Microsc. Microanal. 16, 779–784, 2010
doi:10.1017/S1431927610093931

Microscopy AND Microanalysis

Effect of the Insertion and Polymerization Technique in Composite Resin Restorations: Analysis of Marginal Gap by Atomic Force Microscopy

Marcos Aurélio Bomfim da Silva,[1] Guilherme José Pimentel Lopes de Oliveira,[2,*] Josealdo Tonholo,[3] José Ginaldo da Silva Júnior,[3] Lucineide de Melo Santos,[4] and José Ivo Limeira dos Reis[4]

[1]*Departamento de Odontologia Restauradora, Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, 13414-903, Piracicaba-SP, Brazil*
[2]*Departamento de Diagnóstico e Cirurgia, Faculdade de Odontologia de Araraquara, Universidade Estadual Paulista, 14801-140 Araraquara-SP, Brazil*
[3]*Instituto de Química e Biotecnologia, Universidade Federal de Alagoas, Campus A. C. Simões, Tabuleiro do Martins, 57072-970 Maceió-AL, Brazil*
[4]*Faculdade de Odontologia, Universidade Federal de Alagoas, Campus A. C. Simões, Tabuleiro do Martins, 57072-970 Maceió-AL, Brazil*

Abstract: This *in vitro* study evaluated the marginal gap at the composite tooth/resin interface in class V cavities under the influence of two insertion techniques and a curing system by means of atomic force microscopy (AFM). Forty enamel and dentin cavities were prepared on the buccal surface in bovine teeth with quadratic forms measuring 2 mm × 2 mm and depth of 1.5 mm. The teeth were then divided into four groups: group A, 10 cavities were restored in one increment, light cured by halogen light; group B, 10 cavities filled with bulk filling, light cured by the light emitting diodes (LED); group C, 10 cavities were restored by the incremental technique, light cured by halogen light; group D, 10 cavities were restored by the incremental technique, light cured by the LED. The teeth underwent the polishing procedure and were analyzed by AFM for tooth/restoration interface evaluation. The data were compared between groups using the nonparametric Kruskall-Wallis and Mann-Whitney tests ($p < 0.05$). The results showed a statistically significant difference between groups A and B and groups A and C. It was concluded that no insertion and polymerization technique was able to completely seal the cavity.

Key words: dental marginal adaptation, dental curing light, atomic force microscopy, composite resin, lasers, dental surface analysis

Introduction

One of the most studied phenomena in dentistry in recent years is the polymerization shrinkage of resin composites. It is currently known that several factors influence the tension of contraction, such as the cavity configuration factor (Factor C) (Feilzer et al., 1987), chemical composition, speed of polymerization, curing system, and elasticity modulus of the restorative material (Braga et al., 2005).

The chemical reaction triggered in the organic resin produces the conversion of monomers into polymers, resulting in the approximation of these molecules and consequently the contraction. In the process, the tension generated in the composite concentrates on the adhesive interface between the tooth and restorative material compromising the integrity in this region, which may cause marginal defects, cleft formation, deflection of cusps, and postoperative sensitivity (Braga & Ferracane, 2004; Braga et al., 2006).

There are several types of dental light curing units in the market with different types of energy source, wavelength, and light intensity for the polymerization of photoactivated restorative materials (Cavalcante et al., 2007). These materials' polymerization has an influence on its properties, as it depends on the particle size, composition, color, translucency, light intensity, length of exposure to radiation, as well as the monomer composition and concentration of polymerization initiators (Lindberg et al., 2005).

Several techniques have also been proposed in an attempt to overcome problems resulting from the polymerization shrinkage (Beznos, 2001; Moszner & Salz, 2001). The insertion techniques of the composite cavity are widely

Received May 25, 2010; accepted July 28, 2010
**Corresponding author*. E-mail: guioliveiraodonto@hotmail.com
This research was performed at the Universidade Federal de Alagoas.

known as a modifying factor in the tensions of the composite to preserve the sealing for better adaptation to the cavity and to provide high conversion rates (Versluis et al., 1996; Yap, 2000).

