Title
Low Current Electrical Enhancement of a Dental Unit Waterline Cleaner - Effect on Planktonic Bacteria and Bacterial Biofilm in Simulated Dental Unit Waterline

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Low Current Electrical Enhancement of a Dental Unit Waterline Cleaner – Effect on Planktonic Bacteria and Bacterial Biofilm in Simulated Dental Unit Waterline

By

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THESIS

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Low Current Electrical Enhancement of a Dental Unit Waterline Cleaner – Effect on Planktonic Bacteria and Bacterial Biofilm in Simulated Dental Unit Waterline

Christina Gasper, DDS, MS

ABSTRACT

Purpose: The purpose of this investigation was to study the effect of application of low electric current in combination with dilute chlorine dioxide in removal of planktonic bacterial counts and bacterial biofilm from a simulated dental unit waterline (DUWL). Our hypothesis was that bioelectric effect would greatly enhance the efficacy of antimicrobials in removal of bacteria from DUWL. This study was to assess the effect on biofilms of naturally occurring multiple species from actual DUWL.

Materials and Methods: A model DUWL was set-up to simulate a dental unit. Test and control tubings were connected to a peristaltic pump and then to a 10L reservoir. A current was applied along the length of the test DUWL. The 10L carboy was filled with softwater (Arrowhead) and DUWL microorganisms recovered from the planktonic 3-way air-water syringe (AWS) flow of 5 contaminated DUWLs were inoculated to create a biofilm on the inner wall of the tubings. Every Monday and Thursday, samples were collected from the effluent at the ends of the test and control waterlines and plated. One sample from each waterline was collected and plated in triplicates. Low current (10 mA) was applied to the test tubing in both experiments. During the second experiment dilute chlorine dioxide was added to the water. At the end of each experiment, the waterlines were disassembled and tubing sections were assessed for counts of viable bacteria.

Results: In experiment 1 we compared the effect of electric current to no current. After current was applied to the test tubing, the only time the counts from the effluent of the

test sample ($2.8x10^5$ CFU/ml) were significantly different (lower) than the control sample ($4.1x10^5$ CFU/ml) was at day 38. Samples of biofilm bacteria after 28 days of current were collected from the barb-end and mid-section of the test and control tubing. The bacterial counts from the barb-end of the test tubing ($9.9x10^3 \pm 2.2x10^3$ CFU/cm²) were significantly lower (P=0.009) than those from the barb-end of the control tubing ($3.2x10^4 \pm 7.9x10^3$ CFU/cm^2. The bacterial counts from the mid-section of the test tubing ($3.0x10^4 \pm 2.6x10^3$ CFU/cm²) were also statistically lower (P=0.004) than the bacterial counts from the mid-section of the control tubing ($1.8x10^5 \pm 4.4x10^4$ CFU/cm^2). In experiment 2 we compared the effect of current in the presence of ClO_2 to current alone. Effluent samples collected from the test tubing fluctuated from 10^1 to 10^3 CFU/ml. Effluent samples from the control tubing fluctuated from 10^2 to 10^4 CFU/ml. The differences were statistically significant at days 32, 40, 53, and 56. Samples of biofilm bacteria after 28 days of current were collected from the barb-end and mid-section of the test and control tubings. The bacterial counts from the barb-end of the test tubing (0) were significantly less (P=0.004) than those at the barb-end of the control tubing ($1.0x10^4 \pm 3.1x10^3$ CFU/cm^2). Similarly, the bacterial counts from the mid-section of the test tubing (0) were significantly less (P=0.003) than those from the mid-section of the control tubing ($8.8x10^3 \pm 2.2x10^3$ CFU/cm^2)

Conclusion: The combination of dilute chlorine dioxide and low current was effective in eradicating bacterial biofilm from a simulated DUWL. Current alone was not effective in eradicating bacterial biofilm from a simulated DUWL.

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Introduction

Dental unit waterline (DUWL) contamination has become a concern in clinical dentistry. This concern arises from heterotrophic bacteria sloughed from established biofilms in dental unit waterlines. Heterotrophic organisms must ingest biomass to obtain their energy and nutrition. Heterotrophic bacteria, therefore, are largely responsible for the process of organic matter decomposition. Even though many species of these bacteria are abundant in the environment and are frequently part of normal oral flora, others are pathogenic (disease-causing) bacteria. A biofilm is an aggregate of such microorganisms in which cells tend to adhere to each other and to a surface. Bacteria sloughed from such biofilm can increase colony forming counts in water exiting DUWLs and expose patients to millions of potential pathogens during routine dental treatment and periodontal surgery.

The existence of contaminated water in dental units was first reported in 1963 in Great Britain by Dr. G.C. Blake [1]. What led Dr. Blake to investigate the quality of dental treatment water is not clear; however, his finding that large numbers of bacteria were present in aerosols and water reservoirs was later confirmed by other researchers during the last 47 years [2]. The susceptibility of the host, whether it's a patient or health care worker, and the pathogenicity of the organisms are the links over which we have the least control. For that reason, a lot of time, effort, and expense have been invested in reducing the number of organisms in the clinical environment.

Dr. G. C. Blake also was the first to test and report on the effectiveness of chemical germicides as a possible solution to the problem [1]. Since Blake's publication,

many research teams have not only described the existence of microbial contamination in dental water systems but also investigated ways to control it. Different methods and devices have been designed for DUWL treatment, which include waterline flushing [3], filtration [4], independent water reservoir [5], chemical treatment [6], and the use of antimicrobials [7]. In a study by Tanahashi *et al*. [8], water was circulated in a simulated dental unit water line with a small electric current. Destruction of biofilms by electrification was observed using scanning electron microscopy (SEM), in addition to deformation and hypertrophy of the bacteria. By naked eye observation, small pieces, which were possibly exfoliated biofilms, were detected in electrified water. A study by Blenkinsopp *et al*. [9], demonstrated that several industrial biocides exhibited enhanced killing action against *Pseudomonas aeruginosa* biofilms grown on stainless steel studs when a low electric current was applied.

The aim of this study was to investigate the efficacy of applying a low voltage and low amperage current together with antimicrobials in the removal of bacterial biofilms in DUWL. Our hypothesis is that current applied will greatly enhance the efficacy of an antimicrobial, in this case chlorine dioxide, in the removal of bacterial biofilms from a simulated DUWL.

Biofilm

A biofilm is an aggregate of microorganisms in which cells are stuck to each other and/or to a surface. Most biofilms are heterogenous (diverse) in species and morphology and are enveloped in a polysaccharide slime layer known as glycocalyx. The glyocalyx protects the organism within. Biofilms may form on a living or non-living surfaces and

represent a prevalent mode of microbial life in natural, industrial and hospital settings [10]. The sessile cells of a microorganisms growing in a biofilm are physiologically distinct from planktonic cells of the same organism. Planktonic cells are single-cells that may float or swim in a liquid medium. When a cell switches to the biofilm mode of growth, it undergoes a phenotypic shift in behavior [11].

There are five stages of biofilm development:

1. Initial attachment
2. Irreversible attachment
3. Maturation I
4. Maturation II
5. Dispersion

Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface initially through weak, reversible van der Waals forces. If not separated immediately from the surface, these microorganisms can anchor themselves more permanently using cell adhesion structures. The pioneer colonists help the arrival of other cells by providing more diverse adhesion sites and begin to build the matrix that holds the biofilm together. This becomes an irreversible attachment. It is during this colonization that the cells are able to communicate via quorum sensing. Quorum sensing is a method of coordinating activities among bacteria. Bacteria communicate with one another through the release of signaling molecules or autoinducers. When the signals

reach a certain concentration, a “quorum” is established. This cell-cell communication is called quorum sensing.

Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. Once the biofilm is established it may only change in shape and size [12].

Dispersal of cells from the biofilm is an essential stage of the biofilm lifecycle. Dispersal enables biofilms to spread and colonize new surfaces [12]. Enzymes that degrade the biofilm extracellular matrix may play a role in biofilm dispersal [13].

