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Review Article

A Review on Control Methods for Bacteriological Water Quality and Biofilm in Dental Unit Water Systems

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Abstract: Contamination of dental unit water systems (DUWS) to a variety of microorganisms and biofilm formation is one of the most important problems in dentistry centers against the proper operation of thisunits. This study was conducted to aim a review on identifying control methods for bacteriological water quality and biofilm in DUWLs and introducing effective solutions. A considerable number of articles published from 2000 to 2014 in the Iranian and international scientific journal, final reports of the research projects, articles presented in the Congress and student theses, with using standard and sensitive keywords were reviewed. Then the articles which have inclusion criteria of the study, were selectedand were carefully studied. Review of selected studies indicated that different methods for the control of bacteriological water quality and biofilm in dental unit water systems were used. Preventive measures, design and use of a central system of treatment and disinfection of entering water to the units, use of chemical disinfectants, flashing, genus and size of dental unit water pipe, properly designed unit by manufacturers, anti-retraction device (anti-return) and optimum operation are the main methods that were used in several studies. Certainly, one method cannot be supply the standard for unit water quality and remove biofilm. But using different methods is necessary to reach this objective. The most effective method to reduce the microbial contamination load of water and reduce adverse effects on personal health and patients is preventing of biofilm formation, especially using enter of safe water to the dental unit in combination, with other control methods should be seriously considered. In addition, due to bacterial resistance against bactericides and their side effects, use of properly designed units that their management is easy, can be minimized the problems.
Keywords: Biofilm, Bacteriological water quality, Dental Unit Water System (DUWS), Disinfection, Water management system.

INTRODUCTION

Effective control of pathogens is one of the important indicators in medical and dental centers and shows good performance of these centers [1]. DUWL (dental unit waterline) is an integral part of Dental Unit and supplies the required water for high-speed handpieces, air/water syringe and Ultrasonic Scaler.

This system usually is polluted by different species of microorganisms [2]. The origin of these microorganisms may be patients' oral back fluid into the DUWLs [3], the initial contamination of water entering into the unit and or may be as a result of entering water contact with biofilm [4].

Of course, the main problem of contamination is attached biofilm layer to DUWLs wall [5]. Szymańska *et al*. have introduced the release and spread of bacteria from the biofilm as the main source of infection and contamination of consumable water and air of dental centers unit in their own study [6].

The factors such as high flow rate of water in the center of the tube, low flow rate of water in the circumference and surface of tubes and stop of units activitycause to provide the conditions for more adhesion of microorganisms to tubes wall and biofilm formation and increasing the contamination load of units' consumable water [7]. In several studies, the effects of units' consumable water contamination have been proved with air pollution of wards.

Masoumbeigi *et al*. showed that there is a significant correlation in moderate limit between the mean of HPC (Heterotrophic Plate Count) of the water and air in the restoration and periodontal surgery wards [8]. In many studies the positive effect of reduction in the air microbial pollution following consumable water contamination control has been reported.

ADA (American Dental Association) has determined the permissible value of units' consumable water HPC, the maximum 200 CFU/ml at any time of units' activity and has expressed essential the use of sterile water in surgeries, especially, dental and periodontal surgery of people withimmunodeficiency, the children and individuals at risk [9].

The results of several studies show that bacterial contamination amount of units consumable water is usually more than the recommended limit (200 CFU/ml) [10].

This polluted water may be swallowed by the patient as unwanted while receiving dental health care and or enter into the air of the ward through bio-aerosols generated by high-speed hand pieces and as a result entered to the patient's respiratory tract [11]. Bacteria contained in the units water can cause to occur infection of the respiratory tract, especially in susceptible individuals such as those with immunodeficiency, pregnant women, elderly, children, smokers and people undergoing a transplant operation or radiation therapy [12].

The results of different studies suggest that due to the presence of such contaminations, the treatment and disinfection of units' consumable water are essential [13]. Until now, the preventive activities that lead to reduce the contamination load up to guidelines level and or several methods of water treatment and disinfection have been used.For example, water disinfection before water entering into the unit has been carried out by using the chemical disinfectants such as chlorination and using the physical methods such as filtration, sterilization by autoclave, flushing, using anti-retraction valves, using independent tanks before water entering into the unit and using the electrochemical method is used [14].

In spite of disinfection,20-120secondflushing can also be effective after services to each patient, patient's mouth rinse by Chlorhexidine before work, performing the manufacturers' recommendations of dental units and learning how to make optimal use of the units are effective on water quality and reduction of water contamination [15-20]. Use of variety of chemical disinfectants is one of the most effective and common methods for removal of pathogens in the dental centers environment.

