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Efficacy of Sterisil in the Treatment of Dental Unit Waterlines

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EFFICACY OF STERISIL IN THE TREATMENT OF DENTAL UNIT WATERLINES

by

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ABSTRACT

Introduction: Dental unit waterlines (DUWL) are an ideal home for bacterial microorganisms to grow, multiply, and develop complex living colonies commonly known as "biofilm." The American Dental Association (ADA) and the Centers for Disease Control and Prevention (CDC) have recommendations to maintain DUWL heterotrophic plate count bacteria levels below 500 colony forming units per milliliter (CFU/ml) of water. The aim of this study is to determine whether Sterisil PureTube is able to control the bacterial load in the orthodontic clinic to recommended levels. **Methods:** Waterline samples from the twelve dental chairs in the orthodontic clinic were used in this study. These chairs are isolated from the municipal water system and have historically used distilled water as a supply source of water. After a two-minute flush of the DUWL, 40 ml water samples were collected in sterile 50 ml tubes from the air-water syringe on each of the dental units. Sampling was performed on Monday's before the start of the clinic day. Samples were grown on R2A Agar plates and processed in accordance with the standard heterotrophic plate count method outlined by the American Public Health Association (Standard Methods). Baseline waterline samples were collected and analyzed. Six of the dental units were then randomly selected and converted to the Sterisil PureTube and Antimicrobial Bottle (Sterisil, Castle Rock, CO). The other six chairs served as a control group. All twelve chairs were then supplied with the same source of distilled water. Three months later, samples were collected and

analyzed using the same protocol as baseline collection. The results were statistically analyzed using a mixed model ANOVA ($p<0.0004$).

Results: Baseline DUWL samples showed plate counts approximating 240,000 CFU/ml. After treatment with Sterisil PureTube and Antimicrobial bottle the samples showed plate counts of 0 CFU/ml. While the control group of untreated chairs increased to over 300,000 CFU/ml.

Conclusions: Sterisil PureTube and Antimicrobial bottle effectively reduced the bacterial counts in the DUWL samples.

Keywords: Sterisil.

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LIST OF ABBREVIATIONS

ADA	American Dental Association
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Units
DUWL	Dental Unit Waterlines
EPA	United States Environmental Protection Agency
EPS	Extracellular Polysaccharide
FDA	United States Food and Drug Administration
HIV	Human Immunodeficiency Virus
JADA	Journal of the American Dental Association
OSHA	Occupational Safety and Health Administration
UVGI	Ultraviolet Germicidal Irradiation
WHO	World Health Organization

INTRODUCTION

Efficacy of Sterisil in the Treatment of Dental Unit Waterlines

Over recent years, biofouling has been recognized as the prime source of microbial contamination in dental unit waterlines (DUWL). While few dentists refute the existence of microbial contamination in the DUWL and the water that is used on patients, many are still uncertain as to what to do about it. There are a number of factors that may lead to this uncertainty. First of all there is an absence of well-documented links to health problems in health care workers and patients who have been exposed to these aerosols. In addition, there is a lack of consensus among the experts about the best way to resolve this issue.[1] Regardless of opinions, there are regulations concerning this matter and there are also solutions that enable compliance. This paper will discuss how the microbial contamination develops, what the effects are, what the regulations are, and what can be done to resolve this issue—highlighted by the treatment of DUWL with Sterisil.

In 1963, Dr. G. C. Blake first reported the existence of contaminated water in dental units after the installation of new high-speed air-rotor handpieces. The handpieces were equipped with their own water reservoirs and Dr. Blake determined that large numbers of bacteria were present in water and aerosols. This finding has been confirmed by dozens of published articles since then.[2]

In the years since Blake's publication, there have been many articles that have appeared in dental journals detailing the existence of microbial contamination and the potential methods of controlling it. Even though most of the documented articles and methods to control DUWL contamination have been around since the 1980's, there is little evidence that these articles have had an impact on the management of waterlines in the dental office.[3]

In the early 1990's, the dental profession became more sensitized to infection control issues as the worldwide human immunodeficiency virus (HIV) epidemic came to the forefront of the news. Reports of waterline contamination by *Legionella* and other potential pathogens increased overall awareness.[4] Research also began to make clear the role that biofilms played in the presence and persistence of the phenomenon.[5] This led the Centers for Disease Control and Prevention to first address the topic of water quality in its 1993 infection control guidelines.[6] In 1995, the American Dental Association's Council on Scientific Affairs gathered an expert panel on DUWL's. This panel established a formal statement that was published in the Journal of the American Dental Association (JADA) in 1996. The ADA's statement encourages a consolidated effort to improve water quality in the dental office and set out an aggressive, proactive research agenda for the control and prevention of biofilm prevention in dental unit water lines.[7]