To evaluate the crack originated from the marginal contraction, using a methodology that enables estimating its length and depth is of great interest. Some methodologies are applied, such as the use of colorants that penetrate the tooth/composite interface and further evaluation using a stereomicroscope (Niu et al., 1998; Hardan et al., 2008) or by using a scanning electron microscope (SEM) (Waldman et al., 2008; Xie et al., 2008). However, the atomic force microscope (AFM) allows three-dimensional (3D) evaluation of this interface, making it possible to assess the length and depth of the marginal gaps by quantitative data.

The AFM has been used in some studies in dentistry; however, none has evaluated the formation of marginal gaps (Eliades et al., 1997; Saeki et al., 2001; Oliveira et al., 2003; Silva et al., 2006; Batista et al., 2007). The aim of this study was to evaluate *in vitro* by AFM, of which the insertion technique and the polymerization system are more appropriate to minimize the formation of marginal gaps.

Material and Methods

Selection and Preparation of Tooth Cavities

Forty bovine incisors were extracted, cleaned, and stored in saline at 37°C until the beginning of the dental cavities preparation. Subsequently, 20 mm were sectioned from the apex/cementum-enamel junction with an Isomet 1000 cutting machine (Buehler, Lake Bluff, IL, USA). The cavities were performed on the midface vestibular using a high speed turbine (Dabi Atlante, Ribeirão Preto, São Paulo, Brazil), under air/water coolant with diamond burs (KG Sorensen 1090, Barueri, Sao Paulo, Brazil). An acrylic resin “stop” was fixed at 2 mm from the edge of the active point of these tips, standardizing the wells with regard to its depth. At the end of the preparation stage, the cavities had quadrangular shapes measuring 2 mm in the mesiodistal direction, 2 mm in the cervical-incisal, and 1.5 mm in depth and enamel cavo-superficial angle.

Restoring Procedures

The teeth were divided into four groups ($n = 10$), a total sample volume of 40 teeth. A three-step adhesive system was applied to all (Alloybond, SDI, Victoria, Australia) with the following clinical sequence: total conditioning of the cavity with phosphoric acid at 37% for 20 s; rinsing in running water for 15 s and air drying for 5 s; primer application and light curing for 10 s; application of lightweight jet air for 5 s; adhesive application and curing for 10 s. Then, the hybrid composite resin (Glacier, A3, SDI, Victoria, Australia) was inserted into the cavity with a titanium spatula. The insertion and polymerization technique was performed as follows: group A, 10 cavities were restored by the bulk filling technique, and light cured for 60 s with the unit Optilight Digital–Halogen Light (Gnatus, Ribeirão Preto, São Paulo, Brazil), with an intensity of 700 mW/cm^2; group B, 10 cavities were filled by the bulk filling technique of resin and photoactivated for 60 s with an Optilight II LD–LED (light-emitting diodes Gnatus, Ribeirão Preto, São Paulo, Brazil), with an intensity of 470 mW/cm^2; group C, 10 cavities were filled by the incremental technique, inserted in three increments: The increment A was activated for 30 s with a Digital Optilight (Gnatus, Ribeirão Preto, São Paulo, Brazil), with an intensity of 700 mW/cm^2, increment B was activated for 30 s and increment C for 60 s; and group D, 10 wells were restored by the incremental technique, the same as group C; however, the polymerization was carried out with the Optilight II LD (Gnatus, Ribeirao Preto, São Paulo, Brazil), with an intensity of 570 mW/cm^2.

After the restorations, all samples were stored in saline at 370°C for 7 days. Next, they underwent the finishing procedure using decreasing granulation Soflex discs (3M ESPE Dental Products, St. Paul, MN, USA) and polishing with circular felt discs without water cooling, soaked in Diamond Excel paste (FGM, Joinville, Santa Catarina, Brazil) and Diamond R (FGM, Joinville, Santa Catarina, Brazil) and placed on a counter angle (Dabi Atlante, Brazil). In each grained pulp a new felt disc was used to prevent the granules from mixing with different granules on one disc. The samples were positioned parallel and held in position by exerting gentle pressure to the granule and the disk spinning for 60 s for each different grain intermittently.

