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ORIGINAL RESEARCH

Efficacy of preprocedural mouth rinse containing chlorine dioxide in reduction of viable bacterial count in dental aerosols during ultrasonic scaling: A double-blind, placebo-controlled clinical trial

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ABSTRACT

Background: The risk to dentists, dental assistants, and patients of infectious diseases through aerosols has long been recognized. The aim of this study was to evaluate and compare the efficacy of commercially available preprocedural mouthrinses containing 0.2% chlorhexidine (CHX) gluconate, chlorine dioxide (ClO_2) mouthwash, and water in reducing the levels of viable bacteria in aerosols. **Materials and Methods:** This single-center, double-blind, placebo-controlled, three-group parallel-designed study was conducted over a period of 4 months. One hundred twenty patients with chronic periodontitis were divided randomly into three groups (A, B, and C) of 40 patients each to receive the ClO_2 mouthwash, water, and 0.2% CHX gluconate respectively as preprocedural rinse. The aerosol produced by the ultrasonic unit was collected at five standardized locations with respect to the reference point, i.e., the mouth of the patient. The blood agar plates were incubated at 37°C for 48 h, and the total number of colony-forming units (CFUs) was counted and statistically analyzed. **Results:** The results showed that CFUs in groups A and C were significantly reduced compared to group B, and $P < 0.001$ [analysis of variance (ANOVA)]. CFUs in group C underwent the highest reduction, but statistically there was no significant difference between the mean values of postprocedural CFUs in groups C and A (i.e., $P > 0.05$). The numbers of CFUs were the highest at the patient's chest area and lowest at the patient's front i.e., the 6 o'clock position. **Conclusion:** This study proves that a regular preprocedural mouthrinse could significantly eliminate the majority of aerosols generated by the use of an ultrasonic unit, and that ClO_2 mouthrinse was found to be statistically equally effective in reducing the aerosol contamination to 0.2% CHX gluconate.

Key words: Aerosols, chlorhexidine (CHX), chlorine dioxide (ClO_2), mouthrinses, ultrasonic scaling

Introduction

Many routine dental procedures produce aerosol and splatter composed of various combinations of the following: Water; organic particles, such as tissue and tooth dust; and organic fluids, such as blood and saliva.[1] The microbial aerosol peak concentrations in dental treatment rooms were associated more with scaling procedures and to a lesser extent with cavity preparation.[2] Aerosols generated by dentists in their work may contain solid particles and chemicals or gases, as well as bacteria and viruses. Bacterial cells with diameters of approximately 0.2-2.0 μm or viruses with diameters 20-400 nm may be found in aerosols arising from an operative procedure or from subsequently altered splatter. Within a general dental practice, numerous procedures are performed on a daily basis that result in the production of aerosols and splatter.[3] These aerosols may be inhaled into the lungs and reach the alveoli, or they may come in contact with the skin or mucous membranes. Most of the aerosols produced during treatment procedures have diameters ≤5 μm,

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and these can cause respiratory or other health problems as they can penetrate into and remain within the lungs.[4] One of the methods of reducing overall bacterial counts produced during dental procedures is preprocedural rinsing with a product containing an antimicrobial agent.[5]

Chlorhexidine (CHX) gluconate, a bisbiguanide, is considered to be the most effective antiplaque agent, but it also has some side effects, notably tooth staining, taste alteration, enhanced supragingival calculus formation, and, less commonly, desquamation of the oral mucosa.[6] In an earlier study, rinsing with CHX mouthwash led to a 94.1% reduction in recoverable colony-forming units (CFUs) compared to the nonrinsed control, while the control rinse produced a 33.9% reduction.[7] Studies have stated that 1% povidone/iodine used as a preprocedural mouthrinse has a bactericidal effect on the microorganism, resulting in the reduction of surviving microorganisms for up to 4 h.[8] In another study, it was observed that 0.05% cetylpyridinium chloride was found to be equally effective as CHX in reducing splatter bacteria during ultrasonic scaling.[9]

