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Detection of Marginal Leakage of Class V Restorations *In Vitro* by Micro–Computed Tomography

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Clinical Relevance

Micro–computed tomography can provide *in vitro* nondestructive detection of leakage around a composite restoration to display entire three dimensional dentin leakage patterns with accuracy comparable to that of the conventional dye-penetration method.

SUMMARY

This *in vitro* study evaluated the efficacy of micro–computed tomography (CT) in marginal leakage detection of Class V restorations. Standardized Class V preparations with cervical margins in dentin and occlusal margins in enamel were made in 20 extracted human molars and restored with dental bonding agents and resin composite. All teeth were then immersed in 50% ammoniacal silver nitrate solution for 12 hours, followed by a developing solution for eight hours. Each restoration was scanned by micro-CT, the depth of marginal silver leakage in the central scanning section was measured, and the three-dimensional images of the silver leakage around each restoration were reconstructed. Afterward, all restorations were cut through the center and examined for leakage depth using a microscope. The silver leakage depth of each restoration obtained by the micro-CT and the microscope were compared for equivalency. The silver leakage depth in cervical walls observed by micro-CT and microscope showed no significant difference; however, in certain cases the judgment of leakage depth in the occlusal wall in micro-CT image was affected by adjacent enamel structure, providing less leakage depth than was observed with the microscope ($p<0.01$). Micro-CT displayed the three-dimensional image of the leakage around the Class V restorations with clear borders only in the dentin region. It can be concluded that micro-CT can detect nondestructively the leakage around a resin composite restoration in two and three dimensions, with accuracy comparable to that of the conventional microscope method in the dentin region but with inferior accuracy in the enamel region.

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INTRODUCTION

Marginal adaptation of dental restorations is an important factor that influences the longevity of dental restorations. Poor marginal adaptation will result in microleakage along the tooth-restoration interface, leading to marginal staining and increasing the risk of postoperative sensitivity and secondary caries.[1] Microleakage has been defined as passage of substances, such as saliva, ions, compounds, or bacterial by-products, between a cavity wall and the restorative material.[2] The investigation of microleakage is, therefore, important in the assessment of restorative materials. A variety of *in vitro* methods have been introduced into the investigation of microleakage, including compressed air, electrochemical approaches, bacteria infiltration, scanning electron microscopy (SEM), and perhaps most common of all, the dye-penetration method.[2,3] The conventional dye-penetration method, also known as the microscope method, typically involves soaking a restored tooth in a dye solution, cutting through the center of the restoration, and assessing the leakage visually detectable on the section under a microscope. The extent of leakage may be quantified using a length scale, but it is often more subjectively evaluated using a system whereby the degree of leakage is graded by operators using a predetermined range of values.[4] However, this methodology has been known to have a number of shortcomings, among which the major issue is that the results obtained are two-dimensional (2D) in nature and do not take the whole tooth-restoration interface into account, as only a few sectioned planes are measured for leakage. Furthermore, some studies have shown that the dye leakage in different sections taken from different places on the restoration varied significantly.[5]

Although some other methods, such as the compressed air method,[6] are capable of producing results revealing the whole tooth-restoration interface, the path and location of leakage cannot be demonstrated, which is problematic because these are helpful for understanding the mechanism and etiology of microleakage and its predilection site.

To overcome this drawback, Gale and others,[7] Youngsen,[8] and Iwami and others[9] reported a three-dimensional (3D) method that revealed the dye penetration at the adhesive interface of resin restorations. With their method, images of the sectional surface of the resin restorations are taken with continuous reductions, and 3D images are finally created from the obtained images using computer software. However, this method is destructive and time consuming, and the 3D images obtained are rough because of the low spatial resolution (more than 100~300 μm between two sections).

Micro-CT uses X-rays to create cross sections of a 3D object that can be used to recreate a virtual model with a spatial resolution at a micron level without destroying the original specimen. Micro-CT has been used widely in material research to detect the inner structure of materials.

