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MICROBIAL CONTAMINATION OF DENTAL UNIT WATER LINE

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ABSTRACT

Dental unit waterlines (DUWL) are an integral part of dental surgery equipment, supplying water as a coolant, primarily for air turbine and ultrasonic scalers. Dental unit water lines (DUWL) used to irrigate the oral cavity during routine dental practice is highly contaminated. Concern about indoor air quality is increasing, and more attention is being laid on the aerosol and splatter produced during dental procedures [1]. The source of bacterial contamination within the dental unit water supply is thought to be caused by micro-colonies of proliferating bacteria, fungi and protozoa on the inner surface of the water lines, forming a biofilm[2].The unique feature of dental chair water lines is the capacity for rapid development of a biofilm on the dental water supply lines combined with the generation of potentially contaminated aerosols. The present review emphasizes the underlying biological cause of waterline colonisation by microorganisms, the evidence of potential health consequences and possible means of improving the quality of dental water.

KEYWORDS: Dental unit water line, Contamination, Biofilm

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INTRODUCTION

The various apparatuses used by modern dentists in their offices in treating patients includes dental chair units, triple syringe system, high speed handpieces, ultra sonic scalers etc [1]. Water from dental units (DU), used for cooling and clearing the field of dental operations, is frequently contaminated by microorganisms and provides suitable habitat for various forms of organisms including microbes and fungi [2]. Modern dental chair units consist of a network of interconnected narrow-bore plastic tubes called dental unit waterlines (DUWLs) [1]. One common condition that these dental units possess is that they are always in contact with water to provide a suitable condition for the organisms. In order to work properly water-linings of these dental instruments are installed throughout the clinic from the city-water output to the primary filtering system to each dental chair unit, which has the secondary filtering system [3]. Theseunits, however are left unused and their powers are off during the night, which aggravates the condition of the dental water line units or facilitates the growth of bacteria in these parts [4]. Such conditions lead to biofilm formation, which is nothing but a community of microcolonies. Contamination in dental water line is mostly due to biofilm formation [5]. The small diameter of dental water lines, accompanied by periods of prolonged water stagnation, provides a suitable ecological niche for biofilm development and the proliferation of microorganism[6].

The water delivered by these DUWLs acts both as a coolant for a range of instruments and an irrigant during dental treatments. The quality of water is of considerable importance because both patients and dental team are regularly exposed to water and aerosols generated by dental equipment[7]. The provision of dental unit waterline (DUWL) that is safe for use with all categories of patients is now an essential issue world-wide that units both Dental Governing Bodies and dental equipment manufactures. Several studies [8,9,] have indicated that dentists and dental staff have higher rates of respiratory infection than the general public. Contaminated handpieces are believed to be at least partially responsible for these higher rates of respiratory disease [10]. Appropriate procedures to decontaminate handpiece, includingautoclaving and handpiece replacement between patients; have been developed and implemented in dental practices [11, 12]. These procedures are implemented to reduce the likelihood of aerosol dissemination of pathogens. However, the dental handpiece requires the adoption of strict decontamination procedures to ensure its continued safe use. Dental handpieces recognized as a means of cross infection accumulates contaminated debris which acts as carrier that favours patient–patient transmission[13].

CONTAMINATION OF DUWL

More than 25 different bacterial species as well as several species of fungi, and protozoa have been isolated from DUWL; the actual species depends on the geographic location was evidenced from a study observed in dental unit water samples from 150 operatories by JF Williams *et al.,* [14] revealed widespread and unacceptably high levels of contamination due to biofilm formation along the walls of the fine-bore waterlines. Predominant are the environmental Gram negative bacteria. DUWL may also harbour smaller numbers of opportunistic pathogens which are responsible for respiratory disease, namely *Pseudomonas aeruginosa*, *Legionella pneumophila* was evidenced from the study of Atlas *et al* ., in dental unit water. Contamination is not thought to be entirely environmental, as retrograde flow from the oral cavity may contribute to a lesser degree to the microbial biofilm in the waterlines. In the past, oral bacteria such as Streptococci were commonly isolated from dental waterlines due to retrograde movement of irrigant water and saliva into the handpiece on releasing the foot pedal, whereas modern handpieces normally incorporate an anti-retraction valve, which prevents suck-back of oral microbes and hence reduces the risk of contamination from this source. If the valve is

