Acta Odontologica Scandinavica, 2006; 64: 141–145

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Dye staining gap test: An alternative method for assessing marginal gap formation in composite restorations

ROBERTA CAROLINE BRUSCHI ALONSO[1], GISELE MARIA CORRER[1], LEONARDO GONÇALVES CUNHA[1], ANA FLÁVIA SANCHES BORGES[1], REGINA MARIA PUPPIN-RONTANI[2] & MÁRIO ALEXANDRE COELHO SINHORETI[1]

[1]*Dental Materials Area, Department of Restorative Dentistry, Piracicaba Dental School, UNICAMP, Piracicaba, SP, Brazil and* [2]*Pediatric Dentistry Area, Department of Pediatric Dentistry, Piracicaba Dental School, UNICAMP, Piracicaba, SP, Brazil*

Abstract
Objective. The aim of this study was to evaluate and compare marginal adaptation of composite restorations assessed by a dye staining method and by scanning electron microscopy (SEM) analysis. ***Material and methods.*** Twenty bovine incisors were selected and ground flat to expose dentin. Two cylindrical cavities were prepared on the central area of flattened surfaces. Single bond adhesive system was applied in accordance with the manufacturer's instructions and the cavities were filled with Filtek Z250 or Filtek Flow. The specimens were polished and replicas were obtained in epoxy resin. The replicas were observed by SEM for marginal quality/quantity evaluation. Caries detector was then applied on each specimen for 5 s to verify marginal adaptation through dye staining of the formed gaps on the outer margins. Images of the stained gaps were transferred to a computer measurement program to determine gap length. The length of the gap was expressed as the percentage of total length of the margins observed. Data were submitted to two-way ANOVA and Pearson correlation. ***Results.*** Filtek flow showed 36% and 34% and Filtek Z250 27% and 29% of gap in the margins when evaluated by SEM analysis and by the dye staining test, respectively. There was no difference between the composites, regardless of the evaluation technique. There was a strong positive correlation ($r = 0.83$) between the results obtained with the tested methods to assess marginal gap. ***Conclusion.*** Dye staining the gaps can be used with good reliability to evaluate the gap formation in composite restorations.

Key Words: *Composite restoration, dye, gap, marginal adaptation, SEM*

Introduction

Composite restorative materials undergo significant volumetric shrinkage when polymerized. The shrinkage that accompanies the polymerization of resin composites generates stress at the tooth-restoration interface and may lead to marginal gap formation [1].

The dimensional stability of the tooth/restoration interface is challenged from the very beginning of the polymerization reaction. The polymerization stress inside and around the restoration can be relieved rapidly by deformation of the cavity walls around the restorative materials [2] by plastic flow, or, over longer periods of time, by water sorption of the material [3]. It can also be reduced by different rupture mechanisms, cohesive inside the material or tooth, or adhesive at the interface. Adhesive failures open margins at the periphery of the restorations [4].

The main factors that determine shrinkage stress and, consequently, gap formation in composite restorations are polymerization shrinkage level [5,6], elastic modulus, and flow capacity of the composite [5]. Other factors that also regulate the gap formation are the configuration factor of the cavity in which the composite is inserted and the bond strength of the resin composite to the cavity walls [6].

The marginal gap formation does not necessarily correspond to microleakage. However, it is accepted that the detectable marginal gap leads to interfacial microleakage [7]. *In vitro* microleakage measurements have not been accepted as predictive of restoration failure. Some researchers believe that

Correspondence: Roberta Caroline Bruschi Alonso, Dental Materials Area, Department of Restorative Dentistry, Piracicaba Dental School – UNICAMP, Av. Limeira, 901. Piracicaba, SP, Brazil 13414-018. Tel: +55 19 3412 5374. Fax: +55 19 3412 5218. E-mail: robalonso@yahoo.com

(Received 12 September 2005; accepted 14 November 2005)
ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.1080/00016350500474565

in vitro microleakage studies overestimate the amount of leakage that will occur clinically, since molecular size and molecular weight of the tracers are smaller than bacteria or endotoxines that would be responsible for pathosis and secondary caries [8]. The criticism leveled at microleakage studies includes the necessity of destroying the specimen each time a dye assessment is made and difficulties in interpreting staining patterns. The presence of gaps is considered to be more reliable, once it is considered as the first sign of restoration failure [8]. It can be clinically evidenced by marginal staining [9]. Thus, the identification of marginal gaps could enable easier prognosis of the longevity of the composite restorations.