Kakaboura and others[10] investigated the gap along the interface between resin composite restorations and tooth structure using micro-CT. To display the effects of polymerization contraction of resin composite on the gap, they did not apply any conditioner and bonding agents prior to filling the cavity with the composite (which was not in agreement with clinical protocol). Consequently, the gap imaged by the micro-CT would be different from the true situation. Sun and others[11] investigated the volume contraction of cylindrical fillings of resin composite in a model cavity prepared in a PMMA block using the micro-CT. They used rhodamine B dye solution as a tracer to penetrate the gap between the resin composite restoration and the cavity wall and referred to the stained area as microleakage. Both of these two studies demonstrated the gap leakage created by resin polymerization contraction but did not take adhesion into account. In fact, according to the definition of microleakage,[3] it is caused not only by gaps resulting from contraction but also by defects located in the bonding interface, especially in the hybrid layer—the concept of nanoleakage introduced by Sano and others,[12] which even shows gap-free margins around restorations.

It is possible to use micro-CT to detect the leakage around a bonded restoration. However, it is unclear whether the micro-CT is comparable to the conventional dye-penetration method in displaying the leakage. Therefore, in this *in vitro* study, we used the micro-CT and dye-penetration method to examine the leakage around the same Class V resin composite restorations and compared the difference in the results in leakage depths obtained by the two methods in order to evaluate their equivalency in measuring marginal leakage. In addition, the efficacy of micro-CT in demonstrating the 3D shape of the leakage was evaluated.

MATERIALS AND METHODS

Twenty caries-free third molars were selected within six months of extraction. After surface debridement with a hand scaling instrument and cleaning with a rubber cup and pumice, a standardized Class V cavity was prepared on the buccal or lingual surface of each tooth (3 mm in diameter and 1.5 mm in depth), with the cervical margin located in the dentin and the coronal margin in the enamel. The cavities were prepared first using a cylindrical diamond bur with a high-speed handpiece and air-water coolant spray and were then finished using a carbide bur with a straight flat fissure head without cross-cuts.

The cavities were then checked for cracks at the margins using a stereomicroscope. This step is necessary to eliminate those cavities with defects that may allow tracer solution to ingress through microscopic spaces of the tooth/restoration interface, thereby giving false-positive results. The teeth were randomly assigned to four groups (n=5) for four adhesive systems, as follows: 1) Prime & Bond NT (Lot No. 506001515; Dentsply, Kanstanz, Germany), 2) Clearfil SE Bond (Lot No. 71167; Kuraray, Okyama, Japan), 3) Adper Prompt (Lot No. 255379; 3M ESPE, St Paul, MN, USA), and 4) iBond (Lot No. 10069; Hereaus, Hanau, Germany). The adhesive systems were applied according to the manufacturers' instructions, and the cavities were then restored with a resin composite (Clearfil AP-X, Lot No. 1089AA, Kuraray) and cured for 40 seconds with a light at 800 mW (LED D, Woodpecker Co, Guilin, China). After the teeth were stored in distilled water for 24 hours at 37°C, the surface of each restoration was finished with a diamond-coated abrasive bur to remove possible resin flash overlaying the enamel so that a butt joint was formed; this was followed by finishing with a rubber cup with abrasive paste and then polishing with polishing disks (Soflex, 3M ESPE).

To prevent silver penetration in areas other than the exposed margins, the root apices were etched and sealed with a light-curing fissure sealant (Clinpro, 3M ESPE). The tooth surfaces were then sealed with two layers of nail varnish to be within 1 mm of the restoration margins.

All of the teeth were immersed in 50% ammoniacal silver nitrate solution in the dark for 12 hours, rinsed with running water for two minutes, immersed in photo-developing solution, and exposed to light for eight hours. Then, the teeth were cleaned with a toothbrush and polished slightly with Soflex disks to remove silver depositions on the surface.

