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Reliability of In Vitro Microleakage Tests: A Literature Review

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Purpose: The literature contains conflicting data about in vitro microleakage evaluations and their usefulness and reliability. No standardization has yet been established. Here we consider features of published studies that might affect the results of the in vitro microleakage tests.

Materials and Methods: We reviewed 144 in vitro microleakage studies, published in 14 international reviews between 1992 and 1998, which comprised 917 sets or groups of experiments. The published studies were entered in a database and compared using selected literature criteria: sample, cavities, restoration procedures, thermocycling and mechanical cycling, evaluation method.

Results: The methods employed vary widely. The most frequent methodological choices (%) were (1) specimen storage after extraction: duration (unspecified, 59.2), medium (distilled or deionized water, 33.8), temperature (unspecified, 52.2), additives (none, 47.0); (2) aging method (79.1): duration before aging (< 24 h, 35.9); medium and temperature of storage before aging (distilled or deionized water, 26.8; 37°C, 54.3); (3) medium of cycling (tap water, 50.5), number of cycles ([250-500], 34.6), number of baths (2, 84.0), bath temperature (5°C to 55°C, 60.6), immersion dwell time (30 s, 44.3); (4) tracer: type (basic fuchsin, 40.7), time of immersion (after thermocycling and/or mechanical cycles, 64.1), immersion duration (basic fuchsin: 24 h, 59.5); assessment of dye penetration of sections (91.7): direction (perpendicular, 88.5), number (1, 47.1).

Conclusion: The great variability in the methods used in these 144 studies prevented meta-analysis and comparison of the results, thus reducing the value of these methods.

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The interface between restorative material and tooth structure is known to be an area of clinical concern that can result in marginal discoloration,

secondary caries, and pulpal pathology.¹ The turnover of restorative materials, especially dentin bonding agents and composite resins, is substantial, and it is therefore impossible to test all these materials clinically. In vitro tests remain an indispensable method of initial screening of dental materials and set a theoretical maximal amount of leakage that may or may not occur in vivo.²⁷ The most common method of assessing the sealing efficiency of a restorative material is microleakage evaluation. Between 1992 and 1998, more than 300 studies (Medline) on microleakage were published in the literature. Unfortunately, these studies have generally given contradictory results which are all the more difficult to interpret since each study evaluated a limited number of materials. These contradictions are probably due to differences in technical procedures and, as yet, no standardiza-

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Table 1 Inclusion and exclusion selection criteria

	Inclusion criteria	Exclusion criteria
Study	Microleakage in vitro tests	Literature review
Sample	Human or bovine teeth	
Cavity preparation	Operative and prosthetic dentistry	Endodontics
Materials	Resin composite, glass ionomer, amalgam, compomer, ceramic,...	

Table 2 Alphabetical list of publication journals

Publication journals
American Journal of Dentistry
Australian Dental Journal
Caries Research
Dental Materials
International Journal of Prosthodontics
Journal of the American Dental Association
Journal of Dentistry
Journal of Dental Research
Journal of Oral Rehabilitation
Journal of Prosthetic Dentistry
Operative Dentistry
Pediatric Dentistry
Quintessence International
Schweiz Monatsschrift Zahnmedizin

MATERIALS AND METHODS

We applied the five steps described by Cohen³ for meta-analysis:

1. Specifying inclusion criteria: Criteria for the first selection of studies are listed in Table 1.
2. Locating studies: The Medline database was searched for potentially relevant studies.
3. Selection of publications: Studies on in vitro microleakage tests published between 1992 and 1998 were included. Fourteen journals were chosen and are listed in Table 2.
4. Coding study features: Restorative materials, techniques used, and methodological factors potentially affecting in vitro microleakage results were recorded in a database using selected literature criteria (Table 3).
5. Final exclusion: Studies suffering from methodological problems were excluded from this review.

RESULTS

We recorded 144 studies comprising 917 groups of experiments (range: 1 to 33 groups/study). The most frequent choices for restorative materials, techniques, and methodological factors are presented in Table 3.

Sample Characteristics

The most frequent choices were human teeth (96.0%), molars and third molars (49.6%), and ten samples per group (44.0%) (Fig 1). The tooth stor-

tion has been established. Retief in 1991 and Stanley in 1993 pointed out the same problem with the bond strength studies.^{24,30} In the same way, Hilton has recently grouped together and identified some experimental conditions that could affect the microleakage evaluations.¹⁶

Consequently, an overview of restorative procedures is difficult to establish, thus decreasing the reliability and value of these microleakage tests.

