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Marginal integrity and microleakage of direct and indirect composite inlays – SEM and stereomicroscopic evaluation

Integridade e microinfiltração marginal de restaurações diretas e indiretas de compósitos – avaliação por estereomicroscopia e MEV

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ABSTRACT: The aim of this study was to evaluate the microleakage of direct and indirect composite inlays by stereomicroscopy and scanning electron microscopy (SEM). Thirty bovine incisors were ground to obtain an incisal platform, simulating the occlusal surface of a human molar. Each tooth received two 8° proximal cavities with cervical finishing line prepared in dentine or enamel. One of the cavities was filled with Filtek Z250/Single Bond, using the direct technique, and the other was filled with with Solidex/Rely X ARC/Single Bond, using the indirect technique. The samples were stored in water at 37°C for 24 hours and placed in a 50% silver nitrate solution for 6 hours in a dark container. Next, the samples were washed under running water, immersed in a developing solution and exposed to fluorescent light for 12 hours. The teeth were then severed and evaluated for dye penetration by stereomicroscopy and SEM. There were no significant differences between the direct and indirect techniques for the cervical finishing line in enamel, but for the finishing line in dentin, the indirect technique allowed less microleakage than the direct technique. SEM analysis showed leakage similar to that observed by stereomicroscopic analysis. The use of stereomicroscopic and SEM evaluations improves microleakage analysis.

DESCRIPTORS: Composite resins; Dental restoration failure; Dental leakage; Marginal adaptation (dentistry).

RESUMO: O objetivo deste estudo foi avaliar a microinfiltração em restaurações diretas e indiretas em resina composta por meio de estereomicroscopia e microscopia eletrônica de varredura (MEV). Trinta incisivos bovinos foram lixados de forma a produzir uma plataforma incisal, simulando a face oclusal de um molar humano. Cada dente recebeu dois preparos proximais com 8º e término cervical em esmalte ou dentina. Uma cavidade foi restaurada pela técnica direta com Filtek Z250/Single Bond, e a outra pela técnica indireta com Solidex/RelyX ARC/Single Bond. As amostras foram armazenadas em água a 37°C por 24 horas e então imersas em solução de 50% de nitrato de prata por 6 horas em recipiente escuro. As amostras foram lavadas em água corrente, imersas em solução reveladora e expostas à luz fluorescente por 12 horas. Os dentes foram seccionados para avaliação da penetração do marcador em lupa estereoscópica e MEV. Não houve diferença entre as técnicas direta e indireta para o término em esmalte, porém, para o término em dentina, a técnica indireta resultou em menor infiltração. MEV mostrou infiltração similar à da lupa estereoscópica. A análise por meio de lupa estereoscópica e MEV melhora a avaliação da microinfiltração.

DESCRITORES: Resinas compostas; Falha de restauração dentária; Infiltração dentária; Adaptação marginal (odontologia).

INTRODUCTION

Adequate polymerization of resin composite is considered to be a very important factor for assuring appropriate physical and biological properties. Shrinkage stress, however, is one of the inherent disadvantages that occur when visible light-activated resin composites are submitted to light polymerization. Stresses arising from post-gel polymerization shrinkage may produce defects in the composite to tooth bond, leading to failure associated with microleakage, postoperative sensitivity,

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and recurrent caries. These problems are the most frequent consequence of fluid penetrating along cavity walls toward the pulp[23]. Soft-start polymerization with short-pulses of light energy[12,15], in association with glass ionomer as the gingival increment[17], and incremental techniques have been used in an endeavor to minimize this effect.

Different resin-composite inlay systems were also developed and direct composite resin is one of the most popular[3]. However, because of the complexities associated with insertion and finishing techniques in large direct composite restorations, many clinicians have difficulty in establishing proper anatomic form, proximal contour and contact[25]. Indirect inlay systems became popular to overcome this limitation of direct restorations. Shrinkage stress should be minimized with indirect restoration, since polymerization occurs before the restoration is cemented. However, indirect resin restorations require internal adjustment, which could result in poor marginal fit[25].

