NEW DIRECTION FOR MEASUREMENT OF MICROLEAKAGE IN CARIOLOGY RESEARCH

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Abstract

The present study introduces the feasibility of a different new direction to determining the microleakage volume associated with dental restorations and the relative marginal adaptation deficiency of teeth in in vitro conditions. Our method utilizes the molecular adsorption characteristics of Methylene Blue. It was concluded that investigations showed the microleakage volume measurement method looking as a valuable new technique for the in vitro study of microleakage dynamics around dental restorations.

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Introduction

Main principle in restorative dentistry states that the transition between the restorative material and the dental hard tissue must be continuous to increase the survival probability of the restoration[1].

One of the most important problems of restorative dentistry today is the failure of restorative materials to completely bond to enamel and dentin, causing microleakage. Microleakage has been defined as the passage of ions, molecules, fluids or bacteria between a cavity wall and the applied restorative material. Microleakage has been reported as the cause of hypersensitivity of restored teeth, discoloration at the margins of cavities and restorations, recurrent caries, pulp inflammation and failure of endodontic treatment[2-4].

It is also affected by a number of other factors: for example, the operative techniques used, including the size of the cavity, the angle at which the enamel prisms and dentin tubules are cut depending on their location, the method used to condition dental hard tissues[1].

Microleakage is determined today by many *in vitro* techniques with or without thermal cycling, such as staining; scanning electron microscope; bacterial activity; decay; air pressure; chemical agents; markers; neutron activation analysis; radioisotope; ionization; autoradiography, reversible radioactive adsorption and electrochemical technique[2-9].

The significant differences between these models and materials suggest that an ideal method for the determination of microleakage has not yet been established.

The aim of some researchers is to develop an *in vitro* model to replicate microleakage at a tooth/restoration interface[3,10-12].

Laboratory tests and clinical trials must also be added to the process of evaluating whether the microleakage measurement methods fulfill the objectives of evidence-based dentistry. It is only possible to obtain sufficient evidence in the natural sciences if proven and correct evaluation methods are applied and if those methods are subjected to the process of validation. If the test method confirms by examination and the provision of objective evidence that the particular requirement for a

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specific intended use can be consistently fulfilled, the method can be called "validated" and the results are "internally valid" [1].

The purpose of present study was to introduce valuable an *in vitro* model to determine the microleakage volume in *in vitro* marginal adaptation research studies.

Materials and Methods

Primary human teeth were obtained from the Department of Maxillo-Facial Surgery of Dental Faculty, they were selected by Binocular Stereo Microscope (Olympus Co., Japan) as without caries and cracked for this study.

The teeth species were used for microleakage volume study. Before the cavity preparation and restorative procedure, all teeth were cleaned. Class V cavity prepared on the buccal surface of each tooth. The cavities had a mesio-distal width of 3mm, an occluso-cervical length of 2mm and a depth of 1.5 mm.

The teeth were restored in the following way: The type 2 light-hardening powder and liquid glass ionomer cement (Variglass VLC, Dentsply, USA) were prepared in accordance with the manufacturer's instructions, applied to the bottom of the preparations and polymerized. (Astralis3, Vivadent, Australia). Following polymerization, all preparations were filled with a high copper amalgam (Cavex Avalloy, Cavex Co., Holland) and 24 hours later finishing and polishing were performed.

The specimens were subjected to thermo cycling between $5^{o}C \pm 4^{o}C$ and $55^{o}C \pm 4^{o}C$ for 500 cycles (Guidance on substrate selection, storage, handling, and execution of bond strength tests according to the ISO Technical Specification 11405103).

After thermo cycling, the surface of the teeth, up to approximately 1.5 mm to the restoration, were coated with a layer of nail varnish, melted utility wax and a second layer of nail varnish[3,10,12-15].

The methylene Blue(MB) solution was prepared to a concentration of MB 4.75 g/l. A stock solution was prepared using a buffer of $H_2PO_4^-$ / HPO_4^{-2} (phosphate / biphosphate) with a pH of 6.98 and 24 hours did storage the specimens in the MB solution.