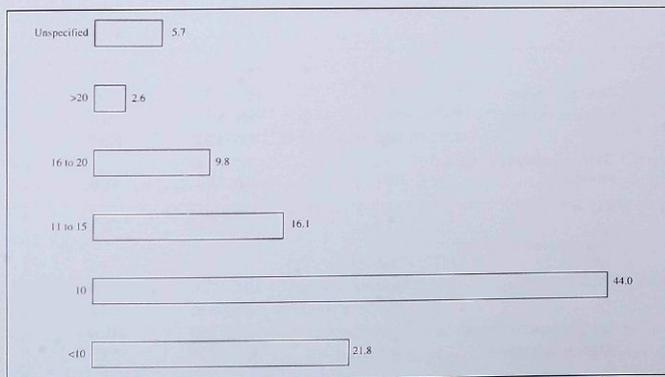
The present literature review was designed to inventory restorative materials and techniques, and to identify methodological factors that might potentially affect the results of in vitro microleakage tests.

Table 3 Most frequent choice of the restorative materials, techniques, and methodological factors registered

Most frequent choice		Groups			
		Number	%		
<i>Samples</i>					
Substrate and tooth morphology	Human	880	96.0		
	Molars and third molars	455	49.6		
Reason for extraction	Unspecified	790	86.2		
Number of teeth in each sample	10	403	44.0		
Storage medium after extraction	Distilled or deionized water	310	33.8		
	No additive	431	47.0		
Temperature tooth storage after extraction	Unspecified	479	52.2		
Tooth storage duration after extraction	Unspecified	543	59.2		
<i>Cavity</i>					
Cavity form	Class V (U, C or V-shaped)	573	62.5		
		U-shaped	410 / 573	71.6	
Dimensions	Class V	Height: 2 mm	233 / 573	40.7	
		Width : 3 mm	249 / 573	43.5	
		Depth : 1.5 mm	277 / 573	48.3	
Location	Cemento-enamel junction	600	65.4		
Bevel	No	617	67.3		
<i>Restorative procedure</i>					
Direct	Restorative material	Resin composite	803	87.6	
		Dentin bonding agents	526 / 803	65.5	
		Yes	581 / 803	72.4	
		Base or liner	None	668 / 803	83.2
Light curing	Materials tested	608	66.3		
		Resin composite	526 / 608	86.5	
		Total duration	> 60 s	195 / 608	32.1
		Filling technique	Increments	382 / 608	62.8
Cervical matrix	Class V (n = 573)	Increments	362 / 526	68.8	
		(resin composites)	362 / 526	68.8	
		None	439 / 573	76.6	
		Class II (n = 213)	Yes	108 / 213	50.7
Aging	Yes	725	79.1		
<i>If no aging</i>	Clinical service	No	192	21.0	
		Medium storage	None	68 / 192	35.4
		Temperature:37°C	77 / 124	62.1	
		Duration after specimen fabrication	No	61 / 192	31.8
<i>If aging</i>	Storage medium before cycling	725	79.1		
		Distilled or deionized water,	194 / 725	26.8	
		Temperature:37°C	342 / 630	54.3	
		Duration before aging	< 24 hours	260 / 725	35.9
Medium of cycling	Tap water	366 / 725	50.5		

Table 3 (continued) Most frequent choice of the restorative materials, techniques, and methodological factors registered

Most frequent choice	Groups			
			Number	%
Thermal cycling			705 / 725	97.2
		Number of cycles [250-500]	244 / 705	34.6
		Number of baths 2	592 / 705	84.0
		Dwell time of immersion 30 s	312 / 705	44.3
		Bath temperature 5°C and 55°C	427 / 705	60.6
Mechanical cycling			59 / 725	8.1
		Number of cycles 5000	17 / 59	28.8
		Force 125 N	18 / 59	30.5
Duration after aging		None	638 / 725	88.0
<i>Dye / tracer</i>				
Type		Basic fuchsin	373	40.7
		Concentration 0.5 %	251 / 373	67.3
		Immersion duration 24 h	222 / 373	59.5
pH		Unspecified		> 95.0
Moment of immersion		After aging	588	64.1
<i>Microleakage evaluation</i>				
Evaluation method		2 dimensions	813	88.7
Evaluation criteria		Scores	660	72.0
Sections		Yes	837	91.3
		Number 1	396 / 837	47.3
		Direction Perpendicular	744 / 837	88.9
		Unspecified	282	30.8
<i>Statistical test</i>				
Nonparametric			604	65.9

