Growth-Inhibitory Effect of Antibacterial Self-Etching Primer on Mutans Streptococci Obtained from Arrested Carious Lesions

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ABSTRACT

Background: An antibacterial self-etching primer has been developed to inhibit bacterial attachment and plaque accumulation on the tooth surfaces. The purpose of this study was to evaluate the growth-inhibitory effect of an antibacterial self-etching primer on mutans streptococci obtained from arrested carious lesions.

Materials and Methods: Softened dentin specimens were obtained from arrested carious lesions with an excavator or a round steel bur. The effect of a commercial antibacterial self-etching primer and a commercial self-etching primer on the total streptococci on Mitis-Salivarius (MS) agar plates and on *Streptococcus mutans* on MS agar plates containing 0.02 M bacitracin (MSB) was evaluated. Specimens with no primer were used as controls. The number of colonies of both total streptococci and *S. mutans* were compared statistically with two-way analysis of variance and Fisher's Protected Least Squares Differences (PLSD) test ($p < .05$).

Results: The number of colonies of total streptococci (CFU/mL) for the two methods (excavator and round steel bur) were as follows: for the control, 5.0×10^6 and 5.0×10^6; for the self-etching primer, 1.0×10^5 and 1.0×10^5; and for the antibacterial self-etching primer, 0 and 0. The number of colonies of *S. mutans* for the two methods (excavator and round steel bur) were as follows: for the control, 5.0×10^6 and 1.8×10^5; for the self-etching primer, 3.5×10^4 and 5.0×10^3; and for the antibacterial self-etching primer, 0 and 0. Regardless of the method of softened dentin removal, the antibacterial effect was significantly higher for the group that had antibacterial self-etching primer compared with that of the control group and the group that had commercial self-etching primer ($p < .05$).

CLINICAL SIGNIFICANCE

The antibacterial self-etching primer showed a high level of antibacterial activity against mutans streptococci obtained from the arrested carious lesions.

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A considerable amount of research has demonstrated that antibacterial agents in oral rinses can control bacterial plaque.[1,2] In vitro studies have investigated the effects of adding antibacterial agents to dental materials to control bacteria associated with carious lesions.[1,3] These studies have shown that adding antibacterial agents to dental materials resulted in increased antimicrobial action but led to a reduction in the inherent strength of the material.[1,2,4]

Several self-etching adhesive systems, in which the acid etching and priming steps are combined, have been developed and are commercially available. These adhesive systems have reduced the number of bonding steps and the "chair time" by omitting the etching and rinsing steps. In the case of an arrested caries lesion, even though arrested carious dentin is characterized as being darkly pigmented with a hard leathery surface, it is sometimes difficult to distinguish clearly the boundary between arrested dentin and normal dentin.[5] It has also been suggested that bacteria may remain in dentin even after a caries-detection dye solution is used.[6] In these situations, residual bacteria pose a threat to the long-term success of the restoration. In many types of dentinal caries, mutans streptococci, *Streptococcus mutans* and *S. sobrinus*, have been detected.[7] Residual bacteria may cause pulp sensitivity and pulpal inflammation.[8,9]

12-Methacryloyloxydodecylpyridinium bromide (MDPB) is an antibacterial agent incorporated into a self-etching adhesive system to inhibit bacterial attachment and plaque accumulation on the tooth surface.[10–14] The addition of this antibacterial monomer to the adhesive system has been shown not to affect the inherent strength of the material.[15–17] Some in vitro studies have demonstrated an inhibitory effect of this agent against bacterial growth using the disk-diffusion method, in which antibacterial agents contact the microorganisms directly.[1,16,18] In the clinical setting, this antibacterial effect might occur within restored hard tissues in the preparation. As mentioned above, for the treatment of an arrested carious lesion, it is difficult to clearly distinguish the boundary between carious and normal dentin, and residual bacteria may remain within the prepared dentinal surface. In these situations, it may be beneficial to incorporate an antibacterial agent into the self-etching primer to eliminate any residual cariogenic bacteria.

