ORIGINAL ARTICLE

Microleakage Evaluation in Teeth Restored with Experimental Dental Nanocomposite

Rana K. Al-Bayati[1], Gururajaprasad Kaggal Lakshmana Rao[1], Dasmawati Mohamad[2], Azman Seeni[3], Norehan Mokhtar[1]

[1] Craniofacial and Biomaterial Science Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam 13200, Pulau Pinang, Malaysia
[2] School of Dental Sciences, Universiti Sains Malaysia, Kubang Kerian 16150, Kelantan, Malaysia
[3] Integrative Medicine Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam 13200, Pulau Pinang, Malaysia

ABSTRACT

Introduction: The aim of this study was to evaluate the experimental nanocomposite (Kelfil) against microleakage when bonded with two different types of bonding systems. **Methods:** Sixty sound extracted human permanent incisors were divided into six groups randomly. Each tooth was prepared with standardised Class III cavity on each proximal surface. The teeth were immersed in Rhodamin B dye for ten hours and sectioned to analyse the depth of dye penetration. The depth of the dye penetration was measured in micrometres by using confocal laser scanning microscope (CLSM). **Results:** The depth of the dye penetration in the groups was compared by ANOVA test. The results showed comparable microleakage between different types of composites. **Conclusion:** The experimental nanocomposite (Kelfil) in comparison to the commercially available nanocomposite and micro-hybrid composite has comparable microleakage when bonded using self-etching and total-etched adhesive systems.

Keywords: Adhesive system, Microleakage, Nanocomposite, Polymerisation shrinkage, Scanning electron microscope

Corresponding Author:
Norehan Mokhtar, MOrth RCS
Email: norehanmokhtar@usm.my
Tel: +604-5622003

INTRODUCTION

Aesthetic dental fillings have become a routine dental procedure as the demand for tooth coloured dental fillings is on the rise (1). The patient awareness of the availability of aesthetic tooth coloured dental fillings has led to the development of various tooth coloured materials such as composite restorative materials and glass ionomer cement (2). The growing patient demand for cosmetic dental care and procedures has provided the dental community with an impetus to innovate and develop new materials with properties of improved aesthetics and better physical properties. To satisfy the growing aesthetic concerns of the patients, research and development of aesthetically sound and pleasing restorative materials is a solution which is being explored. The science of nanotechnology has led to a significant improvement in the evolution of dental composites (3). Composite restorations have become the most widely used dental materials for aesthetic dental restorations. The advantages of better aesthetics, smoother surface finishes and higher translucency and polishability in comparison to other restorative materials have made composites the material of choice. However, dental composites exhibit certain limitations in their physical properties such as wear resistance and microleakage as seen in Ceram X Mono (Dentsply Caulk, Milford, DE, USA) methacrylate-based nanohybrid composite and Filtek P6O (3M ESPE, St Paul, Min, USA) methacrylate-based microhybrid composite (4). In order to overcome the limitations of current dental composites, an experimental nanocomposite has been developed by the School of Dental Sciences, Universiti Sains Malaysia. The nanosilica fillers were synthesised using a sol-gel process. The filler particle size ranged from 10-20 nm. The newly developed nanocomposite Kelfil aims to reduce the polymerisation shrinkage thereby enhancing the physical properties of the material. In this study, the experimental nanocomposite Kelfil will be used as a direct composite filling material on 40 Class III cavities prepared on 20 extracted teeth in comparison to two other commercially available composites (Filtek Z350 and Spectrum TPH3). The restored teeth will be assessed for microleakage by measuring the depth of dye penetration in micrometres (μm) on the incisal and cervical walls of sectioned teeth. The results highlight our newly developed Kelfil's property of microleakage resistance to be in par with the commercially available nanocomposites.

MATERIALS AND METHODS

The *in-vitro* study was approved by the Research Ethics Committee (Human) of Universiti Sains Malaysia, USMKK/PPP/JEPeM [235.3. (01)]. The samples for the study were collected from the dental clinic, Advanced Medical and Dental Institute, Universiti Sains Malaysia. The sample size was determined using the PS software which compared two means (5). A total of 60 extracted sound human permanent incisor teeth were selected for this study. Anticipating a five per cent tooth breakage during sectioning, the sample size was decided as 20 cavities per group summing up to 120 cavities for all six groups (Table I).

