

**MICROBIAL AND BIOFILM CONTAMINATION OF DENTAL UNIT WATERLINES- VARIOUS APPROACHES TO RISK REDUCTION**

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**ABSTRACT**

The unique feature of dental chair water lines is that they rapidly develops a biofilm on the dental water supply lines combined with the generation of potentially contaminated aerosols. The quality of dental unit water is of considerable importance because patients and dentist are regularly exposed to aerosols and water generated from the dental unit. Dental water may become heavily contaminated with

opportunistic respiratory pathogens such as Legionella. The main aim of infection control is to reduce the risk from exposure to potential pathogens and to create a safe working environment in which to treat patients. This review article is aimed to evaluate the range of currently available infection control methods and prevention strategies which are designed to reduce the impact of the biofilm on dental water contamination.

**KEYWORDS:** Dental unit waterlines, Biofilm, Contamination, flushing.

**INTRODUCTION**

Safety of dental patients and dental personnel requires an appropriate microbiological quality of water used in dental units, flowing from working handpieces; water cools dental

equipment and rinses oral tissues. During dental treatment, patients and personnel are exposed both to direct contact with bacteria-contaminated water in the form of splatter and with contaminated water aerosol emitted during work by unit handpieces, including rotating and ultrasonic instruments.<sup>[1]</sup> The aim of providing dental unit water that can be used safely with all patients has become a priority issue world-wide uniting dental governing bodies, research scientists and dental equipment manufacturers. There is a lack in public confidence in municipal water quality which has been illustrated by an exponential increase in sales of bottled water in many countries.<sup>[2, 3]</sup> A high level of microbial contamination, presence of opportunistic microorganisms and bacterial endotoxin associated with Gram-negative bacteria are the most important health risk factors transmitted by water from dental units.<sup>[4]</sup> Dental water may be ingested, inhaled in the form of aerosols or directly contaminate surgical wounds. Dentists have a duty of care to their staff and patients. It is indeed ethically unacceptable to expose patients to contaminated water knowingly. Microbial composition of water exiting from unit of working handpieces depends on the microbiological quality of water flowing into a unit, but also, as many researchers stress, by the biofilm present on the walls of tubing that constitutes dental unit waterlines.<sup>[5]</sup> Guidelines on preventive measures for reducing dental unit water contamination have been issued by government agencies such as the CDC Atlanta, USA, the main aim of which is flushing of dental units.<sup>[6]</sup> The overall opinion is that water used for restorative procedures should be of the same quality 'as for drinking water'. Separate sterile water supplies are advised for surgical procedures.<sup>[7]</sup> Coolant water or saline used for surgical procedures should be sterile, and should not be contaminated during delivery. Devices used to deliver the sterile water must be sterilized before use for invasive procedures. This review article aim is to bring in the notice that why contamination of the dental unit water occurs, as well as it is also helpful in assessing the relative risk of contaminated water and aerosols to dental surgery staff and patients.

### **Biofilms in Dental Unit Water**

The term 'biofilm' refers to the development of microbial communities on submerged surfaces in aqueous environments. The growth of biofilm is considered to be a result of complex processes facilitated by production of extracellular polymeric substances (EPS), often referred to as glycocalyx or slime.<sup>[8]</sup> The patterns of dental water lines are such that they lead to stagnation of the entire water column within the waterlines for extended periods during the day, which promotes further undisturbed bacterial proliferation. Dental units are equipped with microbore (approximately 1mm diameter), and this narrow bore tubes are

inaccessible to mechanical debridement processes which is generally used to maintain the functional integrity of wide bore industrial style water supply lines. Bacteria adhere more readily to hydrophobic polymeric plastic tubing like polyvinyl chloride, polyurethane which are utilized in dental equipment, than to those composed of glass or steel.<sup>[9]</sup> Susceptibility of medical equipment such as catheters to biofilms has been reduced by coating with heavy metals or incorporating biocides into the fabric of the tubing that inhibit bacterial growth.<sup>[10]</sup> Biofilm are predominantly derived from the incoming mains water. Once a new dental unit water system is connected to mains water supply, even when it is not used for patient treatment, a biofilm will form within 8 hours.<sup>[11]</sup> The biofilm will develop to reach a climax community of micro colonies embedded in a protective extracellular amorphous matrix by 6 days<sup>[11]</sup>. Bacteria shed from the biofilm during use maintain the bio-burden of planktonic (suspended) organisms detected in dental unit water. Characteristically biofilm bacteria exhibit greater resistant to surfactants, biocides and antibiotics than organisms floating freely in fluids.<sup>[12]</sup> The physics of laminar flow of dental unit water passing through the waterlines results in maximum flow at the centre of the lumen and minimal flow at the periphery, encouraging deposition of organisms onto the surface of the tubing.<sup>[13]</sup>

