How Does Time-Dependent Dental Unit Waterline Flushing Affect Planktonic Bacteria Levels?


Abstract: The purpose of this study was to evaluate how time-dependent waterline flushing affects the presence of biofilm in otherwise-untreated dental unit waterlines (DUWLs). Water samples were obtained from twelve highspeed handpiece DUWLs located in the undergraduate treatment clinic at the University of Missouri-Kansas City, School of Dentistry. Baseline water samples (50 cc) were collected prior to the start of continuous flushing. Additional 50 cc samples were collected after two-, three-, and four-minute flushing intervals from the baseline. The levels of planktonic bacteria in DUWLs were quantified by counting colony forming units (CFUs). In addition, segments of water tubing from each of the highspeed handpiece waterlines were examined by scanning electron microscopy, which confirmed the presence of a residual biofilm in the lumen of each dental unit waterline. A one-factor repeated measures ANOVA showed a statistically significant (p< 0.01) reduction in CFUs at all intervals compared to baseline and between each successive time interval. Indeed, after four minutes of continuous flushing, all waterlines still harbored CFU levels that exceed current American Dental Association (ADA) recommendations. It was concluded that water flushing of DUWLs produced a statistically significant reduction in planktonic bacteria at each time interval compared to the baseline and between each successive time interval. However, the level of CFUs after four minutes of continuous water flushing still exceeds the current ADA recommendations for acceptable levels of microorganisms.

Bacterial contamination of dental unit water lines (DUWLs) has become a growing concern for clinical dentistry. The capacity of bacteria to colonize the lumen of waterlines has been well documented.1,2 The possible formation of aerosols of these bacteria as well as direct transmission raises the question of potential risk to both the health care provider and patient. However, due to a lack of epidemiological research, there is still little evidence of significant health problems associated with the use of contaminated DUWLs, in spite of the large number of patient visits per day in both private practice and institutional settings.

In the dental operatory, biofilms (or microbial communities) are formed over time as microorganisms adhere to the luminal surface of waterlines, coalescing into a film containing multiple species and morphotypes. DUWLs offer a large surface area for potential microbial growth where colony forming units (CFUs) can reach levels of one million per ml.3 Biofilm microbes are imbedded in a polysaccharide slime layer, or glycocalyx, that facilitates adherence and also protects the biofilm from desiccation and chemical insult. The reported list of bacterial genera isolated from DUWLs is long and varied. For example, a variety of opportunistic pathogens belonging to the following genera have been identified: Legionella,4 Klebsiella,4 Staphylococcus,4 Enterococcus,5 Mycobacterium,6 Pseudomonas,7 Moraxella,8 Sphingomonas,9 Brevundimonas,9 and others.3,8,11

Despite the lack of epidemiological data demonstrating a positive correlation between contaminated DUWLs and significant patient health problems, it has been suggested that the dental profession...
take proactive steps to limit microbiological contamination of water used in all forms of dental treatment. Currently, there are several methods of reducing the numbers of CFUs in the DUWL, including flushing lines with water, intermittent or continuous use of bactericidal chemicals, radiation, self-contained independent water reservoirs, and filtration.\(^\text{11}\) Indeed, published guidelines from the U.S. Department of Health and Human Services and the Centers for Disease Control and Prevention (CDC) suggest that high-speed handpieces should be run to discharge water and air for a minimum of twenty to thirty seconds after use on each patient.\(^\text{12}\) In addition, the guidelines state that “microbial accumulation in waterlines can be reduced substantially by allowing water lines to run and to discharge water for several minutes at the beginning of each clinic day.”\(^\text{13}\)

Large, multichair dental clinics such as those associated with schools of dentistry, military installations, and public health clinics are confronted with unique problems with regard to control of contaminated DUWLs. Some dental units may remain unused for several consecutive days, for example, thereby allowing undisturbed biofilm to increase in mass due to the lack of shearing force from laminar water flow.\(^\text{14,15}\) There are extensive logistics associated with maintaining filtration units, independent water reservoirs, chemical supplies, and automated treatment devices, along with quality assurance and monitoring. Lastly, there is considerable expense associated with the equipment, supplies, and personnel needed for these sophisticated treatment modalities.

