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ORIGINAL ARTICLE

Influence of the methodology and evaluation criteria on determining microleakage in dentin–restorative interfaces

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Abstract This in vitro study compared microleakage along the dentin–restorative interface using a spectrophotometer protocol and two conventional single-surface methods (scores and percentages), using an organic dye (0.5% buffered methylene blue) or a tracer (50% silver nitrate). Occluso-proximal preparations with gingival margins in dentin were made in 40 human teeth. The teeth were divided into four groups (*n*=10) according to the solution dyes and adhesive system used: group 1, single bond/methylene blue; group 2, single bond/silver nitrate; group 3, Clearfil SE Bond/methylene blue; and group 4, Clearfil SE Bond/silver nitrate. The dye penetration measurements were made in all groups, using scores and percentages. Groups 1 and 3 were also assessed by UV spectrophotometer. For percentage measurement, the data were submitted to ANOVA and Tukey's test. For the material factor, there was a statistically significant difference between groups 1 and 3. For the dye factor, there was a statistically significant difference between groups 3 and 4. The score results were submitted to Kruskall–Wallis test and showed differences between groups 1 and 3 and groups 2 and 3. For spectrophotometer measurement, no significant difference was observed between groups 1 and 3. The results of dye penetration suggest that there was a difference between dyes and measurement methods, and this should change the interpretation of microleakage tests.

Keywords Dye · Microleakage · Composite · Methodology

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Introduction

The anatomic and physiologic restoring of the dental element, after loss of mineralized dental tissue either through caries disease signs or through fractures, has been studied frequently. To characterize the efficiency of these restoring measures, tests that qualify and specify the quantity of its efficacy were created. One of these tests is the analysis of microleakage to obtain data regarding the marginal sealing on the dentin–restorative interface [9, 24]. Nevertheless, some doubts have been raised about the efficacy of the microleakage test in evaluating the marginal sealing, resulting in the requirement for studies questioning the applied methodology to improve accuracy and adapt to this new reality.

The test of microleakage along the dentin–restorative interface using organic and inorganic dyes has been widely used due to its speed in obtaining results and its ease of execution [4, 26]. The results of this test, however, can be partly or totally influenced by the variations of the methodology applied [24]. In addition, these variations make the comparison of results obtained difficult [32] and even lead to doubt and erroneous results.

The usage of organic dyes is a common method for the detection of microleakage along the dentin–restorative interface [9]. In general, the most commonly used dye is methylene blue, employing varying concentrations and immersion times [1]. Tracers and radioisotopes can also be used [13]. Despite being used as dyes, these substances can be employed in different manners with particle sizes and solution pH, making knowledge of these characteristics essential [5, 26, 32].

The manner of interpretation of the results more employed is the use of previously established scores, probably due to their ease of use and low cost. Another form of interpretation is the quantification of the dye penetration measurements and their transformation into percentages, turning this analysis objective but requiring specific machines [7, 30].

The spectrophotometer allows the measurement of dye penetration in volume and is another method of interpretation [11, 14, 21, 22]; however, it requires a specific machine and professional qualification.

Thus, this study has the objective of evaluating different dyes and methods to compare their influence on microleakage results in dentin–restorative composite restorations. The null hypothesis tested was that the dyes and methods used in microleakage tests could not make influence on results.

Materials and methods

Forty sound human third molars were selected just after being pulled out and they were immersed in distilled water under refrigeration, for a maximum period of 4 months.

To create positioning conditions similar to the mouth, a sample made of orthoftalic resin (T-208 Redefibra, São Paulo, Brazil) was created, where the natural tooth was positioned between two teeth made of acrylic resin [4, 5]. The samples were then distributed into two random groups (n=20).

In each tooth, occluso-proximal preparations with gingival margins in dentin were made with the following measurements: cervical occlusal extension=6 mm, buccal lingual extension=4 mm, and depth=2 mm.

