

Ozonated Liquids in Dental Practice – A Review.

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Part 5: Dental Unit Water Lines (DUWL's). A Review of The Problem & Solutions.

Abstract: In Part 5 of *Ozonated Liquids in Dental Practice*, the formation of biofilms in water pipes is examined. The research examining contamination from mains water and ingress of micro-organisms from patients into Dental Unit Water Lines (DUWL's) is discussed. Published research suggests biofilms and their potential for cross-infection present health hazards to the health care worker and other patients during treatment. Detachment of micro-organisms 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 (*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. 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.

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 micro-organisms from the water. Once they become sessile, the micro-organisms 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 micro-organisms can signal one another, transfer nutrients, and exchange genetic material. The insoluble exopolysaccharides shield the micro-organisms from displacement and from penetration by predator organisms, antibiotics, and disinfectants. The external surface layer of micro-organisms is faster growing and may detach as "swarmer" cells. Detachment of micro-organisms 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- 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 (*Barbeau & Nadeau 1997*):

- 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.

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, cross-infection and survival.

DUWL's are ideal environments for the growth of micro-organisms 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 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 micro-organisms 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*) and puts a barrier between the clinical staff and the patient and carer when present.

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

In Ireland, Al Shorman *et al* (*Al Shorman et al 2003*) and in Jordan Al-Hiyasat *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 *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 *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 *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 *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-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 *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 (*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 *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.

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 *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 *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 O_3 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 *et al* (*Campbell et al 2003*) study were illustrated in the Holmes 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 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 *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 ozone 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 and prevent 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. However, employee compliance with instructions is an issue, in the same way the dental profession whinge about patient compliance to oral hygiene instructions and dietary advice.

In Jordan, Al-Hiyasat *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 micro-organisms.

Wirthlin *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.

A study published by Nagayoshi, Fukuizumi *et al* (Nagayoshi, Fukuizumi *et al* 2004) examined the effect of ozonated water on oral micro-organisms and dental plaque. Almost no micro-organisms were detected after being treated with ozonated water (4 mg/l) for 10 s. When the experimental dental plaque was exposed to ozonated water, the number of viable *S. mutans* remarkably decreased. These researchers noted that ozonated water strongly inhibited the accumulation of experimental dental plaque *in vitro*. After the dental plaque samples from human subjects were exposed to ozonated water *in vitro*, almost no viable bacterial cells were detected. These results suggest that ozonated water should be useful in reducing the infections caused by oral micro-organisms in dental plaque.

A further study by Nagayoshi, Kitamura *et al* (Nagayoshi, Kitamura, *et al* 2004) examined the effect of ozonated water against *Enterococcus faecalis* and *Streptococcus mutans* infections *in vitro* in bovine dentin. After irrigation with ozonated water, the viability of *E. faecalis* and *S. mutans* invading dentinal tubules significantly decreased. These researchers concluded that used with ultrasonic instrumentation, ozonated water application may be useful for endodontic therapy.

Kohno *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 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 *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 (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 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 *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 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 Dr Edwin Parr, 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* (Cardon *et al* 2002) 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.

In 2004, Young and Setlow (Young and Setlow 2004) determined that ozone does not kill spores by DNA damage. Rather, ozone seems to render the spores defective in germination, perhaps because of damage to the spore's inner membrane. They reported that ozone does not cause damage to the spore's DNA, as wild-type spores were not mutagenised by ozone and wild-type and recA spores exhibited very similar ozone sensitivity. Spores (termed alpha-beta-) lacking the two major DNA protective alpha/beta-type small, acid-soluble spore proteins exhibited decreased ozone resistance but were also not mutagenised by ozone, and alpha-beta- and alpha-beta-recA

spores exhibited identical ozone sensitivity. Killing of spores by ozone was greatly increased if spores were chemically decoated or carried a mutation in a gene encoding a protein essential for assembly of the spore coat.