Biofilm

Biofilms are microbial communities that adhere to solid surfaces wherever there is sufficient moisture. Upon immersion of any solid surface in an aquatic environment, macromolecules and other low-molecular-weight hydrophobic molecules in the water

immediately begin to adsorb to the surface to form conditioning films.[8] These conditioning films alter the characteristics of the surface, which, in turn may enhance the efficiency of bacterial adhesion. Biofilms consist primarily of bacteria and often exhibit complex communal architecture. Most biofilms are heterogeneous in species and morphology and are enveloped by a polysaccharide slime layer know as a glycocalyx. The fundamental process leading to biofilm formation is the result of the initial bacterial adhesion and may be either passive or active. Some microorganisms may already possess the necessary attachment structures to immediately form a firm passive attachment to a surface. Other bacteria require prolonged exposure to a surface to attach firmly. This is a time-dependent process called active adhesion which is initially reversible. Irreversible adhesion and colonization is achieved through the secretion of an extracellular polysaccharide (EPS) layer and subsequent microbial multiplication. The eventual production of a continuous fixed biofilm on the now-colonized surface is a function of cell division within the EPS matrix and the physical inclusion of other bacteria, fungi, and parasitic agents from the free-floating population in the surrounding water.[8, 9] Biofilms provide an environment conducive to the proliferation of a wide variety of microscopic life, including fungi, algae, protozoa, and nematodes.[8] Minus the algae and nematodes, this description of biofilm should be familiar to dentists, as dental plaque is a classic biofilm.[9]

Biofilm formation is recognized as the leading culprit in many life-threatening infections in patients who are treated using medical devices. Identifying the role of biofilms has been much easier, however, than discovering a way to control them. Most known pathogenic organisms have the potential of using biofilms. Once, established,

biofilms are difficult to eliminate from the surfaces of such devices. Many medical device manufacturers need to prevent biofilm formation. One method under investigation incorporates antimicrobial properties into the devices themselves. The theory is that the antimicrobial treatment might discourage the establishment of biofilms. However, manufacturers are faced with identifying which antimicrobial agent will be effective against a wide range of organisms and yet will be tolerated next to healthy tissue.[5, 40]

Biofilms that form in dental units result in contamination of the water that passes through the unit. Organisms recovered from dental chairs are typically gram-negative non-coliform water bacteria.[5] Most of the species recovered have limited pathogenic potential, however, the Safe Drinking Water Act sets a standard for non-coliform bacteria in drinking and recreational water at 500 colony-forming units per milliliter (CFU/ml).[10] A colony forming unit (CFU) is a standard measure of microbial contamination that represents a single colony grown on solid media. A single cell may consist of a single cell or many bacterial cells clumped together. The American Public Health Association recommends the same standard for recreational waters such as swimming pools and spas.[11] By comparison, DUWL contamination in untreated systems often exceeds 1000 CFU/ml. Counts ranging between 10,000 and 100,000 or more may be common place.[12]

Consequences of Biofilm in the Dental Setting

DUWL provide an ideal environment for microbial colonization and proliferation, primarily due to the high surface/volume ratio in the tubing and the character of fluid dynamics in narrow, smooth walled water lines. Microorganisms in dental waterlines can

come from a variety of sources. Most experts suggest that the public water supply is the primary source.

It is important to note that the microbial species colonizing the dental units are mainly bacterial, fungal, and free-living protozoan agents. Viruses, such as HIV, cannot multiply in DUWL. It is possible that the patient body fluids can be aspirated back into the waterlines during treatment, however, current infection control recommendations minimize the likelihood of this occurrence. Current recommendations include the installation and proper maintenance of anti-retraction valves and thorough flushing of DUWL after treatment of each patient.[7] Biofilm in DUWL enables elevated concentrations of microorganisms in the water emitted from the high-speed handpieces and air-water systems. Microbial counts in the range of 1 million microorganisms per milliliter of water have been reported.[7]

There is currently no scientific documentation that establishes that biofilm in DUWL represents a definable public health risk. The lack of evidence may reflect the absence of, or at least a very low rate of, disease transmission and is reassuring, as water is used during most dental procedures. The lack of evidence may also reflect the difficulty in establishing epidemiological links between infections with extended incubation times and antecedent dental procedures. Studies in the scientific literature are limited, but do suggest that dental unit water may contain significant concentrations of *Pseudomonas* and *Legionella* species, both of which are potentially pathogenic to the susceptible host.[7]

In 1987, two case reports were published in the *British Dental Journal* describing the placement of large amalgam restorations using matrix bands in two patients with

cancer.[13] The patients returned to the office three to five days after the fillings were placed complaining of pain and swelling in the areas where the matrix bands were placed. Microbiologic culture of the infected sites recovered *P. aeruginosa*, the same pyocin type of *P. aeruginosa* was subsequently isolated from the dental unit water lines in both cases. The author speculated that both infections were a result of direct inoculation of traumatized tissue with contaminated dental water. There is the possibility that the microorganisms of concern may have originated from the patients.