The teeth were then cut again to produce compatible samples for evaluation in the AFM. Next, square forms measuring 6 × 6 × 2 mm were marked on each sample using a dermographic pencil (Pilot, Cubatao São Paulo, Brazil). To standardize the exposed surfaces, the samples were subjected to a finish using a sheet of sandpaper under refrigeration, so that the front and back of the sample were parallel to each other, and this planning was made on the opposite face to the restored face.

AFM Analysis

All images were obtained through the AFM Shimadzu SPM-9500J3 model (Shimadzu® Corp., Japan) coupled to an optical microscope with a 0.8–5× zoom lens with light source (Kyowa Optical, Kanagawa, Japan), a Shimadsu Micro-CDD-S2 video camera, and a TM-A14S video monitor. All images shown were obtained in contact mode. Si^3N^4 tips were used in the Olympus (Tokyo, Japan) of 200 μm length, controlled by the AFM J3 Shimadzu software (Version 2.59). The microscope scanner allows scanning of up to 125 μm in the horizontal directions (x and y) and up to 8 μm in the orthogonal direction (z direction), with regular image resolution of 10 to 100 nm. The mapping procedure produced images of 50 × 50 μm, with a frequency of 1 Hz. The

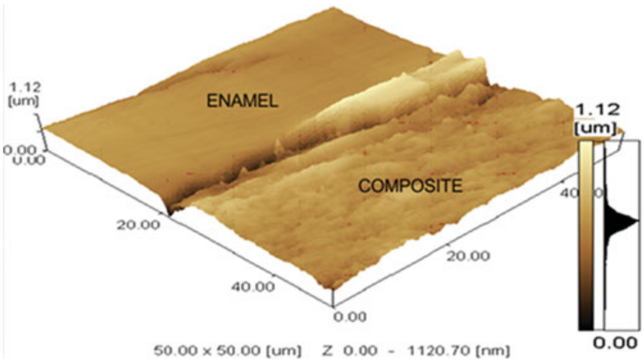

Figure 1. Marginal gaps image obtained by AFM.

images were generated from regions at the tooth/resin interface randomly chosen (Fig. 1). Some images were subjected to the flattening procedure in the *x* and *y* axes to correct the angle discrepancies in the sample. The cracks were measured by the Profile system (Fig. 2).

Statistical Analysis

The Bioestat 5.0 (Belém, PA, Brazil) computer program was used for the statistical analysis. The Shapiro-Wilk normality test was used to evaluate the distribution of data in relation to the theorem of central distribution, and it was observed that the data are distributed in the nonnormal way. The results relating the type of growth and type of light curing unit with the length of marginal gaps were subjected to statistical analysis using nonparametric Kruskal-Wallis and Mann-Whitney tests. All the tests used in this study were applied with a significance level of 95%.

Results

In evaluating the marginal gaps formed in each group, we verified that the group that experienced the largest cracks was group A, where the resin was inserted into the cavity by a single increment and the light curing unit used was the halogen light (2.38 μm ± 0.50), followed by group D, where the cavity was filled with three increments and the light curing unit used was an LED (2.22 μm ± 0.57). In group B, where the cavity was filled by a single increment in the cavity and was light cured through the LED (1.98 μm ± 0.29), and group C, where the resin was inserted in three increments in the cavity and was light cured through the LED (1.85 μm ± 0.32), had the lowest marginal gaps