**Fig 1** Number of teeth in experimental groups (%).

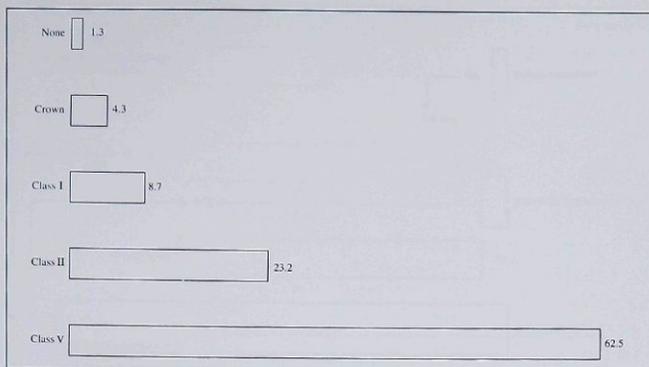


Fig 2 Cavity type (%).

age medium after extraction was distilled or deionized water (33.8%) and no additives were used (47.0%). Tooth storage temperature and duration after extraction were unspecified in 52.2% and 59.2% of groups, respectively.

Cavity Type

Class V cavities (Fig 2) were used (62.5%) (U-shaped: 71.6%). Cavities were preferentially located at the cementoenamel junction (65.4%) and no bevel (67.3%) was made at the enamel margins.

Class V dimensions were 2 mm high (40.7%), 3 mm wide (43.5%), and 1.5 mm deep (48.3%).

Restorative Procedure

Direct restorative procedures were tested in 87.6% of groups ($n = 803$), and resin composites (65.5%) and dentin bonding agents (72.4%) were the most tested materials. No base or liner was used (83.2%).

Among light-cured materials ($n = 608$; 66.3%), total light-curing duration was > 60 s (32.1%) and the materials were applied in increments (62.8%). Only light-cured resin composites were used and the incremental technique (cf. bulk technique) for this material was applied in 62.8% of groups.

No matrices were used for Class V (76.6%) or for Class II (32.4%) cavities.

Aging

79.1% of the groups included an aging technique.

Without aging ($n = 189$)

12.5% of groups were realized and evaluated after clinical service. The restorations were either evaluated directly with no storage (35.4%) or after storage at 37°C (62.1%).

With aging

97.2% of groups were thermally cycled (Fig 3). Storage medium before cycling was distilled or deionized water (26.8%) and storage temperature was 37°C (54.3%). Before aging, the teeth were kept in the storage medium for less than 24 h (35.9%). Teeth were generally thermally or mechanically cycled in tap water (50.5%). Among the thermocycled groups, more than 250 and fewer than 500 cycles of thermal stress (Fig 4) were used in 34.6% in two baths (84.0%) with a dwell time of immersion of 30 s (44.3%). The minimal and maximal bath temperatures were 5°C and 55°C (60.6%). Only 8.1% (11 studies) of groups used mechanical cycling (Fig 3). The number of cycles ranged from 100 to 500,000 and the forces applied on the restorations varied between 72.5 and 340 N.

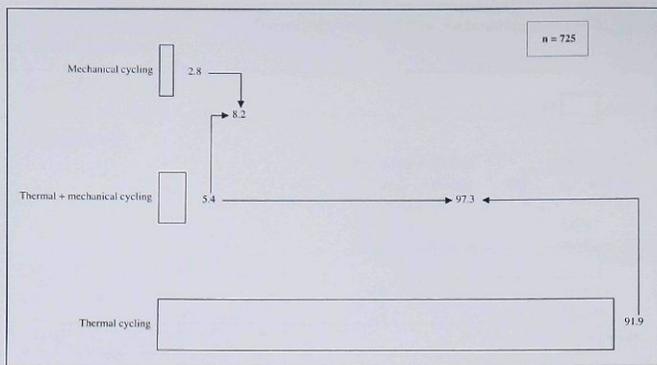


Fig 3 Type of aging (%).