For the restorative procedure from group 1, the Single Bond adhesive system (3M/ESPE) was used, and all the recommendations from the manufacturers were followed regarding the acid conditioning (15 s of application and 30 s for washing), the kind of acid to be used (phosphoric acid 35%), and the application (two coats). For group 2, the Clearfil SE Bond adhesive system (Kuraray) was applied; the cavity was cleaned thoroughly with water followed by drying. The adhesive system was applied afterwards according to the manufacturer's instructions.

In both groups, Tofflemire steel strips were used and adapted with a wood wedge (TDV, Pomerode, Santa Cruz, Brazil). The insertion of the restorative composite Z250 (3M/ESPE) was performed according to the horizontal incremental technique, and three increments were necessary for the complete filling of the cavities; thus, each increment was about 2 mm wide.

The restorations were photo-activated through the occlusal face, with a XL 3000 (3M Dental Products, St. Paul, MN, USA), with an intensity of about 550 mW/cm^2, controlled by a radiometer (Demetron, Danburry, CT, USA). Each increment was photoactivated for 40 s. After filling the whole cavity, the matrix band was removed, and two additional photo-activations of 20 s were made directing the light first to the buccal site and then to the lingual site.

For the finishing and polishing of the restoration, diamond points were used (golden series no. 1990F, KG Sorensen). Enhance (Dentsply De Trey, Konstanz, Germany) was used afterwards for polishing the occlusal face. The proximal faces were finally manipulated using scalpel blade no. 12 and polishing was performed with finishing strips (3M).

Teeth preparation for the microleakage test

After the restorative procedure, the 40 restorations of each adhesive system/composite combination were immersed and kept in distilled water, at room temperature for a week, before they were dried and isolated with two layers of colored nail varnish (Revlon, São Paulo, Brazil) to allow the dentin–restorative interface in the cervical region and a margin of 0.5 mm to remain free from contact with the dye. After preparation, the groups were divided into two, according to the kind of dye applied, as shown in Table 1.

For groups 1 and 3, the samples were immersed in buffered methylene blue solution, 0.5%. After 2 h of immersion, the samples were removed, washed in running water, dried, and sectioned in a machine used for slicing (South Bay Technology, model 650, San Clement, CA, USA).

For groups 2 and 4, where 50% silver nitrate dye was applied, the dye immersion procedure was similar. After removal from the dye and washing, the samples were immersed in radiographic developing solution for 4 h, washed, and dried. The protection was then removed and the samples were sectioned as described previously.

Measurement of dye infiltration in percentages

The slice with the greatest penetration of the dye was chosen independent of which dye was used. The extent of dye penetration was analyzed by an Olympus optic (Olympus Optical, Tokyo, Japan), with a magnification of ×30.

Table 1 Division of the groups according to the adhesive system and dye solution used

	Adhesive system	Dye solution	Complement	Number of samples, *n*
Group 1, SB/MB	Single Bond	Methylene blue 0.5%, 2 h, pH 6.89		20
Group 2, SB/SN	Single Bond	Silver nitrate 50%, 2 h, pH 3.98	Developing solution, 4 h	20
Group 3, CSE/MB	Clearfil SE Bond	Methylene blue 0.5%, 2 h, pH 6.89		20
Group 4, CSE/SN	Clearfil SE Bond	Silver nitrate 50%, 2 h, pH 3.98	Developing solution, 4 h	20

The actual extent of the cervical and the axial preparations was obtained. The extent of dye penetration was measured in a quantitative manner. This measurement was converted into percentage using the formula described by Sano et al. [23]: $I=p/L\times100$, where I=microleakage, p=width of dye infiltration along the interface, and L=sum of the total width of the cervical and axial walls of the preparation. These percentage values were submitted to variance analyses (ANOVA) and to Tukey's test.

Score measurement

Scores, as described in Table 2, were used to analyze microleakage.

The same samples analyzed in percentages were also analyzed by three professionals with an optic Leitz 40 magnification (Leitz, Wetzlar, Germany) using the score above. These data were submitted to Kruskall–Wallis nonparametric test.