After being placed and fixed into the specimen holder, each tooth was scanned individually using a micro-CT (Inveon, Siemens, Germany) at a resolution of 20 μm with an integration time of 2500 ms. The micro-focus X-ray source was set at 80 kV and 500 μA. After completion of the x–y scans, the 2D and 3D images of each restoration were reconstructed using image analysis software of the instrument—Inveon CT Research Workplace. The leakage depths along the cervical wall and coronal wall of each restoration, including their respective extensions at the axial wall, were measured on the section image scanned longitudinally through the center of the restoration. In addition, the 3D image of each restoration and the leakage around the restoration were reconstructed with the software. Furthermore, to display the leakage more clearly in 3D, the images of silver leakage were picked up digitally according to a radiopaque value that was just higher than the value of enamel using the software.

All teeth were then sectioned longitudinally through the center of the restoration in a bucco-lingual direction with a slow-speed diamond saw (SYJ-150, MTI Corporation, China) under water-cooling. The thickness of the cutting blade was 0.2 mm. The black leakages along the cervical and coronal walls, as well as their extensions in the axial wall of each restoration on the section, were photographed and measured for the leakage depth with a measuring microscope at 10× magnification.

Statistical Analysis

Statistical comparisons were made between the results of the micro-CT and microscope methods for equality in measuring the leakage depths. The data for leakage depths at the coronal and cervical walls of each restoration, measured by the micro-CT and microscope, were compared with the Wilcoxon paired-rank test. All data were submitted for statistical analysis at the $p < 0.05$ level of significance.

RESULTS

Table 1 lists the leakage depths along the coronal and cervical walls of each restoration measured with the micro-CT and microscope. The Wilcoxon paired-rank test revealed no significant difference between the two methods in measuring the depth of leakage at the cervical margin, but there was a significant difference ($p<0.01$) at the coronal margin, with the depths from micro-CT being lower than those from the microscope.

Figure 1 shows images of the leakage at the mid-longitudinal sections of four typical restorations demonstrated with the micro-CT and microscope. It

Table 1: *Leakage Depths in Cervical and Coronal Margins Measured With Micro–Computed Tomography (micro-CT) and Microscopy*

Bonding Agents	Restoration	Leakage Depth, mm			
		Cervical[a]		Coronal	
		Micro-CT	Microscope	Micro-CT	Microscope
Prime & Bond NT	1	3.22	3.14	0	0.06
	2	0.66	0.69	1.32	1.22
	3	1.19	1.11	0	0
	4	0.09	0.14	1.01	1.22
	5	1.43	1.44	0	0.30
Clearfil SE Bond	1	0	0	0.24	0.33
	2	0	0	0.74	0.99
	3	0.14	0.13	0.23	0.26
	4	0.45	0.42	0.2	0.26
	5	0.18	0.17	0.36	0.45
Adper Prompt	1	1.11	1.06	0.47	0.52
	2	1.51	1.51	0.52	0.66
	3	1.23	1.23	0.86	1.51
	4	1.62	1.66	0.16	0.18
	5	1.48	1.49	0.7	0.91
iBond	1	0.22	0.19	0.33	0.9
	2	1.03	1.03	0	0.98
	3	0.12	0.15	0	0.26
	4	1.47	1.33	0.82	0.86
	5	0.23	0.24	0	0

[a] *Including the extension of the leakage in the axial wall.*

is obvious that silver leakage depths in the cervical margin of the same restoration presented in the micro-CT image and microscope image are comparable, whereas the leakage depths in the coronal margin of the same restoration are different in some restorations, with deeper leakage visible in the microscope images compared with those in the micro-CT image, such as those illustrated in Figures 1e and f.