The aim of this study was to evaluate the growth-inhibitory effect of this newly developed antibacterial self-etching primer on mutans streptococci obtained from arrested carious lesions. The hypothesis was that the antibacterial primer would eliminate any residual cariogenic bacteria.

TABLE 1. DESCRIPTION OF SPECIMENS.

Case*	Location of Tested Caries Lesion	Weight of Specimen (mg)	
		Excavator Used	Round Steel Bur Used
Case 1: right lower first premolar, female, 51	Buccal root surface	1.2	0.8
Case 2: right lower second premolar, male, 39	Distal proximal side	2.3	1.6
Case 3: left lower second premolar, male, 42	Buccal root surface	0.8	1.9
Case 4: left upper second premolar, female, 37	Mesial proximal side	1.7	1.2
Case 5: right lower canine, male, 47	Buccal root surface	1.1	0.8
Case 6: right upper first premolar, male, 34	Buccal root surface	1.4	0.9
Case 7: right lower canine, female, 54	Distal proximal side	1.6	1.3

*Tooth and patient's sex and age.

MATERIALS AND METHODS

Specimens of arrested carious dentin were obtained from seven healthy adult patients (four males, three females; average age 43.4 yr) who had given verbal consent to their use for research purposes. Because the tested carious dentin had a hard leathery surface and dark pigmentation, it was classified as arrested caries.[5] A rubber dam was applied to isolate the tested tooth. The superficial softened dentin was removed from the tested teeth using only an excavator, and then the residual deep softened dentin was removed using a round steel bur (Table 1 and Figure 1). Finally, a caries-detection dye (Caries Detector, Kuraray Medical Inc., Tokyo, Japan) was used to check that all the softened dentin had been removed.

The excavated caries dentin was treated with a commercially available self-etching primer (Clearfil MEGA Bond®, Kuraray Medical Inc.) or an experimental antibacterial self-etching primer (Clearfil PROTECT Bond®, Kuraray Medical Inc.) (Table 2). (Note: Clearfil MEGA Bond is the brand name used in Japan. In the United States, the name is Clearfil SE Bond.) After treatment of the recovered arrested caries material with either 0.02 mL MEGA Bond primer or PROTECT Bond primer for 20 seconds, the specimens were each placed individually in 4 mL of Brain Heart Infusion (BHI) (Difco Laboratories, Detroit, MI, USA) broth. Excavated specimens not exposed to either self-etching primer were used as controls. After anaerobic culture at 37 °C for 24 hours, the BHI broth was shaken vigorously and poured onto Mitis-Salivarius (MS) agar plates or MS agar plates containing 0.02 M bacitracin (MSB) in a volume of 50 μL using the spiral plate system (EDDY JET® spiral system, Gunze Sangyo Inc., Tokyo, Japan).[19] The susceptibility of the microorganisms and the antibacterial activity of the two self-etching primers were evaluated using blood agar plates. This medium was chosen to facilitate the observation of the biochemical reactions, growth, and changing characteristics of the microbes and dental materials. After anaerobic culture for 48 hours, the number of total streptococci colonies on the MS agar plates and

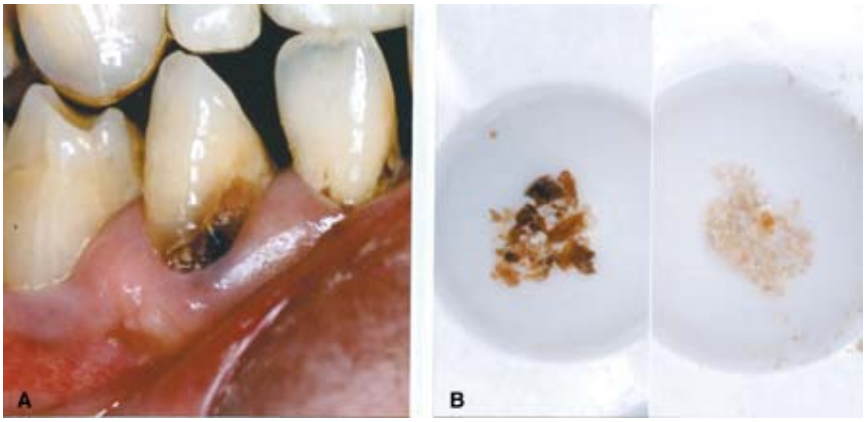

Figure 1. Case 5. A, *Buccal root surface caries of the right lower canine in a male patient, age 47 years.* B, *The removed softened dentin shown on a weighting vessel:* left, *using an excavator;* right, *using a round bur.*

TABLE 2. SELF-ETCHING PRIMERS USED.