Several reports have shown that *Legionella* species can colonize DUWL and may pose a risk of occupational exposure through aerosolization of contaminated water.[14] It should be noted that *Legionella* species are found in the environment, and it is difficult to establish a definitive relationship between the presence of serum antibody and the source of exposure without comprehensive epidemiological investigations. However, one such study analyzed samples of from 107 dentists, dental assistants and dental technicians for antibodies to seven different *Legionella* species.[14] Thirty-four percent of the dental personnel showed a positive reaction to the polyvalent *L. pneumophila* antigen SG1-SG6. *L. pneumophilia* is the species considered to be most pathogenic to humans. Only five percent from a control group (nonmedical workers) tested positive. Dentists demonstrated the highest prevalence (50 percent) of antibodies, followed by assistants (38 percent), and technicians (20 percent).

Higher seroprevalence rates for *Legionella* antibodies among dental personnel have not been correlated with higher rates of disease. Investigators have speculated that the higher prevalence of antibodies may reflect continuous exposure to small numbers of the organism, resulting in mild (Pontiac fever) or inapparent infections.[15]

While there are no definitive data linking exposure of contaminated dental water with specific disease incidents, several retrospective studies among dental staff may suggest occupational exposure to potential pathogens. Even though exposure is not the same as infection or disease, avoiding unnecessary exposure is a sound basis for decision making.

Dental Unit Water –vs- Drinking water.

Even though there have been high-profile incidents of contaminated drinking water in the United States such as the *Cryptosporidium* outbreak that sickened thousands in Milwaukee in 1994, most drinking water meets established standards on a day-to-day basis.[16] Many dentists are confused as to how dental units can become so heavily contaminated when they are supplied with well-maintained municipal water systems or even distilled water. The answer to this question deals with a combination of biology, fluid dynamics, and geometry. It can be summarized into the following components: surface colonization, laminar flow, and surface/volume ratio.[1]

The materials commonly used to deliver water to dental handpieces and air/water syringes are great substrates for the initial attachment of bacteria and the subsequent growth and colonization of a biofilm. Confounding this issue is the fact that most treated drinking water contains minerals, such as calcium carbonate, that can be deposited on the water-side of the dental tubing. Carbon-based organic molecules then concentrate in these areas and promote adhesion of bacteria suspended in the municipal water system. The cells that attach multiply over time to form microcolonies that eventually coalesce to form a continuous sheet of bacteria.

The second component of DUWL contamination is fluid dynamics and specifically laminar flow. Fluids moving through narrow tubing characteristically assume a hydrodynamic pattern known as laminar flow. The frictional forces along the inside of the tubing are higher than in the middle of the tubing. This causes the water to slow down and stabilize which creates an environment that is conducive to the formation of a biofilm.[17] Within laminar flow-type situations, biofilm can fluorish without being disturbed or dislodged. Flushing of waterlines can help removed suspended microorganisms, but is usually not effective in removing biofilms.[18]

Another factor in the development of biofilms in DUWL is the geometry of waterlines in the dental unit. As the diameter of a waterline decreases, the surface area for a given volume of water increases. Most plastic dental tubing has an inside diameter of 1/16" to 1/8". This creates a very large ratio of surface area to water volume of narrow bore tubing. The total combined volume of waterline tubing in most dental units is approximately 100 mL or less. 100 mL of water covers approximately four square inches in a ten inch water main. This same volume of water covers more than 400 square inches in a 1/16" diameter dental unit water line. This large amount of surface area is an ideal environment for the formation of a biofilm which helps explain why there can be an extreme buildup of biofilms in DUWL, but the microbial load is not nearly as significant in an adjacent faucet even though it is fed from the same supply.[1]

During the process of biofilm maturation, the environment becomes hospitable for fungi, protozoa and other organisms that survive in drinking water systems.[8] Most of these organisms have minimal pathogenic potential in immunocompetent hosts, but some

protozoa serve as hosts for proliferation of parasitic bacteria such as *Legionella pneumophilia* [19, 20] and *Pseudomonas aeruginosa* [21].

