***Received:* 28th January 2015 *Accepted:* 20th April 2015 *Conflicts of Interest:* None**

***Source of Support:* Nil**

Original Research

Microleakage in Resin Composite Restoration following Antimicrobial Pre-treatments with 2% Chlorhexidine and Clearfil Protect Bond

Hisham Hameed[1], Biju P Babu[2], V M Mohammed Sagir[3], Kennet J Chiriyath[2], Jones Mathias[4], A P Shaji[5]

Contributors:
[1]Senior Lecturer, Department of Conservative Dentistry, Royal Dental College, Chalissery, Palakkad, Kerala, India; [2]Professor, Department of Conservative Dentistry, Royal Dental College, Chalissery, Palakkad, Kerala, India; [3]Professor and Head, Department of Conservative Dentistry, Royal Dental College, Chalissery, Palakkad, Kerala, India; [4]Reader, Department of Conservative Dentistry, Royal Dental College, Chalissery, Palakkad, Kerala, India; [5]Reader, Department of Orthodontics, Royal Dental College, Chalissery, Palakkad, Kerala, India.

Correspondence:
Dr. Hameed H, Department of Conservative Dentistry, Royal Dental College, Chalissery, Palakkad, Kerala, India. Phone: +91-9605515544. Email: hameedhishamn@gmail.com

How to cite the article:
Hameed H, Babu BP, Sagir VM, Chiriyath KJ, Mathias J, Shaji AP. Microleakage in resin composite restoration following antimicrobial pre-treatments with 2% chlorhexidine and clearfil protect bond. J Int Oral Health 2015;7(7):71-76.

Abstract:
Aim: To evaluate microleakage in resin composite restorations after antimicrobial pre – treatments

Materials and Methods: Forty freshly extracted non carious human premolars were procured. In all forty premolar specimens, class V preparation of standard dimension were prepared and were randomly divided into three experimental and one control group. In all control and experimental groups the class V preparations were restored with FILTEK Z350 composite restorative material. The experimental groups included different self etching primers and 2% Chlorhexidine gluconate. The control group included Xeno III and no antimicrobial pre-treatment was done for the control group. Thereafter these specimens were thermocycled, dried and sealed with nail varnish, leaving 1mm around the restoration and immersed in 0.5% basic fuchsin for 24 hours and then the specimens were subjected for microleakage evaluation. The results were statistically analyzed by Kruskal Wallis Test and Mann Whitney 'U' test.

Results: Results indicate that group II (2% chlorhexidine gluconate group) had the minimum mean value (15.05) and group III(Clearfil protect Bond group) and IV(control group) had the maximum mean microleakage at the enamel margin (23.00). At the gingival margin the lowest mean microleakage values were obtained with group I (Clearfil SE bond group) and group II (2% chlorhexidine gluconate) (20.25) and highest with group III and group IV (20.85). The difference was not statistically significant both at the enamel margin and the dentin margin (p>0.05).

Interpretation & Conclusions: Within the limitations of this in-vitro study, we conclude that:

1. None of the materials tested in this study completely eliminated microleakage at the enamel and at the gingival margin.
2. All of the tested materials provided better sealing at the enamel margin than at the gingival margin.

Key Words: Antimicrobial pre-treatment, class V cavity, microleakage, self-etching primers

Introduction

Dental caries is one of the most common problems in dentistry today. In addition, recurrent or secondary dental 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 for recurrent or secondary dental caries, pulpal inflammation and necrosis.