### **Risks to dental surgeons**

In general all the attention and precaution are given to the patient but the clinical members of the dental team inhale aerosols generated by dental equipment on a daily and long-term basis. Unfortunately, in common with the public, working dentists also have increased susceptibility to infection due to underlying disease or drug therapy. Abnormal nasal flora in dental personnel has been linked to water system contamination.<sup>[14]</sup> Studies have indicated that the clinical dental team experience an increased prevalence of respiratory infections compared to the general population or their medical colleagues.<sup>[15]</sup> Employing PCR methodology *Legionella* spp have been detected in 68 per cent of dental unit water samples and *L. pneumophila* in 8 per cent. High concentrations of *Legionella* are usually required to initiate infection. Rates of seropositivity for *Legionella* antibodies are demonstrably higher amongst dental personnel than in the general Population.<sup>[16]</sup> Additionally, the magnitude of *Legionella* antibody titres correlated directly with the duration of time spent carrying out clinical work, suggesting that aerosols generated from dental unit water are the likely source.<sup>[17,18]</sup> Conversely, a survey carried out by Central Public Health Laboratory, London found no evidence that previous dental treatment was a risk factor in patients with legionellosis.<sup>[19]</sup> A single case of fatal pneumonia in a dentist from *L. dumoffi* has been

reported. However, the possibility still remains that dental unit water -associated infections have gone unrecognised or unreported because of the failure to associate exposure to dental unit water or aerosols with the development of specific infections. Sporadic infections not requiring hospital admission, such as Pontiac fever also caused by *Legionella*, are less likely to be investigated or notified to health authorities.

### **Risks to patients**

There is no evidence of a widespread public health problem from exposure to dental unit water. Nevertheless, the goal of infection control is to minimise the risk from exposure to potential pathogens and to create a safe working environment in which to treat patients. The ever increasing number of patients who are either immunocompromised or immunosuppressed due to drug therapy, alcohol abuse or systemic disease has produced a cohort of patients susceptible to environmental waterborne opportunistic pathogens such as those prevailing in dental unit water. The organisms recovered from dental unit water vary with geographic location.

They include fungi like *Cladosporium*, free living amoebae, protozoa like *Microsporidium* and nematodes as well as the consistently reported recovery of saprophytic and opportunistic gram negative pathogens such as *Pseudomonas*, *Klebsiella* and *Flavobacterium*.<sup>[20]</sup> The latter species are capable of thriving in low temperature and low nutrient environments including distilled water. Only *Pseudomonas aeruginosa* derived from dental unit water has definitely been shown to cause infection. Two patients with solid tumours were unwittingly exposed to dental unit water contaminated with *P. aeruginosa*. Both patients subsequently developed oral abscesses which pyocine typing confirmed were caused by the same strain isolated from the dental unit water.<sup>[21]</sup> Of particular concern are the primary respiratory environmental pathogens found in dental unit water that can cause pneumonia, milder flu-like respiratory infection and, less commonly, wound infections for example, *Legionella pneumophila* and non-pneumophila spp as well as *Mycobacterium* spp including *Mycobacterium avium*. *M. avium* can cause disseminated infection in HIV seropositive patients following ingestion and colonisation of the gut.<sup>[22]</sup> Numbers of non-tuberculous mycobacteria in dental unit water exceeded that of drinking water by a factor of 40018. High numbers of non-tuberculous mycobacteria may be swallowed, inhaled or inoculated into oral wounds during dental treatment with the potential for colonisation, infection or immunisation. Priming of the immune system by exposure to environmental non-tuberculous mycobacterium helps to

maintain the anti-tuberculin immune response.<sup>[24]</sup> The true extent of the risk posed by non-tuberculosis mycobacteria in dental unit water to the immunocompromised patient has yet to be fully elucidated. Similarly, the primary pathogen *Acanthamoeba* is recovered from dental unit water and biofilms. They are reputed to cause amoebic keratitis in contact lens wearers who clean their lenses in tap water.<sup>[25]</sup> It is unknown whether they present a risk in the dental setting but routine use of protective eye wear by both the dental team and patients should shield the eyes from any possible exposure.

### **Approaches to risk reduction**

#### **A) Filtration**

Recent publications and original research have demonstrated that high levels of recontamination of dental unit water occur within 24 hours as a result of trapping and growth of bacteria on the filters.<sup>[26]</sup> The use of filters on the dental waterline to eliminate bacteria from the water entering the handpiece was first described 20 years ago to reduce planktonic bacteria.<sup>[27]</sup> Therefore disposable filters are recommended, which must be changed daily.<sup>[28]</sup> For maximum efficiency, filters should be inserted just distal to the point of entry of water into the handpiece. Filters have no impact on biofilm formation. While disposable filters are a promising method of improving water quality their clinical effectiveness has not yet been fully established. Nevertheless, prevention of planktonic bacteria from entering the handpiece from the waterline will reduce patient exposure to harmful pathogens. Filters should also reduce retrograde contamination.

