Assessment of antibacterial properties of newer dentin bonding agents: 
An in vitro study

PAVITRA B. SAMPATH, MITHRA N. HEGDE¹, PRIYADARSHINI HEDGE¹

Abstract

Aim: To evaluate and compare the antibacterial activity of newer dentin bonding agents on Streptococcus mutans using the direct contact test. 

Materials and Methods: Streptococcus mutans was used as test organism and a direct contact test was performed. The dentin bonding agents to be tested were grouped as Group I, Clearfil Protect Bond, Group II, Adper Easy One, and Group III, Prime and Bond NT. For the direct contact test, three microtiter plates consisting of 96 wells each were taken (288 wells). These wells were divided into three groups of 96 wells; 16 wells of a microtiter plate were utilized, of which four were designated as 'A' wells (with the dentin bonding agent and bacterial suspension), another four as 'B' wells (without the dentin bonding agent, but with the bacterial suspension), another four as the 'C' wells (with the tested material, but without bacteria, which served as the negative control), and the remaining four as the 'D' wells (without the dentin bonding agent, which served as the positive control). Each group was treated with their respective bonding agents as per the manufactures instructions. Broth of 15 µL was then transferred from the A wells into an adjacent set of B wells containing fresh medium (215 µL). This resulted in two sets of four wells for each tested material containing an equal volume of liquid medium, so that bacterial growth was monitored both in the presence and in the absence of the tested material. The plate was placed for incubation at 37°C in the microplate reader and the optical density in each well was measured at 600 nm. The readings were taken at regular intervals. (Every 30 minutes for 16 hours).

Results: The Dentin bonding agents evaluated in this study showed different inhibitory effects. Clearfil Protect Bond and Prime and Bond NT were most effective, and Adper Easy One was least effective against Streptococcus mutans. Interpretation and Conclusion: The Dentin bonding agents evaluated in this study showed different inhibitory effects. Clearfil Protect Bond and Prime and Bond NT were most effective, and Adper Easy One was the least effective against Streptococcus mutans. Hence, the incorporation of antibacterial agents into the dentin bonding agents may become an essential factor in inhibiting residual bacteria in the cavity and secondary caries.

Keywords: Antibacterial property, dentin bonding agents, direct contact test, optical density, Streptococcus mutans

Introduction

The development of adhesive systems that have enabled variable cavity designs to preserve intact tooth structure and treatment of caries has recently been shifted from the traditional method to that with downsized cavities. However, when attention is focused on less removal of the tooth structure, it is possible that some active bacteria reside in the cavity.[1]

In spite of considerable improvement in the recent years, polymerization shrinkage and resultant contraction gaps in the tooth restoration interface continue to be a significant problem associated with composite resin restorations. Poor adaptation to the surrounding tooth substance may predispose to discoloration and bacterial colonization.[2] It is well known that the bacteria that invade along the tooth restoration interface are the main cause of secondary caries and damage to the pulp.[3]

Dentin adhesives are currently available as three-step, two-step, and single-step systems, depending on how the three cardinal steps of etching, priming, and bonding to tooth substrate are accomplished or simplified. In the three-step system, etching, priming, and bonding are carried out in three different steps. Two-step systems are sub-divided into the self-priming adhesives that require a separate etching step, and the self-etching primers that require an additional bonding step. The recently introduced all-in-one adhesives further combine these three bonding procedures into a single step application.[4]

Departments of Conservative Dentistry and Endodontics, A.J Institute of Dental Sciences, Kuntikana, Mangalore, ‘A. B. Shetty Memorial Institute of Dental Sciences, Rajiv Gandhi University of Health Sciences, Mangalore, Karnataka, India

Correspondence: Dr. Pavitra B. Sampath, Departments of Conservative Dentistry and Endodontics, A. J Institute of Dental Sciences, Rajiv Gandhi University of Health Sciences, Kuntikana, Mangalore – 575 004, Karnataka, India.
E-mail: pamma16@rediffmail.com

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These current concepts have proved themselves both scientifically and clinically. The concepts reduce the clinical steps, can be placed inexpensively, provide adequate bonding to the enamel and dentin, and most importantly, ensure the patients’ postoperative comfort.\[^{[3]}\]

To provide resin-based materials with antibacterial activity a new monomer, methacryloyloxydodecylpyridinium bromide (MDPB), has been developed (Imazato \textit{et al.}, 1994). The incorporation of MDPB is considered to be a potential method of providing dentin adhesive systems with antibacterial activity before and after curing.\[^{[6]}\]

The antibacterial effects of non-polymerized and immediately polymerized adhesives are beneficial in the eradication of residual bacteria in the oral cavity. The long-lasting antibacterial activity of polymerized adhesives may be effective in inactivating the bacteria that invade the tooth-adhesive interface by microleakage.\[^{[7]}\]

Hence, the aim of the present study is to assess the antibacterial activity of newer dentin bonding agents against \textit{Streptococcus mutans}.

**Materials and Methods**

**Tested materials**

**Materials used**

Three commercially available dentin bonding agents were used in this study [Table 1].

