

Literature Review

REAL TIME REFERENCE CONTROLLED REVERSE DIFFUSION QUANTITATION OF MICROLEAKAGE: JUSTIFICATION

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من أهم أولويات البحوث في مجالات طب الأسنان هو، محاولة إيجاد طرق لمنع التسرب الدقيق للسوائل حول الحشوات السنية، وقد بُذلت جهود كثيرة في هذا المجال ولكن لعدم وجود طريقة تقيس كمية التسرب أدى ذلك إلى صعوبة اتخاذ خطوات سريرية تمنع التسرب. يناقش هذا المقال البحوث المنشورة في هذا المجال ويلقي الضوء على الطرق المستخدمة فيها وعن الحاجة إلى إيجاد طريقة دقيقة لقياس مقدار التسرب الدقيق حول الحشوات السنية ويتعرض للطريقة غير المدمرة التي استخدمها المؤلفان لقياس كمية التسرب حول الحشوات السنية.

Among the priorities for dental research is preventing microleakage. Despite many efforts to achieve this goal, the phenomenon continues to intrigue researchers. Perhaps, the lack of a definitive method to objectively quantitate microleakage is among the primary handicaps preventing its control. This paper asserts that as of today all restorations badly suffer from microleakage irrespective of the material and restorative technique used. The paper further substantiates that, despite their excellence, all prior efforts in quantitating microleakage and claims to such an achievement are not sound. This paper reviews and analyzes prior studies on microleakage, including those of the author, and points out their nominal nature in quantitating microleakage. Further, based on the literature review and experimental evidence a well standardized non-destructive *in vitro* testing method for real time quantitation of microleakage is presented.

Introduction

One of the fundamental goals of a conservative (intracoronar, extracoronar, endodontic) dental treatment is to eradicate the disease and restore the tooth to its "original" functions. Prosthodontist, endodontists and dental materials specialists have been most innovative and hard working at introducing new treatment modalities, tooth/root preparation designs and materials to achieve that goal.

Received 24.06.95; accepted 16.07.95

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+ This investigation was supported by King Abdulaziz City for Science and Technology, Research Grant AT-12-57, P.O. Box 6086, Riyadh 11442.

Techniques and materials capable of withstanding the most challenging conditions that may be present in the oral cavity have been successfully developed. Many of the problems barring tooth restoration to its "original" functions have been resolved. Dentistry as a science and an art has achieved phenomenal progress during the recent decades. However, the great achievement of researchers continued to be threatened by one phenomenon. That phenomenon is microleakage. The phenomenon has also been described as marginal permeability, micro-marginal leakage, fluid exchange, liquid diffusion and capillary penetration.

Microleakage is the result of the fact that current conservative materials do not chemically bond to cavity walls to the extent of forming a hermetic seal. Lack of a hermetic seal at the interface means a gap that allows the seepage of oral fluids between the restoration and the prepared tooth surface.

Microleakage is probably the only phenomenon that can be singled out as the most harmful to con-

servative dental practice. Hundreds of scientific papers addressing the issue of microleakage have been published. Many of these articles used one of the numerous experimental procedures that compares sealing ability of a given tooth preparation design or a restorative material *vs* another. The data generally embodied terms such as "It appears that" material A or tooth preparation design A caused less leakage than that of B. Since all conservative materials and tooth/root preparation design techniques, did leak, it is important to know how much leakage and at what point in time did such leakage occur. Thus, prior experiments, despite the excellent efforts of their respective investigators, lacked one essential characteristic, that is, true objective quantitation of microleakage.

Some attempts at quantitation were considered. The experimental procedure in these attempts lacked reasonable clinical simulation even as an *in-vitro* effort. In other studies attempting quantitation, the standard deviations given were greater than the means themselves and inaccurate assumptions were made relative to the experimental reference used. Furthermore, other experimental techniques were so complex that they required the use of a nuclear reactor with adequate neutron flux. Accordingly, despite the real threat of microleakage to dental practice, there is no means available, heretofore, to quantitate it. Without proper quantitation of microleakage, proper understanding of the factors that enhance or reduce it, hence its control, is not possible.