Each individual sample was quantitatively measured for volume of the marginal gaps using the chemical molecular characteristic properties of MB. Theoretically, the volume measurement method was created and applied as described below.

The MB molecule is made up of an acid combined with an organic base. Its molecular weight is (M_A=319.868g.mol^{-1}) and a single piece of the absorbed covers an area of (σ)=120 $A^{0\,2}$ on the surface[16]. (Figure 1).

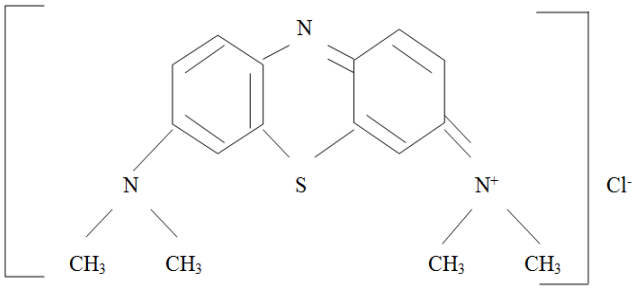

Figure 1. **The molecular structure of methylene blue.**

Adsorption is the accumulation of dissolved molecules over the surface of a solid matter, the dissolved molecules could be atoms or ions of matter present in any solution of a gas, vapour or liquid phase. In the phase, which allows the accumulation to occur between surface, is known as the adsorbent (the teeth), the matter which accumulates, is known as the adsorbate (MB) [16-18].

Adsorption, in the liquid form, is usually measured using an indirect method. After the experiment, the teeth were dissolved in a 50% solution of nitric acid, the MB that filled the microleakage gaps dissolves into the solution and it's the MB concentration is determined.

To draw the calibration graph, a part of the MB solution was taken and determined to have a wavelength of 664 nm in a spectrophotometer λ_{max} (maximum absorption wavelength).

Some of the MB stock was taken and diluted (10 different concentrations were prepared using 100 milliliters of distilled water in each beaker to dilute the 2% MB, ranging from 0 mL added to 180 mL added in 20 mL increments) to form a series of solutions of varying concentration. These varying concentrations were measured for their absorption wavelengths. These measurements were then used to construct the calibration graph (Figure 2).

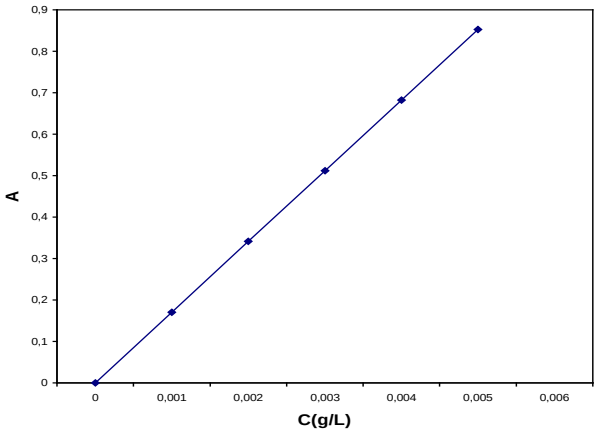

Figure 2. The calibration curve of Methylene Blue (A; Absorbance, C; Concentration).

A=E C (A: Absorbance, E:molar absorption coefficient, C: concentration)

The molar absorption coefficient was determined to be 170.57 $dm^3.cm^{-1}.g^{-1}$.

After the calibration graph was drawn, the concentrations were calculated using the absorption values.

In the absorption experiments of our study, the teeth were placed in joje balloons along with 100ml of MB solution. The primary teeth were subjected to MB dye penetration at 37°C for a 24-hour period, to allow dye penetration into any possible existing gaps between the tooth substance and the restorative material[3,8,10].

Afterwards, the tooth tissue around the restoration surfaces was removed in a block by making cuts 1.5 mm around the restored area (Figures 3,4).

