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Reliability of marginal microleakage assessment by visual and digital methods

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ABSTRACT

Objective: The aim of this study was to investigate the reliability of visual and digital methods to assess marginal microleakage *in vitro*. **Materials and Methods:** Typical Class V preparations were made in bovine teeth and filled with composite resin. After dye penetration (0.5% basic fuchsin), teeth were sectioned and the 53 obtained fragments were assessed according to visual (stereomicroscope) and digital methods (Image Tool Software®-ITS) (University of Texas Health Science Center-San Antonio Dental School, USA). Two calibrated examiners (A and B) evaluated dye penetration, by means of a stereomicroscope with ×20 magnification (scores), and by the ITS (millimeters). The intra- and inter-examiner agreement was estimated according to Kappa statistics (κ), and intraclass correlation coefficient (ρ). **Results:** In relation to the visual method, the intra-examiner agreement was almost perfect ($\kappa_A = 0.87$) and substantial ($\kappa_B = 0.76$), respectively to the examiner A and B. The inter-examiner agreement showed an almost perfect reliability ($\kappa = 0.84$). For the digital method, the intra-examiner agreement was almost perfect for both examiners and equal to $\rho = 0.99$, and so was the inter-examiner agreement value. **Conclusion:** Visual (stereomicroscope) and digital methods (ITS) showed high levels of intra- and inter-examiner reproducibility when marginal microleakage was assessed.

Key words: Assessment, composite resin, microleakage, reproducibility of results

INTRODUCTION

Longevity of dental restorations is dependent on the interface between the restorative material and tooth structures.[1] The oral cavity creates a rather severe challenge for tooth composite bonds, that may lead to the formation of gaps at the tooth-restoration interface,[2] that are favorable to the passage of fluids, molecules, ions, and mainly bacteria, a process known as marginal micro leakage (MM).[3,4]

Failures are, usually, related to having cervical margins bellow the cemento-enamel junction (CEJ),[5-7] making the bonding of the composite to dentin more difficult than to enamel.[7,8]

The assessment of MM in dental restorations has been performed *in vitro* as the *in vivo* model is difficult to simulate.[1] Several techniques have been used to measure the level of MM, such as the application of compressed air, bacteria, chemical, and radioactive markers, as well as electrochemical investigations, scanning electron microscopy, dye penetration,[9] and recently micro-CT images.[10]

Regarding dye penetration technique, MM classification can be measured by qualitative assessment in scores[11,12] or by quantitative assessment.[13] Despite the use of qualitative assessment, some authors have investigated and recommended the application of specific software for the detection of MM[14-16] through digital images,[12,17-20] which are measured in millimeters, micrometers or percentages.

However, one question is "how reliable is MM assessment to predict marginal gaps?" A fundamental

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concept is related to reproducibility, defined as the consistency of the results when the measurement or examination is repeated under identical conditions. A test or measurement is reproducible or reliable if the results are identical or closely similar every time it is conducted.[21]

In order to attribute a score or to establish the specific marks to perform an MM measurement, it is important to mention that there is considerable subjectivity in the classification process. Regardless the methods used to assess MM, the study of reproducibility is recommended. Another important issue is that a lack of standardization may lead to variability among examiners.

If it is admitted that there may be discrepancy between classifications and measurements taken by the same examiner in two distinct occasions (intra-examiner agreement), or by different examiners at the same moment in an independent way of classification (inter-examiner agreement), it is necessary to be aware of the limitation with which a MM result is interpreted, whichever the method chosen for assessment.

It is extremely relevant to provide the level of intra-and inter-examiner reproducibility of the MM assessment so that the research can be considered reliable. Therefore, the present study aimed to evaluate the MM reliability when assessed by the visual examination under stereomicroscope, and by the digital examination, using the Image Tool Software 3.0® (University of Texas Health Science Center-San Antonio Dental School, USA).

MATERIALS AND METHODS

Sample preparation

A pool of 110 bovine incisor teeth was examined for defects and micro cracks, and 61 teeth were selected for the study. Teeth were cleaned to remove residual organic tissue, and they were stored in distilled water for 7 days. A typical Class V preparation was made in the buccal surface on each tooth with a #1092 cylindrical diamond bur (KG Sorensen, São Paulo, SP, Brazil) in an air/water-cooled high-speed turbine. A rubber topper was used on the bur so that its cutting tip would precisely penetrate 1.5 mm into the tooth. The occlusal margin of the cavity was in enamel, and the cervical margin was in cementum. Only one operator prepared the standard cavities and checked their dimensions (4 mm wide, 3 mm high and 1.5 mm deep) with a periodontal explorer in order to assure its configuration.