Resin composite bonding to the acid-etched enamel surface is a clinically well-established treatment procedure in restorative dentistry[26]. However, dentin is a complex, heterogenic, hydrated biological substrate[14]. From this standpoint, adhesion to dentin is not as reliable as adhesion to enamel, although the new adhesives have shown high values in laboratorial tests[20]. When caries occurs in the proximal surface of a posterior tooth, it is not uncommon to have to place the cavity preparation finishing line in dentin, which is another factor that produces poor adhesion and sealing in Class II composite resin restorations. In addition, some studies have demonstrated poorer marginal fit in proximal surfaces than in buccal or lingual surfaces[25]. Studies have shown that indirect restorations exhibited more gap formation and leakage at the gingival margin in dentin than in enamel[16]. Achieving a perfect marginal quality with inlay restorations, when gingival margins are located in dentin, continues to be critical even when the new adhesive systems are used[9].

It has been hypothesized that direct or indirect techniques and different cervical margin cavity types would have an effect on the occurrence of microleakage and gap formation in proximal resin composite inlays. The aim of this study was to investigate microleakage in cervical proximal cavities with the finishing line placed in enamel or dentin, restored with direct or indirect composite resins, as well as to observe the micro-morphology of the hybrid layer.

MATERIALS AND METHODS

Thirty freshly extracted bovine incisive teeth were used for microleakage and SEM analysis. The teeth were cleaned, with calculus and soft tissue deposits being removed with a hand scaler, and then stored in a 0.2% thymol solution (Pharmácia Biopharma Ltda., Uberlândia, Brazil). In order to facilitate manipulation, preparation and restoration of the proximal cavities, every tooth was individually fixed in a cylinder so that the roots were resin-embedded up to 5 mm below the apex.

The coronal portion of the tooth was ground with #600 silicon carbide paper (Norton, Recife, Brazil) to create a surface with dimensions similar to those of a human molar, 3.0 mm (margin in dentin) or 6.0 mm (margin in enamel) short of the cementum-enamel junction in the proximal surface. The pulp canal was exposed and filled with composite resin. A medium-grained diamond bur with an 8-degree taper was used in a standardized fashion, to make the two proximal inlay preparations with convergent angles. The dimensions of the cavity preparation for the inlays were: 4.0 mm in buccolingual width, 4.0 mm of axial wall height and 2.0 mm of gingival wall width (Figure 1).

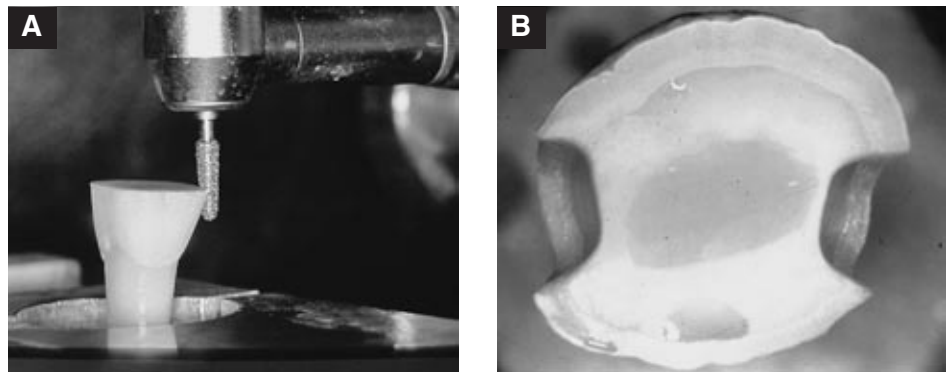

FIGURE 1 - A. Diamond bur positioned for preparing proximal cavities in bovine incisor, which had its incisal portion removed and its pulp canal filled with resin; **B**. Proximal cavities with gingival margin in enamel. Each cavity was restored either with direct or indirect technique.

A one-stage impression was taken using a double viscosity polyvinyl-siloxane (Aquasil, Dentsply DeTrey, Konstanz, Germany) in a stock plastic tray. After 2 hours, the impressions were poured with type IV dental stone (Velmix, Kerr, Romulus, USA). One of these two cavities in the stone dies was randomly selected and used to make the indirect composite inlay, which was manufactured with laboratory composite resin (Solidex, Shofu, Kyoto, Japan), polymerized in a multi-focal laboratory source (Solidilate, Shofu, Kyoto, Japan).

