

Efficacy of Preprocedural Rinsing With an Antiseptic in Reducing Viable Bacteria in Dental Aerosols

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THIS DOUBLE-BLIND, CONTROLLED, CROSS-OVER, CLINICAL study evaluated the effect of preprocedural rinsing with an antiseptic mouthrinse on the level of recoverable viable bacteria in an aerosol generated during a typical dental procedure. Eighteen subjects participated. Following 24 hours of abstention from all oral hygiene procedures, subjects received a 10-minute ultrasonic scaling of a randomly selected one-half of their mouth which served as the unrinsed control. They were then randomly assigned either antiseptic mouthwash or a control rinse and rinsed with 20 ml for 30 seconds, after which the remaining half mouth (experimental side) was scaled ultrasonically for 10 minutes. During each 10-minute scaling period aerosolized bacteria were collected on a sterile filter using a modified vacuum air-sampling device. Microbes captured on the sterile filter were quantitated by overlaying the filters onto trypticase soy agar, incubating the filters aerobically at 37°C for 24 to 72 hours, and counting the resulting colony forming units (CFU). Preliminary experiments had confirmed that neither the collection method nor residual antiseptic mouthwash in the aerosol adversely affected the number of viable bacteria recovered from the filter. Rinsing with the antiseptic mouthwash produced a 94.1% reduction in recoverable CFUs compared to the non-rinsed control, while the control rinse produced a 33.9% reduction. The difference between the mouthwash and control was statistically significant ($P < .001$). This study indicates that preprocedural rinsing with an antiseptic mouthwash can significantly reduce the microbial content of aerosols generated during ultrasonic scaling and may have potential in-office use as part of an infection control regimen. *J Periodontol* 1992; 63:821-824.

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The aerosolization of oral microbes which occurs during certain dental procedures^{1,2} can potentially result in cross contamination in the dental operatory and transmission of infectious agents to both dental professionals and their patients. It is reasonable to assume, therefore, that any stratagem for reducing the viable bacterial content of these aerosols could lower the risk of such cross contamination. For example, it has been shown³⁻⁵ that preprocedural use of an antiseptic mouthrinse significantly reduced the level of viable bacteria in the backspray derived from an air turbine handpiece. More recently, antiseptic mouthrinses have been shown to produce a significant reduction in salivary bacteria^{6,7} providing additional support for the use of these agents preprocedurally. The purpose of this investigation

was to determine the efficacy of preprocedural rinsing with an antiseptic mouthrinse[†] in reducing the level of viable bacteria contained in aerosols generated by ultrasonic scaling. This study utilized a new method for quantitation of aerosolized bacteria to improve the sensitivity of these assessments. Preliminary experiments confirming the validity of the sampling methodology are also reported.

MATERIALS AND METHODS

Eighteen healthy adult subjects meeting the following criteria entered and completed the study: A.D.A. Periodontal Case Type I or II, as determined by clinical probing and radiographs; negative history of blood dyscrasia, renal or hepatic disease, or immunosuppression; negative history of rheumatic fever, heart murmur or defect, or any other condition requiring prophylactic antibiotics prior to invasive

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dental procedures; negative history of current antibiotic, anticoagulant, or steroid therapy; negative history of antibiotic therapy and/or dental scaling, root planing, or prophylaxis during the previous 6 months; and a minimum of 20 sound natural teeth; a modified Quigley-Hein Plaque Index ≥ 2.0 ;⁸ and a Modified Gingival Index ≥ 1.5 .⁹ The study protocol was reviewed and approved by the Institutional Review Board of the Columbia Presbyterian Medical Center. Subjects completed an informed consent form prior to entry into the study.

Following the medical and dental history and clinical screening examination, Modified Gingival Index and modified Quigley-Hein Plaque Index scores were recorded. Each qualifying subject then received a supragingival scaling and rubber cup prophylaxis to reduce the Plaque Index to 0.

Subjects returned 7 days after the prophylaxis, having abstained from all oral hygiene procedures for 24 hours, and received an ultrasonic scaling⁸ of a randomly selected half mouth (right or left side) for 10 minutes to provide the baseline for that day's test. Subjects were then randomly assigned either the antiseptic mouthwash or a 5% hydroalcohol control rinse, and rinsed under supervision with 20 ml of the assigned rinse for 30 seconds. The remaining half of the mouth was then scaled for 10 minutes using the ultrasonic scaler. Each subject was scaled by the same clinician (CM) at each of the test sessions, using the ultrasonic unit with a medium power setting. During each 10 minute scaling period, the aerosol produced by the ultrasonic scaler was sampled extraorally using a vacuum air sampling device¹⁰ which captured bacteria contained within the aerosol on a sterile filter membrane. The air sampling device was modified by inserting a 3-piece filter 4.2 cm diameter cassette¹¹ containing a sterile 0.45 μm filter in a specially adapted intake tube. For aerosol sampling, the filter cassette attached to the intake tube was directed at the subject's mouth at a distance of 2" with the air flow vacuum set at 55 cubic feet per hour. Bacteria were collected on the front surface of the filter membrane. This basic filtration and sampling system has been used extensively to monitor airborne contamination in the food industry.¹⁰

Subjects repeated this identical regimen of ultrasonic scaling and rinsing 1 week later using the alternate rinse. The side (left or right) selected for baseline collection the first week was used for baseline collection the second week.