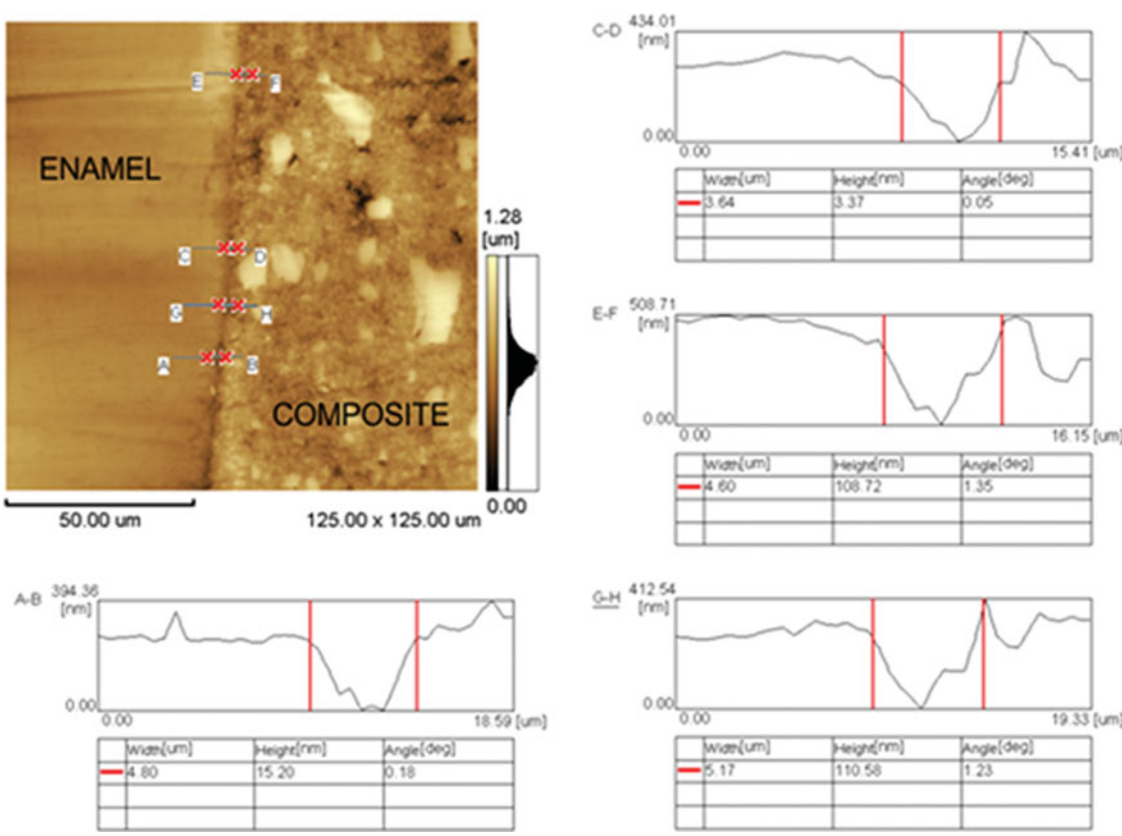

Figure 2. Marginal gap measurements executed by profile system of the AFM.

Table 1. Average and Standard Deviation of the Marginal Gap Measurements.

Group	*n*	Average (μm)	*SD*
A. Bulk filling technique + halogen light	10	2.38	0.50
B. Bulk filling technique + LED	10	1.98	0.29
C. Incremental technique + halogen light	10	1.85	0.32
D. Incremental technique + LED	10	2.22	0.57

(Table 1). The Kruskal-Wallis test showed significant statistical differences between groups ($p \leq 0.05$). The Mann-Whitney test revealed significant differences only between groups A and B ($p = 0.02$) and between groups A and C ($p = 0.01$).

Discussion

In this study, we used only one type of composite resin and bonding system to avoid the influence of the composition of the restorative material and analyze only the restorative technique and type of light used for photoactivation. None of the insertion techniques or light curing units used were able to prevent the formation of cracks, which corroborates the literature (Kubo et al., 2004; Duarte et al., 2007). The resin insertion technique in small increments in cavities has been proposed by some authors (Félix et al., 2007; Park et al., 2008) and is based on the possibility of a material having less contact with the cavity walls, greater depth of light penetration, and lower tension generated by the reaction (Pfeifer et al., 2006).

In this study the incremental insertion technique was superior to the single increment technique when halogen light was the light curing unit used. This probably occurred due to the lower increment thickness, hence leaving the free areas wider, enough to allow the relaxation of the tension caused by polymerization shrinkage during curing. However, these results disagree with other studies (Sensi et al., 2005), where no significant statistical differences were found between the single incremental technique and the increased technique when halogen light was the curing light unit. The results concerning the unity of LED curing light showed no statistically significant difference between the restorative techniques, which disagrees with the studies that advocate the incremental insertion technique as a factor in reducing polymerization stress (Félix et al., 2007; Lazarchik et al., 2007).

