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Marginal Leakage of Class V Composite Restorations Assessed Using Microcomputed Tomography and Scanning Electron Microscope

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Clinical Relevance

Microcomputed tomography (micro-CT) provides uninterrupted inspection of the tooth-restoration interface and detection of the deepest leakage point. As a nondestructive technique, micro-CT allows further testing of the specimen to relate sealing ability with other clinically relevant properties of the restorative materials.

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SUMMARY

Objective: The aim of the study was to compare in Class V composite restorations marginal leakage measurements obtained with microcomputed tomography (micro-CT) and scanning electron microscopy (SEM) observations.

Methods: Class V cavities were prepared on 10 human molars and restored using Optibond FL (Kerr, Orange, CA, USA) and Premise Flowable (Kerr). Sealing ability was evaluated by assessing silver-nitrate penetration depth along enamel and dentin margins. Leakage was quantified using a scoring system. Micro-CT analysis provided 502 cross-sectional images for each tooth. Microleakage evaluation was performed first on three cross-sections corresponding to the sections examined by SEM, then on all 502 of the obtained micro-CT images. SEM observations were performed first at 20× magnification, then, if showing a zero score, at 80× magnification. Enamel and dentin microleakage scores assigned to corre-

sponding sections through micro-CT and SEM (20×) were compared (Wilcoxon signed-rank test, α=0.05).

Results: No statistically significant difference in leakage scores emerged between micro-CT and 20×-magnification SEM. Eight tooth sections that were given a zero score under SEM at 20× magnification showed to be infiltrated at the higher magnification (80×). For five teeth a higher score was assigned following scanning of 502 cross-sections than based on the observation of three sections.

Conclusions: Micro-CT presents as a valid, nondestructive *in vitro* method to quantitatively evaluate marginal leakage of adhesive restorations.

INTRODUCTION

Marginal leakage is considered a major cause of restoration failure, being responsible for over 50% of replacement procedures.[1,2] Polymerization shrinkage, factors related to adhesive procedures, or inadequate tooth isolation are some of the factors that may cause a failure in adapting the material to the cavity walls,[3] leading to frequently described clinical outcomes such as postoperative sensitivity, secondary caries, marginal tooth discoloration, or pulp pathology.[4-6] Increasing the marginal adaptation of restorations and their durability is one of the main efforts of modern adhesive dentistry.[7] The sealing ability of adhesive materials, which is a property of clinical relevance, can be assessed *in vitro* with microleakage tests.[4,8,9] Most of these methods require the use of a tracer or a dye and cutting the tooth into a series of sections to visualize the extent of staining along the tooth-restoration interface with scanning electron microscopy (SEM), light microscopy, or digital imaging. The depth of dye penetration along the margin can be measured or graded with a scoring system.[4,8,9] A shortcoming of these tests is that they provide a two-dimensional (2D) and semiquantitative evaluation of leakage because interfacial staining is visualized on a limited number of tooth slabs, and some tooth structure is inevitably lost with sectioning.[10] Furthermore, tooth sectioning is a time-consuming and destructive procedure that prevents further testing of the specimen.

Over the last decade the use of x-ray micro-computed tomography (micro-CT) has had considerable development in dental research, finding numerous applications, as described in a recent review.[11] Micro-CT is a nondestructive method that, starting from a series of 2D images, produces a three-dimensional (3D) reconstruction of the observed specimen.[12,13] Lately, the technique has been proposed for the evaluation of marginal leakage in adhesive restorations and pit and fissure sealing.[7,13-19] However, micro-CT has been compared with the conventional section method only in the assessment of marginal leakage at the interface between enamel and pit and fissure sealant.[15] Therefore, the objective of the present study was to comparatively evaluate the two techniques in the evaluation of Class V composite resin restorations. Specifically, the aim was to test the null hypothesis that leakage measurements obtained with micro-CT and with the section method do not differ significantly.

