©Operative Dentistry, 2009, **34-3**, 321-327

Microleakage in Resin Composite Restorations After Antimicrobial Pre-treatments: Effect of KTP Laser, Chlorhexidine Gluconate and Clearfil Protect Bond

HS Siso• A Kustarci • EG Göktolga

Clinical Relevance

Although none of the procedures tested in this study completely eliminated microleakage, KTP laser irradiation exhibited the lowest microleakage scores for both enamel and gingival margins.

SUMMARY

The current study evaluated the influence of KTP (Potassium-Titanyl-Phosphate) laser irradiation, 2% chlorhexidine gluconate and Clearfil Protect Bond on the microleakage of Class V composite restorations.

Thirty human premolars were selected for cavity pretreatment. After Class V cavity restorations on the buccal and lingual surfaces, the teeth were randomly divided into four groups: Group I–Clearfil SE Bond; Group II–KTP laser + Clearfil SE Bond; Group III–2% chlorhexidine gluconate + Clearfil SE Bond; Group IV–Clearfil Protect Bond. The cavities were restored with resin composite. The teeth were then thermocycled for 500 cycles, dried and sealed with nail varnish, leaving 1 mm around the restorations and immersed in 0.5% basic fuchsin for 24 hours. They were then rinsed, dried and sectioned, and microleakage was assessed by dye penetration at the occlusal and gingival surface of the teeth using a stereomicroscope (30x).

There were no significant differences among the four groups at the gingival surface (*p*>0.05).

Hergüner Seyda Siso, DDS, PhD, assistant professor, Department of Conservative Dentistry, School of Dentistry, Cumhuriyet University, Sivas, Turkey

*Alper Kustarci, DDS, PhD, assistant professor, Department of Endodontics, School of Dentistry, Cumhuriyet University, Sivas, Turkey

Emine Gülsah Göktolga, DDS, research assistant, Department of Conservative Dentistry, School of Dentistry, Cumhuriyet University, Sivas, Turkey

*Reprint request: 58140, Campus, Sivas, Turkey; e-mail: akustarci@hotmail.com

DOI: 10.2341/08-96

Microleakage at the occlusal margins of all the groups was compared; differences between the KTP laser and chlorhexidine gluconate group and the KTP laser and Clearfil Protect Bond group were found to be statistically significant (*p*<0.05).

INTRODUCTION

Dental caries is one of the most common problems in dentistry today. In addition, recurrent caries has been proven to be one of the most common complications following tooth restoration.[1] Many investigators have identified microleakage as the primary cause of recurrent caries, pulpal inflammation and necrosis.[2] The problems associated with microleakage can be magnified by incomplete sterilization of the preparation from failure to mechanically remove infected tooth structure. Bacteria left in the cavity floor of dentin can live for a long time; complete removal of the infected dentin is desirable in order to prevent the recurrence of decay.[3] Leung and others[4] found the number of residual bacteria in cavity preparations capable of doubling within one month of the restoration being placed. To reduce the potential for residual caries, sensitivity, microleakage and laser irradiation, antibacterial solutions and dental adhesives with antibacterial properties could be used after cavity preparation.

The use of lasers in restorative dentistry has been studied extensively as a substitute for burs during cavity preparation, both as a treatment for dentin hypersensitivity and as a dentin pretreatment before the application of adhesive systems. Investigation of the laser application continues, due to its capacity to seal dentin tubules and form tags that could assist in mechanical adhesion, removal of the smear layer, opening of the dentin tubules or, even in sealing dentin, depending on the energy level used.[5]

The Nd:YAG laser, with a wavelength of 1064 nm, has been reported to be effective on hard dental tissues and offers a significant advantage for clinical use.[6-7] When its pulp effects were proven to be far less aggressive than the effects of the ruby laser,[5] the KTP laser emitting 532 nm and representing a frequency-doubled Nd:YAG device has been introduced primarily for tooth-bleaching procedures in dentistry, and it can be delivered through a wide range of fibers in a constant or pulsed mode.[8-9] This laser has also been used for other dental applications, similar to the Nd:YAG laser, including root canal disinfection, treatment of dentin hypersensitivity and soft tissue surgery.

