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Original Research

The Comparative Evaluation of Antibacterial Activity of Methacryloxydodecyl Pyridinium Bromide and Non-methacryloxydodecyl Pyridinium Bromide Dentin Bonding Systems Using Two Different Techniques: An *In vitro* Study

Prashanth Deshpande[1], Mohan Thomas Nainan[2], Kiran Kumar Metta[3], Vasundhara Shivanna[4], Ramkrishna Ravi[5], B R Prashanth[6]

Contributors:
[1]Reader, Department of Conservative Dentistry & Endodontics, SB Patil Dental College & Hospital, Bidar, Karnataka, India; [2]Professor and Head, Department of Conservative Dentistry & Endodontics, Vydehi Dental College & Research Centre, Bengaluru, Karnataka, India; [3]Assistant Professor, Department of Restorative Dentistry & Endodontics, Ibn Sina National College for Medical Studies, Jeddah, Saudi Arabia; [4]Principal, Professor and Head, Department of Conservative Dentistry & Endodontics, College of Dental Sciences, Davangere, Karnataka, India; [5]Professor and Head, Department of Conservative Dentistry & Endodontics, SB Patil Dental College & Hospital, Bidar, Karnataka, India; [6]Reader, Department of Conservative Dentistry & Endodontics, KLE Dental College, Bengaluru, Karnataka, India.

Correspondence:
Dr. Deshpande P. Department of Conservative Dentistry & Endodontics, SB Patil Dental College and Hospital, Bidar, Karnataka, India. Email: endodeshpande@yahoo.com

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Abstract:

Background: Adhesive systems have enabled clinicians to preserve more tooth structure by changing cavity designs. However, because of the polymerization shrinkage adhesive systems are not capable of totally prohibiting the gap formation between the cavity and restorative material of composite resin leading to colonization of oral microorganisms from saliva. One possible solution for this serious problem is to use dental materials with antibacterial properties. So the development of such agents has initiated for successful restorations. Hence, the purpose of this study was to compare the antibacterial activities of two dentin bonding systems: Clearfil protect bond (CPB) and prime & bond NT using agar well technique and tooth cavity model.

Materials and Methods: CPB and prime and bond NT (PBNT) were evaluated in this study using agar well technique and tooth cavity model. In the agar well technique, the materials were filled in the wells of Muller-Hinton agar plates inoculated with *Streptococcus mutans* NCTC 10449M and the diameter of inhibition zones produced around the materials were measured after 24 h of incubation. For the tooth cavity model test, 3 cavities (of diameter - 1 mm and depth - 2 mm) were prepared in the flat occlusal dentin of human extracted molar. After sterilization, the teeth were left in the culture of broth of *S. mutans* at 37°C for 72 h for allowing bacteria to invade the cavity for 72 h. The dentin bonding systems were applied separately to each of the two infected cavities, and the third cavity was not applied and used as control. After sealing the occlusal surfaces, the teeth were kept in sterile physiological saline at 37°C for 72 h. The standardized amounts of dentin chips (120 + 5 mg) were obtained from the cavity walls, and the numbers of bacteria recovered were determined. The results were analyzed using one-way ANOVA, Kruskal–Wallis and Mann–Whitney's U-tests.

Results: The primer of CPB and PBNT produced similar inhibition zones ($P > 0.05$), but the bonding resin of CPB did not produce any inhibition. When tested by the tooth cavity model technique, the application of CPB resulted in significantly less bacterial recovery than the PBNT ($P < 0.05$), demonstrating substantial antibacterial effects.

Conclusion: The CPB that employs the antibacterial primer containing methacryloxydodecyl pyridinium bromide, was effective in inactivating the bacteria in the cavity compared to little antibacterial activity shown by PBNT. The tooth cavity mode test used in the present study is a reliable method to evaluate the antibacterial effects of dentin bonding agents simulating clinical situations.