Available disinfectants depending on the effect potency are divided into three levels: high, medium and low that can include phenolic compounds, alcoholic and chlorinated compounds with the average potency level and glutaraldehyde compounds,H_2O_2, formaldehyde, peracetic acid with the high potency level can be noted [21, 22].

Zanetti's *et al*. study about infection control methods in 226 dental clinics in Italy introduced glutaraldehyde as one of the most common disinfectants for the surface of the equipment, Shank cutters, hand pieces and dental instruments [23].

Bhatnagar *et al*. study about infection control strategies in dentistry clinics has emphasized on the repairs, development and promotion of sterilization level and updating existing equipment and facilities and sufficient training of personnel [24].

Due to increasing the contamination load of DUWLs and spread of pathogenic agents through it and the necessity to control the spread of infection in dental centers, this study was conducted to aim a review on identifying control methods for bacteriological water quality and biofilm in DUWLs and introducing effective solutions.

METHODOLOGY

This study is a descriptive-review. Papers published in English and Persian sources from 2000 to 2014 and existing theses in databases include Science Direct, Elsevier, PubMed, Google Scholar ,Scopus , Web of Science, Medicos /WHO/EMdR, Open Access Journal Directory, Irandoc, SID, Medlib, Iranmedex, Magiranwere examined to access to the results of the latest researches.

Searching for papers was conducted by using the searchable keywords in the mesh such as bacteriological water quality, biofilm, disinfection and DUWLs in English and Persian sources. A total of 12,800 paperswas obtained. After content review of the titles, abstracts and text articles, the 26 articles that relate to the subject of the present study selected and were grouped and analyzed.

Inclusion criteria of these papers for this study were English and Persian original articles of 2000 year since then which has investigated a control method of microbial load and or removal of biofilm in DUWLs. Exclusion criteria were the absence of the above conditions.

RESULTS AND DISCUSSIONS

Health of dental centers personnel requires health of dental units' consumable water quality. Patients and personnel directly are exposed to contact with the contaminated water and productive bio-aerosols caused by work with high speed handpieces

while offering therapeutic services in dental centers [16, 25].

A high level of microbial contaminants, including opportunistic microorganisms and bacterial endotoxins associated with gram-negative bacteria are among pathogenic agents which are transmissible by dental units' consumable water that provided in the follow [5, 25-28].

Formation of biofilm

Formation of microbial biofilm on the inner wall of DUWLs is one of the major problems against the optimal operation of dental units and is caused by the high contamination of entering water and lack of water appropriate disinfection and contains microbial diverse population that attached to DUWLs wall and they can grow and proliferate.

Pathogenic agents are preserved against disinfection effect by sheltering in layers of biofilm and even cause rising the bacteria resistance against disinfection. When water exposed to biofilm, the bacteria from biofilm enter into the water and polluted it.

These bacteria are usually released from biofilm surface during offering therapeutic services and are added continuously to water.

For this reason, using suitable disinfectants especially those which have a better retention effect in water are highly regarded and are very effective to remove suspense and free microorganisms and even contained in DUWLs biofilms.

Based on the results of different studies, using disinfectants such asH_2O_2, ozone and or UV radiation can be useful and keep microbial load in level less than the permissible limit of 200 CFU/ml [29]. Even products such as Alperon, Strilex ultra and H_2O_2 exist that can completely eliminate the planktonic cell and remove biofilm [30].

Despite disinfection by different methods, operation problems and limitations of dental units greatly provide conditions for the growth of biofilm. Therefore, the particular attention to educating the staff, especially in the optimal operation of units can have an effective role in reducing the microbial load of units' consumable water.

New methods of treatment and disinfection of units' consumable water and biofilm control in internal layer of DUWLs are updating. The best action is to prevent the biofilm formation.Because complete elimination of formed biofilm using bactericidal and disinfectants will not practical and affect the effectiveness of other control activities.

Implementing the methods of disinfection and biofilm control should be easy and economical and have the least adverse effect on the health of personnel and equipment.

Prevention of biofilm formation in DUWLs

Based on results of different studies, the best, the most practical, the easiest and the most economical of methods with minimal adverse effects on the health of personnel and equipment for biofilm control and units consumable water quality control is preventive measures, particularly the prevention of biofilm formation that must be the first priority in any dental center.