not fitted or malfunctioning, it is estimated that approximately 1 ml of fluid containing 25,000 oral bacteria could contaminate the handpiece each time the air turbine is stopped with the risk of these organisms being transmitted to subsequent patients, unless further precautions are taken [15]. Bloodborne viruses such as Hepatitis B and HIV that are secreted in the saliva have been shown experimentally to be sucked back into the handpiece and have been recovered distally in the dental waterlines, transmitted to the dental personnel through needlesticks, percutaneous injuries and the aerosolization of blood, saliva and/or gingival secretions that can cause asymptomatic acute or chronic infections, leading to cirrhosis or liver cancer. Cottone JA *et al.,* illustrated that viral antigen (HbsAg) was found in 76% of saliva samples taken from the virus carriers, and the site of the highest concentration of the virus in the oral cavity is the gingival sulcus. He also examined the patient samples harbouring Hepatitis C using the PCR test which showed the presence of HCV RNA in 35% of saline samples and 59% of gingival secretions [16].HIV is much less infectious than Hepatitis B and C but due to the mortality rate, dental personnel should exercise extreme caution regarding it [17].However, they are unable to proliferate outside the human host and are rapidly diluted out and removed by flushing the waterline and by autoclaving of the handpiece between patients. Therefore, the species that are important with respect to DUWL contamination are those which can divide and grow within the biofilm [18].

Another potential source of bacterial contamination is from independent bottled water systems. These systems isolate the dental unit from the municipal water supplies which introduces chemical agents to waterlines and permit the use of water of known microbiologic quality. If they are not used with sterile water, or the bottles and waterlines are not disinfected on a daily basis and then stored dry, the interior of the bottle becomes colonised, with both skin microbe such as *Staphylococcus* and water microbes like *Escherichia coli, Salmonella typhi, Vibrio cholera, Shigella dysenteriae, Clostridium botulinum and Campylobacter jejuni*. The proliferation of these microbes in the bottle occurs in the stagnant room temperature water and contaminates the waterline [19]. Methods and instructions on the cleaning of independent reservoirs should form part of the risk management of DUWL [12].

Organisms entering from any of these main routes described above colonise the dental waterlines to form a biofilm along the length of the tubing. The biofilm acts as a reservoir for continued long-term contamination of the waterline. Counts of upto 10^{46} cfu/ml are commonly detected in the water from poorly maintained waterlines. A mature biofilm will form in a new dental unit plumbed to the mains within 120 days. This process occurs due to stasis and stagnation of the water in the microbore tubing that comprises the DUWL, permitting unheeded growth and proliferation of the bacterial micro-colonies within the biofilm. This rapid amplification may result in pathogens that originally occurred only in very low concentration in the mains reaching their infective dose for humans [13].

TRANSMISSION

The main route of transmission for most of the pathogenic respiratory pathogens is by aerosolisation of the dental unit water. Multitudes of pathogenic and infectious microbes are known to spread via contaminated aerosols. There are four basic routes of entry for spreading infectious microorganisms in a dental setting: blood-borne, saliva-droplet, direct contact and water-droplet. The patient is generally the source of the blood-borne and saliva-droplet routes, but the dental hygienist could be a source as well. It has been estimated that 7-30% of individuals sustaining needlestick puncture injuries from patients who are positive for hepatitis B virus surface antigen (HBsAg) became infected, compared with less than 0.5% of individuals sustaining needlestick injuries from HIV infected persons The third, direct contact, may be from the patient and/or contaminated equipment[17]. Though there have been several rare cases of care-provider-to-patient HBV transmission, there has only been one recent report of possible HIV transmission

