

Dental Unit Water Lines (DUWL's) – A Review of The Problem & Solutions.

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Date; February 2007.

Abstract

This review and position paper discusses the formation of biofilms, the contamination from mains water and ingress of micro-organisms from patients into Dental Unit Water Lines (DUWL's). These present hazards to the health care worker and other patients during treatment. Detachment of microorganisms or their DNA from dental unit biofilm flushed into the oral cavity could theoretically infect the patient. Splatter and aerosols from dental procedures may possibly infect health care personnel (*Wirthlin et al 2003*). The safety of dental waterlines has been recently questioned on national TV in the USA (*PubMed 2000*). Szymanska J (*Szymanska 2005*) identified moulds, bacteria and yeasts in biofilms. Some of these in certain circumstances, especially in people with immunological disorders, may be a cause of opportunistic infections (*Szymanska 2005*). The role of various decontamination agents is discussed. It is suggested that the state-of-the-art is the use of ozone. Incorporation of ozone generating units into the dental treatment unit would be the logical extension of this technology. The integration of the use of ozone into a dental unit extends a system of disinfection and sterilisation for DUWL's, into the clinical management and patient care arena.

Key Words; biofilms, microorganisms, cross infection, disinfection, dental unit water lines

Introduction

The quality of dental unit water is of great importance since patients and dental staff are regularly exposed to water from aerosols generated during work, and Dental Unit Water Line (DUWL) contamination has become a concern (*Putnins et al 2001, Wirthlin et al 2003*). Biofilms are a natural occurrence in aquatic environments, including community drinking water systems. The interior of small-diameter tubing in dental unit waterlines are also sites of biofilm formation. In the lumen of the tubing, the flow is minimal, and progressively decreases to stasis at the interior wall surface of the tubing. Water becomes stagnant when the units are not in use. Molecules precipitate from the water onto the interior wall and promote the adherence of planktonic microorganisms from the water. Once they become sessile, the microorganisms change their phenotype. After adherence, there is a so-called surface-associated lag time, and the organisms then enter a growth phase and produce exopolysaccharides that coat the organisms in a slime layer. Within the biofilm, the microorganisms can signal one another, transfer nutrients, and exchange genetic material. The insoluble exopolysaccharides shield the microorganisms from displacement and from penetration by predator organisms, antibiotics, and disinfectants. The external surface layer of microorganisms is faster growing and may detach as "swarmer" cells. Detachment of microorganisms from dental unit biofilm flushed into the oral cavity could theoretically infect the patient. Splatter and aerosols from dental procedures may possibly infect health care personnel (*Wirthlin et al 2003*). DUWL contamination has become a concern to clinical dentistry (*Putnins et al 2001*). In one study, a viability staining technique identified significantly more bacteria in water than could be cultured (*Putnins et al 2001*). The mean LPS levels in water collected from high-speed and air & water spray lines in use were 480 and 1,008 endotoxin units (EU)/ml. This was significantly higher than the mean level of 66 EU/ml found in water samples collected from adjacent clinic sinks (*Putnins et al 2001*). In order to satisfy water regulations and comply with health and safety legislation dentists should institute infection-

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control measures to maintain the dental unit water at the standard of less than 200 colony-forming units per ml of aerobic bacteria (*Pankhurst 2003*). However, this may be inadequate with groups of immuno-compromised patients.

Manufacturers are instituting water quality standards for dental units at a time when certain fundamental questions remain unanswered;

- What should be measured and what methods should be used?
- Do certain disinfection procedures have an opposite effect to the one desired?
- Finally, the question of health risks linked to the colonization of waterlines has not been adequately addressed by researchers (*Barbeau & Nadeau 1997*).

Bacteria have been around for millions of years, and are not without a trick or two of their own when survival is threatened. The vast majority of anti-microbial products act over a period of time. This window of opportunity is used by micro-organisms to evolve new species, termed 'resistance', to these disinfection products.

