**In vitro antibacterial activity of various adhesive materials against oral streptococci**

Emre Ozel, Fetiye Kolayli, Elif Bahar Tuna and Doganhan Er

*Department of Restorative Dentistry, Faculty of Dentistry, University of Kocaeli, Kocaeli, Turkey; Department of Medical Microbiology, Faculty of Medicine, University of Kocaeli, Kocaeli, Turkey; Department of Pediatric Dentistry, Faculty of Dentistry, University of Istanbul, Istanbul, Turkey*

**ABSTRACT**

The purpose of this study was to investigate the antibacterial activity of various adhesive materials against five different oral streptococci. The antibacterial activity of the adhesive systems was evaluated using agar diffusion tests. In each section of each plate, 6-mm-diameter wells were created with sterilized glass cylinders. Ten microlitres of self-etch adhesives and control materials were applied into the shallow holes. After incubation at 37°C for 24 h, the growth inhibition zones were measured in millimetres. Statistical analyses were performed by using two-way analysis of variance and the Tukey's multiple range test ($p < 0.05$). A statistically significant difference was found between inhibition zones of oral streptococci cultivated with different adhesive systems ($p < 0.01$). Clearfil Protect Bond exhibited larger inhibition zones than the other materials that were used against the oral streptococci. The antibacterial effects observed for the different tested adhesive systems may be related to 12-methacryloyloxy dodecyl-pyridinium bromide and the acidic nature of the materials.

**KEYWORDS**

Agar diffusion test; antibacterial adhesives; oral streptococci

**Introduction**

In order to prevent both cariogenic bacterial colonization and growth of remaining bacteria in cavity preparation, an antimicrobial effect of resin composites and adhesive materials is desired.[1–3] This effect can be achieved by incorporating antimicrobial agents, such as glutaraldehydes, fluorides or antibacterial monomers in the adhesive systems’ formulation.[4–7] It is well known that adhesive monomers of self-etching adhesive systems present a hydrophilic group at one end of the molecule which is usually an acid, such as hydrogen phosphate or carboxylate.[3] These traits provide the materials with low pH and possible antibacterial properties.[3] Therefore, not only antimicrobial agents but also other substances, such as adhesion-promoting monomers that are acidic in different degrees and can be commonly found in the adhesive systems’ formulas, might be able to exert some activity against bacterial growth.[3,8,9]

In the last decade, self-etch adhesive systems have gained widespread attention from researchers and clinicians.[3,10–12] These systems, composed of aqueous mixtures of acidic functional monomers that are generally phosphoric acid esters, require neither a separate acid etch component nor subsequent rinsing procedures.[13–16] It is a scientific fact that ideal phosphoric acid enamel etching patterns and classical dentin surface hybrid layer formation are necessary.[17,18] Acidic monomers partially dissolve the hydroxyapatite constituent by incorporating the smear layer into the demineralized dentin substrate (i.e. collagen fibres and resin monomers). These monomers simultaneously infiltrate the collagen network with primers and eventual resin monomer attachment to achieve both consequential occlusion of dentinal tubules and decrease of the levels of post-treatment sensitivity.[19–23] Self-etch adhesive systems are therefore frequently used in the restorative dentistry.[22]

To make microbiota unviable after cavity preparation, the literature has suggested incorporating antimicrobials into bonding systems.[2] Imazato et al. [2] has reported obtaining an antibacterial adhesive system by incorporating the monomer 12-methacryloyloxy dodecyl-pyridinium bromide (MDPB) to observe strong bactericidal activity against oral bacteria. MDPB is a compound of antibacterial agent quaternary ammonium with a methacryloyl group.[2,24–26] Although the antibacterial effect of the MDPB monomer has been described, very little is
known about the antimicrobial effect of the other self-etching adhesive systems.[3,27]

The antimicrobial effects of adhesive materials is a very important topic in terms of cariogenic bacterial colonization under resin composite restorations, and colonization with oral streptococci may be responsible for the formation of dental caries. It is reported that a reduced amount of this bacteria during the tooth/restoration interface should be expected to influence the incidence of dental caries.[28,29] Anti-bacterial activity is a key issue for the proper use of materials during successful restorations. The purpose of this in vitro study was to investigate the antibacterial activity of various adhesive materials against five different oral streptococci.