Prior to sampling on each test day, the dental unit water lines were flushed for 1 minute followed by a 10-minute aerosol sampling period in which the spray was collected from the ultrasonic scaler tip with the filter/intake apparatus positioned as described above. The number of organisms collected during this sampling period was so small as to be virtually uncountable and therefore was insignificant compared to the number collected during scaling.

A computer-generated random code was used to maintain double-blinding with respect to the rinse schedule. Neither the patient, the clinician, nor the laboratory technician was aware of the treatment code. Additionally, personnel dispensing the test rinses did not otherwise participate in the examinations in order to avoid potential bias.

Following ultrasonic scaling, the filters were removed aseptically from the sampling device, overlaid on enriched trypticase soy agar¹¹ and incubated aerobically at 37°C for 24 to 72 hours. Colonies were counted with the aid of a dissecting microscope.

Colony counts were transformed to \log_{10} scores for statistical analysis. Analysis of variance was used to test for treatment differences, with the model employed taking into account potential interactions of order of rinse usage as well as right/left jaw effects.

Collection Methodology

Prior to the clinical study, in order to confirm the appropriateness of the methodology, preliminary experiments were carried out to establish whether the collection method *per se* or the presence of residual antiseptic rinse in the aerosol significantly affected the viability of bacteria collected on the filter.

An ampicillin-resistant strain of *S. sanguis* was grown anaerobically at 37°C in trypticase soy broth containing 25 $\mu\text{g}/\text{ml}$ ampicillin* to an optical density of 0.700 at 660 nm, which corresponds to a concentration of 1×10^7 cells/ml. One hundred μl aliquots of this culture were placed either in a tube containing 9.9 ml of the antiseptic rinse or a tube containing 9.9 ml 0.85% sterile saline for 15 seconds. One hundred μl aliquots from each tube were then diluted with 9.9 ml sterile saline. This step effectively terminates the antiseptic action of mouthwash while merely further diluting the control sample. From each diluted sample, 1.5 ml aliquots were gently pipetted onto a MSI 3-piece filter cassette containing a 0.45 μm filter and connected to the vacuum air sampling device as described above with air flow set 55 cubic feet/hour.

In addition to this procedure, 1.5 ml aliquots of cell suspensions treated as described above were delivered to the filter using an atomizer designed to mimic the aerosol coming from the oral cavity. In both cases, the filters were removed from the cassettes with sterile forceps and placed on mitis salivarius agar** containing 25 $\mu\text{g}/\text{ml}$ ampicillin. The plates containing the filters were incubated anaerobically at 37°C for 24 hours followed by aerobic incubation for 24 hours, after which CFUs were counted. For purpose of analysis, CFUs derived from specimens gently delivered to the filter by pipette were compared to CFUs from the respective specimen delivered by the atomizer. A student *t*-test was used to evaluate differences in the two methods of delivery.

*Cavitron Model 3000, Dentsply International, York, PA.

**Model 200, Mattson-Garvin Co., Homosassa Springs, FL.

**MSI Clinical Monitor Cassette, Fisher Scientific, Springfield, NJ.

*Sigma Chemical Co., St Louis, MO.

**Difco, Detroit, MI.

An ampicillin-resistance strain of *S. sanguis* was grown anaerobically at 37°C in trypticase soy broth containing 25 µg/ml ampicillin for 24 hours to reach a concentration of 1×10^7 CFU/ml as previously described. This starting culture was diluted to achieve a working culture concentration of approximately 300 CFU/ml. A series of preseeded filters were prepared by placing 1 ml of the working culture on sterile 0.45 µm filters. For each clinical sampling period described below, a preseeded filter was placed in a 3-piece MSI cassette to which a vacuum with air flow of 55 cubic feet/hour was applied using the air sampling device. A subject rinsed for 30 seconds with 20 ml water and a 10-minute half-mouth ultrasonic scaling was performed. The subject then rinsed for 30 seconds with 20 ml of the antiseptic mouthwash and the remaining half mouth was scaled ultrasonically for 10 minutes. A different preseeded filter was used for each scaling period. This procedure duplicated the design of the clinical study and determined whether residual antiseptic mouthwash in the aerosol affects viability of cells already collected on the filter.