Evaluation of microleakage associated with dental restorations was first reported by Harper in 1912. According to Blackwell,¹ the phenomenon have been described in 1895. Research effort aimed at evaluating microleakage has, perhaps, continued since that time. Today, such investigations continue in abundant numbers.

Tests for evaluating microleakage

The experimental methods used to evaluate microleakage are numerous (Going, 1979). Each method has been applied many times by various investigators to assess leakage associated with a dental material or a technique. This combination led to the generation of many scientific articles.

One widely used method of evaluating microleakage is the use of radio-isotopes.²¹⁸ The method was introduced in 1951 by Armstrong and

Simon.¹⁹ The *in-vitro* isotope experimental procedure involves several steps. Tooth is restored with the appropriate material. The tooth is coated with wax or nail enamel such that only the narrow areas between the tooth and the restoration, are exposed. The coated tooth, or portion therefrom including the restoration surface, is placed in a liquid containing an isotope. The isotope containing liquid seeps into the capillary between the tooth and the restoration. The preparation portion of the tooth is sliced such that the boundary between the restorative material and the tooth walls is exposed. This section of the tooth is placed on a dental radiograph. The radioactivity in the interface exposes the radiographic film in the areas where a radioactive material is present (autoradiography). When the radiographic film is developed, the areas where radio-activity penetrated in the gap (between the tooth and the restoration) appear radiopaque.

The distance of radiopacity is measured as an indicator of how deep the isotope leaked between the tooth walls and the restoration. Deeper radiopacity is an area where microleakage is considered pronounced.

At what point in time did this leakage occur relative to a standard, a different technique, or material, cannot be determined. Leakage rate change by time cannot be measured either since the sample has been destroyed already.

The isotopes used are ⁴⁵Ca, ⁵⁵Mn, ¹³¹I, ²²Na, ³⁵S and Dysprosium. Calcium was the most popular since it is readily available. Besides being qualitative in nature, isotope testing in this manner has the additional disadvantages of being unsuitable for monitoring over a long period of time; a test sample is lost every time a reading is taken (destructive method of testing); and calcium being the most popular, has an affinity to tooth structure. Another major disadvantage of the method is the absence of controls.

Another widely used method of evaluating microleakage is the dye penetration method.¹²¹⁹ⁿ ⁵⁷ The method was used as early as 1933 by Fish.⁵⁸ The dye penetration experimental procedure is almost identical with the one for the isotope method. The exception is that the restored tooth or root is immersed in a dye instead of an isotope bearing liquid. The tooth is then sliced and the depth of dye penetration is determined by micro-

scopic examination. Deeper dye penetration suggested more microleakage. Some of the dyes used were methylene blue, eosin, india ink, methyl violet, hematoxylin, prontosil soluble red, basic fuchsin, fluorescein, rhodamine blue and procaine brilliant green. Methylene blue was the most popular.

Besides being qualitative in nature, the dye penetration method is destructive; a test sample is lost everytime a reading is taken and monitoring over a long period of time is not possible. The method can't be used when an oil base material (e.g. ZOE) is used since the oil chemically quenches the dye and saturates it within the material.⁵⁹

A modification of the dye penetration method is the use of 50% silver nitrate as a chemical marker.⁶⁰⁻⁶⁵ After immersion in silver nitrate, the tooth or root is immersed in a developing solution under bright light. Tooth or root is then sectioned and examined under a microscope.