Materials and methods

The antibacterial activity of the dentin bonding systems Clearfil S3 Bond Plus, Clearfil Protect Bond and Clearfil SE Bond was evaluated. The manufacturers, lot numbers, types, pH values and compositions of the adhesive systems used in this study are presented in Table 1. A 37% phosphoric acid gel or a 0.12% chlorhexidine solution were used in the control groups. Antibacterial activities of the self-etching adhesives were evaluated against five Streptococcus strains: *Streptococcus salivarius* isolated from the saliva of a healthy person, *Streptococcus gordonii* ATCC 10558, *Streptococcus sobrinus* B13, *Streptococcus sanguinis* ATCC 10556 and *Streptococcus mutans* HF676.

The agar diffusion test method was used to determine the antimicrobial activity of the adhesives. The strains were cultured on brain heart infusion (BHI) agar and incubated at 37 °C for 24 h. Inoculum of each bacterial strain was introduced into test tubes containing 5 mL sterile phosphate buffer solution (pH 7.0), to produce a turbidity of 0.5 on the McFarland scale. An amount of 975 μL of each bacterial cell suspension was mixed with 65 mL of melted BHI agar at 50 °C and poured onto a plate. After the agar solidified, the plate for each bacterial strain, was divided into five sections. In each section of each plate, 6-mm-diameter wells were created with sterilized glass cylinders, and the holes were filled with 150 μL of melted BHI agar. Shallow holes were thus obtained in the surface agar. Ten microlitres of the self-etch adhesives and control materials were then applied in the shallow holes. The plates were incubated at 37 °C for 24 h. The whole experiment was repeated 10 times for each isolate.

### Measuring the zone of inhibition

Following the incubation period, the size of the microbial inhibition zones produced around the holes containing the tested materials were measured and recorded in mm.

### Statistical analysis

Data were assessed using Statistical Package for Social Sciences version 15.0 for Windows. To assess the appropriateness of the data, the distribution parameters were assessed by Kolmogorov–Smirnov test and data were found to be normally distributed within the parameters. Statistical analyses were performed by using two-way analysis of variance and the Tukey’s multiple range test at a 5% confidence level.

### Results and discussion

Mean diameters and standard deviation values of antibacterial inhibition zones are presented in Table 2. Statistically significant difference was found between the levels of bacterial inhibition according to adhesive systems (p < 0.01). According to the bacterial type, statistically significant difference was observed between the levels of bacterial inhibition of adhesive materials (p < 0.01).

This study evaluated the in vitro antimicrobial activity of three commercially available self-etch adhesive materials (Clearfil S3 Bond Plus, Clearfil Protect Bond and Clearfil SE Bond) against *S. salivarius*, *S. gordonii*, *S. sobrinus*, *S. sanguinis* and *S. mutans*. The bacterial species used in this study are related to dental caries, whose

### Table 1. Manufacturer, lot number, type, pH value and composition of the adhesive systems used in this study.

| Adhesive system       | Manufacturer            | Lot number | Type                        | pH  | Composition                                                                 |
|-----------------------|-------------------------|------------|-----------------------------|-----|-----------------------------------------------------------------------------|
| Clearfil S3 Bond Plus | Kuraray Medical Inc.,   | 0010A      | One-step self-etch adhesive | 2.3 | MDP, Bis-GMA, HEMA, photoinitiator, ethanol, water, silanated colloidal silica |
| Clearfil Protect Bond | Kuraray Medical Inc.,   | 00107A     | Two-step self-etch adhesive | Primer: 2.0 | MDPB, MDP, HEMA, water, camphorquinone                                        |
| Clearfil SE Bond      | Kuraray Medical Inc.,   | 01093A     | Two-step self-etch adhesive | Primer: 2.0 | MDP, HEMA, water, camphorquinone                                              |