from a dentist to his patients[20].Montebugnoli L *et al.,*2004 in his study observed direct person-to-person transmission of periodontal bacteria through saliva. 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 [20].The sources of the water-droplet route are biofilms and other microorganisms in the DUWL. Dental personnel are exposed to contaminated dental unit waterlines by inhaling the aerosol generated by the ultrasonic scalers, handpiece and the air-water syringe. Dental water may be ingested, inhaled in the form of aerosols or directly contaminate surgical wounds. Dental handpieces generate 5 m particles, which are large enough to transport microbes but small enough to bypass the nasal baffle and pass into the alveoli of the lungs. The numbers of microbes in aerosols generated by air turbines are considerably higher than from conventional speed handpieces. Reports from Larato and William *et al.,* [21] have shown that when heavy droplets fall to floor they become part of the floor dust. Aerosol particles that remains suspended in air forms droplet nuclei that reaches the respiratory passage of dental personnel. Findings of Mills *et al.,*[22] observed microbial growth from the layer of turbine handpiece after following standard cultural procedures. Reduction of microbial contents of bioaerosols can be achieved by following a preprocedural oral rinsing with an antiseptic mouth wash during dental operations as evidenced from the findings of Fine *et al.,* [23] Finally, to complete the route of infection, the host has to be susceptible. Dentists are now treating a more susceptible and vulnerable population than in the past. The geriatric population, patients on inhaled and systemic steroids, diabetics, post surgical cases for example post gastrectomy, those on antibiotics, smokers, patients with cancer and chronic diseases are medically compromised increasing the number of people with high risk.

RISK TO DENTIST

The clinical members of the dental team inhale aerosols generated by dental equipment on a daily and long term basis. Abnormal nasal flora in dental personnel has been attributed to water system contamination. Pathogenic bacteria easily penetrate from the nasopharynx to the oral cavity,and next with saliva droplets to the breathing zone air, creating a direct threat to a dentist. A potential threat to dentists is also some of the Gram-negative bacteria, commonly inhabiting the oral cavity and is known to be harmless saprophytes: *Eikenella corrodens*, *Moraxella catarrhalis*, *Neisseria flavescens*.[23]

It has been suggested that *Legionella* spp. within dental lines may contribute to respiratory illnesses among dentists and dental staff . Higher rates of seropositivity for *Legionella* antibodies have been found among dental personnel than among the general public, suggesting that aerosols generated in dental operatories are a source of exposure to *Legionella* spp. Water-cooled, high-speed handpieces generate stable aerosols that may contain *Legionella* spp [24].The complex design of dental-chair equipment results in the stagnation of water within the water lines, where bacteria, including *Legionella* spp., can proliferate within a biofilm. PCR detection to estimate concentrations of *Legionella* spp., indicates high levels of *Legionella* contamination of water in dental operatories was evident from the study of Mahbubani *et al.,* [25,26]

RISK TO PATIENTS

The increase in the number of immunosuppressed or chronic disease patients under dental treatment, parallels an increase in the number of patients that are susceptible to opportunistic pathogens, found in the dental unit waterline [6].The presence of high concentrations of microorganisms in DU water (up to 106 cfu/mL has been recorded) is a potential risk of infection for dental patients and staff and is incompatible with

good cross-infection control practices (Smith *et al*., 2002).[27]

MICROBIAL LOAD IN DUWL

DUWL's are ideal environments for the growth of microorganisms entering dental units from the municipal water supply (Barbeau *et al .,*2000)[28]. Retrograde flow from previously treated patients was shown by (Montebugnoli *et al .,* 2004)[29] to be the next most important contributor, who concluded 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. The initial biofilm which is nothing but a layer of microbial communities,thickens through replication of the organisms that make up the biofilm, as well as adherence of free-floating microrganisms from the water source. Studies addressing dental unit (DU) water supply (DUWS) contamination have confirmed that the high bacterial count is due to the shedding of biofilm bacteria from the lumen surface of dental waterline tubing into treatment water.The relatively high surface area/volume ratio associated with the DU waterline (DUWL) tubing, periods of stagnation (when the DU is not in use) of water in the lines, and laminar flow conditions with low shear forces near the lumen wall of the waterlines provide the opportunity for the development of bacterial biofilms. The organisms recovered from dental unit water vary with geographic location. They include fungi, free living amoebae, protozoa and nematodes as well as the frequently isolated saprophytic and opportunistic gram negative pathogens such as *Pseudomonas spp, Klebsiella spp* and *Flavobacterium spp*. The latter species are capable of thriving in low temperature and low nutrient environments including distilled water. Only *Pseudomonas aeruginosa* derived from DUW has definitely been shown to cause infection was shown by Monarca *et al.,* who analysed DUWL water for *Pseudomonas aeruginosa* showed that this bacterial species was present in 15-30% of all the samples taken from air-water syringes, while in the samples from turbines and microengines the concentration of these bacteria was very high [30] .