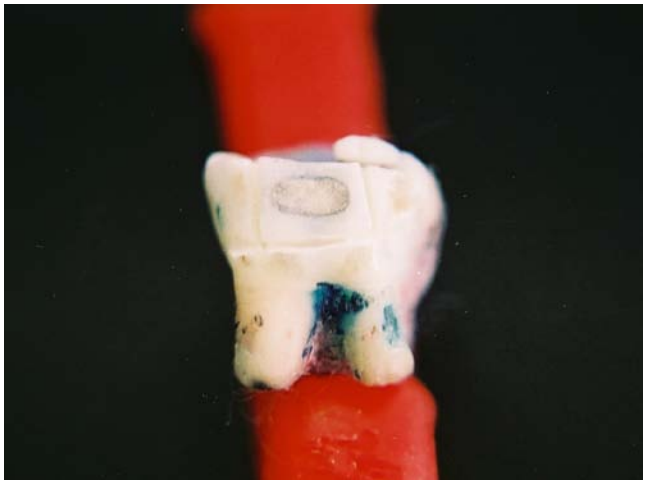

Figure 3. Appearance of cutting model of tooth tissue 1.5 mm around the restoration surfaces was marked.

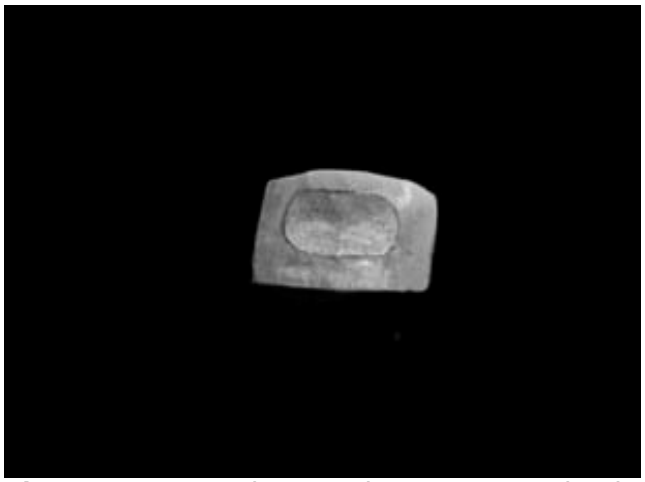

Figure 4. Teeth specimens around the restoration surfaces were removed in a block by making cuts 1.5 mm (MagnificationX10).

These blocks were then dissolved separately in 50% nitric acid (Figure 5) and the amount of MB absorption was calculated using the previously created calibration graph. These values were converted to volume values (V=m/d) and the individual tooth volume values are reported in Table 1.

Figure 5. Removed blocks were then dissolved separately in 50% nitric acid.

Results

Dye penetration for group calculated according to the present method. Measurement values of the volume measurements of the group and the MB molecular counts equivalent to median values are shown in Table 1.

In the present study we didn't use control group due to that study introducing a way for microleakage research. Different specimens

groups, could be compare and evaluate statistically for evaluation of microleakage volume using One Way ANOVA Test and statistically significant differences could be revealed.

Specimens n=10 N	Amalgam+ GIC (Human Teeth) Volume mm^3/tooth(10^{-3})
1	1.91
2	2.10
3	2.16
4	2.19
5	2.18
6	2.20
7	2.23
8	2.25
9	2.27
10	2.33

Table 1. Measurement values of the volume measurements of the group as median values human teeth which restored by Amalgam.

Discussion

The purpose of microleakage tests is to evaluate the seal of restorations placed in extracted teeth and to give a prognosis about the clinical performance with regard to the occurrence of postoperative sensitivity and/or secondary caries[19].

The *in vitro* microleakage phenomenon and the adaptation of filling materials into the cavity walls under clinical and laboratory conditions constituted the focal points of researchers for many years and a variety of methods have been used to research this[4,6,7,10,19-22]. Some of these laboratory models have been successfully used to in order to determine microleakage, but they are not quantitative methods. Also comparison of the marginal adaptation results using

Due to its ease and simplicity, the most frequently used method is the measurement of the microleakage of a specific dye after sectioning teeth that have been restored[1].