But with the association of scientifically proven side effects with CHX (0.2%), newer, tissue-friendly mouthrinses with the power of stabilized chlorine dioxide (ClO_2) need to be evaluated. ClO_2 is widely used in various fields for its safe and highly antibacterial activity. This compound has the ability to effectively clean oral tissues on a daily basis without causing harmful side effects.[10]

The aim of this study was to evaluate and compare the efficacy of bacterial aerosol contamination generated by ultrasonic scalers following preprocedural rinse with commercially available ClO_2, 0.2% CHX, and water.

Materials and Methods

This clinical trial is registered under Clinical Trials Registry — India (CTRI) no. REF/2014/06/007100. The present study was conducted at the Department of Periodontology, Loni, India after it was approved by the Technical and Ethical Committee of the Pravara Institute of Medical Sciences University, Loni, Ahmednagar, Maharashtra, India. This single-center, double-blind, placebo-controlled, three-group parallel-designed study was conducted over a period of 4 months. The subjects enrolled in this study were selected from the Outpatient Department of Periodontology, Pravara Institute of Medical Sciences, Loni, India. The patients were initially screened for their plaque index (PI) (Silness and Loe) and gingival index (GI) (Loe and Silness) scores, and 120 subjects from both the sexes and with ages ranging 18-55 years, willing to participate in the study, and having a PI score of 2-3 and a GI score of 2-3 were selected for this study after informed consent was taken from them.

In relation to rinse schedules, a double-masked protocol was maintained in this study; the patients were recruited in chronological order by systematic sampling and were randomly allotted to one of the three groups by the examiner. They were then moved to a separate clinical operatory, where they were examined by the examiner for inclusion parameters. The preprocedural rinse was given to the participants, and once the patients performed the rinse, the operator performed scaling. The operator was not involved in any evaluations before or after. The treatment group was concealed from the patient, the operator, and the microbiologist.

Inclusion criteria included:

a. Having a minimum of 20 permanent teeth,
b. Not having undergone any dental treatment for the past 3 months,
c. Moderate to severe gingivitis, i.e., a GI score of 2-3,
d. Systemically healthy patients.

The exclusion criteria for the study were

a. The presence of any systemic disease,
b. The presence of a disease with possible effects on the immune system,
c. Received antibiotics or nonsteroidal anti-inflammatory drugs (NSAIDs) in the past 9-11 weeks,
d. Oral prophylaxis with last 3 months,
e. Pregnant and lactating mothers,
f. Smokers.

Blood agar plates were used to sample the air during the experimental procedure. Blood agar was chosen because it is a general purpose, nonselective and enriched medium that promotes the growth of microorganisms, such as those sampled from air. Table 1 shows the five standardized locations of the blood agar plates placed

Table 1: Standardized distances of plates

Plate no.	Plate position
Plate 1 (P1)	1 ft from the reference point (Patient chest)
Plate 2 (P2)	1 ft from the reference point (Operator position)
Plate 3 (P3)	1 ft from the reference point (Assistant position)
Plate 4 (P4)	2 ft from the reference point (12 o' clock position)
Plate 5 (P5)	8 ft from the reference point (6 o' clock position)

Reference point: Mouth of the patient

in operatory room for each treatment group and fixed distances of the plates were also maintained with respect to the reference point, i.e., the mouth of the patient [Figure 1].

A closed operatory with the facility to fumigate the room was used for all treatment procedures. Prior to the procedure the surfaces of the operatory were disinfected with ethyl alcohol (70%). Only one subject was treated per day, and the treatment ended on the same day. Prior to the procedure, the ultrasonic unit was switched on and flushed for 2 min, as directed by the manufacturer, in order to get rid of contaminated water due to overnight stagnation in waterlines. 45 min prior to the procedure, a blood agar plate was positioned on the plate 2 spot for a period of 15 min. This was further subjected to microbial assessment in order to check for environmental contamination, if present in the operatory.