Primer	Manufacturer and Location	Batch No.	Material Composition
Clearfil MEGA Bond*	Kuraray Medical Inc., Tokyo, Japan	00261B	MDP, HEMA, water, hydrophilic-dimethacrylate
Clearfil PROTECT Bond†	Kuraray Medical Inc.	010327	MDP, MDPB, HEMA, hydrophilic-dimethacrylate, water

Information provided by the manufacturer: HEMA = 2-hydroxy-ethylmethacrylate; MDP = 10-methacryloyloxydecyl dihydrogen phosphate; MDPB = 12-methacryloyloxydodecylpyridinium bromide.
*Called SE Bond in the United States.
†Experimental material.

TABLE 3. NUMBER OF TOTAL STREPTOCOCCI COLONIES.

	No. of Streptococci (CFU/mL)	
Group	Excavator Used	Round Steel Bur Used
Control	5.0×10^6	5.0×10^6
MEGA Bond primer	1.0×10^5	1.0×10^5
PROTECT Bond primer	0	0

S. mutans colonies on the MSB agar plates were counted. The number of colonies of total streptococci and *S. mutans* were compared statistically using two-way analysis of variance (material vs type of excavation) and Fisher PLSD test ($p < .05$).[20]

RESULTS

The number of total streptococci colonies on the MS agar plates are shown in Table 3. For the control group, the number of total streptococci colonies was 5.0×10^6 CFU/mL for specimens extracted with the excavator and with the round steel bur (see Figure 1B). After mixing the excavated material with MEGA Bond primer (MDPB 0%) treatment, the number of total streptococci colonies was 1.0×10^5 CFU/mL for specimens extracted with the excavator and with the round steel bur. However, total streptococci colonies were not observed in the specimens mixed with PROTECT Bond primer treatments for specimens extracted with the excavator and with the round steel bur. There was a statistically significant difference in the number of total streptococci present when the MEGA Bond primer was used compared with the PROTECT Bond primer ($p < .05$).

The number of colonies of *S. mutans* on the MSB agar plates is shown in Table 4. For the control group, the number of colonies of *S. mutans* was 5.0×10^6 CFU/mL for the specimens extracted with an excavator and 1.8×10^5 CFU/mL for the specimens extracted with a round steel bur (Figure 2A and D). After MEGA Bond primer treatment, the number of colonies of *S. mutans* was 3.5×10^4 CFU/mL for the specimens extracted with an excavator and 5.0×10^3 CFU/mL for the specimens extracted with a round steel bur (Figure 2B and E). Colonies of *S. mutans* were not observed in the PROTECT Bond-treated specimens extracted with either an excavator or a round steel bur (Figure 2C and F). There was a statistically significant difference in the number of colonies of *S. mutans* present on specimens treated with MEGA Bond primer as opposed to those treated with PROTECT Bond primer ($p < .05$).

TABLE 4. NUMBER OF *STREPTOCOCCUS MUTANS* COLONIES.

	No. of *Streptococcus mutans* (CFU/mL)	
Group	Excavator Used	Round Steel Bur Used
Control	5.0×10^6	1.8×10^5
MEGA Bond primer	3.5×10^4	5.0×10^3
PROTECT Bond primer	0	0

DISCUSSION

Dentin caries is a mixed infection caused by indigenous pathogenic microbiota and biofilm formation.[21] *S. mutans* is considered to be the main oral bacteria that produces a biofilm on the tooth surface.[22] Several biochemical attempts to remove *S. mutans* from the oral cavity have been performed.[19,23] The antibacterial monomer MDPB tested in the current study was developed to inhibit bacterial attachment to and plaque accumulation on the tooth surface.[10–17] However, the bacterial activity of clinically treated lesions is an important factor. We used arrested carious materials in this study because we did not want to cause patients severe pain, and yet we needed sufficient carious specimens to evaluate several antibacterial effects of adhesive systems.