Table I: Summary of Experimental Groups, Adhesive Systems and Nanocomposite used

Group	Number of teeth	Number of cavities	Adhesive system	Nanocomposite
A1	10	20	G-Bond (GC, Japan) One step self-etching	Kelfil
A2	10	20	Adper Single Bond 2 (3M ESPE, USA) Two step etch and prime	Kelfil
B1	10	20	G-Bond (GC, Japan) One step self-etching	Filtek Z350 (3M ESPE, USA)
B2	10	20	Adper Single Bond 2 (3M ESPE, USA) Two step etch and prime	Filtek Z350 (3M ESPE, USA)
C1	10	20	G-Bond (GC, Japan) One step self-etching	Spectrum TPH3 (DENTSPLY, De-trey, Germany)
C2	10	20	Adper Single Bond 2 (3M ESPE, USA) Two step etch and prime	Spectrum TPH3 (DENTSPLY, De-trey, Germany)

Prior to sample collection, suitable written consent was taken from all the patients. The sample selection followed an inclusion and exclusion criteria.

Inclusion criteria: Sound human permanent incisors which were periodontally compromised and otherwise healthy were chosen for the study.
Exclusion criteria: Permanent incisors with fractured crown, permanent incisors with caries, permanent incisors with existing restorations and permanent incisors subjected to bleaching were excluded from the study.

Following extraction, the extracted teeth were placed and stored in a disposable plastic container containing normal saline. Once the entire sample was collected, the teeth were cleaned to remove calculus and any tissue remnants using a scaler (Cavitron). A Class III restoration cavity was prepared on all the teeth. The Class III restoration cavity preparation included both the mesial and distal proximal surfaces of the teeth prepared using a No. 2 sized round bur on the water-cooled high-speed handpiece. The prepared shape of the cavity was rectangular with a mesiodistal width of 1.5-2.5mm, incisogingival height of 2.5-3mm with a depth of 1.5mm in enamel (6).

The next step was to randomise the teeth into three main groups. The three main groups were divided according to the different nanocomposite they received as a filling material. The groups were randomised using randomiser software (7). Each of the three main groups contained 20 extracted teeth with 40 prepared cavities in total. All the teeth were restored according to the assigned protocol as described in Table 1. The composite was light-cured using Elipar (Free light 2 LED, 3M ESPE) unit for the duration of 40 seconds through the celluloid matrix. The tip of the curing light unit was maintained at 1 – 2 mm distance from the composite surface. The restored teeth were then immersed in artificial saliva (Biotene mouthwash, Laclede, USA) for a period of two weeks at room temperature to simulate oral conditions. The teeth were placed in separate bottles containing 100 ml of artificial saliva per bottle. Each bottle consisted of 10 teeth. After two weeks of immersion, the teeth were removed from the artificial saliva and rinsed with water and air dried. To prevent ingression of dye material into the root canals, sticky wax (Kemdent, UK) was placed at the root apices of the prepared teeth. Further dye penetration into the dentinal tubules and the lateral canals was prevented with the application of two layers of nail varnish over the teeth excluding an area of roughly 1mm around the gingival and incisal margins of the restorations. The teeth were then left to air dry for a period of 12 hours. After 12 hours of air drying, the teeth were placed in 0.5 per cent of Rhodamin dye solution. The dye was buffered to pH 7 for 10 hours. Following dye immersion, the teeth were rinsed under water and air dried. Once dry, acetone solution was used to remove any remnants of the nail varnish. The teeth were once again rinsed thoroughly under tap water before sectioning. The next step was sectioning of the teeth. To analyse the extent of the leakage in both the prepared restoration walls, all the teeth were sectioned longitudinally in a mesiodistal direction to achieve two sections. A water-cooled diamond disc on a microtome (Exakt hard material cutter, Germany) was used for sectioning teeth. The specimens were then placed on the glass slide and fixed by moulding plastic, ready for inspection. The specimen was inspected on a CLSM (Leica Microsystem, Heidel Gmbh, Germany) at low magnification (10 x objective) The CLSM image of the dye penetration into the tooth structure is shown in Figure 2. The above procedures were followed for all 60 teeth with each sample assessed for microleakage. The assessment involved a measurement of the depth of dye penetration on the images from the CLSM in micrometre (µm) on both the incisal and cervical walls using screen calipers. The images with measurements were later saved on a compact disc for future retrieval and analysis.

