fluid replacement within the lines will be required as the active ingredient gets used-up or inactivated by the biofilm.

Some microbes could perish and others thrive in the presence of only low-grade antimicrobials leading possibly to growth of monocultures without periodic cleaning or decontamination with stronger germicides. The growth of monocultures could be due to less penetration of the weaker antimicrobial into the deeper levels of the biofilms and other inorganic contaminants.²⁷ Therefore, maintaining good treatment water quality or irrigant quality requires a combined effort of periodically shocking/treating the water system with an intermediate to high-level germicide or a proven biofilm cleaning/removal agent as well as physically treating the incoming municipal water, using a low-grade antimicrobial irrigant or a microfilter, the latter with or without endotoxin retention capabilities. Relying on antiretraction systems within the dental unit was ineffective with respect to cross contamination.^{38,39}

Personnel time required for cleaning or disinfection of the water system is a very important issue with respect to compliance when considering the use of germicides for periodically shocking/disrupting the biofilms and inorganic contaminants and use of an acceptable irrigant. Some low-grade antimicrobial devices that generate silver (Sterisil[®], Castle Rock, CO, USA) or iodine (Dentapure[®], River Falls WI, USA) can be set inline with the water system with the devices being replaced anywhere between 1 month to 1 year and have been shown to control planktonic microbes.^{64,65}

Our investigation supports the observation that a very consistent method in controlling microbial biofilms in water systems as well as providing dental treatment water of 'zero' or very low microbial counts can be accomplished using the autosteril device with TAED perborate for between-patient decontamination and sterile or decontaminated water as a coolant. The use concentration of TAED perborate in this study showed promise at least as an intermediate level hospital disinfectant. Table 2 demonstrates 1.43×10^{10} *M. smegmatis* kill, and Table 1 shows that common hospital infection organisms and microbes found in the oral cavity, such as *P. aeruginosa*, *E. coli*, *S. aureus*, *C. albicans*, were killed in less than 5 minutes of contact using standard laboratory tests. In addition to killing vegetative microorganisms, *B. subtilis* var niger 2.5×10^6 and *B. stearothermophilus* 2.5×10^5 were also killed in 5 minutes of contact (Table 3). This antimicrobial potency of TAED perborate as seen in laboratory tests also showed promise by killing/removing biofilms in the water lines as seen in the LSCM images in all treatment groups while the controls showed continued presence of biofilms (Figs 5 and 6).

Furthermore, the use concentration of TAED perborate was not corrosive to the metals used in the Autosteril system and the latter found to be an easy-to-use automated waterline system that could be disinfecting/cleaning the lines between patients while the barriers of the dental unit are being changed. In this study, we evaluated the efficacy of TAED perborate in both biofilm and treatment water contamination control, and the effective use of an engineering control, namely the Autosteril. Results from this study show the efficacy of the germicide and the device.

CONCLUSION

TAED perborate and utilization of sterile water or municipal water with 2 ppm of ClO₂ removed biofilms and deposits in the simulated dental unit water system. Heterotrophic plate counts were maintained within the 200 cfu/ml mark. Sample metals of autosteril challenged with TAED showed no visible corrosion. TAED perborate showed potential as an intermediate level, hospital disinfectant. The autosteril system was easy to use and effective in the control of dental unit waterline biofilms.

Decontamination dental unit water systems is very important as there has been a recent death reported due to exposure of a dental patient to *Legionella pneumophila*.⁶⁶

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