Note: 10-methacryloyloxydecyl dihydrogen phosphate (MDP); bisphenol A diglycidyl methacrylate (Bis-GMA) 2-hydroxyethyl methacrylate (HEMA); 12-methacryloyloxy dodecyl-pyridinium bromide (MDPB).
development has been an important topic in oral microbiology research for multiple decades.[30] The initial colonization process is dominated by oral streptococci which form over 80% of the early biofilm constituents. [30,31] Though a freshly cleaned tooth surface is colonized by pioneer oral streptococci, even this early event is not immune to interspecies competition. S. sanguinis and S. gordonii are among the first species to colonize the tooth surface and can be isolated from the same intraoral sites.[30,31] S. mutans is the main bacterial agent responsible for dental caries, which is usually found in plaque in the early stages of caries formation, [4,28,32] whereas S. sobrinus is related to the progression of carious lesions or to established carious lesions. The presence of both S. mutans and S. sobrinus at localized sites has correlated strongly with the presence of an early caries.[33] S. salivarius generally has a stronger affinity for the oral soft tissues, though strains of this organism are also found in dental plaque.[32,34,35]

The antibacterial properties of self-etching adhesive systems constitute an important issue in restorative dentistry, since viable bacteria can appear even after cavity preparation.[3] It is well known that bacteria, that invade along the tooth-restoration interface, may cause secondary caries and damage to the pulp.[28,36–38] Since dentin bonding systems have been developed to minimize both contraction gap formation and the potential for marginal leakage around composite restorations,[39,40] dentin bonding systems showing antibacterial effect during the placement of the filling would be of significant use to inactivate residual bacteria in the cavity. [8,26] Many dental clinicians routinely use a cavity disinfectant, such as chlorhexidine or peroxide, in the treatment of dentinal caries as they cannot be sure that the lesion has been completely removed.[2] One of the major advantages of a self-etching adhesive system relies on the simple technique. Especially in pediatric dentistry, a reduction in the clinical steps used with self-etch bonding agents suggests an attractive alternative to the acid-etch technique, since children tolerate only limited chair-time during dental treatments. As a result, self-etch adhesive materials pose great potential for the pediatric dentistry.

Self-etch adhesive systems were also developed to overcome possible discrepancies between dentin demineralization and adhesive impregnation along resin-dentin interface observed in etch-and-rinse adhesive systems. In these systems, the etched substrate is fully infiltrated by adhesive, due to specific monomers which simultaneously demineralize and infiltrate the dentin.[41,42] The self-etch strategy caused a less sensitive system since it eliminated the critical wet bonding technique.[40]

The antibacterial activity of dentin bonding agents depends on several factors, including composition and acidity.[43,44] Self-etch adhesive systems contain an acidic resin, which at once etches and primes tooth structure. Modern self-etch adhesive systems contain methacryloyloxydecyl dihydrogen phosphate (MDP) in their formulation, in order to enhance bonding by substituting 2-(methacryloyloxy)ethyl phenyl hydrogen phosphate (Phenyl-P), which was used in early self-etching systems.[45] Clearfil Protect Bond primer also contains an acidic adhesion-promoting monomer MDPB, with a pH value of 2.0. The antibacterial activity of dentin bonding agents depends on several factors,[8,46–48] though the acidity of the self-etching primers has been recognized as one of the major factors that inhibit bacteria. [8,9,49,50] MDPB monomer is synthesized by combining methacryloyloxyethyl phenyl hydrogen phosphate (Phenyl-P), which was used in early self-etching systems.[45] Clearfil Protect Bond primer also contains an acidic adhesion-promoting monomer MDPB, with a pH value of 2.0. The antibacterial activity of dentin bonding agents depends on several factors,[8,46–48] though the acidity of the self-etching primers has been recognized as one of the major factors that inhibit bacteria. [8,9,49,50] MDPB monomer is synthesized by combining a methacryloyl group with a quaternary ammonium that gives antibacterial properties and inhibitory effect against bacterial growth and plaque accumulation. [24,51]

Clearfil S3 Bond Plus which contains an MDP monomer structure has recently entered the dental market. No study in the available literature has determined the antimicrobial activity of these three self-etch adhesive materials against the five common oral streptococci yet. However, no antibacterial effect was observed in this study for Clearfil S3 Bond Plus, except regarding S. salivarius. Primer solutions of self-etching systems show low pH levels to demineralize the smear layer and dentin surface, hence the separate acid-etching step is generally omitted.[37,52] It is possible that some active bacteria reside in the cavity wall and restorative material interface, as a result of a non-rinsing procedure; these