In this study, the total streptococci and *S. mutans* colonies on the detection plates were evaluated to determine the growth-inhibitory effect of the antibacterial self-etching primer. *S. mutans* colonies were not detected in the groups treated with the PROTECT Bond primer in either the excavator- or round steel bur–sampled specimens. *S. mutans*

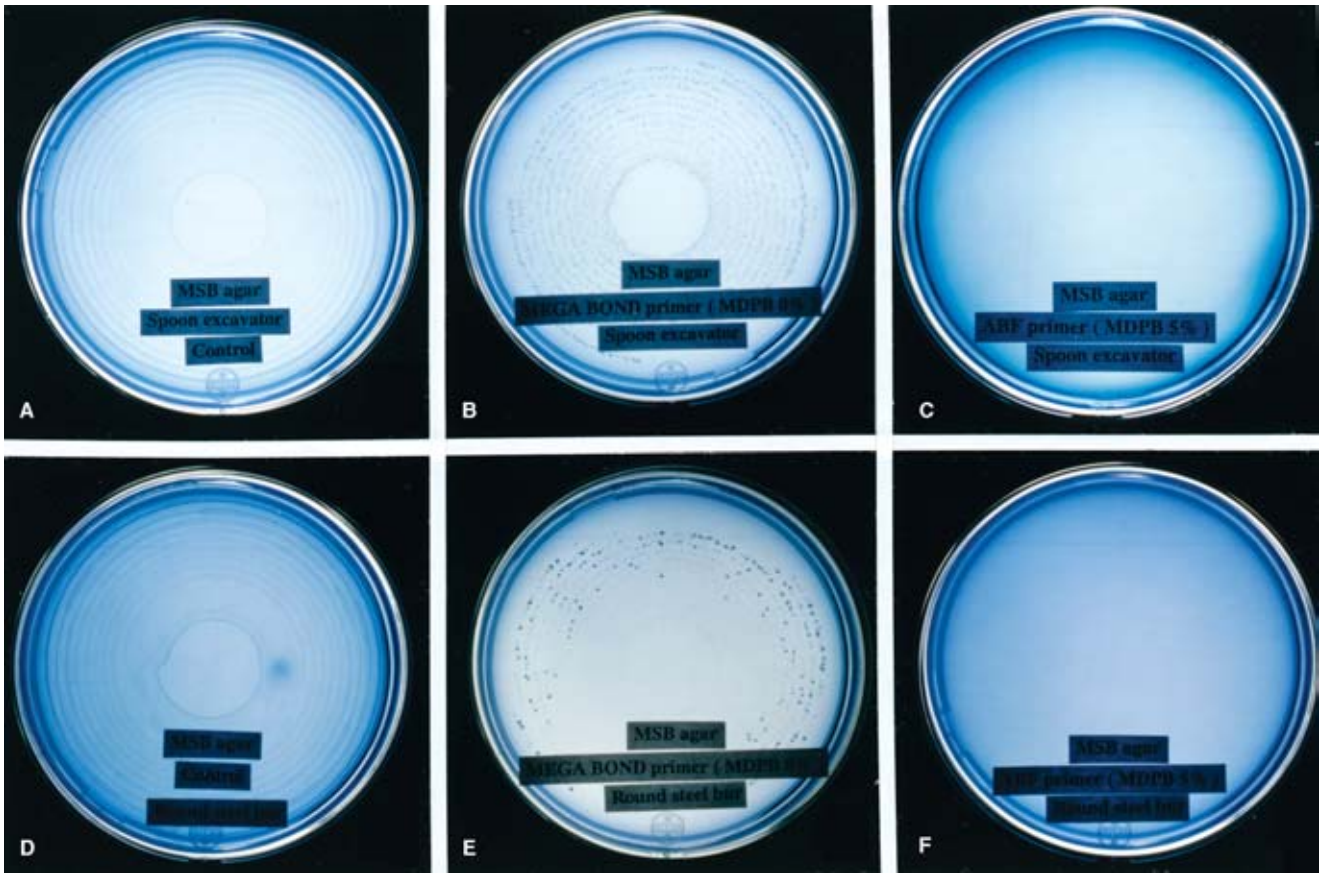

Figure 2. A, *Mitis-Salivarius agar plates containing 0.02 M bacitracin (MSB) with excavator-sampled caries in the no-treatment control group.* B, *MSB agar plate of excavator-sampled caries treated with MEGA Bond primer group.* C, *MSB agar plate of excavator-sampled caries treated with PROTECT Bond primer group.* D, *MSB agar plate of round bur–sampled caries in the no-treatment control group.* E, *MSB agar plate of round bur–sampled carious dentin treated with MEGA Bond primer.* F, *MSB agar plate of round bur–sampled carious dentin treated with PROTECT Bond primer.*

are capable of producing large quantities of acid, even at a relatively low pH. A pH of 5.5 is higher than the threshold for acidogenic stimulation of *S. mutans*.[24] Arrested dentinal lesions were more resistant to proteolytic enzyme attack than active dentinal lesions would be.[25] In the current study, antibacterial activity against total streptococci and *S. mutans* colonies was evaluated using softened dentin specimens obtained from arrested caries lesions. However, characterization of streptococci within an arrested caries lesion is not fully understood. Further bacterial studies are required to characterize streptococci and to investigate acidogenic activity on arrested caries lesions.

Because the antibacterial effect is not strong enough to kill all the bacteria immediately, it is difficult to completely elucidate the growth-inhibitory effect on cultured bacteria. In this study, the self-etching primers were applied in vitro to excavated carious dentin specimens that were anaerobically cultured for 24 hours. The PROTECT Bond primer resulted in a high level of antibacterial activity against mutans streptococci in specimens obtained with either an excavator or a round steel bur after only 24 hours of anaerobic incubation.

Because the primers were mixed with pieces of excavated carious dentin in vitro, they may have had better

