

Various Methods of Detecting Micro-Leakage in Restorative Dentistry

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Abstract

Micro-leakage testing has been used to determine the possible clinical performance of a restorative material. A large number of different techniques have been developed for the investigation of micro-leakage. Many micro-leakage testing materials have been developed and performed through the years. There has been no agreement as to which testing methodology would give the most accurate results. Attempts have been made to simulate the oral conditions and to give a more quantitative representation of micro leakage. The different micro leakage testing methodologies are presented in this paper.

Keywords: Micro-Leakage Testing; Clinical Relevance.

Introduction

Restorative dentistry has modified in several aspects due to the myriad of options available for fabricating indirect restorations which have come up over the past few decades, especially pertaining to ceramics [1] and resin cements.

The objective of restorative dentistry is to restore the tooth to its form and function with fair amount of longevity to determine its clinical success. No restorative work can be done without creating an interface with the tooth structure. For a true restorative material or a luting agent one of the many requisites is its adaptability and bonding or chemically joining to the tooth structure. Failure to do so leads to the gap left between the prepared tooth and the luting cement. Further damage is done by the thermal contraction of the luting cements; masticatory load induced volumetric changes which enhance this gap causing micro - leakage between the interfaces. It has been established that bacterial

leakage and its toxins are a greater threat to the pulp there by to the tooth as well as the restorative work.

Most of the indirect restorations in the oral cavity are meant to provide function and aesthetic without causing any damage to the biological tissues. Three main factors, which determine the success of an all-ceramic restoration is; esthetics, resistance to fracture and marginal adaptation [2]. One of the critical parameter for the indirect restorations to be successful in the patient's mouth is marginal adaptation.

The weakest link in the fixed partial denture treatment is the tooth restorative margin interface. Thus, marginal fit and its evaluation is a critical parameter needed for longitudinal success. McLean has given a clinically acceptable, marginal fit to be within 120 μm^3 . Any gap (horizontal or vertical) exposes the luting agents to the oral cavity which may cause degradation, dissolution, micro-leakage [4] increase plaque accumulation, periodontal inflammation, and secondary caries [5].

Poor marginal adaptation and cementation failure leads to abutment and restoration failure. Studies have stated that All-ceramic restorations failed by 10.9% and 21.7% due to secondary caries [6].

Micro-leakage is an unwanted passage of oral fluid with micro-organisms and ions between the tooth and restorations. Micro-leakage cause the pulpal damage, discoloration, hypersensitivity, recurrent caries, hastening of marginal break down, all of which lead to pulpal pathology and failure of

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the restoration.

Micro - leakage may be caused due to various reasons; some of them are:

- Poor adaptation of restorative materials
- The contraction of restorative or luting agents during setting
- Non adherence of these materials to tooth structure
- Deformation under load and
- Temperature induced volume changes

Micro-Leakage Tests

One of the important methods of testing the clinical performance of a restoration as well as the luting materials has been to determine the micro - leakage. Techniques to detect micro - leakage have been classified in to various categories.

- Air pressure method
- Penetration studies with the help of dyes, chemical tracers, radio isotopes, neutron activated analysis and bacterial studies
- Fluid conduction studies
- Electronic methods
- Microscopic examination

Direct Observation

One of the simplest methods of detecting micro - leakage is direct observation without the help of any agent or tracer. It is based on visual and tactile examination like discoloration of enamel or explorer exposing the lack of marginal integrity finding the gap between the tooth and restoration. However, there is no direct correlation between visible marginal gap and the depth of leakage.

Air Pressure Method

The principle of this technique is to take the tooth with the restoration to be evaluated for micro - leakage and to deliver the air at the junction or the interface between the two and place this whole assembly under water. The detection of air bubbles from any of the margin of the restoration indicates micro - leakage. The technique was evolved by Harper 1912 but is now rarely used.

Penetration Technique (Tracers)

Currently, principle of penetration is utilized to

assess the micro - leakage; it involves luting the indirect restoration over the prepared tooth, followed by immersion of the specimen in a penetrant solution for a specific period of time [7]. Later, the specimen is cleaned, sectioned and examined under magnification to determine the extent of the penetration. A standard criterion is used to determine the extent of micro - leakage.