Measurement by UV spectrophotometer

The methylene blue samples were also analyzed by the UV spectrophotometry method. For this, the teeth from groups 1 and 3 were replaced on the resin object for sectioning in a buccal lingual manner using a diamond disk (KG Sorensen) at low rotation under refrigeration, in a way that both halves of the restoration used in both interpretation manners could be joined again. Each half of the tooth was then pulverized into fine powder with a hard, tissue-grinding Marconi 600 series 985923 machine (Marconi, Piracicaba, Brazil). The resulting powder was placed in a tube containing 3 ml of ethyl ethanol 95% for 60 h to turn the dye into solution. The solution was then centrifuged (Eppendorf 5810, Hamburg, Germany) for 5 min at 3,000 rpm.

The solution was read by a UV–visible recording spectrophotometer (UV-160 A Shimadsu, Tokyo, Japan) with 655.5 nm used as the reference wave length pattern, which was chosen by calibration test, with a 1-cm optic pathway. After the choice of the patterns for the readings of the spectrophotometer, the over surface and clean solution, with no sediments, contained in each sample's tube was applied. The readings were expressed as absorbance. For converting absorbance into concentration, a calibration curve was needed, where the methylene blue dye have been deluded, having concentrations from 0.01 to 0.20 as result.

After the readings in the spectrophotometer, the absorbance values were obtained as described in Table 3.

From the information on concentration and absorbance, these data were turned into a dispersion graph (Fig. 1), where the 'tendency line' can be visualized, with R^2 <1. Thus, after calculation, the resulting value of 1.3599 was obtained, which made the application of the equation for obtaining the concentration of each sample from the experiment possible.

This formula was applied for all the data given by the spectrophotometer, turning the absorbance into concentration. These transformed data were submitted to statistical analysis by *t* test.

Results

The data obtained by the dentin–restorative interface microleakage trial, in percentages, were submitted to

Table 2 Scores applied for qualifying dye penetration

Scores for microleakage	Meaning
0	No dye penetration
1	Dye penetration from the gingival wall to the axial–gingival angle
2	Dye penetration along the axial wall

Table 3 Concentrations of methylene blue dye and respective absorbance values

Concentration X 10^{-3}	Absorbance values
0.01	0.028
0.05	0.07
0.10	0.136
0.15	0.205
0.20	0.27

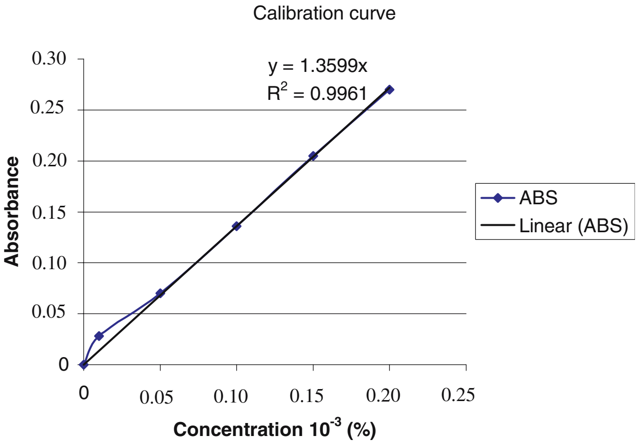

Fig. 1 Dispersion graphic for calibration curve

variance analysis (single surface) considering the factors of material and dye as well as their interaction. The average values were transformed according to arc-sen X/100 and submitted to 5% Tukey's test.

Table 4 shows the comparison between the averages obtained by the two bond systems, using two kinds of dyes. When the methylene blue dye was used, the Single Bond bond system showed a statistically higher average in microleakage compared to the Clearfil SE Bond ($p<0.05$). For silver nitrate dye, there was no statistical difference between the bond systems ($p>0.05$). When the Clearfil SE Bond bond system was used, the silver nitrate dye showed a statistically higher microleakage compared to methylene blue ($p<0.05$). For Single Bond, there was no statistical difference ($p>0.05$) when the averages of microleakage of both dyes were compared.

