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Evaluation of Dental Unit Waterline Disinfection using Glutaraldehyde - Quarternary Ammonium Compound and Sodium Hypochlorite

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Introduction

An intricate network of narrow-bore tubes called dental unit waterlines (DUWLs) supplies water to all of the dental chair units (DCU) instruments, cup-filler and bowl-rinse outlets. Biofilms are aggregations of microorganisms that adhere, attach and, if in an aqueous environment (pipes, tubes etc) secrete a voluminous protective matrix composed of complex polysaccharides which may additionally incorporate inorganic substances from their environment. The microbial quality standards for potable water varies across the world, with the ADA specifying ≤ 200 CFU /mL and the Centre for Disease Control (CDC) recommending ≤ 500 CFU/mL of aerobic heterotrophic bacteria for DCU output water.(2)

Chemical treatment of DUWLs using various disinfectants have been suggested to remove the biofilm attached to these surfaces. Traditionally sodium hypochlorite-based biocides were advocated because of their effectiveness on bacteria and the biofilm matrix, easy availability and low cost. However they are corrosive to the metal components in (DCUs) and pose the risk of producing carcinogenic disinfectant by-products(.3,4) Hence there is a need for a suitable alternative with least possible side effects. Glutaraldehyde-Quarternary Ammonium salts (QAT) is an eco-friendly, multi-component oxidizing biocide which could be used as a chemical disinfectant for dental unit waterline disinfection.(5,6,7)

The objective of the present study was to quantitatively assess and compare the effectiveness of DUWL disinfection using glutaraldehyde -quarternary ammonium compound and sodium hypochlorite-based disinfectants by total viable bacterial count (TVC) using spread plate technique and to assess the efficacy of disinfectants in biofilm removal through scanning electron microscopy.

Material and Methods

The present *Experimental invitro study* aimed to assess the effectiveness of Glutaraldehyde-Quarternary Ammonium salts (QAT) combination and Sodium hypochlorite as dental unit water lines (DUWLs) disinfectants. The study was conducted

in the Dept. of Conservative Dentistry and Endodontics, Govt Dental College, Thiruvananthapuram in collaboration with the Dept. of Microbiology, Govt. Medical College, Thiruvananthapuram and National Centre for Earth Science Studies, Akkulam, Thiruvananthapuram.

Procedure

a. Selection of dental units and obtaining baseline samples

Twenty-one dental units, selected for the study were labelled and divided into three groups.

Group I: 1% glutaraldehyde - quarternary ammonium compound

Group II: 5.25% sodium hypochlorite

Group III: untreated DCU (control)

New plastic tubes were installed in the dental chair and supplied with tap water in the booster bottles. During the observation period, the conservative and endodontic procedures were carried out. Eventually, biofilm was allowed to grow in the dental units for 14 days. Water samples were then collected from each dental unit to assess the baseline bacterial load.

The oral rinse and three-way syringes of these units were disinfected with 70% alcohol to avoid other sources of contamination before sample collection. Baseline 10 mL water samples were collected aseptically in labelled sterile containers on monday morning before the beginning of the working hours. 0.1 mL of 0.1% sodium thiosulphate ($Na_2S_2O_3 \cdot 5H_2O$) was added to neutralize any chlorine present and the samples were immediately transported to the laboratory for quantifying the bacterial load.

b. Disinfection of DUWLs

250mL of respective disinfectants in each group was added to the reservoir bottle of the dental chairs at the end of clinical session and the solution was run through the system for one minute. The unit was switched off and the disinfectant was left overnight. At the beginning of the next work day, the remaining disinfectant solution was discarded from the booster bottle. The bottle was filled with distilled water; each of the DUWLs was flushed until the residual solution was washed out. This procedure was continued for a week. 10 mL water samples from the treated oral rinse and three-way syringe were collected in separate sterile containers under aseptic conditions on the next Monday morning before the commencement of working hours. The samples were labelled and quantified for total viable counts (TVC) in colony forming units per ml (CFU/mL)

c. Microbiological analysis

The TVC of each sample was estimated to assess the microbial load in the DUWL. A 4 mm wide inoculation loop was dipped in the sample and spread over Muller Hinton (MH) agar plate and incubated at 37°C for 48 hours. Colonies were counted manually. The number of CFU/mL of the water sample was calculated by multiplying the number of colonies by 250 (4 mm loop holds 0.004 mL liquid).