Modern health care now faces the problem of bacterial strains which are resistant to a wide variety of products. In a world where the life expectancy has been lengthened by pharmaceuticals micro-organisms are now faced with the ultimate choice of host. The micro-organisms' host is beset with immunological conditions that lower the innate immune system's ability to contain and repel infection. It is an era of opportunistic infection, and as their hosts tend to live in crowded surroundings, conditions are perfect for micro-organism evolution and survival.

DUWL's are ideal environments for the growth of microorganisms entering dental units from the municipal water supply (*Barbeau 2000*) and from previously treated patients (*Montebugnoli et al, 2004*). Very few cases of cross-infection have been linked directly to contamination in DUWL's, but in an era of sociological changes, this risk has grown proportionally (*Szymanska 2005*). Al Shorman H *et al* (*Al Shorman et al 2003*) discussed that *Pseudomonas aeruginosa* found in DUWL's in Belfast Dental School could be a risk for immuno-compromised adults and cystic fibrosis children for example.

Microbiological Studies of DUWL

Detachment of microorganisms from DUWL biofilm flushed into the oral cavity could theoretically infect the patient. Splatter and aerosols from dental procedures may possibly infect health care personnel (*Wirthlin et al 2003*). Health care workers do not want to isolate themselves from patients by donning full-protective suits. The use of face masks, transparent shields and high volume suction is now part of every day clinical practice in a dental clinical setting despite awareness that this creates anxiety (*Domingo et al, 2004*).

A Jordanian study (*Al-Hiyasat et al 2007*) illustrated that stasis in DUWL's during non-working time results in the proliferation of the biofilm and colony forming units (CFU's). Overall, the highest counts (log (10) count CFU ml (-1)) were found at the beginning of the working day (1.38 +/- 1.05), and the lowest counts after flushing for 2 min (1.10 +/- 1.03). An increase in the number of CFU's were seen again at midday (1.15 +/- 1.04) (P < 0.05).

Various studies have looked at DUWL's to categorise the microbiological flora involved in the formation of biofilms. Szymanska J (*Szymanska 2005*) identified moulds: *Aspergillus amstelodami*, *Aspergillus fumigatus*, *Aspergillus* spp. from *Aspergillus glaucus* group, *Aspergillus repens*, *Citromyces* spp., *Geotrichum candidum*, *Penicillium aspergilliforme*, *Penicillium pusillum*, *Penicillium turolense*, *Sclerotium sclerotiorum*; yeast-like fungi: *Candida*

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albicans, *Candida curvata* and other yeasts in a Polish study. Some of them, in certain circumstances, especially in people with immunological disorders, may be a cause of opportunistic infections.

In Ireland, Al Shorman H *et al* (Al Shorman *et al* 2003) and in Jordan Al-Hiyasat A *et al*, (Al-Hiyasat *et al*, 2007) evaluated the extent of *Pseudomonas aeruginosa* contamination of DUWL's at Dental Teaching Centres. Dental units from clinics in conservative dentistry, periodontology, and prosthodontics were examined in the Jordanian study. Al-Hiyasat A *et al* detected *P. aeruginosa* in 86.7% (26/30) of the dental units at the beginning of the working day, and in 73.3% (22/30) after 2 min of flushing and at midday. Conservative dentistry units had the highest counts, followed by periodontology and prosthodontics ($P < 0.05$). Al Shorman H *et al* (Al Shorman *et al* 2003) showed a reduction in the total volume count (TVC) of water from the control unit from 2.3×10^4 (week 1) and 3.4×10^4 CFU/mL after 2 weeks of installation. The primary coloniser was identified (API 20 NE kit) as pure *P. aeruginosa*.

O'Donnell MJ *et al* (O'Donnell *et al* 2006) found the most common bacterial species cultured from the mains water were *Micrococcus luteus* and *Sphingomonas* spp., respectively, the latter of which are known opportunistic pathogens. Montebugnoli L *et al* in their 2004 paper (Montebugnoli *et al* 2004) discussed direct person-to-person transmission of periodontal bacteria through saliva. Dental units have been demonstrated to retract saliva from patients under treatment and to release it into the mouths of subjects undergoing the next operation. A polymerase chain reaction (PCR) based method was used to investigate periodontal pathogenic bacteria inside DUWL's. The presence of DNA of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, *Treponema denticola* was examined, and positive samples of *Prevotella intermedia* DNA found. These findings clearly suggest that dental units have the potential to transmit periodontal pathogens between patients.