Within the limitations of laboratory studies, quantitative marginal analysis by scanning electron microscopy (SEM) has proved to be an exact and reliable assessment method for evaluating the marginal adaptation of adhesive restorations [10]. In contrast to microleakage studies, it is a truly quantitative method by which the entire circumference of the tooth-restoration interface is assessed. It is also a non-destructive method allowing marginal qualities to be assessed before and after exposure of the specimens to aging procedures, such as thermal and mechanical loading [8,10]. This evaluation technique suffers because it may be difficult to distinguish experimental gaps versus 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 [8].

Some SEM studies evaluate the gap width [5,6,11], but the presence or absence of marginal gaps is more relevant than gap width. The gap formed, regardless of width, is an open door to oral fluids and is capable of degrading the dentin-composite interface. It is more reliable to evaluate the marginal gap length along the restorations margins. Reduced gap length could result in reduced length of the margins that are submitted to degradation by oral fluids or penetration of bacteria.

A dye staining gap test was conducted by Ernst et al. [12,13] in class II preparations using 2% methylene blue dye solution for 10 s. A 1% red propylene glycol acid solution to detect marginal gap was used by Yoshikawa et al. [14], Alonso et al. [15], and Correr et al. [16]. This is a simple method for assessing marginal gap formation by dye staining the gaps using reduced dye penetration time (5 s). This short time period of dye penetration allows only a penetration due to capillary action and prevents a diffusion of the dye into the adhesive layer. Longer dye penetration periods would allow a particular look at nano-leakages and not only to marginal gap, but this seems to be only possible in the comparison of adhesives with the same solvent and, therefore, with the same solubility [12,13]. However, there is some concern about the accuracy of this evaluation due to the limited magnification used in the evaluation of the results.

Considering that, the aim of this study was to evaluate the marginal gap formation using a dye staining method and to compare it to the SEM evaluation as a gold standard. The hypothesis tested was that there is no difference between the methods for assessing marginal gap formation in composite restorations.

Material and methods

For this study, we selected a hybrid composite (Filtek Z250, shade A3, batch # 3AM, 3M/ESPE, St Paul, Minn., EUA), a flowable composite (Filtek Flow, shade A3, batch # 1BA, 3M/ESPE, St Paul, Minn., EUA), and an adhesive system (Adper Single Bond, batch # 3HR, 3M/ESPE, St Paul, Minn., EUA).

Specimen preparation

Twenty bovine incisors were selected, cleaned, and stored in a 0.5% chloramine T solution at 4°C for no more than a week. The roots were sectioned off 1 mm under the cement enamel junction using a double-face diamond saw (K. G. Sorensen, São Paulo, SP, Brazil). The buccal surface was ground on a water-cooled mechanical polisher (Metaserv 2000, Buehler, UK Ltd, Lake Bluff, IL 60044 – USA) using 80-, 180-, 320-, and 600-grid silicon carbide (SiC) abrasive paper (Carbimet Disc Set, #305178180, Buehler, UK Ltd, Lake Bluff, IL 60044 – USA) in order to expose a flat dentin area of at least 8 mm. These teeth were observed in a stereomicroscope (Zeiss, Manaus, AM, Brazil), at 25 × magnification, to verify whether the enamel has been completely removed.

Two cylindrical cavities (1.8 mm diameter × 2 mm deep) were prepared on the flattened surface using a cylindrical diamond tip # 2294 (K. G. Sorensen, São Paulo, SP, Brazil) mounted in a high-speed hand piece (Kavo, Joinville, SC, Brazil) under constant cooling with air-water. The diamond tips were replaced after every 10th preparation.

Internal cavity walls had a 90° angle to the dentin surface plan, while the internal cavity angles were rounded with the diamond used. The cavities had an internal area of 13.87 mm^2 and a free surface area of 2.54 mm^2. The C factor of the cavity was 5.4. If any pulp exposure was noted at the axial wall during preparation of the cavities, the specimen was discarded.