| Table 2. Diameter of inhibition zones (mm) produced by each of the tested materials. |
|---------------------------------------------------------------|
| Clearfil S3 Bond Plus | Clearfil Protect Bond | Clearfil SE Bond | Phosphoric acid (37%) | Chlorhexidine (0.12%) |
|-----------------------|----------------------|------------------|-----------------------|-----------------------|
| S. salivarius         | 10.30 ± 1.33<sup>A</sup> | 15.00 ± 0.94<sup>B</sup> | 13.30 ± 0.48<sup>BC</sup> | 10.40 ± 0.97<sup>A</sup> |
| S. gordonii           | 0.00 ± 0.00<sup>A</sup> | 14.60 ± 0.70<sup>B</sup> | 10.50 ± 0.71<sup>BC</sup> | 10.10 ± 0.57<sup>AD</sup> |
| S. sobrinus           | 0.00 ± 0.00<sup>A</sup> | 22.90 ± 0.87<sup>B</sup> | 9.10 ± 0.31<sup>BC</sup> | 9.30 ± 0.48<sup>AD</sup> |
| S. sanguinis          | 0.00 ± 0.00<sup>A</sup> | 12.90 ± 1.20<sup>B</sup> | 9.50 ± 0.53<sup>BC</sup> | 9.30 ± 0.48<sup>AD</sup> |
| S. mutans            | 0.00 ± 0.00<sup>A</sup> | 19.10 ± 2.02<sup>B</sup> | 10.80 ± 1.13<sup>BC</sup> | 9.60 ± 1.09<sup>AD</sup> |

Note: Different superscript letters in the column are significantly different; different capital superscript letters in the row are significantly different. The results were obtained as mean values ± standard deviation (SD).
bacteria increase the risk of secondary caries. The antibacterial activity of the adhesive systems’ primers thus plays an important role in a restoration’s longevity. [28,29,53] In this study, the pH value of Clearfil S3 Bond Plus was 2.3, which might contribute to the limited effect on bacterial counts. Clearfil Protect Bond and Clearfil SE Bond pH values were 2.0, according to the manufacturer (Table 1). Imazato et al. [49] have reported that the bactericidal effects observed in primers mainly arise from their acidic properties. Since bacteria cannot survive in an extremely low pH environment, the acidic property and chemical composition of the adhesives might influence the antibacterial properties of the material. As a result, lower pH values exhibit higher antibacterial activity for adhesive materials. In this study, Clearfil Protect Bond and Clearfil SE Bond showed higher antibacterial properties (Table 2) related to their lower pH value (Table 1).

In this study, all self-etch adhesive systems presented some antibacterial effects with different mean values of the inhibition zones’ diameters. Agar diffusion tests clearly demonstrated that Clearfil Protect Bond showed the best inhibition activity against the oral bacteria when compared to the other adhesive materials tested. These results concur with other studies in the literature that have shown the antibacterial potential of Clearfil Protect Bond, which contains 5% MDPB, to reduce in vitro the oral streptococci. [1–3, 8, 24, 49, 54] This antibacterial effect has also been shown in dentin samples without any detrimental effects on bond strength or degree of conversion, compared to other self-etching adhesives. [54] In this study, the highest inhibition zone was observed in Clearfil Protect Bond for all bacterial groups.

Esteeves et al. [3] have investigated the antibacterial activity of various self-etch adhesive materials against oral streptococci and have reported that Clearfil Protect Bond presented the highest antibacterial activity. Imazato et al. [2] have also reported that Clearfil Protect Bond was the most effective adhesive material in terms of antibacterial activity. The findings of these two studies are similar to ours.

Although the antibacterial activity of adhesive materials is an important factor in terms of reducing the chance of secondary caries, caution is needed, since studies have shown that the incorporation of antibacterial agents could impair mechanical properties, and the release of the agent from the material could result in further changes in physical properties. [3, 24]

Chlorhexidine solution has a broad spectrum of uses against Gram-positive and Gram-negative bacteria. [55] The antimicrobial effect of chlorhexidine is caused by the cationic molecule that binds to the negatively charged bacterial cell walls, thereby altering the cell’s osmotic equilibrium. [56–59] Chlorhexidine is biocompatible and can be absorbed by dental tissues and mucous membranes. [55] In this study, a 0.12% chlorhexidine solution was chosen as a positive control, given its wide range of antibacterial abilities.

Numerous techniques have been proposed for determining the antibacterial activity of dentin bondings. In this study, the agar diffusion test method was implemented to evaluate the antimicrobial activity of self-etch adhesive materials. According to our findings, the agar diffusion test clearly demonstrated that Clearfil Protect Bond could inhibit the three bacteria reportedly associated with dental caries.

