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# Influence of the number of sections on reliability of *in vitro* microleakage evaluations

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**ABSTRACT:** *Purpose:* To test how the number of sections affects the maximum depth of tracer penetration. *Materials and Methods:* This study was simultaneously performed in three different centers. C-shaped Class V cavities were made on 60 human third molars. The cavities were located across the cementum-enamel junction. One restorative system was used: Scotchbond Multi-Purpose and Z100. After restoration, the samples were thermally cycled 3000 times, between 5 and 55°C with a dwell time immersion of 10 seconds. Silver nitrate was used by two centers and basic fuchsin was used by the third. The teeth were longitudinally sectioned with a diamond saw: 5 sections per tooth provided 10 surfaces for evaluation. The penetration of the tracer was recorded on a scale from 0 to 3. The deepest leakage per restoration was identified for comparison with lesser measured values elsewhere in the tooth. The Spearman test was applied to evaluate the relationship between the reference and data from one, two and three sections (*i.e.* two, four and six measurements). The Kruskal-Wallis test was applied to compare the three centers. *Results:* Whatever the study center, the Spearman correlation coefficient ( $r_s$ ) increased as a function of the number of sections (S) up to three: Center 1 (1S, 0.47; 2S, 0.68; 3S, 1.0), Center 2 (1S, 0.60; 2S, 0.99; 3S, 0.99), Center 3 (S1, 0.40; 2S, 0.73; 3S, 1.0). No statistically significant difference was found between the three study centers (*Am J Dent* 2003;16:207-210).

**CLINICAL SIGNIFICANCE:** A single section seemed to be insufficient to reliably detect the maximum tracer penetration at the tooth restoration interface of Class V cervical margin restorations. Three sections may avoid underestimating *in vitro* microleakage. It could be suggested that *in vitro* microleakage assessment utilizing less than three sections is not representative of the efficacy of adhesive systems tested.

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## Introduction

Microleakage is defined as the clinically undetectable passage of bacteria, fluids, molecules, or ions between a cavity wall and the restorative material applied to it.<sup>1</sup> Microleakage is one of the main reasons for restoration failures as it induces marginal discolorations, sensitivity and recurrent caries.<sup>2</sup> Consequently, numerous *in vitro* studies have been conducted over many years to detect and to evaluate the sealing ability of restorative materials at cavity walls. To date, no direct correlation has been established between leakage measured with *in vitro* tests and the marginal quality of restorations evaluated in clinical trials.<sup>3</sup> Nevertheless, these *in vitro* evaluations are needed as first screening tests for materials with a limited commercial life span and frequent reformulation.<sup>4</sup> They are one of the criteria for the selection of restorative materials and techniques.

*In vitro* microleakage tests are numerous and diverse methods have been used to demonstrate the leakage of restorative materials.<sup>5</sup> The consequence of such a large choice of methods is a lack of standardization.<sup>6</sup> Among the different methods, dye penetration measurements on sections of restored teeth is the most common technique for evaluating microleakage of the tooth-restoration interface.<sup>5,7</sup> One of the major drawbacks of this method is the two-dimensional measurement of a three-dimensional phenomenon. Microleakage is not uniform along the interface<sup>8-10</sup> and three-dimensional evaluation seems to indicate more severe leakage than two-dimensional assessments.<sup>9,11</sup> A suitable alternative to cross-section could be the clearing technique: the specimens are decalcified,

dehydrated and then cleared by immersion in methyl salicylate, thus permitting an unobstructed view of the remaining opaque restoration together with the 3-D, black (silver nitrate) microleakage pattern along the dentin-restorative interface.<sup>10</sup> This technique has been demonstrated to be viable for the assessment of Class V restoration microleakage where AgNO<sub>3</sub> had been used as a tracer.<sup>10</sup> Nevertheless, while some authors find this technique simple and inexpensive, others consider this three-dimensional approach as time-consuming and difficult to quantify.<sup>12,13</sup>

Today, most authors still use the sectional technique because it is regarded as less labor intensive. Many studies are based on only one section: in a microleakage literature review of 144 articles published in peer-reviewed journals,<sup>14</sup> it was shown that 47% of the researchers reported using only one section, 20% reported using two sections and 12% reported using three sections. A relevant question is to consider any relationship between number of sections and measurement validity.

This multicenter evaluated the reliability of microleakage assessment according to the number of sections measured and the nature of the dye. Obviously, *in vitro* microleakage assessment involves some variables: the nature of the substrate,<sup>15,16</sup> operator influence,<sup>17-19</sup> and laboratory physical restrictions. Furthermore, different dye medium utilized may influence measurement outcomes.<sup>7</sup> This study also conducted similar experimental protocols in different laboratories to evaluate the laboratory effect on the test variables.

