

# DENTAL UNIT WATERLINE ANTIMICROBIAL AGENTS' EFFECT ON DENTIN BOND STRENGTH

HOWARD W. ROBERTS, D.M.D.; RICHARD I. KARPAY, D.D.S., M.P.H.; SHANNON E. MILLS, D.D.S.

## ABSTRACT

**Background.** In response to concerns of bacterial biofilm colonization of dental unit waterlines, a wide range of commercial intermittent and continuous chemical treatments for dental unit waterlines have been developed and marketed. There has been little research on the possible effect of continuous chemical treatment regimens on dentin-bonding agents. The authors evaluate the effect of four proposed antimicrobial agents used in dental unit waterlines on dentin bond strength.

**Methods.** The authors used a fifth-generation dentin-bonding agent to bond composite cylinders to molar dentin surfaces. They then used selected antimicrobial agents as rinsing agents after conditioning. The composite cylinders were shear tested, and their frac-

ture strengths were compared statistically.

**Results.** All proposed antimicrobial agents reduced dentin bond strength. Proposed waterline treatment regimens of a diluted mouthrinse and chlorhexidine significantly reduced dentin bond strength compared with sodium hypochlorite and citric acid regimens.

**Conclusion.** Dental professionals should be aware of potential interactions between dental unit waterline antimicrobial agents and dentin-bonding agents. Further research in this area is warranted, as the clinical implications are uncertain at this time.

**Clinical Implications.** Dental unit waterline antimicrobial agents may adversely affect dentin bonding strength.

**C**olonization of dental unit water delivery systems with microbial biofilms results in treatment water bacteria levels that range from thousands to millions of colony-forming units per milliliter, or CFU/mL.<sup>1</sup> These levels stand in stark contrast to nationally recognized standards set for potable water of no more than 500 CFU/mL of heterotrophic mesophilic bacteria in potable water.<sup>2</sup> The American Dental Association has urged the dental industry to develop methods of achieving a goal of fewer than 200 CFU/mL in unfiltered output water by the year 2000.<sup>3</sup> To this end, manufacturers have developed a range of equipment, materials and procedures designed to improve dental unit water quality.

Although the use of independent water reservoirs can provide water of known microbiological

quality, the treatment water's quality cannot be improved without addressing the issue of the intrinsically contaminated waterlines. Many manufacturers recommend periodically treating waterline systems with chemical agents and then flushing the systems with fresh water to eliminate residual chemicals. In an alternative approach, practitioners can introduce antimicrobial agents continuously into the system to reduce levels of bacteria in dental treatment water.

Agents that have been evaluated or advocated in both peer-reviewed and non-peer-reviewed dental publications include chlorine at concentrations ranging from 0.5 to 20 parts per million, or ppm<sup>4-7</sup>; chlorhexidine gluconate at concentrations ranging from 1:50,000<sup>6</sup> to 1:5,000<sup>8,9</sup>; copper ions<sup>10</sup>; hydrogen peroxide<sup>11</sup>; and commercial over-the-

TABLE 1

| TEST GROUPS.                                   |                         |  |
|--|-------------------------|--|
| GROUP  | CONCENTRATION           | ACTIVE INGREDIENT(S)   |
| <b>Distilled Water (Control)</b>               | —                       | —  |
| <b>Bio 2000 (Micrylium)</b>                    | Per manufacturer        | 0.12 percent chlorhexidine gluconate, 12.0 percent ethyl alcohol |
| <b>Sodium Hypochlorite</b>                     | Three parts per million | Free chlorine (as hypochlorous acid)                             |
| <b>Listerine (Warner-Lambert)</b>              | 1:10 dilution           | Ethyl alcohol, thymol, eucalyptol, methyl salicylate, menthol    |
| <b>BioClear (Waggoner Product Development)</b> | 0.224 percent           | Citric acid  |

counter mouthrinses.<sup>6,12</sup> The success of reducing bacterial CFU levels in dental waterlines varied with the different chemical protocols, and many studies focused primarily on their ease of use in the clinical setting.<sup>4-12</sup>

Using continuously introduced chemical antimicrobial agents in waterlines both with and without flushing the system with water offers the advantage of potentially suppressing bacterial contamination in coolant and irrigant water, as well as in the aerosols generated by dental rotary and ultrasonic instruments. If these antimicrobial agents are present in dental treatment water, they also will be used to cool and irrigate tooth surfaces when the surfaces are being prepared for adhesive bonding procedures. The possible effect of these

waterline treatment agents on dentin bonding has not been reported in the literature.

The authors conducted a study to measure dentin bond strength to prepared tooth specimens by using four chemical agents developed for the reduction of bacterial contamination in dental treatment water.