Success in conservative dentistry depends on total removal of the infected dentin and achievement of a good seal; however, contemporary procedures for treating dental caries do not always eliminate all cariogenic microorganisms in prepared cavities. Other studies have shown that microorganisms left in the prepared cavity could survive for a long period of time and this problem may be magnified by microleakage of composite resin at margins not ending on enamel. To solve this problem, the use of an antimicrobial solution has been suggested.[5]

Chlorhexidine solutions has been found to be effective in reducing levels of *Streptococcus mutans* found in occlusal fissures and on exposed root surfaces. 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, although, in specific situations, some studies showed an interference of chlorhexidine in adhesion.[6]

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. The clinical procedure is less complicated and time-consuming, because there is no need for rinsing.[6]

Different from etch-and-rinse adhesives, self-etch adhesives do not require a separate etching step, as they contain acidic monomers that simultaneously 'condition' and 'prime' the dental substrate. Consequently, this approach has been claimed to be user-friendlier (shorter application time, less steps) and less technique-sensitive (no wet-bonding, simple drying), thereby resulting in a reliable clinical performance, though this appeared very product-dependent.[2]

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

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

Materials and Methods

40 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. 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 three experimental and one control group, each group containing 10 samples.

Study materials

Product/manufacturer	Type/function	Composition
CPB (Kuraray Medical Inc., Japan)	Self-etching primer	Primer: MDP-MDPB HEMA, (10- methacryloyloxydecyldihydrogen phosphate-12- MDPB HEMA) hydrophilicdimethacrylate, photoinitiator, Water bond: 10-MDP, HEMA, colloidal SiO_2, surface treated sodium fluoride crystals, hydrophilic dimethacrylate
Clearfil SE bond (Kuraray Medical Inc., Japan)	Self-etching primer	Primer: MDP, HEMA, hydrophilic dimethacrylate, photoinitiator, Water bond: 10-MDP, Bis-GMA, HEMA, hydrophilic dimethacrylate, microfiller, photoinitiator
Xeno III (dentsply)	Self-etching primer	Liquid A2-HEMA purified water ethanol BHT highly dispersed silicon dioxide Liquid B Phosphoric acid modified methacrylate (pyro-EMA) Mono fluoro phosphazene modified methacrylate Urethane dimethacrylate BHT Camphorquinone Ethyl-4-dimethylaminobenzoate
Filtek™ Z350 (3M)	Nanotechnology light cure composite	BIS-GMA, BIS-EMA, UDMA with small amounts of TEGDMA. Filler contains a combination of a non-agglomerated/non-aggregated, 20 nm nanosilica filler, and loosely bound agglomerated zirconia/silica nanocluster, consisting of agglomerates of primary Zirconia/silica particles with size of 5-20 nm fillers. The cluster particle size range is 0.6-1.4 μ. The filler loading is 78.5% by weight
2% chlorhexidine gluconate	Antimicrobial agent	
Spectrum® 800 (Dentsply)	Light curing device	
0.5% basic fuchsin dye	For dye penetration test	
Stereomicroscope	To view the specimens	

HEMA: Hydroxyethyl methacrylate, MDPB: Methacryloyloxydodecylpyridinium bromide, BHT: Butylated hydroxy toluene, 10-MDP: 10-methacryloxyloxydecyl dihydrogen phosphate

Group I

Each cavity was washed, dried but not desiccated. Clearfil SE bond (Kuraray Co. Ltd., Japan) two step self-etch adhesive system was applied on cavities according to the manufacturer's instructions. Primer was applied for 20 s and gently air dried. The bonding agent was then applied and light cured for 10 s.

Group II

Each cavity was washed, dried but not desiccated. 2% chlorhexidine gluconate cavity cleanser was applied with a small brush tip, placed for 40 s and then dried with absorbent paper. Clearfil SE bond (Kuraray Co. Ltd., Japan) two step self etch adhesive system similar to Group I was applied on cavities according to the manufacturer's instructions after applying 2% chlorhexidine gluconate.

Group III

Each cavity was washed, dried but not desiccated. CPB (Kuraray Co. Ltd., Japan) self-etching primer was applied to the cavity with a brush and left in place for 20 s. After drying the etched surface with mild air flow, the bonding agent was applied on to the etched primed dentin, gently air dried and light cured for 10 s.