Chlorhexidine solutions are the most widely used oral antiseptic, based on their low toxicity and high spectrum of antibacterial activity.[10] This solution is the most potent antimicrobial agent used to combat Streptococcus mutans.[11-12] It has been found to be effective in reducing levels of S Mutans found in occlusal fissures and on exposed root surfaces.[13-14] The authors of the current study suggest that the use of a 2% chlorhexidine solution to treat the cavity preparation prior to restoration placement could help to reduce residual caries and post-operative sensitivity. Its application does not impair the sealing ability and bond strength of adhesive materials,[15] although, in specific situations, some studies showed an interference of chlorhexidine in adhesion.[16-17]

Self-etching dentin bonding systems that do not require smear-layer removal by acid etching are being developed to avoid this situation. In theory, these self-etching systems simultaneously decalcify the inorganic component of dentin and infiltrate the collagen fibers at the same time through the action of acidic primers that minimize the potential for voids.[18] The clinical procedure is less complicated and time-consuming, because there is no need for rinsing.[19]

To provide resin-based materials with antibacterial activity, a new monomer, 12-methacryloyloxydodecylpyridiniumbromide (MDPB), has been developed.[20-21] MDPB is a compound of an antibacterial agent quaternary ammonium with a methacryloyl group and it exhibits strong antibacterial activity against oral streptococci.[20-22] The incorporation of MDPB has been reported to be effective in providing dentin bonding systems with antibacterial activity before and after curing.[23-25]

The current study evaluated the influence of KTP laser irradiation, 2% chlorhexidine gluconate and Clearfil Protect Bond (which contains the antibacterial monomer 12-MDPB) on the microleakage of Class V composite restorations

METHODS AND MATERIALS

Thirty freshly-extracted caries-free human premolars were selected. The teeth were cleaned of calculus, soft tissue and other debris. They were then maintained in distilled water until testing. Standard Class V cavity preparations (mesiodistal width of 3 mm, occluso-gingival length of 2 mm and a depth of 2 mm) were prepared on the buccal and lingual surfaces using a high-speed handpiece with air-water spray and a diamond fissure bur (Medin, Viachovicka, Czech Republic). New burs were used after every four preparations. Each preparation was designed with the occlusal margin in enamel and the gingival margin in dentin. No bevels were placed. The teeth were randomly divided into four groups (Table 1) and 15 cavities were assigned to each group.

Group 1—Clearfil SE Bond (Kuraray Co Ltd, Osaka, Japan) two-step self-etch adhesive system was applied

Table 1: *Materials Used and Their Composition*

Material	Batch #	Composition	Manufacturer
Clearfil Protect Bond	41164	Kuraray Medical Inc, Okayama, Japan	Primer: MDP-MDPB HEMA, hydrophilic dimethacrylate, photoinitiator, water bond: 10-MDP, HEMA, colloidal SiO_2, surface treated sodium fluoride crystals, hydrophilic dimethacrylate
Clearfil SE Bond	41502	Kuraray Medical Inc, Okayama, Japan	Primer: MDP, HEMA, hydrophilic dimethacrylate, photoinitiator, water bond: 10-MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, microfiller, photoinitiator
Te-Econom (microhybride composite)	G27861	Ivoclar Vivadent Schaan, Liechenstein	Dimethacrylates Barium glass filler, silanized high-dispersed silica, silanized Mixed oxide, silanized Ytterbiumtrifluoride Catalysts and stabilizers Pigments Filler content by weight 81% Filler content by volume 62%

HEMA = hydroxylethyl methacrylate
MDP = methacryloxydecyl dihydrogen phosphate
MDPB = methacryloyloxydodecylpyridinium bromide

to cavities according to the manufacturer's instructions. Primer was applied for 20 seconds and gently dried. The bonding agent was then applied and light-cured for 10 seconds.