Figure 2 shows 3D images of the leakage around four restorations. Obviously, most restorations presented more leakage in the cervical region than in the coronal region in terms of both depth and area (Figure 2a). However, the leakage did not always occur in the cervical region or in the coronal region. In contrast, some leakage occurred primarily in the mesial or distal wall of the cavity (Figure 2e,g), and some even presented no leakage in the center of the cervical region (Figure 2e). In addition, some leakage extended to the axial wall from the mesial or distal wall of the cavity (Figure 2c). The leakage expanded in different shapes, with some leakage extended from the external surface into the cavity through certain passages, as opposed to enlargement of leakage area by continuous degrees from the outside into the inside, such as the leakage marked with an arrow in Figure 2, in which the leakage obviously extends from two sides.

DISCUSSION

The conventional dye-penetration method has been used widely to determine marginal adaptation and is listed as a standard method by the International Organization for Standardization.[13] To evaluate the efficacy of micro-CT in the determination of leakage, the present study compared the leakage depth along the cervical and coronal walls in the mid-longitudinal sections of each restoration obtained with a micro-CT with those measured using the conventional dye-penetration method. The results indicate that leakage depths measured with the micro-CT and microscope at cervical walls of each restoration were comparable, which indicates that micro-CT is reliable in detecting marginal leakage located in dentin when using silver as a radiographic contrast agent.

It is important for the methodology of micro-CT that the radiographic contrast agents used to display

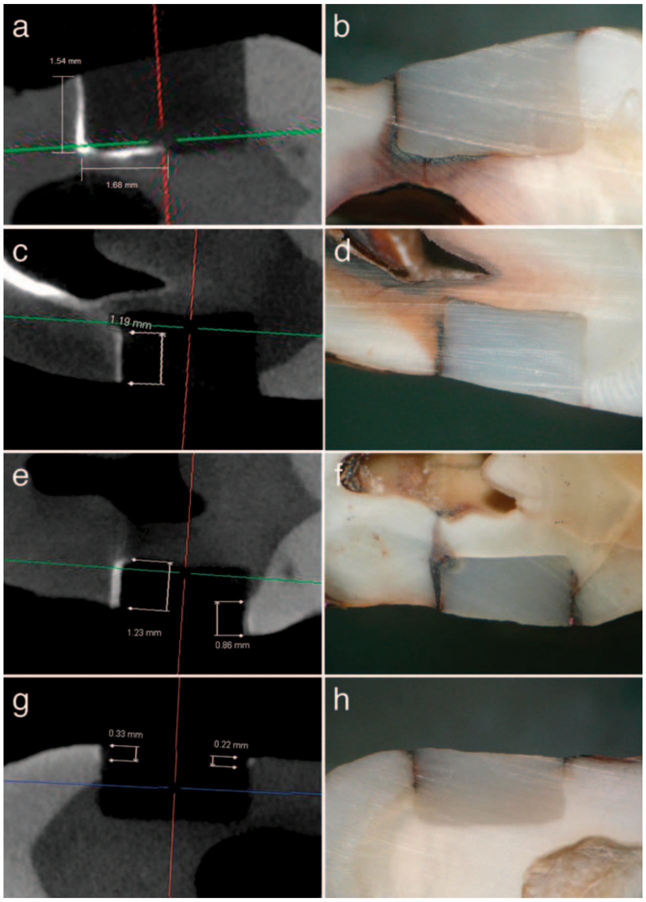

Figure 1. *Marginal leakage on the middle longitudinal sections of four restorations. (a, c, e, and g) Images of the micro-CT; (b, d, f, and h) corresponding images from the microscope after cutting.*