#### **B) Flushing**

Water lines should be flushed through for 'several minutes' at the start of each clinic day and it is recommended by both American Dental Association's and British Dental Association's to substantially reduce microbial accumulation caused by overnight stagnation in the waterlines.<sup>[29]</sup> As well as, in order to minimize exposure to aerosols the procedure can be best done in combination with high velocity evacuation into an enclosed container. Discharging the stagnant water improves the perceived quality of the water and reduces the malodor and bad taste imparted to the water by microbial contamination. It will also draw through low concentrations of chlorine (0.1 to 0.5ppm) normally present in mains water. However, it is recognized that flushing provides only temporary reductions in bacterial load and has no effect on the biofilm. As a result of the physics of the laminar flow in the waterline, the layer in immediate contact with the biofilm is stationary even during flushing. The effectiveness of

flushing has been challenged by a number of authors who report bacterial clearance was both variable and minimal when used for short periods of time (<10 min),<sup>3</sup> and in one report actually increased post-flush.<sup>[31]</sup> In most studies bacterial load was not consistently reduced to the desired standard of less than 200 cfu/ml. Flushing for 20 minutes, which would be impractical in most dental surgeries, will reduce the bacterial count to zero.<sup>[30]</sup> However, the persistent nature of the contamination is demonstrated when 30 minutes later, shedding of bacteria from the biofilm returns the total colony counts to within the pre-flush range.<sup>[32]</sup> Flushing was introduced as a simple and expedient measure that could be instituted immediately as a stop-gap procedure in all dental surgeries of whatever age or type, without the need to purchase additional equipment. As described above flushing is valuable in eliminating retrograde aspiration of oral fluids.

### **C) Biocides and chemical disinfectants**

Biocides (compounds with lethal activity against living organisms) have been used in an attempt to remove the biofilm and eliminate the planktonic bacterial count and their use has met with a limited degree of success. These include povidine iodine.<sup>[33]</sup>, hypochlorite.<sup>[34]</sup>, peroxide<sup>[35]</sup>, chlorhexidine gluconate.<sup>[36]</sup> The intrinsic resistance of the biofilm ecosystem has hampered their value. Ideally, biocides require a broad spectrum of anti-microbial activity, to be non-toxic to individuals and non-pollutant to the environment. In certain States in the USA there are restrictions on dental surgeries discharging their waste water into the municipal sewage system because of the fear of chemical pollution, the effective delivery of approved disinfectants can control the level of microorganisms in DUWs at acceptable levels.<sup>[37]</sup> Such system should also be implemented in India.

### **D) Chlorination**

Sodium hypochlorite (Chlorine) is the most commonly used biocide in water treatment plants which has proven efficacy in cold water hospital systems, specially in controlling Legionella proliferation.<sup>[38]</sup> Chlorine can be added to the water at the central supply intake. This approach is mainly applicable to larger multiple surgery or in Dental Hospitals and universities. Independent reservoir clean water systems can also be used to deliver chlorine flushes to the dental water line. Alternatively a continuous chlorination system can be installed, with an automatic dosing mechanism providing 1ppm chlorine at the chair. Chlorination was found to be effective in maintaining drinking water standards in the storage tank and distribution pipes.<sup>[26]</sup> Equivocal results have been obtained from measurements of

dental unit water with some studies reporting bacterial counts reduced to a few hundred, and others finding only temporary remission in contamination and no elimination of *L. pneumophila*. When legionellae are sequestered within free-living amoebae there is a 30–120-fold increase in chlorine resistance, thus explaining the failure to eradicate the organism from the system.<sup>[39]</sup> Resistance to biocides will develop in the bacterial population with extended exposure. Potentially, higher doses of 3–5ppm could overcome these problems, but high chlorine levels are unpalatable, and long term corrosion damage occurs with free residual chlorine levels as low as 1ppm.<sup>[26]</sup> In addition, high levels of chlorine are associated with in vitro formation of trihalomethanes. However, these problems apply to chlorinated water for clinical use. Higher doses could be used in the independent water systems as a flush to remove planktonic bacteria, as the apparatus is purged of chlorine before patient use. Corrosion of metal components would still be a problem. Glutaraldehyde is available for use with an integral, automated flush system with a contact time of 7 minutes<sup>9</sup>. Glutaraldehyde is a highly effective disinfectant with bactericidal action against most vegetative bacteria, mycobacterium and viruses but it's sensitization of the human lung and skin has severely limited the use of this compound in dentistry except in situations where exhaust ventilation can be assured. Bacteria within the biofilm pose a major stumbling block to the use of biocides. They are 3,000 fold less susceptible to hypochlorite and so they are not readily degraded even by concentrated solutions of bleach or of other disinfectants such as glutaraldehyde. Planktonic organisms will be destroyed but even if the majority of the organisms in the biofilm are eliminated the architecture of the biofilm survives and acts as a pre formed matrix for renewal of the biofilm. Inactivation of biocides is further impaired by interaction with organic material and electro-repulsion caused by surface charges on the biofilm. For the future, 'electro-enhancement' of biocides producing neutralization of the surface charge may be incorporated into medical equipment to resolve the problem of buildup of biofilm.<sup>[40]</sup>

