A CHEMICAL TREATMENT REGIMEN TO REDUCE BACTERIAL CONTAMINATION IN DENTAL WATERLINES

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ABSTRACT

This article describes a pilot study in which the authors used aerobic bacterial cultures to compare the effects of 1:10 mouthwash, 1:20 mouthwash and 2 percent ethanol in reservoir systems with seven conventional water systems. The long-term, low-concentration antiseptic reduced bacteria to within acceptable limits.

In recent years, concerns have been raised about microbial growth in slow-moving waterlines such as those in dental offices. In spite of little documented evidence of disease, the dental profession is understandably interested in preventing any problems that might arise in this area.[1] Dentistry aims to ensure total safety in the dental office, especially as dental patients' immune systems tend to become less vigorous overall because of

- increases in the number of dentulous elderly patients;
- the number of patients taking immunosuppressant drugs;
- the incidence of immunocompromising diseases.

Antiretraction valves in dental waterlines and autoclaved handpieces have limited the problems of contamination from one patient to another, yet there has never been any attempt by public health agencies to provide bacteria-free tap water. The current problem arises from aerobic microorganisms that form a mat of live and dead cells—a biofilm—that adheres to the walls of dental tubing, where the low water-flow rate enhances their growth. Apparently these organisms come from tap water.[2] Biofilms are generally composed of numerous species of bacteria. Current ADA recommendations call for flushing waterlines between patient visits to help dislodge organisms, as well as for the use of sterile water during dental surgery.[3] Use of in-line water filters to trap bacteria is being researched as another means of protecting patients.[4]

An alternative to using water directly from municipal supplies is the independent (self-contained) water system, available from several dental manufacturers. Low-concentration antibacterial agents in such independent water reservoirs have potential as a means of reducing the bacteria that reach patients. The senior author used a 1:10 dilution of Scope mouthwash (Procter & Gamble) in distilled water in two independent reservoir systems from the time the units were new until 42 months later. Periodic culture tests consistently showed no bacterial growth. The pilot study described here compares bacterial growth in these independent systems with growth in other dental offices using a conventional municipal chlorinated water supply or private wells, with and without chlorine.

MIXING ANTIBACTERIAL AGENTS FOR USE IN A 2-LITER RESERVOIR SYSTEM

1:10 Scope From Commercial Source
One part Scope to 10 parts distilled water
180 mL Scope to 1,800 mL distilled water

Concentrated Scope From Manufacturer
55 mL concentrated Scope to 1,945 mL distilled water

2 Percent Ethanol
42 mL ethanol to 1,958 mL distilled water

MATERIALS AND METHODS

The study compared water supplies in seven dental offices vs. independent water reservoir systems containing three different low-concentration antibacterial agents. A 1:10 dilution of Scope was used in the two independent reservoirs from the time they were new till 3½ years later. Either 1:20 Scope or 2 percent ethanol (which approximates 1:10 Scope in alcohol content) was used for 2 weeks before testing. We made 1:10 mouthwash by adding 55 milliliters of concentrated Scope to 1,945 mL of distilled water, and 2 liters of 2 percent ethanol by mixing 42 mL of 95 percent ethanol with 1,958 mL distilled water (Box, "Mixing Antibacterial Agents for Use in a 2-Liter Reservoir System").

Water from dental handpieces sterilized by biotested autoclaves was run for 10 seconds into sterile aerobic brain-heart infusion, or BHI, broth culture tubes (Carolina Biologicals). BHI was chosen because it supports growth of a wide variety of microorganisms. We obtained two samples per day for 4 days. Samples were obtained before patient care began each morning, followed by a repeat sample at midmorning. We determined microbial growth by observing the medium's turbidity after 48 hours of incubation at 34 C. This temperature was used because it is more conducive than room temperature (22 C) to the growth of organisms pathogenic to patients. The results are shown in Table 1. We assessed the accuracy of the 2-day incubation by conducting 4-day incubation at 34 C on a second series of samples from systems in which few positives were found (Table 2).