Legionella pneumophilia are the causative agents for *Legionnaires*' disease and a related condition known as Pontiac fever. Every year there are outbreaks and sporadic cases of Legionnaires' disease that occur in hospitals and community environments and may account for as many as 10,000-15,000 documented cases of pneumonia each year in the United States. The estimated mortality rate of these cases is 5-15%. Risk factors for pneumonia include smoking, pre-existing respiratory disease and age.[21]

Another type of species that has been recovered in DUWL is the aquatic nontuberculos *Mycobacterium* which is associated with pulmonary disease and opportunistic wound infections.[22] Unless specific procedures are in place that can prevent, eliminate, trap or kill biofilms, there is little reason to believe that any dental unit can avoid being colonized by bacteria.[1]

In 1990, a civil suit against a dental unit manufacturer was reported. The plaintiff claimed that bacterial endocarditis and the need for related heart valve replacement surgery was the result of dental treatment with contaminated water. Similar strains of gram-negative water bacteria (*Moraxella*) were isolated from the patient and the DUWL. The plaintiff's argument was that the organism entered the dental unit as a result of retraction of oral flora that occurred because the unit was not equipped with an antiretraction valve. The case was settled out of court for an undisclosed sum.[23]

A second lawsuit was disclosed by Dr. Edward Zinman, a dentist and lawyer. He stated during a two-part "CBS Morning News" broadcast on Oct 11 and 12, 1999, that the plaintiff suffered a brain abscess after undergoing dental treatment. The plaintiff

claimed that the brain abscess was the result of exposure to contaminated water from the DUWL. The case was settled out of court against the dentist.[24]

Gram-negative bacteria, like the ones found in DUWL can produce a wide-range of physiological effects. This is due to the endotoxins that are in the cell-wall of the gram-negative bacteria. Some of the effects include localized inflammation, fever, toxic shock, and possibly even asthma. Recently Puttaiah and Cederburg reported that contaminated dental water may contain levels of endotoxin as high as 500 endotoxin units/mL (EU/mL), with an average of 80 EU/mL.[25] In comparison, the United States Pharmacopeia sets a limit for endotoxin in sterile water for irrigation at only 0.25 EU/mL.

Whatever the true nature of health effects associated with microbially contaminated dental treatment water, there is minimal evidence of a major public health problem. Nevertheless, the evidence suggests reason for concern. As a result, the issue has come to the attention of regulatory agencies and advisory bodies at both state and federal levels.[1]

Recommendations

In 1993 the CDC urged dentists to install and maintain anti-retraction valves to prevent oral fluids from being drawn back into DUWL. In addition, they recommended flushing waterlines daily for 20 to 30 seconds between patients to discharge any fluids that may have entered the lines while treating a patient. The CDC also stated that only sterile solutions should be used for procedures that involve cutting bone.[6]

The ADA's statement on DUWL called for the manufacturers of dental equipment to develop better methods to control biofilms in DUWL. The statement

established a goal of no more than 200 CFU/mL of heterotrophic bacteria in unfiltered output.[7]

In 1999, a panel by the ADA waterline panel was reconvened to review the progress of the 1996 goal. The panel determined that manufacturers had come up with a number of options for the control of microbial growth in DUWLs and that the goal of improved dental water is achievable.[25]

Currently, there are apparently no state or local laws or regulations which specifically address a dentist's obligation to ensure dental treatment water quality. The Safe Drinking Water Act sets limits for heterotrophic water bacteria in drinking water and may be enforceable in clinics. Occupational Safety and Health Administration (OSHA) compliance officers have been advised of potential for occupational exposure to bacteria from contaminated DUWL. Manufacturers of dental units and products intended to improve the quality of dental water are required to comply with laws and regulations which are enforced by the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA).

Infection Control

Clinical infection control procedures concentrate on breaking the chain of infection which consists of potential pathogens in sufficient numbers, a susceptible host, and a portal of entry. Host susceptibility and pathogenicity of the organisms are the links that the clinician has minimal control. Therefore, most efforts to break the chain of infection concentrate on minimizing the numbers of organisms in the clinical environment. This is put into practice in the daily protocols of the dental practice such

as: surface disinfection, hand washing, instrument sterilization, and the use of antimicrobial mouthwashes. Reducing the unnecessary bacterial load on patients through the use of cleaner dental water is consistent with long-accepted infection control principles.[1]

In addition, with the current age of informed consent, dentists have an ethical obligation to provide patients and employees with a safe clinical environment. Patients must be informed of potential risks associated with treatment and provide their consent. Employees have a right to know of potential hazards in the work environment. Even though the incidence of infections with contaminated dental water appears to be low, would patients consent to treatment with water contaminated with thousands or even millions of bacteria?[1]