#### MATERIALS AND METHODS

For this study, we selected 60 extracted, noncarious molars stored in 2 percent formalin. We removed all soft tissue and disinfected the teeth in 5 percent sodium hypochlorite for 48 hours. The teeth were stored in room-temperature distilled water when we were not using them.

To prepare flat dentin surfaces, we removed the occlusal

surfaces of the teeth by sectioning them with a water-cooled diamond saw. We mounted each tooth in autopolymerizing acrylic resin using cylindrical polytetrafluoroethylene molds that positioned the prepared dentin surface approximately 2 millimeters above the end of the resin cylinder. After the resin had completely polymerized, we hand-finished the dentin surface with wet 400- and 600-grit silicon carbide abrasive papers on a polishing wheel. After the finishing process was complete, we used a stereomicroscope to examine the teeth at  $\times 8$  magnification to ensure that we had removed all of the enamel from the bonding area. We then randomly separated the prepared teeth into five groups of 12 teeth each and stored them in room-temperature distilled water before bonding.

To prepare the dentin surfaces for bonding, we dried them for three seconds with oil-free, compressed air but avoided desiccation. We applied a phosphoric acid etchant (Scotchbond Etchant, 3M Dental Products) to the surfaces for 15 seconds and then rinsed the etched surfaces with 50-mL solutions of distilled water mixed with one of the following four waterline antimicrobial agents: 3-ppm sodium hypochlorite; a 1:10 dilution of Listerine (Warner-Lambert) mouthrinse; Bio 2000 (Micrylium Labs), a commercially available chlorhexidine product; and 0.224 percent of BioClear (Waggoner Product Development), a developmental citric acid product that soon will be on the market (Table 1). We used distilled water as the control.

We applied each solution

TABLE 2

| TESTED ANTIMICROBIAL AGENTS' MEAN SHEAR DENTIN BOND STRENGTHS. |  |
|--|--|
| GROUP  | MEAN SHEAR BOND STRENGTH $\pm$ STANDARD DEVIATION (MPa*) |
| <b>Distilled Water (Control)</b>                               | 22.59 $\pm$ 8.93 †                                       |
| <b>Sodium Hypochlorite</b>                                     | 18.13 $\pm$ 6.65   |
| <b>BioClear (Waggoner Product Development)</b>                 | 15.32 $\pm$ 8.95   |
| <b>Listerine (Warner-Lambert)</b>                              | 13.00 $\pm$ 6.84   |
| <b>Bio 2000 (Micrylium)</b>                                    | 12.96 $\pm$ 4.01   |

\* MPa: Megapascal.  
† Vertical lines connect nonsignificant differences at the  $P < .05$  level.

with a 100-mL syringe over a 15-second period. We determined our rationale for using 50 mL of rinse solution during a clinical pilot project we conducted that measured the volume of water expressed during a 15-second rinse from a three-way syringe into a graduated cylinder. We prepared all of the solutions according to the agents' manufacturers' recommendations and immediately blotted excess solution with cotton, leaving a visibly moist dentin surface.

To bond the surfaces, we applied two consecutive coats of Single Bond adhesive (3M Dental Products) and dried them with a gentle stream of air for five seconds. We used a visible light-curing unit (Optilux 401, Demetron Research Corporation) to light-cure the adhe-

sive for 10 seconds. We assessed the adequacy of the light unit intensity (600 megawatts per square centimeter) immediately before use with a Model 100 curing radiometer (Demetron).

We inserted Z-100 composite shade A2 (3M Dental Products) in 2-mm increments into a cylindrical, 4 mm  $\times$  4 mm split polytetrafluoroethylene mold held in place by a positioning ring over the prepared dentin surface. Each 2-mm increment was light-activated for 40 seconds by exposing it to the visible light-curing unit. After the final increment was polymerized, we removed the alignment tube and mold and stored the specimen in 37 C distilled water. At 48 hours after we prepared the specimens, we thermocycled them for 500 cycles between a 5 C and a 55 C water

bath. The dwelling time for each water bath was 30 seconds, and the transfer time was 10 seconds.

At seven days after the specimens were bonded, we performed a shear test by using a perforated steel ring attached by a chain to a universal testing machine (series 1000, Tinius Olsen). The specimens were loaded to failure at a crosshead speed of 0.5 mm/minute. After shear bond strength testing, we examined the specimens with a stereomicroscope at  $\times 8$  magnification to determine the failure mode between the dentin-bonding agent and the dentin. We recorded failures as adhesive (those that occurred between the dentin-bonding agent and the dentin), cohesive (those that occurred within the dentin) or mixed (those that were a combination of adhesive and cohesive). We used analysis of variance, or ANOVA, and Scheffé post hoc tests to compare the bond-strength data and to determine whether there were significant differences between the various waterline disinfectant solutions at the  $P < .05$  significance level.