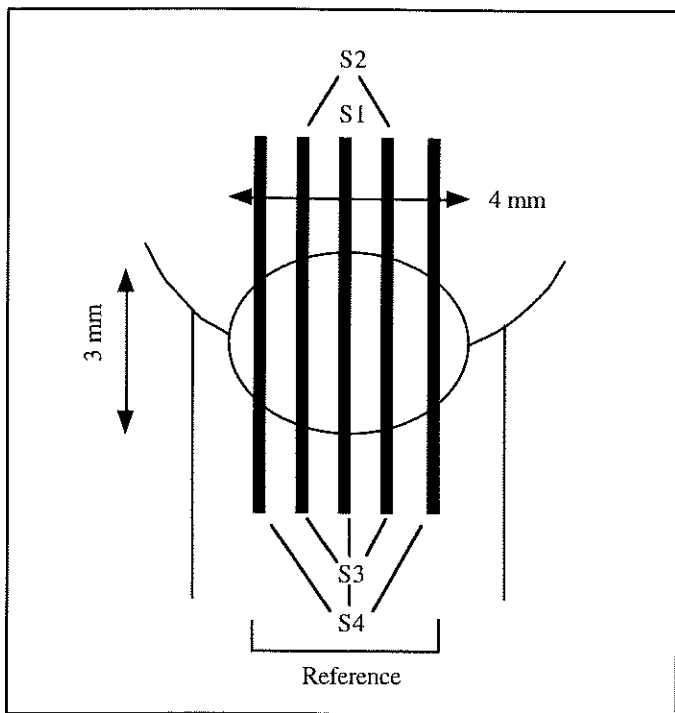


Fig. 1. Localization of the 5 sections. (S1: 1 section/2 measurements; S2: 2 sections/4 measurements; S3: 3 sections/6 measurements; S4: 4 sections/8 measurements; Reference: 5 sections/10 measurements).

## Materials and Methods

This study was simultaneously performed in three different labs (C1, C2, C3) using similar experimental protocols as follow:

**Selection and tooth preparation** - 60 extracted human maxillary or mandibular third molars (20 in each center), free of cracks, caries and restorations, were used in this study. The samples were stored in an aqueous 1% chloramine solution<sup>20,21</sup> for no more than 3 months after extraction.

**Cavity preparation** - The teeth were scaled and cleaned with slurry of pumice before cavity preparation. C-shaped Class V cavities were located at the cementum-enamel junction, on the proximal (mesial or distal) surfaces of each tooth. Cavity preparations were made using round diamond burs (ISO 806 314 110524 027<sup>a</sup>) at high speed with air/water coolant, so that the occlusal cavosurface margin was located in enamel with a 45° bevel of 0.5 mm width placed with a fine, flame-shaped diamond stone (ISO 500 314 277072 014<sup>a</sup>). The gingival margin in dentin/cementum was prepared to a butt joint. Cavity dimensions were approximately 1.5 (± 0.15) mm depth, 4 (± 0.4) mm width, and 3 (± 0.3) mm height.

**Restorative procedure** - The same combination of dentin bonding system and resin-based composite was used by the three centers: Scotchbond Multi-Purpose<sup>b</sup> and Z100<sup>b</sup>. *In vitro* performance of these materials is well documented in the dental literature.

Both adhesive and composite were handled according to the manufacturer's instructions. The cavities were filled in two increments, with the first increment being placed obliquely against the gingival, with the second against the occlusal wall of the cavity. Each increment was light-cured for 40 seconds with a light curing unit (XL 3000<sup>b</sup> and Optilux

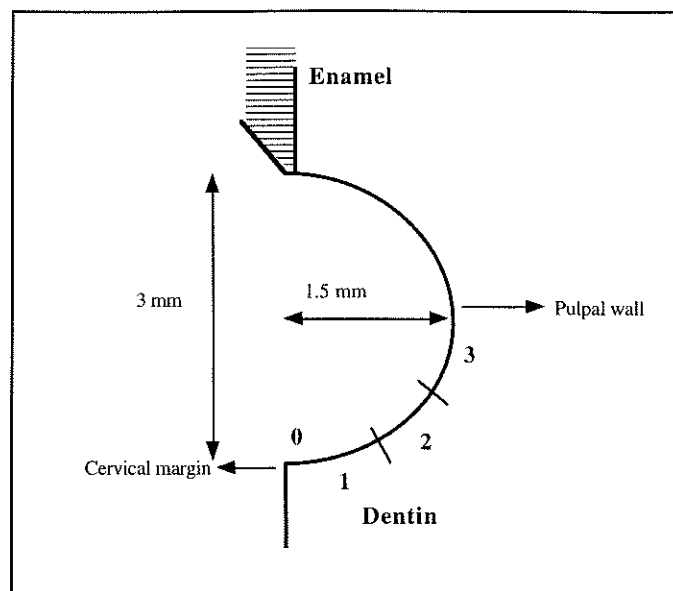


Fig. 2. Tracer penetration score system.