RESULTS

A total of 60 teeth (120 cavities) were prepared in this study with 10 teeth (20 cavities) randomly assigned to each group. Each tooth was prepared with a Class III cavity on both proximal surfaces and restored. However, a total of 5 per cent of the teeth were damaged during sectioning. Due to the limitation of time, no new samples were chosen. Out of 120 cavities, only 82 cavities were evaluated with 12 cavities in group A1 and C2, 13 cavities in group A2, 14 cavities in group B1 and C1, and 17 cavities in group B2. The SEM images of the sectioned teeth reveal the extent of dye penetration providing an understanding of microleakage and the resistance offered by the newly developed nanocomposite (Figure 1). A corresponding CLSM image showing the dye penetration has been shown in Figure 2.

The different groups were examined and statistically analysed. The statistical analysis using One-way ANOVA test indicated no difference in the depth of microleakage between the three composites (Kelfil, Filtek Z350 and Spectrum TPH3) when bonded with self-etching adhesive system G Bond (GC, Japan) and with etch and prime adhesive system Adper Single Bond 2 (3M, ESPE USA) on both incisal (Table II and Table III) and cervical walls (Table IV and Table V). The results highlighted no significant microleakage amongst the tested composites ($P= 0.792$ and $P=0.521$ for the incisal and cervical area). However, there was an inadvertent big standard deviation (SD) which has occurred due to the variability of sample size. The sample size was 120 but we could only manage to evaluate 82 samples, so this has affected the SD value as the decrease in sample size increases the variability. This fluctuation of the data resulted in a higher SD.

DISCUSSION

This study was conducted to analyse the effect of microleakage on different composites bonded with two types of adhesive systems. The comparison of the three different composites bonded with G-Bond one-step self-etching adhesive system (GC, Japan) revealed that there was no significant difference in the depth of microleakage on both incisal and cervical walls with a similar result found in another study (8). The study design only compared the incisal and cervical areas separately and not against each other.

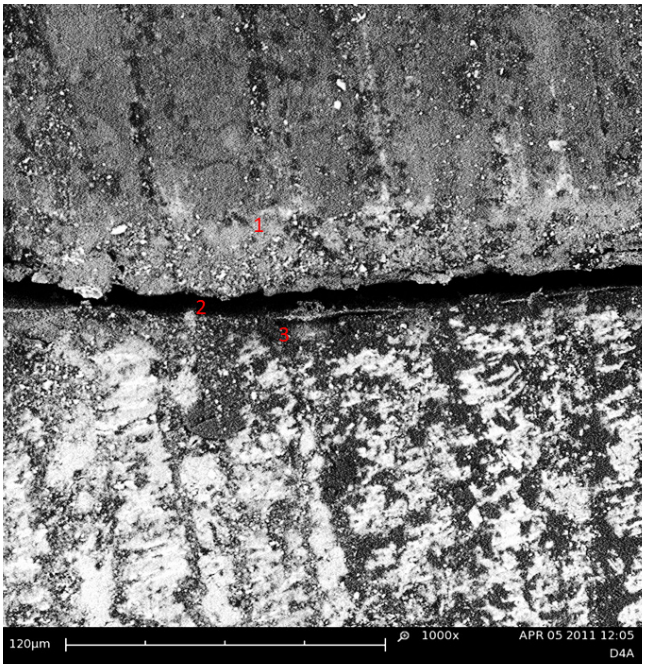

Figure 1: Sectioned tooth showing the composite (1), bonding agent space (2) and the tooth structure (3) as seen under a scanning electron microscope (SEM)