Low-concentration antibacterial agents in independent water reservoirs have potential as a means of reducing the bacteria that reach patients.

We used Millipore HPC Total Count Samplers (Millipore Corp.) to count viable colonies (colony-forming units, or CFU), according to the method described by the manufacturer. Before filling the sample, we added 0.10 mL of 10 percent sodium thiosulfate to 100 mL of handpiece water to inactivate the chlorine added by the water supplier. Eighteen milliliters of water was placed in the sterile sampler case, and the paddle containing dehydrated media beneath a 0.45-micrometer Millipore filter was immersed in the filled case for 30 seconds. The measured amount of media in the paddle allowed absorption of 1 mL of sample through the filter. Because the colonies grew directly on the filter membrane using the media immediately adjacent, it was possible to determine the number of CFU per mL. The water remaining in the case was discarded before the sample underwent stereoscopic microscopy at ×10 magnification. As a control, we tested distilled water obtained from a local drugstore by adding 1 mL to BHI culture tubes, incubating it for 4 days and testing it with the Millipore HPC Sampler as described above.

Water samples taken from the handpiece and from the tap were given numerical codes and analyzed for chlorine by standard municipal laboratory tests. Antiretraction valves were challenged by running a sterile high-speed handpiece partially submerged in a solution of methylene blue (0.2 mL in 10 mL tap water) for 30 seconds. The airflow/water flow was stopped abruptly several times without moving the handpiece. No inoperative valves were used as controls. The handpiece was then removed from the tubing and air and water were sprayed from the handpiece connector onto a white towel. Presence of blue dye on the towel was interpreted as failure of the antiretraction valve.

We measured the water flow through the dental handpiece for 1 minute to determine any correlation between flow rate and incidence of positive cultures.

TABLE 1

TWO-DAY BRAIN-HEART INFUSION BROTH INCUBATION: NO INACTIVATION OF DISINFECTANTS BEFORE CULTURE.

	TYPE OF WATER SUPPLY BY OFFICE									
	Tap Water Into Dental Units							Independent Reservoirs		
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8 2% EtOH	No. 9 1:10 Scope	No. 10 1:20 Scope
2-Day Microbial Growth in BHI Broth										
Day A No. 1*	+	+	-	-	+	-	+	-	-	-
No. 2†	+	+	-	+	+	-	+	-	-	-
Day B No. 1	+	+	-	-	+	-	+	-	-	-
No. 2	+	+	-	+	+	-	+	-	-	-
Day C No. 1	+	+	-	+	+	-	+	-	-	-
No. 2	-	+	-	-	+	-	+	-	-	-
Day D No. 1	+	+	-	-	+	-	+	-	-	-
No. 2	+	+	-	+	+	+	+	-	-	-
Chlorine Samples (ppm-100 mL)										
Handpiece	< 0.20	0	0.2	< 0.2	< 0.2	0.2	0.2	N/A	N/A	N/A
Tap water	0.30	0	0.26	0.26	0.5	< 0.2	0.2	N/A	N/A	N/A
Dye Test of Antiretraction	-	-	-	-	-	-	-	-	-	-
Flow Rate at Handpiece (mL/min.)	29.0	30.0	5.5	40.0	11.7	7.7	25.0	30.0	30.0	27.0
Millipore Colony Count (CFU/mL)‡	TNTC§	TNTC	11	TNTC	TNTC	480	TNTC	0	0	0
Routine Flush of Waterlines	-	+/-	-	+	+	+	+	-	-	-
Water Source S: Small water system with cholorinator P: Private well, no chlorinator C: City water I: Independent reservoir	S	P	C	C	C	C	C	I	I	I

* No. 1: Initial morning sample after flushing of waterline, taken before patient treatment.
† No. 2: Midmorning sample after use of handpiece lines and flushing of waterline.
‡ Millipore samplers incubated for 4 days, unless multiple colonies were observed after 2 days' incubation.
§ TNTC: Too numerous to count.