The incorporation of antibacterial agents into dentin bonding agents may become an essential factor in inhibiting residual bacteria in the oral cavity following a cavity disinfection procedure. [60] The application of self-etching adhesive materials could contribute toward completely eliminating or at least minimizing the bacteria during tooth preparation. [61] The self-etching adhesive containing the MDPB molecule showed an important antibacterial activity and could thus be recommended in situations in which the total disinfection of cavity remains incomplete, due to lack of accessibility. However, further studies are needed to determine whether the in vitro antibacterial effects of adhesive systems are sufficient to increase the longevity of dental restorations by long-term in vivo studies.

Conclusions

In order to avoid cariogenic bacterial colonization in the cavity preparation, the adhesive systems should have an antimicrobial effect. The self-etching adhesives or self-etching primers, used in this study, demonstrated different levels of oral streptococci inhibition. Within the limitations of this study, it may be concluded that among the adhesive systems tested, Clearfil Protect Bond exhibited the most effective antibacterial activity against oral streptococci, whereas Clearfil S3 Bond Plus exhibited no antibacterial activity, except against the S. salivarius’ test group. The antimicrobial capacity of bonding systems is related both to their chemical composition and the bonding agents’ interaction with several microbial strains present in the oral cavity. The antibacterial effects observed for the different adhesive systems tested may be related to the MDPB and the acidic nature of the materials.

Disclosure statement

No potential conflict of interest was reported by the authors.
References

[1] Imazato S, Torii Y, Takatsuka T, et al. Bactericidal effect of dentin primer containing antibacterial monomer methacryloxydodecylpyridinium bromide (MDPB) against bacteria in human carious dentin. J Oral Rehabil. 2001;28:314 – 319.

[2] Imazato S, Kuramoto A, Takahashi Y, et al. In vitro antibacterial effects of the dentin primer of Clearfil Protect Bond. Dent Mater. 2006;22:527 – 532.

[3] Esteves CM, Ota-Tsuzuki C, Reis AF, et al. Antibacterial activity of various self-etching adhesive systems against oral streptococci. Oper Dent. 2010;35:448 – 453.

[4] Peris AR, Mitsui FH, Lobo MM, et al. Adhesive systems and secondary caries formation: assessment of dentin bond strength, caries lesions depth and fluoride release. Dent Mater. 2007;23:308 – 316.

[5] Rodrigues JA, Marchi GM, Serra MC, et al. Visual evaluation of in vitro cariostatic effect of restorative materials associated with dentifrices. Braz Dent J. 2005;16:112 – 118.

[6] Walter R, Duarte WR, Pereira PN, et al. In vitro inhibition of bacterial growth using different dental adhesive systems. Oper Dent. 2007;32:388 – 393.

[7] Karanika-Kouma A, Dionysopoulos P, Koliniotou-Koubia E, et al. Antibacterial properties of dentin bonding systems, polyacid-modified composite resins and composite resins. J Oral Rehabil. 2001;28:157 – 160.

[8] Imazato S. Antibacterial properties of resin composites and dentin bonding systems. Dent Mater. 2003;19:449 – 457.

[9] Ohmori K, Maeda N, Kohno A. Evaluation of antibacterial activity of three dentin primers using an in vitro tooth model. Oper Dent. 1999;24:279 – 285.

[10] Ozel E, Say EC, Yurdaguvven H, et al. One-year clinical evaluation of a two-step self-etch adhesive with and without additional enamel etching technique in cervical lesions. Aust Dent J. 2010;55:156 – 161.

[11] Tekce N, Demirci M, Tuncer S, et al. Microtensile bond strength and sealing efficiency of all-in-one self-etching adhesives. Biotechnol Biotechnol Equip. 2015;29:570 – 578.

[12] Dalli M, Bahsi E, Sahbaz C, et al. A comparison of microleakage scores of five different types of composite resins. Biotechnol Biotechnol Equip. 2010;24:2122 – 2126.

[13] Korkmaz Y, Ozel E, Attar N, et al. Microleakage and scanning electron microscopy evaluation of all-in-one self-etch adhesives and their respective nanocomposites prepared by erbium:yttrium-aluminium-garnet laser and bur. Lasers Med Sci. 2010;25:493 – 502.