Biofilms are generally found on solid substrates submerged in some aqueous solution or exposed to it. Biofilms can contain many different types of microorganisms, e.g. bacteria, archaea, protozoa, fungi and algae. Biofilm bacteria apply chemical weapons in order to defend themselves against disinfectants and antibiotics, phagocytes and our immune system. When bacteria are alone and separated in the water, they can be easily destroyed. However, when they attach to a surface and join other bacteria, they are almost indestructible. Bacteria in biofilms are able to produce effective substances which they cannot produce alone [14].

The biofilm is held together by a matrix of excreted polymeric compounds called exopolysaccharide (EPS). This matrix protects the cells within the biofilm and facilitates communication among the organisms through biochemical signals. Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment is increased resistance to detergents and antibiotics. In some cases antibiotic resistance can be increased 1000 fold [15].

Armitage [16] described four possible mechanisms for bacterial biofilm antimicrobial resistance:

1. Failure to penetrate the biofilm
2. Neutralization or consumption of the drug
3. Presence of drug-resistant bacteria
4. Inability of the drug to affect "dormant" bacteria

It is possible that antimicrobials are not able to penetrate the biofilm matrix. It is also possible population-induced stress results in a biofilm bacterial phenotype that is resistant to antimicrobials [17]. Whiteley *et al.* [18] showed that when *Pseudomonas aeruginosa* in biofilms were exposed to high doses of tobramycin, there were alterations in gene expression for twenty genes. In the biofilm state, expression of a single gene in *P. aeruginosa* results in production of periplasmic glucans, which can protect the bacteria from foreign molecule [19]. Genes for resistance can easily be passed between organisms through horizontal gene transfer because of the close physical proximity of biofilm bacteria.

Lastly, bacteria deep within the biofilm may show reduced growth rates due to low availability of nutrients and resistance of the matrix to oxygen diffusion. These slow-growing or "dormant" bacteria may be unable to be affected by antibiotics and antimicrobials.

Dental unit water lines and biofilm formation

What puzzles most clinicians is how dental units can become so heavily contaminated when they are supplied by well-maintained municipal water systems. There are several contributing factors:

First, most treated drinking water contains minerals. Water described as "hard" is high in dissolved minerals, specifically calcium and magnesium. Hard water is not a health risk, but a nuisance because of mineral buildup on water-bearing surfaces [20].

Second, many materials used to deliver water to dental handpieces and air/water syringes provide excellent substrates for the initial attachment of bacteria and then the subsequent proliferation of biofilm. The adhesion of bacteria is promoted by the concentration of organic molecules from the treated water [2].

Third, fluids passing through narrow tubing assume a hydrodynamic pattern known as laminar flow. Frictional forces closer to the tubing lumen surface slow the movement of fluids until flow at the surface is stabilized, which creates an environment conductive to the formation of biofilm [21]. In such an environment biofilm can flourish and not get dislodged. Such laminar flow systems are one of the principal reasons that flushing of waterlines can eliminate planktonic microorganisms, but is not effective in removing biofilms [3].

Lastly, as the diameter of a cylinder- in this case, the waterline- decreases, an increasingly larger surface area to volume ratio becomes available for colonization. In most dental units, the total combined volume of waterline tubing is about 100mL.

Organisms in the waterlines

The majority of microbes living in the biofilm communities are gram-negative water bacteria of the same varieties that survive in small numbers in municipal water systems [22]. Because most of the organisms recovered in dental treatment water are gram-negative bacteria, some researchers have speculated about the presence of bacterial endotoxins in that water. Endodoxins are lipopolysaccharides in the cell wall of gram-negative bacteria that can produce a wide range of physiological responses, such as localized inflammation, fever and even septic shock [23]. Putnins *et al.* [24] reported that water from dental unit waterlines may have endotoxin levels as high as 500 endotoxin units/mL (EU/mL). In comparison, the United States Pharmacopeia, or USP, has a limit for sterile water endotoxin at only 0.25 EU/mL.. Mathew *et al*. [23, 25] found that there was significant decrease in lung function in 15 percent of 57 children aged 6-18 years who underwent dental treatment. Even though the authors did not suggest that there is an association with contaminants from dental waterlines, bacterial endotoxin is a recognized trigger for asthma. More research is necessary to clarify if such an association actually exists.

One important gram-negative pathogen found in the dental unit waterlines is *Pseudomonas aeruginosa* which is associated with a wide range of opportunistic infections. Martin *et al.* [26] published a case report where *P. aeruginosa* was found in wound infection in two immunocompromised patients. The organism that was isolated from the infected sites was matched by pycocyanin typing to the bacteria that were recovered from the dental unit used to treat the patients.

Other prominent waterline bacteria are *Klebsiella, Acinetobacter,* and *Enterobacter.* Slots *et al.* [27-29] have reported the presence of these bacteria in severe and refractory periodontitis.

Even though most investigations of dental water have focused largely on bacterial contamination, as biofilms mature, they also provide a hospitable environment for fungi, protozoa and other organisms that survive in drinking water systems. In an immunocompetent host these organisms rarely have any pathogenic potential, however, some protozoa serve as hosts for proliferation of parasitic bacteria, such as *Cladosporium* and *Legionella* [30-31]

Aquatic nontuberculous *Mycobacterium* species associated with pulmonary disease and opportunistic wound infections also have been recovered in dental unit water [32].

Lastly, organisms found in the oral flora can be found in the dental water lines if the water is retracted. However, these organisms usually are well-adapted to the warm, nutrient-rich oral environment and do not compete well with the water flora in the waterline biofilms [22].

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative, rod-shaped bacterium which can cause disease in animals, humans, and plants. It is found in soil, water, skin flora and most man-made environments throughout the world and has the ability to colonize many natural and artificial environments. Even though it is classified as an aerobic organism,

P. aeruginosa is considered by many as a facultative anaerobe, as it is well adapted to proliferate in conditions of partial or total oxygen depletion. It is an opportunistic pathogen that infects damaged tissues and typically causes disease in humans when host defenses are compromised. If such colonization occurs in critical body organs such as the lungs, the urinary tract, and kidneys, the results can be fatal [33]. Adaptation to microaerobic or anaerobic environments is essential for certain lifestyles of *P. aeruginosa*, for example, during lung infection in cystic fibrosis patients, where thick layers of alginate matrix surrounding bacterial cells can limit diffusion of oxygen [34-38].

P. aeruginosa secretes a variety of pigments, including fluorescein, pyocyanin, and pyorubin. It is generally identified by its pearlescent appearance and grape-like or tortilla-like odor *in vitro*. However, definitive clinical identification includes its ability to grow at 42ºC and identifying the production of both pyocyanin and fluorescein, the two pigments of *P. aeruginosa*, which impart the blue-green characteristic color seen in laboratory cultures of the species [39].

Cell-surface polysaccharides play diverse roles in the bacterial lifestyle. They serve as a barrier between the cell wall and the environment, mediate host-pathogen interactions and are a structural component of biofilms. These polysaccharides are synthesized from nucleotide-activated precursors, and, in most cases all the enzymes necessary for biosynthesis, assembly, and transport of the completed polymer are encoded by genes organized in dedicated clusters within the genome of the organisms. Lipopolysaccharide is one of the most important cell-surface polysaccharides, as it plays

a key structural role in outer membrane integrity, as well as being an important mediator of host-pathogen interactions [40].

One of the major virulence factors of P. aeruginosa is its ability to produce a thick, exopolysaccharide matrix and to establish a complex biofilm. Chronic opportunistic infections caused by *P. aeruginosa* often cannot be treated effectively with traditional antibiotics. Biofilms seem to protect these bacteria from adverse environmental factors, making them resistant to antibiotic therapy. This low susceptibility can be attributed to the presence of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes as well as to the low permeability of the bacterial cellular envelopes. In addition to this intrinsic resistance, *Pseudomonas aeruginosa* can easily acquire resistance either by mutation in chromosomally-encoded genes or by the horizontal transfer of antibiotic resistance genes [41].

