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Comparison of the Antibacterial Efficacy of Several Dentin Bonding Agents: Two Different in Vitro Studies

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Abstract **Objective:** In this study, we compared the antibacterial effects of several dentin bonding agents with different pH values and active monomers. **Method and Materials:** Infected dentin samples were obtained from depths of approximately 4-6 mm, from isolated and air-dried dental caries. *Streptococcus mitis* was isolated and incubated on sheep blood agar plates at 37°C for 18 h. Impregnated antimicrobial disks were used for the liquids, and the samples were grouped as follows: group 1 was a negative control group with nothing applied; group 2 was a positive control group with 2% chlorhexidine digluconate applied; group 3 had Adper Single Bond Universal applied; group 4 had Clearfil SE Bond 2 (including 10-methacryloyloxydodecyl dihydrogen phosphate[MDP]) applied; group 5 had Clearfil S3 Bond Plus (including [MDP])applied; and group 6 had Clearfil Protect Bond (including 12-methacryloyloxydodecylpyridinium bromide [MDPB])applied. The antimicrobial disks were inserted into the blood agar plates. Inhibition zones were measured on the plates by well-educated specialists. **Results:** The 2% chlorhexidine digluconate solution had a more extensive inhibition zone than the other groups. *S. mitis* was significantly inhibited on the MDPB-impregnated disks applied to the agar plates. **Conclusions:** The results demonstrated that dentin bonding agents including MDPB-containing primer had significant antibacterial effects.

Keywords: *dentin bonding agents, MDPB, antimicrobial effect, agar-disk diffusion, tryptophan-containing broth*

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1. Introduction

The treatment procedures for dental caries do not always eliminate all of the microorganisms in the residual dental hard tissues. Bacteria, which live in the residual tissues and invade along the restoration-tooth interface, constitute the main cause of secondary caries. The presence of secondary caries is the most common reason for the replacement of dental restorations [1,2,3]. Dental materials with antimicrobial properties could offer a solution to this problem. The usage of dental restorative materials with antimicrobial actions could extend the longevity of dental restorations [1].

Dentin bonding agents (DBAs) are used to create a hermetic seal between the filling and the cavity walls. At the same time, DBAs should contain materials with antimicrobial properties, such as 12-methacryloyloxy dodecyl pyridinium bromide (MDPB) chemical monomers [4]. Quaternary ammonium-including monomers have excellent antimicrobial effects on some bacteria, such as Streptococci spp. [2], and the antimicrobial effects of DBAs should not be affected by the light curing stage.

The use of acidic solutions on dentinal structures could be effective in reducing the number of residual bacteria in cavities [5]. All-in-one-bottle DBAs contain acidic monomers, such as itaconic acid, and two-step DBAs have acidic and antimicrobial monomers in the primer bottle. Three-step DBAs include 34% phosphoric acid gel in the set, but the cleansing effects of the acid, followed by water rinsing, are limited and should not be regarded as reliable [6].

In contrast, *Streptococcus viridans* constitutes a large group of commensal streptococcal bacteria species that are either α-hemolytic, producing green coloration on blood agar plates, or non-hemolytic. *Streptococcus mitis*, a member of the *S. viridans* family, is a commensal bacteria that is part of the oral flora and that colonizes mucus membranes and hard surfaces in the oral cavity, such as dental hard tissues [7] It is also one of the "pioneer species" of oral biofilm, and it is responsible for dental caries [8].

Recent studies have used different methodologies to determine the antimicrobial activity of DBAs [9,10]. Simple direct inhibition methods, such as agar-disk diffusion and tryptophan-containing broth diffusion methods, have been used most commonly. However, direct inhibition tests depend on solubility, and the

bactericidal and bacteriostatic monomers in DBAs, such as MDPB, itaconic acid or 10-methacryloyloxydodecyl dihydrogen phosphate (MDP), have limited solubility.

In this study, we compared the antibacterial effects of several dentin bonding agents with different pH values and different active monomers. The MDPB agents included a primer, the MDP agents included all-in-one and two-step bonding systems, and the itaconic acid agents included all-in-one system Bond that were not cured with light.