Bacteria and caries were also used in studying microleakage.^{42,66,71} The bacterial penetration method was introduced by Fraser in 1929.⁷² Inducing caries and measuring its depth as evidence of leakage was introduced by Brown *et al* in 1962.⁷³ Since bacterial penetration and the resulting secondary caries are of prime concern in dental practice the method was considered more relevant than others.^{74,78} The method was criticized for its lack of quantification, limitations due to the size of bacteria, its unsuitability for monitoring marginal leakage over a long period of time, and being tedious and unreliable due to many variables experienced.^{42,60,79}

Electron microscopy has been used in evaluating microleakage through examining the width of the gap existing between the cavity walls and the restoration.^{80,88} Scanning electron micrographic "SEM" examination technique was combined with the radioisotope method by visually observing samples that have been exposed to radioisotope penetration and evaluation.⁷⁹ There was no correlation between the size of the gap observed by SEM and the leakage determined by radioisotope readings. Besides being qualitative and destructive method of testing, the SEM procedure introduces errors and artifacts related to cracks and distortions that occur during sectioning and drying crazes that occur during examination.

Air pressure was also used to evaluate microleak-

age. The technique was first described by Harper in 1912.⁸⁹ The method was used by several investigators.^{90,96} The testing method consists of forcing air through the interface between the cavity walls and the restoration and measuring the pressure causing leak at the interface. While the test measures the degree of ease or difficulty of air passage of the interface, it fails to measure liquid leakage; the diffusion dynamics are quite different between the two situations. The test is also non-destructive in the sense that the specimen is not sacrificed everytime a reading is taken. However, the test is destructive in the sense that when the interface is exposed to such pressure at a given point in time, small bi-products therein (corrosion biproducts for example) are removed at that time. In this manner, the specimen cannot be used to take another reading at a later date to indicate the cumulative effect and the change in rate. Finally, the test method is far removed from the clinical situation even as an *in-vitro* technique. The method does not simulate conditions present in the mouth or in the tooth.

A modification of the air pressure technique is the use of fluid filtration to measure microleakage.^{97,101} The technique consists of making a hole in the floor of the cavity under investigation. A tube is connected, flush with the pulpal floor and extended to be connected at the other end to a pressurized fluid reservoir (fluid filtration apparatus). The cavity is then restored. A fluid under constant pressure is introduced at the cavity floor. A small air bubble in a micropipette situated in the middle of the connecting tubing is then used to indicate fluid movement. While this method is a slight improvement over the air pressure technique, it is destructive since the tooth must be cut so that only the section that contains the restoration remains. The apparatus is complex and sample preparation is cumbersome. Further, microleakage is measured under pressure, which is far removed from the clinical situation.

A most expensive method for evaluating microleakage is one that requires the use of a nuclear reactor, the neutron activation method. The tooth or root is restored and restoration margins are soaked in a non-radioactive solution of Manganese,^{102,103} Vanadium, Indium¹⁰⁴ or Dysprosium.¹⁰⁵ The teeth are then placed in the core of a nuclear reactor and exposed to pulsed neutron flux. The non-radioactive material, within the

tooth, becomes radioactive emitting gamma rays. The emission is measured by a scintillation detector. The higher the counts emitted by a given tooth, the higher the microleakage. This method could be quantitative but has several limitations. The difficulty in using this method emanates from the high cost of processing the samples, the need for the expertise of a highly qualified nuclear engineer to do the laboratory work and the availability of a nuclear reactor with adequate neutron flux. Further, the equipment for analysis and computation of data are generally unavailable.⁸ Additionally, a sample used to determine microleakage at a given time cannot be used again to determine microleakage at a later time. In this sense, the method is destructive.

A fairly recent study of microleakage used an old method, a pH indicating paper.¹⁰⁶ All tooth preparations are coated with a layer of calcium hydroxide, then the respective restorative material is inserted. The restored tooth is soaked in water for one minute then dried with absorbent paper. A small section of pH indicating paper is placed on the restoration margins. At the end of one minute, the pH paper is removed. Paper color conversion will indicate microleakage, while no change in color will mean no microleakage. Clearly, the test is highly quantitative; even pH of free liquids is no longer quantitated with pH indicating paper.