[14] Van Meerebeek B, De Munck J, Yoshida Y, et al. Adhesion to enamel and dentin: current status and future challenges. Oper Dent. 2003;28:215 – 235.

[15] Perdigão J, Geraldeli S, Hodges JS. Total-etch versus self-etch adhesive: effect on postoperative sensitivity. J Am Dent Assoc. 2003;134:1621 – 1629.

[16] Vinay S, Shivanna V. Comparative evaluation of microleakage of fifth, sixth, and seventh generation dentin bonding agents: an in vitro study. J Conserv Dent. 2010;13:136 – 140.

[17] Hobson RS, McCabe JF. Relationships between enamel etch characteristics and resin-based bond strength. Br Dent J. 2002;192:463 – 468.

[18] Miyazaki M, Iwasaki K, Onose H, et al. Enamel and dentin bond strengths of single application bonding systems. Am J Dent. 2001;14:361 – 366.

[19] Gokce K, Aykor A, Ersoy M, et al. Effect of phosphoric acid etching and self-etching primer application methods on dentinal shear bond strength. J Adhes Dent. 2008;10:345 – 349.

[20] Aykor A, Ozel E. Five-year clinical evaluation of 300 teeth restored with porcelain laminate veneers using total-etch and a modified self-etch adhesive system. Oper Dent. 2009;34:516 – 523.

[21] Attar N, Korkmaz Y, Ozcel E, et al. Microleakage of class V cavities with different adhesive systems prepared by diamond instrument and different parameters of Er:YAG laser irradiation. Photomed Laser Surg. 2008;26:585 – 591.

[22] Owens BM, Johnson WW, Harris EF. Marginal permeability of self-etch and total-etch adhesive systems. Oper Dent. 2006;31:60 – 67.

[23] Attar N, Korkmaz Y, Kiliçal Y, et al. Bond strength of orthodontic brackets bonded to enamel with a self-etching primer after bleaching and desensitizer application. Korean J Orthod. 2010;40:342 – 348.

[24] Imazato S, Torii M, Tsuchitani Y, et al. Incorporation of bacterial inhibitor into resin composite. J Dent Res. 1994;73:1437 – 1443.

[25] Imazato S, Ebi N, Tarumi H, et al. Bactericidal activity and cytotoxicity of antibacterial monomer MDPB. Biomater. 1999;20:899 – 903.

[26] Deshpande P, Nainan MT, Metta KK, et al. 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. J Int Oral Health. 2014;6:60 – 65.

[27] Kim SR, Shin DH. Antibacterial effect of self-etching adhesive systems on Streptococcus mutans. Restor Dent Endod. 2014;39;32 – 38.

[28] Başer M, Yazıcı AR, Ozalp M, et al. Antibacterial activity of different generation dentin-bonding systems. Quintessence Int. 2005;36:339 – 344.

[29] Pennemets RKR, Rekha SA, Poppuri KC, et al. An in vitro evaluation of antibacterial properties of self-etching dental adhesive systems. J Clin Diagn Res. 2014;8:ZC01 – ZC05.

[30] Kreth J, Merritt J, Qi F. Bacterial and host interactions of oral streptococci. DNA Cell Biol. 2009;28:397 – 403.

[31] Rosan B, Lamont RJ. Dental plaque formation. Microbes Infect. 2000;2:1599 – 1607.

[32] Alexander WM, Cawson RA. Clinical and oral micro-biology. Washington (DC): Hemisphere Publishing; 1983.

[33] Brailsford S, Sheehy E, Gilbert S, et al. The microflora of the erupting first permanent molar. Caries Res. 2005;39:78 – 84.

[34] Basaran G, Başaran E, Hamamci O. Effects of orthodontic adhesive materials on Streptococcus mutans and Lactobacilli levels in human saliva. Biotechnol Biotechnol Equip. 2006;20:156 – 159.

[35] Arora R, Rao MH. Comparative evaluation of the antibacterial effects of four dentine bonding systems: an in vitro study. J Conserv Dent. 2013;16:466 – 470.

[36] Browne RM, Tobias RS. Microbial microleakage and pulpal inflammation: a review. Endod Dent Traumatol. 1986;2:177 – 183.
Sampath PB, Hegde MN, Hegde P. Assessment of antibacterial properties of newer dentin bonding agents: an in vitro study. Contemp Clin Dent. 2011;2:165–169.