Group IV

Each cavity was washed, dried but not desiccated. The self-etching adhesive (Xeno III) was applied to the cavity preparation with an applicator tip, left undisturbed for 20 s and the excess solvent was removed with a gentle stream of air. Light curing was done for 20 s with a visible light curing unit.

The cavity was restored incrementally with nano composite Filtek Z350 using an oblique incremental insertion technique and each increment was light cured for 40 s respectively. Finishing and polishing of the restorations was carried out with tungsten carbide finishing bur and a series of polishing discs (3M Sof-Lex).

The teeth were stored in 37°C and 100% humidity for 24 h. The specimens were then thermocycled for 500 cycles with baths conducted in-between (5°C and 55°C), a dwell time of 30 s and a transfer time of 3 s. 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 h 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 ×30 original magnifi cation with a stereomicroscope (SMZ 800, Nikon, Tokyo, Japan) using the following scale according to criteria given by Siso *et al*. (2009)[6] (Table 1).

- 0 - No marginal leakage
- 1 - Basic fuchsin penetrates up to the dentinoenamnel 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.

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

According to the result analysis

- None of the procedures tested in the current study completely eliminated microleakage
- The scores of microleakage at the enamel margin (occlusal) of the four groups were compared, and no statistical significant differences were found ($P > 0.05$)
- The lowest mean microleakage values were obtained from Group II (clearfil SE bond + 2% chlorhexidine gluconate group) at the enamel margin (Figure 1b)
- The highest values were obtained with Group III (CPB group) and Group IV (control group) at the enamel margin (Figure 1c)
- When the scores of microleakage at gingival margin of the four groups were compared, there was no statistical significance ($P > 0.05$)
- The lowest mean microleakage values were obtained with Group I (clearfil SE bond group) (Figure 1a) followed by Group II (clearfil SE bond + 2% chlorhexidine gluconate group) at the gingival margin
- The highest values were obtained from Group III (CPB group) and Group IV (control group) (Figure 1d) at the gingival margin

Table 1: Frequency of number of specimens in each microleakage scoring category.

Groups	Enamel scores					Dentin scores				
	0	1	2	3	4	0	1	2	3	4
Clearfil SE bond	3	5	0	1	1	3	0	3	2	2
Clearfil SE bond+2% chlorhexidine gluconate	3	5	0	2	0	1	4	0	3	2
Clearfil protect bond	4	3	0	2	1	3	2	2	1	2
Xeno III	1	3	3	1	2	1	3	1	2	3

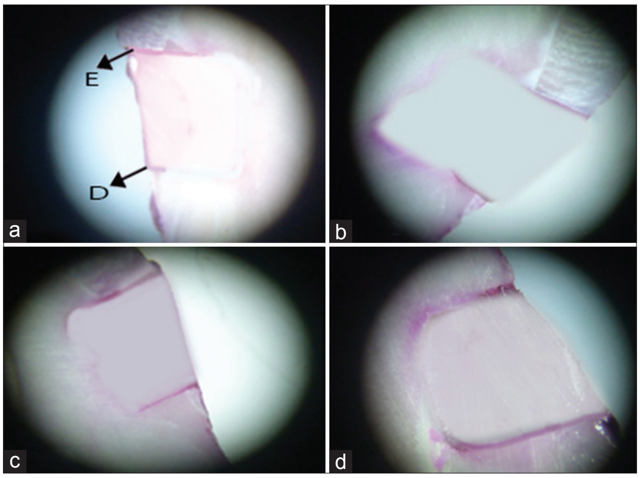


Figure 1: (a) Stereomicroscope photograph of Group I (clearfil SE bond), (b) stereomicroscope photograph of Group II (clearfil SE bond + chlorhexidine), (c) stereomicroscope photograph of Group III (clearfil protect bond), (d) stereomicroscope photograph of Group IV (control group), E - Enamel margin, D - Dentinal margin.