After biofilm formation, its complete elimination is very difficultand will not be practical and affects the effectiveness of other control activities. Certainly just a way cannot remove biofilm and deliver water quality of unit to the standard limit. Furthermore, regardless of the preventive measures that will be introduced follow, water supply with a standard quality is not possible for dental units. These measures including the presence of an automatic treatment and disinfection central system of entering water into the units, personnel training and providing appropriate scientific sources and encouraging to study them, few-secondflushing after each patient and few-minute flushing at the beginning and end of units work [31], complete discharge the DUWLs at the end of units activity for prevention of water stop, installing anti-retraction valves of water, using biological filters at the end of DUWLs before instruments [32], using deionized and distilled sterile water (DDSW) in an independent tank especially in surgeries, regular monitoring and control of consumable water quality and special attention to the recommendations of the units manufacturer company during the operation are in the priority among preventive measures.

Using chemical disinfectants individually or as combined [33] also are including significant and effective solutions and complementary of preventive methods and it is required to being considered both classes of these methods together for unit water management system (WMS), but each have disadvantages.

For example, flushing water to partially reduce microbial density but is not effective in removing biofilm [34].

Need to regular change of filter due to clogging and the weak effect of using DDSW cause of the biofilm presence are disadvantages of the mentioned methods [26]. Bottles also as the independent tank of water usually are polluted by microorganisms such as *Staphylococcus epidermidis* and *Staphylococcus aureus* that eventually cause to consumable and output water contamination of units instruments [35].

Flushing

One of the effective methods that its effectiveness has been proved and often has been used is flushing before the start of therapeutic services and after providing services to each patient. Numerous studies and researches confirm the flushing before starting therapeutic work in reducing the level of bacteria [31, 36].

Watanabe *et al*. reported despite of low HPC (4-15 CFU/ml) of entering water to units, all samples studied from new and old units were contaminated and with flushing HPC reduced in all units, but this reduction was higher in new than old units. Of course, *Escherichia coli* and coliform were not found in none of the samples [37].

This study showed flushing is a feasible and beneficial action for reduce the HPC and should be used routinely. Memarian *et al*. also showed after 120 seconds of flushing, contamination reaches to zero [38]. Gaudie *et al*. compared the effect of flushing for 20 seconds and two minutes and reported that two-minute flushing causes more reduction in the rate of water contamination [39].

ADA has emphasized in its own guidelines on water flushing for a few minute before starting work, 30-20 seconds between two patients and several minutes at the end of the workday. But this method is not enough as only method of controlling units' consumable water contamination load and preventing transmission of the bacterial agents because, flushing reduces the amount of suspension bacteria in water but hasn't effect on the amount of attached bacteria to biofilm in DUWLs that are constantly being released [38].

Lucio *et al*. showed theflushing among patients with the use of Tetra Acetyl Ethylene Diamine (TAED) is effective in microbial contamination control of unit water and delivers the mean contamination from 5.45 $\log_{10}$ CFU/ml to 2.01 log CFU/ml.Also reported flushing after every patient with the use of TAED is effective in microbial contamination control of units' consumable water [40]. Lucio *et al*. reported peracetic acid and 30-second flushing are effective in unit water disinfection and biofilm formation control [41]. Rice *et al*. reported inefficiency of flushing method in decreasing Legionella and protozoa and its being effective in substantial reduce of HPC (1.1-1.5 $\log_{10}$ CFU/ml) [31].

Singh *et al*. in comparison of CHX effect with three methods of flushing in contamination control of DUWLs showed that CHX has been very effective and has delivered the number of bacteria to Zero compared to other studied methods [42]. Under any circumstances, flushing is effective and has been a practical method for reducing the microbial load.

Disinfection

One of the most effective methods and with many applications for bacteriological water quality control of units and to eliminate biofilm is consumable water disinfection by the various chemical disinfectants such as phenol, alcohol, chlorine compounds, formaldehyde, hydrogen peroxide, Gluconate Chlorhexidine, hypochlorite-sodium, alkaline peroxide, citric acid, ozone, chlorine dioxide, peracetic acid and Poidine iodine [43-45].

These compounds individually or with other methods such as thirty-second flushing have been used in the early morning but most of them have been unable whether in the reduction of the contamination load up to permissible value of ADA or have had a temporary effect.

The use of some these materials isn't also economically cost-effective [46] and some disinfectants may an adverse effect on the tooth and bonding resin to enamel or dentin [47-49]. Walker *et al*. also showed that Chlorhexidine causes teeth staining and corrosion of the tubes [33].