## RESULTS

Table 2 gives the mean shear dentin bond strengths of the test groups. The ANOVA revealed a significant difference between groups ( $P = .009$ ). The Bio 2000 and the Listerine groups had significantly lower bond-strength values than did the distilled water, sodium hypochlorite or BioClear groups.

When we analyzed the mode of failure, we found that all of the failures in the distilled water and sodium hypochlorite groups were cohesive failures within the dentin. The Bio 2000

and Listerine groups had adhesive failures wholly at the adhesive-dentin interface. The modes of failure in the BioClear group were mixed, being both cohesive dentin failure and adhesive interfacial failure.

## DISCUSSION

Chemical agents have been investigated for their potential efficacy in controlling or eliminating biofilm formation in dental water systems.<sup>4-12</sup> Antibiofilm agents inactivate biofilm organisms (germicides) or cause detachment of the biofilm matrix (cleaners). Some germicidal agents also may produce biofilm detachment.<sup>13</sup>

Antibiofilm agents can be applied in two basic ways: by periodic treatment that uses high concentrations of an agent, usually a germicidal agent, or by continuous application at lower, and presumably biocompatible, concentrations. In some cases, the same agent can be used at different concentrations for periodic and continuous treatment. Agents that have been tested for use in periodic and continuous treatment at different concentrations include sodium hypochlorite,<sup>3-6</sup> hydrogen peroxide,<sup>11</sup> metallic ions,<sup>10</sup> chlorhexidine gluconate<sup>8,9</sup> and commercial mouthrinses.<sup>6,12</sup>

Taylor and colleagues<sup>14</sup> reported that dental unit waterline antimicrobial agents reduced enamel bond strengths and theorized that dentin bond strengths would be affected as well. Our current study investigated the effect of four proposed dental unit waterline antimicrobial agents—sodium hypochlorite, Listerine, Bio 2000, BioClear—and a control—distilled water—on dentin bond strength using a fifth-generation dentin-

bonding agent. Under the conditions of this evaluation, all four waterline antimicrobial agents affected shear dentin bond strength, with Bio 2000 and Listerine demonstrating significantly lower ( $P < .05$ ) bond strengths than the control or other two antimicrobial agents evaluated.

We do not know the explanation for this study's results. In a bonding study, sodium hypochlorite primarily was used to remove dentinal collagen that showed either no effect on dentin bond strength or an increase.<sup>15</sup> The use of chlorhexidine as a preparation disinfectant or cavity cleanser has been reported not to affect bond strength when used with All Bond-2 (Bisco Dental Products)<sup>16</sup> and Tenure (DenMat Corp.).<sup>17</sup> Chlorhexidine has been reported to have adversely affected the bond strength of Syntac (Ivoclar North America),<sup>17</sup> and it is associated with increased microleakage when used with Syntac<sup>18,19</sup> and with Prime & Bond 2.0 (L.D. Caulk Dentsply).<sup>19</sup> Any theories concerning the interactions of chlorhexidine, mouthwash ingredients and citric acid with either the conditioned dentin surface or the dentin-bonding agent used in our study are speculative and deserve further investigation.

One factor that may have contributed to some of the observed effects in our study is the presence of essential oils that are used as flavoring agents in Bio 2000 and as active ingredients in Listerine. The Bio 2000 package includes printed instructions that advise users to rinse dentin and enamel surfaces with distilled water before applying bonding

agents; these instructions, however, are not printed on the outside of the package. Following this procedure may help eliminate residual oils or other surface contaminants and improve bond strengths.

Another factor that could be investigated in the future is the relative acidity of all potential acidic solutions and their possible interaction with the conditioned dentin. Since we designed this study only to evaluate waterline antimicrobial agents' possible effects on dentin bond strength, future studies could be designed to investigate our current speculative but relevant concerns.

When we prepared the specimens using standard dentin bonding study methods, we irrigated the diamond saw used to section the teeth with distilled water rather than with the antimicrobial agent solutions. This may have affected our results. Future investigators may want to prepare the dentin or enamel surfaces with the experimental antimicrobial solutions to more closely replicate clinical conditions.

Another observation of interest is that the specimens exhibited varying degrees of adhesive and cohesive failure within the dentin or composite. From the standpoint of predicting the clinical significance of these data, this may be of greater importance than the actual measurements of shear bond strength—especially if a simple standardized test for the effects of waterline treatment solutions is to be developed.