- Inter-group comparison of mean microleakage between all experimental Groups (I, II, III) with Group IV (control group) using "Mann–Whitney U" test was done both at enamel margin and gingival margin
- When mean microleakage values of Group I (clearfil SE bond group) were compared with Group IV (control group) at the enamel margin, no statistical difference was found
- Group II (clearfil SE bond + 2% chlorhexidine gluconate group) had the lowest mean microleakage values at the enamel margin, but the mean values were not statistically significant when compared with Group IV (control group) ($P > 0.05$)
- Group III (CPB group) and Group IV (control group) had the same mean microleakage values at the enamel margin (Graph 1), which were not statistically significant ($P > 0.05$) when compared
- For Group I (clearfil SE bond group) and Group II (clearfil SE bond + 2% chlorhexidine gluconate group) the lowest mean microleakage were obtained at the gingival margin but no statistical significant difference ($P > 0.05$) was found when each group was compared with Group IV (control group)
- Group III (CPB group) had the highest mean microleakage at the gingival margin but was same as that of Group IV (control group)

- When the mean microleakage of Group III (CPB group) was compared with Group IV (control group) there was no statistical significant difference ($P > 0.05$).

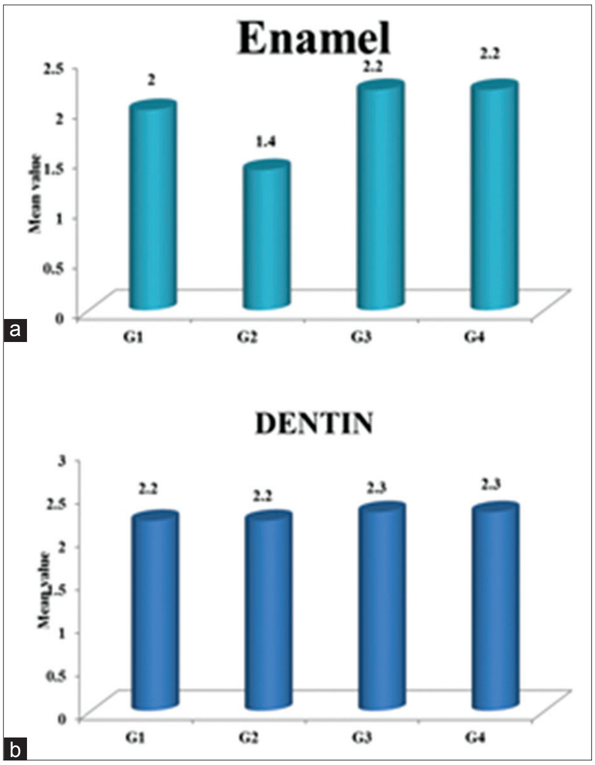
Stereo - microscopic analysis was done to analyze the degree of dye penetration.

Statistical analysis was done using by Kruskal–Wallis test (Table 2) and and Mann–Whitney *U*-test.

Discussion

Microleakage has been defined by Sidhu and Henderson as "the clinically undetectable passage of bacterial fluids, molecules and/or ions between the cavity wall and the restoration material applied to it."[9]

Microleakage at the tooth restoration interface especially with resin restoration is considered, to be a priority issue influencing the retention of dental restorations with no clinical problems. Cavosurface margin of restorations, leak more which may lead to recurrent caries at the tooth restoration interface, post-operative sensitivity and pulpal pathosis.



Graph 1: (a) Scoring of microleakage at enamel margin, (b) scoring of microleakage at dentine margin.

Table 2: Group comparison using Kruskal–Wallis test.			
Ranks			
Class	**Group**	***N***	**Mean rank**
Enamel	1.00	10	20.95
	2.00	10	15.05
	3.00	10	23.00
	4.00	10	23.00
	Total	40	
Dentin	1.00	10	20.05
	2.00	10	20.25
	3.00	10	20.85
	4.00	10	20.85

The ability of a composite to minimize the extent of microleakage at the tooth/restoration interface is an important factor in predicting clinical success.

