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Antibacterial Activities of MDPB and Fluoride in Dentin Bonding Agents

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Abstract - *The aim of this study was to compare in vitro antibacterial activity of MDPB containing bonding system ABF with activities of three Fluoride containing bonding systems (Fuji Bond LC (FBLC), Prime&Bond NT (PBNT), and FluoroBond (FLB). Two bacterial strains were tested: Streptococcus mutans and Lactobacillus acidophilus. The study was performed on Muller Hinton Agar by Agar Well Technique. The bacterial agar was evenly distributed over the surface of petri dishes. Standard wells were punched into the agar. The test materials were placed in the wells of Muller Hinton agar plates, inoculated with Streptococcus mutans NCTC10449 and Lactobacillus acidophilus. The diameters of inhibition zones produced around the materials were measured after 24 h of incubation at 37 ±1°C. For statistical analysis, Duncan's multiple range test was used. The primers of bonding agents were highly effective against to Streptococcus mutans. The MDPB containing primer of ABF Bond produced the greatest inhibition zones against to Lactobacillus acidophilus. It was followed by FLB primer. Lactobacillus acidophilus was resistant to FBLC primer and bonding, ABF, PBNT and FLB bonding agents. The results of this study indicated that, incorporation of MDPB in primer of self-etch system increased its antibacterial activity especially against to Lactobacillus acidophilus. However, all bonding systems except for bonding agent of ABF showed some antibacterial activity against to Streptococcus mutans.*

KEY WORDS: Antibacterial tests, Antibacterial activities, *Streptococcus mutans*, Dentin bonding systems, Dentin primers

INTRODUCTION

Polymerization shrinkage and resultant contraction gaps at the tooth-restoration interfaces continue to be a significant problem associated with composite resin restorations[1]. Therefore, microleakage may be considered as one of the most important factors resposible for clinical failure of restorations. Bacteria which invade along the tooth/restoration interface are the main cause of secondary caries and damage to the pulp.

Streptococcus mutans and Streptococcus *sobrinus* are associated with the initiation of human dental caries, while lactobacilli are associated with the progression of the established lesion. A reduction in the amount of these bacteria at the tooth-restoration interface would be expected to influence the incidence of dental caries. The possibility that composite or bonding resins may have an antibacterial effect is, therefore, attractive[2–4].

Two benefits are derived from the clinical use of filling materials with an inhibitory action on microbial growth; antibacterial substances can extend the longevity of restorations and they can help alleviate postoperative discomfort. However, leaching of the antimicrobials from the materials has several disadvantages; decease in the mechanical properties of the carrier material over time, short-lived effectiveness, and possible toxicity. Because of the need for an antibacterial composite or dentin bonding agents which does not rely on agent release, a new monomer which has antibacterial properties was synthesized and incorporated into resin materials. MDPB (12-methacryloyloxydodecylpyridinium bromide) is a recently developed antibacterial monomer that can be incorporated in to dentin bonding agents. MDPB is a compound of an antibacterial quaternary ammonium agent with a methacryloyl group, and exhibits strong antibacterial activity against to oral streptococci[5,6]. The incorporation of MDPB has been reported to be effective in providing dentin bonding systems with antibacterial activity before and after curing[7–10]. MDPB has a potential to be incorporated into dental resin-based materials such as dentin bonding primer/resin to provide bactericidal activity without causing adverse effect on biocompatibility.

Glass-ionomers and calcium hydroxide have been shown to have an antibacteial effect on various types of oral bacteria *in vitro*[11–13]. But in a further study, Loyola-Rodrigues and Garcia-Godoy[14] observed that small amounts of fluoride released from dental materials might not have any affect on *Streptococci*.

Several studies[15–22] have determined the antibacterial activity of conventional cements, lining materials or dentin bonding systems using different methodologies. *In situ* or *in vivo* test models have been developed to determine the clinical value of the antibacterial effects of the dentin bonding systems. Among them, simple direct inhibition tests such as agar-well techniques have been most frequently used.

The purpose of this in vitro study was to compare antibacterial activitiy of MDPB containing bonding system ABF with the antibacterial activities of three fluoride containing bonding systems (Fuji Bond LC (FBLC), Prime&Bond NT (PBNT), and FluoroBond (FLB) using conventional agar well technique.