One hundred twenty patients who met the minimal criteria for entry were selected. The nature of the procedure and the likely discomforts and risks were fully explained, and informed consent was obtained from each patient. Patients were recruited in chronological order by systematic sampling and were randomly allocated to one of the three following groups: Group A: (ClO_2), group B: (water), and group C: (0.2% CHX). Strict asepsis was followed inside the operatory, and the selected subjects entered the operatory wearing headcaps and autoclaved gowns. All activities such as conversation, sneezing, and coughing were strictly prohibited (if any such action occurred incidentally, then that subject was excluded from the study), and the subjects were instructed to refrain from all actions that generate aerosols. The patient was made to sit in a reclined position with his mouth at a standardized height of 3 ft from the floor of the operatory.

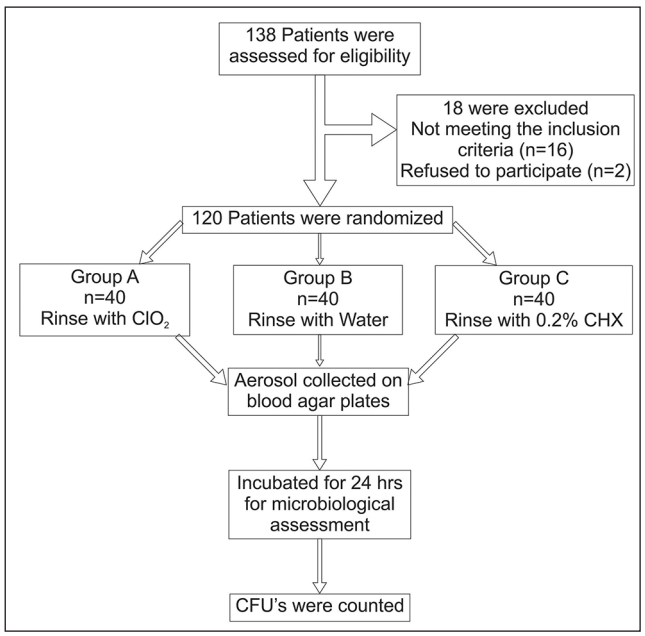

Figure 1: Study phases

Ultrasonic scaling was performed using an EMS ultrasonic scaler. Distilled water was used for all the ultrasonic scaling procedures. Coolant water flow and power settings were adjusted to a medium mode. The amount of water flow from the ultrasonic scaler during 1 min was then measured using a graduated cylinder. Based on these measurements, a water coolant volume of 15 mL per min was used during all the measurements of aerosol contamination. Prior to each trial, the coolant flow of the ultrasonic scaler was adjusted to this volume of water to ensure that coolant volume was consistent for all the trials.

Ultrasonic scaling was done on a randomly selected quadrant (control side) with the ultrasonic scaler for a period of 10 min. Following the 10-min sampling period, blood agar plates were covered and taken off the tray. A gap of 30 min was followed after the scaling procedure so as to allow the aerosols to settle down. After the gap of 30 min, fairly fresh blood agar plates were placed in a similar fixed position with regard to the reference point. The subject was then assigned to 1 min of 10 mL rinse with 0.2% CHX, ClO_2, or water. Ultrasonic scaling was again done with the same ultrasonic scaler on the other side (test side) of the same arch for a period of 10 min. Following the 10-min sampling period, blood agar plates were covered and taken off the tray. The blood agar plates were then transported immediately to the microbiology laboratory for microbial assessment. The blood agar plates were placed in an incubator and incubated at 37˚C for 48 h. After the incubation period, the plates were observed for microbial growth. Using a colony counter, the resulting CFUs were counted for each plate. Each colony was assumed to represent a single viable particle in the air, and the microbial concentration was defined as the number of viable particles per cubic foot of air.

The key ingredients of mouthrinses used in the study are illustrated in Table 2.