Safavi *et al*. study showed that the disinfectant of Bilpron stops the growth of bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus β-hemolytic* and *Escherichia coli* in the samples of studied waters [50]. Mohseni *et al*. showed that in addition to providing strategies for reducing pollution, has emphasized the effect of water disinfection on the contamination reduction of consumable water of unit [51].

Jatzwauk *et al*. reported that H_2O_2 and silver ion are effective to reduce the microbial load of consumable water of unit and prevent the biofilm formation [52]. Zanetti *et al*. reported that determine of the effectiveness of H_2O_2 on pathogens that *Staphylococcus aurous* and *Pseudomonas aeruginosa* reduced from 4 $\log_{10}$ to 6 [53].
Szymańska *et al*. reported that disinfection by H_2O_2-based disinfectants as weekly and continuous can reduce the biofilm [46]. Szymanka *et al*. reported that a 50% decrease of the bacteria in the air after DUWLs disinfection by H_2O_2 [54]. Tuttlebeea *et al*. reported that effect of two H_2O_2-based disinfectants is effective on the bacterial load reduction and delivers it below the standard level of ADA (200 CFU/ml) [55].

O'Donnell *et al*. showed that the evaluating the effectiveness of an advanced water treatment system of units and reported that WMS with using disinfectant of Planosyl Forteh on a weekly basis can maintain output water quality of units in the ADA standard level [56].

Walker *et al*. reported in studying effect of disinfectants on biofilm and the total number of live bacteria that Grotanol, Betadine and Alperon (based-

chlorite) were effective 100% in reduction of total viable count (TVC) caused by biofilm and higher than 90% in reduction of attached biofilm to DUWLs wall [30].

Schel *et al*. in studying the effect of different disinfectants reported reduction in average water TVC from 0.69 (affected by 4-spray ester) to 3.11 $\log_{10}$ CFU/ml (affected by Dentocept). Dentocept and H_2O_2 were more effective than others. Of course, Dentocept had a high durability [57].

Coleman *et al*. performed disinfection by usingH_2O_2, Sanosil containing silver ion and Planosil Forteh with the help of a central system, automatic and continuous disinfection of dental units water source so that the water quality become better than drinking water and according to ADA standards.

WMS due to continuous supply of standard quality for unit output consumable water is better than the waterline cleaning system (WCS) [26]. Liaqat *et al*. showed in studying the effect of biocides types on biofilm that a reduction or elimination of biofilm by using sodium hypochlorite and Gluconate Chlorhexidine is in range of 85% to 98% and is more compared to the other biocides. The combination of sodium hypochlorite with phenol was more effective than either separately and decreased biofilm from 85% to 95% and its use was recommended [17].

Percival *et al*. showed that Tetra sodium EDTA 4% and 8% deliver respectively the output water samples microbial load of air/water syringe and high speed hand pieces to less than 200 and 10 CFU/ml [58]. Ketabi *et al*. showed that the stabled chlorine dioxide has reduced the amount of microbial colonies at the air/water syringe, Dental Piezoelectric Ultrasonic Scaler and turbine respectively equal to 4630, 610 and 5595 [15].

Lin *et al*. while using 2, 3 and 7% H_2O_2 for 12 weeks delivered the amount of heterotrophic bacteria from 400, 000 to less than 500 CFU/ml. 3 and 7% H_2O_2 acted similarly in the removal of biofilm [59].

Pareek *et al*. indicated that the mean TVC after disinfection of units consumable water by 3 disinfectants of Aloe Vera, H_2O_2 10% and sodium hypochlorite 5% is respectively, 5.7, 51.37 and 45.2 [60].

Ramalingam *et al*. revealed during using from a Nano-emulsion in consumable water disinfection of units, the higher exposure to desired disinfectant, the more its effectiveness [61]. Muralidharan *et al*. during own his study showed that biological monitoring of DUWLs, preparation and codification of protocols for controlling contamination and disinfection of DUWLs after the end of each workday is necessary [62].

Vanessa *et al*. showed that none of disinfectants could remove yeast and FLA (Free living amoebae). When assessing the activity of disinfectants, single species or a mixture and attached and floating of such microorganisms should be considered [63]. Disinfectants such as Chlorhexidine and Bio-2000 (Chlorhexidine and ethanol as active agents) can completely eliminate suspense TVC, but not biofilm [17].

Products containing formaldehyde as Tegadoro Giga Cept are unable in biofilm removalfrom surfaces of DUWLs, but can quickly reduce the bacterial load. Use of products containing formaldehyde can be caused the occupational exposure of dental centers personnel if used, should be regularly monitored and controlled [30].