Scanning electron microscopy was used to examine the reservoir and handpiece tubing.

In a follow-up study, an independent reservoir was installed in office no. seven to replace a conventional water system. Supply lines to the dental unit were new, but lines within the unit and handpiece lines were not replaced. In this instance, 1:10 Cepacol (J.B. Williams Co.) was used in the reservoir for 1 week, followed by 1:10 Scope. Samples were taken after one, four and eight cycles of hyperchlorination with 1:10 Chlorox (Chlorox Co.) in the reservoir bottle, run through the lines until chlorine odor was detected, and left for 10 minutes before flushing with water until the chlorine odor was undetectable. The results are shown in Table 2.

TABLE 2

FOUR-DAY BHI BROTH INCUBATION IN SYSTEMS WITH FEW 2-DAY POSITIVE FINDINGS.

SAMPLE	NO. 3	NO. 4	NO. 6	NO. 7A*	NO. 7B†	NO. 7C‡	NO. 8§	NO.10**	DISTILLED WATER
Day E									
No. 1 (Preoperative)	+	+	+	+	-	+	-	-	-
No. 2 (Midmorning)	-	+	+	+	-	-	-	-	-
Day F									
No. 1 (Preoperative)	+	+	+	+	+	+	-	-	-
No. 2 (Midmorning)	+	+	-	+	+	-	-	-	-

* Two weeks after new system with Cepacol 1:10 (after one hyperchlorination with 1:10 Chlorox for 10 min.
† Four weeks after new system with change to Scope 1:10 (after four hyperchlorinations with 1:10 Chlorox).
‡ Six weeks after new system with Scope 1:10 (after eight hyperchlorinations with 1:10 Chlorox).
§ No. 8: 2 percent ethanol.
** No. 10: Scope 1:20.

In another follow-up study, we evaluated the effect of antiseptic action in the culture medium by using eight 10-fold dilutions of log phase growth (mildly turbid) cultures from three conventional-system offices that were incubated for 36 hours at 34 C and pooled immediately before inoculation. One-half–milliliter aliquots of these increasingly dilute bacteria sources were compared with equal-volume samples from the dental handpiece reservoir systems. We used 1.0 mL of 2 percent ethanol, 1:10 Scope, 1:10 Cepacol and sterile water with each dilution, while sterile water served as a control. Additionally, we used 1.0 mL of handpiece effluvium from the original reservoir systems containing 2 percent ethanol or 1:10 Scope to determine any presence of viable bacteria in the reservoirs. Table 3 illustrates that some ingredient beyond 2 percent ethanol (contained in Scope and Cepacol) appears to be the effective ingredient.

We then neutralized antiseptic action by use of Letheen broth (Carr-Scarborough), which deactivates quaternary ammonium compounds, phenolic disinfectants and ethanol by action of lecithin and polysorbate-80.[5] BHI was used as a control. These cultures were incubated at 35 C for 4 days, then at 22 C (room temperature) for 4 additional days. The results are shown in Table 4.

RESULTS

Two-day incubation of culture from conventional water supplies showed positive results, with the exception of cultures from office no. three. Four-day incubation resulted in 75 percent positivity of the conventional systems, which did not show turbidity after 2-day incubation. All disinfectant-independent reservoir systems yielded negative growth after 2- and 4-day incubations (Tables 1 and 2). This was confirmed by Millipore colony counts of zero for the antiseptic reservoir systems after 4-day incubation. Cultures of distilled water showed no growth, and no colonies grew on Millipore samplers. Only one Millipore colony count for the conventional water supply was below the recommended maximum.