Inlay cavities were restored as follows: one of them with the direct composite technique – (Filtek Z250, 3M-ESPE, St. Paul, MN, USA) and Single Bond (3M-ESPE, St. Paul, MN, USA), and the other with the indirect technique – Solidex cemented with Single Bond and dual cure resin cement (Rely X ARC, 3M-ESPE, St. Paul, MN, USA). In the direct procedure, dentin and enamel surfaces were conditioned with 36% phosphoric acid gel (3M-ESPE, St. Paul, MN, USA) for 15 seconds, rinsed with water for 15 seconds and blot-dried, leaving a moist surface. Single Bond was applied according to the manufacturer's directions and light-polymerized. Z250 composite resin was inserted in three increments and then light-polymerized (XL 3000, 3M Dental Products, St. Paul, USA) for 20 seconds for each restoration. During the first 5 seconds, the light was kept 1 cm away from the restorative material. The other cavity was restored following the indirect procedure. Both the enamel and dentin surfaces were conditioned with 36% phosphoric acid gel for 15 seconds, rinsed with water for 15 seconds and blot-dried, leaving a moist surface. Single Bond was applied and light-polymerized. The inner surface of the Solidex inlay was treated with aluminum-oxide 50 μm airborne particle abrasion (CVM Ltda., Cachoeirinha, Brazil) for 5 seconds and rinsed with water for 10 seconds. One-bottle pre-hydrolyzed silane (Ceramic Primer, 3M-ESPE, St. Paul, MN, USA) was then applied for 1 minute. Rely X ARC was mixed and applied to the inner surface of the Solidex inlay, which was inserted in the cavity. Excess material was removed, and then the restoration was light-polymerized for 40 seconds with an XL 3000 light-activation unit. After 15 minutes, the restorations were finished and polished with diamond burs (KG Sorensen, São Paulo, Brazil), and final finishing was done with aluminum oxide disks (Sof-Lex, 3M-ESPE, St. Paul, MN, USA).

The teeth were removed from their cylinders and their apex and coronal flat surfaces were coated with epoxy resin (Polipox, São Paulo, Brazil). All external surfaces of each tooth were coated with two layers of nail varnish (Risqué, Niasi, Taboão da Serra, Brazil), leaving a 1.0 mm-wide margin around the restoration free of varnish. Specimens were placed in a freshly prepared 50wt% aqueous silver nitrate solution (Quimibrás, Rio de Janeiro, Brazil) for 6 hours and kept in the dark. Next, the teeth were rinsed in tap water for 1 minute and immersed in a photo developing solution (Kodak, Rochester, USA) and exposed to fluorescent light for 12 hours[23]. Then the specimens were rinsed in tap water to remove the photo developing solution. After that, the teeth were sectioned in a mesiodistal direction through the center of each restoration. The cut surfaces were polished with 6, 3, 1 and 1/4 micrometer diamond pastes (Arotec, São Paulo, Brazil). Both halves were evaluated blind and independently by three examiners with a stereomicroscope (Olimpus, Tokyo, Japan) at a 16 X magnification, to determine the microleakage at the gingival margins[21]. The criteria used are shown in Table 1.

TABLE 1 - Dye Penetration Scores used as the criterion for microleakage analysis (Saboia *et al.*[22], 2002).

Scores	Characterization of dye penetration
0	No dye penetration
1	Dye penetration up to 1/3 of the length of the gingival wall
2	Dye penetration from 1/3 to 2/3 of the gingival wall
3	Dye penetration greater than in score 2, but not including the axial wall
4	Dye penetration with penetration spreading along the axial wall

For SEM analysis, the samples were acid-etched with 10% phosphoric acid (Pharmácia Biopharma Ltda., Uberlândia, Brazil) for 5 seconds to remove the smear layer produced by the polishing method. Each specimen was desiccated by immersion in a series of different concentrations of alcohol (Super'Sol, Uberlândia, Brazil). Next, the specimen was fixed onto a metal stub, its surface was coated with a thin layer of gold, and then it was observed under a scanning microscope (LEO, Japan), which examined it by means of backscattered electron images[24]. Overall leakage was calculated based on the method of Sano *et al.*[24] (1995), who defined that the overall leakage scores could be calculated as a percentage of the total cut den-

tin surface margins that were penetrated by silver nitrate, using the equation:

Overall Leakage = p/L × 100
Where:
p = length of silver nitrate penetration along the resin dentin interface and
L = total length of the dentinal cavity wall on the cut surface.