2. Material and Method

We chose patients who had not taken any antibiotic drugs for three months prior to the study and who had no systemic health diseases. All patients were informed about this study before dentin caries samples collection. Any extra treatments was not applied expect standard caries treatment procedures. The enamel entrance to the cavity was enlarged with 1036G diamond round burrs (KG Sorensen, Sao Paulo, Brazil). One millimeter of infected dentin was removed from the cavity, and infected dentin samples were obtained from a depth of approximately 4-6 mm in the isolated and air-dried dental caries. *S. mitis* was isolated and incubated on sheep blood agar plates (SBAPs) (Thermo Scientific™ Blood Agar, Thermo Scientific Inc., NY, USA) at 37°C for 18 h.

All of the samples were grouped as follows (n=10 for agar plates and n=10 for tryptophan-containing broth): group 1 was a negative control group with nothing applied; group 2 was a positive control group with 2% chlorhexidine digluconate (CHX) (Cavity Cleanser, Bisco Inc., Schaumburg, IL, USA) applied; group 3 had Adper Single Bond Universal (SiB) (3M ESPE, Neuss, Germany) applied; group 4 had Clearfil SE Bond 2 Primer (SE2) (Kuraray Co., Okayama, Japan) applied; group 5 had Clearfil S3 Bond Plus (S3B) (Kuraray Co., Okayama, Japan) applied; and group 6 had Clearfil Protect Bond Primer (PB) (Kuraray Co., Okayama, Japan) applied. Table 1 shows all of the materials used. We used liquids (60 µl) that were impregnated into blank antimicrobial susceptibility test disks (Oxoid Ltd, Hants, UK). The disks were inserted into the SBAPs. Inhibition zone diameters were measured on the plates by two well-educated specialists.

Table 1. shows all of the materials used

Materials	Composition and pH	Manufacturer
Cavity Cleanser	2% chlorohexidine digluconate pH: 6	Bisco Inc, Schaumburg, IL, USA
Clearfil S3 Bond Plus	15-35% bisphenol A diglycidylmethacrylate, 10-35% 2-hydroxyethyl methacrylate, 10-methacryloyloxydecyl dihydrogen phosphate pH:2	Kuraray Co., Okayama, Japan
Clearfil SE Bond 2 Primer	20-40% 2-hydroxyethyl methacrylate, 10- methacryloyloxydecyl dihydrogen phosphate pH: 2.5	Kuraray Co., Okayama, Japan
Clearfil Protect Bond Primer	25-45% 2-hydroxyethyl methacrylate, 10-methacryloyloxydecyl dihydrogen phosphate 12-methacryloyloxydodecylpyridinium bromide pH: 2	Kuraray Co., Okayama, Japan
Adper Single Bond Universal	Dimethacrylate resins, 25-45% 2-hydroxyethyl methacrylate, Vitrebond copolymer pH: 4.5	3M ESPE, Neuss, Germany

We then used different techniques to count the numbers of surviving bacteria and to control the first test results. We used a liquid medium (tryptophan-containing broth (Dev Tryptophan Broth, EMD Millipore Corp., Darmstadt, Germany)), and we then applied 60 µl of the DBAs, similar to the other test method, in the experimental groups. After 18 h of incubation time, the samples were inoculated and incubated into SBAPs at 37°C for 18 h. Bacterial colonies then were counted using a Colyte3 Colony Counter (Synbiosis, Frederick, MD, USA). The Mann-Whitney's U test, in the SPSS statistical software package (SPSS, version 20.0, IBM, Armonk, NY, USA) was used for the statistical analysis.

3. Results

The diameters of inhibition zones on the agar plates and surviving bacteria counts (CFUs) with tryptophan-containing broth techniques produced by each material are shown in Table 2.