Key Words: Agar well technique, dentin bonding systems, dentin primers, *Streptococcus mutans,* tooth cavity model

Introduction

The development of adhesive systems has enabled clinician to change the most extensive conventional cavity designs to downsized cavity preparation and thus preserving more tooth structure. However, even the most recently evolved adhesive systems are not capable of totally prohibiting the gap formation between the cavity and restorative material because of the polymerization shrinkage of composite resin. Gap between restorations and cavity walls may be colonized by oral microorganisms from saliva. This may result in secondary caries and thereby pulpal inflammation.[1] One possible solution for this serious problem is to use dental materials with antibacterial properties. The use of filling materials with an inhibitory action on microbial growth may be able to help in preventing post-operative discomfort and extend the longevity of restorations. As a consequence, until now many attempts have been made to produce dental materials that may inhibit bacterial growth. Glass-ionomer cements, zinc oxide-eugenol

cements and calcium hydroxide preparations have shown antibacterial activities. For dentin bonding agents, several approaches may be possible to enhance antibacterial activity. First, the incorporation of fluorides into the dentin bonding agents may be one way to inhibit bacterial growth. The next way is to lower the pH of monomers through the addition of specific antibacterial groups to them, which can produce antibacterial effects.[2]

To produce resin-based materials with antibacterial activity, a monomer, methacryloxydodecyl pyridinium bromide (MDPB) has been developed. MDPB is a compound of an antibacterial agent, quaternary ammonium with a methacryloyl group, and exhibits strong antibacterial activity against oral streptococci. *Streptococcus* species, *Actinomyces* and *Veillonellae,* are predominant in dental plaque and among these *Streptococcus mutans* is considered the chief etiological agent for causing dental caries.

The incorporation of MDPB has been reported to be effective in providing dentin bonding systems with antibacterial activity before and after curing.

Several studies have determined the antibacterial activity of conventional cements, lining materials or dentin bonding systems using different methodologies.[3] Among them, simple, direct inhibition tests such as agar-disk diffusion methods have been most frequently used. However, direct inhibition methods are considered to be inappropriate for the comparison of antibacterial activity of different materials since the release characteristics of antibacterial components are not precisely involved. Bactericides, such as chlorhexidine, glutaraldehyde, or even acids present in many dental materials, have limited solubility. Accordingly, the diffusion of antibacterial components from the materials into the dentin may vary significantly, and they may be released at rates slow to be only bacteriostatic and not overly bactericidal. Therefore, it is not possible to determine the clinical valve of the antibacterial effects of the dentin bonding systems only from culturing studies and tests using *in situ* or *in vivo* models are needed to examine its substantial value. Hence, in this study, to simulate more appropriate clinical situations, a new tooth cavity model is designed. The objective of this study was to compare the antibacterial activities of the two dentin bonding systems using two different techniques – agar well technique and tooth cavity model.

Materials and Methods

The two dentin bonding systems used in this study were (Figure 1):

1. Clearfil protect bond (CPB) (Kuraray, Osaka, Japan) a dentin bonding system which consists of single-bottled self-etching primer containing 5% MDPB and a fluoride – releasing bonding resin.
2. Prime and bond NT (PBNT) (Dentsply De Trey, Konstanz, Germany) is a one-step self-etching/priming agent, which contains the pre-reacted glass (PRG)-ionomer filler to release fluoride and equipment's used were autoclave, incubator and microscope.

Methodology

This *in vitro* study was conducted in the Department of Conservative Dentistry and Endodontics, to compare the antibacterial activities of two dentin bonding systems using two different techniques. A total of thirty extracted sound human molars devoid of caries, anatomical variations and fractures were used (Figure 2).