Given the problems associated with large treatment clinics, a simple and inexpensive technique to disturb and reduce biofilm accumulation is highly desirable. Daily flushing of DUWLs remains the least expensive and simplest method for reducing the level of biofilm contamination in the waterlines of large numbers of dental treatment units. Thus, the purpose of this study was to evaluate how time-dependent waterline flushing affects the levels of planktonic bacteria in otherwise untreated and contaminated DUWLs in a large institutional treatment clinic.

**Methods and Materials**

Enumeration of heterotrophic planktonic bacteria was accomplished by random identification of water tubing from twelve highspeed handpieces in the 220 chair-treatment clinic at the University of Missouri-Kansas City, School of Dentistry. The clinic floor plan is divided into three clinical teams and a pediatric dentistry treatment area, essentially representing four quadrants of a rectangular floor. All four treatment areas receive water from a centrally located main waterline that, in turn, is a direct extension of the municipal water supply. Each dental unit has three waterlines, one designated for a highspeed handpiece and one each for a sonic scaling instrument and an air-water syringe. All three waterlines are a polypropylene tubing, and all are extensions of a common “feeder” waterline. Thus, the twelve highspeed waterlines, three from each treatment area of the clinic, constituted a representative sample from the entire clinic floor.

Prior to removal of the selected tubing, at a time when the units had been unused for forty-eight hours, a 50 ml baseline water sample was collected from each line using sterile technique. The sterile technique required the following precautions: use of sterile surgical gloves; wiping the external surfaces of the waterline tubing with sterile cotton gauze soaked in 90 percent ethyl alcohol; cutting of the waterline with sterile instruments; and collection of waterline samples in sterile bottles. Following collection of the baseline sample, additional 50 ml samples were procured after two-, three-, and four-minute intervals of continuous waterline flushing.

All samples were subjected to Vortex mixing for thirty seconds to disperse the planktonic biofilm. Tenfold serial dilutions (10\(^{-1}\) to 10\(^{-6}\)) were prepared with 10 mM sterile phosphate buffered saline (pH 7.3). Duplicate 1 ml volumes of each sample at each dilution were spread with a glass rod on a petri dish of dextrose-enriched trypticase-soy agar (pH 7.2). The agar plates were incubated for seventy-two hours in 5 percent CO\(_2\) at 37\(^\circ\) C. Following the incubation period, colony-forming units (CFUs) were counted with the aid of a colony counter. The mean of the duplicate platings was calculated, corrected for dilution, and data recorded as CFUs/ml. There was no attempt to subculture or identify recovered microorganisms.

Specimens examined by scanning electron microscopy consisted of water tubing from the same highspeed handpieces identified for the bacteriologic sampling. A one-inch section of the tubing was cut from the end that joined the handpiece connector, split longitudinally, using a scalpel, to yield two equal halves, and immediately immersed in ice-cold 2.5 percent glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for four hours. After the initial fixation,
 longitudinal tubing halves were post-fixed in 1 percent osmium tetroxide in 0.1 M cacodylate buffer, pH 7.4 at 4°C for two hours. The two halves were then dehydrated in a series of graded ethanol solutions (20 percent to 100 percent) at fifteen-minute intervals, followed by immersion in hexamethyldisilazane for forty-five minutes. Specimens were then affixed to an aluminum stub, stored in a desiccator overnight, and subsequently sputter-coated with approximately 20 nm of gold-palladium. Specimens were examined in a Philips 515 SEM or a Philips XL 30 ESEM-FEG (Philips Electronic Instruments, Inc., Mahwah, NJ).

Descriptive data analyses were used to characterize CFUs at each flushing time interval. Data were analyzed using a one-factor, repeated measures analysis of variance ANOVA. The Bonferroni method was used to adjust for Type I error rate for post hoc comparisons.