Young and Setlow also reported that ozone-killed spores did not germinate with either nutrients or Ca(2+)-DPA and could not be recovered by lysozyme treatment. These workers concluded the major factor in spore resistance to sterilisation agents appears to be the spore coat. Spore killing by ozone seems to render the spores defective in germination, perhaps because of damage to the spore's inner membrane.

This study underlines that concerns that ozone may cause mutations in cells may be unfounded, despite the production of radicals in water and fluids.

Al Shorman *et al* (Al Shorman *et al* 2002) used O₃ at a concentration of 2100 ppm bubbled into 1 litre of water over a 10-minute time period. O₃ formed from dry air resulted in a bacterial reduction from 5.2*10³ CFU/ml before treatment to 300 CFU/ml 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 and Lin (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 *et al* (Al Shorman *et al* 2003) compared hydrogen peroxide and O₃ DUWL decontamination. Hydrogen peroxide continuously produced water with a Total Viability Count (TVC) of less than 100 CFU/mL. The TVC of water from the control unit was 2.3 x 10⁴ and 3.4 x 10⁴ 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 x 10⁴ CFU/mL after a week with few *Pseudomonas* colonies. After two weeks, TVC was 2.8 x 10³CFU/mL 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).

In 2007, Shenberg *et al* (Shenberg *et al* 2007) showed ozone is extremely reactive towards selected carious dentine biomolecules, and such reactions are likely to be of relevance to its reported microbiocidal activity. High resolution proton (1H) nuclear magnetic resonance (NMR) spectroscopy was used to determine the nature and extent of the oxidation of biomolecules present in carious dentine, plaque and saliva. Experimental samples were treated with ozonated (2 ppm) water. These results mirrored previous studies (Holmes 2003 and Holmes 2003) where ozonated water showed marked reductions in volatile sulphur compounds. In these earlier studies, the Halimeter, a volatile sulphur detection system, was used. In the Shenberg 1H NMR study, ozone was shown to attack:

- a-D-glucose, giving rise to formate as it's by-product
- pyruvate with acetate and CO₂ via an oxidative decarboxylation process
- amino acid volatile sulphur compound precursors cysteine and methionine were oxidatively transformed to their corresponding primary oxidation products, cystine and methionine sulphoxide respectively

These results are similar to the published ¹H NMR studies from previous years where carious tissue samples were treated with ozone. The Shenberg study shows that ozone dissolved in water has the ability to denature bio-molecules seen in active decay and also found in oral saliva.

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 micro-organisms.
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 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, Nagayoshi, Fukuizumi *et al*, 2004) 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 (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.

As part of a dental treatment unit, ozone can easily be integrated into routine dental care. Some detractors have tried to suggest that the regular usage of ozone could cause mutations in human cells. But what if mankind as we understand him/her now *is* that mutation? The energy houses of the human cell, mitochondria, are known remnants from a past symbiotic relationship with bacteria. Should mankind be surprised that we also have mutated in other areas at a cellular level?

In 2004, Young and Setlow (*Young & Setlow 2004*) determined that ozone does not kill spores by DNA damage. Rather, ozone seems to render the spores defective in germination, perhaps because of damage to the spore's inner membrane.

Young and Setlow's published research reported that ozone does not cause damage to the spore's DNA, as wild-type spores were not mutagenised by ozone and wild-type and recA spores exhibited very similar ozone sensitivity. Spores (termed alpha-beta-) lacking the two major DNA protective alpha/beta-type small, acid-soluble spore proteins exhibited decreased ozone resistance but were also not mutagenised by ozone, and alpha-beta- and alpha-beta-recA spores exhibited identical ozone sensitivity. Killing of spores by ozone was greatly increased if spores were chemically deoated or carried a mutation in a gene encoding a protein essential for assembly of the spore coat.

Young and Setlow also reported that ozone-killed spores did not germinate with either nutrients or Ca(2+)-DPA and could not be recovered by lysozyme treatment. These workers concluded the major factor in spore resistance to sterilisation agents appears to be the spore coat. Spore killing by ozone seems to render the spores defective in germination, perhaps because of damage to the spore's inner membrane.