Agar well technique

S. mutans NCTC 10449 was in lyophilized form (powdered form). First the lyophilized form containing *S. mutans* was opened under strict sterile conditions, then it was transferred to individual tube containing glucose broth and tube was kept in an incubator at a temperature of 37°C for 24 h. After 24 h of incubation, the tube containing suspension of glucose broth and *S. mutans* was taken out and it was subcultured on 5% sheep blood agar to obtain the growth of bacterial colonies by keeping agar media in incubator at a temperature of 37°C for 24 h (Figure 3). The growth of *S. mutans* colonies obtained on 5% sheep blood agar was confirmed with staining procedure and using microscope. The obtained *S. mutans* colonies were transferred to glucose

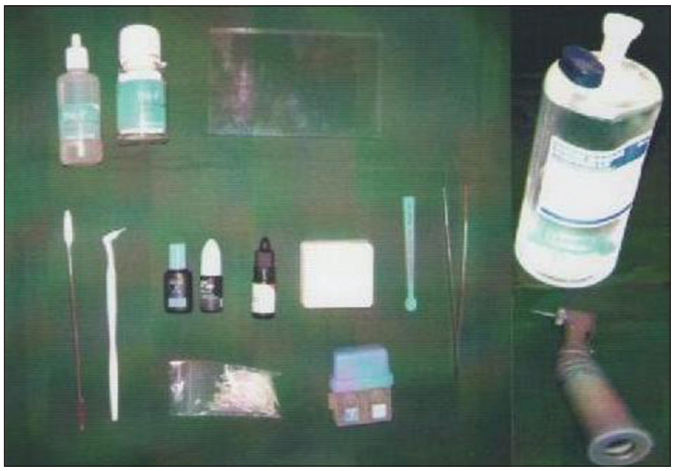


Figure 1: Materials with dentin bonding agents.

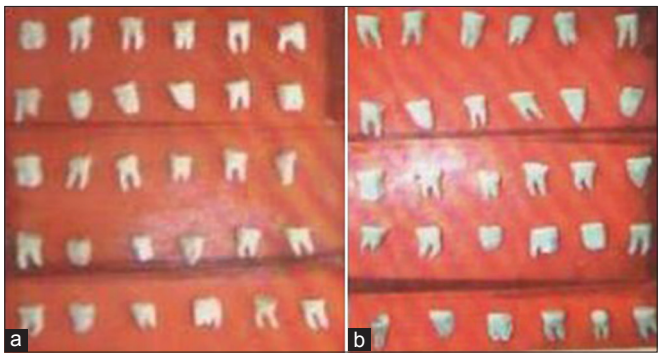


Figure 2: Study samples (a) before (b) after decoronation.

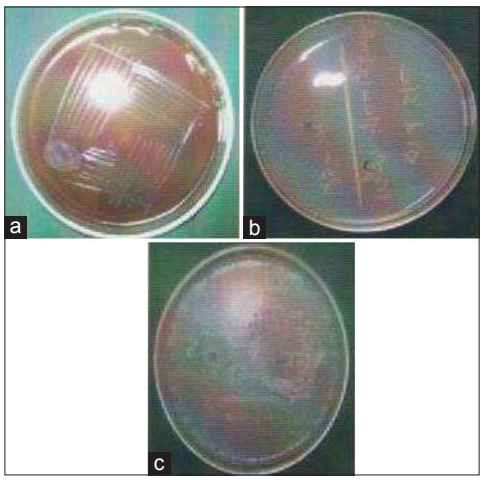


Figure 3: Media (a) 5% sheep blood agar with *Streptococcus mutans* colonies (b) Muller-Hinton agar (MHA) plate with 3 wells and (c) MHA plate with inhibition zones produced.

broth with the help of bacteriological loupes and once again incubated at 37°C for 24 h. Muller-Hinton agar (MHA) was evenly distributed over the surface of 6 cm diameter petri-dishes to thickness of 5 mm and was kept ready for the next step. Standardized wells were punched into the MHA plate with the blunt end of a sterile Pasteur pipette. Approximately 0.5 ml of *S. mutans* suspension (3.6×10^7) was inoculated by swabbing over the MHA surfaces, and the test materials were filled in the wells in two groups. Group 1: Dentin bonding agent containing MDPB: CPB which is available in two bottles, i.e., the primer and bonding resin. The both components were added separately into these wells and tested separately. Group 2: Dentin bonding agent not containing MDPB: PBNT, which is available in a single bottle, was added into the third well. After adding all materials into these well separately, the MHA plate was again incubated for 24 h at 37 + 1°C, diameters of circular inhibition zones produced around the materials were measured after 24 h. The test was repeated 12 times for each material.