When the two insertion techniques were compared, excluding the type of light used for curing, there was no statistically significant difference, which is in agreement with some studies (Kuijs et al., 2003; He et al., 2007), although other studies (Versluis et al., 1996; Chikawa et al., 2006; Park et al., 2008) showed greater stress on the single increment technique. In our study we used cavities with depths less than 2 mm, which is the likely cause for the nonstatistically significant differences between techniques because the minimum thickness for differences in effective curing resin is an increment of 2 mm (Kuijs et al., 2003; He et al., 2007).

One can divide the contraction of the resins in two stages, pre-gel and post-gel, with the contraction of the material involving the combination of both. During the polymerization shrinkage that occurs during the so-called pre-gel, the speed with which a light cured resin reaches its gel is dependent on the light intensity applied in the initial stage of polymerization (He et al., 2007). The light curing emitting high incidence of light induces greater polymerization shrinkage and consequently higher marginal fissure (Takamizawa et al., 2008). Halogen lamps are the most used in the polymerization of composite resin (Santos et al., 2007); however, the high temperature generated during the light emission, damage to the bulb, reflector, and filter can affect the degree of polymerization of the material, without the operator perceiving this (Hasler et al., 2006). In our study two types of light curing units were used, with intensities of different lights. The small difference in the size of the holes can be explained by small thickness of the increments and the proximity of the intensity values of the apparatus.

Thus, the LED can be an alternative for curing composite resins; however, careful insertion techniques and the type of resin used are important as resins using other types of photoinitiators, which are different from camphorquinone, such as the PPD (1-phenyl-1,2-propanodiona) and benzyl (Santos et al., 2007). The action of the LED is better in resins that use camphorquinone as a photoinitiator. In resins in which the camphorquinone is not the photoinitiator, the halogen light is better (Uctasli et al., 2005).

In our study AFM proved to be an appropriate tool for the assessment of marginal gaps formed at the tooth-resin composite tissue interface. The AFM provides 3D high-resolution images because it has a software with great mathematical accuracy for the mapping and characterization of surfaces (Silva et al., 2006; Batista et al., 2007; Kakaboura et al., 2007) providing quantitative data on the length of marginal gaps. Furthermore, samples to be evaluated by AFM need not be fixed or metalized, which reduces the need to create artifacts that affect the analysis (Miles et al., 2003; Batista et al., 2007; Botta et al., 2008).

To our knowledge, this is the first study to evaluate the formation of marginal gaps by means of AFM. Previous studies that evaluated the tooth-restoration interface using tools such as the SEM (Magni et al., 2008; Waldman et al., 2008; Xie et al., 2008), or the analysis of dye penetration using a stereomicroscope (Niu et al., 1998; Hardan et al., 2008; Yazici et al., 2008). For evaluation using SEM, it is necessary to process and metallize the samples, which can cause artifacts that hinder the assessment of cracks, and furthermore, the images obtained by SEM are lower resolution two-dimensional (2D) images than those obtained by AFM. The assessment by dye penetration, like SEM, can produce structural changes in the samples; the images are 2D and the data, unlike those obtained by AFM, are qualitative, thereby providing a lower statistical power.

In the literature, we found that the studies show different results and similar methodologies but different forms of analysis. Thus, for a better comparison of the results of this research, we need more scientific studies using the AFM as a form of analysis.

Conclusion

Thus, the following can be concluded:

- None of the insertion and polymerization techniques were able to completely seal the cavity.
- There was no statistically significant difference between both insertion and polymerization techniques.
- AFM proved to be an appropriate tool for the assessment of marginal gaps formed at the tooth-resin composite tissue interface.

Acknowledgments

The authors thank the granting authority (FAPEAL) for financial support and for fellowships to Guilherme José Pimentel Lopes de Oliveira e Marcos Aurélio Bomfim da Silva. The authors are particularly thankful to Luiz Henrique Carvalho Batista for help in this research.

References

Batista, L.H.C., Silva, J.G., Jr., Silva, M.F.A. & Tonholo, J. (2007). Atomic force microscopy of removal of dentin smear layers. *Microsc Microanal* **13**, 245–250.

Beznos, C. (2001). Microleakage at the cervical margin of composite class II cavities with different restorative techniques. *Oper Dent* **26**, 60–69.