#### **E) Peroxide, ozone and ultraviolet light**

Bacteria from the biofilm are shed continually while the film is in contact with water. Hydrogen peroxide has been used in dentistry as a bleaching agent, and root canal irrigant as well as in dentifrices and mouth rinses. It has found favor as a disinfectant (7 per cent solution) for flexible endoscopes where the efficacy was found to be comparable with that of 2 per cent glutaraldehyde and in the disinfection of contact lens cases it was reported to be more effective against biofilms.<sup>[41]</sup> An FDA approved delivery system for hydrogen

peroxide is commercially available which provides metered, microprocessor controlled, continuous release of stabilized peroxide into the water line. UV treatment of water has been used alone and in conjunction with ozone and other biocides for control of legionellae and reduction of endotoxins in water cooling towers and water treatment plants, for example for swimming pools. UV would appear to be an attractive, non-polluting alternative for point of entry of mains water purification. However, evidence that UV irradiation alone has a significant effect on reducing microbial contamination is equivocal due to the relative resistance of some important waterborne pathogenic species. A major advantage of these systems is that they avoid introducing chemical disinfectants into the effluent water system with the potential for pollution and destructive effects on wildlife.

#### **F) Anti-retraction valves and retrograde aspiration of oral fluids**

Anti retraction valves (also known as check valves) will limit re-aspiration and are most efficacious when fitted immediately distal to the handpiece. As with any component of the water supply line they are subject to clogging due to biofilm deposition and fatigue<sup>13</sup>. In order to ensure adequate mechanical functioning, they require regular maintenance and programmed replacement. Autoclaving of handpieces after use and flushing of units for 30 seconds at the end of patient treatment and for 2.5 minutes at the end of the day will augment the action of the anti retraction valve and should help to eliminate any aspirated fluid.<sup>[42]</sup> Some manufacturers have incorporated anti-retraction valves within the handpiece design permitting autoclaving of the valve between patients. Backflow from dental units to the mains water supply may occur and it may be necessary to install check-valves to prevent this occurring.<sup>[43]</sup> There is little evidence to support this notion as low colony counts at drinking water concentrations are recovered from the plumbing to the water faucets in the dental surgery. However, dentists are required to comply with locally enforced water supply and sewage regulations. Studies have shown that the overwhelming majority of the anti-retraction devices did not prevent retraction when the turbine stopped running, leading to a contamination of the water lines, and to a consequent possible cross-contamination of the patients.<sup>[44]</sup>

#### **G) Autoclavable systems**

In response to the evolving high standards for quality control and prevention of dental unit water contamination, a fully autoclavable assembly of water reservoirs, silicon multi lumen dental unit waterline tubing and fittings to be sterilized between patients has been produced

and has been cleared for marketing by the Food and Drug Administration, in the USA<sup>9</sup>. Such devices should, if manufacturers' instructions are fully adhered to, remain free from biofilm build up, as any contamination from retrograde aspiration or from skin organisms during manipulation should be destroyed during autoclaving. Autoclavable systems may be the solution to providing secure, sterile water systems.

## CONCLUSIONS

Since the origins of dental unit water contamination are now more clearly defined, substantial progress can be made by dental manufacturers and the scientific community in approaches to prevention and control. Due to the multiple ports of entry to the dental unit water system for microbes, no single method or device will completely eliminate the potential for cross infection. Combinations of currently available procedures and equipment, including ant retraction devices, flushing, independent water supplies used in conjunction with biocide purges or fully autoclavable water line circuitry should provide water which is of a higher standard than that of drinking water. All these systems require strict adherence to maintenance protocols to perform to their full potential. Chair side devices for monitoring microbial quality of the dental unit water need to be developed and are an essential component to assure satisfactory water quality. Future research into the prevention of biofilm proliferation is being actively promoted by the American Dental Association and other dental organizations and government agencies around the world saviors.

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