The coefficient of agreement among the three examiners was verified by the Kappa estimate[21]. Subsequently, data were analyzed by the Kruskal-Wallis non-parametric test at the 5% probability level. The SEM data were analyzed by one-way ANOVA and Duncan's multiple range test ($p < 0.05$).

RESULTS

From the stereomicroscopy microleakage analysis, the Kruskal-Wallis test showed that the gingival margin in enamel obtained significantly lower scores than did the gingival margin in dentin. No significant difference was shown between direct and indirect techniques when the gingival margin was placed in enamel. However, the direct technique showed greater microleakage than the indirect technique when the gingival margin was placed in dentin.

Table 2 shows the distribution of microleakage scores and Table 3 shows the mean and standard deviation values of the overall leakage levels and marginal gap openings of each of the restorative techniques and gingival margin type associations. The one-way ANOVA demonstrated that there were differences among these groups. The Duncan test showed SEM results similar to those observed with stereomicroscopic analysis.

DISCUSSION

For many years the dental profession has strived to achieve good adhesion of resin composites to tooth substrates, since reliable bonding should produce less microleakage and more restoration stability[20]. In accordance with Hannig, Friedrichs[10] (2001), a central goal achieved by adhesive dentistry has been to secure intimate adaptation between the restorative materials and the cavity walls in order to resist microleakage. The occurrence of a gap could promote dentinal fluid percolation, and this phenomenon may cause pulpal sensitivity during functional load on the restoration, in addition to intensifying microleakage and bacterial invasion whenever the marginal integrity of the restorations fails[10].

In this study, as in previous studies[2], bovine teeth were used because it is difficult to obtain large numbers of intact, extracted human teeth for conducting bond and microleakage tests, and bovine teeth present a similar number and diameter of coronal dentinal tubules. Unfortunately, micro-

TABLE 2 - Distribution of microleakage scores according to the stereomicroscopic analysis.

Restorative Method	Gingival margin type	Frequency of microleakage scores									
		0		1		2		3		4	
		n	%	N	%	n	%	n	%	n	%
Direct	Enamel	4	26.67	10	66.66	1	6.670	0	0.00	0	0.00
	Dentin	0	0.00	4	26.67	4	26.67	6	40	1	6.67
Indirect	Enamel	6	39.96	8	53.37	1	6.670	0	0.00	0	0.00
	Dentin	0	0.00	7	46.67	7	46.67	1	6.66	0	0.00

TABLE 3 - Marginal gap openings and overall leakage levels by SEM analysis.

Restorative Method	Gingival margin	Marginal Gap		Leakage score (%) mean ± SD	Ranked using Duncan's Test (p < 0.05)
		n	total		
Direct	Enamel	0	15	9.00 ± 3.42	A
	Dentin	3	15	31.50 ± 7.31	C
Indirect	Enamel	0	15	7.79 ± 2.78	A
	Dentin	2	15	21.00 ± 4.20	B