Group 2—The cavities were irradiated at 1 W, 10.7 J/cm² with the KTP laser (Smartlite D, Deka, Calenzano Firenze, Italy) and the laser beam was delivered by 200-µm diameter optical fiber for 60 seconds. The distance between the fiber and specimens was 1 mm with perpendicular position. Then, the Clearfil SE Bond self-etching bonding system, which was similar to Group 1, was applied to cavities according to the manufacturer's instructions.

Group 3—A 2% chlorhexidine gluconate cavity cleanser (Drogsan, Ankara, Turkey) was applied with a mini-brush tip placed for 40 seconds, then dried with absorbent paper. The Clearfil SE Bond self-etching bonding system, which was similar to Group 1, was applied to the cavities according to the manufacturer's instructions after applying chlorhexidine gluconate.

Group 4—Clearfil Protect Bond (Kuraray, Osaka, Japan) self-etching primer was applied to the cavity with a brush and left in place for 20 seconds. After drying the etched surface with mild air flow, the bonding was applied onto the etched-primed dentin, gently air dried and light-cured for 10 seconds.

The operator restored all samples incrementally with shade A2 (TE-Econom, Ivoclar Vivadent) resin-based composite using a visible light-curing unit (Hilux, 40 seconds/1-mm increment, Benlioglu Dental, Ankara, Turkey). The curing light built-in radiometer was used to check for light efficiency before starting each restoration. After immediate finishing and polishing with sequential discs (Sof-Lex Pop-On, 3M ESPE, St Paul, MN, USA), the teeth were stored in 37°C and 100% humidity for 24 hours. The specimens were then thermocycled for 500 cycles with baths conducted in-between (5°C and 55°C), a dwell time of 30 seconds and a transfer time of three seconds. After thermocycling, the apices of the teeth were sealed with sticky wax, and all tooth surfaces except a 1-mm wide zone around the margins of each restoration were sealed with nail polish. To minimize dehydration of the restorations, the teeth were replaced in water as soon as the nail polish dried. The teeth were then immersed in a 0.5% basic fuchsin solution for 24 hours at room temperature.

The specimens were then rinsed in tap water, and each specimen was sliced longitudinally using a low-speed diamond disk (Isomed Buehler, Ltd, Lake Bluff, IL, USA) with water coolant and evaluated for marginal leakage. The primarily stained half of the tooth was used to evaluate the microleakage. The degree of dye penetration was then graded at 30x original magnification with a stereomicroscope (SMZ 800, Nikon, Tokyo, Japan) using the following scale:

- 0 No marginal leakage
- 1 Basic fuchsin penetrates up to the dentinoenamel junction (DEJ) or corresponding length at the dentin wall
- 2 Basic fuchsin penetrates beyond the DEJ or corresponding length at the dentin wall, surpassing half the cavity depth
- 3 Basic fuchsin penetrates beyond half the cavity depth, without reaching the axial wall
- 4 Basic fuchsin penetrates along the axial wall

Table 2: *Distribution of Microleakage Scores Verified at the Enamel and Dentin Margins for All Groups (N=15)*

	Enamel Scores					Dentin Scores				
Groups	0	1	2	3	4	0	1	2	3	4
(G1) Clearfil SE Bond	9	6	0	0	0	4	7	4	0	0
(G2) KTP Laser+Clearfil SE Bond	12	3	0	0	0	7	5	1	1	1
(G3) Chlorhexidine+Clearfil SE Bond	5	8	2	0	0	4	6	2	0	3
(G4) Clearfil Protect Bond	6	7	2	0	0	6	3	4	1	1

