MARGINAL MICROLEAKAGE: CRITICAL ANALYSIS OF METHODOLOGY

Margareth Calvo Pessutti Nunes[1]
Eduardo Batista Franco[2]
José Carlos Pereira[3]

[1]*FOB/USP Restorative Dentistry Specialist.*

[2]*Associate professor. Department of Dentistic. School of Dentistry of Bauru, University of São Paulo.*

[3]*FOB/USP Professor of Dentistry, Edodontics and Dental Materials.*

NUNES, Margareth Calvo Pessutti; FRANCO, Eduardo Batista; PEREIRA, José Carlos. Marginal microleakage: critical analysis of methodology. *Salusvita*, Bauru, v. 24, n. 3, p. 487-502, 2005.

ABSTRACT

The longevity of restorative procedures is related to, among other factors, a perfect adaptation and a strong, long-term union between dental restorative materials and teeth structures. The knowledge of the factors causing marginal leakage is essential to develop proce - dures and materials in order to minimize the problem. In the same manner, the importance of choosing a standardized technique is necessary to analyze microleakage and to relate and discuss the results presented in literature. There are many laboratory tests to measure the marginal adaptation between tooth and restorative material, and each one has its own characteristics and properties. Once there is no standardization of techniques to analyze marginal leakage at literature, the aim of this review was to perform a criti - cal evaluation related to factors influencing some techniques and consequently the results. The subject is of interest to researchers of the methodologies presented in literature.

Received on: July 28, 2004.
Accepted on: May 5, 2005.

KEY WORDS: microleakage; microleakage tests; dental structures

NUNES, Margareth Calvo Pessutti; FRANCO, Eduardo Batista; PEREIRA, José Carlos. Marginal microleakage: critical analysis of methodology. *Salusvita*, Bauru, v. 24, n. 3, p. 487-502, 2005.

INTRODUCTION

The search for restorative materials with the ability to promote dental structure sealing reflects the constant introduction of new products in the market. Although this frequent renewal of products is also a result of the technological evolution and refinement of research, it is a challenge to professionals who test these materials, since up to now there is no material that is restoration groove-free (DAVIDSON; FEILZER, 1997; PRATI et al.,1997). Laboratory studies are used due to the immediate results obtained but they are not complete and do not allow a comprehensive analysis and respective direct extrapolation to the forecast of the clinical behavior of materials. The laboratorial tests used to assess the marginal leakage of lining and/or restorative materials is based mainly in the evaluation of the penetration of colorants and analysis of grooves between the restoration and the dental structure in the attempt to simulate the same alteration that occurs during its behavior in the oral milieu. In this context, the forecast for clinical performance of materials based on its properties seems a complex goal, if not unattainable at all.

Facing the reasonable number of available tests for such evaluation, it is up to the researcher to know the advantages and limitations in order to select the one most appropriated to test the hypothesis of their own study.

Diverging results, due to different methodologies, make it difficult to understantand the issue and do not allow discussions by comparison of data.

The present study aims to list and discuss critically the inherent factors to the techniques that use colorants to evaluate marginal leakage and that may interfere in results and in the comparison and discussion of values.

Definition

Microleakage is defined as the passing, not clinically detected, of bacteria, fluids, molecules or ions between the cavity wall and the restorative material (KIDD, 1976). It is a phenomenon that involves diffusion, thus the knowledge of the dynamic relation between the dental structure and the restorative material is of prime importance (TROWBRIDGE, 1987).

Microleakage is considered as a main factor in the longevity of restorations and can lead to marginal degradation, relapsed

caries, post-operative sensibility, hypersensitivity and even pulp pathologies (ALANI; TOH, 1997).

The infiltration, although refered in many studies as just the in the cavo-surperficial margin, has importance in other regions of the restoration. In this way, some factors may influence in the adaptation of a restoration: type of substrate (BRACKETT et al., 1998; SCHMALZ et al., 2001), form of the cavity (cavity configuration), localization of margins, techniques of insertion of the restorative material, type of restoration material and its way of activation, utilization of base/lining (TAYLOR; LYNCH, 1992) and types of finishings (TAYLOR; LYNCH, 1992; ALANI; TOH, 1997).

Studies on marginal leakage presented many methodologies for its evalution. In this sense, possible intervenient factors will be suggested that may compromise the collection, comparison and discussion of results.