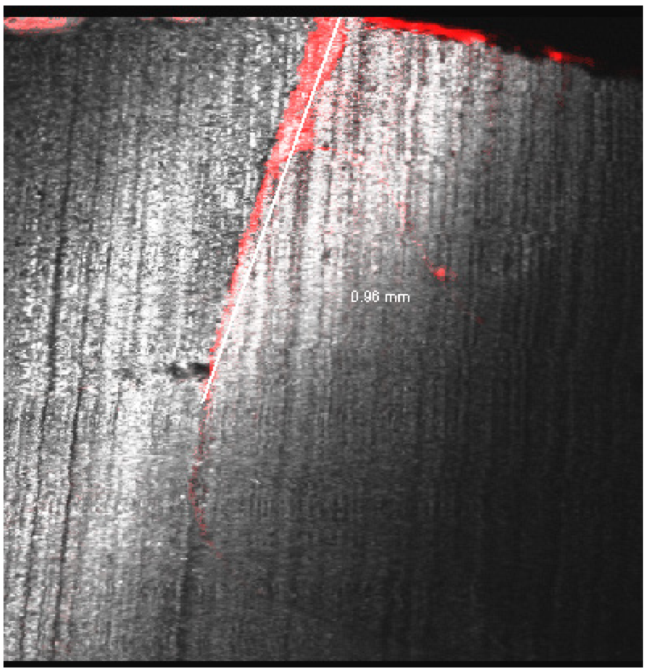

Figure 2: Microleakage evaluation of the sectioned tooth showing the depth of dye penetration as seen under a confocal laser scanning microscope (CLSM)

Table II: Comparison of microleakage between Group A1, Group B1 and Group C1 in the incisal wall

Group	Microleakage (μm)			
	N	Mean (SD)	*f* statistic [a] (*df*)	*P* value [b]
A1	12	1037.40 (273.69)		
B1	14	871.79 (488.92)	2.154 (2; 36)	0.131
CI	14	687.67 (453.43)		

[a] One-way ANOVA tests

Table III: Comparison of microleakage between Group A2, Group B2 and Group C2 in the incisal wall

Group	Microleakage (μm)			
	N	Mean (SD)	*f* statistic [a] (*df*)	*P* value [b]
A2	13	501.46 (585.35)		
B2	17	451.27 (553.17)	0.234 (2; 36)	0.792
C2	12	597.81 (576.28)		

[a] One-way ANOVA tests

Table IV: Comparison of microleakage between Group A1, Group B1 and Group C1 in the cervical wall

Group	Microleakage (μm)			
	N	Mean (SD)	*f* statistic [a] (*df*)	*P* value [b]
A1	12	951.80 (550.95)		
B1	14	809.84 (583.74)	0.249 (2; 36)	0.781
CI	14	858.17 (389.94)		

[a] One-way ANOVA tests

Table V: Comparison of microleakage between Group A2, Group B2 and Group C2 in the cervical wall

Group	Microleakage (μm)			
	N	Mean (SD)	*f* statistic [a] (*df*)	*P* value [b]
A2	13	750.45 (613.09)		
B2	17	518.37 (568.13)	0.663 (2; 36)	0.521
C2	12	717.64 (635.72)		

[a] One-way ANOVA tests

A strong adhesion has to exist between the restorative material and the cavity walls for a proper marginal seal to be effected resulting in less microleakage and a longer life of the restoration. The type of the adhesive system is an important factor that affects microleakage in addition to the restorative material factor (9).

Self-etching adhesive can simplify the procedure of bonding and prove to be time-saving for the clinicians. In contrast, self-etching adhesive system exhibits a lower bond strength with increased microleakage than etch and prime adhesive system. The micromechanical bonding is improved by the process of acid etching which creates a microporosity on the etched surface enabling a deeper penetration of the composite material into the tooth structure.

The sealing ability of the tested composite could be due to the differences in the adhesive system formulations. The composition of the restorative material greatly influences the mechanical properties as a combination of different monomers results in different water absorption properties. A study highlighting the effect of particle filler load showed the lowest shrinkage strain values but exhibited the most stable shrinkage volume (10).
There is a varying degree of dye penetration into the tooth-restorative interface. However, the results stated that there was no significant difference in the depth of microleakage between the three composites bonded with Adper Single Bond 2 two-step etch and prime adhesive system (3M ESPE, USA). Therefore, the research hypothesis that there is a significant difference in the depth of microleakage between the three composites bonded with Adper Single Bond 2 two-step etch and the prime adhesive system was rejected.

Many previous studies have determined microleakage by scoring the degree of dye penetration along the occlusal and cervical walls. This analysis was performed using different criteria. A score of 0 represented no dye penetration, 1 represented dye penetration into half extension of the cervical wall, 2 represented dye penetration into a complete extension of the cervical wall and 3 represented dye penetration into cervical and axial wall towards the pulp.

The length of dye penetration measured in this study in both incisal and cervical walls from the restoration margin to some depth of the cavity wall by CLSM agrees with another previous study (11). The pattern of dye penetration into the dentinal tubules appeared inhomogeneous; therefore, it was not possible to measure the pattern.