Restorative procedure

Each specimen was restored using the single bond adhesive system (SB) applied in accordance with the manufacturer's instructions: the cavity was etched with 35% phosphoric acid (H_3PO_4) gel for 15 s, rinsed for 10 s, and blot-dried. The adhesive system was applied twice with a 5-s interval in between, dried carefully with air to remove the solvent, approximately 15 s (observing a glossy surface), and light cured for 10 s using the photocuring unit Elipar Tri-light (3M/ESPE) with a power density of 800 mW/cm^2.

The cavities were restored using a hybrid composite (Filtek Z250) or a flowable composite (Filtek Flow). The composites were inserted in a single increment and photoactivated for 20 s using the photocuring unit Elipar Tri-light.

After the light curing procedures, the specimens were stored in distilled water at 37°C for 24 h. The restorations were then finished with 600- and 1200-grid SiC paper under water and polished with 1 and 0.5 µm diamond paste using a polish cloth under water and ultrasonically cleaning for 30 min between the steps of the finishing procedures.

Evaluation of marginal adaptation by SEM

Impressions of the restorations were taken with a low-viscosity polyvinyl siloxane material (Aquasil, Dentsply DeTrey, Konstanz, Germany) and the impressions were poured with epoxy resin (Buehler, Lake Buff, Ill., USA). Afterwards, they were gold-sputter coated (Balzers-SCD 050 Sputter Coater, Liechtenstein) and observed by SEM (JEOL, JSM-5600LV, Scanning Electron Microscope, Japan) for evaluation, measurement, and classification of the cavity margins. The measurements and classification were made with 200× magnification directly on the microscope monitor using a multi-point measuring device and observing the entire cavity perimeter in approximately 20 sections [17]. The measurements were recorded and classified in steps of 50 to 150 µm, according to morphologically defined parameters: 1) *Perfect margin*: defined as a continuous, gap-free transition between filling and tooth substrate (Figure 1A), and 2) *marginal gap*: observed as gap formation and loss of interfacial adhesion (Figure 1B). This classification was defined by Kemp Scholte & Davidson [18]. Marginal gap formation was calculated and expressed as a percentage of the cavity perimeter of each specimen.

Evaluation of marginal adaptation by dye staining gap test

In order to determine the marginal adaptation at the surface, by a staining technique, a 1.0% acid red propylene glycol solution (Caries Detector, Kuraray Co., Osaka, Japan) was applied at the restoration margins for 5 s [14–16]. The specimens were then rinsed in tap water and gently dried. This technique stained the gaps such they could easily be quantified. The cavity margins were evaluated using a stereomicroscope LEICA MZ6 (Leica Microsystems Ltd., Heerbrugg, Switzerland) at ×16 magnification. A digital image of each specimen was obtained at this stage (Figure 2A) using a video camera attached to the stereomicroscope and associated to a computer using Pinnacle 9.0 software. The length of dye-stained gaps along the cavity margins was measured (µm) from the images using the UTHSCSA Image tool software version 2.0 (alpha 2 – September 1997) developed by the Department of Dental Diagnostic Science at the University of Texas Health Science Center (San Antonio, Tx. 78210, USA). The length of the gap formed was calculated as a percentage of the entire margin length.

Statistical analysis

Data of marginal gap formation using the SEM evaluation and the dye staining test were subjected to two-way ANOVA at 5% significance in order to compare the marginal gaps among the groups. Correlation between the results of the tested evaluation methods was obtained by the Pearson correlation.