Bovine Teeth X Human Teeth

The use of bovine teeth instead of human teeth has been accepted in many studies on marginal infiltration (BRACKETT et al., 1997; BRACKETT et al., 1998). The advantages of bovine teeth, such as easy availability (SCHMALZ et al., 2001), similarity at the moment of extraction (SCHMALZ et al., 2001) and more resistance against infection transmission (BRACKETT et al., 1997; BRACKETT et al., 1998) make them a choice item for many studies.

Fish in 1933 demonstrated the normal dentin should allow the penetration of colorants into the inner part of the dentinal tubules of human teeth. Later, following the same idea, Swartz and Phillips (1961) in a review on microinfiltration showed that the dental structure is permeable to the diffusion of fluids by natural or acquired defects.

According to Going (1972) many studies conducted to evaluate the permeability of the interface between the teeth and the restoration are sometimes naive in the sense that they place emphasis in the fact that restorations are not fixed conditions, inert and impermeable as clinicians would expect. The authors also comment that the presence of sclerotic dentin, reparative or both still represents a mechanism of defense and reaction to some sort of injury. In the presence of these tissues the permeability decreases significantly. Fresh enamel allow the penetration of fluids what decreases with age by the filling of the intercristaline spaces with ions from saliva. This factor should be questioned or mentioned in studies since most of the time only third molars are indicated for exodonty.

According to Nakamich et al. (1983) the use of bovine incisives is a more appropriate choice than human teeth to conduct adhesion studies, although Retief (1991) reported that bovine teeth show a high degree of infiltration and low resistance values to adhesion in relation to the one obtained with human teeth.

Nakabayashi and Pashley (2000) point out that the human dentin is more resistant to acids that the bovine dentin. These authors also suggest that, despite the availability of bovine dentin, the use of human dentin should be retained.

While the permeability of bovine teeth is questioned as more similar to human teeth, Schmalz et al. (2001) reported that bovine dentin presents low variability, somewhere around half of what is found for human dentin. The number and the diameter of the dentinal tubules in the crown of bovine teeth are similar to the human dentin, even though the density of bovine tubules is significantly higher (SCHILKE et al., 2000). It was observed that the composition and the ultra-structure of resin tags in conditioned bovine and human dentin are similar *in vitro,* and that despite the described three mechanisms for tag resin formation *in vitro*, probably two of them can be observed (TITLEY et al., 1995). Bovine dentin, close to the enamel-cement junction (SCHMALZ et al., 2001), as well as the crown dentin (SCHILKE et al., 2000) are alternatives comparable to human crown dentin for tests on the dentinary permeability. Efes et al. (2003) conducted a study to assess whether bovine teeth are comparable substrates to human teeth in studies on microinfiltrantion in direct restoration with composite resins. Conclusion was that these teeth can be safely used as an alternative substrate to human teeth and that the bovine crown dentine is a reliable substitute of the human teeth in studies on adhesion and microinfiltration.

Restorative procedures

The marginal integrity of the interface restoration tooth depends on many factors, such as the type of restorative material, physical properties of the material, the interaction of materials, the physical properties of dental tissues (enamel, dentin and cement) and the interaction of the oral milieu.

Microleakage also contributes to corrosion, dissolution or discoloring of certain restorative materials (TROWBRIDGE, 1987). This problem is more severe in composite resins mainly due to some properties inherent to the material (such as the polimerization contraction), differences in the coefficient of thermal expansion of the tooth and to the restoration, the adsortion of water of the restorative

material while exposed to the oral millieu, quality of the hybrid layer, technique of insertion and the C factor for configuration of the cavity walls (BOWEN et al., 1982; BULLARD et al., 1988; HANSEN, 1982; RETIEF, 1994; TORSTENSEN; BRÄNNSTRÖM, 1988; YEARN, 1985).

For composite resins, the linear thermal expansion coefficient, in relation to the dental structure, induces a stress that leads to disconnection of the binding between them (ABDALLA; DAVIDSON, 1993). When the linear thermal expansion coefficient of the restorative material is significantly different of the dental structure, the reduction in temperature create a negative interfacial pressure. This action favors the penetration of oral fluids into the margins. The contrary occurs with the increase of the temperature, the interfacial pressure increases and thus the fluids are forced out of this interface. The result of great differences in the linear thermal expansion coefficient is a great change in the internal pressure of the interfacial surface and, as a result, studies suggest a definitive relation between this coefficient and the degree of infiltration.