Table 1
Microorganisms routinely isolated in dental unit water line

Bacteria	Fungi	Parasite
Achromobacter xyloxidans *Acinetobacter spp* *Actinomyces spp* *Alicaligenes dentrificans* *Bacillus spp* *Bacteriodes spp* *Caulobacter spp* *Flavobacterium spp* *Fusobacterium spp* *Klebsiella pnuemoniae* *Lactobacillus spp* *Legionella pneumophila* *Legionella spp* *Micrococcus spp* *Moraxella spp* *Mycobacterium avium* *Mycobacterium spp* *Nocardia spp* *Pasteurella spp* *Proteus vulgaris* *Pseudomonas aeruginosa* *Burkholderia cepacia* *Streptococcus spp* *Staphylococcus aureus* *Xanthomonas sp*	*Phoma spp* *Penicillium spp* *Cladosporium spp* *Alternaria spp* *Scopulariopsis spp*	*Acanthomoeba spp* *Cryptosporidium spp* *Microsporidium spp* *Giardia spp*

Among bacteria that are listed above, the four most commonly discussed bacteria within biofilms of Dental Water Line are *Legionella spp, Mycobacterium spp, Pseudomonas aeruginosa, Staphylococcus spp*. These three are also the major focus of infection control with regard to DUWL as they are the respiratory pathogens capable of proliferating in the biofilm and reaching infective concentrations with potential for inhalation via aerosols or direct contamination and also known to cause disease in immunocompromised patients. Various studies on DUWL's have categorized the microbiological flora involved in the formation of biofilms. Szymanska J in a polish study 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 . Some of them, in certain circumstances, especially in people with immunological disorders, may be a cause of opportunistic infections[30].

ELECTRON MICROSCOPIC EXAMINATION – BIOFILM

DUWL biofilm structure and its properties can be studied with various methods, such as microscopic examination with the use of an electron microscope, fluorescent microscope, transmission electron microscope, scanning electron microscope (SEM) and confocal laser microscope. These study methods make it possible to find whether the biofilm is present, to elucidate the nature of biofilm, its formation stages and maturation. Transmission electron microscopic

examination of bacterial biofilm on the inner surface of the tubing in dental unit water lines was analysed by Jolanta Szymanska [31]. SEM analysis of the luminal surface of tubing obtained from Dental Unit by Singh *et al.,*[32] revealed a dense biofilm matrix. Bacteria of distinct morphotypes (cocci, rods, and filamentous bacteria) observed in the matrix suggested that DU biofilms are complex.