Methods of Dental Unit Waterline Treatment

Numerous methods have been researched and developed that can help to maintain the quality of dental treatment water. Most strategies employ the use of chemical treatment to inactivate microorganisms, induce detachment of biofilms, or both. Other strategies may use microfiltration or even bypass dental unit delivery systems by using autoclavable or disposable methods. Some methods may utilize of combination of options.[1]

Mechanical flushing—while flushing waterlines can temporarily reduce the amount of suspended material in DUWL, there is no predictable effect on the biofilm. The scientific literature does not support the efficacy of mechanical flushing to control contamination in DUWL.[18, 26, 27] Clumps of bacterial biofilm that break free during

treatment have been shown to recontaminate dental unit water during the course of subsequent clinical treatment. Flushing between patients may help remove materials that may have entered during treatment.[18]

Reservoir systems—can be used to isolate the dental units from the municipal water supply so the quality of the water to the dental units can be controlled. However, even with clean water entering the systems, biofilm can still build up and contaminate the dental treatment water.[1]

Chemical treatment—an optimal solution for control of biofilm would be one that can kill bacteria but not hurt humans. In addition, it should be able to dissolve biofilm and prevent reformation while protecting the dental unit from corrosion or degradation. It would have no negative effect on restorative materials and also be cheap and easy to use.[1]

Chemicals can be introduced into the water system intermittently or continuously. The intermittent methods are similar to a "shock treatment" of a swimming pool. The approach is to deliver the chemical agent for a specified contact time and frequency using an independent water reservoir. The active agent is then purged from the system before the patient is treated. Drawbacks of this system include the following: potential for microorganism rebound between treatments; staff exposure to chemicals; and potential for adverse impact on the dental unit materials. Continuous methods may use lower concentrations of potentially biocidal agents in the water. This may be similar to the residual chlorine used to maintain the safety of municipal drinking water.[1]

Another concern about chemical treatment of DUWL is that enamel and dentin bond strengths may also be affected. A study by Roberts and Karpay showed that some

proposed antimicrobial agents can reduce dentin bond strength. Proposed waterline treatment regimens of a diluted mouthrinse and chlorhexidine significantly reduced dentin bond strength compared with sodium hypochlorite and citric acid regimens.[28, 29]

Sodium hypochlorite is a potent germicide with broad-spectrum antimicrobial action that is used to treat both potable and recreational waters. Several dental unit manufacturers now authorize weekly treatment of water systems with household bleach diluted to 1:10 to control biofilm accumulation and improve the quality of treatment water. However, no sodium hypochlorite-based solution has been submitted to the FDA for clearance or registered with the EPA specifically as a waterline biocide.[1] Intermittent rinses reduce the risk of carcinogenic disinfectant byproducts, such as tri-halomethanes, as a result of chlorine's reacting with biofilm organic polymers. Karpay and colleagues detected tri-halomethanes when tap water with three parts per million free chlorine was used in independent reservoirs, none of their samples exceeded the EPA limits.[30] Household bleach does have a down side in that the relative complexity of treatment protocols may result in noncompliance and the reformation of a biofilm or excessive treatment and the oxidation of dental unit components.[31] These effects can be limited by following the manufacturer's recommendations.[32]

Many varieties of DUWL cleaners and disinfectants are on the market to combat microbial contamination of DUWL by using different chemicals and approaches. Chlorhexidine gluconate, hydrogen peroxide, iodophors and commercial mouthrinses are some examples of other agents that have been suggested or evaluated for improving dental unit water quality. A new challenge has emerged due to the increasing number of biocide resistant microorganisms that have been isolated in DUWL.[33] Silver ion has

been widely reported as an effective antimicrobial agent. More specifically, silver has exhibited antimicrobial properties in preventing biofilms on catheters and in other medical equipment, and in water filters and cooling towers. One mechanism though which silver functions as a bactericidal agent is its interaction with disulfide or thiol (sulfhydryl) groups within the amino acids of bacterial cell wall proteins. Silver can bind to DNA, which in turn, interferes with normal metabolic functioning of microorganism, eventually leading to cell death.[34, 35]

Automated treatment devices that can introduce chemical agents into the DUWL automatically are also now available. This approach can reduce the effect of non-compliance variable on clinical success.[1] Numerous automated devices exist such as the Sterisil tube which releases silver ions though a resin matrix. It was used in this experiment and be discussed later. In addition, the Odyssey I (Tuttnauer USA) is a device that generates an ozone and silver germicide via electrolytic action on incoming water. Other devices include the Denta Pure iodinated resin cartridge (MLRB International) which continuously releases two to six ppm free iodine into treatment water to control biofilm; their life ranges from one week to one year.[1]