Simple tests for quantitating microleakage

In 1979, at the University of Florida, an alternate *in-vitro* method for quantitating microleakage was introduced.^{107,108} The method showed promise to be non-destructive, quantitative, simple, inexpensive and subject to minimal human error. Another feature of the new method was the fact that the restoration-cavity interface can be kept intact and undisturbed for any length of time.¹⁰⁹ As such, a minimal number of teeth is needed while conducting a long term study. This latter feature is important in that the investigator can study changes of the tooth-restoration interface over time. Findings of these studies were not published as full articles since the researchers realized that the experimental procedure was not carefully calibrated with respect to radioisotope adsorption by the tooth. The method consisted of depositing a known number of counts of a radioactive material on the prepared tooth surface. The radioactive material is dried.

The tooth is restored and immersed in an appropriate liquid. Microleakage (diffusion) is now reversed relative to all prior studies, *i.e.* radioisotope leaks from tooth to medium. They termed the method reverse diffusion. Samples from the medium are taken at various time intervals and the total radioisotope leaked is determined by using a scintillation counter. The amount leaked at any point in time can be computed as a percent from the original quantity deposited. In 1983, two other investigators from the same department carefully analyzed the method mathematically.¹¹⁰ They concluded that the "reverse diffusion simulation model of the microleakage phenomenon is a new approach towards providing a quantitative analysis of microleakage." An essential factor in proper quantitation that was neglected by the advocates of this quantitative methods was the wrong assumption that the total amount of tracer (in radioactivity) deposited in each cavity is available to be released by microleakage. This assumption, unfortunately, and as is shown below, is not true. Consequently, all the data obtained by the reverse diffusion method may be considered quantitative but inaccurate.

The reverse diffusion method was adopted by three other groups^{111,113} utilizing ¹⁴C, ¹²⁵I and ³H-uridine as tracers in endodontic restorations. Not only did these investigators fall into the same error made by the initiators of the reverse diffusion method but added a new error. The new error was leaving the radioisotope with its liquid carrier in the obturated canals. In this case, they were studying liquid/liquid diffusion which does not represent the clinical situation whereby there is no free liquid inside the restored canal (cavity). Microleakage in the clinical situation is a liquid/solid diffusion phenomenon not a liquid-liquid diffusion one. The diffusion dynamics of these two conditions are quite different. Consequently, it is not surprising that the reported standard deviations of the results exceeded the means themselves.

Another quantitative method exposes the tooth to dye penetration then the tooth is dissolved in nitric acid. The amount of dye is determined by a spectrophotometer.^{114,115} The higher the dye concentration, the more the leakage through the tooth-restoration interface. Besides being destructive, the quantitative ability of the dye method can be questioned since the volume of dye present is extremely

minute relative to the tooth volume. Hence, the tooth tissues can mask the dye. As teeth have different colors, it is expected that this may also alter the spectrophotometer readings. Suffice to state that when the principal author of the method recently published again on microleakage, he used the qualitative silver nitrate dye method.⁵³

The above literature review indicates that the efforts of dental scientists in measuring microleakage have never ceased. Much have been accomplished by the investigators of these prior studies. Yet, a simple, quantitative, objective and non-destructive testing method capable of measuring the rate of change in microleakage over time and for unlimited time period is not yet available. It is the objective of this paper to introduce such a method.

Reference controlled reverse diffusion method

Except for the erroneous assumption that all the radioactivity placed in a cavity is available immediately for microleakage and the lack of careful standardization, quantitating microleakage by the reverse diffusion meets all the requirements of an acceptable method.¹⁰⁷¹¹⁰¹¹⁶ Experimental substantiation that not all radioactivity in a cavity is immediately available for microleakage and introducing a means to account for such inavailability, is presented below.

In the reverse diffusion method an amount of radioactivity (CPM's) is placed in a cavity and dried. The cavity is restored and the tooth is sealed with nail enamel except at the restoration tooth interface. The tooth is placed in artificial saliva. At various time intervals, specimens are taken from the aliquot. The radioactivity in the specimen is determined using a scintillation counter. Through a simple computation, the total amount of leakage that occurred at any of the time intervals tested can be calculated.