In the current study, basic fuchsin was used to detect leakage at the gingival and occlusal surface. 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 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.

Researchers have adopted several methods for estimation of dye leakage under *in-vitro* condition. The available *in-vitro* studies include the use of dyes, chemical tracers, radioactive isotopes, air pressure, bacteria, neutron activation analysis, scanning electron microscopy, artificial caries techniques and electrical conductivity.

The use of organic dye as tracers is one of the oldest and most common methods of detecting leakage *in-vitro*. The review of published literature demonstrates that there have been wide variations in choice of dye used either as solutions or particle suspension of different particle size. The concentration of dyes used also ranges between 0.5% and 10% while the time of immersion of specimen in dye varied between 4 h and 72 h or more.[8]

Christen and Mitchell (1966) found that different concentration of dyes can vary in penetration time between 5 min and over 1 h. Most researchers have used 0.5% basic fuchsin as a standard dye in all *in-vitro* leakage studies.[10]

In the present study, we have utilized 0.5% basic fuchsin and the time of immersion of specimen in the dye was 24 h. This included three experimental groups and one control group. In all the three experimental groups self-etching primers were used as preliminary step while in Group II 2% chlorhexidine gluconate was used before application of the self-etching primer. However in Group III CPB which is supposed to have antiseptic properties was utilized, while in the control group however conventional self-etching primer was used. Filtek Z350 composite resin was utilized according to the manufacturer's instructions for the restoration of the cavities in all the groups.

Results of the present study indicated that the scores of microleakage of the four groups at the enamel margin when compared, there was no statistical significant difference found ($P > 0.05$).

The lowest mean microleakage values at the enamel margin were obtained from Group II when 2% chlorhexidine gluconate was used as a coating.

The highest mean microleakage was obtained from Group III at enamel margin where CPB was used and Group IV the control group.

When the scores at gingival margin of four groups were compared there was no statistical significance ($P > 0.05$).

However the lowest mean values at gingival margin were obtained from Group I (clearfil SE bond) followed by Group II (clearfil SE bond + 2% chlorhexidine gluconate).

The highest mean value were obtained at gingival margin from Group III (CPB) and Group IV (control).

When comparing the enamel and gingival margin in the entire three experimental groups, microleakage was higher in the gingival margin compared to the enamel margin. However there was no statistical significant difference between the groups.

Alani and Toh (1997) observed that dentin permeability is also an important factor to be considered.[8]

In the present study both enamel and gingival margin were studied for microleakage and found to be higher in the gingival margin when compared to enamel margin.

Shafie *et al.* (2010)[3] observed that chlorhexidine gluconate 2% is a well-known disinfectant and has antimicrobial effect when applied prior to placing a restoration. 2% chlorhexidine gluconate functions as a matrix metalloproteinase inhibitor apart from its antibacterial property, which may also prevent collagen degradation and disintegration of the bonding interface over a period of time.

Siso *et al.* (2009) observed in their studies on microleakage in resin composite restorations that the most critical factor affecting the microleakage of resin composite is the polymerization shrinkage. The forces generated by polymerization shrinkage exceeded the bond strength especially in the gingival margin. Polymerization shrinkage in this area is not compensated for by acid etching and the application of dentin adhesive.[6]

Clearfil SE bond is one of the first self-etching primer in the market and belong to the mild group of self-etch adhesive with a hydrogen ion concentration very close to two Clearfil SE bond contains functional monomer 10-methacryloxyloxydecyl dihydrogen phosphate (10-MDP), which has two hydroxyl groups that also may bind to calcium. Moreover, 10-MDP causes minimal dissolution of smear plugs and the limited opening of the tubules, thus reducing dentin permeability. 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.[7]

Others have reported that MDP tightly adheres to hydroxyapatite and that its calcium salt hardly dissolved in water.