Organic Dyes

They are also known as organic tracers which are used in dentistry to detect micro - leakage. They may be used as solutions or particles of suspension. One of the most popular as well as oldest techniques using this principle is by making use of organic dyes. These dyes may be used in many ways with the specimen under examination. If the specimen is dipped in the dye for a specific period of time it is known as passive method. The phenomenon of capillarity is very important to determine leakage. Similarly, the specimen may be dipped in the dye under vacuum/negative pressure or positive pressure (specimen is dipped in epoxy resin and kept in an autoclave under desired pressure).

Amongst the commonly used organic dyes basic fuchsin is the most common [8]. However, there are other dyes also which are made use of like methylene blue, aniline blue, eosin, and crystal violet. The aniline blue dye has a drawback as it becomes transparent at an elevated pH with calcium hydroxide [9].

Most of the dyes are used in the concentration range of 0.5 to 2.0 percent [10,11]. Basic fuchsin dye is commonly used at 0.5 percent solution. It is also used as caries disclosing agent in combination with propylene glycol. Some of these dyes were initially toxic, due to which they were not used in vivo studies. Further, some of these dyes diffused profusely to the enamel or dentin which discolored the tooth, making it difficult to interpret. Despite of having drawbacks, the popularity of the organic dyes has not gone down due to ease of use and low cost.

Many a times the standard criteria are used for evaluation of the results. This has been criticized for being subjective and qualitative. In order to remove this drawback, spectrophotometry was introduced to quantify the results. Dye penetration can also be assessed with the help of stereomicroscope linked to image analysis software [12]. Digital imaging microscopy can also be made use of along the interface to record the actual length of the dye penetration.

Fluorescent Dyes

With the advent of fluorescent dyes many researchers shifted to them for the following reasons:

Fluorescent dyes are non-toxic in nature; therefore they are safer to be used in vivo studies as well as topical applications [13].

They are also detectable in dilute concentrations, as well as sensitive to ultraviolet light. This makes them more popular as it is easy to take photograph and produce reproducible results. The contrast provided by the fluorescent dye against the natural fluorescence of the tooth makes it easy to detect the path of dye penetration under ultraviolet light.

Criticisms have been raised against laboratory testing because of the absence of the effect of pulpal hydrostatic pressure on the dye. The scores obtained from in vivo studies for micro - leakage are much lower than the in vitro studies [14].

Radioisotopes

Radioisotopes became popular primarily for two reasons; their ability to penetrate more deeply than the dyes and due to autoradiography technique which detected even minute amounts of tracers that otherwise was not possible. Commonly used isotopes are ^{45}Ca , ^{14}C , ^{131}I , ^{35}S , ^{22}Na , ^{32}P , ^{86}Rb and ^{14}C . Radioisotopes of ^{45}Ca are being used in autoradiography for detecting micro - leakage [15].

Only the part of the tooth structure with the restoration is left unpainted with varnish. Then the sealed teeth (except the interface to be tested) are immersed in the isotope solution for several hours just like it is done with dyes. Later, the tooth-restoration assembly is rinsed with water and cut into longitudinal sections through the restoration. The cut (flat) surfaces are applied to a photographic film making good contact. After development the film shows the radiolucency around the restoration (due to the presence of radioactive isotopes) if the micro - leakage is present. This technique offers many advantages over the conventional dye technique.

Radioisotope technique requires an exposure time of two hours in comparison to a day required by the dye.

The differences between in vivo and in vitro results are minimal which supports the use of this technique in vitro testing.

However, special training is required to handle and master this technique. Further, the results of

this method are always qualitative.

Bacterial Studies

The more realistic method with more clinical and biological relevance in detecting micro - leakage is with the help of bacterial studies. Usually bacteria's have larger size than the molecular size of dye or isotope, thereby giving more real life results of micro - leakage. The specimen with the interface to be checked is placed in a broth inoculated with bacterial culture. Later this specimen is kept in a sterile broth for culture; if the sterile broth turns cloudy micro - leakage is diagnosed. Commonly *Enterococcus* and *Streptococcus* bacteria are used as tools in this method. *Enterococcus faecalis* is used because it is a part of normal oral flora and exists with other aerobic and anaerobic microorganisms. This technique is qualitative rather than quantitative.