INFECTIONS – DUWL

As biofilms mature, they provide a hospitable environment for fungi, protozoa and other organisms that survive in drinking water systems [33]. While most of these organisms have minimal pathogenic potential in immunocompetent hosts, some protozoa serve as hosts for proliferation of parasitic bacteria including Legionella [34,35]. Pseudomonas aeruginosa has been reported as being present in dental units [28]. This gram negative rod is associated with a wide range of opportunistic infection and is a cause of pneumonia in hospitalized patients. Only pseudomonas aeruginosa derived from DUW has definitely been shown to cause oral infection in patients. High numbers of non-tuberculous mycobacterium may be swallowed, inhaled or inoculated into oral wounds during dental treatment with the potential for colonization, infection or immunization [6]. In the DUWL, extended periods of stagnant water, low chloride concentrations and an average water temperature of 23°C all contribute to the proliferation of *Legionella* spp ., as observed in the findings of Szymanska *et al.,*[30].These weakly staining bacteria thrive as intracellular parasites of protozoa. They are the causative agents for legionnaires disease and a related condition known as Pontiac fever. Transmission of *Legionella* spp. occurs via inhalation of infected/contaminated aerosols or direct inoculation of open wounds. Aerosols are often produced during dental procedures utilizing high-speed handpieces, hence DUWLs are a potential source of *Legionella* spp as was reported from the study of Atlas et *al.,*[14]Aquatic nontuberculous mycobacterium species associated with pulmonary disease and opportunistic wound infections also have been reported in dental unit water[8].

PREVENTIVE MEASURES TO DECONTAMINATE DUWL CHEMICAL TREATMENT

An ideal agent for control of biofilm would be bactericidal but not toxic or irritating to humans. And, of course to be truly ideal, it should be inexpensive and easy to use, should discourage subsequent reformation of biofilm, while protecting the dental unit's internal components from corrosion or degradation. Chemicals may be introduced into the water system either intermittently or continuously. Most intermittent treatment regimens use potentially biocidal concentrations of germicide that also may remove biofilm. A major advantage of intermittent chemical use is that the active agent is purged from the system before patient treatment. Disadvantages include the potential for surviving biofilm organisms to rebound between treatments, potential staff exposure to chemicals, and the potential for adverse impact on metal, rubber and synthetic dental unit components. Although continuous treatment offers less potential for recolonization of waterlines, it still may damage equipment. Since the agent is always present and may be aerosolized, the effects of chronic exposure on the health care worker must be considered.[36,37]

Chlorination

Chlorine, as sodium hypochlorite is the most commonly employed biocide in water and has proven efficacy is hospital cold water system in particular for controlling Legionella proliferation. In some environments Legionellae are able to increase their chlorine resistance by 30-120 folds by living inside amoebae, often resulting in failure to eradicate the organism. Potentially higher doses of 3-5 ppm could overcome this problem[38]. Disadvantage of long term exposure to chlorine include bacteria developing resistance, corrosion damage even at 1ppm, formation of trihalomethanes (potential carcinogens) and that high chlorine levels are unpalatable[28]. Wirthlin *et al.,* 2003 showed that chlorine dioxide waterline cleaners are effective in decontaminating DUWL biofilms. Chlorine dioxide is more

beneficial than other chlorine products as it does not form carcinogenic compounds, has a long shelf-life in comparison with other products, and is not a strong irritant [54].

Biocides and chemical disinfectants

Biocides (compounds with lethal activity against living organisms) are used to remove the biofilm and eliminate the planktonic bacterial count. Their use has met with a limited degree of success[8]. Different biocides are used in dentistry including sodium hypochlorite, chlorhexidine gluconate, povidine iodine, ethanol, peroxide and glutaraldehyde. Integral automated flush system employing glutaraldehyde are commercially available but its sensitization of the human lung and skin have severely inhibited the use of this compound in dentistry[8]. In a study by Liaqat I *et al.,* the potency of eight biocides were analysed to reduce the biofilm microorganisms contaminating dental unit waterlines. Biocides efficacy observed by culture independent and by culture dependent method resulted in NaOCl, EDTA, CHX, SDS as effective biocides. All strains tolerated both 100 µgml-1 and 500 µgml-1 concentration of biocides alone and in combined form. Higher concentration of biocides i.e., 1000 µgml-1were tested. By adding biocides into the media alone at 1000 µgml-1 concentration, NaOCl and EDTA were found to be effective against DUWL biofilm isolates[39].