access to bacteria within the carious debris than would have occurred if the primers had been applied to carious lesions in situ. Clinically, the soft carious material would have to be excavated and the antibacterial primer applied to the caries-affected dentin. Because tubules of caries-affected dentin are often occluded with mineral crystals, the primer might not diffuse into the dentin as well as it did in the soft debris treated in this study. Further studies under more clinically relevant conditions are indicated.

Although both self-etching primers might have some toxic effects on mutans streptococci, the antibacterial mechanisms of MDPB have yet to be elucidated. MDPB is a compound containing quaternary ammonium dodecylpyridinium bromide and methacryloyl groups.[15] Quaternary ammonium compounds are thought to have a broad spectrum of antibacterial activity.[26] It has been suggested that the antibacterial mechanism of an MDPB-containing self-etching primer might be dependent on the antibacterial mechanism of quaternary ammonium.[14]

Modern adhesive bonding systems have been developed to improve the quality of the bond to tooth structure and to simplify clinical procedures. In many situations, adhesive techniques result in greater conservation of the tooth tissue and a reduction in the need to prepare mechanically retentive preparations. An increase in the life expectancy of the general population, coupled with an increase in the number of retained teeth, has led to an increase in the number of root surfaces exposed to the oral environment. As a result, the elderly have a higher risk of root surface caries than existed previously. However, it is difficult to completely remove all the caries-affected dentin when treating root caries, which leads to the possibility of bacteria remaining in the prepared cavity. In this study, four of seven cases involved root caries that were located on the buccal surfaces. Recently, an antibacterial approach for the management and treatment of root caries in the elderly (older than 60 years) has been proposed.[14,27] This antibacterial self-etching system might be useful for treating this caries in this population. Further in vitro and in vivo antibacterial studies with other bacteria are necessary to further develop this antibacterial self-etching system.

CONCLUSION

The antibacterial self-etching primer showed a high level of antibacterial activity against mutans streptococci on the arrested carious lesions.

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