Finishing and polishing procedures

These techniques can be questioned since the study by Brackett et al. (1997), revealed that the finishing of composite resin restoration with carbide drills promotes greater values for infiltration in the dentin margins. In a similar way, Prati et al. (1997) reported that there was a greater number of composite resin restoration with infiltration, in deep areas of the restoration, coming from the dentin margin after the finishing with drills and disks of serial granulation, which promoted a greater wearing in the dentin than in the composite resin, leading to a small step. Some finishing and polishing procedures can create a gap in the tooth restorative material interface and thus facilitate infiltration. Therefore, the delay period for restorative materials to show complete setting time reaction, hygroscopic expansion, particularly in the case of composite resins, in order to allow finishing and polishing procedures, should be respected.

The majority of studies on marginal leakage are conducted *in vitro*. It is questioned if the adhesion procedures would lead to the same results of preservation of the restoration if they were conducted *in vivo*. It is known that the adhesive procedures lead to the formation of hybrid layers and tags with different characteristics if used in *in vivo* or *in vitro* dentin due to the differences inherent to the substracts, such as the presence of fluid and hydrostatic pressure form the dentinary tubules (TITLEY et al., 1995). The marginal gingival integrity,

in vivo and in vitro, of composite resin restorations was investigated by Abdalla and Davidson (1993) and the results showed that in all *in vivo* groups there was a greater infiltration if compared to the similar groups *in vitro*. Only 60% of the *in vitro* specimens suffered infiltration whereas there was infiltration in 100% of the *in vivo* specimens. In this sense, the results of the studies *in vitro* can not be directly compared to those conducted clinically or *in vivo*.

Obtaining specimens

There are variations in the literature in what concerns the technique for obtaining the specimens for analysis of marginal infiltration. In this sense, the discussion of results is jeopardized. The cut of specimens is the most used method for the measurement of the microinfiltration, although the site where the cut is made and if it would be coincident with the real site of the infiltration are open to discussion (YOUNGSON, 1992; WIBOWO; STOCKTON, 2001) in order to determine the pattern of infiltration in that specimen (GWINNETT et al., 1995). Some authors do the cut in the central part of the restoration (BRACKETT et al., 1997; SANTINI et al., 2001) producing only two interfaces for evaluation. Others, (PRATI et al., 1997; CAMPOS et al., 2002) make several cuts allowing the selection of the more infiltrated one among the various exposed facets by different sections. However, the hemi-section of the restoration is also suggested with its latter removal with an excavation instrument aiming to evaluate the penetration of the colorant observing gall the margins of the cavity (WIBOWO; STOCKTON, 2001).

According to Gwinnett et al. (1995) the sectioning of specimens from its middle point does not allow an adequate evaluation of the infiltration, even when analyzed under stereomicroscopy, revealing lesser values for infriltration in relation to methods that use multiple sections analyzed by software and in relation to methods with demineralization of the specimens and determination of the pattern of infiltration under stereomicroscopy.

Déjou et al. (1996) determined the influence of five different evaluation criteria in the statistical analysis of a study on marginal leakage using 13 different restorative systems. For that the specimens where sectioned in 3 slides of 300 µm, creating six interfaces that where examined on microscopy (100x) in order to select the slide with greater infiltration in extension from the cavo-superficial margin to the deeper part. For evaluation, five criteria were used: mean infiltration for the six slides by each tooth, median of these data; mode of these data, maximum penetration of colorant by tooth

and percentage of the teeth without infiltration by the colorant as measured in the six slides. It was demonstrated that the results of infiltration depend on the different characteristics of the used materials as well as of the criteria of evaluation. Despite the fact that the penetration of colorant can be represented by carious criteria, the maximum penetration in each tooth seems to be the best since, theoretically, it is the most relevant *in vivo*.

Thermal and mechanical cycling

The thermal and mechanical cycling have been included in the studies of microleakage as variables with the purpose of stressing the dental/restoration simulating thermal changes or/and mastigatory stress that occurred in the intra-oral milieu and that can the life of restoration decrease.

In 1970, Jörgensen introduced the term "mechanical percolation" to indicate mechanical factors in the oral cavity that may induce symetric pressures in the restoration and in the liquid in the space between the restoration and the dental structure and, therefore, stress its margins and compromise the structural and marginal resistance.