The teeth were not subjected to any polishing or finishing procedures due to the possibility of heat generation interfering with the restoration's ability to resist microleakage. The effect of the thermal and mechanical loading on microleakage and bond strength at the cervical margin of the restoration was shown in another study. They stated that the application of thermal cycling and mechanical load cycling did not influence microleakage and bond strength (12). In contrast, it was found that thermal cycling significantly reduced the bond strength between the restoration and the tooth structure (13). For this reason, it was decided not to carry out thermal cycling in this study design. However, there was no effect of thermocycling on the amount of nanoleakage and the same results were reported by 2 studies (11 and 14) on the amount of microleakage.

The application of composite restoration as an ideal filling material is still restricted by a few limitations. Microleakage at the tooth-restorative interface being one of them results in marginal deterioration, cusp movement, debonding, sensitivity, enamel cracks and recurrent caries (15). The composite resin contracted throughout the depth of the material. This contraction, because of polymerisation shrinkage leads to the gap formation enabling microleakage (16). These factors may influence the clinical longevity of the composite restoration. Polymerisation shrinkage, stress and marginal integrity in Class V has been shown to demonstrate a direct relation having a negative impact on the clinical performance of bonded restorations (17). This type of shrinkage has been measured as the volume concentration of free test specimens which varies between 1.7 per cent and 5.7 per cent. There are many important factors in determining the level of shrinkage. It is believed that with a greater number of monomers the resultant shrinkage is larger (18).

A dilatometer for assessment of polymerisation shrinkage of setting materials has been developed (19). A larger amount of the shrinkage occurs in the viscous and early plastic stages after initiation. Several factors responsible for causing polymerisation shrinkage have been discussed in another study (18). Therefore, nanotechnology has been used to produce composite restorations with nanofillers to reduce polymerisation shrinkage. Kelfil is an experimental nanocomposite which has been produced with nanofillers. It is characterised by a particle size of 10-20 nm synthesised by the sol-gel process (20).

In-vitro studies have evaluated the tooth-restoration interface by using a light microscope equipped with an in-built video camera linked computer to evaluate the dye penetration ability (21). Other studies have analysed microleakage by using stereomicroscope and scoring the extent of dye penetration (11). SEM evaluation of the restorative material and dentin interface to correlate the results with the microleakage scoring have been previously used in another study (22).

The bonding interface between the dentin and the composite restorative material when seen under a SEM exhibited a morphology typical to the penetration of the bonding agent into the submicrometer hiati (23). The phenomenon of nanoleakage was studied by another study (11). The depth of dye penetration was measured at the base of the hybrid layer by quantifying the amount of nanoleakage by using CLSM which is characterised by high-resolution microscopy with fluorescence technique producing three-dimensional images from thick samples. This study however could not quantify the nanoleakage pattern into dentinal tubules towards the pulp as the penetration was inhomogeneous.

CONCLUSION

The present study has developed an experimental composite (Kelfil) by utilising nanotechnology. The study found comparable results to the commercially available micro-hybrid and nanocomposite on the physical properties of enamel bonding. The enamel bonding analysed through microleakage was found to be within acceptable range in comparison to the commercially available Filtek Z350 and Spectrum TPH3. Due to the limitations of the study, not all the mechanical and physical properties of the newly developed nanocomposite were analysed. However, in a successive study, the physical and mechanical property of Kelfil will be examined and analysed to understand the usability and clinical application of Kelfil.

ACKNOWLEDGMENT

This work has been supported by the Advanced Medical and Dental Institute (AMDI) student research fund (2011), Universiti Sains Malaysia, Malaysia.

REFERENCES

1. Gouveia THN et al. Esthetic smile rehabilitation of anterior teeth by treatment with biomimetic restorative materials: a case report. Clinical, Cosmetic and Investigational Dentistry 2017:9 27–31
2. Khan, A. S., Azam, M. T., Khan, M., Mian, S. A., & Rehman, I. U. An update on glass fiber dental restorative composites: A systematic review. Materials Science and Engineering: C, 2015: 47, 26-39.
3. Saba, N., Tahir, P., & Jawaid, M. A Review on Potentiality of Nano Filler/Natural Fiber Filled Polymer Hybrid Composites. Polymers, 2014: 6(8), 2247.
4. Sivakumar, J. S. K., Prasad, A. S., Soundappan, S., Ragavendran, N., Ajay, R., & Santham, K. (2016). A comparative evaluation of microleakage of restorations using silorane-based dental composite and methacrylate-based dental composites in Class II cavities: An in vitro study. Journal of Pharmacy & Bioallied Sciences, 8 (Suppl 1), S81–S85.
5. William D. Dupont and Walton D. Plummer, Jr.