401<sup>c</sup>). The output was checked to be higher than 500 mW/cm<sup>2</sup> with a radiometer.<sup>c</sup> The excess was removed with a diamond bur (ISO 806 314 249514 010<sup>b</sup>) with water spray and the restorations were slightly finished by wet grinding with a series of abrasive discs (Sof-Lex<sup>b</sup>) with decreasing grain size.

**Thermal cycling and microleakage preparation** - The samples were first stored for 1 day in saline solution at 37°C and then thermally cycled in water baths: 3000 cycles, between 5 and 55°C with a dwell time immersion of 10 seconds.<sup>22</sup>

In preparation for dye penetration testing, all specimen root apices were sealed with Fuji II LC<sup>d</sup> to prevent tracer from penetrating through the apex. All remaining surfaces of the teeth were coated with two layers of red nail varnish to within 1 mm of the restoration margins. Silver nitrate was used by two centers (C1, C2): all specimens were placed in a 50% by weight aqueous solution of silver nitrate for 2 hours in darkness. They were then rinsed with water and placed in a radiographic developing solution (Ilford multigrade<sup>e</sup>) for 6 hours under fluorescent light. Basic fuchsin dye was used by the third center (C3): the teeth were stored at room temperature in 0.5% basic fuchsin dye for 24 hours.

The teeth were thoroughly washed with water and acetone to remove nail varnish and embedded in slow-curing epoxy resin (Araldite<sup>f</sup>). After embedding, the teeth were longitudinally sectioned with an Isomet low-speed saw. Five sections per restoration of approximately 500 µm thickness provided 10 surfaces for evaluation (Fig. 1).

**Microleakage evaluation** - Tracer penetration was evaluated under stereomicroscopes at x125 and recorded as depth of penetration along the cervical cavity walls (Fig. 2):

- 0 = No penetration,
- 1 = Leakage extending within the first 1/6 of the cavity wall, that is to say within the part considered as the middle of the cervical wall,
- 2 = Leakage extending beyond 1/6, but less than one-third, of the cavity wall, the third corresponding to the junction between the cervical and the pulpal wall,
- 3 = Leakage extending beyond 1/3 of the cavity circumference, that is to say on the pulpal wall.

Table 1. Microleakage score frequency (max score) in each center (C1,C2,C3) and the whole sample (C1+C2+C3) according to the number of sections.

Score	1 section				2 sections				3 sections				5 sections (Reference)			
	C1	C2	C3	C1+C2+C3	C1	C2	C3	C1+C2+C3	C1	C2	C3	C1+C2+C3	C1	C2	C3	C1+C2+C3
0	2	7	5	14	0	1	2	3	0	1	0	1	0	0	0	0
1	12	4	9	25	11	6	10	27	6	6	8	20	6	7	8	21
2	4	8	3	15	7	7	3	17	8	7	5	20	8	7	5	20
3	2	1	3	6	2	6	5	13	6	6	7	19	6	6	7	19

Table 2. Medians of the maximum scores in each center and p values relating to the "center effect" (Kruskal-Wallis test) and the "section effect" (Friedman test).

	C1	C2	C3	"center effect"
(Kruskal-Wallis test)				
1 section	1	1	1	P= 0.88
2 sections	1	2	1	P= 0.36
3 sections	2	2	2	P= 0.96
Reference	2	2	2	P= 0.98
"Section effect"				
(Friedman test)	P= 0.017	P= 0.009	P= 0.05	

Groups with the same vertical bars letter are not significantly different

The maximum score found among the 10 surfaces evaluated per tooth was taken as the reference. This reference was compared with the maximum score found with two, four and six measurements representing respectively one central, two and three sections (Fig. 1). The maximum score was selected since only one channel of leakage is sufficient to affect the quality of the restoration, especially if the channel is deep.<sup>23</sup>

The Friedman non-parametric test<sup>24</sup> was used to compare the experimental groups (one, two, and three sections) at each center. The statistical significance was fixed at the 0.05 level. The Spearman test<sup>24</sup> was applied to evaluate the correlation ( $r_s$ ) between the reference and data of studied variables (one, two, and three sections). These two statistical analyses were performed with the data from each center separately and then on the overall sample. The results of the three labs were then compared using the Kruskal-Wallis non-parametric test.<sup>24</sup>

### Results

The frequency of maximum interface microleakage depth at cervical dentin margins up to five sections (reference) is listed in Table 1. The maximum score increased with the number of sections up to three. There were fewer restorations without any leakage as the number of sections increased. With five sections, all restorations showed microleakage at the gingival margins, whatever the center.