### Results

Table 1 displays mean values of CFUs for each flushing time interval. For each successive flush, there was a statistically significant (p < 0.01) reduction in CFUs. The Eta squared value of 0.963 suggests a very strong effect of flushing over time on CFU reduction. Initial bacterial recovery averaged 15,320 CFUs/ml (±4,211). Following four minutes of line flushing, the bacterial recovery averaged a mean of 3,160 CFUs/ml (±1,890). Statistical analysis of the difference between the mean CFUs/ml prior to flushing versus that after a four minute line flush showed a significant reduction in CFU counts following the four-minute flush (p < 0.01).

All twelve samples of handpiece waterline tubing exhibited a confluent layer of biofilm covering the lumen surface (Figure 1). Higher magnifications revealed a compact microbial mass characterized by an undulating surface topography and a dense extracellular matrix (Figure 2). With the aid of low angle views, it was determined that the biofilm was of variable thickness, ranging from 10 µm to 50 µm. Vari-

<table>
<thead>
<tr>
<th>Line #</th>
<th>Baseline CFUs/ml</th>
<th>2 min flush CFUs/ml</th>
<th>3 min flush CFUs/ml</th>
<th>4 min flush CFUs/ml</th>
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<td>9.7</td>
<td>5.8</td>
</tr>
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<td>5.9</td>
<td>4.2</td>
<td>3.1</td>
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<td>5.5</td>
<td>3.7</td>
<td>2.2</td>
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<tr>
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<td>1.0</td>
<td>1.0</td>
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</tbody>
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| Mean   | 15.32            | 8.25                | 4.74                | 3.16                |
| s.d.   | ±4.21            | ±3.47               | ±2.92               | ±1.89               |

Note: A statistically significant difference between the means (p < 0.001) was achieved between baseline and each time interval and between all time intervals.
ous microbial morphotypes were observed, such as cocci, short and medium length rods, and spiral-shaped rods (Figures 2 and 3). In addition, multiple oval-shaped spore-like structures, ranging in diameter from 10 to 15 μm (Figures 4 and 5), were frequently noted and assumed to represent mycotic spores.

Discussion

Although the research literature involving time-dependent waterline flushing of DUWLs offers mildly conflicting results, the findings of this study suggest that time-dependent flushing for as few as two to four minutes will produce a statistically significant reduction in planktonic CFUs. This result appears to be consistent with most of the previously reported research literature. For example, Scheid et al.16 found that a four-minute waterline flushing through attached high-speed handpieces reduced CFUs to zero. In contrast, a six-minute waterline flushing reduced the number of CFUs from 188,600 to 6,018 per milliliter, a 96.8 percent reduction.17 Williams et al.18 reported that a ten-minute waterline flush significantly reduced the number of CFUs in DUWLs. Whitehouse et al.19 reported that twenty minutes of flushing reduced the number of detectable bacteria in water from dental school lines to zero, but the lines were repopulated again twenty-four hours later. Although these extended flushing times resulted in large drops in CFUs, flushing for excessive time periods is impractical in large, multichair treatment clinics. Indeed, it must be emphasized that while waterline flushing results in statistically significant reductions in the number of planktonic microbes, most studies that use waterline flushing alone do not show complete removal of all accumulated biofilm.15,17,18

Although the CDC recommends the use of sterile saline or water for surgical procedures, it is unrealistic to assume that all clinicians performing minor surgical procedures (such as simple tooth extraction) are compliant with the suggested guidelines. Thus, the finding by Putnis et al.20 of relatively high levels of bacterial endotoxin (LPS) in
DUWLs is significant, particularly if one considers the potentially deleterious effects on wound healing resulting from endotoxin interactions with the host immune response. The authors reported significant levels of LPS in DUWLs ranging from 480 to 1,008 endotoxin units (EU)/ml. The authors also reported that LPS levels of »2,560 EU/ml were reduced by 70 percent with a one-minute waterline flush. However, waterline flushing of five to ten minutes did not reduce LPS levels to zero. To understand why DUWLs appear impossible to sterilize, one must look at the nature of a biofilm.