(2015). PS: Power and Sample Size Calculation (version 3.1.2, 2014) [computer software]. Retrieved on July 30, 2018, from http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize
6. Robertson TM, Heymann HO, Swift Jr EJ. Sturduvent's Art & Science of Operative Dentistry. 5th Ed. Mosby. 2006; 538-540.
7. Urbaniak, G. C., & Plous, S. (2013). Research Randomizer (Version 4.0) [Computer software]. Retrieved on July 30, 2018, from http://www.randomizer.org/
8. Guньlol N, Ertaş E, Yılmaz A, Зankaya S. Effect of thermal aging on microleakage of current flowable composite resins. Journal of Dental Sciences. 2015;10(4):376-82.
9. Ozgul BAYGIN, Fatih Mehmet KORKMAZ and Ipek ARSLAN. Effects of different types of adhesive systems on the microleakage of compomer restorations in Class V cavities prepared by Er,Cr:YSGG laser in primary teeth. Dental Materials Journal 2012; 31(2): 206–214
10. Charton C, Petit A, Colon P, Pia F. Thermal Expansion Polymerization of Composite Resins: Influence on Shrinkage Stress and Interest for The Qualitative Degree of Conversion Analysis. European Cells and Materials 2005; 9:14-16.
11. Dorfer CE, Staehle HJ, Wurst MW, Duschner H, Pioch T. The nanoleakage phenomenon: influence of different dentin bonding agents, thermocycling and etching time. European Journal of Oral Sciences 2000; 108: 346-351.
12. Bedran-de-Castro AKB, Cardoso PEC, Ambrosano GMB, et al. Thermal and Mechanical Load Cycling on Microleakage and Shear Bond Strength to Dentin. Operative Dentistry 2004; 29: 42-48.
13. Eakle WS. Effect of thermal cycling on fracture strength and microleakage in teeth restored with a bonded composite resin. Dental Materials 1986; 2: 114-117.
14. Prati C, Chersoni S, Acquaviva GL, et al. Permeability of marginal hybrid layers in composite restorations. Clinical Oral Investigations 2005; 9: 1-7
15. Park J, Chang J, Ferracane J, Bog I. How should composite be layered to reduce shrinkage stress: Incremental or bulk filling?. Dental Materials 2008; 24: 1501-1505.
16. Sun J, Eidelman N, Lin-Gibsona S. 3D mapping of polymerization shrinkage using X-ray micro-computed tomography to predict microleakage. Dental Materials 2009; 25: 314-320.
17. Braga RR, Ballestera RY, Ferracane JL. Factors involved in the development of polymerization shrinkage stress in resin-composites: A systematic review. Dental Materials 2005; 21: 962-970.
18. Goldman M. Polymerization shrinkage of resin-based restorative materials. Australian Dental Journal 1983; 28: 156-161.
19. De Gee AJ, Davidson CL. A modified dilatometer for continuous recording of volumetric polymerization shrinkage of composite restorative materials. Journal of Dentistry 1981; 9: 36-42.
20. Mohamad D, Rahim NA, Ismail AR, Akil HM. Synthesis of Nanosilica Fillers for Experimental Dental Nanocomposites and Their Characterisations. Journal of Physical Science 2011; 22: 93-105.
21. Celiberti P, Lussi A. Use of a self-etching adhesive on previously etched intact enamel and its effect on sealant microleakage and tag formation. Journal of Dentistry 2005; 33: 163-171.
22. Korkmaz Y, Ozel E, Attar N, Bicer C, Firatli E. Microleakage and scanning electron microscopy evaluation of all-in-one self-etch adhesives and their respective nanocomposites prepared by erbium:yttrium–aluminum–garnet laser and bur. Lasers in Medical Science 2010; 25: 493-502.
23. Pioch T, Sorg T, Stadler R, Hagge M, Dorfer C. Resin Penetration through Submicrometer Hiatus Structures: A SEM and CLSM Study, Journal of Biomedical Materials Research Part B: Applied Biomaterials. 2004; 71: 238-243.