The results obtained for the microleakage test based on scores are expressed on Table 5. Comparing the scores obtained by groups 1 and 2, 2 and 4, and 3 and 4, the difference found was not statistically significant. For groups 1 and 3, the difference was statistically significant ($p<0.05$).

The method for evaluating microleakage, spectrophotometry, was applied only for groups 1 and 3, where the dye used was methylene blue. The data were submitted to statistical analysis, applying the *t* test with independent samples. There were no statistically significant differences between these two groups ($p<0.05$). The results are shown in Table 6.

Table 4 Averages of microleakage (%) for each adhesive system, according to the dye solution used

Material	Dye solution	
	Methylene blue, 0.5%	Silver nitrate, 50%
Single Bond	22.98 a, A (17.85)	27.56 a, A (16.41)
Clearfil SE Bond	4.98 b, A (7.69)	19.74 a, B (15.92)

Means followed by the same small letter in the column and capital letter in the line, do not differ from each other based on 5% Tukey's test

Table 5 Statistical analysis (Kruskal–Wallis) for reading of dye penetration using scores

Groups	G1-SB/MB	G2 -SB/SN	G3-CSE/MB	G4-CSE/SN
Group 1	–	0.4542 (NS)	0.0263 (S)	–
Group 2		–	–	0.1704 (NS)
Group 3			–	0.1098 (NS)
Group 4				–

NS $p>0.05$, *S* $p<0.05$

Discussion

The results found for these materials, utilizing methylene blue dye, varied according to the methodology in reading applied. In percentages and scores, there was a statistically significant difference between the two materials. However, the data obtained using spectrophotometry did not demonstrate any statistically significant difference. With silver nitrate, there was no difference between the two materials, in either percentages or in scores.

In contrast, when the dyes were compared, it was observed that there was no difference between the two kinds of dyes for Single Bond in percentages or in scores. With the material Clearfil SE Bond, methylene blue dye, in percentages, demonstrated lower penetration levels than silver nitrate, differing statistically. This difference was not observed when the analysis was performed by scores, where dyes did not differ from each other.

Methylene blue is able to demineralize dentin due to its acidic pH [32] besides being extremely soluble and able to easily penetrate into the tooth spaces that contain water without being adsorbed into the dental matrix or crystal apatita [16]. However, in this study, the pH of the dye was buffered from 3.86 to 6.89, reducing its capacity for demineralizing and, therefore, its ability to penetrate dentin [5].

Table 6 Average of dye concentrations (%) for each restorative material

Materials	Dye solution (methylene blue)
Single Bond	0.0132 (0.024) a
Clearfil SE Bond	0.0040 (0.039) a

Averages followed by the same letter in the column do not differ fromeach other based on 5% Tukey's test

In groups 1 and 3, where this dye was applied, a statistically significant difference was detected for the tested materials; both presented dye penetration, demonstrating the existence of a gap in relation to the marginal sealing/occlusion, allowing the formation of a gap between the cavity wall and the restorative material. The size of the gap created after using Clearfil SE Bond (group 3) was possibly a limiting factor for the methylene blue dye to penetrate the interface in a more obvious manner, as it has a high molecular weight [17].

Silver nitrate is obtained by the action of nitric acid on silver; it has no smell, no color, and presents a pH of 3.98. When it is pure, it is not sensitive to light and only an organic material turns it gray or black as the silver ions can combine with proteins, causing denaturation or precipitation [17, 20].

For groups 2 and 4, the action of the silver nitrate dye possibly affected the results obtained for the two materials used. Although the CSE Bond system did not cause extensive exposition of the collagen, the dye was able to penetrate due to the low pH and small size of particles (0.059 nm) [29] with a molecular weight of 169.87 [17, 20], creating a situation of equilibrium between these groups (27.56 and 19.74%, respectively) and making it impossible to establish a statistically significant difference, not even when using scores (0.1078).