The quantity of microleakage is then expressed as a percent of the radioactivity placed in the cavity. This is achieved by dividing the radioactivity counts leaked during a given time interval by the radioactivity counts deposited in the cavity before it was restored. The initiators of this experimental method.^{107109, 116} and those who applied it¹¹¹ⁿ¹¹³¹¹⁰ assumed, that all the radioactivity (counts) placed in the cavity is available to immediately leak

out. This assumption will be accurate only if the radioactivity will not physically or chemically be attached to the cavity floor/walls. Should the radioactivity become bound to the cavity completely or partially, permanently or for a time period, then it will not be available to leak. Such lack of leakage will not be due to the sealing ability of the restoration; rather, it will be due to the inability of the radioactivity to leak.

To illustrate the serious problem with the reverse diffusion method for quantitating microleakage, an example is used. Assume that 100 counts of radioactivity is placed in a cavity. The cavity is restored and the tooth is placed in the medium. If the 100 counts were permanently held to tooth structure, then, microleakage will not cause any release. A sample taken from the aliquot 12 months after immersion will show no radioactivity. The conclusion drawn in this event will be that the restorative material prevented microleakage. In fact, however, the restoration could have been leaking severely.

Assume now that 50 counts of the radioactivity were permanently held to tooth structure while the other 50 counts remained unbound. A sample taken from the aliquot 12 months after immersion will show 50 counts had leaked. The conclusion drawn in this event will be that the restoration leaked only 50% after one year. However, the fact is that the restoration leaked all the radioactivity it is capable of leaking and its microleakage should be 100%.

To substantiate that all the radioactivity placed in a cavity/root canal is not available to be released immediately, an experimental determination was made.

Two groups of six specimens each (roots and crowns) were prepared. The roots were endodontically prepared as canals ready to receive restorations. The roots were then split longitudinally so that half of the root canal became exposed. One side of each 6 roots was taken as a specimen. The 6 crowns has standardized Class V cavities (2 mm occluso- gingivally, 2 mm deep and 5 mm mesio-distally). The external surfaces of all roots and the whole teeth for the crowns group except the cavities were coated with 2 coats of nail enamel. Thus, only the root canals and the cavities were exposed. In each root canal and in each Class V

cavity, 5 μ L of ^3H -Alanine* was deposited and carefully dried with a visible light cure dental unit. Each specimen, received 3.17×10^5 counts of ^3H -Alanine. Each specimen was placed in a 50 mL conical flask containing 20 mL artificial saliva. The conical flasks were sealed with rubber stoppers and placed in water-bath adjusted to 37°C with gentle shaking (80 times/minute). At time intervals starting from 0.25 hr up to 42 days, 10 μ L aliquot samples were collected. The aliquot removed was replaced with equal amounts of saliva so that saliva volume remained as 20 mL. The 50 μ L aliquot samples were mixed with 10 mL Optiphase scintillation cocktail. Radioactivity of all collected samples together with standard dose were counted using a liquid scintillation counter.**

Table 1 and Figure 1 show the mean radioactivity release for the 6 open roots and the 6 open cavities together with their respective standard deviations. The values are expressed as percent of the amount of radioactivity deposited initially. It is clear that up to 42 days the radioactivity placed in either a cavity or a root canal is not available for leakage even

Table 1. Rate of radioactivity release from open root canals and open crown cavities.

Time	% CPM Release from open root canals	% CPM Release from open crown activities
	Mean \pm SD	Mean \pm SD
0.25 hr	12.93 \pm 7.55	27.1 \pm 8.0
0.5hr	17.76 \pm 5.1	34.56 \pm 16.8
1.0hr	30.7 \pm 13.43	41.1 \pm 8.2
2.0hrs	62.8 \pm 1.79	36.1 \pm 14.8
3.0hrs	63.3 \pm 5.35	40.9 \pm 14.64
1 day	71.5 \pm 2.11	40.3 \pm 9.37
2 days	64.4 \pm 3.25	44.2 \pm 10.85
3 days	70.8 \pm 1.19	35.4 \pm 11.6
4 days	70.1 \pm 5.17	52.4 \pm 8.28
7 days	71.7 \pm 5.35	50.9 \pm 8.23
9 days	77.3 \pm 6.43	60.4 \pm 8.21
11 days	81.9 \pm 7.31	59.6 \pm 9.6
14 days	78.5 \pm 7.1	60.7 \pm 10.8
17 days	80.8 \pm 4.24	64.2 \pm 6.22
28 days	81.3 \pm 6.56	71.03 \pm 13.48
31 days	83.4 \pm 5.26	76.7 \pm 5.19
42 days	85.4 \pm 1.55	81.3 \pm 6.34

Radiochemical Centre, Amersham, England.