METHODS AND MATERIALS

Sample Preparation

Ten sound human molars were stored in 0.5% chloramine-T solution under refrigeration (4°C) and used within one month after extraction. The preparation of Class V cavities was performed on the buccal aspect of each tooth, using a cylindrical diamond bur (305L, Intensiv, Switzerland) mounted on a high-speed handpiece under abundant water cooling. The cavities were round, about 1.5 mm deep and 4 mm in diameter. Cavities were restored using the three-step etch-and-rinse adhesive Optibond FL (Kerr, Orange, CA, USA) and the resin composite Premise Flowable (Kerr). Light curing was performed with a halogen curing device (VIP, Bisco Inc, Schaumburg, IL, USA; 600 mW/cm^2). Table 1 reports chemical composition, batch numbers, and instructions for use of the materials. After storage in distilled water at 37°C for one day, teeth were prepared for microleakage testing.[19] The entire tooth surface was covered with two layers of fast-setting nail varnish within 1 mm of the bonded interface. In order to allow the varnish to dry, the specimens were left undisturbed for one day. Then, teeth were immersed in a 50% weight/weight silver-nitrate aqueous solution for 24 hours at room temperature. The silver-impregnated teeth were thoroughly rinsed with distilled water, placed into a photo-developing solution for eight hours (Dental X-Ray Developer, Kodak Co, Rochester, NY, USA), and again abundantly rinsed with water. Teeth were to be viewed first with micro-CT, then under SEM. On each tooth crown three indentations, 1 mm in depth (Figure 1), were made using a water-cooled, slow-speed Isomet saw (Buehler, Lake Bluff, IL, USA). At each indentation a micro-CT scan was performed.

Table 1: *Chemical Composition, Batch Numbers, and Instructions for Use of the Tested Materials*					
Material	**Type**	**Lot (Batch) No.**	**Manufacturer**	**Composition**	**Application Mode**
Gel Etchant	37.5% phosphoric acid	3213200	Kerr, Orange, CA, USA	37.5% orthophosphoric acid, silica thickener	Apply for 15 seconds; rinse with water for 15 seconds; gently air dry for a few seconds.
Optibond FL	Light-cure total-etch adhesive bonding system	Primer: 3215398 Adhesive: 3215399	Kerr, Orange, CA, USA	Primer: HEMA, GPDM, PAMM, ethanol, water, photo initiator; Adhesive: TEGDMA, UDMA, GPDM, HEMA, bis-GMA, filler, photo initiator	Apply primer with light scrubbing motion for 15 seconds; gently air dry 5 seconds; apply adhesive; light application of air; light cure for 20 seconds.
Premise Flowable	Light-cured, flowable composite	3433707 Shade: A3	Kerr, Orange, CA, USA	Prepolymerized filler (PPF), barium glass, silica filler, ethoxylated bisphenol-A-dimethacrylate, TEGDMA, light-cure initiators and stabilizers, organophosphate dispersant	Apply 2-mm-thick layer; light cure for 20 seconds.

Abbreviations: HEMA, hydroxyethylmethacrylate; GPDM, glycerol phosphate dimethacrylate; PAMM, phthalic acid monoethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate; bis-GMA, bisphenol A glycidyl methacrylate.

Micro-CT

Micro-CT scanning was performed using SkyScan 1072 (SkyScanb.v.b.a., Aartselaar, Belgium) with the following settings: 10 W, 100 kV, 98 μA, a 1-mm thick aluminum plate, 15× magnification, 4.9-second exposure time, 0.45° rotation step. The acquisition procedure involved several 2D lateral projections of the specimens during a 180° rotation around the vertical axis. The digital data were processed using a reconstruction software (NRecon V1.4.0; SkyScan).