Recommendations to decrease contamination of DUWL

The Dental Board of California requires a course in infection control for license renewal of DDS, RDH, and RDA. There are also numerous recommendations to maintain dental treatment water at least at the same level as the drinking water quality.

In 1993, the Centers for Disease Control urged dentists to install and maintain anti-retraction valves to keep oral fluids from being drawn into DUWLs. They also recommended that dental waterlines need to be flushed daily for several minutes and for 20 to 30 seconds between patients to eliminate any oral fluids that may have entered the lines during treatment. For surgical procedures, such as cutting bone, they urged the dentists to use sterile solutions only [42].

In 1996, American Dental Association came up with the guideline that dental water should not contain more than 200 CFU/mL of heterotrophic bacteria [43]. That same year, the Organization for Safety and Asepsis Procedures issued a statement supporting both the CDC and the ADA guidelines. Furthermore, the statement included additional guidelines on controlling waterline contaminants and the use of sterile irrigants in surgery [44].

In 2003, the CDC recommended that during nonsurgical procedures dentists adhere to the EPA standards for drinking water at less than 500 CFU/ml for heterotrophic bacteria, and for surgical procedures, the use of sterile water was recommended [45].

Ways to improve and maintain quality of water in DUWLs

Over the last 47 years studies of waterline treatment have investigated different chemical agents that are able to either inactivate microorganisms, induce detachment of biofilms, or both. Other studies examined the flushing of waterlines and the use of filters. A number of products have been developed on the basis of this research. Most strategies to improve the quality of water provided by conventional dental units employ the use of chemical treatment either alone or in combination with other technologies, including microfiltration. Another alternative is to entirely bypass the conventional dental water delivery system and use either autoclavable or disposable pathways.

Waterline flushing

Mechanical flushing alone is not an efficient way to control waterline contamination, and it is not well-supported by the scientific literature [3, 46-49].

Flushing alone can temporarily reduce the number of suspended organisms, however, its effect on adherent biofilm is less than predictable – bacterial aggregates breaking free from the biofilm have been shown to re-contaminate dental unit water during the course of subsequent clinical treatment [3]. The only time that flushing may be useful is between patients to remove materials that may have entered the water system during treatment.

Independent reservoirs

By using independent reservoirs and, therefore, isolating the dental unit from the municipal water supply, the clinician is able to control the quality of water that is introduced into the system. However, without treatment with chemical agents to inactivate or detach biofilm, these reservoirs alone cannot improve the quality of treatment water.

Chemical Treatment

An ideal chemical agent for use in DUWL would be inexpensive and easy to use. It should be bactericidal but not toxic. It should detach biofilm and discourage subsequent reformation and also protect the dental unit components from corrosion and degradation. Unfortunately, such an agent does not exist. There are, however, products that have some of these desired characteristics.

Chemicals may be introduced either intermittently or continuously. If the chemicals are introduced intermittently, the usual practice is to deliver the agent for a specific period of time and frequency. Most of these agents use potentially biocidal

concentrations of germicide that also may remove biofilm. One major advantage of intermittent chemical use is that the active agent is purged from the system before patient treatment. One disadvantage is the potential for surviving biofilm organisms to rebound between treatments. Additional disadvantages are potential staff exposure to chemicals and adverse impact on metal, rubber and synthetic dental unit components.

On the other hand, continuous treatment uses either lower concentrations of potentially biocidal agents or biostatic substances in the water used for patient treatment. This is similar to the way municipal water systems rely on residual chlorine to maintain the safety of drinking water. Although continuous treatment offers less potential for recolonization of waterlines, it still may damage equipment. Also, since the chemical agent is always present and may be aerosolized, the effects of chronic exposure on the health care worker must be considered. Enamel and dentin bond strength of dental adhesive material also may be affected [50-51].

Chlorine compounds have been studied more extensively than any other class of chemical agents intended to control or eliminate biofilm in dental unit water systems. Sodium hypochlorite (usually in the form of diluted household bleach in varying concentrations) has been used by most investigators [4, 48, 52-56], however, chloramines T [57] and elemental chlorine [58] also have been evaluated. Sodium hypochlorite is a potent germicide with broad-spectrum antimicrobial action which has shown promising results as a means to improve the quality of treatment water in numerous clinical trials. Although some investigators have voiced concern about the formation of potentially carcinogenic disinfectant byproducts, such as tri-halomethanes, as a result of chlorine's reacting with biofilm organic polymers, the use of intermittent protocols minimizes the

exposure risk for both the patients and the staff. However, the use of chlorinated water in the presence of residual biofilm may increase this risk. Although Karpay and colleagues [52] detected tri-halomethanes with rechlorinated tap water when three parts per million free chlorine was used in independent reservoirs, none of their samples exceeded EPA limits.

Sodium hypochlorite, as an oxidizing agent, can corrode metal components and damage rubber or synthetics. Nevertheless, these effects can be limited by following the manufacturer's recommendations [54].

Wirthlin and colleagues [59] reported significant reductions in hetertrophic bacterial counts when DUWLs were treated with two types of chlorine dioxide solutions. The same study showed reductions in some scanning electron microscopy images of biofilm coverage of the DUWL after 10 days – none of the SEM findings were statistically significant. Wu and Wirthlin [60] performed a 6-month trial of chlorine dioxide treatment of DUWL biofilms using a "shock" treatment of chlorine dioxide followed by continuous use of a 10:1 dilute chlorine dioxide solution in the dental unit reservoir, and compared results to a tap water control. The initial shock treatment reduced planktonic bacterial counts to zero overnight. Using SEM, significant reductions in biofilm coverage were shown between the control and experimental groups, and within the experimental group after a period of 6 months. Although the biofilms were significantly reduced by chlorine dioxide, they were not completely eliminated.

Devices to introduce chemical agents into the water system automatically also are available. This approach potentially could reduce the effect of compliance variables on clinical success.

Whatever method is used to control or eliminate biofilm in the water system, the quality of unfiltered output can be no better than that of the water entering the system. Using water of known microbiological quality is the best way to eliminate uncertainty and ensure consistent delivery of high-quality treatment water. For example, bottled sterile water for irrigation is not only free of viable microorganisms but also has very low levels of minerals and organic compounds that can encourage re-establishment of biofilm. However, unless the water pathway is sterile as well, no conventional dental unit can deliver sterile irrigating solution.

Filters

Micropore membrane filters are used to remove microorganisms from water and solutions in a wide range of medical and industrial applications. Filtration is even used to sterilize heat-labile sterile pharmaceutical solutions [61]. However, relatively few studies in peer-reviewed journals have evaluated the efficacy of filtration in dental units. Studies conducted to date suggest that filters can produce water that meets or exceeds the 200CFU/ml goal established by the ADA for nonsurgical procedures when used according to the manufacturer's recommendations. Two independent evaluations of microfiltered water (0.22-um filters) used in dentistry showed that 80 percent of output water samples were free of bacteria, and none of the remaining specimens exceeded 200 CFU/ml of heterotrophic plate count bacteria [61-62]. These studies also found that there were fewer numbers of organisms observed by SEM in postfiltration tubing sections than in prefiltration sections [61-62]. A research group led my Mayo and Brown found that there were no detectable organisms in water samples taken immediately downstream

from 0.2-um proprietary filters. However, when they collected samples after increasing the distance at which the filter was placed from the air water syringe, they noticed that the levels of bacteria in effluent water increased. They concluded that the latter observation was due to the formation of biofilm in the post-filtration waterlines [63].