Silver Nitrate Technique

Silver nitrate is also very commonly used as a stain to detect micro - leakage because it provides a good optical contrast because of silver [16]. A 50% solution of silver nitrate is used to keep the specimen dipped in it for two hours. Later, they are washed and exposed to developing solution to precipitate the silver particles. Specimens are cut in the desired section to detect the micro - leakage. The degree of micro - leakage is determined in a similar fashion to one used for organic dyes.

Neutron Activation Analysis

Micro - leakage can also be quantified by using neutron activation analysis [17]. This technique may be used for determining micro - leakage in vivo and in vitro. However, the in vitro uptake is always greater than the in vivo uptake. ^{55}Mn was used for neutron activation but the presence of manganese in the tooth or the restorative material can alter the results. Therefore, it has been replaced now by dysprosium as tracer.

For in vivo testing the teeth are soaked in an aqueous solution of a nonradioactive ^{55}Mn salt after isolating for one hour. Later, they are extracted and placed in a nuclear reactor and irradiated at 1 megawatt for two minutes where the ^{55}Mn is activated to ^{56}Mn . The gamma-ray emission of ^{56}Mn is measured with a scintillation detector and a germanium crystal linked to a gamma-ray spectrometer.

Fluid Conduction Studies

This technique was evolved by Pashley to quantify the micro-leakage. The restoration is placed on the tooth and sectioned. The sectioned tooth with the restoration forms a working model are connected to a plastic tube. The working in this model is based on the fluid transport under positive pressure, which displaces the air bubble in a capillary tube filled with deionized water between the model under test and the pressurized chamber. The air bubble is created and adjusted in the capillary by sucking the water. The model with the attached capillary are kept in water bath at a contrast temperature. Now the pressure is applied from the inlet to force the water through the void along the restoration, which displaces the air bubble in the capillary. The volume of fluid transported is measured by observing the displacement of the air bubble which is expressed in ml/day

Electronic Monitoring

This technique permits the quantification of the micro - leakage. It allows the micro - leakage to be recorded over a period of time during which several reading may be taken. Hence, leakage as a function of time can also be recorded.

The micro - leakage detecting device consists of two main parts.

- Constant pressure reservoirs
- Micro-pressure sensor

Constant pressure is maintained in the steel reservoir by means of manual air pump and electronic micro-pressure sensor. With the help of a valve a constant pressure is supplied to the specimen to be tested. The sensor is sensitive to the pressure changes as small as 0.05 mm of Hg. A polyvinyl chloride tubing with known inner and outer diameter is connected between a pressure reservoir and the pressure sensor and calibrated with 1 ml of water to check the variation in the pressure.

Water and specimen to be tested is injected into the sensor end of the tube. A constant air pressure is then applied to the water bolus and changes in air pressure on the sensor side of the specimen are recorded. With the help of proportionality factor, the changes in pressure measures as the result of micro - leakage can be converted to volume of leakage per unit time.

Microscopic Examination

Scanning Electron Microscopy (SEM)

With advancement scanning electron microscopy (SEM) was made use of to observe the accuracy of marginal integrity between the tooth and the restoration. This technique had the advantage over the microscopic examination like introduction of artifacts during its preparation for imaging¹⁸. SEM works with a very thin layer of heavy metal like gold or palladium being coated on the surface of the specimen to be examined. Before that the specimen is coated with carbon which provides a conducting base. These heavy metal alloys usually serve as secondary source of secondary electrons. An electron beam is focused into a fine probe. The probe is scanned over the surface of the specimen. When a beam of electron hits the surface of the specimen it interacts up to 1 μm of the surface, some electrons are reflected (scattered back) and others are ejected (Secondary electrons) released from the heavy metal. The secondary electrons are collected by detectors and interpreted and displayed as a 3D image on a monitor. SEM is commonly used to measure gap which occurs between the restorations and the axial walls or the floor of the reparations. The defects can be observed at required magnification such as 200 x or 1000 x at the submicron level.

Replication Scanning Electron Microscopy

In replication SEM replicas are used which permit marginal defects to be evaluated on a longitudinal basis clinically as well as in vitro. In vivo technique replicas are prepared of the experimental restoration after finishing or at any desired intervals of time. The surfaces are cleaned with a 5% of NaOCl solution for the impression to be made with silicone. The replicating material should be fluid enough to record all the fine details, shouldn't react with the material used to fabricate the positive and also it should be able to record the details of wet as well as dry surfaces. These impressions are used to make cast with epoxy resin. The casts are prepared for SEM by coating of gold and examined. For in vivo assessment of marginal gap and associated micro - leakage replica technique provides a satisfactory way.