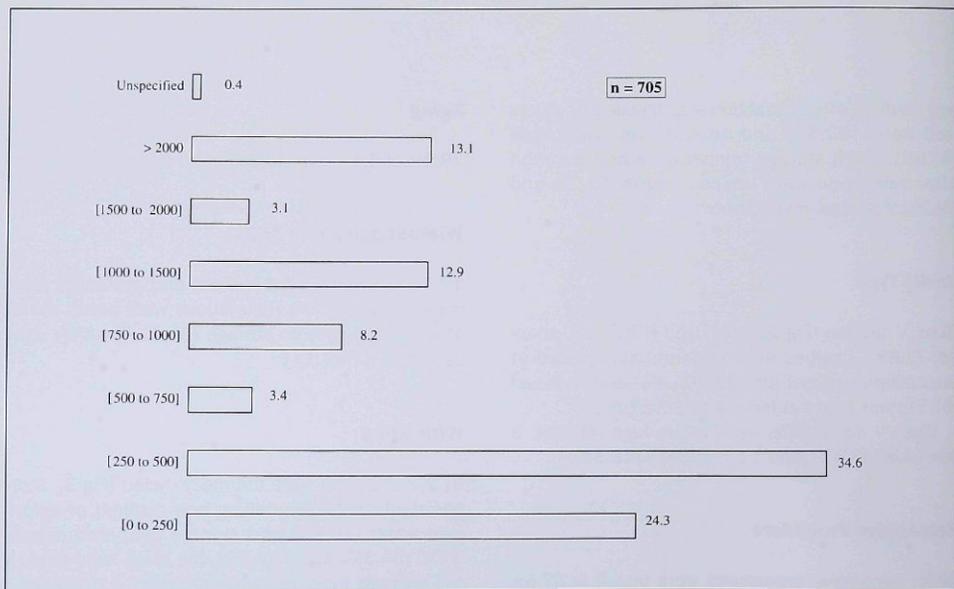


Fig 4 Number of thermal cycles (%).

Dye/Tracer

In 64.1% of cases, teeth were immersed in dye/tracer solution after thermal and/or mechanical cycling (Fig. 5). The three most frequently used dyes/tracers were basic fuchsin, methylene blue, and silver nitrate in 40.7%, 22.0%, and 17.0% of the

groups, respectively (Fig 6). Immersion duration was generally 24 h for fuchsin, 2 hours for silver nitrate, and 4 hours for methylene blue. The pH of the dye solution was unspecified in more than 95% of the groups.

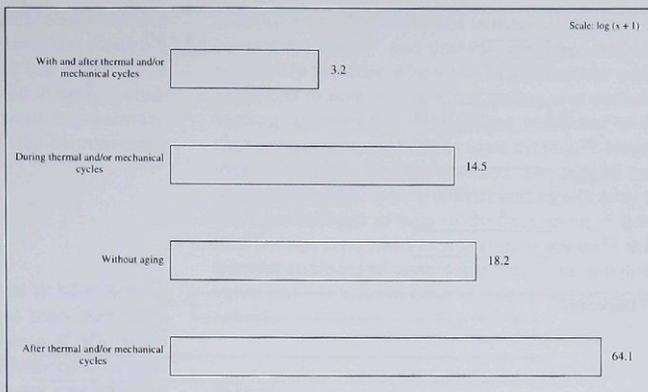


Fig 5 Time of immersion in dye/tracer (%).