Tooth cavity model

Non-carious thirty extracted human molars were used in this study. The enamel is decoronated from the occlusal part of the teeth to obtain flat dentinal surfaces by using a low-speed diamond saw. Three cylindrical cavities (diameter of 1 mm and 2 mm depth) were prepared in the flat surfaces of each tooth without causing pulpal exposure. The teeth were sterilized by an autoclave for 15 min at 121°C. To confirm sterility, the teeth were put into the bottle containing plain brain heart infusion (BHI) broth and incubated for 24 h at 37°C. If turbidity was found in a bottle, then it is indicated that the teeth were not sterile, and sterilization procedure was repeated, until the teeth are absolutely sterile.

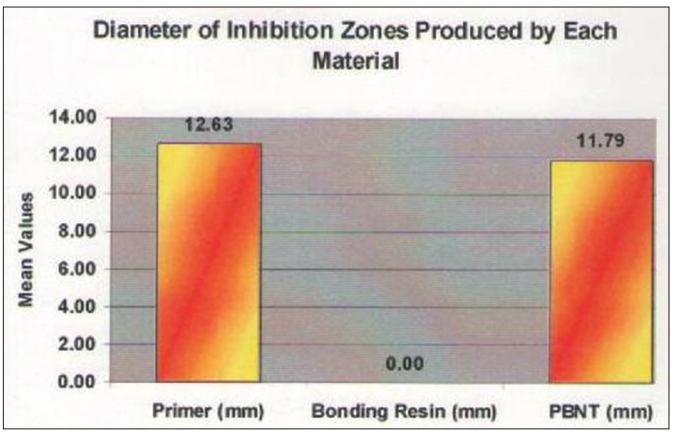
Then, all teeth were transferred to bottle containing sterile physiological saline (SPS) and stored for 24 h at 37°C to wash out culture medium and to avoid dehydration. After drying with sterile paper points under laminar flow, all teeth were placed in a bottle containing suspension of BHI broth and *S. mutans* NCTC 10449 and incubated at 72 h at 37°C to establish infected cavity. Following incubation, the teeth were taken out from the bottle and dried again with sterile paper points and a gentle stream of air. Each dentin bonding system was applied to cavities in the teeth according to the manufacturer's instructions.

Grouping for tooth cavity model done as, Group 1: Dentin bonding agent containing MDPB (CPB). The primer of CPB was applied using a sterile brush, left undisturbed for 20 s and evaporated with an air-syringe. The bonding agent was applied with another sterile brush spread gently with an air-syringe and light cured for 10 s. Group 2: Dentin bonding agent not containing MDPB (PBNT). The etchant gel was applied for 15 s rinsed with water for 20 s to remove the agent and reaction products of acid and mineral hydroxyapatite. The etched site was air dried with oil free compressed air. Then, the Dentin bonding agent, i.e., PBNT is applied with a sterile brush to the etched site and was left undisturbed for 20 s and light-cured for 10 s and Group 3: left as a control.