Chlorine dioxide is one of the other germicidal agents that effectively removes the biofilm and reduce the number of bacteria in consumable water to less than 200 CFU/ml. It can also prevent the corrosion of metals and sediment in reverse osmosis membrane [63].

Of course, bacteria resistance to such disinfectants is another important issue and failing some of them in disinfection has been emphasized in many reports [64]. Bio-oxides dependon the type and concentration used, can be damage DNA, protein or enzyme, cytoplasmic membrane and cell wall and cause the death of microbes.
This mechanism also depends on environmental conditions and type of microorganism [65]. Among disinfectants, sodium hypochlorite compound with phenol also is very effective for units consumable water disinfection with over 98% efficiency and its effectiveness is more than either individually [17].

According to obtained the results of the application H_2O_2 2%, using it as a periodic cleaner is recommended to remove of the contamination from units' consumable water. Based on results from different studies, design a central automatic treatment and disinfection system of entering water into the dental centers units, because of the continuity in the consumable water supply with proper quality for dental units is more efficient and effective than a specific disinfection system in DUWLs and can even supply a quality according to the presented standards and guidelines by ADA [26].

DUWLs Material

Using proper material for DUWLs by the manufactures of units, such as copper, poly tetra fluor ethylene (PTFE) and polyvinylidenedifluorid (PVDF) and design of an independent water tank for dental unit water supply, so that carefully and regularly is disinfected and after cleaning their wall is sterilized by

autoclave is very effective and can have an important role in water pollution control of units.

Some studies reported that the material of DUWLs in control and biofilm formation rate and contamination load have known effective. Yabuneet al. showed that PVDF material is effective in biofilm control and reduction of bacterial density [66]. Sacchetti *et al*. reported that amount of aerobic heterotrophic bacteria is less in tubes output of poly Tetra Fluor ethylene than polyethylene [67].

Anti-retraction valve

The patient's oral microbial flora is main source and origin of the consumable water contamination of units. For this reason, the several studies have showed the positive effect of installing anti-retraction valve in the reduction of the contamination load. DUELs and handpieces are equipped with anti-retraction valve and their maintenance, control and monitoring can prevent from patient's oral water back into DUWLs [68], but there is convincing evidence such as identify the oral bacterial species in units consumable water showed that the anti-retraction valves also sometimes haven't had a successful performance [55, 69-71].

Berlutti *et al*. in study of 5 units with 18 produced different models in 6 factories reported that anti-retraction valves have not acted successfully in 74% of cases. Also reported that prevention of oral water back occurred only in two cases (3.7 %) [69].

Other Methods

Based on results from different studies for removal of bacterial and viral pollution from turbine that soaked in saliva of patients and dental handpieces sterilization using autoclave is recommended [72, 73]. Marais *et al*. reported that the electrochemical method can be reduced the number of bacteria to <1 CFU/ml the electrochemical method can be reduced the number of bacteria to <1 CFU/ml and remove the biofilm completely [74]. This method is not practical. Jatzwauk *et al*. reported that the using the filter was effective on the reduction of microbial contamination load, but had no effect on reduction of the biofilm formation rate [52].

Coleman *et al*. reported that dental units' producers can be have an important role in control of biofilm problems with units' proper design [68]. Using the appropriate mouth washes like chlorine dioxide 0.1%, H_2O_2 before treatment, one-time use of parts that are directly related to the patient's mouth and using herbal germicidal instead of chemical herbicide can help to us for achieve the high water quality of dental units and biofilm removal.

CONCLUSION

Based on the results of different studies reported in this paper, preventive measures and simultaneous disinfection are complement together and effective in reduction of contamination load and removal or decreasing of the biofilm formation. In addition, complete and continuous disinfection of DUWLs especially with usingfromdisinfectants based on H_2O_2 and chlorine dioxide have good effectiveness. Therefore biofilm formation control and consumable water quality of units, required to preventive measures, using from disinfectants with enough durability and without adverse effects on the health of personnel, patients and equipment and then, attention of designer engineers and manufactures to modify the design of dental units and designing an appropriate disinfection system.

Comprehensive review of methods for consumable water quality control of units and biofilm and introduce of effective methods and measures is the strengths of this study.The lack of study of papers before of 2000 is for this reason that there is no excellence methods in these studies. Under any circumstances, proper management, optimal operation of dental units and regular control and monitoring by health manager on the bacteriological water quality of dental units is essential until with implementation of the mentioned methods constantly, always supplied the ensure level for health of personnel and patients.

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