Table 2. Diameter of inhibiton zones (mean ± SD) and surviving bacteria counts (CFUs) produced by each material

Test materials	Diameter of inhibition zones (mm)	Survived bacteria counts (CFUs)#
Cavity Cleanser	21 ± 1.23 [ab]	None
Clearfil S3 Bond Plus	26 ± 1.62 [ab]	None
Clearfil SE Bond 2 Primer	30 ± 0.76 [ab]	None
Clearfil Protect Bond Primer	30 ± 0.34 [b]	None
Adper Single Bond Universal	14 ± 2.5 [b]	7×10^4

[a]There were no significant differences between these materials. ($p>0.05$).
[b]There were significant differences between these materials. ($p<0.05$).
# 1.2×10^7 colony-forming units (CFUs) *S. mitis* proliferated in the negative control group with no inhibition zones.
n=10 for all groups and testing techniques.

The Clearfil Protect Bond primer produced a mean inhibition zone of approximately 34 mm, and a statistically significant difference was observed in the sizes of the inhibition zones ($p<0.05$). *S. mitis* was significantly

inhibited on the Clearfil Protect Bond-impregnated disks applied to the agar plates. For the Adper Single Bond Universal, there was a significantly smaller inhibition zone, compared to the other groups. No significant differences were measured between groups 4 and 5.

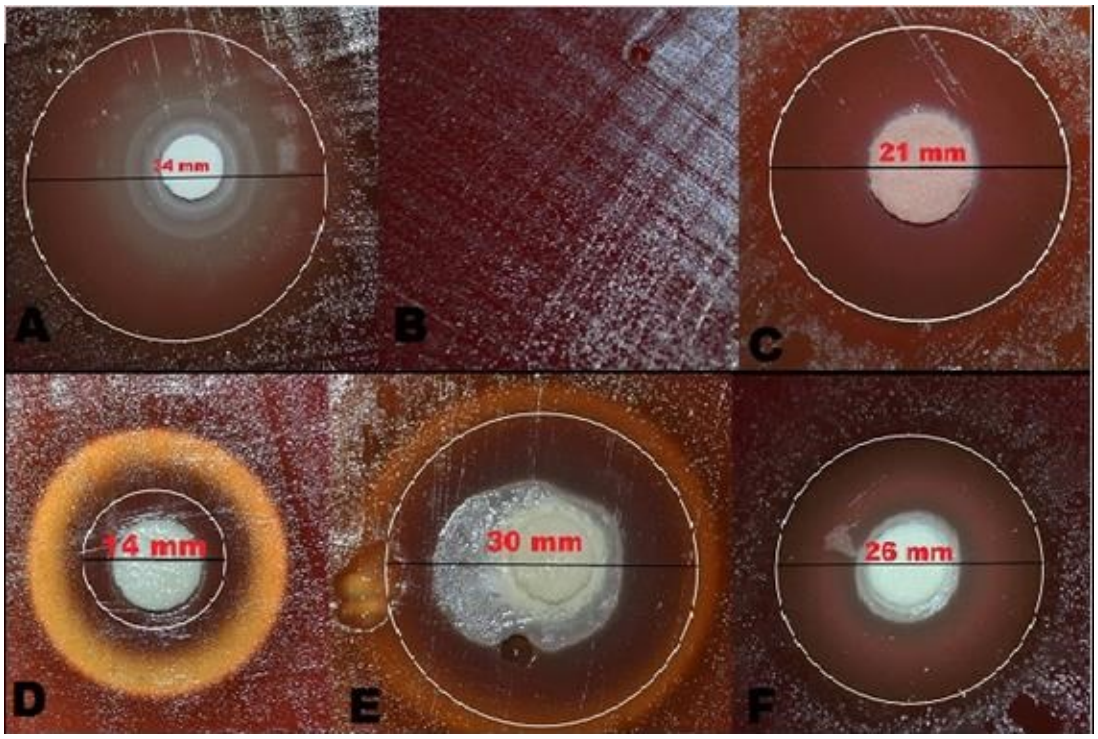

Figure 1. showed inhibition zones in the agar plates

In the liquid medium test $1.2x10^{6}$ colony-forming unit (CFUs) of *S. mitis* proliferated in the negative control group, which had no inhibition zones on the sheep blood agar-disk diffusion test. In group 3, $7x10^{4}$ CFUs of *S. mitis* survived, representing a smaller inhibition zone than with the other dentin bonding agents. We also determined the number of surviving bacteria in all of the experimental groups.