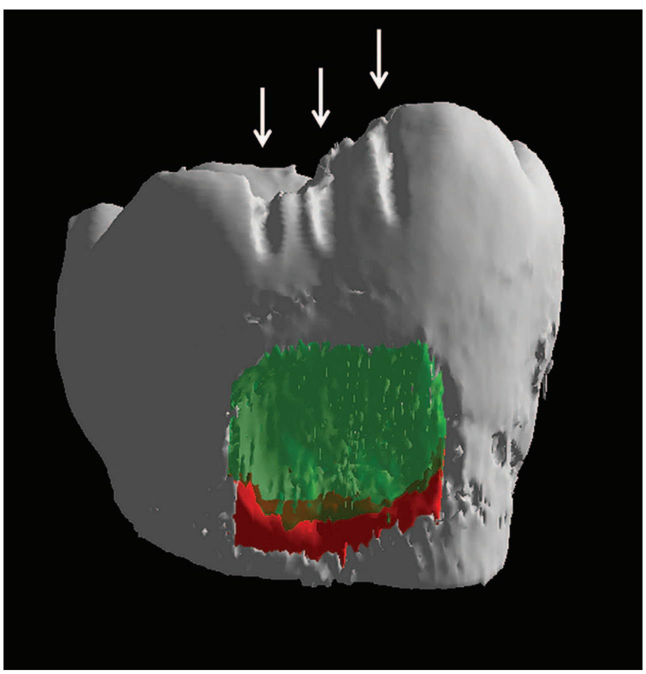

Figure 1. *Image of a restored and stained tooth obtained through 3D reconstruction of micro-CT scans. Silver nitrate infiltration (red) is visible along the cervical margin and wall of the composite resin restoration (partially transparent green). Indentations were made (arrows) to provide landmarks for longitudinal sectioning of the crown into slabs that were then viewed under SEM.*

3D Reconstruction—The software also provided axial cross-sections of each tooth with a pixel size of 19.1 × 19.1 μm. The distance between each cross-section was 38 μm. Images of 502 slices per tooth were acquired. After cone-beam reconstruction, the raw data were converted to 16-bit grayscale picture files with a resolution of 1024 × 1024 pixels. The cross-sectional images were imported into a 3D visualization software package (Mimics software; Materialize, Leuven, Belgium).

Microleakage Assessment

Two experienced investigators (C.R., G.S.) independently evaluated microleakage at the enamel (occlusal) and the dentin (cervical) margins on SEM micrographs and micro-CT images (Figures 2-4).

For microleakage assessment, the exact length of the entire enamel-composite and dentin-composite interfaces as well as the length of the stained interface were measured in pixels using the image analysis software Digimizer V.3.0.0 (MedCalc Software, Mariakerke, Belgium). The percentage of microleakage was thus calculated: (Length of stained interface / Total interfacial length) × 100 and converted to a scoring system[20] from 0 to 4, as presented in Table 2. Scoring differences between the evaluators were discussed until a final score was reached by consensus.

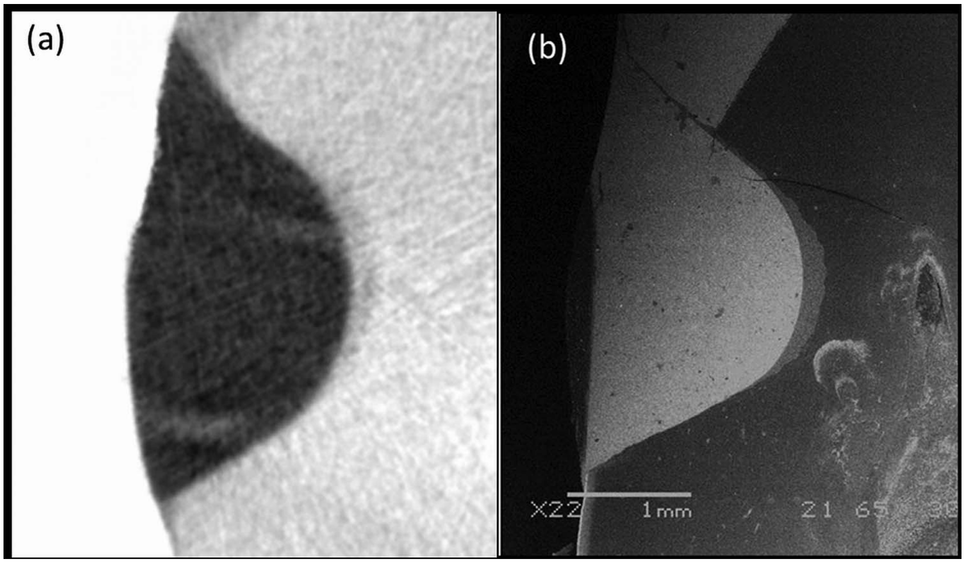

Figure 2. *(a): Micro-CT scan of a tooth showing margins free from dye infiltration and (b): corresponding section observed under SEM.*