Table 2
***Effective concentration of biocides*[39]**

Product name	Active agent(s)	Effective concentration. (%)	Application
Alpron BRS	Sodium hypochlorite, citric acid	1–2, 70	Once
Alpron BRS	Sodium-*p*-toluol-sulfonechloramide, EDTA	0.2, 1–5	Continuous
BioBlue	Ethanol, chlorhexidine	12, 0.12	Intermittent
Dentosept P	Hydrogen peroxide, silver ions	0.014	Continuous
Betadine	Povidone iodine solution	10%	_____
Grotanat Bohrerbad	Calcium hydroxide, propanol, ethylhexanol	Undiluted	_____
Sterilex Ultra	Alkaline peroxide	5%	Intermittent
Ster4Spray	Sodium perborate, EDTA	2	Intermittent

Alpron disinfectant

The disinfectant system marketed as Alpron comprises a three component system specifically designed for the removal and control of biofilm formation within the narrow bore plastic water lines of a dental unit. The initial biofilm removal solution consists of a 1–2% sodium hypochlorite solution applied to the DUWLs at an initial temperature of 50°C for a period of 30 minutes. This is followed by a second solution containing alkylamines, complexing agents, tensides and defoamers applied to the DUWS at an initial temperature of 60°C for 30 minutes. The third solution, a 1% solution of sodium-p toluolsulfonechloramide and sodium ethylenediamine tetra actetic acid (1% Alpron) was added to the reservoir that supplies the water to the dental handpieces and triple syringe. From the studies of A.J.Smith, it was suggested that Alpron considerably disrupted the biofilm lining DUWLs as evidenced by the material ejected from dental units after treatment, maintaining low microbial counts over a reasonable time period. This indicates that the course of treatment followed may be effective for a prescribed period of time under the operating conditions, followed by the reassessment of microbial counts after 6 weeks from a treated dental unit[40].

Figure 2
Alpron – disinfectant in duwl

Recommendations for DUW

Regular culturing of dental unit water lines remains the only method to ensure that a system meets the CDC & US Environmental Protection Agency (EPA) standards. The goal of American Dental Association recommends a total colony count of <200 cfu/ml in the dental unit water, whereas the European Union strives for < 100 cfu/ml [41]. Researchers beginning with Black [42] in 1963 have investigated treatment options intendedtomaintain the dental treatment water. Currently, there is no single method or device is available that will completely eliminate contamination of DUW or prevent the risk of cross infection

To reduce contamination a combination of methods need to be used. The recommendations for DUW are:

1. Water supplying dental units should have a total colony count of <200 cfu/ml and comply with local drinking water standards.
2. For surgical procedures use sterile irrigant water or saline provided from a separate and preferably single use source.
3. Existing recommendations for flushing through of water lines between patients and at the beginning and end of the working day, eliminate oral flora entering the water lines via suck-back but only partially and temporally reduces the existing bio-burden in the DUW.
4. Anti-retraction valves should be incorporated on all hand pieces or waterlines and must be regularly monitored and maintained.
5. Independent water reservoirs when used with sterile water are capable of delivering water with <200 cfu/ml total count. This can only be achieved if manufacturer's instructions regarding disinfection by purging the line with biocide are adhered to.
6. To reduce biofilm proliferation and overnight water stagnation, drain down the waterlines at the end of the day.

Waterline flushing

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[8]. Although flushing can temporarily reduce the number of organisms suspended in DUWL's there is no predictable effect on adherent biofilm[43]. Barbeau *et al.,* demonstrated in his findings that the effectiveness of flushing in bacterial clearance was both variable and minimal when used for short periods of time (<10 min) [28].Whitehouse *et al.,* described in his study that bacterial load was not consistently reduced to the desired standard of less than 200 cfu/ml, unless extended flush times were employed [44]. Flushing for 20 minutes, which would be impractical in most dental surgeries, will reduce the bacterial count to zero [28]. Al-Hiyasat A *et al*, *2007* identified in his findings that flushing the dental unit for 2 minutes significantly reduced the counts of *P. aeruginosa* [45]. ADA's and BDA's recommendations state that water lines should be flushed through for several minutes at the start of each clinic day to substantially reduce microbial accumulation caused by overnight stagnation in the

waterline[8]. Discharging the stagnant water improves the perceived quality of the water by reducing the malodour and bad taste imparted to the water by microbial contamination, flushing is valuable in eliminating retrograde aspiration of oral fluids[8]. The efficacy of mechanical flushing alone to control microbial contamination in dental unit water line is not well supported by the scientific literature. Although flushing can temporarily reduce the number of organisms suspended in DUWL's, there is no predictable effect on adherent biofilm since the flushing of dental water lines has been shown to decrease the levels of planktonic bacteria in the water, but this practice has not been shown to affect the biofilm that accumulates in the water lines [46].