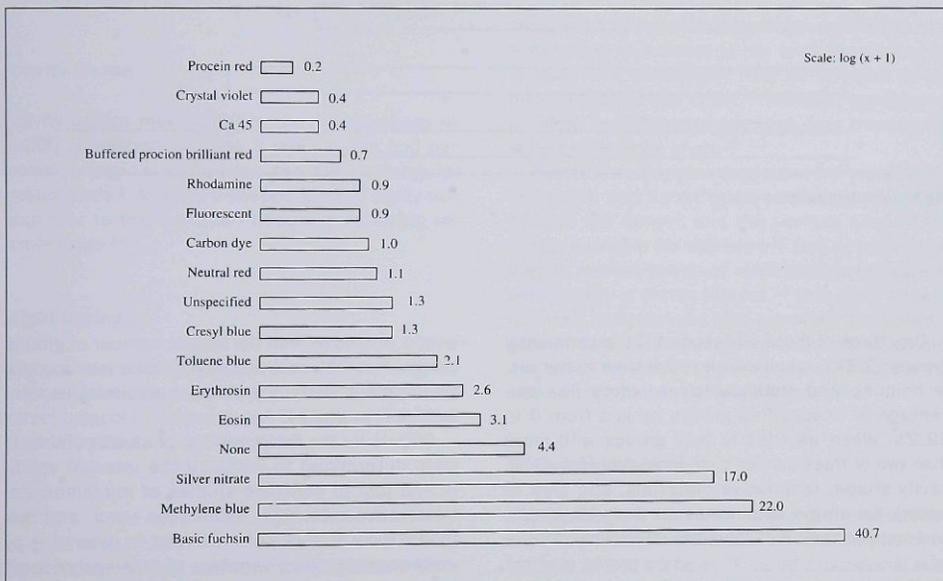


Fig 6 Type of dye/tracer (%).

Microleakage Evaluation

The most common method was two-dimensional evaluation (88.7%), using ordinal and dichotomic data (72.0%). Sections of restored teeth were made in 91.7% (n = 841) of the groups. Only one section was made in 47.1% of groups and two or three sec-

tions were made in 20.0% and 12.7% of groups, respectively (Fig 7). Sectioning was perpendicular to the restoration in 88.5% of the groups.

The statistics (ie, mean, maximum) of dye/tracer penetration were unspecified in 30.8% of cases. Nonparametric statistical analysis was applied in 65.9% of cases.

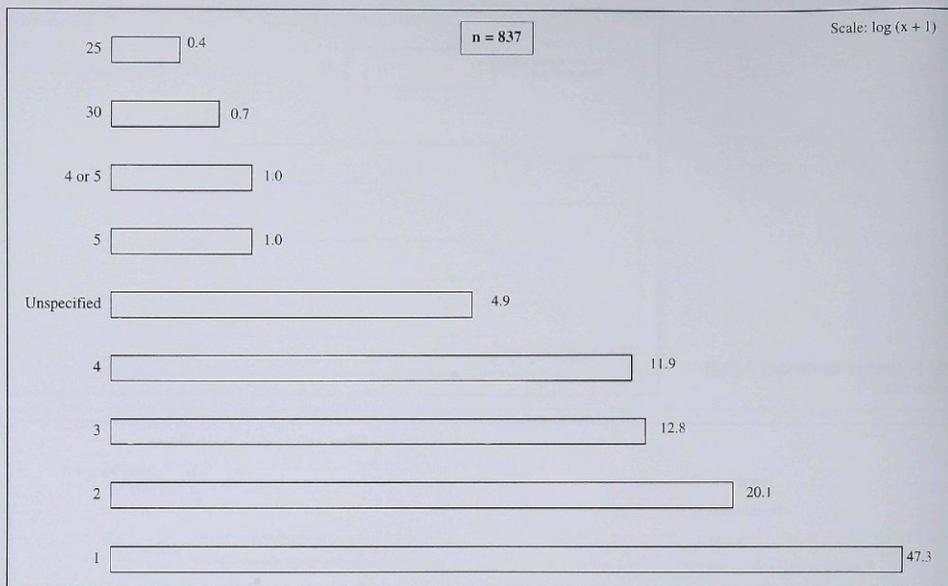


Fig 7 Number of sections (%).

Only three of these 144 studies (21 experimental groups; 2.3%) specified all restorative materials, techniques, and methodological factors. The percentage of unspecified groups ranged from 0 to 59.2%. When we tried to pool groups with more than two or three similar methodological factors (ie, cavity shape, restorative materials, and type of tracer), we always obtained fewer than 15 groups, and meta-analysis was therefore impossible.¹⁰

DISCUSSION

It is difficult to affirm that the 144 studies reviewed here constitute all studies published between 1992 and 1998 in the 14 journals considered. Nevertheless, the search procedure (Medline database) likely yielded most of the published studies.

These 144 studies comprised 917 groups of experiments. The results were expressed in percentages of groups and not in percentages of studies, firstly to avoid underestimating the statistical

weight of studies with the largest number of groups (range: 1 to 33), and secondly to take into account the different study objectives, methodologies, and materials evaluated.