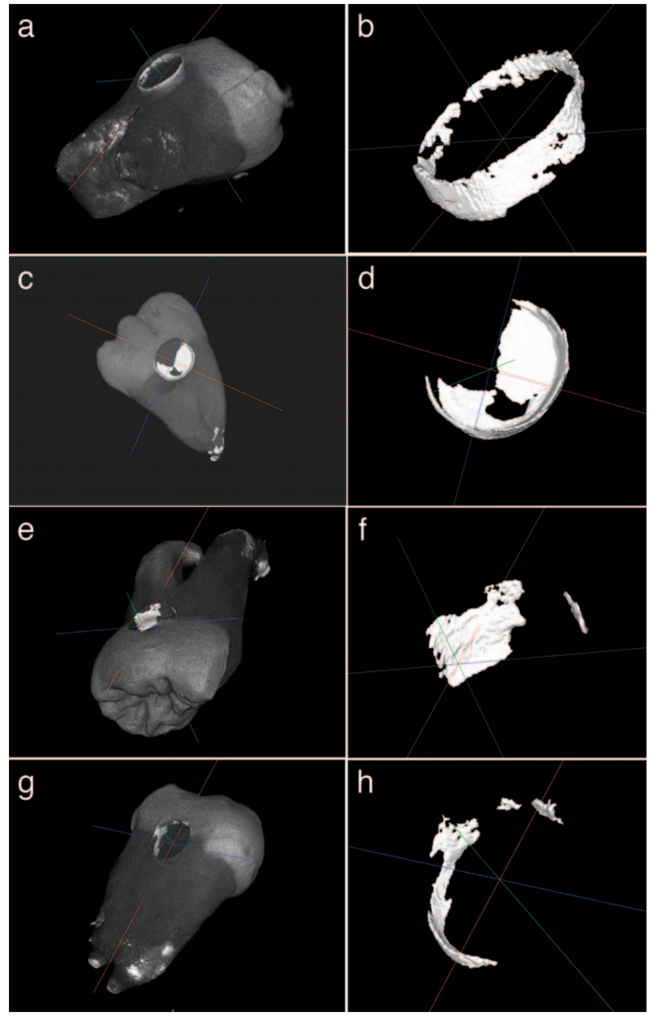

Figure 2. *3D images of silver leakages around four Class V restorations. (a, c, e, and g) Leakage in the cavity after removal of restorations digitally; (b, d, f, and h) corresponding images of silver leakage after removal of both restorations and tooth structures digitally.*

leakage should be highly radiopaque in relation to the restorative material and tooth structure so that even a small amount of leakage can be differentiated. The two main factors contributing to a material's radiopacity are atomic number and density.[14] Silver has a much higher atomic number than do the elements that exist in tooth hard tissues and composite resin fillings; thus, silver is capable of presenting a good radiopaque contrast when it is dense. Dentin contains less hydroxyapatite compared to enamel, which makes it less radiopaque than enamel and much less radiopaque than silver and thus provides a good contrast to silver leakage. Accordingly, silver solution, such as 50% ammoniacal silver nitrate, has broadly been used as a tracer in nanoleakage studies on dentin bonding interface under transmission electron microscopy or SEM.[12,15] Therefore, it was easy to determine the border of silver leakage in the dentin region in the present study.

As mentioned above, the density of silver leakage affects its radiopacity. In the present study, heavy silver leakage presented a good radiopaque contrast to enamel structure and demonstrated a clear border in the enamel region. However, slight silver leakage presented a decreased radiopacity and thus a poor radiopaque contrast to enamel structure, leading to difficulty in identification of the border of silver leakage on the CT image. Even a small portion of some slight leakage presented a similar radiopacity to the adjacent enamel structure and therefore cannot be identified, which resulted in a measured depth of silver leakage that was less than its true depth. To reconstruct the 3D image of silver leakage, the software picked up the silver leakage according to a radiopaque value that was just higher than the value of enamel. Thus, some peripheral parts of the silver leakage next to the enamel structure cannot be picked up as a result of their lower radiopaque value, which resulted in a 3D image of the leakage that was smaller than it should have been.

To improve the differentiability of leakage in the CT method, there is a need for a better leakage tracer that presents a preferable radiopaque contrast to enamel structure. In addition, micro-CT with a higher resolution can present a clearer margin of leakage, which is conducive to the identification of silver leakage.

To present accurately the leakage around a restoration, the leakage tracer should be nondestructive of tooth tissue. Previous studies[16] demonstrated that the nonbuffered silver nitrate solution at pH 3.5 is highly acidic and thus erosive to tooth structures, producing its own path into the tooth/restoration interface. However, the pH of silver nitrate solution can be buffered with ammonia to a pH value of about 7; therefore, a buffered silver nitrate solution is suitable to trace the leakage around a restoration.