Tubing samples were taken and fixed in 2% Glutaraldehyde and washed in phosphate buffer solution for 10 – 15 minutes. Dehydration was carried out for 10 minutes through 30%, 50%, 70%, 90% and 100% series of alcohol and final treatment was done with hexamethyldisilazane and air dried overnight. Specimens were mounted on SEM aluminium mounts and coated with gold-palladium.

Results

Wilcoxon signed rank test for intragroup comparison showed statistically significant difference between pre- and post- treatment bacterial count. Kruskal Wallis test was used to compare the mean percentage change in bacterial count between the groups and was found to be statistically significant. Pair-wise comparison using Wilcoxon rank sum test revealed statistically significant difference between the two biocides and the control group, while there was no statistically significant

Difference between the two disinfectants. Scanning electron microscopic image of the untreated tubings shows extensive mature microbial colonization indicating biofilm formation whereas the inner lumen of the cut section of treated dental unit waterline was smooth suggestive of absence of biofilm formation.

Table A

Table shows intragroup comparison of mean bacterial count among three-way syringe and oral rinse samples. There is statistically significant difference in mean bacterial count in group I and group II pre- and post-treatment three-way syringe and oral rinse samples.

	Variable	N	Mean	SD	Std. error	P-value
Group I	Pre-treatment (S)	7	5178.57	1443.08	545.58	**0.017***
	Post treatment(S)	7	214.28	114.80	43.40	
	Pre-treatment (O)	7	6392.85	1631.03	616.63	**0.018***
	Post-treatment(O)	7	500.00	218.21	81.28	
Group II	Pre-treatment (S)	7	4625.00	245.49	92.81	**0.012***
	Post treatment (S)	7	281.25	99.52	37.62	
	Pre-treatment (O)	7	4093.75	210.95	79.75	**0.011***
	Post treatment (O)	7	500.00	176.77	66.83	
Group III	Pre-treatment (S)	7	9107.14	2455.47	928.34	**0.352**
	Post treatment (S)	7	9178.57	1793.43	678.04	
	Pre-treatment (O)	7	10178.57	1598.65	604.40	**0.176**
	Post treatment (O)	7	11892.85	1032.01	390.17	

*significant at the 0.05 level using Wilcoxon signed rank test.

Table-B

	Variable	N	Minimum	Maximum	Mean	Std. Deviation	p value
Percentage change (s)	Group I	7	78.57	100.00	95.08	8.33	0.001*
	Group II	7	78.57	100.00	93.10	7.65	
	Group III	7	-50.00	26.88	-8.95	24.67	
Percentage change (O)	Group I	7	68.75	100.00	92.11	11.37	0.001*
	Group II	7	72.22	100.00	88.75	10.48	
	Group III	7	-42.10	29.73	-36.03	69.28	

*significant at the 0.05 level using Kruskal Wallis test.

Table shows comparison of the percentage change of bacterial count of the 3 study groups. There is statistically significant difference in mean percentage change in bacterial count (P=0.000) in both three-way syringe and hand piece coupling of all the study groups.

Table C:

	(I) variable	(J) variable	P-Value
Percentage change (S)	Group I	Group II	0.630
		Group III	**0.001***
	Group II	Group III	**0.002***
Percentage change (O)	Group I	Group II	0.627
		Group III	**0.001***
	Group II	Group III	**0.002***

*significant at the 0.05 level using Wilcoxon rank sum test

Table shows pair wise comparison of percentage change in bacterial count. Group III shows statistically significant difference in mean percentage change (P=0.001) with that of Group I and II in both three-way syringe and oral rinse samples. But no statistically significant difference was observed between Group I and II.