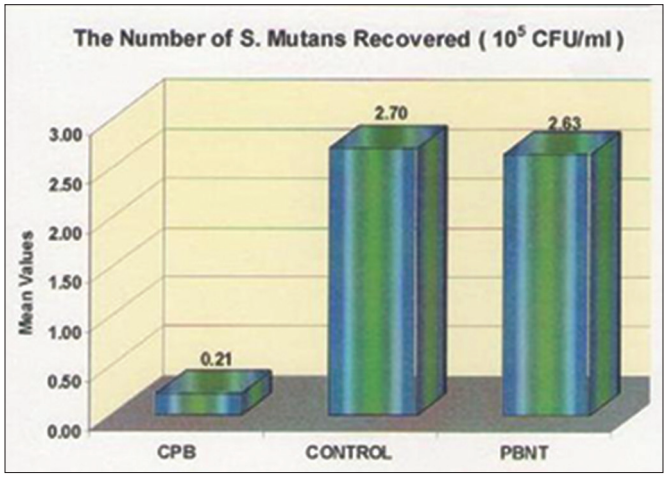
The occlusal surfaces of all the teeth in each group were sealed with a temporary restorative material, like zinc polycarboxylate cement. The teeth were kept in bottle containing SPS at 37°C for 72 h. Then, the teeth were removed from SPS and kept in a freezer at −25°C for 1 h for cooling. The standardized amounts of dentin chips (120 + 5 mg) were collected from the circumferential cavity walls (except pulp floor) into sterile petri-dishes by using carbide fissure burs mounted to a low-speed contra-angle hand piece. The sterile bur was used to prevent overheating of dentinal walls during cutting action. The suspension with dentin chips collected were diluted in 2 ml SPS, and serial dilutions of 10^{-1}, 10^{-2} and 10^{-3} were obtained. The number of *S. mutans* recovered was determined by the classical bacterial counting method using bacterial colony counter method on 5% sheep blood agar media. The data tabulated, and statistical analysis performed using Kruskal–Wallis, one-way ANOVA and Mann–Whitney's U-test.

Results

In agar well-technique (Table 1 and Graph 1) the bonding resin of CPB (Group 1) shows no inhibition zone, whereas the primer of CPB (Group 2) produced mean value of 12.63 mm, which is slightly more than the PBNT (Group 2) (11.79 mm). In agar well technique, the pairwise comparison of study groups showed no statistical significant difference between the primer of CPB and PBNT, when the diameter of inhibition zones produced by each material was taken into account. In tooth cavity model, the pairwise comparison of study groups showed highly statistically significant difference between CPB and PBNT when the number of *S. mutans* recovered from each group was taken into consideration. There was highly statistical



Graph 1: Diameter of inhibition zones produced by each material of both the groups in agar well technique.



Graph 2: The mean value of number of *S. mutans* recovered from all the three groups in tooth cavity model.

significant difference between CPB and control. However, there was no statistical significant difference between PBNT and control. The result of the present study showed that in the agar well-technique, the primer of CPB and PBNT exhibited production of inhibition zones with similar zones. However, in the tooth cavity model tests, the antibacterial effects of CPB were significantly greater than the PBNT, which showed no inhibition of bacteria. The mean value of CPB produced was very less bacterial recovery (0.21 × 10^5 CFU/ml) (Graph 2 and Table 2).

Table 1: Diameter of inhibition zones produced by each material in agar well technique.

Test number	CPB (primer) (mm)	CPB (bonding resin) (mm)	PBNT (mm)
1	11	0	12
2	14	0	10
3	15	0	13
4	10.5	0	15
5	13	0	9
6	12	0	11
7	13.5	0	10
8	14.5	0	12
9	11	0	14
10	12.5	0	11.5
11	14	0	14.5
12	10.5	0	9.5

CPB: Clearfil protect bond, PBNT: Prime and bond NT

Table 2: The number of *S. mutans* recovered from test materials of dentin bonding agents.