When the material and the dyes were compared, it was observed that the union Single Bond system was less influenced by the dye than the Clearfil SE Bond. During the use of the Single Bond system, 35% phosphoric acid is used (pH 0.6), whilst the adhesive has a pH of 5.0. Thus, the substrate is submitted to intense demineralization. For methylene blue dye, whose power of demineralization was reduced due to its buffer state, an infiltration of 22.98% was observed, making the gap in the marginal sealing evident. For the silver nitrate dye, which might have found a substrate with low capacity of demineralization, a slight but non-significant increase of 27.56% dye penetration was seen, even when presenting a potential of demineralization (pH 3.98).

The Clearfil SE Bond system has a pH of 2.0, which allows the dental structure to become enjoined as its penetration occurs, creating a hybrid layer of smaller thickness [3]. When this system was used with methylene blue dye, the penetration index was the lowest of all the groups (4.98%). However, with the silver nitrate, there was a significant rise in dye penetration (19.74%), which may be explained by the contact of a potentially demineralized dye with a low mineralized permeable substrate, raising the gap in marginal sealing.

According to Tay and Pashley [25], the Clearfil SE Bond adhesive system is considered to be a low-aggressiveness self-etching system. When treated with this system, the smear layer, whether thin or thick, is not completely dissolved and joins the hybrid layer [6]. The smear layer can be dissolved through the contact with substances that present a pH of 6.0 or less [18]. It is possible that the NP dye (pH=3.98), when in contact with this hybrid layer, destroyed the smear layer there. This occurrence is less probable with the MB dye (pH=6.89), possibly explaining the results obtained for groups 3 and 4.

The results obtained with silver nitrate dye, regardless of the system tested, were higher than the ones obtained with methylene blue, possibly due to the differing characteristics of the dyes which may influence their penetration abilities. Although silver nitrate has nanometric particles, with molecules of high molecular weight, it is only possible to observe and measure its infiltration with a radiographic revealer. Despite this technical difficulty, its ability to penetrate dentin remains the same, although it is higher when compared to buffered methylene blue dye [5]. In addition, silver phosphate crystal deposition also occurs inside some of the dentin tubules, which may be a limiting factor for the penetration of this tracer [29].

The methylene blue dye also presents limitations for its use; for example, in contact with reducing agents that exist in restorative materials, it may be reduced to a colorless substance [31]. The use of this dye in tests containing alkalis is not recommended as methylene blue is hydrolytic, becoming methylene purple and then Tyonal, which is colorless. Thus, previous knowledge of limitations and pilot studies with dye solutions are essential for the success of this research [32].

With regard to the various studies investigating directed marginal leakage, little attention has been given to the evaluation and comparison of the different methods that exist [27], and the factor that promotes the greatest disagreement is the reading of the results [8].

In the present study, the three kinds of methodology employed presented different degrees of sensitivity. The reading in percentages in relation to quantity is easier to evaluate, and achieving results and statistical analysis are also easier, as the reading is linear and only related to the dye penetration at the interface. Furthermore the measurement employs a comparative optic, with a resolution of ×30, making a clear reading possible when the dye penetration in the near dentin is not considered. The only difficulty found in this method is the angle between the axial and the gingival wall, which is a curve, requiring the use of a bisector for an approximate reading.

The reading in scores is subjective [13] and, therefore, more than one observer with previous experience is necessary. The choice of the limits of the scores is considered important for reducing the divergent situations. In this study, the scores 0, 1, and 2 represent a good sealing, with gaps, and unacceptable, respectively, clearly limited to the walls of the cavity preparation and also not considering the penetration in the near dentin.

The scores that include the penetration in dentin depend more on the infiltration ability of the dye and on dentin permeability than on the interface sealing, leading to a result of difficult penetration. Thus, the measurement receives commonly statistic treatment, not parametric, which compares the data in a more interactive manner but reduces the sensitivity for the reduced number of samples.