Table 3: *Kruskal-Wallis Test Results of Comparison of the Experimental Groups*

Enamel Margins		**Mean Rank Chi-Square**	**Asymp Sig**
Groups		27.70 8.959	0.030
Clearfil SE Bond		22.10	
KTP Laser + Clearfil SE Bond		37.03	
Chlorhexidine + Clearfil SE Bond		35.17	
Clearfil Protect Bond			
Gingival Margins			
Groups	**Total (N)**	**Mean Rank Chi-Square**	**Asymp Sig**
Clearfil SE Bond	15	30.67 1.454	
KTP laser + Clearfil SE Bond	15	26.50	0.693
Chlorhexidine + Clearfil SE Bond	15	33.73	
Clearfil Protect Bond		31.10	

The results of the staining measurements were analyzed using the Kruskal-Wallis and Mann-Whitney U-tests for independent samples and the Wilcoxon test for dependent samples. All the tests were run at a significance level of $p<0.05$.

RESULTS

None of the procedures tested in the current study completely eliminated microleakage. Data showing the extent of leakage scored for the enamel and gingival margins of the restorations are shown in Table 2.

When the scores of microleakage at the gingival margins of the four groups were compared, there were no statistical differences found ($p>0.05$) (Table 3). However, the lowest mean microleakage values were obtained from Group 2 (KTP laser group). The highest values were obtained from Group 3 (chlorhexidine gluconate group). The mean microleakage values of the other two groups, respectively, were from lower to higher for Group 1 (Clearfil SE Bond group) and Group 4 (Clearfil Protect Bond group).

When the scores of microleakage at the enamel margins of the four groups were compared, the differences among the groups were found to be statistically significant ($p<0.05$) (Table 3). The mean microleakage values of the four groups, respectively, from lower to higher, were Group 2 (KTP laser group), Group 1 (Clearfil SE Bond group), Group 4 (Clearfil Protect Bond group) and Group 3 (chlorhexidine gluconate group). The differences between Group 2 (KTP laser group) and Group 3 (chlorhexidine gluconate group), and Group 2 (KTP laser group) and Group 4 (Clearfil Protect Bond group) were found to be statistically significant ($p<0.05$).

Comparing the gingival and enamel margins in each group, statistically significant differences existed in the KTP laser and the Clearfil SE Bond group ($p<0.05$), and no significant differences were exhibited at the other groups ($p>0.05$).

DISCUSSION

Microleakage has been defined by Sidhu and Henderson[26] as "the clinically undetectable passage of bacterial fluids, molecules and/or ions between the cavity wall and the restoration material applied to it." The ability of a composite to minimize the extent of microleakage at the tooth/restoration interface is an important factor in predicting clinical success. Failure of the restoration may contribute to marginal staining, adverse pulpal response, postoperative sensitivity and recurrent caries.[27]

In the current study, basic fuchsin was used to detect microleakage at the gingival and occlusal surface position. Different methods have been employed to disclose microleakage around the restorations. Dye leakage is probably the most common method used. The principal advantages of this technique are its low cost and ease of application. Disadvantages include subjective evaluation of the results[28] and low molecular weight of the dye, which is less than that of bacteria. Also, tests using dyes could sometimes detect leakage where bacteria could not penetrate.[29]

When comparing the gingival and enamel margins of the same pretreatment groups, the scores were higher at the gingival margins compared to the enamel margins for all the groups, and statistically significant differences existed in the KTP laser and Clearfil SE Bond group for the current study. Perhaps the most critical factor affecting the microleakage of resin composites is resin polymerization shrinkage. The forces generated by polymerization shrinkage exceeded the bond strength, creating marginal gaps, especially on the gingival margin. Polymerization shrinkage in this area is not compensated for by acid etching and the application of dentin adhesives. The substrate of the gingival margin can also contribute somewhat to retention with the available materials. Dentin contains a substantial proportion of water and organic materials; it presents a moist surface that impairs the bonding mechanism. There is always an increase in leakage on restoration margins located on dentin/cement.[30]

In the current study, the use of pulsed KTP laser energy showed a decrease in microleakage around the restorations. Obeidi and others[2] stated that the level of microleakage was significantly less in laser-treated cavities compared to non-lased cavities. Also, White and others[31] showed similar results. Goodis and others[6] stated that a significant decrease was reported to be achieved in the intratubular fluid flow due to closure of tubule orifices following melting after Nd:YAG laser irradiation. Miserendino and others[7] reported that a lower dye permeability of dentin is seen when the prepared dentin surface is treated by Nd:YAG laser energy. It seems that the deposition of glass-like material seals dentin walls with partial to total closure of the dentinal tubules.