There are many advantages to filters such as reduction or elimination of reliance on chemicals, less potential for damage to dental units and possible staff exposure to chemical residues. Installation can also be done at minimal cost and requires only the placement of filter housing on water-bearing lines. In addition, the units may remain connected to the municipal water supply. While the advantages are great, and filters have been shown to be effective in removing suspended bacteria from dental treatment water, they will not have any effect on the biofilm that will continue to build up in pre-filtration segments of waterlines, nor in DUWL downstream from filter location, unless some other treatment to remove them is performed at the same time. Persistence of biofilm in the dental unit water system carries many risks, such as biofouling, clogging and elution of endotoxin in treatment water.

When considering the use of filters, one important point to remember is that even when water produced by filtration in the dental clinic is bacteria-free, since conventional dental units cannot be sterilized, they cannot reliably provide sterile water. Therefore, filtered or chemically treated water should not be used in place of sterile water in surgical procedures.

Electrical enhancement of antimicrobials

The bioelectric effect, in which electric fields are used to enhance the efficacy of biocides and antibiotics in killing biofilm bacteria, has been shown to reduce the very high concentrations of these antibacterial agents needed to kill biofilm bacteria to levels very close to those needed to kill planktonic (floating) bacteria of the same species. A study by Barranco, *et al.* revealed that when currents were applied to electrodes incorporated into the plates, zones of inhibition for plated bacteria were seen [64]. Another research group studied 24-hour old *P. aeruginosa* biofilms growing on stainless steel surfaces in a modified Robbins device. The group reported decreases in CFUs when the biofilms were treated with combinations of low current and industrial biocides [9]. Several *in vitro* studies demonstrated some degree if inhibition of bacterial growth in urinary and intravenous catheters when low current was applied to the wires incorporated into the catheters [65-67].

A study by Tanahashi *et al.* [8], reported on the effect of a small electric current applied to dental unit waterlines. In this study, they developed a simulated dental waterline where the fluid was cycled through the line with the aid of a pump. An electric current generator was incorporated into the loop, which created a current potential downstream from the pump. The results showed that there was an initial decrease in CFUs from effluent samples during the first week. This initial decrease was then followed by a gradual increase in CFU counts for the remaining five weeks of the study. The group also looked at the SEM images of some of the tubing sections – the observations suggested that there was an effect on the matrix or surface of bacteria, however, this was not quantified or tested through culture data.

Hypothesis

The purpose of this project is to study the efficacy of applying a low voltage and current together with antimicrobials in removal of bacterial biofilms in DUWL. Our hypothesis is that bioelectric effect will greatly enhance the efficacy of antimicrobials in removal of bacteria from DUWL. The theses of Gunstream and Liu [68-69] demonstrated the effect on a single species of *Pseudomonas aeruginosa.* This study is to assess the effect on biofilms of multiple species from actual DUWL.

Materials and Methods

Model DUWL Set-up

A model DUWL was designed and constructed to simulate an aDec dental unit water line (Figure 1). The water line simulated is from the water reservoir to the 3-way air-water syringe(AWS) (a 15´distance). The test and control experiments were conducted at the same time by utilizing a parallel system- one length of tubing was used for the test and [the] another for the control. Three 5-foot sections of 1/8" outside diameter polyurethane tubing (#036.005.04, aDec, Newberg, OR) were used for each tubing. The three sections were connected with three "barb" fittings similar to those found in the dental unit. The tubing was in a new, clean condition.

The two parallel test and control tubings were connected by a Y-fitting to a neoprene tube. The neoprene tube was connected to a peristaltic pump (Manostat 72-410-014, Cole-Palmer, Vernon Hills, IL), which was connected to a 10-liter carboy reservoir. The Y-fitting, neoprene tube connections, and the peristaltic pump tubing were autoclaved prior to set-up.

A platinum wire (15' long, 0.2mm dia.) was autoclaved prior to set-up. It was used as one electrode and was inserted into the test tubing through a 20ga. needle in a 3 inch long section from an I.V. line injection port. The wire was continued along the length of the test water line trying not to introduce any kinks or nicks in its surface. The needle was then removed allowing the rubber of the port to seal itself against the wire.

A standard dental unit uses straight 1/16" plated brass barb fittings. However, in the model DUWL, the barbed fitting connectors were modified. Two 10-32 x ¼"

hex/male- 1/16" barb Nylon plastic fittings (#101K, E-Z Air, C&OR, Rogue River, OR) were connected back-to-back in a metal sleeve with 10-32 female openings at each end (#MF-1010-316, Beswick Engineering Co. Inc., Greenland, NH). By doing this, a 1/16" gap of metal was created inside the sleeve between the ends of the barb fittings to serve as opposite electrodes to the platinum wire electrode in the test system. This way the wire was able to pass through the barb fittings without shorting out. A one inch length of electrical wire was silver-soldered to the outside of the sleeve for connection to the current source. Both the control and the test tubings had similar barb fittings, however, no current was applied in the control water line. The metal sleeves were autoclaved prior to set-up, and the Nylon male barb fittings were used in new clean condition.

In order to simulate daily use of a dental operatory unit, water was run with a flow rate of 25ml/min continuously for 5 minutes every 30 minutes except for the first run, which was 2 minutes. The capacity of the 15' of tubing is ~30ml so it was emptied with each run. It was run for 7 hours per day (9AM to 5PM, no runs from 12 to 1), and 5 days per week by a peristaltic pump (Manostat 72-410-014), controlled by a programmable timer (ChronTrol XT, ChronTrol Corp., San Diego, CA). The flow rate and effluent output were determined primarily by the internal diameter of the tubing used in the peristaltic pump, which runs at 14rpm. The action of the peristaltic pump introduces an intermittent fluid shear as in prototype DUWL. There was an outflow of about 125ml from the two tubings each time the pump ran – every 30 minutes for 5 minutes. Since the first outflow of the day was slightly less, water samples for testing were taken after 09:30AM. The 10L carboy was refilled every 3-4 days.

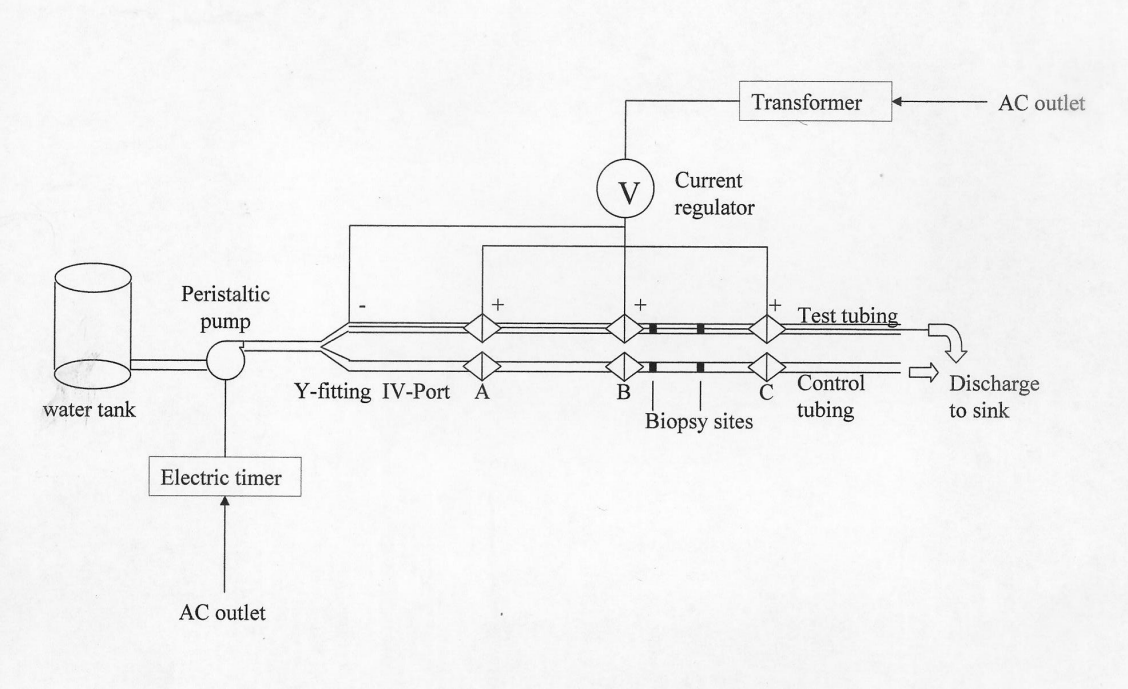

Figure 1. Model DUWL set-up.