Scanning Electron Microscopy

Starting from the indentations preliminarily cut in the crown as landmarks (Figure 1), each tooth was sectioned longitudinally with the saw in a buccolingual direction to provide four slabs with three specular surfaces. For each pair of slabs offering specular surfaces, only one slab selected at random was processed for SEM observations of interfacial morphology. The slabs were polished with a series of silicon carbide papers of increasing grit from 600 to 1200 under water irrigation and subsequently treated with silica-free 32% phosphoric acid gel (Uni-Etch, Bisco) for 60 seconds. A 120-second deproteinization using 2% sodium hypochlorite solution was followed by dehydration of the specimens in an ascending series of aqueous ethanol solutions to absolute ethanol. The specimens were then dried using hexamethyldisilazane (Carlo Erba, Rodano, Italy). Each specimen was observed under the SEM JSM-6060LV (JEOL, Tokyo, Japan) at different magnifications. Specimens were first viewed at 20×, which was the highest possible magnification in relation to the total field of view of the tooth-restoration interface,[15] and scores were assigned. Specimens that received a zero score were further observed at a higher magnification (80×) to detect any silver nitrate infiltration that was perhaps invisible at 20×.

Statistical Analysis

Separate statistical analyses were applied to data recorded at the enamel (occlusal) and at the dentin (cervical) site. The Wilcoxon signed-rank test was applied to compare microleakage scores assigned to corresponding sections on micro-CTs and 20×-magnification SEM images. Calculations were handled

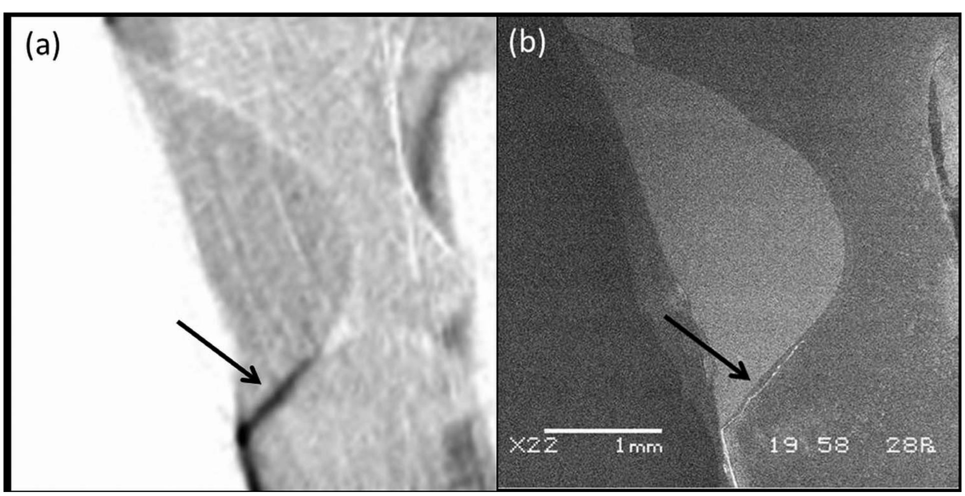

Figure 3. *(a): Micro-CT scan of a tooth showing dye penetration along the dentin-restoration interface and (b): corresponding section observed under SEM.*