It has been demonstrated that the mechanical cycling of restored tooth can increase the amount of permanent mechanical deformation or only while teeth are under stress (JÖRGENSEN et al., 1976). The difficulty, however, is the lack of standardization in regarding the value and the dynamic of the load and the number of cycles used.

In the thermal cycling the extreme differences of temperature, compatible to the ones of the oral cavity, induce differences in expansion and contraction of the restorative material and dental structure leading to "percolation" and, therefore, microinfiltration (COY, 1953; NELSEN et al., 1952; PAFFENBARGER et al., 1953).

Thermal cycling influences in the marginal infiltration of restoration that have a high coefficient of linear expansion and high thermal difussibility, that promotes the contraction and expansion of the restoration in a different way than when it occurs in the dental structure, weakening the restoration dental interface. Despite of that, it is suggested that the thermal cycling and the mechanical cycling, on their own, do not influence the microleakage of composite resin, what can be seen in the study by Rigsby et al. (1992), in which the microinfiltration of restorations was significantly higher in the margins in cement when they used the thermal and mechanical cycling conjointly. Studies that compare the marginal infiltration between thermocycled and non-thermocyled groups (WENDT et al., 1992; CHAN; GLYN JONES, 1994; ROSSOMAN-

DO; WENDT JR, 1995; BIJELLA et al., 2001) with different number of thermal cycles (CRIM et al., 1985; CRIM; SHAY 1987; CRIM; GARCIA-GODOY, 1987; VERONEZI, 2000) carried results with statistically non significant differences. In the same way, the number of thermal cycles does not influence directly the increase of the marginal leakage in composite resin restoration as demonstrated by Campos et al. (2002), which did thermal cycling va rying the number of cycles (zero, 500, 1000, 2500 and 5000) and found results with non-significant statistical differences between the groups. This result suggests that the technique of thermal cycling does not influence in the marginal infiltration of composite resin restoration and that there is no reason to use them in the laboratory aiming to simulate a clinical condition. However, in some studies (CRIM; MATTINGLY 1981; CRIM et al., 1985; LITKOWSKI et al., 1989; SCHUCKAR; GEURTSEN, 1997; HAKIMEH et al., 2000) there are reports of significant differences between the restoration margins of termcyled groups and non-thermo cycled groups.

Therefore, it can be understood that the literature carries a great variety of results obtained in relation to the effectiveness of the thermal cycling in the study of marginal microleakage (CHAN; GLYN-JONES, 1994; CRIM, 1989; CRIM; MATTINGLY, 1981; CRIM et al., 1985; KIDD, 1976; TORTENSEN; BRANNSTRON, 1988), and various are the factors suggested as reason for these differences: the different temperatures used the thermal cycling, period of each bath, type of substance in which the bodies are immersed and the number of cycles.

This variety of methodologies and contradictory results in what regards thermal cycling reveals the lack of a consensus on this subject and the necessity for standardization of microleakage tests (RASKIN, 2000; SHORTAL, 1982; TAYLOR; LYNCHY, 1992).

Colorants and chemical tracers

The diameter of the dentinal tubules and the number of tubules by surface unit increase with the convergence of tubules toward the pulp.

Virtually, this is not desirable in order to use any colorant particle that has a diameter greater than the inner diameter of the dentinal tubule (1 to 4 μm). Therefore, studies on dentin infiltration show some degree of pigmented dentin, what should be differentiated from real microleakage between the cavity and restoration. This has suggested that dentinal involvement can be used as relative indicator for the marginal infiltration (GLY N -JONES et al., 1988).

There are many techniques to evaluate *in vivo* and *in vitro* forms of sealing of the restoration-tooth interface. Methods for *in vivo* assessment include radioactive isotopes, chemical tracers, bacteria, scan electronic microscopy, artificial caries, colorants and other (SHORTALL, 1982; ALANI; TOH, 1997). The use of organic colorants is one of the oldest methods in use, it is affordable and detects infiltration *in vivo* without need of a chemical reaction or radiation as is the case of chemical tracers. However, disadvantages are the subjectivity in the quantification of the penetration of the colorant (KIDD, 1976), the need to destroy the specimen for evaluation (YOUNGSON, 1992; WIBOWO; STOCKTON, 2001) and the possibility of variation in the dentin permeability in the different specimens, which could affect the results (SHORTALL, 1982; GALE; DARVELL, 1999). A literature review showed a great variability in the choice of the colorant, of solutions or suspensions of particles of different sizes. The colorant concentration also varied from 0.5% to 10%, whereas the period of immersion of the specimen in the colorant varied from 4 to 72 hours or more. This showed that in fact different concentrations of colorant can vary in time of penetration from 5 minutes to 1 hour (CHRISTEN; MITCHELL, 1966).