Another factor in the accumulation of biofilm is the type and quality of source water used in the dental unit. The quality of unfiltered output can be no better than that of the water entering the system. Some offices may use water from the tap that may meet drinking water standards, but usually contains some viable bacteria and organic molecules that accumulate on waterline surfaces and form biofilm. The best method to assure quality output is to ensure consistent delivery of high-quality of treatment water.[1]

Bottled sterile water for irrigation is a great source of water since it is free of viable microorganisms and also has very low levels of minerals and organic compounds that can cause re-establishment of biofilms. An autoclave with a fluid-sterilization cycle can prepare sterile water as can boiling water, but this type of water may still have a significant amount of minerals and organic compounds that can create biofilm. Another method of reducing bacteria in supply water is called continuous ultraviolet germicidal irradiation (UVGI). Bulbs in these systems must be replaced at determined times since they lose germicidal efficacy over time. Distillers and deionizers can reduce mineral and organic content, but do little for bacterial contamination.[1]

Filtration is another option, but if connected to municipal water supplies, the water may contain impurities such as minerals, organic compounds, and endotoxin that may not be able to be removed by filters. Two independent evaluations of microfiltered water used in dentistry found that 80 percent of output was bacteria-free, and none of the remaining specimens exceeded 200 CFU/mL.[36, 37] Mayo and Brown found no detectable organisms in water samples taken immediately downstream from 0.2 micrometer proprietary filters; however, when they increased the distance at which the filter was placed from the air water syringe, levels of bacteria in effluent water increased—probably owing to the formation of biofilm in the post-filtration waterlines.[1, 38] The Denta Pure point-of-use filter employs an iodinated resin in combination with a 0.22 micrometer filter. The release of small amounts of iodine is supposed to retard the growth of biofilm formation in the post-filtration tubing segment.[1] These methods still rely on frequent staff compliance to change filters and perform system maintenance.

Present Study

While it is apparent that there are numerous products available to treat water in DUWL, many require staff compliance to ensure recommended water quality is maintained. The Sterisil PureTube and Antimicrobial bottle offer a unique approach in that once installed, there should be no compliance issues, other than changing it out yearly. The aim of this study is to determine whether Sterisil PureTube is able to control the bacterial load in DUWL to recommended levels.

MATERIALS AND METHODS

Study Design

Waterline samples from the twelve dental chairs in the orthodontic clinic were used in this study (Dexta Model #MK12XE/330E, Napa, CA). These chairs are isolated from the municipal water system, each have their own water bottle as a supply, and have historically used distilled water as a supply source of water.

Sampling was performed on Monday's before the start of the clinic day. After a two-minute flush of the DUWL, 40 ml DUWL samples were collected in sterile 50 ml tubes using the air-water syringe on each of the dental units. 100 microliters of sample were pipetted and plated using serial dilutions to target plate counts in the range of 5-500 CFU/ml. Duplicate plates were constructed for each sample to verify accuracy. Samples were grown on R2A agar plates (18.2 grams Difco R2A Agar/1 liter sterile water) and processed in accordance with the standard heterotrophic plate count method outlined by the American Public Health Association (Standard Methods). The agar plate samples were then incubated at 37 degrees Celsius for seven days (Thelco GCA Precision Scientific Model 6M Incubator, Chennai India). After incubation, the colonies on each plate were counted and recorded.

After pre-treatment waterline samples were collected and analyzed, six of the dental units were then randomly selected (Table 1) and converted to the Sterisil PureTube and Antimicrobial Bottle (Sterisil, Castle Rock, CO). The other six chairs served as a

control group. All twelve chairs were then supplied with the same source of distilled water. Three months later, samples were collected and analyzed using the same protocol as baseline collection.

Table 1

Randomization of Treated and Control Dental Units

Chair	1	2	3	4	5	6	7	8	9	10	11	12
Control			X	X	X		X		X		X	
Treated	X	X				X		X		X		X

Statistical Analysis

This experiment utilized a repeated measures design with a control. The null hypothesis for this study was that there was no difference between the six randomly selected treated dental units and the six untreated (control) dental units. The results were statistically analyzed using a mixed model analysis of variance (ANOVA). The power for this comparison was 0.996 with a p value < 0.0004.

RESULTS

As seen in Table 2, Pre-treatment DUWL samples showed plate counts approximating 240,000 CFU/ml.