The results of the current study indicate that using a cavity cleaning solution of 2% chlorhexidine prior to the application of Clearfil SE Bond did not affect the sealing ability of this bonding system. No statistical difference in microleakage was found among all groups on the gingival surface. When the scores of microleakage at the enamel margins of the four groups were compared, the difference between the KTP laser group and the chlorhexidine gluconate group was found to be statistically significant. However, differences between the chlorhexidine gluconate group and the other two groups were not found to be significant.

Analysis of the average amount of microleakage obtained for each group revealed the chlorhexidine group to present higher values at the enamel and gingival margins compared with the other groups. The scanning electron microscopy examination by Meiers and Kresin[32] showed that cavity disinfectants applied to dentin surfaces were resistant to acidic conditioning. This acid-resistant layer might inhibit the ability of hydrophilic resin to impregnate the dentin surface. This may account for the increased microleakage level in 2% chlorhexidine gluconate. In an *in vivo* study by Tulunoglu and others,[17] the investigators found that chlorhexidine cavity disinfectant increased microleakage when used prior to the two adhesive system applications. They reported that there might have been some negative interaction between the cavity disinfectants and dentin bonding agents.[17]

Sung and others[33] evaluated the microleakage of Class V composite restorations after conditioning preparations with various irrigation solutions (tap water, sterile water, sodium chloride solution, filtered water, chlorhexidine, sodium hypochlorite and distilled water). Although they applied the antiseptic solutions after a 37% phosphoric acid etch (total-etch technique), the results were the same as the current study. However, Owens and others[34] and Piva and others[29] stated that chlorhexidine had no adverse effects on microleakage, and the results of these previous studies were the same as the current study, except for a comparison with the KTP laser group at the occlusal surface position.

Self-etch adhesive systems have become increasingly popular in the last decade. The combination of etchant and primer into one system is advantageous in that it reduces application time and technique-related sensitivity.[18] On the other hand, there is an ongoing debate regarding the efficacy of bonding to enamel with self-etch adhesive systems. Some authors support the manufacturers' recommendations that the adjunctive use of phosphoric acid etching is necessary when bonding to uncut enamel,[35] while others argue that the bond strength of self-etch adhesives is equal to the bond strength of total-etch adhesives to unground enamel.[36-37]

Clearfil SE Bond is a mild two-step self-etch adhesive with a pH very close to 2. Mild self-etch adhesives produce a hybrid layer that is thinner than total-etch systems. As dentin demineralization is less pronounced, and smear plus occludes the orifice of the dentinal tubules, which are partially infiltrated by resin, a reduced resin tag formation occurs with these systems.[38-39] Clearfil SE Bond contains the functional monomer 10-methacryloxyloxydecyl dihydrogen phosphate (10-MDP), which has two hydroxyl groups that also may bind to calcium.[40] Moreover, 10-MDP causes minimal dissolution of smear plugs and the limited opening of tubules, thus reducing dentin permeability.[41] The functional monomer 10-MDP also facilitates penetration, impregnation, polymerization and the entanglement of monomers with demineralized dentin to form a relatively thick hybrid layer.[42] Recently, Yoshida and others[43] reported that MDP tightly adheres to hydroxyapatite and that its calcium salt hardly dissolved in water. The investigators suggested that the lower dye penetration observed in the samples bonded with Clearfil SE Bond could be attributed to the difference in chemical composition of the self-etch adhesives.