The second most frequent method is the quantitative marginal analysis of replicas of restored teeth with the scanning electron microscope (SEM) and an appropriate software for length measurements, is used less often, because it is more time consuming, and complex[1,19] but SEM analysis, is semi quantitative method within samples allocated from the study groups.

In fact, in the studies of dye penetration, the dentin staining was observed to be more different than the actual gaps between cavity walls and restoration materials. This resulted in the use of a dye with a particle diameter equal to the bacterial size or smaller by researchers (around 2μm) [3].

Dye penetration is a diffusion phenomena and the consequences are that the results are not obtained immediately, they are semi-quantitative, and the defect is evaluated on a section (two-dimensional evaluation) [23].

For microleakage studies a popular dye is Methylene Blue [19,24].

In present study, a 2.00% solution of the MB molecule was used (one MB molecule=1.2nm^2=120 $A^{0\ 2}$) since the particle size is less than that of the bacterial one. MB molecules were used because the also dissolve as monomer and bimer in an aqueous environment in which the pH is adjusted to 6.98 with a phosphate and biphosphate buffer [3,17].

Another important issue in microleakage studies arises from the scoring systems. Since the evaluation in those studies largely depends on the observer's interpretation, the leakage scoring is at best a semi-measurable method[3].

Various studies performed show that the dye leakages in different sections taken at different places of the restorations may show significant differences[6,23]. For this reason, the accuracy of a leakage study based on a single section made from a tooth may be negligible.

As of today, there are no quantitative methods applicable and valuable for the

microleakage determination; we have above indicated the amount of microleakage through quantification.

In the stereo microscopic studies, the method is based on the interpretation of the leakage of dye on the cavity wall and is defined as a semi-quantitative approach where the leakage is calculated solely at the surface where the section is made[3,6,7].

In that method, the researcher's observation and interpretation do not come into play in the determination of microleakage volume quantity and all surfaces where a leakage occurs between tooth/restoration materials is quantitatively measured by a chemist.

The volumetric measurement method leads to the immediate behavior of the whole interface and not only the sealing ability of the margin (volumetric evaluation).

The groups can be comparing as a statistically for microleakage volume measurements using the One Way Anowa Test.

Conclusions

It was also concluded in that preliminary and our previous investigations[25, 26] show that the method of measuring the microleakage volume can be best a valuable tool for the *in vitro* study of microleakage dynamics around dental restorations, and this method can be use as a new technique for the determination of microleakage volume.

The use of a measurement of volume to detect microleakage in magrin of restoration appears to be effective, although it is not suitable for simulating *in vivo* clinical circumstances.

It is necessarily more further work to establish the true scope of the model, but this preliminary investigation shows promise and its supported with previous experimental study.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

References

1. Siegward D. Heintzea, Systematic Reviews: I. The Correlation Between Laboratory Tests on Marginal Quality and Bond Strength. II. The Correlation Between Marginal Quality and Clinical Outcome *J Adhes Dent 2007; 9: 77-106.*

2. Taylor, M.J; Lynch, E. Microleakage. *J. Dent.* February 1992, vol. 20, p. 3-10.

3. Yavuz, I; Aydin H. New Method For Measurement of Surface Areas of Microleakage at The Primary Teeth By Biomolecule Characteristics of Methilene Blue. *Biotechnol. & Biotechnol. Eq.* 2005, vol. 19, p. 181-187.

4. Tjan, A. and Tan, D. Microleakage at gingival margins of class V composite resin restorations rebonded with various low-viscosity resin systems. *Quintessence Int.* July 1991, vol. 22, p. 565-573.

5. Pradelle-Plassea N, Wengerb F, Picarda B, Colona P.Evaluation of microleakage of composite resin restorations by an electrochemical technique: the impedance methodology Dental Materials (2004) 20, 425–434.

6. Yavuz, I and Atakul, F. Evaluation of microleaking in different in vitro restorations of cow permanent teeth. *Balk J Stom.* March 2001, vol. 5, p. 42-45.

7. Yavuz, I and Atakul, F. The comparison of amount of leaking occurred in different sections of in vitro prepared restorations in cow permanent teeth. *Balk J Stom*. July 2001, vol. 5, p. 104-107.