Porteous NB *et al* (Porteous *et al* 2004) found non-tuberculosis mycobacteria in DUWL at a hospital dentistry clinic where immuno-compromised patients are seen. *Mycobacterium simiae* was isolated from one of the four pre-treatment samples and from two of the four post-treatment samples. *Mycobacterium mucogenicum* was isolated from one of the four post-treatment samples.

Microbiological Studies of Turbine and 3-in-1 Spray

A study in 2005 by Szymanska J (Szymanska 2005) examined bacterial endotoxin concentration in the water flowing from a high-speed hand piece of a dental unit and in the air contained in the bio-aerosol formed during dental conservative treatment. The air was collected in the space between the patient and dentist. The study was conducted on 25 operative units and had two stages: before application of a DUWL disinfectant and after a 2-week application of disinfection procedure. The research showed that the mean concentration of bacterial endotoxin in the water flowing from high-speed hand pieces was significantly reduced after the use of a disinfectant. The mean concentration of bacterial endotoxin in the air was similar at both stages - before and after application of waterline decontamination procedure. The study showed that in dental air-water aerosol, water is the main source of bacterial endotoxin contaminating the aerosol during the work with dental hand pieces.

In Japan, Kohno S *et al* (Kohno *et al* 2004) found that the mean viable bacteria count was 910 \pm 190 CFU/ml at the end of dental hand pieces, and 521 \pm 116 CFU/ml at the end of three-way syringes.

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Contamination of DUWL with Cleaning Fluid Residue and Resin Bonding Issues

In response to concerns of bacterial biofilm colonization of DUWL, a wide range of commercial intermittent and continuous chemical treatments for DUWL have been developed and marketed. Roberts HW *et al* (Roberts *et al* 2000) researched the possible effect of continuous chemical treatment regimens on dentin-bonding agents. Four proposed antimicrobial agents for use in DUWL on dentin bond strength were examined. The authors used a fifth-generation dentin-bonding agent to bond composite cylinders to molar dentin surfaces. They then used selected antimicrobial agents as rinsing agents after conditioning.

The composite cylinders were shear tested, and their fracture strengths were compared statistically. All proposed antimicrobial agents reduced dentin bond strength. Proposed waterline treatment regimens of a diluted mouth rinse and chlorhexidine significantly reduced dentin bond strength compared with sodium hypochlorite and citric acid regimens. The clinical implications of this 2000 research were that DUWL antimicrobial agents may adversely affect dentin bonding strength.

A 2001 study showed there was no significant difference in shear bond strengths of resin-based composite to tooth structure when rinsed with distilled water mixed with mouthwash, distilled water or water from a municipal source (Knight *et al* 2001). The clinical implication is that DUWL disinfected using a diluted mouthwash solution may be used while bonding resin-based composite to either enamel or dentin. However dental mouth wash is not a particularly good sterilizing medium.

Another 2001 study suggested that DUWL biocides may adversely affect adhesion of resin to enamel (Taylor-Hardy *et al* 2001). This study evaluated the effects of chemical biocides used to control dental unit waterline biofilm on the bond strength of resin to enamel. Sixty bovine teeth were randomly assigned to six treatment groups. One-way ANOVA revealed a significant difference in means ($p < 0.001$) and Tukey's multiple range test indicated that three of the experimental groups had significantly lower mean shear bond strengths than the control ($p < 0.05$).

A 2004 study examined the effects of biocide contamination of DUWL (von Fraunhofer *et al* 2004). In their closing discussion, they comment on the varying reports on the effects of such agents on the bond strength of restorative dental materials and, particularly, between these agents and dental hard tissues.

Failure of the enamel-resin bond can lead to marginal micro-leakage around the periphery of the restoration. Failure of this margins results in staining – the ingress of diet-related chromogenic organic molecules, establishment of the acid-niche environment, and eventual failure of the restorative care.