Results

Filtek flow showed 36 (±27)% and 34 (±25)% and Filtek Z250 27 (±26)% and 29 (±27)% of gap in the margins when evaluated by SEM analysis and by dye staining test, respectively. There was no difference in the marginal gap formation between

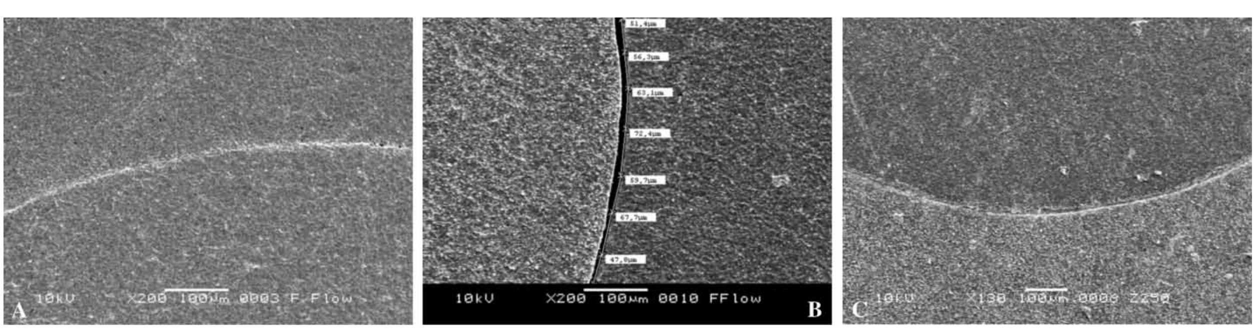

Figure 1. SEM analysis. A. Perfect sealing of the margin. B. Marginal gap measured using a multi-point measuring device of the scanning electron microscope. C. Marginal irregularity.

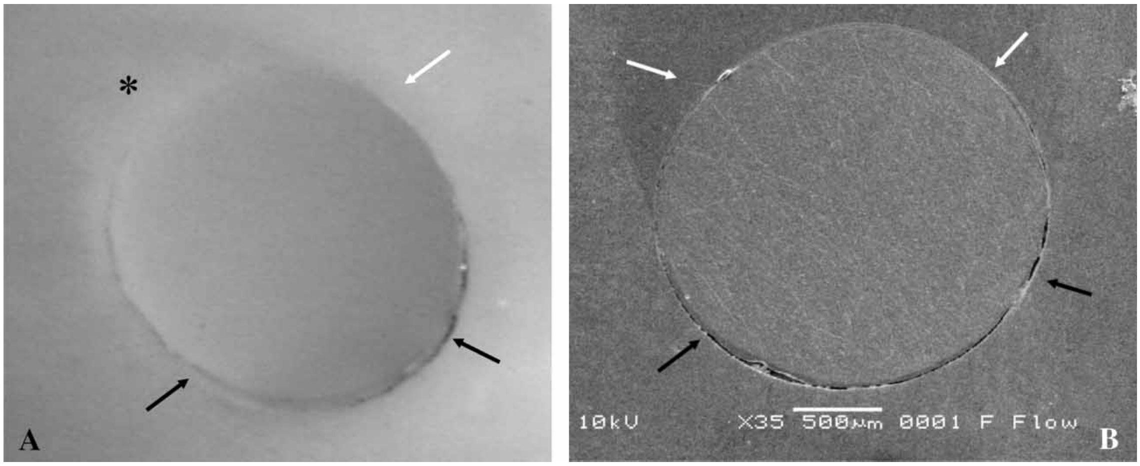

Figure 2. Images of the perimeter of the restorations. A. Image from dye staining gap test. B. Image from SEM analysis. Black arrows indicate marginal gap and white arrows marginal sealing. Staining of the surrounding dentin is indicated by an asterisk (*). A common finding in marginal adaptation study is the semi-circumferential gap.

the resin composites, regardless of the evaluation method. Furthermore, the evaluation method did not influence the results, because there was no difference between the methods, regardless of the resin composite used.

The validation of the dye staining gap test was verified by the concordance between the results of the two methods. There was a strong positive correlation between the methods (SEM analysis using replicas and dye staining gap test). The Pearson correlation coefficient was 0.83. The linear correlation is shown in Figure 3.

Discussion

The work hypothesis was accepted. Marginal adaptation can be evaluated using the dye staining method or the SEM analysis. There is no difference between the results of gap formation observed in these two tests.

An important factor to be considered in the evaluation of marginal gap using any technique is to define exactly what will be considered gap.