For statistical analyses, individual measurements were summarized within each individual and then analyzed.

Statistical analysis was performed by applying mean, standard deviation (SD), Student's unpaired *t*-test, Probability *P*, analysis of variance (ANOVA), and Tukey-Kramer multiple-comparison tests.

Results

One hundred twenty participants, 40 in each group, were analyzed, and analysis was according to intention to treat, as illustrated in Figure 2; the noncompliance rate for our study was 0 and there was no one who dropped out after randomization. Table 3 displays the age- and sex-wise distribution of the subjects in each group. The mean age of the subjects was 32 years and no significant differences in age or sex were found among groups for any demographic variables. Figure 3 shows the comparison of mean values of the GI and the PI for all three groups. By applying the one-way ANOVA test for repeated measures; the variations among column means were not significantly greater than expected than chance and by applying the Tukey-Kramer multiple-comparison test there was no significant difference between the mean values of GI and PI in Group A, B, and C when compared together, where the value of F = 1.184, (F=variance of the group means / mean of the within group variances), $P = 0.3177$, not statistically significant.

The mean and SD values of CFUs for each of the three treatment groups at the five standardized plate locations are summarized in Table 4. This analysis revealed that the 0.2% CHX group showed the maximum reduction of CFUs at all the five plate locations, followed by ClO_2. Compared with the control group (water), both the test groups (0.2% CHX and ClO_2) showed the efficiency of those products in reducing the number of CFUs. The numbers of CFUs were the highest at plate P1 (patient's chest) and the lowest at plate P5 (6 o'clock position). Comparison of the mean and SD values of CFUs in group A: (ClO_2), group B (water), and group C (0.2% CHX) by applying Student's paired *t*-test there showed a highly significant difference between the mean values of CFUs values from preprocedural to postprocedural in group A and C where $P < 0.01$, and no significant difference was observed in group B where $P > 0.05$ at all the plates, i.e., P1 to P5.

Comparison of mean postprocedural values of CFUs in group ClO_2 versus water, ClO_2 versus 0.2% CHX, and water versus 0.2% CHX were illustrated in Tables 5-7. On applying the Student's unpaired *t*-test, there was found a highly significant difference between the mean values of postprocedural CFUs in group A versus B and group B versus C (i.e., $P < 0.001$), while there was no significant difference between the mean values of postprocedural CFUs in group A versus C (i.e., $P > 0.05$).

Table 2: Ingredients of mouthrinse

Mouthrinse	Trade name	Ingredients
ClO_2	Power Rinse Oxyfresh®, USA	Deionized water; zinc acetate; sodium citrate; chlorine dioxide concentrate (15% solution); xylitol; sucralose; aloe powder; sodium hydroxide and citric acid. In addition, it is a nonalcoholic preparation, with no dye or color
CHX	Hexidine ICPA, India	CHX gluconate solution I.P. diluted to CHX gluconate 0.2% in aqueous base

ClO_2 = Chlorine dioxide; CHX = Chlorhexidine

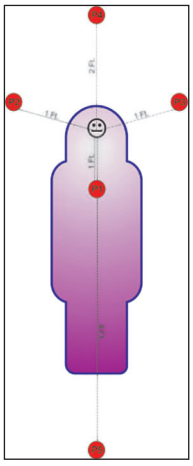

Figure 2: Culture plate locations (P1 to P5)

Table 3: Age- and sex-wise distribution of subjects in groups A, B, and C

Age (years)	Group A: ClO_2 (n = 40)		Group B: Water (n = 40)		Group C: 0.2% CHX (n = 40)	
	M	F	M	F	M	F
<20	2	0	1	0	1	0
20-30	2	5	3	4	5	2
30-40	11	11	10	9	12	12
40-50	4	4	4	6	6	2
>50	1	0	3	0	0	0
Total	20	20	21	19	24	16
Mean±SD	31.24±11.24	32.24±10.24	30.95±11.32	32.58±12.04	33.25±14.25	33.06±11.25