The preseeded filters were placed on mitus salivarius agar containing 25 µg/ml ampicillin and the plates were incubated at 37°C anaerobically for 24 hours and then aerobically for 24 hours following which CFUs were enumerated. For purpose of analysis, the number of viable preseeded *S. sanguis* exposed to the aerosol following the control rinse were compared to the number exposed to the aerosol following the antiseptic mouthwash rinse using a student *t*-test.

RESULTS

In the preliminary experiments, no significant differences were observed ($P > .05$) when cells delivered to the filter in the aerosol were compared to cells gently delivered to the filter. The respective aerosolized and liquid-layered *S. sanguis* counts were similar for both the antiseptic and control treated cells (Fig. 1), indicating that the impact of an aerosolized cell on the filter does not affect cell survival. Irrespective of the method of delivery, there were significantly fewer antiseptic-treated organisms recovered than control-treated ($P < .001$).

Similarly, no significant differences ($P = .161$) were found when filters preseeded with ampicillin-resistant strains of *S. sanguis* were exposed to aerosols produced by ultrasonic scaling after subjects rinsed with either antiseptic or a control rinse (Fig. 2). It appears, therefore, that the residual antiseptic mouthwash contained within the ultrasonically generated aerosol does not influence the survival of cells already trapped on the filter. Taken together, the results of these experiments indicate that the collection method *per se* will not affect the quantity of viable bacteria recovered from the filter.

Analysis of the clinical study data indicates that rinsing with the antiseptic mouthwash prior to ultrasonic scaling resulted in a 1.23 log reduction in recovered CFUs compared to baseline levels, while the control rinse produced

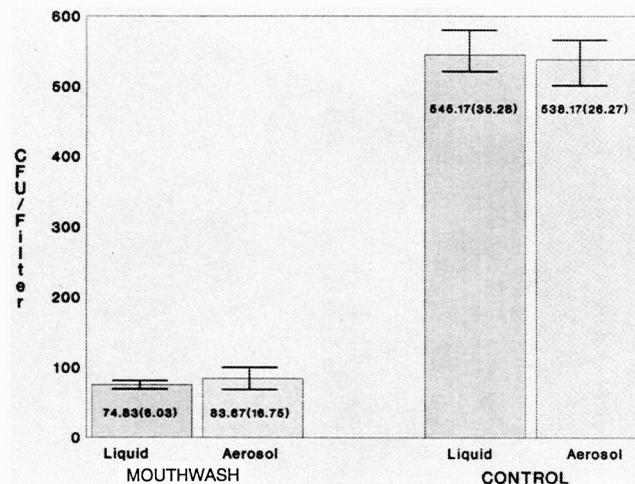


Figure 1. Effect of impaction on cell viability. Mean (S.D.) recoverable counts of *S. sanguis* following exposure to mouthwash or control and either delivered to the filter gently by liquid or impacted from an aerosolized mist.

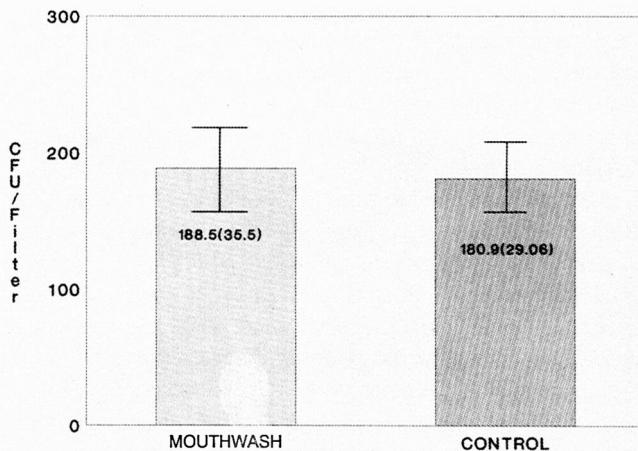


Figure 2. Effect of mouthwash carry over on cell viability. Mean (S.D.) recoverable counts of preseeded *S. sanguis* following exposure to aerosols generated by ultrasound scaling after rinsing with either mouthwash or control.

less than a 0.2 log reduction in recovered CFUs (Fig. 3). These results correspond to a 94.1% reduction for the mouthwash and a 33.9% reduction for the control. This reduction is significant at the $<.001$ probability level. There were neither treatment order effects nor significant differences in baseline counts between the left and right sides.