All materials were handled and polymerized in strict compliance with the manufacturers instructions.

**Test microorganism and growth conditions**

\textit{Streptococcus mutans}, the primary etiological agent for caries is widely used to test the antibacterial activity of the dentin bonding agent. A clinical isolate of mutans streptococci 27351M, naturally resistant to bacitracin, was grown aerobically from a frozen stock culture in the Brain–Heart infusion (BHI) broth containing 8 µg / mL bacitracin, for 48 hours, at 37ºC, before being applied to the samples according to the experimental design described a little later in the text.

### Table 1: Dentin bonding agents used in study

| Bonding agent          | Manufacturer | Type                      |
|------------------------|--------------|---------------------------|
| Prime and Bond NT      | Dentsply     | Two-step etch and rinse    |
| Clearfil Protect Bond  | Kuraray      | Two-step self-etch        |
| ADPER Easy One         | 3M ESPE      | One-step self-etch        |
| **Group 1**            |              |                           |
| **Group 2**            |              |                           |
| **Group 3**            |              |                           |
| Experimental group     |              |                           |
| (With the dentin       |              |                           |
| bonding agent)         |              |                           |
| Two-step etch and      |              |                           |
| rinse                  |              |                           |
| Experimental group     |              |                           |
| (coated with dentin    |              |                           |
| bonding agent)         |              |                           |
| Two-step self-etch     |              |                           |
| Experimental group     |              |                           |
| (coated with dentin    |              |                           |
| bonding agent)         |              |                           |
| One-step self-etch     |              |                           |

**Method of collection of data**

Data were collected by recording the optical density, a measurement of turbidity that is based on the kinetics of bacterial growth, with the help of a spectrophotometer.

**Direct contact test**

The direct contact test (DCT) is based on the turbidometric determination of bacterial growth in the 96-well microtiter plates. The kinetics of the outgrowth in each well are monitored at 600 nm at 37ºC and recorded every 30 minutes, using a spectrophotometer.

**Schematic representation of the direct contact test [Figure 1]**

Three microtiter plates consisting of 96 wells each were taken (288 wells). These wells were divided into three groups of 96 wells. Sixteen wells of a microtiter plate were utilized, of which four were designated as ‘A’ wells (with dentin bonding agent and bacterial suspension), another four as ‘B’ wells (without the dentin bonding agent, but with bacterial suspension), another four as ‘C’ wells (with the tested material, but without bacteria, which served as the negative control), and the remaining four as the ‘D’ wells (without the dentin bonding agent, which served as the positive control).

### Figure 1: Schematic representation of direct contact test performed on 96-well microtiter plates. Bacteria are brought in direct contact with the tested material for one hour, followed by addition of the growth medium
Fifteen microliters of broth was then transferred from the ‘A’ wells to an adjacent set of ‘B’ wells containing fresh medium (215 µL). This resulted in two sets of four wells for each tested material, containing an equal volume of liquid medium, so that bacterial growth was monitored both in the presence and in the absence of the tested material. Following the outgrowth of the microorganism in the presence of the tested material (‘A’ wells) is equivalent to measuring both the direct contact effect and the effect of those components that are capable of diffusing into the liquid medium, whereas, following bacterial growth in the absence of the tested materials (‘B’ wells) measures the effect of only the direct contact incubation period. Four wells coated with the tested material, but without bacteria served as the negative control (‘C’ wells). Four uncoated wells in the same microtiter plate served as the positive control (‘D’ wells) and it was processed as the experimental ‘A’ and ‘B’ wells. The plate was placed for incubation at 37°C in the microplate reader and the optical density in each well was measured at 600 nm. The readings were taken at regular intervals (every 30 minutes for 16 hours). The whole experiment was carried out under aseptic conditions and was repeated six times to ensure reproducibility.

The data was recorded and the values were obtained from the negative control (‘C’ wells), which were considered as the baseline values, and were subtracted from experimental values. The data was then plotted and statistically analyzed using ANOVA and the Tukey’s multiple comparison test.

**Results**

Table 2 – Shows intergroup comparison between the bonding agents at different time intervals using Tukey’s multiple comparison test. It showed statistically significant difference when Group I (Clearfil Protect bond) was compared with Group II (Adper easy one) \( P < 0.05 \), and Group II (Adper easy one) was compared with Group III (Prime and Bond NT) \( P > 0.05 \). No statistical significant difference was seen when Group I (Clearfil Protect bond) was compared with Group III (Prime and Bond NT) \( P > 0.05 \), showing that the antibacterial activity of Clearfil Protect Bond and Prime and Bond NT were comparable [Figure 2].

The results infer that the two-step etch and rinse (prime and Bond NT) and two-step self-etch adhesive (Clearfil Protect Bond) are better than the one-step self-etch adhesive (Adper easy one) in their antibacterial activity.