leakage is still one of the most frequent problems associated with composite restorations, especially in the Class II cervical margin[3]. Several studies have reported that indirect inlay composite restorations result in less microleakage than direct composite resins[11,16] depending on the interaction between the dentin system and the restorative used. Other studies showed similar behavior between direct and indirect composite techniques[1]. In this study, the restorative method used produced a significant effect only when the gingival margin was placed in dentin. The shrinkage produced by the polymerization process inherent to the composite resin is greater for direct insertion in a cavity when the direct technique is used, than the shrinkage of the resinous cement layer used to fix the indirect inlay; this fact resulted in a greater magnitude of stress in the gingival wall, thus facilitating microleakage. Other important aspect is that the volumetric shrinkage of the luting composite resins is compensated by the deformation of the cavity walls. Alavi, Kianimanesh[1] (2002) reported that, when bonding agents are properly applied, there is no advantage to the indirect technique in small class V cavities, but when large Class II cavities are restored, the effect of the shrinkage stress at the cervical margin placed in dentin-cementum is most significant. Liberman *et al.*[13] (1997) related that the indirect procedure resulted in a significantly reduced microleakage when compared to that produced by the semi-direct inlay technique. When bonding adhesives are polymerized before inlays are cemented, adhesion between the tooth surface and the composite luting cement can be improved, however this causes an increase in the resin cement thickness between the inlay and the cervical margin[8]. Several studies have shown greater marginal gaps after inlay cementation[6]. However this aspect did not produce any significant effect when specimens were analyzed by SEM.

Irrespective of the restorative technique used, this study showed a significant difference between dentin and enamel margins, which is in agreement with the findings of Alavi, Kianimanesh[1] (2002), Gerdolle *et al.*[7] (2005). Adhesive bonding of composite to dentinal surfaces is far more complex and less reliable[5,19]. Dentin is a substrate with a highly oriented microstructure, dominated by tubules that converge from the dentine-enamel junction in the crown and from the cementum in the root. The orientation of the tubules toward the cavity wall depends on its location[4]. In the gingival wall the tubules are perpendicular to the interface, but the influence of their direction on bond strength to dentin is still unclear. The direction of tubules appears to be an important variable in determining bond strength. This may determine the intrinsic wetness of the surface[18]. Irrespective of the presence of marginal gap (Figures 2 and 3), the penetration of silver between the hybrid layer and into the dentin is easily observed, the amount of leakage being determined by the restorative method used. SEM analysis showed no difference in the formation of gap-free enamel margins with direct or indirect inlays (Table 3), but the microleakage was greater in dentin with the direct technique. In addition, when the overall leakage in the same method was analyzed, microleakage was greater in the dentin (Figure 4) than in the enamel gingival margins (Figure 3).

The professional's initial intention should always be to analyze the possibility of using the direct technique. However, the indirect composite

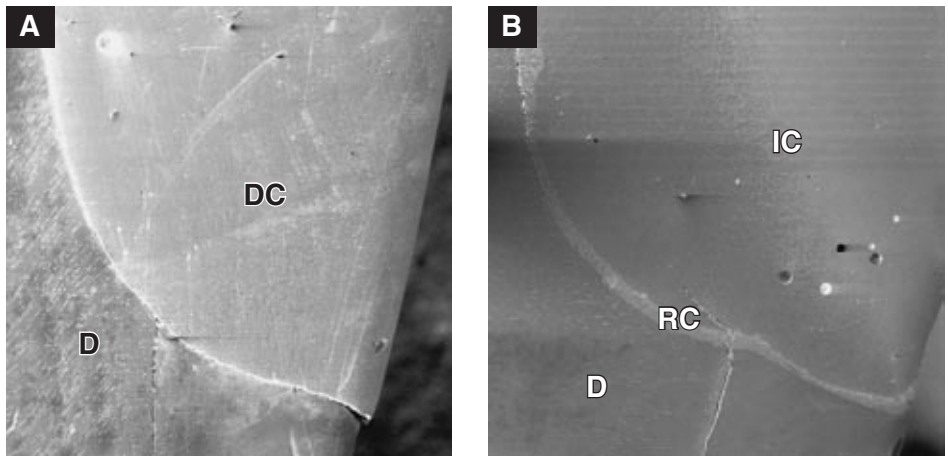

FIGURE 2 - A: SEM of the dentin gingival margin cavity restored with direct composite showing the marginal gap and large secondary electron image along the dentin/adhesive interface (magnification 200 X). **B**: SEM of the enamel gingival margin cavity restored with indirect composite inlay with intact external margin and absence of the secondary electron image along the dentin/adhesive interface (magnification 200 X). DC - direct composite restoration; IC - indirect composite inlay; RC - Resinous cement; D - Dentin.