Peroxide, Ozone, UV

Bacteria from the biofilm are shed continually while the film is in contact with water. Thus UV disinfection, H2O2 and ozone have advantages in that they can be introduced continuously into the water line during patient treatment thus maintaining low levels of planktonic counts throughout the working day[8]. That UV irradiation alone has a significant effect in reducing microbial contamination is equivocal due to the relative resistance of some important waterborne pathogenic species. Ozone (O_3) has been used for purification of water due to its efficiency and lack of side effects[8]. Al Shorman *et al* (*2002*) used O_3 at a concentration of 2100 ppm. O_3 formed from air resulted in a bacterial reduction from 5.2^{*} 10^{5} 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[47]. Puttaiah and Lin *et al.,* 2006 reported from their study that use of 0.8 ppm of ozonated water as irrigant has reduced the counts to > 500 cfu/mL in the dental units at the end of fourth week [48] . They concluded that an initial cleaning with 60 ppm ClO2 and use of 0.8 ppm O_3 mixed in water as irrigant controlled contamination up to 30 days. In 2005, Szymanska J (*2005*) concluded that the application of 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 [49]. In a study by A. J. Schel et al., (2006) it was very effective in reducing TVCs and maintaining the microbial load to levels well below 200 CFU / m[50]. 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. UV would appear to be an attractive, non-polluting alternative for point of entry of mains water purification [51].

Filtration

Using filters on the dental waterline was first described 20 years ago to reduce planktonic (suspended) bacteria. Micropore membrane filters are used to remove microorganisms from water and solutions in a wide range of medical and industrial applications. If the units are connected to municipal water supplies, the water also may contain impurities including minerals, organic compound and endotoxin, that are not always removed by filters. Therefore, even when water produced by filtration in the dental clinic is bacteria free, it should not be used in place of sterile water in surgical procedures[28]. Highlevels of recontamination of DUW occurs within 24 hours as a result of trapping and growth of bacteria on the filters. Therefore disposable filters are recommended, which must be changed daily. A pore size of 0.2 microns is recommended by US Federal Drug Administration[7]. Murdoch-Kinch and colleagues found that use of 0.22 µm filters resulted in few numbers of organisms observed on scanning electron microscopy in post filtration tubing sections than in pre-filtration sections[52]. Mayo and Brown found no detectable organisms in water samples taken immediately downstream from 0.2 µm proprietary filters; however, when they increased the distance at which the filter was placed from the air water syringe, levels of bacteria in effluent water increased, probably owing to the formation of biofilm in the post filtration waterlines[53].

CONCLUSION

The concerns about dental unit waterline contamination are justified by scientific evidences and the dentists should find the best way to avoid or overcome this problem. Substantial progress in preventive and control measures can be made by dental manufacturers and the scientific community since the origins of dental unit water contamination are vividly defined. Due to the multiple ports of entry to the DUW system for microbes, no single method or device will completely eliminate the potential for cross infection. The long-term solution to the problem lies in redesigning the water supply system within dental units to eliminate stagnant areas and biofilm build up. Combinations of currently available procedures and equipment, including anti retraction devices, flushing, independent water supplies used in conjunction with biocide purges or fully autoclavable water line circuitry will provide water of a higher standard than drinking water. All these systems require strict adherence to maintenance protocols to perform to their full potential. Future research into the prevention of biofilm proliferation is being actively promoted by the American Dental Association and other dental organisations and government agencies around the world. Hence DUWL contamination can be controlled when dental personnel use available technologies and adhere to recommended maintenance protocols.

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