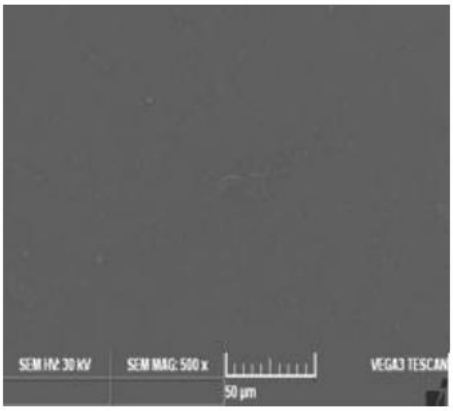

Fig 1. Scanning electron microscope images of the inner luminal wall of the cut section tube collected from GROUP 1

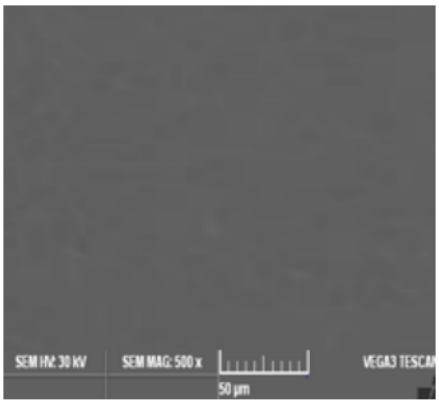

Fig 2. Scanning electron microscope images of the inner luminal wall of the cut section tube collected from GROUP II

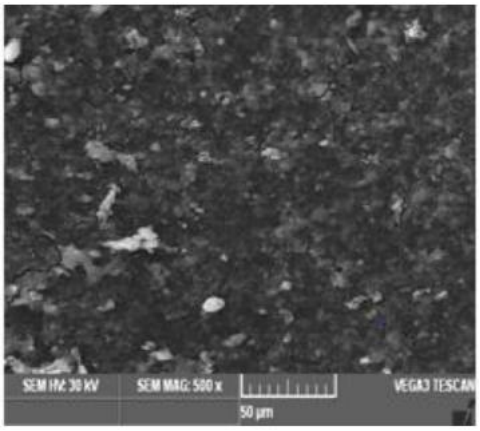

Fig 3. Scanning electron microscope images of the inner luminal wall of the cut section tube collected from GROUP III

Scanning electron microscopic images shows the efficacy of disinfectant or disinfection procedure. Figure 1 and 2 show the inner lumen of the cut section of treated dental unit waterlines (Group I and II) as a smooth tubing wall with no biofilm. In Figure 3, the inner lumen wall of the cut section from the untreated dental waterlines (Group III) records extensive mature microbial colonization with abundant organic matrix material

Discussion

DUWL treatment agents are generally divided into two categories - agents for intermittent DUWL treatment (e.g. once-weekly) and continuous or residual DUWL treatment agents. The intermittent methods are similar to a "shock treatment" of a swimming pool. The approach is to deliver the chemical agent for a specified contact time and frequency using an independent water reservoir. The active agent is then purged from the system before the patient is treated. Drawbacks of this system include potential for microorganism rebound between treatments; staff exposure to chemicals; and potential for adverse impact on the dental unit materials. Continuous methods may use lower concentrations of potentially biocidal agents in the water. This may be similar to the residual chlorine used to maintain the safety of municipal drinking water. A variety of DUWL disinfectants are available for use, but laboratory and clinical studies have shown wide variation in their efficacy. Chlorine dioxide, chlorhexidine gluconate, hydrogen peroxide, silver peroxide, sodium hypochlorite, povidone-iodine or electrochemically activated water were some of the suggested biocides to remove biofilm from DUWLs.[8]

Sodium hypochlorite is the most commonly used DUWL disinfectant. Previous studies of various Glutaraldehyde- quarternary ammonium salt based DUWL disinfectants have reported the ability of these disinfectants to reduce effluent microbial contamination. However there is no study in literature comparing the combinations of glutaraldehyde - quarternary ammonium salt and and Sodium hypochlorite.

Monarca *et al* evaluated the effectiveness of methods of chemical decontamination using different disinfectants (peracetic acid, hydrogen peroxide, silver salts, chloramine T, glutaraldehyde T4) and methods of physical decontamination using synthetic membranes for the filtration of water and glutaraldehyde T4 seems to be the best disinfectant only if integrated with periodic biofilm removal for the maintenance of the water quality.[9]

Ramalingam *et al* investigated the level and composition of bacterial contamination of dental chair syringe waterlines and investigates the efficacy of a cetylpyridinium chloride-containing nanoemulsion disinfectant in reducing bacterial loads. The findings indicate that nanoemulsion effectively disinfects waterlines to consistently meet the American Dental Association (ADA) recommendation[10]