Schmalz G, Ergüçü Z, Hiller KA. Effect of dentin on the antibacterial activity of dentin bonding agents. J Endod. 2004;30:352–358.

Ozel E, Soyman M. Effect of fiber nets, application techniques and flowable composites on microleakage and the effect of fiber nets on polymerization shrinkage in Class II MOD cavities. Oper Dent. 2009;34:174–180.

Espejo LC, Simionato MR, Barroso LP, et al. Evaluation of three different adhesive systems using a bacterial method to develop secondary caries in vitro. Am J Dent. 2010;23:93–97.

Breschi L, Mazzoni A, Ruggeri A, et al. Dental adhesion review: aging and stability of the bonded interface. Dent Mater. 2008;24:90–101.

Mozner N, Salz U, Zimmermann J. Chemical aspects of self-etching enamel dentin adhesives: a systematic review. Dent Mater. 2005;21:895–910.

Pinheiro SL, Soares HH, Ribeiro MC. Microbial contamination and inhibitory effect against Streptococcus mutans from fifth-generation bonding systems. J Appl Biomater Biomech. 2010;8:52–55.

Korkmaz Y, Ozalp M, Attar N. Comparison of the antibacterial activity of different self-etching primers and adhesives. J Contemp Dent Pract. 2008;9:57–64.

Pashley DH, Tay FR. Aggressiveness of contemporary self-etching adhesives. Part II: etching effects on unground enamel. Dent Mater. 2001;17:430–444.

Emilson CG, Bergenholtz G. Antibacterial activity of dentinal bonding agents. Quintessence Int. 1993;24:511–515.

Meiers JC, Miller GA. Antibacterial activity of dentin bonding systems, resin-modified glass ionomers, and polyacid-modified composite resins. Oper Dent. 1996;21:257–264.

Fraga RC, Siqueira JF Jr, de Uzeda M. In vitro evaluation of antibacterial effects of photo-cured glass ionomer liners and dentin bonding agents during setting. J Prosthet Dent. 1996;76:483–486.

Imazato S, Imai T, Ebisu S. Antibacterial activity of proprietary self-etching primers. Am J Dent. 1998;11:106–108.

Imazato S, Kuramoto A, Kaneko T, et al. Comparison of antibacterial activity of simplified adhesive systems. Am J Dent. 2002;15:356–360.

Imazato S, McCabe JF. Influence of incorporation of antibacterial monomer on curing behavior of a dental composite. J Dent Res. 1994;73:1641–1645.

Amin S, Shetty HK, Varma RK, et al. Comparative evaluation of antibacterial activity of total-etch and self-etch adhesive systems: an ex vivo study. J Conserv Dent. 2014;17:266–270.

Pashley DH, Carvalho RM. Dentin permeability and dentin adhesion. J Dent. 1997 25:355–372.

Imazato S, Kinomoto Y, Tarumi H, et al. Incorporation of antibacterial monomer MDPB into dentin primer. J Dent Res. 1997;76:678–772.

Ferraz CC, Gomez BP, Zaia AA, et al. In vitro assessment of the antimicrobial action and the mechanical ability of chlorhexidine gel as an endodontic irrigant. J Endod. 2001;27:452–455.

Pacios MG, Silva C, López ME, et al. Antibacterial action of calcium hydroxide vehicles and calcium hydroxide pastes. J Investig Clin Dent. 2012;3:264–270.

Quadir F, Amin F, Shahbaz U. Comparison of intracanal medications for the assessment of pain after root canal treatment. P Oral Dent J. 2015;35:286–289.

Mohammadi Z, Abbott PV. The properties and application of chlorhexidine in endodontics. Int Endod J. 2009;42:288–302.

Hamed SJ, AL-Yasiri IK, Ali NT, et al. Antibacterial activity of calcium hydroxide combined with chlorhexidine or sodium hypochlorite against gram positive and gram negative bacteria. J Nat Sci Res. 2014;4:55–61.

Hegde MN, Hedge P, Shetty V, et al. Assessment of antibacterial activity of self-etching dental adhesive systems: an in vitro study. J Conserv Dent. 2008;11:150–153.

Kakar S, Goswami M, Nagar R. Dentin bonding agents—II recent trials. World J Dent. 2012;3:115–118.