ClO_2 = Chlorine dioxide; CHX = Chlorhexidine; SD = Standard deviation; M = Male; F = Female

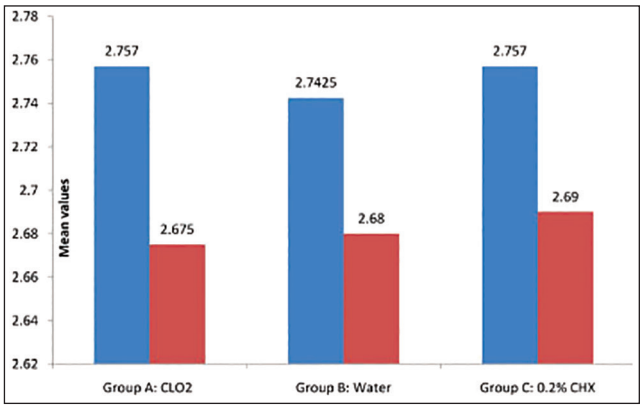

Figure 3: Comparison of the mean values of the GI and the PI in all groups

Discussion

Effective infection control is one of the cornerstones of good dental practice. However, due to many drawbacks, infection control has not been achieved to the greatest level of satisfaction. A marked increase in the airborne organisms was demonstrated in the samples collected from dental clinics where ultrasonic scalers were in use.[11] So there remains a risk of transmission of potentially harmful infectious agents to dentists and patients through several vectors including instruments and the air.[12] Many dental procedures such as ultrasonic scaling, air polishing, orthodontic debonding, and cavity preparation are known to produce viable bioaerosols. To effectively minimize the formation of bioaerosols, many protective barriers have been suggested, from the use of the mouth mask, to preprocedural rinse, to high-volume evacuators, to high-efficiency particulate air room filters.[13] Thus, the present study was designed to compare the efficacy of preprocedural rinse with ClO_2 and 0.2% CHX in reducing the quantity of bacterial contamination in bioaerosols generated during oral prophylaxis using ultrasonic scalers.

The results of this study showed that there was a highly significant reduction of bacterial CFU in both group A (ClO_2) and group C (0.2% CHX); however, there were no significant results in the reduction of bacterial CFU in group C (water), as seen in Figure 4.

In this study, 0.2% CHX pre-procedural rinse significantly reduced CFUs at all the five locations, compared to no preprocedural rinse. This finding is in accordance with data reported by Feres M *et al*.[9] These results are also consistent with those reported by Muir *et al*.,[14] who found that a preprocedural rinse with CHX to be more effective than no rinsing, in reducing aerosols generated by ultrasonic scaler. The enhanced efficacy of 0.2% CHX in reducing the CFUs could be because of the reason

Table 4: CFUs (mean ± SD) according to test group and location

Plate location (P1 to P5)	Mean and SD (Pre-Rinse)	Mean and SD (Post-Rinse)	*P* value (Pre-Post)	Significance
P1				
CLO_2	93.325±3.83	13.625±1.61	*P*<0.001	HS
Water	92.50±3.01	90.6±2.84	*P*>0.05	NS
0.2% CHX	92.325±3.43	11.12±2.10	*P*<0.001	HS
P2				
ClO_2	89.35±4.31	12.75±1.373	*P*<0.001	HS
Water	92.32±3.45	90.37±2.72	*P*>0.05	NS
0.2% CHX	92.12±3.61	11.36±1.84	*P*<0.001	HS
P3				
ClO_2	89.425±2.84	13.175±1.13	*P*<0.001	HS
Water	90.63±3.06	88.56±3.36	*P*>0.05	NS
0.2% CHX	90.76±2.78	10.78±1.69	*P*<0.001	HS
P4				
ClO_2	74.325±4.33	10.65±1.63	*P*<0.001	HS
Water	74.36±3.03	71.51±3.30	*P*>0.05	NS
0.2% CHX	70.57±3.00	8.78±1.10	*P*<0.001	HS
P5				
ClO_2	55.85±2.38	6.025±1.35	*P*<0.001	HS
Water	56.27±2.95	54.35±3.13	*P*>0.05	NS
0.2% CHX	54.34±3.90	3.90±1.18	*P*<0.001	HS