Fluorescent colorants revealed themselves to be particularly useful as markers due to their ability to be detected in diluted concentration, their low cost and also to the fact that they are non-toxic and, therefore, can be used in clinical and laboratorial studies (GOING, 1972).

Fluorceine is a non-toxic colorant and shows good contrast. However, some used materials can jeopardize its effect, such as zinc oxide and eugenol (CHRISTEN; MITCHELL, 1966).

Little emphasis has been placed on the difference of the size of molecules and the colorant particles and its behavior when used under testing conditions. Some colorants, such as basic fucsin, preferably binds to decayed dentin. Methilen blue has affinity to glycosaminoglycans present in dentin (FARNDALE et al., 1986) and the silver nitrate, with its very low molecular weight (0.059 nm), when compared to the size of a tipical bacteria (0.5-1.0 µm), is more penetrative in this way (ALANI; TOH, 1997). Studies that used chemical tracers had problems similar to studies with colorants, particularly in the interpretation of results.

Colorants move to be bound to dental structure or restoratiove material under investigation can potentially reveal a more ample or deeper groove than the real situation.

Once the dentinal permeability acts as factor that should be bore in mind in the moment of the evaluation of the degree of infil-

tration, it is necessary to differentiate the dentin staining by the colorant to the real microleakage between the cavity wall and the restorative material (ALANI; TOH, 1997; GALE; DARVELL, 1999). For this reason Kidd (1976) and Trowbridge (1987) suggested the study of microinfiltration in the marginal defects by bacterian invasion, which would be more clinically realistic than the method of diffusion with colorants or isotopes.

EVALUATION OF THE RESULTS OF MICROLEAKAGE

Analysis of the microleakage can be done by qualitative or quantitative methods. The qualitative method is the most used one (BRACKETT et al. 1997; BRACKETT et al., 1998; ZANATA et al., 1998) because it is easy, needs only slide projector, magnifying glasses and a low-magnification microscope. Through a score system, calibrated evaluators analyze individually the infiltration in the restorative dental material interface in relation to the cavity walls and the results are compared. When there is disagreement, a new evaluation is conducted and the consensus is searched. In the quantitative method (YOUNGSON, 1992; FRIEDL et al., 1997; WIBOWO; STOCKTON, 2001) the use of a microscope with gauged oculars is needed (CRIM; SHAY, 1987) or a software (GWINNETT et al., 1995; CAMPOS et al., 2002) and some method to copy images so the infiltration can be measured in metric units or in percentage (PRATI et al., 1997). It was also reported a quantitative method by absorbance, using the volumetric measure of the colorant infiltration (MAGALHÃES et al., 1999) and another measuring the infiltration in a 3-D mode (YOUNGSON, 1992). The quantitative method is more expensive and detailed but allows parametric statistical analysis making the study of values and results easier. The choice of method may not interfere in the process of analysis of the infiltration to the extend of modifying its final result. However, the use of standardized procedures and of quantitative methods, as well as qualitative measures under scan electronic microscopy for the evaluation of the adaptation of the restorations in the cavity (TAYLOR; LYNCH, 1993) would allow the attainment of more reliable results and would be liable to discussion in the studies of microleakage.

In preliminar studies Campos et al. (2002) observed that the analysis of microleakage, even by quantitative method, is somewhat empirical and subjective. This happens when softwares are used to

measure the penetration of the colorant into the specimens in which the evaluator is the one that controls the extension that the infiltration has attained. It is understood that the evaluator has a visual limitation when he/she observes the colorant penetration only through the computer monitor because, despite the image being digitally amplified, there is always some distortion making the real analysis of the infiltration difficult. These authors verified that values for infiltration differs in the same specimens under 0.5% basic fucsin when analysied in software and under stereomicroscopy. Therefore, a previous analysis of the specimen is suggested under microscopy in order to outline the infiltration which, later on, is calculated by the software. It is also important that this previous analysis confirms if the penetration of the colorant occurred through the restorative dental material interface and not through another area of the tooth, such as a not impermeabilized area around the restorations (GALE; DARVELL, 1999) or through the cement wear and dentin exposition caused by finishing and polishing procedures (PRATI et al., 1997), which can interfere in the results.