Experiment 1

Microorganisms were recovered from the planktonic AWS flow of 5 contaminated DUWL over several weeks. The 10L carboy was filled with tap water and those DUWL microorganisms were inoculated to create a biofilm on the inner wall of the tubings; and the system was turned on.

Inoculated water was initially pumped through the system. The aim was to create a biofilm on the internal wall of the DUWL tubing. Samples (5ml) were collected from the effluent at the ends of the waterlines. Samples were serially diluted, plated on R2A agar plates in triplicate, and were cultured at room temperature for 3-4 days. The resulting bacterial colonies were then counted at 12X with a dissecting microscope. A

steady state was assumed when two sample counts were within a log of one another. The biofilm required about 5-7 days to reach steady state. Establishing steady state was necessary because bacteria in earlier phases are more sensitive to anti-microbial agents. In a steady state our model better resembled conditions in DUWL. At this point the experiment was started.

A current regulator was designed similar to that of Mims but with an added potentiometer. The current regulator had an input of 15V DC from the wall transformer, protected by a 1A fuse. The input was directed to three current regulators, one for each barb fitting anode. In order to limit the current to no more than 400mA, the current regulators used a 7812T 12V positive voltage regulator, and a 30 ohm fixed resistor. The current regulator was applying a voltage of 12V DC and a current of 10 milliamperes. It was connected to the platinum wire and to the barb fitting sleeves via alligator clips. The platinum wire was the cathode and the barb fittings were the anodes.

The current to each anode was set by manipulation of the potentiometer and was verified with an ammeter. This was done by connecting the ammeter between the leads from current regulator with test lead clips until the current was set correctly, then the ammeter and the test clips were removed and current regulator was connected to the barbed fitting sleeve anodes and the platinum wire cathode by its alligator clips

The carboy was replaced after total viable counts (TVC) showed a satisfactory inoculation. A new carboy was autoclaved and was filled with bottled Arrowhead brand water and run until sample counts indicated achieving a steady state. The electric current was then turned on to start the test.

The effect on planktonic bacteria was assessed by collecting 5 ml samples from both tubings at the start and at different time intervals. Those samples were then serially diluted and plated on R2 agar plates. The plates were incubated aerobically at room temperature for three-four days. Colonies were then counted at 12x magnification. Results were expressed as CFU/ml.

At the termination of the experiment the effect on the sessile biofilm was assessed by sectioning 2.1cm long samples of the tubing at different locations. The tubings were then split and the biofilm on the lumen scraped into 1ml sterile saline. The scrapings and tubing sections were vortexed, sonicated and homogenized. The samples were then serially diluted for plate counts. One tubing sample was taken near the barb fitting anode and another from the middle of a 5' tubing section. Results were expressed as CFU/cm² because the 2.1 cm long tubing has a lumen surface area of 1 cm².

The activity of the experimental current was assessed by testing the water samples from the test and control effluent. The water was tested for pH and oxidation-reduction potential (ORP) by meter electrodes (Oyster 10, Extech, Waltham, MA). The temperature of the barb fitting metal sleeves was measured by an infrared-sensing thermometer.

Experiment 2

Both the set up and the sequence of this trial were the same as those for experiment 1 and 2. The exception was the introduction of a phosphate-buffered, 0.1% chlorine dioxide dental unit waterline cleaner (MicroClear, Rowpar Pharmaceuticals, Inc., Scottsdale, AZ) at the same time as the current was started. This solution was used at a 10:1 dilution (1 part chlorine dioxide and 10 parts Arrowhead water) to see if the

antimicrobial effect was enhanced by the electrification. The samples were plated in triplicate to allow the calculation of mean ± variance.

Analysis of Data

Data were entered onto a standardized form. Total viable counts (TVC) were compared between the control and experimental groups to look for differences at all time points. Comparison of counts between test and control at each sampling day was by unpaired Student's *t*-test with $\alpha=0.05$.

Results

Experiment 1

In the 1st experiment, biofilm was established and low current was applied. Water collected from dental units was passed through the test and control tubings and samples were collected every Monday and Thursday morning for 14 days (Table 1).

Table 1: Cultivable effluent bacteria (CFU/ml) from samples collected when dental unit inoculated water was flushed though control and test tubings
CFU=colony forming units

Day	CFU Test Current	Mean	CFU Control Current	Mean
3	**Off**	**$1.5x10^7$**	**Off**	**$2.1x10^7$**
7	**Off**	**$2.3x10^6$**	**Off**	**$2.5x10^6$**
10	**Off**	**$4.2x10^6$**	**Off**	**$3.6x10^6$**
14	**Off**	**$4.3x10^6$**	**Off**	**$3.8x10^6$**

Inoculated dental unit water was switched to Arrowhead brand water on day 17. Once the steady state was established, on day 35, and the counts from both tubings were within one log difference in terms of the bacterial counts, the electricity was turned on and applied to the test setup (Table 2).

Table 2: Cultivable effluent bacteria (CFU/ml) from samples collected when Arrowhead water was flushed though control and test tubings

Day	CFU Test Current	Mean	CFU Control Current	Mean
17	**Off**	**$3.7x10^5$**	**Off**	**$6.2x10^5$**
21	**Off**	**$1.0x10^6$**	**Off**	**$6.1x10^6$**
24	**Off**	**$7.0x10^5$**	**Off**	**$1.5x10^6$**
29	**Off**	**$3.6x10^5$**	**Off**	**$6.0x10^5$**
31	**Off**	**$3.6x10^5$**	**Off**	**$4.4x10^5$**
35	**Off**	**$3.0x10^5$**	**Off**	**$5.7x10^6$**

A low current of 10mA was applied to the test tubing continuously for 24 hours per day, 7 days per week on an established biofilm. Samples were regularly taken twice per week and showed no significant changes from the samples taken prior to the application of low-current. The only time the counts from the test sample were significantly different (lower) than the control sample, was at day 38. That day the counts were $2.8x10^5$ CFU/ml vs. $4.1x10^5$ CFU/ml (Table 3).

Table 3: Cultivable effluent bacteria (CFU/ml) from samples collected when current was applied to the test system during working hours and after biofilm formation. Standard deviation (SD) was based on cultivating bacteria in triplicates form a single dilution.

Day	CFU Test			CFU Control		
	Current	Mean	SD	Current	Mean	SD
*38	On	$2.8x10^5$	$2.1x10^4$	Off	$4.1x10^5$	$6.0x10^4$
42	On	$2.3x10^5$	$7.5x10^4$	Off	$3.0x10^5$	$2.0x10^5$
45	On	$3.2x10^5$	$8.1x10^4$	Off	$4.0x10^5$	$4.0x10^4$
49	On	$3.5x10^5$	$9.5x10^4$	Off	$2.1x10^5$	$2.1x10^4$
52	On	$3.3x10^5$	$2.5x10^4$	Off	$4.7x10^5$	$9.1x10^4$
56	On	$2.6x10^5$	$6.1x10^4$	Off	$2.0x10^5$	$3.2x10^4$
59	On	$4.2x10^5$	$1.0x10^5$	Off	$3.2x10^5$	$3.1x10^4$
63	On	$2.7x10^5$	$5.6x10^4$	Off	$2.2x10^5$	$2.3x10^4$

* Statistically significant at $P<0.05$

The pH, oxidation-reduction potential (ORP), and temperature readings were taken throughout the experiment each time a sample was collected. The pH readings of both the test and the control samples remained similar but varied from 6.90 to 7.77. The ORP readings fluctuated between 258 and 511 for the test samples and between 305 and 504 for the control samples. The ORP readings were declining for both samples without any significant difference between the two (Table 4). Every time new jug of arrowhead water was used to fill up the carboy, a sample was collected, pH and ORP reading were noted, and the sample was plated. The pH range was 6.92 to 7.2, and the ORP range was

305 to 482. Arrowhead CFU's/ml fluctuated from 610 to 3600 (Table 5). A couple of times during the experiment we also measured the current at the barb-fitting- 3 readings were recorded and varied between 0.7μA and 176μA (Table 6).