Table 2

Pre-treatment Plate Counts (CFU/ml)

Dental Unit	Plate A	Plate B	Average
1	305,000	268,000	286,500
2	71,000	68,000	69,500
3	300,000	300,000	300,000
4	39,000	46,000	42,500
5	300,000	300,000	300,000
6	300,000	300,000	300,000
7	54,000	52,000	53,000
8	300,000	300,000	300,000
9	300,000	300,000	300,000
10	300,000	300,000	300,000
11	300,000	300,000	300,000
12	300,000	300,000	300,000
Source (Distilled)	0	570	285
Tap Water	90	120	105

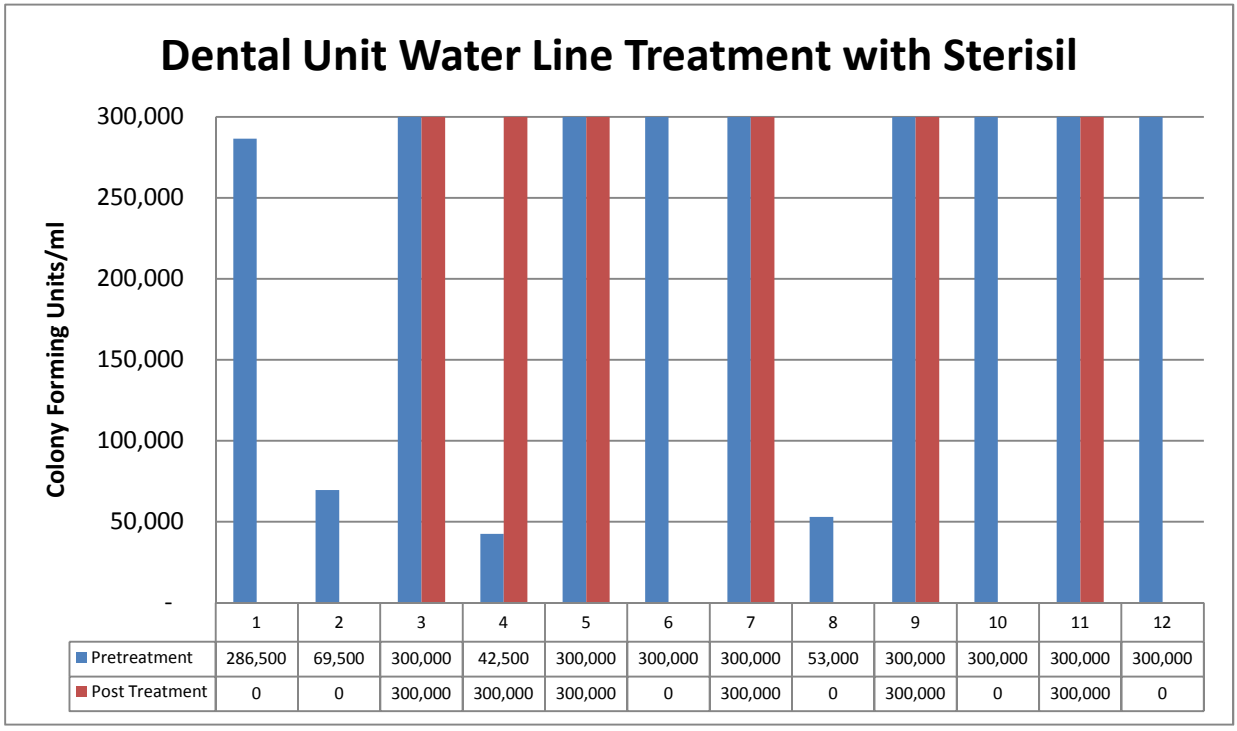
As seen in Table 3, after treatment with Sterisil PureTube and Antimicrobial bottle the samples showed plate counts of 0 CFU/ml. While the control group of untreated chairs were all in excess of 300,000 CFU/ml.

Table 3

Post-treatment Plate Counts (CFU/ml)

Dental Unit	Plate A	Plate B	Average
1	0	0	0
2	0	0	0
3	300,000	300,000	300,000
4	300,000	300,000	300,000
5	300,000	300,000	300,000
6	0	0	0
7	300,000	300,000	300,000
8	0	0	0
9	300,000	300,000	300,000
10	0	0	0
11	300,000	300,000	300,000
12	0	0	0
Source (Distilled)	40	100	70
Tap Water	10	0	5

Figure 1 is a graphical representation of pre-treatment and post-treatment plate counts. The units treated with Sterisil PureTube and Antimicrobial bottle were #1, 2, 6, 8, 10, and 12. As seen in Figure 1, in the dental units treated with Sterisil, the colony forming units were all reduced to zero while the non-treated chairs were all in excess of 300,000 CFU/ml.