In this investigation, the results obtained by percentage and scores were considered interchangeable, similar to the result found by Witzel et al. [30]. However, it presents a bi-dimensional perspective of three-dimensional phenomena as disadvantage [10].

The spectrophotometer method of measurement was the least sensitive; there was no statistically significant difference between groups 1 and 3, which presented differences in the results obtained with the other kinds of methodology applied.

Spectrophotometry is an efficient method for measuring the quantity of dye [19]; however, it needs specific sophisticated equipment, requiring a device for trituration of the samples, laboratory glasses, centrifuge, spectrophotometer, and a qualified professional for performing the test, thus, increasing the cost of this research.

The manner of obtaining the data depends upon the measurement of the dye in volume, which includes the dye that penetrates the interface, the dye that penetrates dentin, and often the dye on the outer tooth surface, which is not considered an advantage.

Thus, the difference in dentin penetration of the samples and the size of the gap in the sealing (nail varnish), which allows dye penetration, can be determined and alter the results [8]. The requirement for converting the spectrophotometer absorbance values into concentration increases the complexity of the method but is necessary for allowing objective and quantitative results and for the performance of statistical analyses.

Alternative methods using scanning electron microscopy (SEM) or dye staining gap test have been used for evaluation of the marginal adaptation of adhesive restorations [15]. In contrast to microleakage studies, it is a truly quantitative method that assesses the entire circumference of the tooth–restoration interface. It is also a non-destructive method, but this evaluation technique suffered because it may be difficult to distinguish experimental gaps vs specimen damage artifacts formed as a result of sectioning or dehydration, heat, and vacuum required for SEM imaging. However, these latter problems can be overcome to some extent by utilizing replicas [12].

A dye staining gap test has been used in preparations using 1% red propylene glycol acid solution for 5 s to detect marginal gap [2, 28]. This is a simple method to assess marginal gap formation and the short time period of dye penetration allows only a penetration due to capillary action and prevents a diffusion of the dye into the adhesive. However, there is some concern about the accuracy of this evaluation due to the limited magnification used in the evaluation of the results.

The increasing interest in the development of union agents has augmented the necessity for creating a test that evaluates the dentin–restorative interface with security. Adding this need to the interaction of the items presented makes the microleakage test a complete one. The utilization of more than one method for evaluating microleakage may be the best procedure for obtaining real results.

Conclusion

Under the conditions applied in this research, it can be concluded that:

1. The reading methods influenced the results obtained.
2. The dyes used influenced the results found for marginal leakage.
3. When using methylene blue, the Single Bond system presented a higher level of marginal leakage and differed statistically from Clearfil SE Bond for reading in scores and percentage; this did not occur when silver nitrate was used.
4. The bond systems, Single Bond and Clearfil SE Bond, presented mean microleakages that did not differ statistically from each other when reading was done by using a spectrophotometer.

References

1. Alani AH, Toh CG (1997) Detection of microleakage around dental restorations: a review. Oper Dent 22:173–185
2. Alonso RC, Cunha LG, Correr GM, De Goes MF, Correr-Sobrinho L, Puppin-Rontani RM, Sinhoreti MA (2004) Association of photoactivation methods and low modulus liners on marginal adaptation of composite restorations. Acta Odontol Scand 62:298–304
3. Arrais CA, Giannini M (2002) Morphology and thickness of the diffusion of resin through demineralized or unconditioned dentinal matrix. Pesqui Odontol Bras 16:115–120
4. Camargo DA, Sinhoreti MA, Consani S, Sobrinho LC (2002) In vitro evaluation of marginal leakage on class II composite restorations. Braz J Oral Sci 1:84–88
5. Camargo DA, Sinhoreti MA (2002) In vitro evaluation of the penetration capacity of dyes and tracers used in microleakage studies. J Dent Res 81(Special Issue B):119p (Abstract 628)
6. Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C, Tay FR (2005) A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives. Biomaterials 26:1035–1042

