



RESEARCH ARTICLE

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EVALUATION OF EFFICACY OF VARIOUS DISINFECTANTS IN REDUCING MICROBIAL CONTAMINATION OF DENTAL UNIT WATER LINES

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ABSTRACT

Background: Many studies have shown extensive contamination of dental unit water lines. This poses a potential risk of infection for patients as well as dental professionals.

Objective: To compare and evaluate the efficacy of different commonly available disinfectants in reducing the microbial colony count in water derived from dental unit waterlines.

Method: Four dental units were selected and samples were collected before and after intervention using 2 disinfectants (0.02% H₂O₂ continuously), (1:50 Original Listerine overnight treatment), Air Purge technique and control group for 14 days. Samples were cultured in Brain Heart infusion agar.

Result: Study showed that all units were heavily contaminated with microbes. After intervention, counts reduced progressively in consecutive days.

Conclusion: Within the limitations of the present study, it was found that all types of intervention were effective in reducing the microbial colony count of the DUWLs. Continuous Hydrogen peroxide and air purge were most effective.

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INTRODUCTION

Dental units are the basic functional component of a clinical set-up. The three-way syringe, high speed air turbine hand-pieces, ultrasonic units are connected to the dental unit by a network of small bore plastic tubes. Air and water pass through the tubes for either activating or cooling these devices. Many studies have been conducted that have reported these waterlines to be highly contaminated with microbial biofilms.

[1] Microorganisms of at least 40 different species have been identified. The list includes oral streptococci, *Pseudomonas* spp., Enterobacteria, *Candida albicans*, *Legionella pneumophila* and non-tuberculous *Mycobacterium* spp.

Planktonic forms of microbes and shreds of biofilm are transferred directly into the oral cavity of patients during procedures. This represents a potential source of infection for both patients as well as dental health care personnel (Orrù, 2010). Municipal water, contaminated independent reservoirs and back flow patient's saliva are direct sources of DUWL contamination (Garg, 2012; Ozawa, 2010 and Lewis, 1992). Also, biofilm developing in small-bore plastic tubing constitutes for indirect source of contamination. Flushing or purging the waterlines have been recommended to improve the quality of dental water lines (Szymanska, 2003 and Watanabe, 2008). Chemical treatments using agents like hydrogen peroxide, chlorhexidine and sodium hypochlorite have been studied by various authors (Orrù, 2010; Ozawa, 2010;

Rodrigues, 2005). Despite this situation, there is still lack of awareness and management techniques. Thus this study was performed to compare and evaluate efficacy of various disinfectants that are both cost effective and commonly available for reducing the microbial colony count in DUWLs.

MATERIALS AND METHODS

The study was conducted to evaluate and compare the efficacy of different disinfection techniques and chemicals in reducing the microbial colony count in water collected DUWLs through 3-way syringe and air-rotor device at the Department of Conservative Dentistry and Endodontics, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh, India. Before initiating the disinfection protocol, samples were collected from all the marked unit for baseline count. Four units with independent reservoirs were labelled as group A, B, C and D according to the type and method of disinfection.

Group A: 1:50 Original Listerine overnight treatment. (Prepared by dissolving 20 ml of original listerine in 1L of distilled water) (Listerine, Johnson & Johnson Ltd, India) The unit was flushed with the solution for 2 minutes so that it reaches till the length at the exit points and was allowed to stand overnight. In the morning, the solution was replaced by flushing for 2 minutes using sterile water.

Group B: Dental water-lines were allowed to remain dry overnight after air purge, to prevent biofilms to thrive in moist environment. In the morning a 2 minute flush was done to eliminate patent material in the pipes.

Group C: 0.02% Hydrogen Peroxide. The solution was prepared by dissolving 3.33ml of 6% H₂O₂ in 1L of distilled water and was added in the independent reservoir and was used continuously.

Group D: Control group. It received no intervention and was used as a baseline to compare groups and assess the effect of intervention.

Microbiological Assessment: Sterilized and labelled borosilicate glass test tubes were used for collecting pool of sample water. Five samples were collected at each instances. Samples were taken on zero day (before intervention), first day, seventh day and fourteenth day. A total of 80 samples were collected for the study.

After collection, the tubes were sealed and transported immediately to the Department of Microbiology, Himachal Institute of Dental Sciences, Paonta Sahib. The samples were serially diluted (dilution factor- 100) and was cultured on Brain Heart Infusion Agar (Himedia Laboratories, Pvt. Ltd. Mumbai, India) using spread plate method. Samples were incubated at 37 °C for 24 to 48 hours. Afterwards the cultured plates were taken out and the colony forming units were counted manually using microbial colony counter. (Spectronics, India). The results were charted for each samples in terms of colony forming units per ml (cfu/ml) and were evaluated statistically using one-way ANOVA and paired t-test.