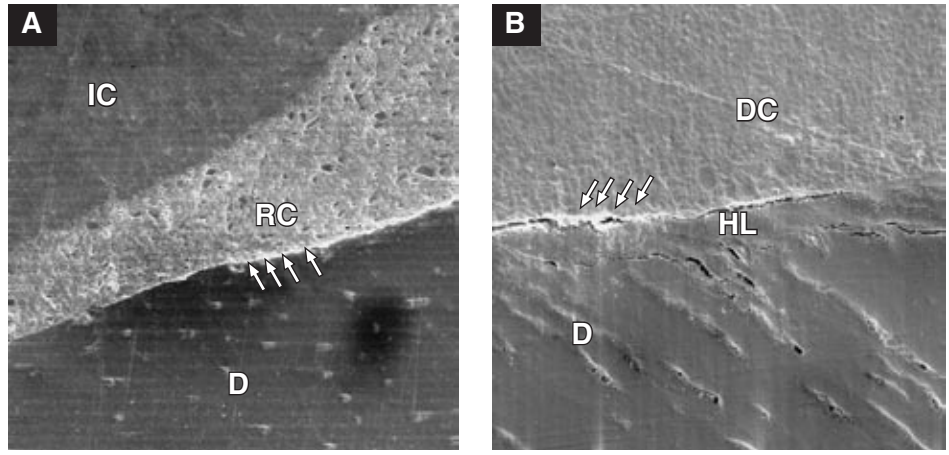

FIGURE 3 - SEM of the enamel gingival margin cavities restored with indirect composite (**A**) and direct restoration (**B**) with little silver penetration. (magnification 200 X). The white arrows indicate the silver infiltrated in the hybrid layer. IC - indirect composite inlay; RC - Resinous cement; DC - direct composite restoration; D - Dentin; HL - Hybrid layer.

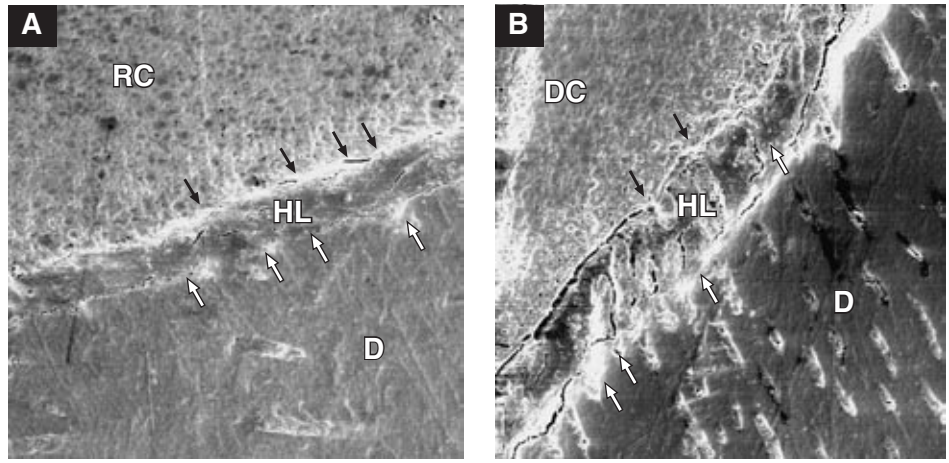

FIGURE 4 - SEM of the dentin gingival margin cavities restored with indirect composite inlay (**A**) and direct composite restoration (**B**) with great silver penetration. Note the silver penetration indicated by black arrows between restorative material and top of hybrid layer, and white arrows indicating the silver infiltrated between bottom of hybrid layer and dentin (magnification 200 X). RC - Resinous cement; DC - direct composite restoration; D - Dentin; HL - Hybrid layer.

restoration, which has shown a close fit at the margins[26], may become an important alternative for restoring complex and extensive cavities in posterior teeth.

CONCLUSION

Within the limits defined in the experimental design, the following conclusions may be drawn:

- Direct and indirect restorations showed similar behavior in relation to leakage when the preparation's gingival margin was placed in enamel.
- Indirect restorations showed smaller leakage than direct restorations when the preparation's gingival margin was placed in dentin.
- Both evaluation methods showed similar capacity to analyze marginal leakage, but their combined use increased accuracy.

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