HS = Highly significant; NS = Not Statistically significant; SD = Standard deviation

Table 5: Comparison of mean and SD values of CFUs in ClO_2 vs water

P	Group A (ClO_2) (*n* = 40) Mean ± SD	Group B (Water) (*n* = 40) Mean ± SD	Student's unpaired t-test and *P* value with significance
P1	13.625±1.61	90.6±2.84	*t*=149.18, *P*<0.001, HS
P2	12.75±1.373	90.37±2.72	*t*=357.69 *P*<0.001, HS
P3	13.175±1.13	88.56±3.36	*t*=347.39 *P*<0.001, HS
P4	10.65±1.63	71.51±3.30	*t*=280.64 *P*<0.001, HS
P5	6.025±1.35	54.35±3.13	*t*=222.70 *P*<0.001, HS

P = Plate locations (P1-P5); SD = Standard deviation; HS = Highly significant

Table 6: Comparison of mean and SD values of CFUs in ClO_2 vs 0.2% CHX

P	Group A (ClO_2) (*n* = 40) Mean ± SD	Group C (0.2% CHX) (*n* = 40) Mean ± SD	Student's unpaired *t*-test and *P* value with significance
P1	13.625±1.61	11.12±2.10	*t*=1.87, *P*>0.05, NS
P2	12.75±1.373	11.36±1.84	*t*=1.74, *P*>0.05, NS
P3	13.175±1.13	10.78±1.69	*t*=1.39 *P*>0.05, NS
P4	10.65±1.63	8.78±1.10	*t*=1.54, *P*>0.05, NS
P5	6.025±1.35	3.90±1.18	*t*=1.77, *P*>0.05, NS

P = Plate locations (P1-P5); SD = Standard deviation; NS = Not statistically significant

Table 7: Comparison of mean and SD values of CFUs in water vs 0.2% CHX

P	Group B (Water) (*n* = 40) Mean ± SD	Group C (0.2% CHX) (*n* = 40) Mean ± SD	Student's unpaired *t*-test and *P* values with significance
P1	90.6±2.84	11.12±2.10	*t*=79.4, *P*<0.001, HS
P2	90.37±2.72	11.36±1.84	*t*=141.59, *P*<0.001, HS
P3	88.56±3.36	10.78±1.69	*t*=139.39, *P*<0.001, HS
P4	71.51±3.30	8.78±1.10	*t*=112.41, *P*<0.001, HS
P5	54.35±3.13	3.90±1.18	*t*=90.41, *P*<0.001, HS

P = Plate locations (P1-P5); SD = Standard deviation; HS = Highly significant

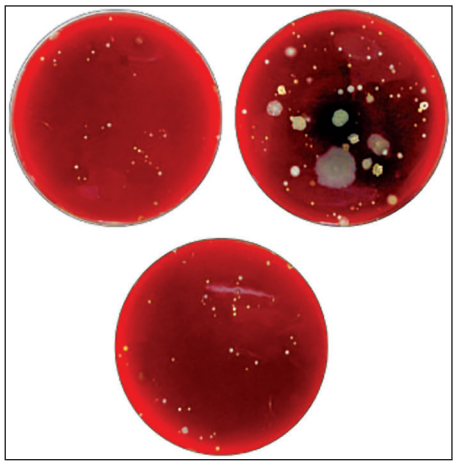

Figure 4: Microbial colonies formed on agar plates in all groups: A: ClO_2; B: water; C: 0.2% CHX

that CHX starts its antimicrobial action at the point of generation of aerosols and also at the time of onset of formation of aerosol. The antiplaque activity of CHX appears to be due to the retention of the drug in oral tissues and its subsequent slow release in an active form.