In SEM analysis, the gap was considered loss of interfacial adhesion (Figure 1A) due to polymerization shrinkage. This analysis allows distinguishing marginal gaps from marginal irregularities (Figure 1C) or tooth fractures. SEM analysis was conducted in very high magnification (from 37 × to 1000 ×). Doubtful areas can be evaluated in higher magnification, making the evaluation more accurate.

In the SEM evaluation, a counterbalancing relationship of marginal gap and dentin fracture was observed, demonstrating that the stress due to shrinkage can be relieved when marginal gap formations or fractures of the surrounding dentin occur. In the images of the dyed restorations, the dentin fracture could not be distinguished from the marginal gap formation. The image is not as clear as the SEM photomicrographs. In Figure 2A and 2B, a comparison between the restoration images obtained in the dye staining test (Figure 2A) and in SEM analysis (Figure 2B) can be done. The SEM image is clearer than the dye staining image.

In the dye staining gap test, it was decided that all parts of the margin into which the dye had penetrated to turn it dark would be considered a shrinkage gap (Figure 2A). Staining of the surrounding dentin was distinguished from gaps because it was a more diffuse staining (Figure 2A). However, this differentiation is difficult to carry out rigorously and the dye staining gap test needs a trained evaluator. In addition, in cases when the restoration margins became only slightly stained, these were considered marginal irregularities and not gaps. Consequently, only a trained evaluator could reliably estimate the marginal gaps. Marginal irregularity was not included in the analysis of this study because it could not be distinguished definitively in the staining gap test. Therefore, the irregularities were consid-

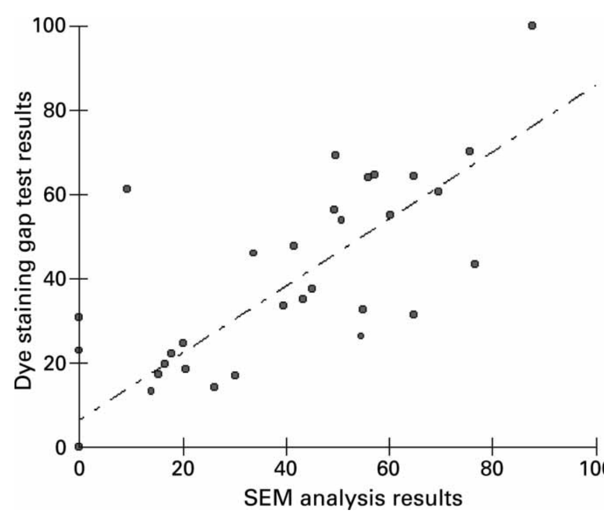

Figure 3. Scatterplot of results of dye staining test and SEM analysis. Significant correlation was observed ($r = 0.83$, $p < 0.001$).

ered as a perfect seal in both evaluation methods. However, the dye staining test is easier and faster to execute than the SEM analysis because there is no need for SEM or impression and casting procedures.

In the dye staining gap test a digital image can be obtained using the video camera attached to a stereomicroscope and associated to a computer using Pinnacle 9.0 software, as in this study, or using a high resolution scanner with 1200 dpi. Less resolution could reduce the quality of the image and make visualization and interpretation of the gaps more difficult.

Both methodologies can be adapted to evaluate internal gap formation. In the dye staining gap test, the specimens must be sectioned and the dye applied in the internal margins of the restorations. This method was used by Yoshikawa et al. [14] and by Correr et al. [16]. For SEM analysis, the specimen must be sectioned and a replica confectioned from the section.

The marginal adaptation test, regardless of the method used, shows high variability data, revealed by the high standard deviation observed in all groups. This seems to be a characteristic of this test, especially on dentin substrate, since other studies show the same pattern [14–16]. The high variability could be associated with the bonding process, which is influenced by many variables. Within the same group, specimens without gaps and specimens with almost 100% of gaps were found.

In this study, two different types of composite, a flowable and a hybrid, were selected. There was no statistical difference between them, regardless of the evaluation method used.

Polymerization shrinkage of composite restorations may induce mechanical stresses on tooth structure via bond to enamel and dentin. The magnitude of the polymerization stress depends on the composition of the material, stiffness and flowability of the composite, rate of polymerization, the volume of the material to be polymerized, and the geometry of the restorations [19]. The volumetric shrinkage and the elastic modulus have opposite effects on the total stress on tooth structure. High polymerization shrinkage could explain why the flowable composite, although it has low elastic modulus, showed restorations with similar marginal gap formation compared to the restorations using the hybrid composite [20].