Botta, A.C., Duarte, S., Jr., Paulin Filho, P.I. & Gheno, S.M. (2008). Effect of dental finishing instruments on the surface roughness of composite resins as elucidated by atomic force microscopy. *Microsc Microanal* **14**, 380–386.

Braga, R.R., Ballester, R.Y. & Ferracane, J.L. (2005). Factors involved in the development of polymerization shrinkage stress in resin-composites: A systematic review. *Dent Mater* **21**, 962–970.

Braga, R.R., Boaro, L.C.C., Kuroe, T., Azevedo, C.L.N. & Singer, J.M. (2006). Influence of cavity dimensions and their derivatives (volume and 'C' factor) on shrinkage stress development and microleakage of composite restorations. *Dent Mater* **22**, 818–823.

Braga, R.R. & Ferracane, J.L. (2004). Alternatives in polymerization contraction stress management. *Crit Rev Oral Biol Med* **15**, 176–184.

Cavalcante, L.M.A., Peris, A.R., Ambrosano, G.M.B., Ritter, A.V. & Pimenta, L.A.F. (2007). Effect of photoactivation systems and resin composites on the microleakage of esthetic restorations. *J Contemp Dent Pract* **8**, 70–79.

Chikawa, H., Inai, N., Cho, E., Kishikawa, R., Otsuki, M., Foxton, R.M. & Tagami, J. (2006). Effect of incremental filling technique on adhesion of light-cured resin composite to cavity floor. *Dent Mater J* **25**, 503–508.

Duarte, S., Jr., Dinelli, W. & da Silva, M.H. (2007). Influence of resin composite insertion technique in preparations with a high C-factor. *Quintessence Int* **38**, 829–835.

Eliades, G., Palaghias, G. & Vougiouklakis, G. (1997). Effect of acid conditioners on dentin morphology, molecular composition and collagen conformation *in situ*. *Dent Mater* **13**, 24–33.

Feilzer, A.J., De Dee, A.J. & Davidson, C.L. (1987). Setting stress in composite resin in relation to configuration of the restoration. *J Dent Res* **66**, 1636–1639.

Félix, S.A., González-López, S., Mauricio, P.D., Aguilar-Mendoza, J.A. & Bolanos-Carmoña, M.V. (2007). Effects of filling techniques on the regional bond strength to lateral walls in Class I cavities. *Oper Dent* **32**, 602–609.

Hardan, L.S., Amm, E.W. & Ghayad, A. (2008). Effect of different modes of light curing and resin composites on microleakage of class II restorations. *Odontostomatol Trop* **31**, 27–34.

Hasler, C., Zimmerli, B. & Luss, A. (2006). Curing capability of halogen and LED light curing units in deep class II cavities. *Oper Dent* **31**, 354–363.

He, Z., Shimada, Y. & Tagami, J. (2007). The effects of cavity size and incremental technique on micro-tensile bond strength of resin composite in class I cavities. *Dent Mater* **23**, 533–538.

Kakaboura, A., Fragouli, M., Rahiotis, C. & Silikas, N. (2007). Evaluation of surface characteristics of dental composites using profilometry, scanning electron, atomic force microscopy and gloss-meter. *J Mater Sci Mater Med* **18**, 155–163.

Kubo, S., Yokota, H., Yokota, H. & Hayashi, Y. (2004). The effect of light-curing modes on the microleakage of cervical resin composite restorations. *J Dent* **32**, 247–254.

Kuijs, R.H., Fennis, W.M., Kreulen, C.M., Barink, M. & Verdonschot, N. (2003). Does layering minimize shrinkage stresses in composite restorations? *J Dent Res* **82**, 967–971.

Lazarchik, D.A., Hammond, B.D., Sikes, C.L., Looney, S.W. & Rueggeberg, F.A. (2007). Hardness comparison of bulk-filled/transtooth and incremental-filled/occlusally irradiated composite resins. *J Prosthet Dent* **98**, 129–140.

Lindberg, A., Peutzfeldt, A. & Van Dijken, J.W. (2005). Effect of power density of curing unit, exposure duration and light guide distance on composite depth of cure. *Clin Oral Investig* **9**, 71–76.