Table 4: pH, ORP (oxidation-reduction potential), and Temperature readings
T=test, C=control

Day	pH (T/C)	ORP (T/C)	Temperature (T/C)
3	**7.7/7.4**	**493/464**	**73/73.3**
7	**7.8/7.4**	**507/491**	**73.9/73.6**
10	**7.7/7.4**	**508/495**	**73.2/73.3**
14	**7.5/7.1**	**487/504**	**74.1/73.9**
17	**7.2/7.1**	**511/497**	**73.9/73.6**
21	**7.8/7.5**	**493/477**	**73.7/73.6**
24	**7.3/7.3**	**456/455**	**74.6/74.8**
29	**7.0/6.9**	**436/424**	**73.1/73.5**
31	**7.0/7.0**	**434/424**	**73.2/73.4**
***35**	**7.3/7.1**	**350/350**	**73.3/74.2**
38	**7.0/7.0**	**303/325**	**73.3/73.7**
42	**7.2/7.2**	**266/301**	**73.3/73.3**
45	**7.0/7.1**	**288/306**	**73.3/73.1**
49	**7.1/7.1**	**263/300**	**73.1/73.1**
52	**7.1/7.1**	**258/305**	**73.6/73.4**
56	**7.0/7.0**	**241/304**	**73.4/73.3**
59	**6.9/6.9**	**242/306**	**73.5/73.5**
63	**7.0/7.2**	**239/305**	**73.5/73.4**

*** Turned on current**

Table 5: pH, ORP, and CFUs (CFU/ml) from Arrowhead samples

Day	pH	ORP	CFU
17	**Not measured**	**Not measured**	**$3.2x10^4$**
21	**Not measured**	**Not measured**	**$3.6x10^4$**
24	**7.2**	**482**	**Not plated**
35	**6.9**	**365**	**$4.2x10^4$**
42	**7.0**	**318**	**$6.1x10^2$**
49	**7.0**	**305**	**$1.3x10^4$**
56	**7.0**	**310**	**$1.2x10^5$**
63	**7.0**	**305**	**$3.5x10^5$**

Table 6: Current readings at the barb-fittings

Day	Barb-fitting 1 (µA)	Barb fitting 2 (µA)	Barb fitting 3 (µA)
35	**74**	**156**	**109**
42	**11.1**	**0.7**	**141**
49	**52.3**	**43.7**	**125.4**
56	**176**	**55**	**83**
63	**17.6**	**39.4**	**7.5**

On day 63, after the last effluent water samples were collected, the apparatus was disassembled. Samples of biofilm bacteria after 28 days of current were collected from the barb-end and mid-section of the 5' test and control tubings. The bacterial counts from the barb-end of the test tubing were $9.9x10^3 \pm 2.2x10^3$ CFU/cm². The bacterial counts from the barb-end of the control tubing were $3.2x10^4 \pm 7.9x10^3$ CFU/cm^2 (P=0.009) (Figure 1). The bacterial counts from the control tubing were at least one log higher when compared to the ones from the test tubing – the findings were statistically significant.

When analyzing at the counts from the mid-sections of the tubings, the results were also significant. The bacterial counts from the test tubing were $3.0x10^4 \pm 2.6x10^3$ CFU/cm² and the bacterial counts from the control tubing were $1.8x10^5 \pm 4.4x10^4$ CFU/cm^2 (P=0.004) (Figure 2).

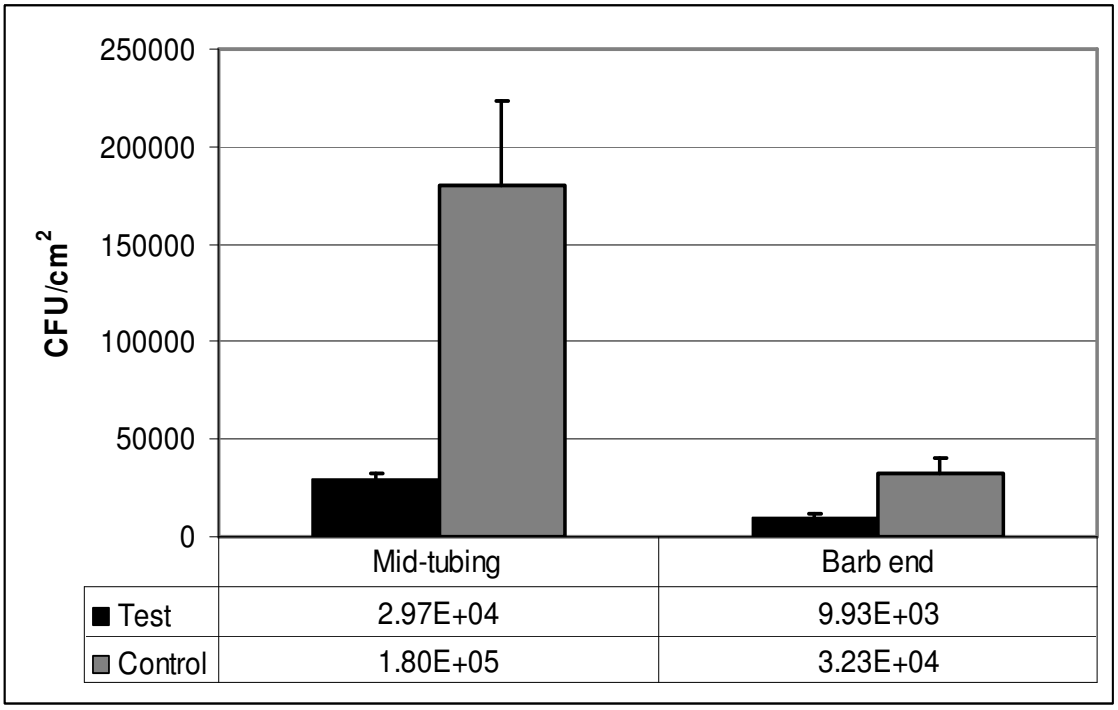

Figure 2. Cultivable biofilm bacteria counts (CFU/cm²) when current alone was applied to the test system for 28 days after steady state was established. No current was applied to the control. Error bars represent standard deviation from the mean.

Experiment 2

In the second experiment, in addition to applying current of 10 mA on the test tubing, a chlorine dioxide solution (MicroClear, Rowpar Pharmaceuticals, Inc., Scottsdale, AZ) diluted 10:1 was used in both the test and the control. First, just as in experiment 1, inoculated dental unit water was circulated for 14 days (Table 7).

Table 7: Cultivable effluent bacteria (CFU/ml) from samples collected when dental unit inoculated water was flushed though control and test tubings

Day	CFU Test Current	Mean	CFU Control Current	Mean
4	**Off**	**$2.1x10^7$**	**Off**	**$2.4x10^7$**
7	**Off**	**$2.5x10^7$**	**Off**	**$2.6x10^7$**
10	**Off**	**$2.3x10^7$**	**Off**	**$2.1x10^7$**
14	**Off**	**$4.0x10^7$**	**Off**	**$3.9x10^7$**

Inoculated dental unit water was switched to Arrowhead brand water on day 18. Once the steady state was established, on day 32 (Table 8), and the counts from both tubings were within one log difference in terms of the bacterial counts, the reservoir was changed to 0.01% chlorine dioxide and electricity was turned on and applied to the test tubing.