The bonding system Clearfil Protect Bond is a two-step self-etch adhesive system composed of a self-etching primer containing the antibacterial monomer MDPB and a fluoride-releasing adhesive.[20,44] The antibacterial monomer MDPB is a polymerizable biocide and has strong bactericidal activity against oral bacteria. The antibacterial agent is immobilized in the polymer network by copolymerization of MDPB, and the cured resin-containing MDPB exhibits the inhibition of bacterial growth.[20-21] Therefore, a dentin bonding system incorporating MDPB can show antibacterial effects before and after the curing process.[23,45]

Although Clearfil Protect Bond is derived from Clearfil SE Bond with modifications in the components, mean microleakage values were higher than with Clearfil SE Bond in the current study. However, no statistical difference in microleakage was found among all groups in the gingival surface position and, when the scores of microleakage at the occlusal surface position of the four groups were compared, the difference between only the KTP laser and the Clearfil Protect Bond group was found to be statistically significant. Comparing the gingival and the occlusal surface position in the Clearfil Protect Bond group, no significant difference was exhibited.

The authors stated that Clearfil Protect Bond contains crystal-like structures, and these are likely to be the NaF crystals.[46] Although the filled adhesive resins have been said to have greater mechanical properties, differences in the filler content and composition of the adhesive may account for variations between them.[47] The authors speculate that the non-uniform distribution of the nanometer-sized fillers observed may contribute to a relatively poor mechanical property in some regions.[46] Hence, microleakage values could be higher than Clearfil SE Bond in the current study.

CONCLUSIONS

Within the limitations of the current study, the following conclusions may be drawn:

- none of the procedures tested in this study completely eliminated microleakage.
- all of the tested procedures provided better sealing at the enamel margins than at the dentinal margins.
- KTP laser irradiation prior to the use of Clearfil SE bond following cavity preparation reduced microleakage at the enamel and dentinal margins. Also, the lowest microleakage scores were obtained from the KTP laser group.
- analysis of the average of the amount of microleakage obtained for each group revealed the chlorhexidine gluconate group presented the highest scores at the occlusal and dentinal margins.

(Received 21 July 2008)

References

1. Sturdevant CM (1995) *The Art and Science of Operative Dentistry* 3rd edition Mosby, St Louis.
2. Obeidi A, Ghasemi A, Azima A & Ansari G (2005) Effects of pulsed Nd:YAG laser on microleakage of composite restorations in Class V cavities *Photomedicine and Laser Surgery* **23(1)** 56-59.
3. Boston DW & Graver HT (1989) Histological study of an acid red caries-disclosing dye *Operative Dentistry* **14(4)** 186-192.
4. Leung RL, Loesche WJ & Charbeneau GT (1980) Effect of Dycal on bacteria in deep carious lesions *Journal of the American Dental Association* **100(2)** 193-197.
5. Ribeiro CF, Anido AA, Rauscher FC, Yui KC & Gonçalves SE (2005) Marginal leakage in Class V cavities pretreated with different laser energy densities *Photomedicine and Laser Surgery* **23(3)** 313-316.
6. Goodis HE, White JM, Marshal SJ, Marshal GW & Lee F (1994) Measurement of fluid flow through laser-treated dentine *Archives of Oral Biology* **39(Supplement 1)** 128.
7. Miserendino LJ, Levy GC & Rizoiu LM (1995) Effects of Nd:YAG laser on the permeability of root canal wall dentin *Journal of Endodontics* **21(2)** 83–87.
8. Machida T, Wilder-Smith P, Arrastia AM, Liaw LH & Berns MW (1995) Root canal preparation using the second harmonic KTP:YAG laser: A thermographic and scanning electron microscopic study *Journal of Endodontics* **21** 88-91.
9. Schoop U, Kluger W, Dervisbegovic S, Goharkhay K, Wernisch J, Georgopoulos A, Sperr W & Moritz A (2006) Innovative wavelengths in endodontic treatment *Lasers in Surgery and Medicine* **38(6)** 624-630.
10. Johnson BT (1995) Uses of chlorhexidine in dentistry *General Dentistry* **43(2)** 126-140.
11. Emilson CG (1981) Effect of chlorhexidine gel treatment on Streptococcus mutans population in human saliva and dental plaque *Scandinavian Journal of Dental Research* **89(3)** 239-246.
12. Järvinen H, Pienihäkkinen K, Huovinen P & Tenovuo J (1995) Susceptibility of Streptococcus mutans and Streptococcus sobrinus to antimicrobial agents after short-term oral chlorhexidine treatments *European Journal of Oral Science* **103(1)** 32-35.
13. Fure S & Emilson CG (1990) Effect of chlorhexidine gel treatment supplemented with chlorhexidine varnish and resin on mutans Streptococci and Actinomyces on root surfaces *Caries Research* **24(4)** 242-247.
14. Schaeken MJ, Keltjens HM & Van Der Hoeven JS (1991) Effects of fluoride and chlorhexidine on the microflora of dental root surfaces and progression of root-surface caries *Journal of Dental Research* **70(2)** 150-153.