The results obtained up to three sections compared to the reference were significantly different, regardless of the center (Friedman test, Tables 2, 3). Statistical analysis using the Spearman test revealed that whatever the center, the correlation between the reference and the maximum scores increased with the number of sections, up to three ( $r_s \pm 1.0$ , Table 4). Pooling the three centers did not affect the results *i.e.* the reliability of the Spearman correlation evaluation increased up to three sections ( $r_s = 0.99$ , Table 4).

No significant difference was noted (Kruskal-Wallis test) between the three centers for up to five sections (Table 2, P> 0.36).

Table 3. Medians of the maximum scores in the overall sample and p value according to the number of sections (Friedman test).

	C1+C2+C3
1 section	1
2 sections	1.5
3 sections	2
Reference	2
	P ≤ 0.0001

Groups with the same vertical bars letter are not significantly different.

Table 4. Spearman correlation ( $r_s$ ) between the data obtained with up to three sections and the reference.

Sections	C1		C2		C3		C1+C2+C3	
	$r_s$	p	$r_s$	p	$r_s$	p	$r_s$	p
1	0.47	0.015	0.60	0.005	0.40	0.059	0.48	≤0.0001
2	0.68	0.002	0.99	≤0.0001	0.73	0.001	0.78	≤0.0001
3	1.0	≤0.0001	0.99	≤0.0001	1.0	≤0.0001	0.99	≤0.0001

### Discussion

Under our experimental conditions, one or two sections (*i.e.* 2 or 4 measurements of the criterion) did not find with sufficient reliability the deepest tracer penetration at the cervical dentin margins of adhesive restorations. Three or more sections (*i.e.* 6 measurements) provided this reliability ( $r_s = 1.0$ , P= 0.0001).

Numerous factors influence the marginal sealing of restorations<sup>5,16,25</sup> and the lack of standardization of operative procedures allow only relative rather than absolute comparisons of results.<sup>4</sup> Nevertheless, *in vitro* testing is essential for developmental purposes. Among the available methods, the three-dimensional techniques could be expected to reveal more clinically-relevant microleakage patterns than two-dimensional sectioning techniques. However, the three-dimensional techniques are time-consuming and expensive.<sup>12</sup> Consequently, simple sectioning techniques are frequently used, but a single section may not be representative of the restoration's actual microleakage.<sup>11</sup> Several sections of each tooth must be examined with several measurements accomplished to increase the probability of detecting the deepest leakage site.

In this evaluation, we chose an ordinal criterion (score index) to evaluate tracer penetration, in accordance with more than 70%<sup>14</sup> of *in vitro* microleakage studies. The maximum score registered on each restoration was chosen because it seems to be the most relevant criterion in evaluating microleakage.<sup>23</sup>

The maximum number of sections that is technically feasible with 4 mm wide restorations was five, due to the 300 μm thickness of the diamond saw. Therefore, observation of these five sections (10 measurements) afforded the best and most accurate representation of the situation under our experi-

imental conditions. Nonetheless, our data indicates that whatever the center (*i.e.* three operators and two tracers), three sections were sufficient to reach the same results as the reference. The tracer dye currently used by the laboratories was chosen for this study. If different correlations among the centers or interactions between tracer dyes would have been observed, then both dyes would have been used at all three study centers.

In a microleakage literature review,<sup>14</sup> it was found that 47% of the authors utilized only one section and more than 65% examined fewer than three sections. Since increasing the number of sections seems to increase the strictness of the evaluation and the reliability of the results (Tables 2, 3), it is suggested that tests with fewer than three sections underestimate tracer depth, and may not represent the actual microleakage occurring.<sup>26</sup> Also, clinical operators and tracer type are suggested to be significant variables that may affect results of microleakage studies. Under conditions of this study, neither operator (different laboratories) nor tracer type appeared to affect the results (Table 4). Further microleakage investigations involving different adhesive restorative systems should be performed in order to analyze effect and significance of different adhesive systems, operators, and tracers.