Table 1. *Materials used in the study*

Materials	*Composition*
ABF primer (ABP) (Experimental)	MDP, HEMA, MDPB, water
ABF bonding (KBF) (Experimental)	MDP, HEMA, NaF, microfiller
FBLC conditioner	Mild polyacrylic acid
FBLC bonding	Powder: Alumina-silicate glass Liquid: HEMA, Polyacrylicacid, resins, tartaric acid, distilled water
PBNT bonding	Di-and trimethacrylate resins, amorphous silica PENTA, Photo initiators, hydrofluoride, stabilisers, acetone
FLB primer	HEMA, water, 4-AET, 4-AETA catalyst
FLB bonding	Pre-Reacted Glass-ionomer, UDMA resin

Table 2. *Diameter of inhibition zones produced by each material (mean ± SD)*

Materials	*S. mutans*	*L. acidophilus*
ABF Primer (ABP)	16,66 ± 0,64 b	19,08 ± 0,33 a
ABF Bonding (KBF)	0,00 ± 0,00	0,00 ± 0,00
FBLC Conditioner	20,83 ± 0,40 a	0,00 ± 0,00
FBLC Bonding	11,25 ± 0.60 c	0,00 ± 0,00
PBNT	11,58 ± 0,69 c	0,00 ± 0,00
FLB Primer	18,16 ± 0,53 b	14,83 ± 0,47 b
FLB Bonding	7,92 ± 1,10 c	0,00 ± 0,00

Note: There was no significant difference between same letters (p>0.05).

MATERIAL AND METHODS

The dentin bonding systems evaluated in this study are shown in *Table 1*. ABF (Kuraray, Osaka, Japan) is an experimental dentin bonding system which consists of a single-bottled self-etching primer containing 5% MDPB. The antibacterial activity of primers and bonding resins of adhesive systems were observed by means of agar plate diffusion method that has been described previously[15–17]. The antibacterial action of both the primer and adhesive agents of the bonding systems were evaluated against following bacteria: *Streptococcus mutans (S. mutans)* (NCTC 10449) and *Lactobacillus acidophillus (L. acidophillus)* (human isolate provided by O. Erganis, Selcuk University). The lyophilized *S. mutans* was rehydrated and their purity and viability were confirmed in ideal culture media. The bacteria were then inoculated on to plates containing brain-heart infusion broth (Difco Laboratories, Deroit, MI, USA) and incubated for 48 hours in the appropriate atmosphere from there cultures, bacterial suspensions were prepared in sterile brain-heart infusion broth (using McFarland opacity standards) to a concentration of $1{,}5x10^8$ (bacteria/ml).

The Muller Hinton Agar was evenly distributed over the surface of 15cm-in-diameter petri dishes in a thickness of 3mm. Standard wells with a diameter of 5mm were punched into the agar with the blunt end of a sterile Pasteur pipette. The agar plate was inoculated by swabbing over the agar surfaces with a bacterial suspension (1 ml). The test materials were filled in the wells under a laminar flow hood. For ABF, FBLC, PBNT and FLB, the primer/conditioner and bonding components were tested separately. The bonding agents were not irradiated with a visible light curing unit after placement into the agar wells. The petri dishes were incubated at 37 ± 1°C and 24 h. Diameters of circular inhibition zones around the materials were measured in mm. The test was repeated 12 times. For statistical analysis of the results, Duncan's test in SPSS for Windows 10.0 statistical program was used.

RESULTS

Table 2 shows the diameter of inhibition zones produced by each material as means ± SD. FBLC-conditioner gave the largest zone of inhibition against to *S. mutans*. FLB primer and ABF primer had smaller zones of inhibition against to *S. mutans* than FBLC conditioner although the differences were not significant (p<0.05). PBNT, FBLC and FLB bondings produced inhibition zones against to *S. mutans* but the bonding resin of ABF did not produce an inhibition zone against to *S. Mutans* or *L. acidophillus*. ABF primer was the most inhibitory agent against to *L. acidophillus*. However, ABF bonding, FBLC conditoner, FBLC bonding, PBNT and FLB bonding agents failed to produce inhibition halos against to *L. acidophillus*.