The outstanding results of the Al Shorman H *et al* (Al Shorman *et al* 2003) paper, where the bacterial count of samples collected showed a bacterial reduction from 5.2×10^3 CFU/ml before treatment to 300 CFU/ml after the first O₃ application and then to 0 CFU/ml after the second application onwards, points to the use of ozone as the DUWL sterilisation method of choice and state-of-the-art. Con-current studies have shown that ozone does not interfere with dental material bond strengths or material retention (Campbell *et al* 2003 and Abu-Naba'a *et al* 2004).

The findings from the Campbell D *et al* (Campbell *et al* 2003) study were illustrated in the Holmes J study (Holmes 2004) where these bonding issues were further examined. After ozone treatment, resin bonding was carried out over *soft, previously-infected dentine*. This flies in the

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face to all conventional teaching with regards to cavity preparation and dentine bonding protocols. The results from the Holmes J study at 6-months showed that ozone treatment returned *alpha* scores on the UHPS criteria for all restorations placed in this way. It is argued that the incorporation of ozone into a dental unit will have a major impact on the standard of care delivered by a dentist with either limited investment in instrumentation, or those of mediocre skills.

Implications in Dental Surgery Wound Healing

In the Putnins EE *et al* (*Putnins et al 2001*) paper in 2001, the role of the infective biofilm in DUWL's was discussed with relation to surgery. As it was not possible to reduce the CFU count to zero, the authors concluded that the presence of high heterotrophic bacterial counts, sloughing biofilm, and high LPS levels posed a real risk to periodontal wound healing biology. This can be widened to include *any* form of dental surgery from tooth removal, to implant placement. Most dental practices in the western world use sterile saline, but in other areas of the world this is not available for routine use. The incorporation of an ozone generator would of course revolutionise not only the pre- and post- surgical aspects, but the surgical process itself. Ozone is known to encourage wound healing as well as control opportunistic infection (*Bocci 1994*).

Solutions to the Infective Biofilm

In a study from the United States of America in 1997 (*Murdoch-Kinch et al 1997*) the effectiveness of American Dental Association (ADA) recommended approaches for reducing DUWL contamination were investigated using newly installed dental units. Over a 2-month period, the authors studied independent water reservoirs, a sodium hypochlorite disinfection regimen, daily draining and purging of DUWL's and point-of-use filters by assessing microbial contamination and biofilm development using scanning electron microscopy. The findings demonstrate that DUWL contamination can be controlled when dental personnel use available technologies and adhere to recommended maintenance protocols.

In Jordan, Al-Hiyasat A *et al*, (*Al-Hiyasat et al 2007*) found that flushing the dental unit for 2 minutes significantly reduced the counts of *P. aeruginosa*, but flushing with infected water is not going to eliminate the biofilm, nor will it reduce the CFU count to zero.

Another study from the USA in 2002 (*Cobb et al 2002*) concluded that after four minutes of continuous flushing (the current ADA recommendation), all waterlines still harboured CFU levels that exceed current ADA recommendations. Cobb CM *et al* concluded that water flushing of DUWL's 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 CFU's after four minutes of continuous water flushing still exceeded the current ADA recommendations for acceptable levels of microorganisms.

Wirthlin MR *et al* (*Wirthlin et al 2003*) in their 2003 paper showed that chlorine dioxide waterline cleaners are effective in decontaminating DUWL biofilms. Chlorine dioxide has advantages over other chlorine products; it does not form carcinogenic compounds, has a long shelf-life in comparison with other products, and is not a strong irritant. These authors concluded that controlling DUWL biofilm would have beneficial effects on nosocomial infections.

Kohno S *et al* in 2004 (*Kohno et al 2004*) published their results that indicated acidic electrolyzed water could be applied as an appropriate measure against bacterial contamination of the DUWL. Montebugnoli L *et al* concluded in their 2004 paper that dental manufacturers should be invited

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to design dental units that incorporate automated devices to disinfect DUWL's between patients with minimal effort by dental staff (*Montebugnoli et al 2004*). Porteous NB *et al* urged dental practitioners in 2004 (*Porteous et al 2004*) to understand the limitations of available DUWL treatments, and to consider the use of sterile water for non-surgical, as well as surgical, treatment of immuno-compromised patients to reduce the risk of cross infection..