Table 8: Cultivable effluent bacteria (CFU/ml) from samples collected when Arrowhead water was flushed though control and test tubings

Day	CFU Test Current	Mean	CFU Control Current	Mean
18	**Off**	**$2.9x10^5$**	**Off**	**$1.7x10^5$**
21	**Off**	**$4.5x10^5$**	**Off**	**$4.2x10^5$**
25	**Off**	**$5.3x10^5$**	**Off**	**$3.3x10^5$**
28	**Off**	**$4.9x10^5$**	**Off**	**$3.1x10^5$**
32	**Off**	**$3.9x10^5$**	**Off**	**$5.7x10^5$**

When the steady-state biofilm was established, the mean counts from the effluent were $3.9x10^5$ CFU/ml (test) and $5.7x10^5$ CFU/ml (control). The chlorine dioxide solution was added to the carboy and was used in both the test and the control lines in addition to applying current of 10 mA on the test tubing only. The current was of alternating polarity with a cycle time of 70 seconds. Four hours after the current was turned on, samples were

collected again – the mean counts from the effluent were $1.6x10^2 \pm 1.2x10^2$ CFU/ml (test) and $1.7x10^4 \pm 3.6x10^3$ CFU/ml (control). This difference was statistically significant (Table 6). Effluent samples collected from the test samples fluctuated from 10^1 to 10^3 CFU/ml. Effluent samples collected from the control samples fluctuated from 10^2 to 10^4 CFU/ml. The differences were statistically significant at days 32, 40, 53, and 56 (Table 9).

Table 9: Cultivable effluent bacteria (CFU/ml) from samples collected when chlorine dioxide solution was applied to the test and control system and low current was applied to the test system continuously after biofilm formation. Standard deviation (SD) was based on cultivating bacteria in triplicates form a single dilution.

Day	CFU Test			CFU Control		
	Current	Mean	SD	Current	Mean	SD
†*32	On	$1.6x10^2$	$1.2x10^2$	Off	$1.7x10^4$	3.710^3
35	On	$1.4x10^3$	$3.8x10^2$	Off	$1.0x10^4$	$1.6x10^4$
*40	On	$4.6x10^1$	$3.0x10^1$	Off	$9.0x10^2$	$4.4x10^2$
42	On	$2.9x10^2$	$3.0x10^1$	Off	$2.9x10^3$	$1.7x10^3$
46	On	$2.6x10^1$	$1.5x10^1$	Off	$3.5x10^3$	$5.8x10^3$
49	On	$8.5x10^2$	$5.8x10^2$	Off	$9.4x10^3$	$5.8x10^3$
*53	On	$5.4x10^2$	$1.6x10^2$	Off	$9.6x10^3$	$1.5x10^3$
*56	On	$2.7x10^2$	$7.5x10^1$	Off	$8.2x10^3$	$2.8x10^3$
60	On	$5.5x10^2$	$2.9x10^2$	Off	$9.0x10^2$	$2.0x10^1$

† Four hours after turning on current
* Statistically significant at $P<0.05$

The pH, oxidation-reduction potential (ORP), and temperature readings were taken throughout the experiment each time a sample was collected. The pH readings of both the test and the control samples remained similar but varied from 6.9 to 7.7. The ORP readings fluctuated between 207 and 639 for the test samples and between 224 and 638 for the control samples. The ORP readings were increasing for both samples without any significant difference between the two (Table 10). Every time new jug of arrowhead water was used to fill up the carboy, a sample was collected, pH and ORP reading were

noted, and the sample was plated. The pH range was 6.5 to 6.8, and the ORP range was 253-568. Arrowhead CFU's/ml fluctuated between $7.8x10^3$ and $8.1x10^4$ (Table 11). A couple of times during the experiment we also measured the current at the barb-fitting- 3 readings were recorded and varied between 21.5μA and 170.4μA (Table 12).

Table 10: pH, ORP (oxidation-reduction potential), and Temperature readings
T=test, C=control

Day	pH (T/C)	ORP (T/C)	Temperature (T/C)
4	**7.5/7.7**	**207/225**	**74.4/73.4**
7	**7.1/7.0**	**231/239**	**73.2/72.4**
10	**7.1/7.2**	**213/229**	**72.5/72.6**
14	**6.9/7.0**	**236/224**	**74.9/74**
18	**7.1/7.0**	**235/247**	**73.7/74.2**
21	**6.9/7.0**	**238/247**	**74.1/72.8**
25	**7.0/7.1**	**248/237**	**73.3/72.9**
28	**6.9/6.8**	**312/312**	**74/73.7**
***32**	**7.2/7.3**	**310/298**	**74.9/74.2**
35	**7.1/7.2**	**545/532**	**73.6/73.9**
40	**7.0/7.0**	**587/600**	**74/73.2**
42	**7.0/7.1**	**632/638**	**72.6/72.8**
46	**7.0/7.2**	**639/629**	**76.4/76.4**
49	**7.1/7.2**	**631/621**	**74.8/74.9**
53	**7.2/7.2**	**610/627**	**75.2/74.6**
56	**7.4/7.3**	**604/628**	**74.5/74.7**
60	**7.0/7.1**	**621/628**	**75.9/75.8**

*** Turned on current**

Table 11: pH, ORP, and CFUs (CFU/ml) from Arrowhead samples

Day	pH	ORP	CFU
18	**Not measured**	**Not measured**	**$2.4x10^4$**
25	**6.8**	**253**	**Not plated**
28	**7.0**	**319**	**$8.1x10^4$**
32	**6.8**	**319**	**$1.4x10^4$**
35	**6.5**	**491**	**Not plated**
46	**6.7**	**494**	**$7.8x10^3$**
56	**6.5**	**568**	**$9.4x10^4$**

Table 12: Current readings at the barb-fittings

Day	Barb-fitting 1 (µA)	Barb fitting 2 (µA)	Barb fitting 3 (µA)
35	**25.9**	**21.5**	**170.4**

On day 60, after the last effluent water samples were collected, the apparatus was disassembled. Samples of biofilm bacteria after 28 days of current were collected from the barb-end and mid-section of the 5' test and control tubings. The bacterial counts from the barb-end of the test tubing were 0. The bacterial counts from the barb-end of the control tubing were $1.0x10^4 \pm 3.1x10^3$ CFU/cm^2 (P=0.004) (Figure 3). The bacterial counts from the control tubing were at least one log higher when compared to the ones from the test tubing – the findings were statistically significant.

When analyzing at the counts from the mid-section of the tubings, the results were also significant. The bacterial counts from the test tubing were 0, and the bacterial counts from the control tubing were $8.8x10^3 \pm 2.3x10^3$ CFU/cm^2 (P=0.003) (Figure 2).