Statistical Analysis: Results were charted and statistically evaluated using one-way ANOVA and paired t-test.

RESULT AND STATISTICS

The results of the study were based on the number of colony forming units per milliliter. To evaluate the efficacy, the results were subjected to paired t-test and to compare the efficacy of each disinfectant, data was subjected to one-way ANOVA. There was a statistically significant difference in mean colony count among samples 1, 2, 3 and 4 at day 0 ($p=0.008$), day 1 ($p=0.011$), day 7 (0.001) and day 14 ($p < 0.001$). Although reduction in microbial count was observed in all the groups, clinically and statistically significant difference in colony count from day 0 to day 14 was observed in air purge and hydrogen peroxide groups.

Sl No	GROUPS	I	II	III	IV	Total
		Day 0	Day 1	Day 7	Day 14	
1	1:50 Original Listerine overnight	5	5	5	5	20
2	Air-purge	5	5	5	5	20
3	0.02% H2O2	5	5	5	5	20
4	Control	5	5	5	5	20
TOTAL NUMBER OF SAMPLES						80

Figure 1. Sample Grouping

Sl. No.	GROUPS	MEAN COUNT(CFU)			
		Baseline	Day 1	Day 7	Day 14
1	Listerine	475	150	63	50
2	Air Purge	720	326	145	40
3	H2O2	650	95	75	15
4	Control	350	300	210	200

Figure 2. Mean values of colony count (CFU/ml) after culture of DUWL samples

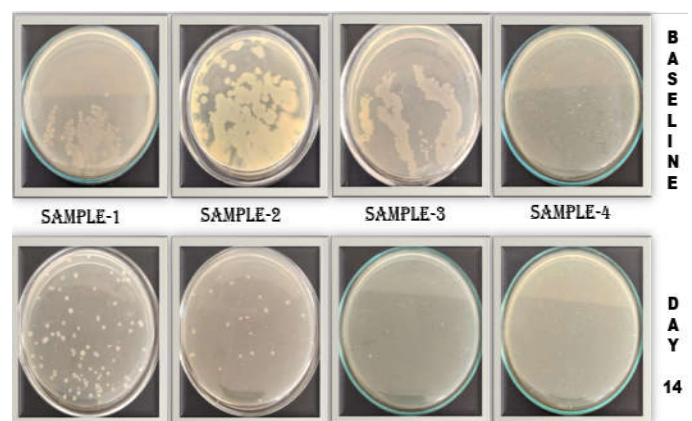


Figure 3. Brain heart infusion agar culture plates of all groups

DISCUSSION

Reduction of microbial count in the dental office is very important for patient as well the dental health care giver. Infection control regimen has to be incorporated for the same. Potential source of pathogens is usually from patients to practitioners, also air and water plays a role (Coan, 2007). In dentistry, many procedures are performed using water from municipal sources or from over-head tanks.

		N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
			1					
Day0	Listerine	2	475	35.35534	157.3449	792.6551	450	500
	Air Purge	2	720	28.28427	465.8759	974.1241	700	740
	Hydrogen Peroxide	2	650	70.71068	14.6898	1285.31	600	700
	Control	2	350	70.71068	-285.3102	985.3102	300	400
	Total	8	548.75	160.8404	414.284	683.216	300	740
Day1	Listerine	2	150	0	150	150	150	150
	Air Purge	2	325	35.35534	7.3449	642.6551	300	350
	Hydrogen Peroxide	2	95	7.07107	31.469	158.531	90	100
	Control	2	300	70.71068	-335.3102	935.3102	250	350
	Total	8	217.5	108.3315	126.9326	308.0674	90	350
Day7	Listerine	2	62.5	17.67767	-96.3276	221.3276	50	75
	Air Purge	2	145	7.07107	81.469	208.531	140	150
	Hydrogen Peroxide	2	75	7.07107	11.469	138.531	70	80
	Control	2	210	14.14214	82.938	337.062	200	220
	Total	8	123.125	63.97195	69.6431	176.6069	50	220
Day14	Listerine	2	50	14.14214	-77.062	177.062	40	60
	Air Purge	2	40	0	40	40	40	40
	Hydrogen Peroxide	2	15	7.07107	-48.531	78.531	10	20
	Control	2	200	0	200	200	200	200
	Total	8	76.25	77.81618	11.194	141.306	10	200