In 2005, Szymanska J (*Szymanska 2005*) concluded that the application of a disinfection product containing hydrogen peroxide caused a significant decrease both in the number of total fungi and individual fungal species. This confirmed his assertion that hydrogen peroxide was effective for fungal decontamination of DUWL's. In another paper from 2005 titled 'Microbiological Studies of Turbine Spray', Szymanska J commented that the application of a user-friendly water disinfectant to significantly decrease endotoxin concentration in the aerosol, is one of recommended methods to reduce health risk.

O'Donnell MJ *et al* (*O'Donnell et al 2006*) discussed a Water Management System, described as 'an integrated and automated DUW cleaning system'. This was investigated over a 12-month period. The system uses hydrogen peroxide- and silver ion-containing disinfectants in a once-weekly disinfection protocol.

Ozone (O₃) has been used for purification of water due to its efficiency and lack of side effects. It has been used in the medical profession since the late 19th Century to treat infections and aid wound healing. In the 1920's a Swiss dentist started to use O₃ as part of his disinfection system. The use of O₃ mushroomed until the inter-war periods, when the advent of cheap chlorine saw the use of O₃ decline. The pharmaceutical industry began to flood the market with the wide variety of anti-microbials we know today.

The vast majority of anti-microbial products act to kill micro-organisms over a period of time. This window of opportunity is used by bacteria to evolve resistance to these disinfection products, and modern health care now faces the problem of bacterial strains which are multiple-product resistant. The micro-organisms' host is beset with immunological conditions that lower the innate immune system's ability to contain and repel infection. And there is a trend to increased life span that requires pharmaceutical products for continued survival. The risk of cross-infection into this group of the population cannot be over looked.

Looked at in terms of; 'What is the perfect anti-microbial agent?' ozone would seem to fit the required profile. Ozone acts instantly, by oxidising bacteria, fungi, viruses, prions, and their effluent bio-molecules. Micro-organisms cannot evolve fast enough to develop resistance to O₃, so it remains the 'perfect' disinfection and sterilisation product to use. However, O₃ is not without its own issues. From a physical property perspective, O₃ is a very unstable gas, and has to be manufactured at the point of use. The equipment to deliver O₃ has an associated cost. But it is a one-time investment that is still more economical than disinfectant use. Ozone leaves no biocidal traces so the risk of contamination in bonding procedures is removed. The potential health risk with free O₃ in the oral cavity and the work place must be addressed, and this would be carried out as part of the risk assessment and design of the ozone system integrated into the dental unit.

In an early paper from 2002 (*Cardon et al 2002*) Cardon BE *et al* concluded that an ozonation system evaluated appeared to have no long-term benefit on DUWL biofilm control. However on closer reading, the concentration of O₃ used, 0.01 to 0.06 ppm, would not have been sufficient to lower high CFU levels or eliminate the DUWL biofilm. Not surprising, CFU values *after* O₃ treatment of excess 10,000 CFU were reported.

Al Shorman *et al* (*Al Shorman et al 2002*) used O₃ at a concentration of 2100 ppm. O₃ formed from air resulted in a bacterial reduction from 5.2×10^3 CFU/ml before treatment to 300 CFU/ml

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after the first O₃ application and then to 0 CFU/ml after the second application onwards. The authors commented on how low the concentration could be lowered and retain efficacy. Puttaiah R and Lin S (*Puttaiah and Lin 2006*) in an IADR abstract published in 2006 used 0.8 ppm of ozonated water as irrigant. At the end of week four all Units showed counts > 500 cfu/mL. They concluded that an initial cleaning with 60 ppm ClO₂ and use of 0.8 ppm O₃ mixed in water as irrigant controlled contamination up to 30 days.