- a. Komet, Lemgo, Germany.
- b. 3M/ESPE, St. Paul, MN, USA.
- c. Kerr Corp., Orange, CA, USA.
- d. GC America, Chicago, IL USA.
- e. Ilford Ltd, Mobberley, Cheshire, England.
- f. Ciba-Geigy Corp., New Jersey, NJ, USA.
- g. Buelher Ltd, Lake Bluff, IL, USA.

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## References

1. Kidd EAM. Microleakage: a review. *J Dent* 1976; 4:199-206.
2. Brännström M. The cause of postoperative sensitivity and its prevention. *J Endod* 1986;12:475-481.
3. Prati C. What is the clinical relevance of *in vitro* dentine permeability tests? *J Dent* 1994;22: 83-88.
4. Roulet JF. Marginal integrity: Clinical significance. *J Dent* 1994;22: S9-S12.
5. Taylor MJ, Lynch E. Microleakage. *J Dent* 1992;20: 3-10.
6. Stanley HR. Guest editorial: An urgent plea for a standardized bonding (adhesion) test. *J Dent Res* 1993;72: 1362-1363.
7. Alani AH, Toh CG. Detection of microleakage around dental restorations: A review. *Oper Dent* 1997;22: 173-185.
8. Mixson J, Eick JD, Chappell RP, et al. Comparison of two surface and multiple-surface scoring methodologies for *in vitro* microleakage studies. *Dent Mater* 1991;7: 191-196.
9. Gwinnett JA, Tay FR, Pang KM, et al. Comparison of three methods of critical evaluation of microleakage along restorative interfaces. *J Prosthet Dent* 1995;74: 575-585.
10. Tay FR, Pang KM, Gwinnett AJ, et al. A method for microleakage evaluation along the dentin/restorative interface. *Am J Dent* 1995;8: 105-108.
11. Gale MS, Darvell BW, Cheung GSP. Three-dimensional reconstruction of microleakage pattern using a sequential grinding technique. *J Dent* 1994;22: 370-375.
12. Hilton TJ, Schwartz RS, Ferracane JL. Microleakage of four class II resin composite insertion techniques at intraoral temperature. *Quintessence Int* 1997;28: 135-144.
13. Hilton TJ, Ferracane JL. Cavity preparation factors and microleakage of Class II composite restorations filled at intraoral temperatures. *Am J Dent* 1998;11: 123-130.
14. Raskin A, D'Hoore W, Gonthier S, et al. Reliability of *in vitro* microleakage tests: Literature review. *J Adhes Dent* 2001;3:295-308.
15. Rueggeberg FA. Substrate for adhesion testing to tooth structure - Review of the literature. *Dent Mater* 1991;7: 2-10.
16. Söderholm KJM. Correlation of *in vivo* and *in vitro* performance of adhesive restorative materials: A report of the ASC MD 156 Task Group on Test Methods for the Adhesion of Restorative materials. *Dent Mater* 1991;7: 74-84.
17. Ciucchi B, Bouillaguet S, Holz J. The battle of the bonds 1995. *Rev Mens Suisse Odontostomatol* 1997;107: 32-36.
18. Finger WJ, Balkenhol M. Practitioner variability effects on dentin bonding with an acetone-based one-bottle adhesive. *J Adhes Dent* 1999;1: 311-314.
19. Sano H, Kanemura N, Burrow MF, et al. Effect of operator variability on dentin adhesion: Students vs. dentists. *Dent Mater* 1998;17: 51-58.
20. Jörgensen KD, Itoh K, Munksgaard EC, Asmussen E. Composite wall-to-wall polymerization contraction in dentin cavities treated with various bonding agents. *Scand J Dent Res* 1985;93: 276-279.
21. Finger WJ. Dentin bonding agents. Relevance of *in vitro* evaluations. *Am J Dent* 1988;1- Special Issue: 184-188.
22. Gale MS, Darvell BW. Thermal cycling procedures for laboratory testing of dental restorations. *J Dent* 1999;27: 89-99.
23. Déjou J, Sindres V, Camps J. Influence of criteria on the results of *in vitro* evaluation of microleakage. *Dent Mater* 1996;12: 342-349.
24. Siegel S, Castellan NJ. Nonparametric statistics for the behavioral sciences. 2nd ed, Singapore: McGraw-Hill International Editions, 1988; 180, 206-216,235-245.
25. Chohayeb AA. Bonding to tooth structure: Clinical and biological considerations. *Int Dent J* 1988;38: 105-111.
26. Pashley DH. Clinical considerations of microleakage. *J Endod* 1990;16: 70-77.