Tooth sample number	CPB (primer) (10^5 CFU/ml)	CPB (bonding resin) (10^5 CFU/ml)	PBNT (10^5 CFU/ml)
1	0.25	2	4
2	0.2	4	2
3	0.5	3	3
4	0.2	2	4
5	0.2	1	4
6	0.15	3	2
7	0.25	1	2
8	0.15	2	2
9	0.15	4	2
10	0.15	3	2
11	0.25	2	1
12	0.25	3	3
13	0.25	4	1
14	0.25	2	4
15	0.2	3	3
16	0.25	1	4
17	0.25	2	1
18	0.25	4	2
19	0.2	2	3
20	0.25	4	2
21	0.2	3	3
23	0.25	4	2
24	0.2	2	3
25	0.25	2	4
26	0.2	3	4
27	0.15	4	3
28	0.15	4	2
29	0.2	2	4
30	0.25	2	2

CPB: Clearfil protect bond, PBNT: Prime and bond NT, *S. mutans: Streptococcus mutans*

Discussion

The dentin bonding systems have been developed to minimize the contraction gap formation, which is the main potential for marginal leakage around composite resin restorations. Dentin bonding systems, which show any antibacterial effects during the placement of the filling, would be of better use to inactivate residual bacteria in the cavity.[4]

Adhesion-promoting acidic monomers are elements that support antibacterial effects of dentin primers. Addition of these monomers in a large amount to self-etching primers helps to kill or at least inactivate the bacteria. MDPB monomer, an antibacterial agent, was developed by Imazato *et al*. by containing, i.e. quaternary ammonium and a methacryloyl group. It has been claimed that when applied to the cavity, unpolymerized MDPB contained in the primer of the adhesive system has bactericidal effects. Thus in the present study, self-etching dentin bonding agent

containing the antibacterial agent, MDPB in the primer (CPB) was used.

The earlier study evaluated the bonding of an experimental antibacterial fluoride-releasing adhesive system (ABF, previous name of CPB) to normal and carious dentin in human teeth with Class V root caries. The results showed that the bond strengths of ABF to caries-affected and caries-infected dentin were significantly lower than those to normal coronal and root dentin. The clinical significance of this study is that although the bond strength of ABF adhesive system to root carious dentin is lower than that of normal dentin, the antibacterial and fluoride-releasing properties of ABF may contribute to prevent caries progression and inhibit secondary caries.[5]

PBNT is a one-step adhesive with simultaneous priming and bonding effects, which contains monomer, i.e., PENTA (Dipentaerythritol penta acrylate, monophosphate) and loaded with PRG-ionomer filler. The previous studies reported that a reduction in the amount of bacteria at the tooth-restoration interface could be expected to influence the incidence of dental caries. Dental caries is an infectious disease of bacterial aetiology. *S. mutans* is the main bacterial agent responsible for dental caries. Therefore, antibacterial activity is an important property of materials for successful restorations.[6] Thus in the present study; *S. mutans* was used to compare the antibacterial activity of dentin bonding agents.

The earlier studies used various semi-quantitative methods for determining the antibacterial activity. They are agar-disk diffusion test (ADT), survival time, dilution in broth, growth curves and direct contact test (DCT). Among them, the most standard and commonly used one is ADT. A wide inhibition zone in ADT was interpreted as a potent antibacterial property. It also indicates the material solubility and the relative amount of antibacterial agent released, in the first 24 h. ADT is not a viable test for dental materials which may react with the agar media or those which will disintegrate after curing in oral conditions, as they are undetectable in the test.

Thus, in the present study, in addition to the conventional agar well technique, a newly designed tooth cavity model is used to test the antibacterial effects of two dentin bonding systems. The production of inhibition zones in a larger size for CPB primer and PBNT may be derived due to the similarity in the acidity of both materials. However, the results of tooth cavity model test clearly demonstrate that only CPB is effective in inactivating the bacteria in the cavity. The antibacterial activity of CPB is obviously dependent upon the primer solution because the bonding resin did not show any antibacterial effects in the agar well technique. The present results support the finding that the MDPB monomer containing primer has greater antibacterial activity than other self-etching primers.