FINAL CONSIDERATIONS

The marginal integrity of restoration is essential to attain clinical success, in the absence of which the onset of infiltration in the restorative dental material interface takes place that can lead to secondary and injuries to the dentin-pulp complex.

The literature shows that it is not possible to reproduce *in vitro* the conditions found *in vivo* in the oral cavity considering that patients are different in terms of the risk for caries, since they have diverse diets and dental plaque control. However, laboratorial studies can focus their result on the clinical performance of the evaluated materials. *In vivo* studies have been largely used to evaluate microleakage in the restoration dental interface.

In this respect, a review in the literature shows a variety of methodologies for the studies of marginal infiltration that have advantages and/or limitations. No method used in the evaluation of microinfiltration can be considered ideal.

In spite of the fact that the analysis of marginal infiltration by colorants is still largely used in research, it presents difficulties proper of the method and there is need for standardization. An adequate methodology leads to a correct evaluation and interpretation of the result, allowing in this way comparisons and discussions.

There are many studies on marginal infiltration. However, most of them do not mention the difficulties proper to the methodology of

analysis as well as do not question the differences found in values of infiltration in the different studies that use the same material and operative technique.

REFERENCES

1. ABDALLA, A. I.; DAVIDSON, C. L. Comparison of the marginal integrity of in vivo and in vitro class II composite restorations. *J. Dent.,* v. 21, n. 3, p. 158-162, June 1993.
2. ALANI, A. H.; TOH, C. G. Detection of microleakage around dental restorations: a review. *Oper. Dent.*, v. 22, p. 173-185, 1997.
3. BIJELLA, M. F. B. et al. In vitro quantitative evaluation of marginal microleakage in class II restorations confected with a glass ionomer cement and two composite resins. *Pesq. Odont. Bras.*, v. 15, n. 4, p. 277-282, out. /dez. 2001.
4. BOWEN, R. L. et al. Hardening shrinkage and hygroscopic expansion of composite resins. *J. Dent. Res.*, v. 61, n. 5, p. 654-682, May 1982.
5. BRACKETT, W. W. et al. Effect of finishing method on the microleakage on class V resin composite restorations. *Am. J. Dent.*, v. 10, n. 4, p. 189-191, Aug. 1997.
6. _____. Microleakage of class V compomer and light-cured glass ionomer restorations. *J. Prosthet Dent.*, v. 79, n. 3, p. 261-263, Mar. 1998.
7. BULLARD, R. H. et al. Effect of coefficient of thermal expansion on microleakage. *J Amer. Dent. Ass.*, v. 116, p. 871-874, June 1988.
8. CAMPOS, B. B. et al. Efeito do número de ciclos térmicos na infiltração marginal de restaurações de resina composta. *Pesquisa Odontológica Brasileira*, Águas de Lindóia, 19, 2002. Anais, Águas de Lindóia, Resumo n. Pa 239, p.152, 2002.
9. CHAN, M. F. W. Y.; GLYN-JONES, J. C. Significance of thermal cycling in microleakage analysis of root restorations. *J. Dent*., v. 22, n. 5, p. 292-295, Jan. 1994.
10. CHRISTEN, A. G.; MITCHELL, D. F. A fluorescent dye method for demonstrating leakage around dental restorations. *J. Dent. Res.*, v. 45, n. 2, p. 1.485-1.492, 1966.
11. COY, H. D. Direct resin fillings. *J Amer. Dent. Ass.,* v. 47, n. 5, p. 532-537, Nov. 1953.
12. CRIM, G. A.; GARCIA-GODOY, F. Microleakage: the effect of storage and cycling duration. *J. Prosth. Dent.,* v. 53, n. 5, p. 574-576, May 1987.