## CONCLUSION

Dental unit waterline antimicrobial agents have the potential to affect dentin bond

strengths when continuously introduced in dental treatment water. Under the conditions of this study, all of the tested dental unit waterline antimicrobial solutions reduced dentin shear bond strength. Diluted Listerine mouthwash and Bio 2000 produced a significantly greater reduction in dentin bond strength than did Bio-Clear, a distilled water control or a comparable sodium hypochlorite solution. All of the failures with the latter two materials were cohesive within the dentin and suggest possible clinical significance.

To obtain optimal results, clinicians should conscientiously follow the manufacturers' instructions when using antimicrobial solutions and dentin-bonding agents. As new products intended to improve the quality of dental treatment water enter the marketplace, there may be strong incentives to develop standardized test methods to evaluate the possible effects on adhesive dental materials. ■

The authors would like to acknowledge the donation of composite and bonding agents from the 3M Corporation.



**Dr. Roberts is the director, Technical Evaluations, USAF Dental Investigation Service, USAF School of Aerospace Medicine, 2509 Kennedy Circle, Building 125, Room 215, Brooks AFB, Texas 78235-5117. Address reprint requests to Dr. Roberts.**



**Dr. Karpay is the director, Hillsborough County Dental Research Clinic, Tampa, Fla.**

The opinions expressed in this article are those of the authors and do not reflect the official opinions or position of the United States Air Force or the Department of Defense.

1. Shearer BG. Biofilm and the dental office. *JADA* 1996;127:181-9.
2. Clesceri LS, Greenberg AE, Eaton A, Closer L, eds. Standard methods for the examination of water and wastewater. 20th ed. Washington: American Public Health Association, American Water Works Association and the Water Environment Federation; 1998.
3. American Dental Association. ADA statement on dental unit waterlines. *JADA* 1996;127:185-6.
4. Abel LC, Miller RL, Micik RE, Ryge G. Studies on dental aerobiology, IV: bacterial contamination of water delivered by dental units. *J Dent Res* 1971;50(6):1567-9.
5. Fiehn NE, Henriksen K. Methods of disinfection of the water system of dental units by water chlorination. *J Dent Res* 1988;67(12):1499-504.
6. Pankhurst CL, Philpott-Howard JN, Hewitt JH, Casewell MW. The efficacy of chlorination and filtration in the control and eradication of *Legionella* from dental chair water systems. *Hosp Infect* 1990;16(1):9-18.
7. Eleazer PD, Schuster GS, Weathers DR. A chemical treatment regimen to reduce bacterial contamination in dental waterlines. *JADA* 1997;128:617-23.
8. Blake G. The incidence and control of bacterial infection in dental spray reservoirs. *Brit Dent J* 1963;115:413-6.
9. McEntegart MG, Clark A. Colonisation of dental units by water bacteria. *Brit Dent J* 1973;134(4):140-2.
10. Hesselgren S, Nedlich U. Microbial growth in dental units. *Quintessence Int* 1979;9:103-7.
11. Kellett M, Holbrook W. Bacterial contamination of dental handpieces. *J Dent* 1980;8(3):249-53.
12. Kelley J, DePaola L, Baqui A, et al. Reducing the risk of infectious transmission in bioaerosols in the dental operatory (abstract 9803). Abstract presented at: 1998 Organization for Safety and Asepsis Procedures Annual Symposium; June 11, 1998; Providence, R.I.
13. Srinivasan R. Biofilm parameters influencing biocide efficacy. *Biotechnol Bioeng* 1995;46:553.
14. Taylor TL, Leonard RH, Mauriello SM, Swift EJ. Effect of dental unit waterline biocides on enamel bond strengths (abstract 9813). Abstract presented at: 1998 Organization for Safety and Asepsis Procedures Annual Symposium; June 11, 1998; Providence, R.I.
15. Vargas MA, Cobb DS, Armstrong SR. Resin-dentin shear bond strength and interfacial ultrastructure with and without a hybrid layer. *Oper Dent* 1997;22:159-66.
16. Perdigao J, Denehy GE, Swift EJ Jr. Effects of chlorhexidine on dentin surfaces and shear bond strength. *Am J Dent* 1994;7(2):81-4.
17. Meiers JC, Shook LW. Effect of disinfectants on the bond strength of composite to dentin. *Am J Dent* 1996;9(1):11-4.
18. Meiers JC, Kresin JC. Cavity disinfectants and dentin bonding. *Oper Dent* 1996;21(4):153-9.
19. Tulunoglu O, Ayhan H, Olmez A, Bodur H. The effect of cavity disinfectants on microleakage in dentin bonding systems. *J Clin Pediatr* 1998;22(4):299-305.



**Dr. Mills is medical inspector, Air Force Inspection Agency, Kirtland AFB, N.M.**