DISCUSSION

The evaluations of antimicrobial properties of primers are of interest since it is in direct contact with the cavity. Especially, in the case of self-etching/self-priming systems with no water-rinsing procedure, the demineralized smear layer is incorporated into the resin-impregnated layer and any residual bacteria in the smear layer may be left at the interface of the tooth and restorative material[18]. In this study the FBLC conditioner and FLB, ABF primers were more effective on the growth of *S. mutans* than the bonding agents of the systems. The primers in the materials contain acidic monomers with a low pH that exhibit etching effects. This may be the reason for this result. Especially the FBLC conditioner which produced the greatest inhibition halos against *S. mutans*. FBLC conditioner did not produce any inhibitory effect against *L. acidophillus*. This bacteria may be resistant to mild the polyacrylic acid composition of the conditioner. In a study by Settembrini *et al.*[19], it was indicated that phosphoric acid etchants demonstrated antimicrobial activity against to *S. mutans* and *S. salivarius*. But they did not test *L. acidophillus* in their study.

Adhesion-promoting acidic monomers are elements which support antibacterial effects of dentin primers[20]. Addition of these monomers in large amount for self-etching primers decrease pH values of the materials enough to kill or at least inactivate the bacteria. ABF is a two-step self-etching system and the primer of ABF contains adhesion-promoting monomer MDP (10-methacryloyloxydecyl dihydrogen phosphate). The pH values of ABF primer and FLB primer are 2.0. Accordingly, production of inhibition zones of similar size against to S mutans for ABF primer and FLB primer may be derived from the similarity in the acidity of both materials. ABF primer and FLB primer produced inhibition zones against to *L. Acidophillus* but the inhibition zones of FLB primer was smaller than that of ABF primer. MDPB containing the primer of ABF produced the highest inhibition zones against to *L. acidophillus*. Our results support the findings that incorporation of antibacterial monomer MDPB was effective to provide substantial antibacterial activity and the bactericidal effect of MDPB-containing primer was greater than other self-etching solutions[7,10,21]. However, in this study, fluoride containing PBNT, FBLC and FLB bonding agents were found to be more effective against to *S mutans* than ABF bonding agent. ABF bonding did not show any inhibition zones against to both *S mutans* and *L. Acidophillus*. ABF bonding agent does not contain any MDPB and does not have low pH.

Imazato *et al*.[20] indicated that a dentin primer incorporating MDPB showed inhibition of growth of *S mutans, A. viscosus* and *L. casei* and a little bactericidial effect on *S. mutans* after being cured. Özer *et al*.[17] reported that the experimental dentin bonding system ABF, which employs the antibacterial primer containing MDPB, proved to be effective in inactivating the bacteria in the test cavity model. They indicated that the tooth model test used in their study was a reliable method to evaluate the antibacterial effects of dentin bonding agents simulating clinical situation.

Fluoride-releasing restorative materials such as glass ionomers were reported to show inhibitory effects against *S. mutans* in many previous investigations[12,16,22–25]. Prati *et al* [15] also showed that glass ionomer cements produced marked antibacterial activity with their fluor content. Likewise, in our previous study[17], we reported that Reactmer Bond, which includes PRG filler to release fluoride, demonstrated inhibitory effects against *S. mutans* in the agar well technique, but it was not effective to inactivate the bacteria in the cavity. It was speculated that the antibacterial effects shown by Reactmer Bond may have been mostly dependent upon its acidity rather than the leaching of fluoride ion. But the results of the present study indicated that fluoride containing bonding resins of FBLC, PBNT and FLB had an inhibitory effect on *S. mutans*. The antibacterial properties of these resins may be due to their fluoride ingredients. Therefore, it would be appropriate to test their antibacterial effectiveness in the tooth cavity model used in our previous study. Fluoride released from the restorations possibly inhibits recurrent caries formation[26–28], and also it appears to play some role in exhibiting substantial antibacterial effects.

CONCLUSIONS

The results of this presented study indicates that, incorporation of fluoride and MDPB into bonding systems may play an effective role to increase antibacterial performance of these materials and also fluor incorporation in to bonding materials is beneficial to activate antibacterial effectiveness of bonding agents.

MANUFACTURERS' DETAILS

- Experimental ABF Bond, Kuraray, Osaka, Japan
- Fuji Bond LC, GC Corp., Japan
- Prime &Bond NT and SureFil,Dentsply -Caulk,Milford, USA
- Fluoro Bond, Shofu Inc.,Kyoto, Japan.

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