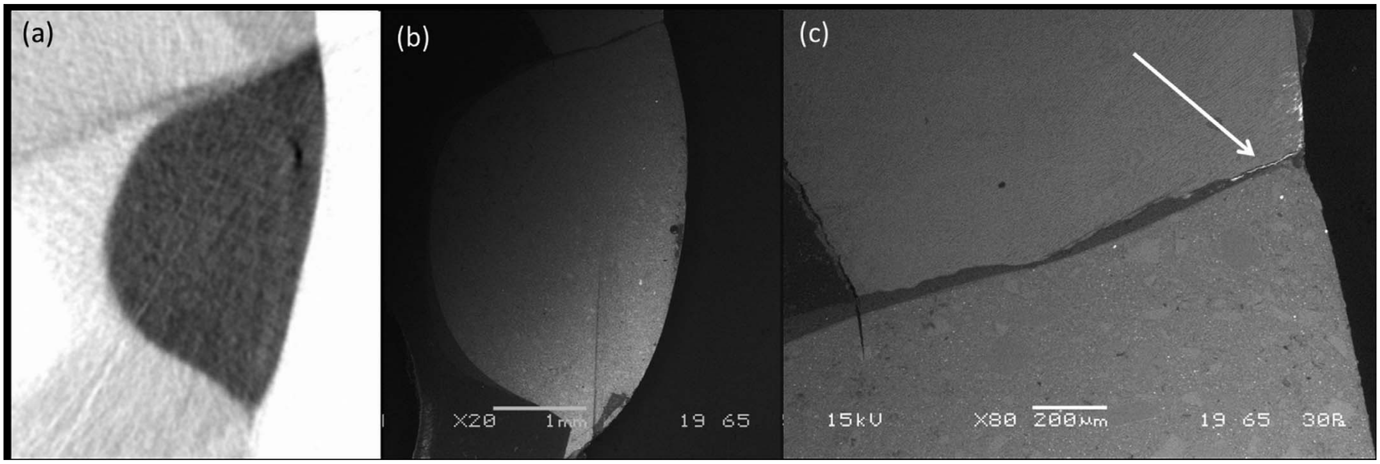

Figure 4. *(a): Micro-CT scan and (b): corresponding SEM micrograph at 20× magnification of a tooth apparently free of infiltration, but (c): showing silver nitrate deposition at the enamel-restoration margin when observed under SEM at 80× magnification.*

by SPSS software, version 18.0 (SPSS Inc, Chicago, IL, USA).

RESULTS

Microleakage scores assigned to SEM micrographs and to micro-CT images of corresponding tooth sections are presented in Table 3. Silver nitrate infiltration was detected as white stains along the material-tooth interface (Figures 2-4). Agreement between 20×-magnification SEM and micro-CT in assessing leakage was observed for the great majority (93.3%) of the observed specimens, both at the enamel and at the dentin margin (Figure 3). Table 4 reports the descriptive statistics of microleakage scores. The statistical analysis revealed that scores assigned using micro-CT images and 20× SEM micrographs did not differ significantly either at the enamel ($p=0.5$) or at the dentin ($p=1.0$) site (Table 4).

Eight tooth sections that were given a zero score at 20× magnification showed staining along up to one-third of the interface at the higher magnification (score 1, Table 3). For three of these sections such discrepancy occurred both at the enamel and at the dentin site.

With specific regard to micro-CT analysis, for five teeth (No. 1, 4, 6, 9, and 10 of Table 3) a higher score was assigned following scanning of 502 cross-sections than based on the observation of three sections. The difference emerged also in comparison with SEM observations. For most of those teeth (No. 4, 6, 9, 10 of Table 3), scanning of all the cross-sections allowed to spot a score 1 leakage that was indeed undetected when viewing only three sections or SEM images. For one tooth (No. 1 of Table 3), scans of all the cross-sections revealed that the dye had penetrated along the interface deeper than it had appeared from the observation of only three sections or of SEM micrographs.

Table 2: *Score System to Quantify Microleakage at the Enamel-Composite and Dentin-Composite Interfaces*

SCORE	MICROLEAKAGE
0	Absence of infiltration
1	Infiltration up to 1/3 of the interface
2	Infiltration up to 2/3 of the interface
3	Infiltration higher that 2/3 of the interface

DISCUSSION

Lately, micro-CT has been proposed as a nondestructive method to assess three-dimensionally the sealing ability of adhesive materials.[7,12-16,21] With the purpose of validating the new technique, in the present study the ability of micro-CT to investigate leakage in Class V restorations was comparatively assessed with reference to the commonly used section method.[8] The finding that microleakage measurements produced by micro-CT and 20×-magnification SEM did not differ significantly leads to acceptance of the formulated null hypothesis. Moreover, the high agreement noted between the two methods points out that micro-CT is as reliable as low-magnification SEM in assessing interfacial leakage of adhesive restorations. The 20× magnification was selected as adequate for viewing the entire tooth-restoration interface. However, such magnification in eight of 30 sections failed to reveal leakage that was instead visible when the specimens were observed at 80× (Figure 4c). This is in agreement with Chen & others[15] who advocated

Table 3: *Microleakage Scores of the Tooth Sections Observed Under SEM and With Micro-CT*