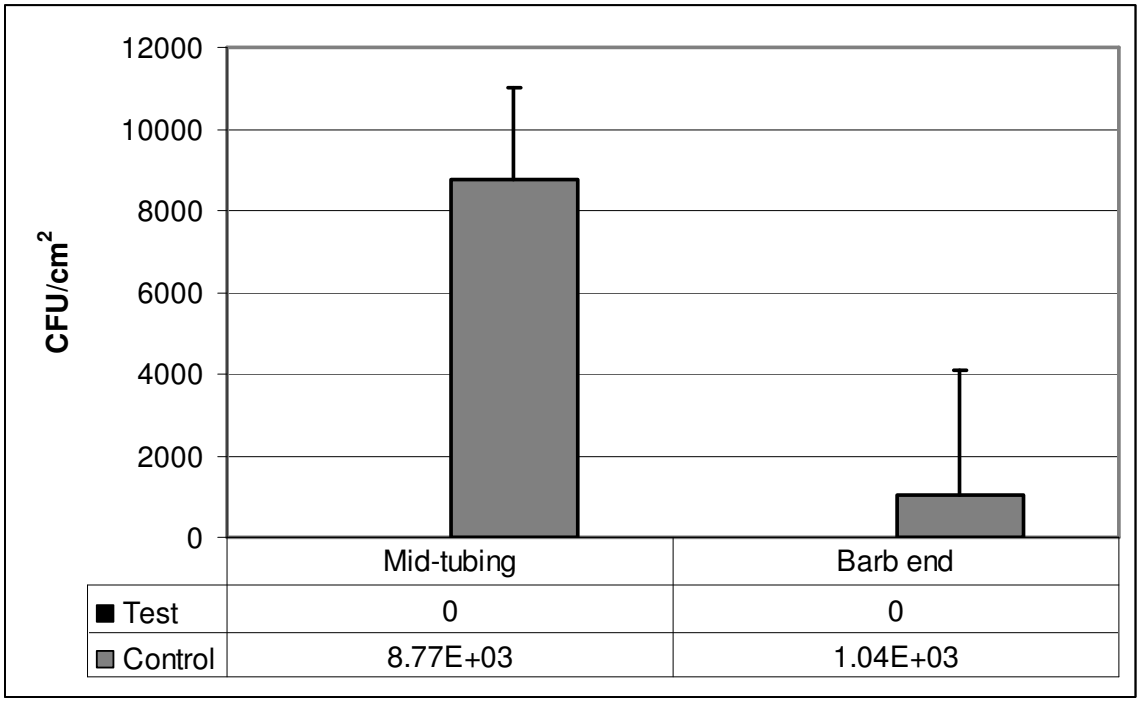

Figure 3. Cultivable biofilm bacteria counts (CFU/cm²) when current and dilute chlorine dioxide were applied to the test system for 28 days after steady-state was established. Dilute chlorine dioxide without current was applied to the control system. Error bars represent standard deviation from the mean.

Discussion

This study used microbiological methods to monitor the planktonic effluent and biofilm formation from a simulated dental unit waterline inoculated with bacteria collected from dental unit waterlines.

In the first part of this study, bacterial biofilm steady state was achieved and low electric current was applied to the test tubing continuously for 24 hour per day, 7 days per week, for 28 days. The results showed that the effluent counts between the test and control tubings were very similar to each other. Counts of effluent reflect the bacteria dispersing from the biofilm and those in the Arrowhead supply water. The only time the counts from the test sample were significantly different (lower) than the control sample, was at day 38. One explanation for it could be that when current was first applied, it eliminated some of the planktonic bacteria; however, the equilibrium was then re-established as the current was continuously applied. From the first part of this experiment, it seems that low electric current alone applied with no disturbances may not be enough to create any damage or significantly affect the bacterial counts in the effluent. The ORP of test effluent was reduced from that of control effluent and Arrowhead supply, but was not of a magnitude to effect a significant difference in CFU/ml.

After 28 days of current, the apparatus was disassembled and samples of biofilm bacteria were collected from the barb-end and mid-section of the test and control tubings. The results showed that the bacterial counts from the control tubing were at least one log higher when compared to the ones from the test tubing. Those findings were statistically significant. The results also showed that the counts from the test mid-section of tubing were at least one log lower than the bacterial counts from the control mid-tubing. Those

findings were also statistically significant. The results in this case indicated that low current did affect the biofilm formation.

In the second experiment, a different approach was used to eradicate the biofilms. A 10:1 dilution of chlorine dioxide was added to both the control and the test tubings, after bacterial biofilm steady state was established, and then a low electric current was added continuously to the test tubing only. The current, just like in the first part of the experiment, was applied for 24 hours per day, 7 days per week, for 28 days. Effluent counts from the test samples fluctuated from 10^1 to 10^3 CFU/ml and effluent samples collected from the control samples fluctuated from 10^2 to 10^4CFU/ml. The results showed statistically significant differences at comparison on days 32, 40, 53, and 56. One explanation for fluctuating counts is the possibility that phenotype of various waterline bacteria kept changing after achieving steady state. Some bacterial colonies may get dislodged, travel to a different spot and start new biofilm formation. *Pseudomonas aeruginosa*, in particular, has been shown to lose their flagella once they adhered to surfaces and formed established biofilms [70]. In addition to establishing colonies, *P. aeruginosa* can also change its morphology during the disturbance start developing flagella and actively traveling in the effluent. This, in turn, can elevate effluent for a short period of time, before it got back to steady state again. Effluent counts reflected not only the dispersal from respective biofilms, but also the total viable counts of the Arrowhead supply. None of the Arrowhead counts were below the CDC criterion of 500CFU/ml for potable water, while in the test line effluent counts on 5 out of 9 days after current was turned on and chlorine dioxide was added, showed counts under 500 CFU/ml.

The results of this experiment show that application of low current with the addition of chlorine dioxide may be more effective in reducing bacterial counts than application of low current alone. However, the results did not show complete eradication of planktonic counts. Such treatment of dental waterline units is not capable of consistently producing CFU counts at or below the ADA recommended 200 CFU/ml for the duration of this experiment. When the peristaltic pump is operating, the Arrowhead water is flowing through, and there is apparently insufficient time for the combination current/chlorine dioxide to complete its activity. Since we found the test line biofilm was eradicated in the sites we chose to biopsy, there was probably little biofilm dispersal in test line effluent counts. Yet, the test line counts were less than the Arrowhead counts. We did not do Arrowhead counts in triplicate so a *t* test cannot be done to ascertain a significant difference from test counts. It is conceivable that had we not been alternating polarity every 70 second, but used a continuously positive anode at barb fittings, that test counts would have been even lower.

When the apparatus was disassembled and samples of biofilm bacteria after 28 days of current and chlorine dioxide were collected from the barb-end and mid-section of the tubing of the test and control tubings, the results were much more remarkable. The colony forming units in the test system dropped to zero. The bacterial counts from the control tubing were much higher when compared to the ones from the test tubing. The findings were statistically significant. No viable bacteria were cultured from the test waterline tubing. These observations provide strong evidence for combined effects of low current and dilute chlorine dioxide solution in eliminating biofilms. Because biofilms are extremely resilient and very difficult to eradicate, these results are significant.

One possible explanation for the effectiveness of the combined effect of chlorine dioxide and low electric current is the generation of transient chlorine dioxide radicals from the waterline cleaning solution. At 10:1 dilution, there would normally be 3 to 4 parts per million (ppm) of active chlorine dioxide. The low current might have increased the concentration of active radicals so that the combination of ClO_2 and current was more lethal than ClO_2 alone in the control line. These active radicals are known to affect methionine and prevent bacterial protein synthesis [71]. Methionine is an amino acid that is used in the synthesis of all proteins in prokaryotes and archaea but is not synthesized in humans [72]. Methionine is the lead triplet in messenger RNA. Chlorine dioxide removes methionine – that means no mRNA can be decoded at the ribosome and no protein can be made. Therefore, without this important amino acid, bacterial protein synthesis will be interrupted. Using chlorine dioxide test strips on the effluent of test and control lines showed no difference in chlorine dioxide concentration. It may have been consumed before it got to the end of the 15' tubings.

This study demonstrated that current alone was incapable of eliminating all established biofilms in the dental unit waterline tubings. At the low current used the salinity of the water may not have been high enough to create much electrolysis. However, when combining low current and dilute chlorine dioxide solution, eradication of bacterial biofilm was successful. Since the above combination was not able to have similar results with the planktonic bacteria due to the load of Arrowhead TVC, it would be interesting to see the result on the effluent counts with a combination of dilute chlorine dioxide in sterile water, low current and filters.

Conclusion

This study evaluated the effect of 10 mA low current with and without dilute chlorine dioxide solution in removal of bacterial biofilms from a simulated dental unit waterline. The conclusion is that in a simulated dental unit waterline apparatus, application of low current alone is not able to lower bacterial counts from the effluent and the biofilm to acceptable levels. However, a combination of low current with dilute chlorine dioxide is very effective at complete eradication of bacterial biofilm, but less so at eliminating planktonic counts.

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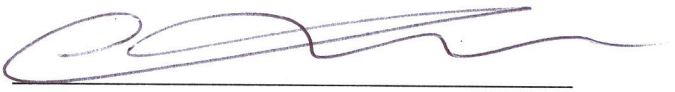

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