15. Perdigão J, Denehy GE & Swift EJ (1994) Effects of chlorhexidine on dentin surfaces and shear bond strengths *American Journal of Dentistry* **7(2)** 81-84.
16. Meiers JC & Shook LW (1996) Effect of disinfectants on the bond strength of composite to dentin *American Journal of Dentistry* **9(1)** 11-14.
17. Tulunoglu O, Ayhan H, Olmez A & Bodur H (1998) The effect of cavity disinfectants on microleakage in dentin bonding systems *The Journal of Clinical Pediatric Dentistry* **22(4)** 299-305.
18. Tay FR & Pashley DH (2001) Aggressiveness of contemporary self-etching systems, I: Depth of penetration beyond smear layers *Dental Materials* **17(4)** 296-308.
19. Geraldo-Martins VR, Robles FRP & Matos AB (2007) Chlorhexidine effect on sealing ability of composite restorations after Er:YAG laser cavity preparation *The Journal of Contemporary Dental Practice* **8(5)** 26-32.
20. Imazato S, Torii M, Tsuchitani Y, McCabe JF & Russell RR (1994) Incorporation of bacterial inhibitor into resin composite *Journal of Dental Research* **73(8)** 1437-1443.
21. Imazato S, Russell RR & McCabe JF (1995) Antibacterial activity of MDPB polymer incorporated in dental resin *Journal of Dentistry* **23(3)** 177-181.
22. Imazato S, Ebi N, Tarumi H, Russell RR, Kaneko T & Ebisu S (1999) Bactericidal activity and cytotoxicity of antibacterial monomer MDPB *Biomaterials* **20(9)** 899-903.
23. Imazato S, Kinomoto Y, Tarumi H, Torii M, Russell RR & McCabe JF (1997) Incorporation of antibacterial monomer MDPB into dentin primer *Journal of Dental Research* **76(3)** 768-772.
24. Imazato S, Ehara A, Torii M & Ebisu S (1998) Antibacterial activity of dentine primer containing MDPB after curing *Journal of Dentistry* **26(3)** 267-271.
25. Imazato S, Torii Y, Takatsuka T, Inoue K, Ebi N & Ebisu S (2001) Bactericidal effect of dentin primer containing antibacterial monomer methacryloyloxydodecylpyridinium bromide (MDPB) against bacteria in human carious dentin *Journal of Oral Rehabilitation* **28(4)** 314-319.
26. Sidhu SK & Henderson LJ (1992) Dentin adhesives and microleakage in cervical resin composites *American Journal of Dentistry* **5(5)** 240-244.
27. Kidd EAM (1976) Microleakage: A review *Journal of Dentistry* **4(5)** 199–206.
28. Alani AH & Toh CG (1997) Detection of microleakage around dental restorations: A review *Operative Dentistry* **22(4)** 173-185.
29. Piva E, Martos J & Demarco FF (2001) Microleakage in amalgam restorations: Influence of cavity cleanser solutions and anticariogenic agents *Operative Dentistry* **26(4)** 383-388.
30. Araujo RM, Eduardo CP, Duarte Junior SL, Araujo MA & Loffredo LC (2001) Microleakage and nanoleakage: Influence of laser in cavity preparation and dentin pretreatment *Journal of Clinical Laser Medicine and Surgery* **19(6)** 325-332.
31. White JM, Goodis HE, Setcos JC, Eakle S, Hulscher BE & Rose CL (1993) Effects of pulsed Nd:YAG laser energy on human teeth: A three-year follow-up study *Journal of the American Dental Association* **124(7)** 45–51.