Tooth No.	Section	SEM 20×		SEM 80×		Micro-CT		Micro-CT 502 Sections (Highest Score Recorded Per Tooth)	
		Enamel	Dentin	Enamel	Dentin	Enamel	Dentin	Enamel	Dentin
1	1	0	1	0	N/E	0	1		(2)
	2	0	1	0	N/E	0	1		
	3	0	1	0	N/E	0	1		
2	1	*0*	*0*	*1*	*1*	0	0		
	2	**3**	**1**	N/E	N/E	**0**	**0**		
	3	**4**	1	N/E	N/E	**0**	1		
3	1	0	1	0	N/E	0	1		
	2	0	1	0	N/E	0	1		
	3	0	2	0	N/E	0	2		
4	1	0	0	0	0	0	0		(1)
	2	0	0	0	0	0	0		
	3	0	0	0	0	0	0		
5	1	0	1	0	N/E	0	1		
	2	0	0	0	0	0	0		
	3	0	0	0	0	0	0		
6	1	0	0	0	0	0	0	(1)	
	2	*0*	*0*	*1*	*1*	0	0		
	3	*0*	0	*1*	0	0	0		
7	1	0	1	0	N/E	0	1		
	2	*0*	0	*1*	0	0	0		
	3	0	0	0	0	0	0		
8	1	0	1	0	0	0	1		
	2	*0*	1	*1*	N/E	0	1		
	3	*0*	*0*	*1*	*1*	0	0		
9	1	1	0	N/E	0	1	0		(1)
	2	0	0	0	0	0	0		
	3	0	0	0	0	0	0		
10	1	0	1	0	N/E	0	1	(1)	
	2	0	*0*	0	*1*	0	0		
	3	0	***0***	0	*1*	0	**1**		

Abbreviation: N/E, not evaluated.
[a] *The sections that were assigned a 0 score in 20×-magnification micrographs were observed also at 80×. The italic character labels sections that were given a higher score at 80× than at 20× magnifications. The bold character labels sections that were assigned different scores under SEM and with micro-CT. Values in brackets indicate the highest score recorded in the tooth when 502 micro-CT scans were performed.*

Table 4: *Descriptive Statistics of Microleakage Scores Assessed on Three Sections Per Tooth With 20× SEM and Micro-CT*[a]

Substrate	Technique	n	Median	25%	75%
Enamel	20× SEM	30	0.000	0.000	0.000
	Micro-CT	30	0.000	0.000	0.000
Dentin	20× SEM	30	0.000	0.000	1.000
	Micro-CT	30	0.000	0.000	1.000

[a] *No statistically significant difference emerged within each substrate between the two methods.*

that the ability to increase image magnification is an advantage of SEM over micro-CT. Conversely, in micro-CT, magnification depends upon the distance between the specimen and the x-ray source and therefore upon the specimen size: The smaller the distance, the larger the magnification and the greater the detail of the image.[7] On the other hand, micro-CT has the benefit of obtaining an uninterrupted inspection of the interface that enables detection of the deepest leakage point.[7,15] As a matter of fact, in the present study, for half of the teeth, scanning all the micro-CT cross-sections led to a higher leakage score than that determined from the assessment of three sections or of SEM specimens. Such occurrence may reasonably be related to the "hit-or-miss" characteristic of the section method[18] which provides only partial information. Particularly in microscopic investigations, the loss of tooth structure with slicing makes the inspection of the tooth-restoration interface inevitably defective. The thickness of the cutting blade itself limits the number of sections that can be obtained. Consequently, restorations of clinically relevant size and shape may yield very few specimens for observations.[18] Moreover, the section method precludes further testing of the specimen and therefore the possibility to relate leakage to other properties of the materials, such as shrinkage, elastic, or mechanical behavior, that might be predictive of the clinical outcome of the restoration.[18]

No conclusive evidence has yet been provided regarding the ideal concentration of silver nitrate and the optimal time of tooth immersion for micro-CT observations. Eden and others[7] stated that a four-hour immersion in a 50% silver nitrate solution allowed accurate and reliable measurements of microleakage in Class II composite resin restorations performed *in vivo*. Later, Chen and others,[21] having compared the outcome of three-hour and four-hour immersion in 50% silver nitrate solution, suggested the shorter time interval, although admitting that repeating the experiment with a larger sample size would be advisable. In the present study specimens were immersed in 50% silver nitrate solution for 24 hours. This is indeed the commonly followed protocol for the observation under SEM, to which the same specimens were to be subsequently subjected. Eden and others[7] reported that after a 14-hour immersion in 50% silver nitrate of primary teeth that had been restored *in vivo*, not only interfacial staining but also silver nitrate scattering in adjacent caries-affected dentin was observed. Differently from the mentioned study,[7] in the present investigation only caries-free teeth were included. This may explain why, despite the longer immersion in the staining solution, no dye diffusion within the dentin underlying the adhesive interface was seen in this study's specimens.