Based on the results, dye staining of gaps can be used to assess marginal gap formation with results similar to those found in the SEM analysis.

References

[1] Ferracane JL. Developing a more complete understanding of stresses produced in dental composites during polymerization. Dent Mater 2005;21:36–42.

[2] Suliman A, Boyer DB, Lakes RS. Polymerization shrinkage of composite resins: comparison with tooth deformation. J Prosthet Dent 1994;71:7–12.

[3] Davidson CL, De Gee AJ. Relaxation of polymerization contraction stresses by flow in dental composites. J Dent Res 1984;63:146–8.

[4] Ciucchi B, Bouillaguet S, Delaloye M, Holtz J. Volume of the internal gap formed under composite restorations in vitro. J Dent 1997;25:305–12.

[5] Peutzfeldt A, Asmussen E. Determinants of in vitro gap formation of resin composites. J Dent 2004;32:109–15.

[6] Irie M, Suzuki K, Watts DC. Marginal gap formation of light-activated restorative materials: effects of immediate setting shrinkage and bond strength. Dent Mater 2002;18: 203–10.

[7] Dietschi D, Magne P, Holz J. An in vitro study of parameters related to marginal and internal seal of bonded restorations. Quintessence Int 1993;24:281–91.

[8] Hilton TJ. Can modern restorative procedures and materials reliably seal cavities? Part 2. Am J Dent 2002;15: 279–89.

[9] Alani AH, Toh CG. Detection of microleakage around dental restorations: a review. Oper Dent 1997;22: 173–85.

[10] Manhart J, Chen HY, Mehl A, Weber K, Hickel R. Marginal quality and microleakage of adhesive class V restorations. J Dent 2001;29:123–30.

[11] Loguercio AD, Reis A, Ballester RY. Polymerization shrinkage: effects of constraint and filling technique in composite restorations. Dent Mater 2004;20:236–43.

[12] Ernst CP, Cortain G, Spohn M, Rippin G, Willershausen B. Marginal integrity of different resin-based composites for posterior teeth: an in vitro dye-penetration study on eight resin-composite and compomer-/adhesive combinations with a particular look at the additional use of flow composites. Dent Mater 2002;18:351–8.

[13] Ernst CP, Streicher S, Willershausen B. Marginal adaptation of self-etching adhesives in Class II cavities. J Adhes Dent 2002;4:223–31.

[14] Yoshikawa T, Burrow MF, Tagami J. A light curing method for improving marginal sealing and cavity wall adaptation of resin composite restorations. Dent Mater 2001;17: 359–66.

[15] Alonso RCB, Cunha LG, Correr GM, Góes MF, Correr-Sobrinho L, Puppin-Rontani RM, et al. Association of photoactivation methods and low modulus liners on marginal adaptation of composite restorations. Acta Odontol Scand 2004;62:298–304.

[16] Correr GM, Alonso RCB, Puppin-Rontani RM, Correr-Sobrinho L, Sinhoreti MAC. Marginal and internal adaptation of composite restorations using a resin liner on deproteinized substrate. Acta Odontol Scand 2005;63:227–32.

[17] Montes MAJR, Góes MF, Ambrosano GMB, Duarte RM, Correr-Sobrinho L. The effect of colagen removal and the use of low-viscosity resin liner on marginal adaptation of resin composite restorations with margins in dentin. Oper Dent 2003;28:378–87.

[18] Kemp-Scholte CM, Davidson CL. Marginal integrity related to bond strength and strain capacity of composite resin restorative systems. J Prosthet Dent 1990;64:658–64.

[19] Carvalho RM, Pereira JC, Yoshiyama M, Pashley DH. A review of polymerization contraction: the influence of stress development versus stress relief. Oper Dent 1996;21: 17–24.

[20] Alomari QD, Reinhardt JW, Boyer DB. Effect of liners on cusp deflection and gap formation in composite restorations. Oper Dent 2001;26:406–11.