13. CRIM, G. A.; MATTINGLY, S. L. Evaluation of two methods for assessing marginal leakage. *J. Prosth. Dent.,* v. 45, n. 2, p. 160-163, Feb. 1981.
14. CRIM, G. A. Effect of immediate versus delayed thermal stress on two adhesives. *Quintessence Int*., v. 20, n. 7, p. 517-519, July 1989.
15. CRIM, G. A. et al. Comparison of four thermocyclin techniques. *J. Prosth. Dent.,* v. 53, n. 1, p. 50-53, Jan. 1985.
16. CRIM, G. A.; SHAY, J. S. Microleakage pattern of a resin-veneered glass-ionomer cavity liner. *J. Prosth. Dent.*, v. 58, n. 3, p. 273-276, Sept. 1987.
17. DAVIDSON, C. L.; FEILZER, A. J. Polymerization shrinkage and polymerization shrinkage stress in polymer-based restoratives. *J. Dent.*, v. 25, n. 6, p. 436-440, Nov. 1997.
18. DÉJOU, J. et al. Influence of criteria on the results of in vitro evaluation of microleakage. *Dent. Mater.*, v. 12, n. 6, p. 342-349, Nov. 1996.
19. EFES, B. G. et al. Microleakage of composite materials in bovine and human teeth. *J. Dent. Res.,* v. 82, Spec. Issue, Abstract #1957, p. 256, 2003.
20. FARNDALE, R. W. et al. Improved quantification and discrimination of sulphated glycosaminoglycans by use of dimethyl methylene blue. *Biochim. Biophys. Acta*, n. 883, p. 173-177, 1986.
21. FISH, 1933. In: SWARTZ, M.; PHILLIPS, R. W. In vitro studies on the marginal leakage of restorative materials. *J. Amer. Dent. Ass.*,v 62, p. 141-151, Feb. 1961.
22. GALE, M. S.; DARVELL, B. W. Dentine permeability and tracer tests. *J. Dent.*, v. 27, n. 1, p. 1-11, Feb. 1999.
23. GLYN-JONES, J. C. et al. Marginal leakage associated with three posterior restorative materials. *J. Dent.,* v. 16, p. 130-134, 1988.
24. GOING, R. E. Microleakage around dental restorations: a summarizing review. *J. Amer. dent. Ass.,* v. 84, p. 1.349-1.357, June 1972.
25. GWINNETT, J. A. et al. Comparison of three methods of critical evaluation of microleakage along restorative interfaces. *J. Prosthet. Dent.,* v. 74, p. 575-585, Dec. 1995.
26. HAKIMEH, S. et al. Microleakage of compomer class V restorations: Effect of load cycling, thermal cycling, and cavity shape differences. *J. Prosth. Dent.,* v. 83, n. 2, p. 194-203, Feb. 2000.

27. HANSEN, E. K. Visible light-cured composite resins: polymerization, contraction pattern and hygroscopic expansion. *Scand. J. Dent. Res*., v. 90, p. 329-335, 1982.
28. JÖRGENSEN, K. D. Some observations on silicate cement. *Acta. Odont. Scand.*, v. 28, p. 117-127, 1970.
29. JÖRGENSEN. et al. Deformation of cavities and resin filling in loaded teeth. *Scand. J. dent. Res.*, v. 84, p. 46-50, 1976.
30. KIDD, E. A. M. Microleakage: a review: *J. Dent.*, v. 4, n. 5, p. 199-206, Sept. 1976.
31. LITKOWSKI, L. J. et al. comparison of thermal cycling methods for evaluation microleakage. *J. Dent. Res.*, v. 68, n. 2, p. 207, 1989.
32. MAGALHÃES, C. S. de. et al. Volumetric microleakage assessment of glass-ionomer-resin composite hybrid materials. *Quintessence Int,* v. 30, n. 2, p. 117-121, Feb. 1999.
33. NAKABAYASHI, N.; PASHLEY, D. H. Properties of dentin. In: _____. Hibridização dos tecidos dentais duros. *Quintessence*, São Paulo, Chapter II, p. 35, 2000.
34. NAKAMICHI, I. et al. Bovine teeth as possible substitutes in the adhesion test. *J. Dent. Res.*, v. 62, p. 1.076-1.081, 1983.
35. NELSEN, R. J. et al. Fluid exchange at the margins of dental restoration. *J. Amer. Dent. Ass.,* v. 44, n. 3, p. 288-295, Mar. 1952.
36. PAFFENBARGER, G. C. et al. Direct and indirect filling resins: A review of some physical and chemical properties. *J. Amer. Dent. Ass*., v. 47, p. 516-524, 1953.
37. PRATI, C. et al. Marginal morphology of class V composite restorations. *Am. J. Dent.,* v. 10, n. 5, p. 231-236, Oct. 1997.
38. RASKIN, A. et al. Microleakage in vitro tests: evaluation criteria and statistical methods. *J. Dent. Res.*, v. 78, Special Issue, Abstract n. 1680, p. 353, 2000.
39. RETIEF, D. H. Do adhesives prevent microleakage? *Int. Dent. J.,* v. 44, n. 1, p. 19-26, 1994.
40. RETIEF, D. H. Standardizing laboratory adhesion tests. *Amer. J. Dent.,* v. 4, n. 5, p. 231-236, Oct. 1991.
41. RIGSBY, D. F. et al. Effect of axial load and temperature cycling on microleakage of resin restorations. *Am. J. Dent.*, v. 5, n. 3, p. 155-159, June 1992.
42. ROSSOMANDO, K. J.; WENDT, S. L. Thermocycling and dwell times in microleakage evaluation for restoration for bonded restorations. *Dent. Mat.,* v. 11, p. 74-151, Jan. 1995.