Kathariya *et al* evaluated the bacterial contamination of dental unit water lines and the efficacy of commercial disinfectants in eliminating biofilms from them random water samples were collected from water booster, air-turbine, air water syringe of two dental units and were subjected to bacteriological analysis. Commercially available disinfectants - 0.2% chlorhexidine and 3% sodium hypochlorite, were used to treat the two dental unit water lines

respectively. there were no bacterial isolates after treatment with disinfectants for a period of 15 days.[11]

Shajahan *et al* studied the efficacy of anew disinfection solution based on hypochlorous acid for use as a cleaning agent in dental unit waterlines. Disinfection treatment procedure was carried out and the dental unit waterlines were removed and analyzed using the scanning electron microscope. SEM images showed no evidence of slime layer or bacterial cells in the cut sections obtained from the treated dental waterlines indicative of absence of biofilm formation. Untreated DUWLs showed a microbial colonization with continuous filamentous organic matrix indicating significant biofilm formation. The tested disinfectant was found to be effective in removing biofilms from dental unit waterlines.[12] A similar inference was noted from samples in our study.

Meiller *et al* conducted multiple trials in overnight treatment of dental unit waterlines with Listerine Antiseptic (LA). The presence or absence of biofilm within the dental unit waterlines was evaluated pre- and post-treatment by scanning electron microscopy. One-month long follow-up clinical trials have demonstrated that a maintenance solution of a 1:50 concentration of LA and sterile distilled water in self-contained dental units with new tubing is effective for prolonged periods in maintaining the effluent within the American Dental Association's recommendation for the year 2000 of < 200 CFU per ml.[13]

Our analysis shows statistically significant differences in mean bacterial count between three way syringe and oral rinse samples in both group I (Gluteraldehyde -Quarternary ammonium compound) and II (Sodium hypochlorite) pre and post treatment Pair wise comparison of percentage change in bacterial count shows statistically significant difference in mean percentage change (P=0.001)between group III and Group(I & II) in both three-way syringe and oral rinse samples. But there was no statistically significant difference in mean percentage bacterial change between Sodium hypochlorite (group II) and Glutaraldehyde -quarternary ammonium compound (group I). Hence the latter could be considered as a bio friendly alternative to sodium hypochlorite as disinfectant in dental unit waterlines.

Scanning electron microscopic images of the treated samples show no evidence of biofilm in group I and II which confirms the efficacy of disinfectants in eliminating microorganisms in DUWLS. Extensive and adherent biofilm was found in untreated sample as expected .This highlights the need for routine disinfection of dental unit waterlines.

In our analysis of the DUWLs it was revealed that not all of the dental chairs have water in their DUWLs that meet the recommendations of drinking water at the start of the study. As expected untreated water contained higher bacterial loads than the other treated water groups. In some of the tested samples, there was high concentration of heterotrophic organisms that use a carbon source for survival and anaerobic bacteria. Some of the samples contained more than 200 colonies of heterotrophic bacteria and more than 400 colonies of anaerobic bacteria. Colonies of Acinetobacter baumannii were incidentally noticed in some of the samples and confirmed using biochemical test.

Even though the study has shown high levels of bacteria, we cannot conclude or rule out that dental unit water be considered a risk for immunocompromised patients as the type of bacteria that are present in this water has not been investigated.

Conclusion

Within the limitations of the present study, it can be concluded that Glutaraldehyde - quarternary ammonium salts (QAT) combination and sodium hypochlorite-based disinfectants showed a statistically significant reduction in bacterial load within one week of disinfection procedure compared to untreated DCUs. Based on this

concluded thatit can be suggested that Gluteraldehyde -quarternary ammonium compound is a more safe and suitable alternative agent to Sodium hypochlorite for routine dental unit waterline disinfection. The high microbial loads found in the untreated dental unit water line are in accordance with the literature and illuminate the necessity of supplying them with disinfection systems. Biofilms were scarcely visible in both disinfectant treated waterlines, while they were adherent in untreated systems.

Furthur long follow up studies are needed to assess the effect of this new combination over time. Identification and isolation of the prominent bacterial species along with their significant role in nosocomial infections are to be observed. Regular disinfection protocols of DUWL has to strictly followed in everyday clinical practice so as to provide a safe environment for the practitioner and patient.

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