Dissimilar environments tend to dictate differing characteristics in biofilms. Thus, it is not valid to extrapolate patterns of adherence (that is, sequence of successional colonization and intrageneric coaggregation) from one biofilm system to another.14,21,22 Given that caveat, however, all biofilms consist of microbe-to-microbe and microbe-to-substrate adherence patterns. Microbial adherence is mediated through complex interactions of extracellular matrix proteins and polysaccharides with adhesion molecules, collectively referred to as a glycocalyx. In addition, the glycocalyx may facilitate neutralization of disinfecting agents23 and trap nutrients for future survival.24 The biofilm itself has been shown to be a source of planktonic bacteria as surface microbes are continuously being shed into the flow of water.17,19,25,26 Just as rinsing alone without mechanical debridement cannot remove all adherent bacteria from a tooth surface, water flushing of a unit’s lines cannot be expected to remove all bacteria from the interior lumen.

Paradoxically, the physics behind water flow through the lumen of a waterline is partly at fault for the inability of flushing to remove biofilms. The phenomenon of laminar flow provides for rapid infusion of water through the central corridor of the line, while streaming at the periphery is significantly less, approaching zero. This creates stagnation of water near the already complacent biofilm. Thus, instead of enhancing removal of adherent bacteria, the pattern of laminar flow actually encourages growth and accumulation of the biofilm.27

Due to a lack of research, there is little evidence that contaminated DUWLs pose a health hazard for patients or dental health care providers. Martin24 has reported two postoperative infections caused by Pseudomonas aeruginosa that were believed to have originated from dental unit water. Atlas et al.29 report a single case involving an orthodontist who was thought to have died from a case of Legionnaires’ pneumonia, resulting from exposure to Legionella-contaminated dental unit water. Indeed, if contamination of DUWLs places the patient and dental health care provider at a significantly higher risk for contraction of disease, one would expect the number of incidence reports to be considerably greater than a few isolated cases. In addition, the cases of related illnesses in environments such as dental schools and public health clinics, where numerous medically compromised patients are treated daily, would be noticeably higher than in other settings. To date, however, there is no evidence to support increased rates of illness in medically compromised patients as a result of exposure to DUWL biofilm.

Given the previously cited problems inherent to large dental treatment clinics, what methods can be employed to decrease the number of CFUs expressed from DUWLs? Currently available options include independent water reservoirs, daily draining, flushing, and air-purging regimens, point-of-use filters and anti-retraction valves, ultraviolet irradiation, use of commercial sterile deionized water, and chemical disinfection with or without the use of separate water reservoirs. Chemical agents suggested for use include chlorhexidine gluconate,3 hydrogen peroxide,27,30 povidone iodine,31 Tween 80, a laboratory surfactant,8 and several types of chlorine compounds.3,15,17,32-35 Data that supports the use of any of these options is limited. Although some of the choices are promising, further evaluation of their applicability in a large dental treatment facility must be assessed. For example, existing evidence indicates that simple water flushing of DUWLs for as long as four to ten minutes, a time that becomes excessive given the potential number of treatment units, does not achieve the recommended ADA standard of £ 200 CFUs/ml. In addition, such an assessment must include the cost in terms of dollars, time, and staff allocation to fully implement an effective biofilm control procedure.

The ADA statement regarding DUWLs requested that manufacturers produce systems capable of reducing bacterial levels to less than 200 CFUs/ml by the year 2000. Other current ADA guidelines recommend running high-speed handpieces for a minimum of twenty to thirty seconds after each patient use, routine compliance with manufacturer instructions regarding maintenance of waterlines, giving consideration to commercial options for improving water quality, and the use of sterile water as a coolant or lubricant for surgical procedures that...
involves cutting of bone. The ADA recommends decreasing the levels of waterline bacteria, while admitting that “scientific evidence does not demonstrate any serious health effects from patient or practitioner exposure to dental unit water.” Clearly, large institutional dental treatment clinics face a seemingly difficult task of balancing ethical obligations with the potential of excessive expenditures that may be required to limit patient exposure to potential pathogenic microbes.

REFERENCES