8. Yavuz, I; Atakul, F. The comparison of amount of microleaking for in vitro restorations in permanent teeth of human, dog and cow. *Balk J Stom.* July 2000, vol. 4, p. 103-106.

9. Sano, H *et al.* Nanoleakage: Leakage within the hybrid layer. *Operative Dent.* January-February 1995, vol. 20, p. 18-25.

10. Yavuz, I ; Aydin, H and Kaya, S. New method for measurement of microleakage. *T Klin J Dent Sci.* July 2003, vol. 9, p. 79-85.

11. Matharu, S *et al.* A new *in vitro* model for the study of microbial microleakage around dental restorations: A preliminary qualitative evaluation. *Int End J*. October 2001, vol. 34, p. 547-553.

12. Iwamı, Y; Yamamoto, H; Ebısu, S. A new electrical method for detecting marginal leakage of in vitro resin restorations. *J Dent.* May 2000, vol. 28, p. 241-247.

13. Olmez, A; Oztas, N and Bodur, Haluk. The effect of flowable resin composite on microleakage and internal voids in class II composite restorations. *Oper Dent.* November-December 2004, vol. 29, p. 713-719.

14. Derhami, K; Coli, P and Brannstrom, M. Microleakage in class 2 composite resin restorations. *Oper Dent.* May-June. 1995, vol. 20, p. 100-105.

15. Gungor, H C; Turgut, M D; Attar, N and Altay, N. Microleakage evaluation of a flowable polyacid-modified resin composite used as fissure sealant on air-abraded Permanent teeth. *Oper Dent.* 2003, vol. 28, p. 267-273.

16. Aydin, H and Tez, Z. Comparative determination of the adsorption capacities and possible fields of usage of active carbons prepared from the shells of walnut and hazelnut through some commercial active carbons. *Turk J Chem*. 1996, vol. 16, p. 51-58.

17. Nelsen, R J.; Wolcoltt, RB and Paffenbarger, GC. Fluid exchange at the margins of dental restorations. *J Am Dent Assoc*. March 1952, vol. 44, p. 228-295.

18. Davies, RG. The use of benzene adsorption isotherms in evaluating the surface and pore characteristics of activated carbons. *Chemistry and Industry.* February 1952, vol. 23, p. 160-165.

19. Roulet JF, Reich T, Blunck U, Noack M. Quantitative margin analysis in the scanning electron microscope. Scanning Microsc 1989;3:147-58.

20. Turgut, M D.; Tekcicek, M and Olmez, S. Clinical evaluation of a polyacid-modified resin composite under different conditioning methods in primary teeth. *Oper Dent.* September-October 2004, vol. 29, p. 515-523.

21. Ersin, N K. and Eronat, N. The comparison of a dentin adhesive with calcium hydroxide as a pulp-capping agent on the exposed pulps of human and sheep teeth.*Quintessence Int.* 2005, vol. 36, p. 271-280.

22. Kelsey, WP; Latta, MA; Meara, JD; Barkmeier, WW; Hammesfahr, PD and Jefferies, SR. An accelerated in vitro model for adhesive testing. *Am J Dent.* October 2004, vol. 17, p. 323-326.

23. Prati C, Tao L, Simpson M, Pashley DH. Permeability and microleakage of class II resin composite restorations. J Dent 1994;22:49-56.

24. Raskin A, D'Hoore W, Gonthier S, Degrange M, Dejou J. Reliability of in vitro microleakage tests: a literature review. J Adhes

Dent 2001;3:295-308.
25. Yavuz, I., Aydın H., Ulku R., Kaya S. ve Tümen C., "A New Method: Measurement of Microleakage Volume Using Human, Dog and Bovine Permanent Teeth," Electronic Journal of Biotechnology, 9(1), 8-17 (2006).
26. Yavuz, I.,. Aydın A.H, Ulku R., Dulgergil T.C. ve Akdag M.Z., "New Technique: Measurement of Microleakage Volume in The Marginal Gaps of The Dental Restorations," Biotechnol. & Biotechnol. Eq, 19 (3), 184-191 (2005).