Handpiece water chlorine levels were generally slightly lower than the chlorine concentration at the tap, but they were within the acceptable range for municipal systems. Antiretraction valves all functioned properly. Low flow rates through the handpiece correlated with lower Millipore colony counts and with negative 2-day cultures. Routine flushing of waterlines did not correlate with either low bacterial counts or negative cultures. SEM studies of independent reservoir systems with disinfectant showed no biofilm in the reservoir pick-up tubes or in handpiece lines.

Hyperchlorination of a conventional system converted to an independent reservoir in office no. seven did not yield bacteria-

TABLE 3

BRAIN-HEART INFUSION BROTH INCUBATION.*

	1:10 DILUTION SERIES								CONTROL	HANDPIECE WATER RESERVOIR	
	1×	2×	3×	4×	5×	6×	7×	8×	Sterile Water	1:10 Scope	2 Percent Ethanol
2 percent ethanol	+	+	+	+	+	+	+	-	-	-	-
1:10 Scope	-	-	-	-	-	-	-	-	+	-	-
1:10 Cepacol	-	-	-	-	-	-	-	-	-	-	-
Sterile water	+	+	+	+	-	+	-	-	-	-	-

* Incubation for 4 days at 35 C, then 4 days at 22 C.

free water, even with 6 weeks of dilute mouthwash usage. Three additional hyperchlorinations performed before weekend shutdown showed reduction of positive 4-day cultures from 100 to 50 percent.

Table 3 shows suppression of bacterial growth by dilute Scope and dilute Cepacol in BHI, but no suppression by 2 percent ethanol alone, even in the presence of large numbers of bacteria. When Letheen broth inactivated the mouthwash antiseptics, even diluted bacterial inocula caused turbidity of cultures (Table 4). In both BHI and Letheen media, samples from reservoir systems showed no growth.

DISCUSSION

Using dilute mouthwash or equivalent ethanol in independent reservoirs appears to be an efficient and easy method of ensuring safe water for dental treatment. This system involves some expense in terms of equipment and solution purchase and periodic testing for bacteria and function of the antiretraction valve.

However, this method saves some time in that flushing appears unnecessary to maintain safe water at the handpiece. Our results—consistently negative cultures for more than 3 years—make routine flushing appear unnecessary.

While no single culture method discloses all bacteria, the screening techniques used here are likely to grow most aerobic microbes. In spite of the prevalence of anaerobes in many dental infections, anaerobic culture techniques were not warranted due to the high oxygen levels in water. Apparently, 2-day incubation is inadequate for accurate results; 4-day incubation appears better.

Our results—consistently negative cultures for more than 3 years—make routine flushing appear unnecessary.

The water in office no. three was the only conventional water source within the acceptable limit of 200 CFU/mL.[3] Two-day cultures from this office were negative. The reason may be the small size of innoculum due to the low flow rate (10 sec. sampling × 5.5 mL/min. = 0.9 mL). In office no. six, where the flow rate was low (at 7.7 mL/min.), there was also a low number of positive 2-day cultures. However, both offices had 75 percent positivity at 4-day incubation. No handpiece lubricant was used in office no. three or office no. six, so the possible antibacterial action of a lubricant was not a factor. The age of equipment and chlorine levels in these offices were within the range of those in other offices. In future studies, use of equal volumes of sample would seem advisable.

A factor not currently addressed in the literature is the time of patient exposure to dental water spray during various procedures. Use of lower flow rates that allow adequate cooling and debris removal should be investigated. Similarly, effectiveness of water removal with various suction techniques and devices is another possibility for limiting patient exposure. Use of the rubber dam should be considered as another means of protecting patients from possible adverse effects of dental water.