7. Federlin M, Thonemann B, Hiller KA, Fertig C, Schmalz G (2002) Microleakage in class II composite resin restorations: application of a clearing protocol. Clin Oral Investig 6:84–91
8. Gale MS, Darvell BW (1999) Dentine permeability and tracer tests. J Dent 27:1–11
9. Going RE (1972) Microleakage around dental restorations: a summarizing review. J Am Dent Assoc 84:1349–1357
10. Gwinnett JA, Tay FR, Pang KM, Wei SH (1995) Comparison of three methods of critical evaluation of micoleakage along restorative interfaces. J Prosthet Dent 74:575–585
11. Hasegawa T, Retief DH (1993) Quantitative microleakage of some dentinal bonding restorative systems. Dent Mater 9:114–117
12. Hilton TJ (2002) Can modern restorative procedures and materials reliably seal cavities? In vitro investigations, part 2. Am J Dent 15:279–289
13. Kidd EA (1976) Microleakage: a review. J Dent 4:199–206
14. Mandras RS, Retief DH, Russell CM (1993) Quantitative microleakage of six dentin bonding systems. Am J Dent 6:119–122
15. Manhart J, Chen HY, Mehl A, Weber K, Hickel R (2001) Marginal quality and microleakage of adhesive class V restorations. J Dent 29:123–130
16. Matloff IR, Jensen JR, Singer L, Tabibi A (1982) A comparison of methods used in root canal sealability studies. Oral Surg Oral Med Oral Pathol 53:203–208
17. Merck Index (1996) 20th edition, C6, p 8653
18. Pashley DH (1990) Clinical considerations of microleakage. J Endod 16:70–77
19. Paulillo LA, Coradazzi JL, Lovadino JR, Serra MC (1997) Surface finishing of glass ionomer. Am J Dent 10:137–140
20. Gennaro AR (2000) Remington: the science and practice of pharmacy, C65, 20th edn. Lippincott Williams & Wilkins, Baltimore, MD, p 1272
21. Retief DH, Mandras RS, Russell CM (1994) Shear bond strength required to prevent microleakage of the dentin/restoration interface. Am J Dent 7:44–46
22. Rigsby DF, Retief DH, Russell CM, Denys FR (1990) Marginal leakage and marginal gap dimensions of three dentinal bonding systems. Am J Dent 3:289–294
23. Sano H, Takatsu T, Ciucchi B, Hornen JA, Matthews WG, Pashley DH (1995) Nanoleakage: leakage within the hybrid layer. Oper Dent 20:18–25
24. Shortall AC (1982) Microleakage, marginal adaptation and composite resin restorations. Br Dent J 153:223–227
25. Tay FR, Pashley DH (2001) Aggressiveness of contemporary self-etching systems. I. Depth of penetration beyond dentin smear layers. Dent Mater 17:296–308
26. Taylor MJ, Lynch E (1992) Microleakage. J Dent 20:3–10
27. Von Fraunhofer JA, Adachi EI, Barnes DM, Romberg E (2000) The effect of tooth preparation on microleakage behavior. Oper Dent 25:526–533
28. Yoshikawa T, Burrow MF, Tagami J (2001) A light curing method for improving marginal sealing and cavity wall adaptation of resin composite restorations. Dent Mater 17:359–366
29. Youngson CC, Jones JC, Manogue M, Smith IS (1998) In vitro dentinal penetration by tracers used in microleakage studies. Int Endod J 31:90–99
30. Witzel MF, Grande RH, Singer JM (2000) Bonding systems used for sealing: evaluation of microleakage. J Clin Dent 11:47–52
31. Wu MK, Kontakiotis EG, Wesselink PR (1998) Decoloration of 1% methylene blue solution in contact with dental filling materials. J Dent 26:585–589
32. Wu MK, Wesselink PR (1993) Endodontic leakage studies reconsidered. Part I. Methodology, application and relevance. Int Endod J 26:37–43