In previous micro-CT investigations of pit and fissure sealants, the occurrence of "background noise" due to x-ray scattering from the tooth surface and sometimes interfering with visualization of sealant and enamel was reported.[15,16,21] No such difficulty was experienced in the present study, possibly in relation to the use of a flowable resin composite, which is a higher density material than a sealant. Moreover, in this study micro-CT images could be colored for a clearer differentiation of the substrates and the stain (Figure 1).

Beside pit and fissure sealants, micro-CT has also been used to assess marginal leakage of Class II restorations in primary molars[7] and standardized cylindrical cavities in permanent molars.[18] In the present study, noncarious Class V adhesive restorations were chosen for testing, given that they have been considered ideal for assessing bonding effectiveness for several reasons.[22] First, preparation of Class V cavities is minimal and their restoration is relatively easy, thereby reducing technique-sensitivity and operator-related variability. Additionally, different from sealants, Class V cavities have margins located both in enamel and in dentin. Moreover, Class V lesions have a relatively small configuration factor; consequently, the mechanical properties of the composite resin used are less influential, and the bonding potential of the adhesive determines the outcome of the restoration to a greater extent.[22]

It should also be considered that in the available studies validating micro-CT against microscopy,[15,18] for experimental purposes, it was decided to prevent the development of a bond to the dental substrate by omitting the etching procedure and the adhesive application. It could be argued, however, that such experimental conditions did not reflect the clinical situation. In contrast, in the present investigation, the bonding agent was applied before composite resin layering, as done in the clinical setting.

As far as the cost of image production is concerned, micro-CT devices are more expensive than the equipment for the conventional section method. However, it should be considered that micro-CT has applications in other research fields beside leakage testing.[18,23-28]

In conclusion, in the present *in vitro* investigation, micro-CT was demonstrated to be similarly adequate to 20×-magnification SEM at assessing marginal leakage of Class V composite resin restorations. The possibility to adjust magnification to gain a more detailed view of the interface was precluded with micro-CT, because the magnification depended upon the distance between the x-ray source and the specimen. On the other hand, SEM allowed leakage evaluation only in the plane of sectioning and with inevitable loss of information at the site of cutting. Conversely, micro-CT enabled a 3D mapping of the tooth-restoration interface and detection of the deepest leakage level. It was plausibly due to the hit-or-miss characteristic of the section method that for five of 10 tested teeth, a higher leakage score was determined from the observation of all the micro-CT scans than when only three tooth sections were viewed either with micro-CT or under SEM.

It could be argued that, in order to fully assess the potential of the techniques and whether their outcomes were similar, the whole range of scores should have been covered, whereas a prevalence of low scores was reported in the present investigation. The predominance of low scores, however, was related to the reliability of the tested adhesive.[22,29-31] Also, in previous microleakage studies Optibond FL (Kerr) demonstrated limited infiltration both at the enamel and at the dentin margins.[20,32-34] In the current investigation, Optibond FL (Kerr) was chosen because it has been considered as the gold-standard bonding agent in dental adhesion.[22,29,30]

Whenever a lack of statistically significant between-group differences is observed, the power of the test and the sample size should be considered as a possible alternative explanation for the finding. Nevertheless, it should be pointed out that the sample size used in the present investigation, 30 specimens per group, was chosen with reference to a previous study,[35] where it was reported that such a sample size was adequate to find statistically significant differences in microleakage between two groups, with a power of 80% and a significance level of 0.05.

Finally, it is known that the correlation between the findings of *in vitro* leakage tests and clinical outcomes has recently been debated[36,37] and that the comparison of results among studies is questionable due to the great variability in investigating methods.[22] It is also agreed that, although *in vitro* tests retain the potential to promptly provide some useful information on materials' properties, the ultimate evidence on the sealing ability of adhesive restorations should be gathered through long-term clinical trials.

CONCLUSIONS

The finding that scores recorded with the two compared techniques did not differ significantly indicated that micro-CT was as reliable as low-magnification SEM in assessing interfacial leakage of adhesive restorations. Being a nondestructive method, micro-CT has the added value of allowing further testing of the specimen for the assessment of other clinically relevant properties of the materials besides sealing ability.

Conflict of Interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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