	1	2	3	4	5	6	7	8	9	10	11	12
Pretreatment	286,500	69,500	300,000	42,500	300,000	300,000	300,000	53,000	300,000	300,000	300,000	300,000
Post Treatment	0	0	300,000	300,000	300,000	0	300,000	0	300,000	0	300,000	0

Figure 1. Pre-treatment and Post-treatment plate counts for each dental unit (CFU/ml).

Based on this data, the null hypothesis of no difference between the control and treated groups can be rejected. The alpha was 0.05 and the p-value was 0.0004. Since the p-value was less than alpha, this is statistically significant.

DISCUSSION

Dental unit waterlines are an ideal place for bacterial microorganisms to grow, multiply, and develop into complex living colonies commonly known as “biofilm.” The ADA and CDC have recommendations to maintain DUWL heterotrophic plate count bacteria levels below 500 colony forming units per milliliter of water. There are many available methods and technologies on the market that can achieve these recommendations. Most require daily or weekly compliance by staff to keep the engineering systems in place and working so that bacterial counts can remain under control.

A better technology could be one that is easily installed and used without the staff having to perform frequent maintenance on that system. The Sterisil PureTube and Antimicrobial Bottle utilize a relatively simple approach of installing a treated straw and water bottle on the dental unit. The apparatus releases silver ions as water passes through the tube and the water is treated to quality that exceeds governmental standards. After one year of use, the bottle and tube are replaced with a new Sterisil bottle and tube on each dental chair. The changeover process takes approximately two minutes per chair. This type of system takes the day to day requirements out of maintaining the waterline and reduces the need for daily staff compliance.

Manufacturers are increasingly looking toward silver as the answer to controlling biofilms on medical equipment. Silver is one of the oldest known antimicrobials.

Antimicrobial silver is now used extensively to combat organisms in wounds and burns. It works because pathogens cannot mutate to avoid its antimicrobial effect. In the process of developing burn and wound silver technologies, researchers have studied the ability of silver's antimicrobial properties to remain effective in the face of virulent pathogens. When mobilized from its reservoir in aqueous fluids, silver provides an antimicrobial action. The positively charged ionic form is highly toxic for microorganisms, but has relatively low toxicity for human tissue cells. The US Environmental Protection Agency (EPA) and World Health Organization (WHO) have indicated that silver levels less than or equal to 0.1 mg/L for drinking water are safe.[39, 40] Studies using silver ionization systems have reported silver concentrations of 0.04 mg/L, less than half the EPA limit.[41] In a dental clinic, water is used solely for irrigation purposes, therefore there is little potential for ingestion. Pathways into the patient's bloodstream are often made through the use of dental tools, such as high speed handpieces, or air/water coolants when preparing the subgingival tooth structure. Thus, it is important considering the mode of clinical application and the level of exposure to correctly evaluate the potential risks to patients.

Silver works in a number of ways to disrupt critical functions in a microorganism. For example, it has a high affinity for negatively charged side groups on biological molecules. This binding action alters the molecular structure of the macromolecule, rendering it worthless to the cell. Silver simultaneously attacks multiple sites within the cell to inactivate critical physiological functions such as cell-wall synthesis, membrane transport, nucleic acid synthesis, protein folding and function, and electron transport, which is important in generating energy for the cell. Without these functions, the

bacterium is either inhibited from growth or, more commonly, the microorganism is killed.[42]

In the present study, the bacterial loads in the six chairs treated with the Sterisil PureTube and Antimicrobial bottle dropped from an average of 218,000 CFU/ml to zero CFU/ml within three months of Sterisil installation. The untreated control chairs began the study with an average of 257,000 CFU/ml. At the three month timepoint sample, the bacterial counts on the untreated chairs had all increased to numbers greater than 300,000 CFU/ml. These results indicate that the Sterisil PureTube and Antimicrobial bottle were effectively able to reduce the bacterial load on the treated chairs to meet and exceed the standards established by the ADA and CDC while the bacterial load in the untreated, control chairs all increased with time.

Previous studies of various silver type DUWL disinfectants have reported that these cleaners and disinfectants were able to reduce effluent microbial contamination. In 2006, Schel and colleagues examined the efficacy of various disinfectants. It was reported that Dentosept, Oxygenal and Sanosil, all of which contain hydrogen peroxide or silver ion as active agents, were able to effectively reduce biofilm total viable counts below CDC guidelines.[43] These results were consistent with the findings of this study.

CONCLUSIONS

Under the conditions of the present study, the following conclusion was made:

1. Sterisil PureTube and Antimicrobial bottle effectively reduced the bacterial counts in the DUWL samples.

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