Criteria for the first selection of studies (Table 1) were determined to increase the internal validity and just to compare studies of microleakage. Restorative materials, techniques used, and methodological factors were recorded to determine by meta-analysis which variables of these procedures could influence the results, and to compare the materials used in these studies. However, the studies showed a pronounced degree of non-homogeneity. Furthermore, restorative materials, techniques used, and methodological factors were not always specified (0 to 59.2% of unspecified groups). Consequently, small groups were obtained when trying to gather studies with two or three similar methodological factors, and the conditions needed for the statistical tests of meta-analysis were not met.^{6,7}

This being so, it was impossible to know which steps of the procedure should be taken into ac-

count in the variations of the results recorded in the different studies and when comparing the materials tested. The only thing we know is the influence of some methodological factors tested in several studies; unfortunately, these were tested separately, and the results were also sometimes contradictory, as discussed in the following.

Sample Size

Sample size was less than or equal to 10 in 60% of cases, and less than 20 in more than 90%. This small sample size limits the choice of statistical tests that might be used. Indeed, only nonparametric tests could be used, and these are less powerful than parametric tests.¹⁷

Cavity Shape

Cavity design may influence the microleakage results. The spherical Class II restorations had narrower marginal gaps than the did rectangular restorations,⁹ and the U-shaped Class V cavity was superior to the V-shaped design in reducing microleakage.¹⁴

Light Curing

Light-curing conditions (variable light intensity, exposure times, increments) significantly influenced direct composite restorations^{5,21,29,33} and were seldom described and controlled.

Aging

To simulate clinical conditions, restorations were generally subjected to thermal stress and/or occlusal loads. The most frequently used method was thermal cycling, which simulates temperature variations *in vivo*. The influence of aging was tested by several authors and the results were conflicting. Some authors demonstrated that neither thermocycling nor occlusal stress increased the microleakage of the restorations.^{8,22,28,34,36,39} Others reported that the need for thermocycling is dependent either on how thermally conductive the restorative is in relation to its mass,²⁶ or on the materials.^{4,19,23} In contrast, some authors showed

that thermocycling increases leakage at the dentin-cement interface³⁷ and that microleakage was significantly greater when the restorations were subjected to both temperature cycling and occlusal loading, compared to restorations subjected to either temperature cycling or load cycling.²⁵ Nevertheless, one study demonstrated that tensile and compressive load cycling did not add significantly to the microleakage effects of thermocycling.¹⁴ Gale and Darvell concluded in a literature review¹² that thermal stressing of restoration interfaces is only of value when the initial bond is already known to be reliable.

Tracer

The great majority of microleakage studies examine penetration of a tracer at the tooth/restoration interface. This penetration may be affected by the tracer's particle size or pH.³⁵ However, Youngson et al noted no difference between four tracers, despite a wide range of pH.³⁸

Penetration may also depend on the tracer's concentration and its diffusion coefficient, the thickness of the dentin, and the surface area of the dentin available for diffusion.²⁰ Use of tracer may lead to overestimation of microleakage because of permeability of dentin tubules.¹² One study showed that the radioisotope test generally indicated a greater degree of leakage than did the ultraviolet dye test,² whereas another study showed a close agreement between the two methods.³¹

Evaluation Method

Microleakage is not uniform along the circumference of a restorative margin,^{13,32} and may be more extreme at end surfaces.¹⁸ Three-dimensional evaluation revealed more severe leakage than a conventional, single longitudinal, midline sectioning technique¹³ or a few sections.¹¹ However, three-dimensional evaluations are not easy, and the technique is user sensitive (ie, clearing protocol) and time consuming.¹⁵

Evaluation using sections

Single sections seem to be insufficient for reliable detection of the deepest tracer at a tooth-restora-

tion interface of Class V cervical margin restorations. The use of three sections may avoid underestimation of in vitro microleakage.²³

Despite the contradictions, it seems evident that the sealing ability of restorative materials is highly dependent upon the procedure employed, and it is impossible to evaluate the role of each study feature. Consequently, results from different studies should not be compared, since conclusions regarding the value of one product compared with another would be invalid. Meta-analysis was unfortunately precluded because of considerable variability in the methods used in the 144 studies reviewed, and a comparison of the materials was not possible.

CONCLUSION

Our literature review shows that we are faced with a choice between standardizing tests, thereby enabling meaningful comparisons, or accepting that unstandardized tests are of limited use.

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