Similarly, when ClO_2 was used as a preprocedural rinse, fewer CFUs were developed than without preprocedural rinse. The enhanced efficacy of ClO_2 in reducing the CFUs could be because ClO_2 may act as a strong component in the obliteration of the microbiota via oxygenation and neutralization of toxins produced by the bacteria in the oral cavity. The stabilized ClO_2-based products also destroy the volatile sulfide compounds (VSCs), which further reduces the triggering of gingival inflammation. *In vitro* studies demonstrated stabilized ClO_2-based oral rinse microbicidal activity against various oral pathogens.[15-19] These studies showed that ClO_2-based oral rinse kills oral bacteria associated with the development and/or progression of oral diseases up to 99% in 10 s, and that the oral rinse is less toxic than CHX to human gingival cells *in vitro*.

In this study, it was also observed that on applying Student's unpaired *t*-test, there was no significant difference between the mean values of postprocedural CFU in ClO_2 and 0.2% CHX (i.e., $P > 0.05$) as seen in Table 5. Thus, by using any of the preprocedural mouthrinses, both will be equally potent in reducing the bacterial aerosols during ultrasonic scaling.

There was a lot of research support, and newer, higher versions of medicated combinations are available in the market, compared to the traditional follow-up of alcohol-based mouthwash. The product with the key ingredient of sodium chlorite (i.e., liberates ClO_2) has been confirmed to have equal potential in terms of reducing aerosol contamination with minimal side effects and more tissue compatibility.

The highest bacterial counts were detected on the plate 1, positioned at the patient's chest. These findings agree with those of Bentley *et al*.,[20] who observed that the larger salivary droplets generated during dental procedures settle rapidly from the air with heavy contamination on the patient's chest. Next-higher counts were found on the plate 2, positioned toward the operator, followed by plate 3, positioned toward the assistant's side. In addition, moderate bacterial contamination was found on plates 4 and 5 respectively.

This study demonstrated that a sufficient amount of aerosol and spatter from the patient is ejected far enough to come in contact with the dental personnel performing the treatment. Though the results underlined the need for mouth rinsing before dental procedure, ironically, few dentists put it into practice as a regular regimen in their dental practice. One of the barriers to such implementation is the strong bitter taste, brown discoloration of teeth, dryness, and burning sensation associated with the traditionally used alcohol-based mouthrinses. In such situations, ClO_2-based mouthrinses would be a genuine alternative, as these products showed equal efficiency in reducing the aerosol contamination compared to the alcohol-based products. But the advantage and edge over the traditional alcohol-based mouth rinse is that the ClO_2-based mouthrinses are more tissue-friendly, with no side effects such as burning sensations, dryness, taste alterations, or staining.[10]

The limitations of this study should be considered in the interpretations of the results. The CFUs that were counted here are the values that represent the bacteria capable of growing on blood agar plates. No attempt has been made to identify the bacteria: Either pathogen or nonpathogen. However, viruses, fungi, and specific bacteria require specialized media that were not cultured in this study. Future studies are needed to investigate the viable pathogenic microorganisms generated during the use of ultrasonic scaling devices.

Conclusions

Aerosol production during ultrasonic scaling is very hazardous to the patient, the operator, and the public at large. Hence, preprocedural rinsing should be made a regular practice in all dental setups, along with high-

vacuum evacuation and other barrier techniques.[21] ClO_2 mouthrinse was found to be statistically equally effective in reducing the aerosol contamination produced by ultrasonic scaling to, though slightly less potent than, 0.2% CHX. However, considering the disadvantages of alcohol based mouthrinse (strong bitter taste, brown discoloration of teeth, dryness, and burning sensation), ClO_2-based mouthrinses can serve as effective and safe agents for aerosol control during professional ultrasonic scaling in a dental setup.

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