Restorative procedure

Each cavity was etched for 30 s in enamel and 15 s in dentin with 37% phosphoric acid, then 40 s water rinse, and excess water was blotted using an absorbent paper. This was followed by the application of 2-coats of single bond 2 adhesive (3M ESPE Dental Products) for 15 s with a micro brush and gentle air dry. Light cured for 40 s. In order to avoid that the adhesive system reached the margin around the restorations, the excess of the adhesive system was removed of the micro brush, and so it could be applied inside the cavity. All cavities were restored with composite resin filling (Z100, #4004, shade B3, 3M Brazil, Ribeirão Preto, SP, Brazil) in oblique increments of 2 mm and each layer was polymerized for 40 s. Then, teeth were stored for 1 week in distilled water at 37°C, and then polished with aluminum oxide-coated flexible disks (Sof-lex, #4109, 3M Brazil, Ribeirão Preto, SP, Brazil).

Thermocycling and microleakage test

The teeth were subjected to thermocycling (500 cycles, 5°C and 55°C, 30 s dwell time) during 10 h. The root apices were sealed with acrylic resin, and each tooth was covered with two layers of a fingernail varnish, with the exception of the area approximately 1 mm away from tooth/restoration interface. Then, teeth were immersed in 0.5% basic fuchsin dye solution. After 24 h at 37°C they were brushed under tap water for 1 min and sectioned through the middle of the restoration using a diamond saw (Isomet 1000, Buehler, Germany), with 300 rpm, and 53 sections were finally obtained.

Pre-test study

The pre-test study was performed in order to calibrate the two examiners (A and B) in MM classification, according to visual and digital methods. Ten bovine incisor teeth, which were not included in the experimental study, were selected. The pre-test study was essential to determine the minimal loss of teeth, during cavity preparation, and to promote the examiner's calibration in the assessment of visual MM scores and digital images. Both examiners performed the measurements and established a consensus. As fuchsin infiltration produces enough contrast with the tooth tissue to allow an automatic segmentation of the scanned images, the subjectivity could be eliminated.

Assessment procedure

Two previously calibrated examiners (A and B) assessed dye penetration in the gingival margin visually, under a stereomicroscope (Model SZX7, Olympus

Corporation, Tokyo, Japan), with ×20 magnification, and evaluated digital images, by using the Image Tool Software 3.0® (ITS), which is of public domain. Each examiner performed two evaluations in two different occasions, independently, and under blind conditions. The interval between the two readings was 15 days. Subjectivity was controlled by means of the pre-test study previously described, where the examiners evaluated teeth fragments under stereomicroscope. Intra- and inter-examiner agreement was obtained according to the visual and digital methods, as follows.

Visual method: Stereomicroscope

The severity of the dye penetration was scored using the following five-point confidence scale, as it was used in a previous study:[22]

- Definitely absent: No leakage
- Probably absent: Leakage extending one-third to the deepest point of the restoration
- Uncertain: Leakage extending two-thirds to the deepest point of the restoration
- Probably present: Leakage extending to the deepest point of the restoration
- Definitely present: Leakage extending beyond the deepest point of the restoration.

Digital method: Image Tool Software 3.0®

Each fragment was digitalized in a scanner (Model HP Scanjet 4670, Hewlet Packard, Palo Alto, CA, USA) and the MM was obtained in millimeters (mm). The ITS showed the measurements automatically.

Statistical analysis

The estimation of intra- and inter-examiner agreement in the MM assessment was done by using:

- Kappa statistics,[23] by point (κ) and by 95% of confidence interval ($CI_{95\%}$) in relation to the qualitative scale according to the visual method. κ values were classified according to the patterns of Landis and Koch[23] and
- Intraclass correlation coefficient,[24] by point (ρ) and by 95% of confidence interval ($CI_{95\%}$), in relation to the quantitative scale according to digital method.

The database file was analyzed using STATA software (StataCorp 2003. Stata Statistical Software: Release 8.0 College Station, TX: Stata Corporation).

RESULTS

Visual method: Stereomicroscope

Table 1 shows the intra-and inter-examiner agreement classification, according to the visual method of MM evaluation.

The intra-examiner agreement by point was almost perfect for examiner A (κ = 0.87), and substantial for examiner B (κ = 0.76). The respective confidence intervals showed a non-significant difference between examiners A and B, as well as an almost perfect inter-examiner agreement (κ = 0.84).

Digital method: Image Tool Software 3.0®

The intra- and inter-examiner agreement for the digital method were obtained as it can be seen in Table 2.

An almost perfect intra-examiner agreement was reached for each one of the examiners (ρ = 0.99). The same value was obtained for the inter-examiner agreement (ρ = 0.99), showing an almost perfect reproducibility with the digital assessment.