The Young and Setlow study would seem to suggest concerns that ozone may cause mutations in cellular DNA may be unfounded, despite the production by ozone of radicals in water and fluids. This study also demonstrates the important role ozone plays in the sterilisation of water, fluid lines and the reduction of the potential for cross infection.

The referenced research for the dental use of ozonated fluids date back to the 1950's. Wuhmann and Meyrath examined the bactericidal effect of aqueous ozone solutions (*Wuhmann and Meyrath 1955*), effectively repeating the observations of Dr Edwin Fisch in 1932. In the 1960's. Onouchi in 1965 (*Onouchi 1965*) examined the bactericidal action of aqueous solution of O₃ in dentistry.

The research into ozonated fluids have examined the effects of drinking ozonated water on gut infections (*vanden Bossche et al 1994*), and Khadre and Yousef (*Khadre and Yousef 2001*) examined the sporicidal action of ozone and hydrogen peroxide. This study built on the earlier work of Vestergard (*Vestergard 1994*) who was looking at establishing and maintaining pathogen free conditions in aqueous solutions using ozone. Vestergard's paper examined the use of ozone in space applications for the elimination of pathogens using ozone. Vestergard's area of research

was creating pathogen free conditions in aqueous solutions containing organic matter. This research, although concerned with hydroponic agricultural systems, can be carried into general potable water studies and water distribution networks, including DUWL's.

Studies to look at increasing the solubility of ozone in fluids have identified that the use of ultrasonics (*Zhang et al 2007*) increases ozone solubility, and allows the use of less powerful ozone generators.

Dental researchers have started to examine the effects of ozonated fluids in periodontal disease. Huth *et al* in two papers in 2006 and 2007 (*Huth et al 2006*, *Huth et al 2007*) examined the effect of ozone on periodontal tissues. The 2007 paper compared traditional periodontal anti-microbial products with the use of ozonated water. Both papers concluded that ozonated water has an excellent anti-microbial effect.

Huth *et al* (*Huth et al 2007*) in their later paper examined the effect of ozone on the influence on the host immune response. These researchers chose the NF-kappaB system, a paradigm for inflammation-associated signaling/transcription. Their results showed that that NF-kappaB activity in oral cells in periodontal ligament tissue from root surfaces of periodontally damaged teeth, was inhibited following incubation with ozonized medium. The Huth 2007 study establishes a condition under which aqueous ozone exerts inhibitory effects on the NF-kappaB system, suggesting that it has an anti-inflammatory capacity (*Huth et al 2007*). The use of ozonated water in dental ultrasonic systems, such as scalers, sonic preparation systems (KaVo Sonic-Sys, KaVo GmbH, Germany) and air abrasion systems would seem to be supported by *Zhang et al 2007*, *Huth et al 2006* and *Huth et al 2007*.

There are many benefits to drinking ozonated water, to control oral hygiene and as a source of sterile water. However, patients should also be informed that there is an interaction of aqueous ozone with anti-microbials. This research has been published, illustrating the importance of potential interactions of dissolved ozone and prescribed anti-microbials. Patients who are taking a course of antibiotics may need to be informed that the use of ozonated water inactivates antibacterial agents (*Dodd et al 2006*) and in particular amoxicillin (*Andreozzi et al 2005*), progesterone (*Barron et al 2006*) and tetracycline (*Dalmázio et al 2007*). For concern to dentists is that ozone may inactivate the anti-microbial effects of triclosan (*Suarez et al 2007*).

A current topic of debate in dental material science and long term potential effects, are endocrine disruptors found in resin-based dental restorative materials. *Deborde et al* (*Deborde et al 2005*) showed endocrine disruptors were destroyed by ozonated water. This paper potentially points towards a pathway to remove these chemicals from the body system after placement of 'modern' tooth-coloured or 'white' fillings.

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