While commercial distilled water cannot be considered sterile, Williams and colleagues[6]

TABLE 4

LETHEEN BROTH INCUBATION: DISINFECTANTS INACTIVATED BEFORE CULTURE.*

	1:10 DILUTION SERIES								CONTROL	HANDPIECE WATER RESERVOIR	
	1×	2×	3×	4×	5×	6×	7×	8×	Sterile Water	1:10 Scope	2 Percent Ethanol
2 percent ethanol	+	+	+	+	+	+	-	-	-	-	-
1:10 Scope	+	+	+	+	+	+	-	-	-	-	-
1:10 Cepacol	+	+	+	+	-	+	-	-	-	-	-
Sterile water	+	+	+	+	+	+	-	-	+	-	-

* Incubation for 4 days at 35 C, then 4 days at 22 C.

found that it contained no bacteria, as did we in this study. Since numerous authors report the presence of bacteria in tap water, it would seem prudent to use distilled water in self-contained systems, with or without antiseptics. Likely, this low or absent bacterial load from the beginning helps the low-concentration antiseptics work more efficiently to maintain an environment hostile for bacteria in the antiseptic-reservoir system we studied.

Chlorine concentration in handpiece waterlines was essentially the same as it was in tap water, indicating that factors other than diminished chlorine allow biofilm formation in slow-moving lines. Vess and colleagues[7] showed that 7 days of continuous exposure to 10 to 15 parts per million continuous chlorine did not eliminate established single-organism biofilm bacteria growing in polyvinyl chloride water pipes. This is more than 50 times the concentration found in the tap water in this study. Thus, installation of chlorinators in the dental office to raise chlorine levels would seem to be of little value in reducing waterline bacteria to acceptable levels. Furthermore, prolonged contact with high-level chlorine can adversely affect internal metal components in dental equipment. Vess and colleagues also noted that repeated hyperchlorination did not produce negative cultures, while 70 percent ethanol was effective in killing test pathogens.[7] Given the difficulty in breaking up biofilm, prevention of buildup seems prudent.

> ***Perhaps the lingering action of detergent, essential-oil flavoring agents or quaternary ammonium in mouthwash played a role in antisepsis.***

That ethanol allowed growth in BHI (Table 3) suggests that 2 percent ethanol alone is not sufficiently powerful to kill large numbers of bacteria. Comparison of the results of dilute Scope and dilute Cepacol in tables 3 and 4 shows that antiseptic in the media should be inactivated to correctly determine water bacteria in antiseptic water systems. Further comparison of these tables shows that handpiece water samples from reservoirs with 1:10 Scope and with 2 percent ethanol do not contain viable bacteria.

We interpreted this technique's effectiveness as being due to the antiseptics in mouthwash and their length of exposure, the probably bacteria-free distilled water and the effectiveness of the antiretraction valves in preventing oral bacteria from entering the system. One cannot rule out the residual effects of mouthwash in dental waterlines. Perhaps the lingering action of detergent, essential-oil flavoring agents or quaternary ammonium in mouthwash played a role in antisepsis.

There is no research regarding possible effects of dilute mouthwash on the bond strength of dental restorative materials. Until such data are available, it may be prudent to use 2 percent ethanol in distilled water, in spite of its lesser ability to kill bacteria (Table 3). Concern remains about patients' possible ingestion of alcohol; without high-velocity suction, the patient could swallow a substantial volume of irrigant. This would be especially pertinent in treating chemically dependent patients and those medicated with drugs capable of interacting with ethanol.

No research has been report-

ed regarding the use of these solutions for surgical procedures. Therefore, the ADA recommendation for sterile water use during surgery should remain unchanged. Because of difficulties in eliminating biofilm in dental unit waterlines, surgical irrigation may be best performed with sterile water delivered by a device other than the dental unit waterlines.

Filters capable of trapping bacteria seriously reduce flow rate and can become clogged easily by biofilm shedding. Furthermore, filters installed at the handpiece coupling can alter the handpiece's balance.

CONCLUSION

Within the parameters of this study, this chemical regimen reduces bacterial levels to within acceptable limits. Bacterial tests should be performed regularly to verify compliance with the ADA recommendation of fewer than 200 CFU per mL of water.[3] Antiseptics, including chlorine, in dental water should be inactivated before cultures are taken. Further study of the effectiveness of using low-concentration, long-term antiseptics seems warranted. ■

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