**Discussion**

Secondary caries is one of the major reasons for restoration failure. Accumulated data from numerous studies have demonstrated that the durability of dental restorations is shorter than generally expected, and secondary caries is a major reason for the replacement of restorations. It has been estimated that the removal and replacement of restorations because of secondary caries occupies as much of the dentist’s time as the treatment of primary lesions. The Federation Dentaire Internationale [1962] defines secondary caries as a ‘positively diagnosed carious lesion, which occurs at the margins of an existing restoration’. The lesion usually consists of two carious regions, an ‘outer lesion’ formed in the enamel or the cementum of the tooth surface, similar in histology to a primary lesion, and a ‘wall lesion’, which is a narrower defect in the enamel and / or dentin along the cavity wall-restoration interface.

Bacterial microleakage has been claimed to be the main cause of pulpal inflammation, necrosis, and the eventual need for endodontic therapy after placement of restoration, and the biological sealing of the prepared dentin is now considered critical for successful restoration. Today, many dentin bonding systems require phosphoric acid etching to condition the instrumented cavity surfaces, by removing the smear layer, as a standard procedure in adhesive dentistry. The infiltration of adhesive resins into such demineralized dentin results in a
resin-dentin impregnation zone that not only provides strong bonding, but also a hermetic seal.[13]

Self-etching adhesives have been introduced in an attempt to simplify the clinical application procedure and reduce the technique sensitivity and risk of the primed surface being contaminated.

In self-etching adhesives systems, the pH of the self-etching primer solution is sufficiently low to demineralize the smear layer and the underlying dentin surface; hence, etching and priming of the cavity can be accomplished simultaneously.[12] Therefore, the separate acid-etching step is generally omitted. On account of the non-rinsing procedure, the residual bacteria may remain at the interface between the tooth and the restorative material. The dentin primer is the component that comes into contact and reacts with the dentin substrate at the first stage of restoration in an adhesive system. If tooth conditioners, such as primers, possessed antibacterial activity, these bacteria could be eliminated, thereby preventing the secondary caries. Thus, the antibacterial activity of these adhesive systems primers, which is directly applied to the dentin, plays an important role in the longevity of the restoration.[13]

In the present study the materials to be tested for antibacterial activity against S. mutans, the main pathogen responsible for the initiation of caries, and in the development of secondary caries[7,14] include the Prime and Bond NT, which is a two-step etch and rinse adhesive, and the Clearfil Protect Bond and Adper Easy one, which are self-etching adhesives.

The direct contact test employed in our study has many advantages over the agar diffusion test, and has been studied previously by Weiss et al., Shalhav et al., and Fuss et al. It is a quantitative assay, which allows water-insoluble materials to be tested. It relies on a direct and close contact between the test microorganism and the tested materials and is virtually independent of the diffusion properties of both the tested material and the media. In addition to its reproducible and quantitative nature, the result of DCT is not affected by the size of the inoculum and is relatively insensitive to the size of the inoculum that is brought in contact with the tested material. It facilitates standardized measurements of a large number of specimens and their respective control, simultaneously, on the same microtiter plate and has the ability to monitor the microorganism’s growth, both in the presence and absence of the tested material.[15]

In the present study, the antibacterial activity of the freshly polymerized dentin bonding agent was examined for 16
hours, because the logarithmic growth phase of a bacterial cycle had a range of up to 14 to 16 hours.

Observations from this study showed that among the tested materials, the Clearfil Protect Bond and Prime and Bond NT showed maximum antibacterial activity.

The antibacterial effect of the Clearfil Protect Bond was due to the incorporation of the antibacterial monomer methacyroylhydroxydodecyl pyridinium bromide. MDPB was synthesized by the reaction of hydroxydodecylpyridinium bromide and methacyroyl chloride.[16]

The mechanism of the antibacterial effect of quaternary ammonium compounds is believed to be due to the cationic and hydrophobic binding to the cell-wall components that disturb membrane function and subsequently induce leakage of the cytoplasmic material.

The results are in accordance with the study done by Imazato et al. and Feuerstein et al., who stated that Clearfil protect Bond had a strong antibacterial activity, based upon MDPB, against S. mutans, and the capability to disinfect cavities containing residual bacteria.[17,18]

The Prime and Bond NT exhibited antibacterial activity, this was in accordance with the study done by Atac et al. and Schmalz et al. who stated that the antibacterial property of the dentin adhesive might depend on the components that were originally incorporated to promote adhesion.[4]

The antibacterial effect of the Prime and Bond NT may be attributed to the fluoride present in the bonding agent.[5]

In the present study, the Adper easy one with a PH of 3.5 did not show any antibacterial activity. It was in accordance with a study done by Imazato et al., in 2003, using a different dentin bonding agent, with a low PH, suggesting that the benefit of the low PH environment exhibited by the dentin bonding agent should be considered as limited.[19]

Harper and Loesche (1984) reported that the pH values of the dentin adhesive were required to completely kill the organisms in three hours, 3.0 for S mutans.[20]

Thus, incorporation of antibacterial agents into the dentin bonding agent may be helpful for the complete elimination of the residual bacteria in the cavity.

Within the limitation of the in vitro study, the dentin bonding agent used, showed different inhibitory effects. The Clearfil Protect Bond and Prime and Bond NT were most effective in their antibacterial activity and Adper Easy One was the least effective against Streptococcus mutans.

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