Confocal Microscopy

This is a Laser Scanning microscope, through which multiple scanning of the specimen is achieved which helps in creating a 3-D image. It differs from

the fluorescent microscopy which uses UV radiations, of which a wavelength is absorbed and emitted at different wavelength which is collected by the objective lens. It has limitations like lack of contrast, sharpness and the thickness. On the contrary, confocal microscope enables the imaging of volume objects in a 3-dimensionally.

Specimen Evaluation

The most critical factor in evaluating the micro - leakage is the scoring method or the number of surface to be considered when used with dyes or tracers. Most of the studies are done including two surfaces scoring by sectioning the specimen longitudinally from its centre [8,19]. However, some researchers also propagate the inclusion of all marginal interfaces for the evaluation of micro-leakage, as it reduced the chances of getting false negative results [13]. As far as the scoring is concerned the most standard method is assigning a numerical value to represent the extent of dye penetration signifying micro - leakage [20,21]. The use of radiographs for evaluation with radioisotopes is based on the subjective analysis which is not very reliable.

Leakage Patterns

It has been an established fact that the micro - leakage is generally more at the cervical margin of the restorations in comparison to the occlusal margins [11,22].

Such a behavior has been attributed to permeability of dentin and the prismatic pattern of enamel at cervical and occlusal surface [22].

The temperature throughout the test specimen may be calculated once the thermal parameters like thermal conductivity, specific heat, density of the material and coefficient of heat transfer of the test are known.

Thermocycling

All the specimens to be tested for micro - leakage must undergo thermal cycling or load cycling or both in order to stimulate the oral conditions. Thermocycling is an in vitro method of exposing the test specimens artificially to hot and cold temperatures simulating the oral conditions.

It is a well-known fact that at the interface of two materials percolation takes place due to the difference in the CTE of these materials when exposed to fluctuating temperatures. In the case of a crown cemented on to the prepared tooth two

interfaces are created; tooth-cement and cement-restorative material through which marginal percolation takes place and fluid drops extrude from the margins of the restorations with the increase in temperature which indicates, micro - leakage of the restorations. Such situations are similar to faced by the natural teeth [23] when taking food items simultaneously at different temperatures. Therefore, it is important to study the behavior of different materials when exposed to the extremes of temperatures (within physiological tolerance). The tolerance limit in humans have been found to be 4°C for the lower thermal limit and 60°C for the upper thermal limit [24]. Along with temperature another equally important parameter is the dwell time; that is the time for which the material is exposed to a temperature. It has been generally agreed upon to expose the specimens for a maximum of 10 seconds as dwell time. It is relevant since it determines the ability of the material to conduct heat in relation to its mass [25]. Thermocycling requires two important things; equipment to ensure constant temperatures in the water baths and shifting of the specimens at the appropriate time.

Thermo-Cycler

Thermo-cycler is particularly designed to conduct laboratory testing of micro leakage in composite filling and adhesive bonding materials in and on tooth structures. Specimens to be tested are submerged in hot and cold baths for a definite duration of time for numbers of cycles. The temperature of the water bath is accurately controlled with microprocessors.

International standards specify immersion times (dwell time, 10 seconds to 30 seconds) and temperatures (5 °C cold water and then in 55 °C hot water) for a defined number of cycles for a specimen to be tested. Thermocycling is a useful method of ageing the materials. The result of subsequent testing invariably shows degradation in adhesive strength. It is important that this loss of bonding is limited. Thermocycling is based on the diffusion of heat, movement of moisture in and out of the porous test materials. A diffusion process always acts to even out differences of temperature gradients or moisture content. It also produces transient mechanical and chemical stresses on the test material.

Conclusion

A variety of methods are currently available to

detect and determine the micro - leakage to the researchers. As mentioned earlier all techniques have their advantages and disadvantages. The goal of all these studies is to make testing clinically relevant. However, the in vitro results do not necessarily correlate to the clinical performance. It has been observed the results of various studies on the same materials do not match some times because of the variation in the testing techniques used in the research.

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