One of the great advantages of micro-CT is that it is nondestructive to the specimens tested because there is no need for cutting, which allows the specimens to maintain their original state after measurement and to be further used in other measurements or studies (eg, for observation under a microscope or a SEM). Another advantage of micro-CT is its ability to display the leakage at any section and to reconstruct a 3D image of the leakage around a restoration. Furthermore, the leakage can be measured quantitatively in length, area, and volume using analysis software. The 3D image of any structure in the restored tooth, such as the leakage or the resin composite restoration, can be picked up digitally to display individually, giving a realistic view.

The 3D mapping of the leakage around Class V restorations in this study reveals that the leakage occurs more often and is more severe at the cervical wall than at the coronal wall, which is in agreement with what was previously reported by Pickard and Gaynford[6] and Gale and others[7] using the conventional dye-penetration method. However, the leakage observed on the mid-longitudinal sections is not inadequate to characterize the entire leakage around the restoration, because the leakage around a restoration does not always develop evenly around the cavity wall (eg, the leakage shown in Figure 2a-d). Some leakage even occurs locally in the mesial or distal wall of the cavity, such as the leakage shown in Figure 2e, in which no leakage is detectable at the cervical wall on the mid-longitudinal section. These findings are in agreement with those of Raskin and others,[17] who tested how the number of sections affected the maximum depth of tracer penetration and revealed that microleakage was seldom uniformly distributed. To characterize the degree of the leakage around a restoration, the percentage of the leakage area to the whole area of the cavity wall will be a more meaningful, quantitative index because it takes the whole tooth-restoration interface and the volume of a restoration into account.

It is unclear how the leakage develops and penetrates along the interface, and, thus, a 3D image of the leakage would be helpful to find the answer. As mentioned above, microleakage is the passage of substances such as saliva, ions, compounds, or bacterial by-products, which can be created not only by the contraction gaps of restorative materials but also by the structure defects located within the joint interface between the restoration and the dental structure.[12] The study by Pushpa and Suresh[18] confirmed that the marginal microleakage of composite restorations bonded with one-step self-etch adhesives is closely related to the hydrophilicity of adhesive layer, which is able to generate structure defects in the bonding interface.[19] In this case, the leakage observed may be created mainly by the structure defects within the bonding interface and therefore shows no correlation to the polymerization contraction of the restorations.[20] Accordingly, it is likely that the leakage in the present study demonstrates more action of the structure defects and less action of polymerization contraction of the restoration. Regardless, all of the leakage can be marked by a tracer such as silver solution.

The leakage depth observed varied considerably within some groups; a possible reason for this is the different storage period of the teeth before testing, which varied from three days to six months. There is increasing evidence that storage condition and storage time of the dentin after extraction may influence dentin permeability, adhesion to dentin, and, consequently, marginal leakage.[21-23] On the other hand, the variation in leakage depth provides an additional opportunity to compare the conformity of the results of micro-CT to those of the conventional dye-penetration method in samples with different degrees of leakage.

CONCLUSIONS

Within the limitations of this *in vitro* study, the following are concluded:

- Micro-CT can be used to detect nondestructively the leakage around a composite restoration in 2D and 3D when using a solution of ammoniacal silver nitrate as the tracer.
- Micro-CT has accuracy that is comparable to that of the conventional dye-penetration method in terms of measuring the leakage in the dentin region, but it offers inferior accuracy in the enamel region as a result of the lower radiopacity contrast of silver tracer to enamel.
- The conventional dye-penetration method has the disadvantage of being unable to display the entire leakage around a restoration, and its results are inadequate in terms of characterizing the leakage around a restoration.
- The leakage around a Class V composite restoration is diverse in shape, location, and distribution (rather than always distributing evenly around the cavity wall).

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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