4. Discussion

Many different techniques and bacteria types have been used for the testing of antimicrobial activity [11,12,13,14]. In this study, the agar-disk diffusion and the tryptophan-containing broth techniques were used to test the antibacterial effects of 4 dentin bonding systems. Similar results were obtained with these two methods. The size of the inhibition zones with the agar well technique was not an appropriate index for the comparison of intrinsic antibacterial activity because it reflected the combination of the amounts of antibacterial components included in the materials and their diffusivity within hydrophilic agar [15]. This method had the more important disadvantage of producing an inhibition zone that was not necessarily indicative of bactericidal action.

The adhesive and antimicrobial properties of DBAs have been studied in vitro and in vivo [6,16]. We used oral *S. mitis* were taken from human dental caries cavities and incubated it under in vitro conditions.

Chlorhexidine was used to obtain the chemical control of bacterial plaque and disinfection of the cavities. Chlorhexidine is a chemical antiseptic that is commonly used due to its ability to kill bacteria, some fungi, and certain viruses. At low concentrations, it damages the outer and inner membranes of bacteria, causing the leakage of important substances out of the cells. At high concentrations, it coagulates the cytosol, which is the liquid found inside of the cell. This coagulation inactivates important functions in the cell and results in its death [17]. Other studies have used chlorhexidine as the positive control group [1,2,10,18,19]. Negative control groups have been used to indicate the presence of bacterial growth in media. This study used both negative and positive control groups to obtain healthy data.

Dentin bonding agents block the micro-leakage between tooth-restoration surfaces, and they have an acidic pH. As a result of these features, we can say that many DBAs could be effective in the inactivation of residual bacteria. Self-etching solutions contain particularly large amounts of acidic monomers that produce an etching effect for enamel and dentin, resulting in pH values less than 3 [20]. Our study and recent studies [1,9,13] demonstrated that lower pH dentin bonding agents could kill oral streptococci when examined using in-vitro test methods. As mentioned in the introduction of this article, *S. mitis*, a member of the *S. viridans* family, is a commensal oral flora bacterium that colonizes the mucus membranes and hard surfaces in the oral cavity, such as the dental hard tissues. It is also responsible for dental caries. Because of these properties of *S. mitis*, we wanted to test the antimicrobial effects of these bonding agents on this bacterium. In this study, the lower pH dentin bonding agents showed more adequate antimicrobial effects than the higher pH dentin bonding agents. Adper Single Bond Universal (3M ESPE, Neuss, Germany) has a more alkaline pH and resulted in smaller inhibition zone diameters than the other DBAs under these study

conditions. The tryptophan-containing broth test results for Adper Single Bond Universal (3M ESPE, Neuss, Germany) demonstrated that 5.38% of the *S. mitis* survived after application. The results of tryptophan-containing broth testing showed that the antimicrobial activity of these materials was dependent on the dilution of the solution by dentinal fluid and on dentin-primer contact values.

Clearfil Protect Bond is a two-step DBA, and its primer contains a type of quaternary ammonium monomer known as MDPB. MDPB has a potent positive charge, and it affects bacteria by changing the electrical balance of the cell membranes, similar to what is seen in bacteriolysis [4]. According to the Clinical Laboratory Standards Institute (CLSI), the inhibition zone diameter of penicillin for this type of organism is 28 mm, and effective antimicrobials being tested must exceed this inhibition zone size. The primer with MDBP showed a larger inhibition zone diameter (34 mm ±1.2 mm) than the other groups in our study. The present results supported the findings that the incorporation of the antibacterial monomer MDPB was effective in providing substantial antibacterial activity and that the bactericidal effects of the MDPB-containing primer were greater than those of the other testing DBAs and of chlorhexidine gluconate.

5. Conclusion

The results of our findings showed that MDPB monomers had significant antibacterial effects. In addition, the two-step DBAs with lower pH values showed adequate antimicrobial effects. Follow-up studies could explain the molecular mechanisms underlying these results.

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Conflict of Interest

None of the authors reports any conflicts of interest.

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