DISCUSSION

Microleakage is a major factor influencing the longevity of dental restorations. The use of composites is still associated with some clinical challenges,[25] such as, recurrent caries and lack of retention, that are related to the main causes of failure of indirect restorations,[26] and failure of extensive composite restoration.[25] Furthermore, studies have shown the increase of MM due to margins located bellow the CEJ,[5-7] and after external bleaching with 10% carbamide peroxide.[27]

Therefore, MM assessment should be as reliable as possible. It is important to assure a level of reliability when different methods are used in such evaluation. The present study investigated the intra-and

Table 1: Intra- and inter-examiner agreement by point (*κ*) and by confidence interval ($CI_{95\%}$) for the evaluation of marginal microleakage, according to qualitative assessment-visual method

Agreement	Examiner	*κ*	$CI_{95\%}$
Intra	A	0.87*	0.74-1.00
	B	0.76**	0.63-0.88
Inter	A-B	0.84*	0.75-0.94

**Substantial: 0.61-0,80; *Almost perfect: 0.81-1.00, CI: Confidence interval

Table 2: Intra- and inter-examiner agreement by point (*ρ*) and by confidence interval ($CI_{95\%}$) for the evaluation of marginal microleakage, according to the quantitative assessment-digital method

Agreement	Examiner	*ρ*	$CI_{95\%}$
Intra	A	0.99*	0.98-1.00
	B	0.99*	0.98-1.00
Inter	A-B	0.99*	0.98-1.00

*Excellent: 0.81-1.00, CI: Confidence interval

inter-examiner reproducibility of MM assessment, according to visual (scores) and digital (numerical scale) methods. It was possible to observe that both methods provided high levels of intra-and inter-examiner agreement. A factor that contributed to this result was related to the calibration procedure, which is indispensable in studies of this nature.

According to Corona *et al*.[13] it is essential to verify the reliability of the study with any kind of the diagnostic method. MM assessment is considered simple to be obtained when visual method is used. However, it represents a subjective method.[28]

Few studies mentioned the level of inter-examiner agreement when qualitative measurement in scores was applied.[29] Literature shows an inter-examiner reliability ranging from 0.77 to 0.99 in a study of the evaluation of the MM in scores using a stereomicroscope[30] what is in agreement with the present findings.

It is important to consider that the classification of intermediate levels of MM by the visual method can be attributed in the same score. In the present study, the intra-examiner agreement was 0.87 and 0.76, respectively to A and B, and the inter-examiner agreement was 0.84, leading to a reliability classified from substantial to almost perfect.[23] Chen *et al*.[31] cited the substantial reliability when they investigated MM with the use of silver nitrate and micro-CT.

Based on technological development, several available software packages are able to provide numerical MM measures, which consists a more objective result. One advantage of working with digital images is the possibility of being easier to manipulate them, by using zooming tools. Regarding the methods that are used to detect MM, some authors have applied the quantitative assessment, which allows the exact measurement of the leakage.[32] Other researches recommended the use of devices, such as Leica Qwin Plus image analysis program,[32] and KS300-v2.0 software Kontron Elektronik, GmbH, Eching bei München, Germany).[22] In the present study, it was applied the ITS device as it showed high accuracy to assess MM in a previous study.[33]

Digital assessment by means of the ITS device provided reliable MM measurements, with an excellent reproducibility ($\rho = 0.99$).[24] Although this software was easily operated, and allowed the analysis of the images in a short time interval, it took longer to get images digitalized, when compared to the visual assessment. This could be considered a limitation of the digital method.

Despite such limitation, the authors suggest a previous calibration procedure to check the intra-and inter-examiner reproducibility regardless the method that is chosen to assess MM.

On the other hand, there is another variable that affects the penetration values measured, and it is related to the number of tooth sections. It is convenient to section the tooth structure in half and look only at the two exposed sides.[1] However, Raskin *et al*.[34] showed higher correlation between the reference value of MM and data obtained from three sections.

Another important issue is related to the stereomicroscope, as an evaluation method for MM. Chen *et al*.[35] compared the micro-CT method against the section method regarding the assessment of MM of sealants, and they concluded the best images were obtained from stereomicroscopy. Therefore, micro-CT is not yet ready to replace the section method, as the gold standard for MM assessment at the sealant-enamel interface.[35]

In this study, we used the traditional section method, although nowadays the micro-CT technique shows advantages, such as no loss through slicing and it is possible to see the margin for inspection without destruction of the fragments.

Futures studies carried out with ITS, micro-CT, and other software packages should be performed with the aim of obtaining reproducibility values to improve diagnostic performance when assessing MM.

Along with the evaluation of the material properties, the study of *in vitro* diagnostic methods is essential to obtain clues about the behavior of a dental material. Not only is the study of diagnostic methods essential, but so is the study of the reliability of such methods.

CONCLUSION

The MM assessment by using visual (stereomicroscope) and digital (ITS) methods featured high levels of intra-and inter-examiner reproducibility.

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