Fluoride-releasing restorative materials such as glass-ionomers were reported to show inhibitor effects against *S. mutans* in many previous investigations. Similarly, PBNT, which contains PRG filler to release fluoride, demonstrated inhibitory effects in the agar well technique, but it was not effective to inactivate the bacteria in the cavity model. The antibacterial effects shown by PBNT may have been mostly dependent upon its acidity rather than the leaching of fluoride ion. The bonding resin of CPB produced no inhibition zone in the agar well technique. Although fluoride released from the restorations possibly inhibit recurrent caries formation, it appears to play a limited role in exhibiting substantial antibacterial effects.

The result of the present study showed that in the agar well technique, the primer of CPB and PBNT exhibited production of inhibition zones with similar zones. However, in the tooth cavity model tests, the antibacterial effects of CPB were significantly greater than the PBNT, which showed no inhibition of bacteria.

The size of the inhibition zones in the agar well technique is not an appropriate index for comparison of the intrinsic antibacterial activity as it reflects the combination of the amount of antibacterial components in the materials and their diffusion within the hydrophilic agar. The agar-disk diffusion method also has disadvantages in the point that the production of inhibition zones is not necessarily indicative of bactericidal action. Ohmori *et al*. reported a bovine tooth model for evaluating the antibacterial activity of primers. In their study, three dentin primers were examined by ADTs and tooth model methods. In both methods, ED Primer of Panavia was the most effective, and the cavity model was suggested to be effective. However, they only examined the effects of the primer application, and over-laid bonding resins, which are applied in usual clinical procedure, was not used. In this study, the tooth cavity model test, the application procedure of dentin bonding systems is conducted completely with light curing. Therefore, the antibacterial effects of the material could be compared under more precise simulation of clinical situations, simulating the duration of contact of the uncured material in the cavity.

The present study was in accordance with similar previous study were the authors compared the antibacterial activities of two dentin bonding systems CPB and Xeno III, using three techniques, that is., by agar well, paper and dentin disks and tooth cavity model.[7] The study concluded that CPB was found to be the most antibacterial material with all the techniques used. Furthermore, CPB was able to inactivate the bacteria in the cavity more effectively than Xeno III.[7] However additional *in vivo* and *in vitro* tests and clinical trials, regarding certain points such as the molecular length of MDPB, long-term durability of CPB and the depth of bacterial invasion into dentinal tubules has to be elucidated in future further studies.

Conclusion

The newly introduced dentin bonding system (CPB) which employs the monomer MDPB in the primer has more effective antibacterial property compared to another dentin bonding system which does not contain MDPB monomer (PBNT). The tooth cavity model test compared to the conventional ADT is a reliable method to evaluate the antibacterial effects of dentin bonding agents simulating clinical situations.

References

1. Imazato S, Kaneko T, Takahashi Y, Noiri Y, Ebisu S. *In vivo* antibacterial effects of dentin primer incorporating MDPB. Oper Dent 2004;29(4):369-75.
2. Schmalz G, Ergücü Z, Hiller KA. Effect of dentin on the antibacterial activity of dentin bonding agents. J Endod 2004;30(5):352-8.
3. Ozer F, Karakaya S, Unlü N, Erganiş O, Kav K, Imazato S. Comparison of antibacterial activity of two dentin bonding systems using agar well technique and tooth cavity model. J Dent 2003;31(2):111-6.
4. Imazato S. Antibacterial properties of resin composites and dentin bonding systems. Dent Mater 2003;19(6): 449-57.
5. Doi J, Itota T, Yoshiyama M, Tay FR, Pashley DH. Bonding to root caries by a self-etching adhesive system containing MDPB. Am J Dent 2004;17(2):89-93.
6. Başeren M, Yazici AR, Ozalp M, Dayangaç B. Antibacterial activity of different generation dentin-bonding systems. Quintessence Int 2005;36(5):339-44.
7. Türkün LS, Ateş M, Türkün M, Uzer E. Antibacterial activity of two adhesive systems using various microbiological methods. J Adhes Dent 2005;7(4):315-20.