Same mean microleakage values were obtained with application of 2% chlorhexidine gluconate as a pre application. With the CPB application almost similar microleakage values were obtained without application of 2% chlorhexidine gluconate and there were no statistical significant difference in leakage values among the experimental group. In the control group Xeno III also similar results were obtained without the application of 2% chlorhexidine gluconate on the dentin margin. Xeno III is again a self etching primer with a two bottle system used as a control.

CM Esteves *et al*(2010)[4], evaluated the antibacterial properties of some self etching/primer solutions against oral streptococci. They concluded that the self etching adhesives or self etching primers used in the current study demonstrated different levels of inhibition for oral streptococci tested. Clearfil Protect Bond self etching primer exhibited the most effective antibacterial activity against oral streptococci.

CPB is a two-step self etch adhesive system composed of a self etching primer containing the antibacterial monomer 12- MDPB and a fluoride releasing adhesive. The antibacterial monomer 12- MDPB is a polymerizable biocide and has a strong bactericidal activity against oral bacteria. Although CPB is derived from clearfil SE bond with modifications in the components mean microleakage values were higher than clearfil SE bond in the current study. This is in agreement with the studies of Siso *et al.* (2009)[6] however, no statistical difference in microleakage were found among all the groups in gingival surface and when the scores of microleakage at the occlusal surface position of the four groups were compared.

CPB contains crystal-like structures, and these are likely to the NaF crystals. 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. The non-uniform distribution of the nanometer-sized fillers observed may contribute to a relatively poor mechanical property in some regions.[6] Hence, microleakage values could be higher than clearfil SE bond in the current study.

Conclusion

Results of the study indicated that the score of microleakage of the four groups at the enamel margin and gingival margin when compared did not have statistical significance ($P > 0.05$).

Under the conditions of this *in-vitro* study the following assessments were made:

- None of the materials tested in this study completely eliminated microleakage at the enamel and at the gingival margin
- All of the tested materials provided better sealing at the enamel margin than at the gingival margin
- The lowest mean microleakage were obtained from Group II (clearfil SE bond +2% chlorhexidine gluconate) followed by Group I (clearfil SE bond) at the enamel
- Group I (clearfil SE bond group) had the lowest mean microleakage values which were same for Group II (clearfil SE bond + 2% chlorhexidine gluconate group) at the gingival margin.

References

1. Ferracane JL. Resin composite – State of the art. Dent Mater 2011;27(1):29-38.
2. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater 2011;27(1):17-28.
3. Shafie F, Doozandeh M, Alavi A. Effect of resin coating and chlorhexidine on microleakage of two resin cements after storage. J Dent (Tehran) 2010;7(1):31-40.
4. Esteves CM, Ota-Tsuzuki C, Reis AF, Rodrigues JA. Antibacterial activity of various self-etching adhesive systems against oral streptococci. Oper Dent 2010;35(4):448-53.
5. Darabi F, Eftekhari M. Effect of chlorhexidine on microleakage of composite restorations. J Dent 2009;6(1):16-22.
6. Siso HS, Kustarci A, Göktolga EG. Microleakage in resin composite restorations after antimicrobial pre-treatments: Effect of KTP laser, chlorhexidine gluconate and clearfil protect bond. Oper Dent 2009;34(3):321-7.
7. Gordan VV, Vargas MA, Cobb DS, Denehy GE. Evaluation of acidic primers in microleakage of Class 5 composite resin restorations. Oper Dent 1998;23(5):244-9.
8. Alani AH, Toh CG. Detection of microleakage around dental restorations: A review. Oper Dent 1997;22(4):173-85.
9. Kidd EA. Microleakage: A review. J Dent 1976;4(5):199-206.
10. Christen AG, Mitchell DF. A fluorescent dye method for demonstrating leakage around dental restorations. J Dent Res 1966;45(5):1485-92.