Magni, E., Zhang, F., Hickel, R., Bosso, M., Polemini, A. & Ferrari, M. (2008). SEM and microleakage evaluation of the marginal integrity of two types of class V restorations with or without the use of a light-curable coating material and of polishing. *J Dent* **36**, 885–891.

Miles, M., Antognozzi, M., Haschke, H., Hobbs, J., Humphris, A. & McMaster, T. (2003). Tour de force microscopy. *Mater Today* **6**, 30–37.

Moszner, N. & Salz, U. (2001). New developments of polymeric dental composites. *Prog Polym Sci* **26**, 535–576.

Niu, W., Eto, J.N., Kimura, Y., Takeda, F.H. & Matsumoto, H. (1998). A study on microleakage after resin filling of class II cavities prepared by Er:YAG laser. *J Clin Laser Med Surg* **16**, 227–231.

Oliveira, S., Pugach, M., Hilton, J., Watanabe, L., Marshall, S.J. & Marshall, G.W., Jr. (2003). The influence of the dentin smear layer on adhesion: A self-etching primer vs. a total-etch system. *Dent Mater* **19**, 758–767.

Park, J., Chang, J., Ferracane, J. & Lee, I.B. (2008). How should composite be layered to reduce shrinkage stress: Incremental or bulk filling? *Dent Mater* **24**, 1501–1505.

Pfeifer, C.S.C., Ruggiero, R.B. & Cardoso, E.C. (2006). Influence of cavity dimensions, insertion technique and adhesive system on microleakage of Class V restorations. *J Am Dent Assoc* **137**, 197–202.

Saeki, K., Marshall, S.J., Gansky, S.A. & Marshall, G.W., Jr. (2001). Etching characteristics of dentin: Effect of ferric chloride in citric acid. *J Oral Rehabil* **28**, 301–308.

Santos, G.B., Medeiros, I.S., Fellows, C.E., Muench, A. & Braga, R.R. (2007). Composite depth of cure obtained with QTH and LED units assessed by microhardness and micro-Raman spectroscopy. *Oper Dent* **31**, 79–83.

Sensi, L.G., Marson, F.C., Baratieri, L.N. & Junior, S.M. (2005). Effect of placement techniques on the marginal adaptation of class V composite restorations. *J Contemp Dent Pract* **4**, 17–25.

Silva, M.F.A., Davies, R.M., Stewart, B., Devizio, W., Tonholo, J., Silva, J.G., Jr. & Pretty, I.A. (2006). Effect of whitening gels on the surface roughness of restorative materials *in situ*. *Dent Mater* **22**, 919–924.

Takamizawa, T., Yamamoto, A., Inoue, N., Tsujimoto, A., Oto, T., Irokawa, A., Tsubota, K. & Miyazaki, M. (2008). Influence of light intensity on contraction stress of flowable resins. *J Oral Sci* **50**, 37–43.

Uctasli, S., Tezvergil, A., Lassila, L.V.J. & Vallittu, P.K. (2005). The degree of conversion of fiber-reinforced composites polymerized using different light-curing sources. *Dent Mater* **21**, 469–475.

Versluis, A., Douglas, W.H., Cross, M. & Sakaguchi, R.L. (1996). Does an incremental filling technique reduce polymerization shrinkage stresses? *J Dent Res* **75**, 871–878.

Waldman, G.L., Vaidynathan, T.K. & Vaidynathan, J. (2008). Microleakage and dentin-to-resin interface morphology of pre-etching versus self-etching adhesive systems. *Open Dent J* **28**, 120–125.

Xie, H., Zhang, F., Wu, Y., Chen, C. & Liu, W. (2008). Dentine bond strength and microleakage of flowable composite, compomer and glass ionomer cement. *Aust Dent J* **53**, 325–331.

Yap, A.U.J. (2000). Effectiveness of polymerization in composite restoratives claiming bulk placement: Impact of cavity depth and exposure time. *Oper Dent* **25**, 113–120.

Yazici, A.R., Celik, C., Dayanqac, B. & Ozqunaltay, G. (2008). Effects of different light curing units modes on the microleakage of flowable composite resins. *Eur J Dent* **2**, 240–246.