DISCUSSION

In this era of concern about infectious diseases in the dental operatory it is of utmost importance to consider all methods that can minimize the risk of transmission of potentially infectious agents to dentists, dental auxiliaries, and patients. Although the use of antiseptic rinses preprocedurally has been recommended,^{2,12,13} there has heretofore been relatively little data presented to demonstrate the efficacy of this method in actual dental procedures. This study clearly

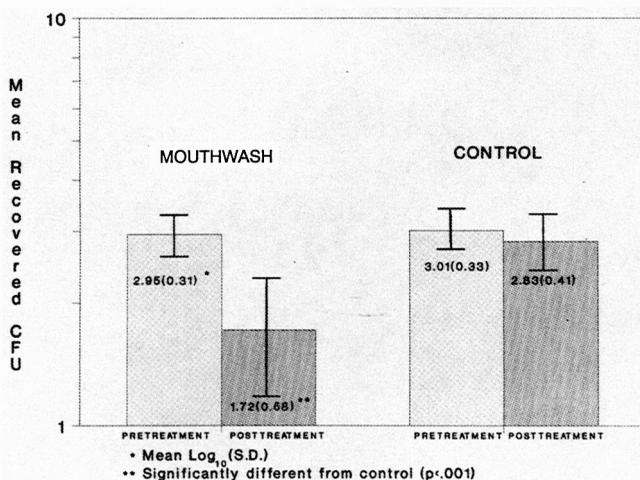


Figure 3. Reduction of viable aerosolized bacteria following preprocedural rinsing. Mean recoverable counts of bacteria in aerosol prior to and following rinsing with either mouthwash or control.

demonstrated the effectiveness of the preprocedural use of an antiseptic mouthrinse in reducing the level of viable bacteria aerosolized in the course of ultrasonic scaling. The cross-over design permitted comparison of both the active and the control rinse to a non-rinsed control. This allowed a distinction between the reduction produced by the physical effect of rinsing *per se* from that produced by the agent's antimicrobial activity, and suggests that mouthwash exerts its effect primarily through antiseptic activity. The comparability of the clinical study design to an actual clinical procedure suggests that this reduction in viable aerosolized bacteria can have significance in a clinical setting.

Additionally, the preliminary experiments described above investigated fundamental questions about the methodology employed to demonstrate clinical efficacy. These experiments demonstrated that neither the method of collection nor the presence of residual mouthwash in the aerosol affect the number of recoverable viable organisms from the collection filter and therefore do not artifactually influence the results.

In conclusion, the results of this study indicate that the antiseptic mouthwash used as a preprocedural rinse can significantly reduce the viable microbial content of aerosols generated during dental procedures. While this study design does not in itself permit an assessment of the decreased risk

of cross contamination, the results indicate that preprocedural rinsing with an antiseptic mouthrinse may potentially have a role in reducing the risk of cross contamination with infectious agents in the dental operatory.

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REFERENCES

- Micik RE, Miller RL, Mazzarella MA, Ryge G. Studies on dental aerobiology. I. Bacterial aerosols generated during dental procedures. *J Dent Res* 1969; 48:143-148.
- Earnest R, Loesche W. Measuring harmful levels of bacteria in dental aerosols. *J Am Dent Assoc* 1991; 122:55-57.
- Mohammed CJ, Manhold JH, Manhold BS. Efficacy of preoperative oral rinsing to reduce air contamination during use of air turbine handpieces. *J Am Dent Assoc* 1964; 69:715-718.
- Litsky BY, Mascis JD, Litsky W. Use of an antimicrobial mouthwash to minimize the bacterial aerosol contamination generated by the high-speed drill. *Oral Surg Oral Med Oral Pathol* 1970; 29:25-30.
- Wyler D, Miller RL, Micik RE. Efficacy of self-administered pre-operative oral hygiene procedures in reducing the concentration of bacteria in aerosols generated during dental procedures. *J Dent Res* 1971; 50:509.
- Veksler AE, Kayrouz GA, Newman MG. Reduction of salivary bacteria by pre-procedural rinses with chlorhexidine 0.12%. *J Periodontol* 1991; 62:649-651.
- DePaola L, Minah G, Overholser C, et al. Effect of an antiseptic mouthrinse on salivary microbiota. *J Dent Res* 1991; 70(Spec. Issue):515(Abstr. 1991).
- Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of vitamin C. *J Periodontol* 1970; 41:41-43.
- Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L. A modified gingival index for use in clinical trials. *Clin Prevent Dent* 1986; 8:3-6.
- Kang Y-J, Frank JF. Biological aerosols: A review of airborne contamination and its measurement in dairy processing plants. *J Food Protect* 1989; 52:512-524.
- Syed S, Svanberg M, Svanberg G. The predominant cultivable dental plaque flora of beagle dogs with gingivitis. *J Periodont Res* 1980; 15:121-136.
- Cottone JA, Molinari JA. State-of-the-art infection control in dentistry. *J Am Dent Assoc* 1991; 122(Aug):33-41.
- Recommended clinical guidelines for infection control in dental education institutions. *J Dent Educ* 1991; 55:621-627.

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