In a follow-up study in 2003, Al Shorman H *et al* (*Al Shorman et al 2003*) compared hydrogen peroxide and O₃ DUWL decontamination. Hydrogen peroxide continuously produced water with a TVC of less than 100 CFU/mL. The TVC of water from the control unit was 2.3×10^4 and 3.4×10^4 CFU/mL after 1 and 2 weeks of installation. After the first O₃ treatment the TVC was reduced to 60 CFU/mL and rose to 3.9×10^4 CFU/mL after a week with few *Pseudomonas* colonies. After two weeks, TVC was 2.8×10^3 CFU/mL and became 0 CFU/mL after the treatment. Repeated sampling of the unit for 9 weeks showed 0 CFU/mL. Flushing with water could not maintain a CFU or TVC value within acceptable potable water standards (200 CFU).

Summary of this Paper Review

1. Manufacturers should be invited to design dental units that incorporate automated devices to disinfect DUWL's between patients with minimal effort by dental staff (*Montebugnoli et al 2004*).
2. After four minutes of continuous flushing, all waterlines still harboured CFU levels that exceed current ADA recommendations. It is concluded that water flushing of DUWL's produced a statistically significant reduction in planktonic bacteria at each time interval compared to the baseline and between each successive time interval (*Cobb et al 2002*). However, the level of CFU's after four minutes of continuous water flushing still exceeds the current ADA and European recommendations for acceptable levels of microorganisms.
3. Choices are available for preventing and controlling waterline contamination, but some of them require a substantial commitment by personnel charged with maintenance of the waterlines. Other approaches and technologies should be developed and tested. If approved by the appropriate agencies, they will offer even more preventive choices. It is fortunate that there are multiple options available, but currently each protocol requires a serious commitment for follow-through (*Molinari 1999*).
4. Dental practitioners need to understand the limitations of available DUWL treatments, and to consider the use of sterile water for non-surgical, as well as surgical, treatment of immunocompromised patients (*Putnins et al 2001, Porteous et al 2004*).
5. The use of ozone as the method of disinfection would offer the best solution as part of an integrated approach to dental care. Indeed, O₃ would seem to offer the opportunity of unit sterilisation which is a very different approach to dental unit care. Ozonated water exceeds all current standards for water quality in DUWL's at high enough concentrations.

Conclusion

Studies from Europe (*Abu-Salem et al 2003; Baysan and Lynch 2001; Holmes 2003; Holmes and Lynch, 2003*) have shown conclusively that the use of O₃ in dental care is effective as a non-destructive method to manage decay and its destructive effects. The use of O₃ has been shown to be the ideal way to manage anxiety of patients – young and old - and their carers (*Dahnhardt et*

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al, 2003; Domingo et al, 2004).

The effects of O₃ reduce tooth destruction in routine preparation (*Clifford, 2004; Holmes, 2004; Holmes and Lynch, 2004*) and O₃ reduces the time and the cost of dental care (*Domingo and Holmes, 2004; Johnson et al, 2003*) and raises the practice income. In Endodontics, O₃ is effective against *Enterococcus faecalis* (*Chang et al, 2003*) which is implicated in endodontic treatment failure. O₃ does not interfere with dental material bond strengths, and there is evidence that it increases material retention (*Abu-Naba'a et al 2004, Campbell et al 2003*).

Bocci V (*Bocci 1994*) has emphasised that the potential toxicity of O₃ should not preclude its employment for medical, dental & veterinary purposes. This statement has been echoed by thousands of health professionals who use ozone in clinical practices around the world, and millions of patients that have been treated. The results of these studies show that ozone reduces the necessity for filling materials of unknown long-term potential toxicity.

The use of ozone as the method of disinfection would offer the best solution as part of an integrated approach to dental care. Indeed, O₃ would seem to offer the opportunity of unit sterilisation which is a very different approach to dental unit care. Ozonated water exceeds all current standards for water quality in DUWL's at high enough concentrations.

The integration of an ozone generator into a dental unit opens the way for the dental profession to access the ozone technologies for the treatment of caries. As part of a dental treatment unit, ozone can easily be integrated into routine dental care. This aspect of dental and health care has been reported in previous papers by Holmes J Lynch E and Filippi A. Over 40 papers and 500 abstracts are presented in an HTML-based book authored by Holmes J and published in 2007 (*Holmes J 2007*).

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