NUNES, Margareth Calvo Pessutti; FRANCO, Eduardo Batista; PEREIRA, José Carlos. Marginal microleakage: critical analysis of methodology. *Salusvita*, Bauru, v. 24, n. 3, p. 487-502, 2005.

43. SANTINI, A. et al. Effect of composite resin placement techniques on the microleakage of two self-etching dentin-bonding agents. *Am. J. Dent.,* v. 14, n. 3, p. 132-136, June 2001.
44. SCHILKE, R. et al. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. *Arch. Oral Biol.*, v. 45, n. 5, p. 355-361, May 2000.
45. SCHMALZ, G. et al. Permeability characteristics of bovine and human dentin under different pretreatment conditions. *J. Endod.,* v. 27, n. 1, p. 23-30, Jan. 2001.
46. SCHUCKAR, M.; GEURTSEN. W. Proximo-cervical adaptation of class II composite restorations after thermocycling: a quantitative and a qualitative study. *J. Oral Rehab.*, v. 20, n. 10, p. 766-775, Oct. 1997.
47. SHORTALL, A. C. Microleakage, marginal adaptation and composite resin restorations. *Brit. Dent. J.,* v. 153, n. 5, p. 223-237, Sept. 1982.
48. SWARTZ, M.; PHILLIPS, R. W. In vitro studies on the marginal leakage of restorative materials. *J. Amer. Dent. Ass.,* v. 62, p. 141-151, Feb. 1961.
49. TAYLOR, M. J.; LYNCH, E. Microleakage. *J. Dent.,* v. 20, n. 1, p. 3-10, Jan. 1992.
50. TAYLOR, M. J.; LYNCH, E. Marginal adaptation. *J. Dent.*, v. 21, n. 5, p. 265-273, Oct. 1993.
51. TITLEY, K. et al. The composition and ultra structure of resin tags in etched dentin. *Am. J. Dent.*, v. 8, n. 5, p. 224-230, Oct. 1995.
52. TORSTENSEN, B.; BRÄNNSTRÖM, M. Contraction gap under composite resin restorations: effect of hygroscopic expansion and thermal stress. *Oper. Dent.*, v. 13, n. 1, p. 24-31, Jan./Feb. 1988.
53. TROWBRIDGE, H. O. Model systems for determining biologic effects of microleakage. *Oper. Dent.*, v. 12, p. 164-172, 1987.
54. VERONEZI, M. C. et al. *Influência da ciclagem térmica e do método de avaliação na determinação da microinfiltração em restaurações de resina composta*. 2000. 222 p. Tese (Doutorado)– Faculdade de Odontologia de Bauru, Universidade de São Paulo, Bauru, 2000.
55. WENDT, S. L. et al. The effect of thermocycling in microleakage analysis. *Dent. Mat.,* v. 8, p. 181-184, May 1992.
56. WIBOWO, G.; STOCKTON, L. Microleakage of class II composite restorations. *Am. J. Den.,* v. 14, n. 3, p. 177-185, June 2001.

57. YEARN, J. A. F. Factors affecting cure of visible light activated composite. *Int. Dent. J.,* v. 35, n. 3, p. 218-225, Sept. 1985.

58. YOUNGSON, C. C. A technique for three-dimensional microleakage assessment using tooth sections. *J. Dent.*, v. 20, n. 4, p. 231-234, Aug. 1992.

59. ZANATA, R. L. et al. Avaliação in vitro da microinfiltração em cavidades de classe V restauradas com diferentes combinações de resina composta e cimento de ionômero de vidro. *Rev. Odontol. Univer. São Paulo,* v. 12, n. 2, p. 113-119, abr./jun. 1998.