Figure 4. Statistical analysis: mean standard deviation

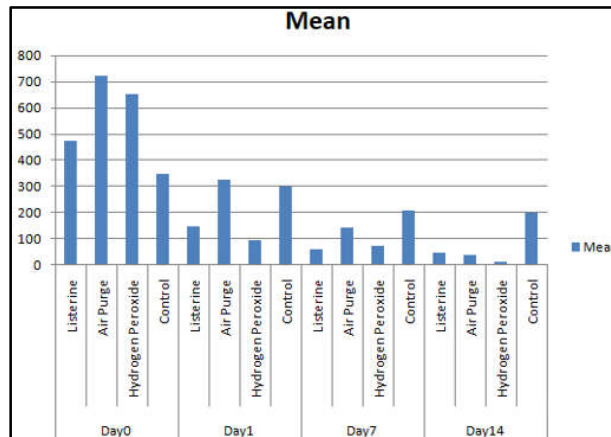


Figure 5. Comparison of mean values of all groups at different time intervals

Paired Samples Test									
	Paired Differences	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
					Pair 1 ListerineDay0 - ListerineDay14	0			
Pair 2 AirPurgeDay0 - AirPurgeDay14	0	680.0000	28.28427	20.00000	425.87591	934.12409	34.000	1	.019
Pair 3 H2O2Day0 - H2O2Day14	0	635.0000	63.63961	45.00000	63.22079	1206.77921	14.111	1	.045
Pair 4 ControlDay0 - ControlDay14	0	150.0000	70.71068	50.00000	-485.31024	785.31024	3.000	1	.205

Figure 6. Paired sample test

Water reaches the oral cavity of the patient via the 3-way syringe or the ultrasonic unit (Coan, 2007). The quality of the water should be at least of drinking water purity, if not better (Warren, 2012). But, in reality, studies have found that untreated DUWLs deliver heavily contaminated water. Historically, attempts have been made by employing various methods and chemicals to reduce the microbial contamination of the water delivered from dental units. Few of the documented techniques being nightly air purging, flushing the DUWL, independent bottle system, in conjugation with filtration, anti-retraction devices to prevent backflow, sterile water treatment and chemical treatment with different chemical agents (Garg, 2012). Original Listerine was evaluated as a potent disinfectant in our study. It was used in the ratio of 1:50 ratio with distilled water for overnight treatment. The microbial count reduced from 475 CFU to 50 CFU as recorded on the baseline day and 14 days after the intervention. Similar results were obtained by Kettering J et al., and Meiller TF et al, in their respective studies (Kettering, 1998; Meiller, 2000).

Listerine constitutes of three phenol derived essential oils: 0.064% thymol, 0.092% eucalyptol, 0.042% menthol combined with 0.60 methyl salicylate. It has bactericidal effect due to cell wall destruction, bacterial enzymatic inhibition and bacterial lipopolysaccharides (Al Habashneh, 2014). Microorganisms in biofilm thrive better in moist environment. So one of the concepts is to empty the pipe-line via air purge at the end of the day. And leaving the unit off overnight to dry to reduce microbial count. Next morning, flushing is done to wash out all residual shreds and debris (Garg, 2012). In this study, colony count reduced from 720CFU to 40CFU after 14 days of intervention. Hydrogen peroxide and products containing the same are known to be effective disinfectants for DUWL treatment (O'Donnell, 2011). It has a wide spectrum of antimicrobial action. It has proven action against bacteria, yeast, fungi and spores. The effectiveness may depend on concentration, pH, temperature, reaction time, use in combination with physical agents. It also depends on microbial concentration, species and their biological phase (spore or vegetative), genetic make-up. It acts on microbes due to the presence of the hydroxyl radical (OH^+), which is said to be the strongest oxidant known. It attacks membrane lipids, DNA and other essential cell components. Some of the biofilm-forming cells are killed by internally produced H_2O_2 (Coan, 2007).

In this study, 0.02% H_2O_2 was used as continuous disinfectant for 14 days. The mean baseline count was quite high (650 CFU). After 14 days, post intervention, effective reduction was recorded (15CFU). Control group was introduced in the study to minimize the effects of variables other than the independent ones, to increase the reliability of the results. No intervention was made for this group. The microbial colony count showed a slight decrease from 350CFU to 200CFU. The chemical agents and techniques employed in this study are cost effective and easily available. Thus this study was conducted to compare and evaluate the efficacy of each of these on microbial reduction of water delivered from the DUWLs.

Conclusion

Within the limitations of the present study, it was concluded that all the intervention showed reduction in microbial colony count, but statistically hydrogen peroxide and air purge groups showed the best outcome.

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