32. Meiers JC & Kresin JC (1996) Cavity disinfectants and dentin bonding *Operative Dentistry* **21(4)** 153-159.
33. Sung EC, Chan SM, Tai ET & Caputo AA (2004) Effects of various irrigation solutions on microleakage of Class V composite restorations *The Journal of Prosthetic Dentistry* **91(3)** 265-267.
34. Owens BM, Lim DY & Arheart KL (2003) The effect of antimicrobial pre-treatments on the performance of resin composite restorations *Operative Dentistry* **28(6)** 716-722.
35. Perdigão J & Geraldeli S (2003) Bonding characteristics of self-etching adhesives to intact versus prepared enamel *Journal of Esthetic and Restorative Dentistry* **15(1)** 32-41.
36. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P & Vanherle G (2003) Buonocore Memorial Lecture. Adhesion to enamel and dentin: Current status and future challenges *Operative Dentistry* **28(3)** 215-235.
37. Tay FR, Pashley DH, King NM, Carvalho RM, Tsai J, Lai SC & Marquezini L Jr (2004) Aggressiveness of self-etch adhesives on unground enamel *Operative Dentistry* **29(3)** 309-316.
38. Hannig M, Reinhardt KJ & Bott B (1999) Self-etching primer vs phosphoric acid: An alternative concept for composite-to-enamel bonding *Operative Dentistry* **24(3)** 172-180.
39. Prati C, Chersoni S, Mongiorgi R & Pashley DH (1998) Resin-infiltrated dentin layer formation of new bonding systems *Operative Dentistry* **23(4)** 185-194.
40. Kubo S, Yokota H, Sata Y & Hayashi Y (2001) Microleakage of self-etching primers after thermal and flexural load cycling *American Journal of Dentistry* **14(3)** 163-169.
41. Dunn WJ & Söderholm KJ (2001) Comparison of shear and flexural bond strength tests versus failure modes of dentin bonding systems *American Journal of Dentistry* **14(5)** 297-303.
42. Gordan VV, Vargas MA, Cobb DS & Denehy GE (1998) Evaluation of acidic primers in microleakage of Class V composite resin restorations *Operative Dentistry* **23(5)** 244-249.
43. Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, Inoue S, Tagawa Y, Suzuki K, De Munck J & Van Meerbeek B (2004) Comparative study on adhesive performance of functional monomers *Journal of Dental Research* **83(6)** 454-458.
44. Imazato S & McCabe JF (1994) Influence of incorporation of antibacterial monomer on curing behavior of a dental composite *Journal of Dental Research* **73(10)** 1641-1645.
45. Imazato S, Imai T, Russell RRB, Torii M & Ebisu S (1998) Antibacterial activity of cured dental resin incorporating the antibacterial monomer MDPB and an adhesion-promoting monomer *Journal of Biomedical Materials Research* **39(4)** 511-515.
46. Sidhu SK, Omata Y, Tanaka T, Koshiro K, Spreafico D, Semeraro S, Mezzanzanica D & Sano H (2007) Bonding characteristics of newly developed all-in-one adhesives *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **80(2)** 297-303.
47. Takahashi A, Sato Y, Uno S, Pereira PNR & Sano H (2002) Effects of mechanical properties of adhesive resins on bond strength to dentin *Dental Materials* **18(3)** 263–268.