* Rackbeta Model 1215, LKB Wallace, Finland.

when a cavity/root canal is fully open. In fact, 100% release of radioactivity from open cavities/canals was not achieved even after one year of free leakage. Accordingly, using the quantity of radioactivity placed initially in a cavity/canal as a reference relative to which the amount of micro-leakage from a restored cavity is compared is erroneous.^{107-113,116}

An effective correction to the above error is the use of reference controls in all reverse diffusion studies for quantitating microleakage. The reference control consists of 6 open cavities, root canals or crown preparations depending on the study being conducted. Everytime aliquot samples are taken from the 6 flasks of the restored "cavities" aliquot samples are also taken from the 6 flasks of the unrestored cavities (reference) at the same time. The mean radioactivity in the latter group at any given time interval will reflect the true radioactivity counts capable of leakage from an open cavity at that time. The difference between the latter count

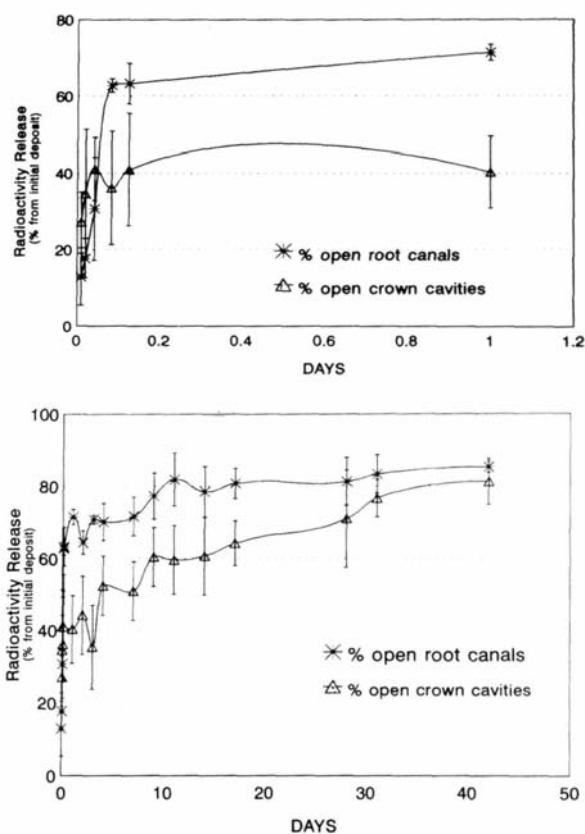


Figure 1. Release of radioactivity from open root canals and open crown cavities as a function of time.

and that released from the restored cavity will be due to the sealing ability of the restoration. Accordingly, the quantity of microleakage (M) at (X) time interval should equal to the mean counts per minute of restored cavities C_r at that time divided by the mean counts per minute of unrestored cavities C_u at the same time, or: $M_x = C_{rx} + C_{ux}$. All prior studies using the reverse diffusion method for quantitating microleakage, erroneously used the relationship

$$M_x = C_{rx} - C_u$$

where C_u is the radioactivity counts initially placed in the cavity before it was restored.

Conclusion

An intensive review of methods attempting to evaluate microleakage showed that a quantitative method is badly needed. The method of quantitating microleakage by reverse diffusion, developed at the University of Florida fails to take in account adherence of the radioisotope to cavity walls. It is proposed, through results of a limited study, that the reverse diffusion method will be capable of quantitating microleakage when a reference control is used.

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