MICROLEAKAGE TESTING

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ABSTRACT

Microleakage testing has been used to determine the possible clinical performance of a restorative material. Many microleakage 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 microleakage. The different microleakage testing methodologies are presented in this paper.

Keywords - Microleakage testing, clinical relevance

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INTRODUCTION

Microleakage is defined as the movement of bacteria, fluids, molecules, or ions between the tooth and restorations of any type(1). Much attention has been focused on the problem of microleakage and its implication in a variety of conditions, including recurrent or secondary caries, tooth discoloration under amalgams, hypersensitivity of restores teeth, pulpal damage and breakdown of certain filling materials(2,3).

Microleakage tests can provide much useful information about the performance of restorative materials. A variety of different techniques for assessing microleakage have been developed and utilized. Most modern techniques utilize different principles involving biological, chemical, electrical, physical or radioactive components. These include the use of dyes, radioactive isotopes, air pressure, bacteria, neutron activation analysis, artificial caries, scanning electron microscopy (SEM), calcium hydroxide and other methods(4-7). All of these techniques have advantages, as well as certain drawbacks. It has been assumed that the different microleakage methodologies would give similar results when used to determine leakage of the same restorative material. However, this has not proven to be the case, and the differences in results have been attributed to the differences in sensitivity of the tests or experimental methods used(8-10).

MICROLEAKAGE TESTS

DIRECT OBSERVATION

The simplest assessment of microleakage involves direct observation of restorations. No agent or tracer is used to detect microleakage. Clinical observation is frequently used to detect macroscopic changes in the marginal integrity of a restoration. This may be done tactilely with the use of an explorer, and/or visually by determining the presence of discoloration in the adjacent enamel or a gap between tooth and restoration. Photographic observation is often used in conjunction with the clinical assessment(11,12). A macrophotographic black and white record of each restoration is made at different time intervals to determine changes in marginal integrity.

Scanning electron microscopy (SEM) provides a more critical means of direct observation of the adaptation of a restorative material to the cavity margin(13). The direct technique involves using the specimen itself for microscopic examination, which is handicapped by the risk of shrinkage and introduction of artifacts during its preparation for imaging(14).

The replica technique overcame this problem and provided a means of in vivo assessment over a period of time and at different time intervals. This method of microscopy is often used together with direct observation(15). These techniques do not quantity diffusion or penetration, and no direct correlation has been demonstrated between visible fissure size and depth of leakage(16).

MICROLEAKAGE TRACERS

Current microleakage test methodology uses the principle of penetration, which involves preparation and filling of a cavity preparation, followed by immersion of the specimen in a penetrant solution for a period of time(17). The specimen is then cleansed, sectioned and examined under magnification to determine the extent and path of the penetration. A standard criterion is used to determine the extent of microleakage.

ORGANIC DYES

The use of organic dyes is one of the most popular techniques using this principle, as well as one of the oldest(17). The initial use of a dye and observation of margins of restorations under magnification were primarily related to studies dealing with dimensional change of amalgam upon setting. Skinner(18) noted that the dimensional change of amalgam was observed by Fletcher (1861) by placing the amalgam in glass tubing and subjecting it to a dye that penetrated between the walls of glass and the amalgam. In the same year, Tomes used the microscope to detect dimensional change of amalgam fillings placed in ivory. Initial microleakage studies were done utilizing glass tubing, steel dies or ivory with roughened internal surfaces to simulate natural tooth structure(1,18,19). Presently, the majority of studies are done using human teeth. Kim et al (20) have introduced the use of machinable glass-ceramic as a tooth replacement.

Some of the organic dyes used include basic fuchsin(21), methylene blue(22), eosin(23), aniline blue(24), crystal violet(25) and erythrosin B(26). The aniline blue dye has not

been found to be suitable for use with calcium hydroxide since it becomes transparent at an elevated pH(27). The basic fuchsin dye is one of the most commonly used dyes today(28). Percentage concentration currently in use ranges from 0.5 to 2.0 percent(29, 30). Some have thermocycled the specimens in the dye solution(31). Others thermocycled the specimens in the dye (25 to 200 cycles), followed by immersion in the dye solution for one to 21 days(32-34). The dye has been used for assessment of different restorative materials used. Basic fuchsin dye at 0.5 percent solution, in combination with propylene glycol, has been used as a disclosing solution for carious dentin(35). Carcinogenic potential of this dye has led to the substitution of acid red dye(36).

Most of the early organic dyes used were toxic, precluding their use in vivo studies(37). There were also problems of diffusibility of the dye, which discolored the walls, making it difficult to interpret. The exposure of the teeth to most dyes is much longer than the other techniques, such as the radioisotope. There were no permanent records, unless photographs were taken of the specimens.

Evaluation of the results using standard criteria has been criticized for being subjective and qualitative. In an attempt to quantify the results, Silva et al(38) used volumetric measurements by spectrophotometry of sections around the restoration. Class V cavities were restored with either cavity varnish, calcium hydroxide base and cavity varnish, or zinc oxide eugenol and cavity varnish. Amalgam was used as the final restoration. After thermocycling, the teeth were immersed in 0.1 percent methylene blue dye. Volumetric leakage was calculated as micrograms of methylene blue per tooth.

Fayyad and Shortall(39) assessed dye penetration by using an image analysis apparatus linked to a stereomicroscope. Digital imaging microscopy was used to record the actual length of the dye penetration along the inteface. Glyn Jones et al(40) measured leakage around different Class II restorations using dye penetration, with 5.0 percent buffered eosin, and image analysis to determine leakage length at the tooth restoration interface and leakage area into the coronal dentin. The area of dye penetration was selected, since this would indicate the amount of dye penetration around the margins of the restoration. The specimens were photographed and color transparencies were made. The transparencies were then evaluated using an image analyzer

In spite of the disadvantages, the popularity of the organic dyes has not diminished due to ease of use and low cost.

FLUORESCENT DYES

Because the fluorescent dye is non-toxic, it offered the advantage of being usable for topical and systemic application for in vivo studies(41). This dye was also detectable in dilute concentrations, sensitive to ultraviolet light, easy to photograph, permitted more reproducible results, and was not expensive. The contrast of the natural fluorescence of the tooth against that of the dye provided a contrast that made it easy to detect the path of dye penetration under ultraviolet light. This has led to the use of the different fluorescent dyes in tagging of restorative materials, such as cavity varnish and glass ionomer cements(42, 43). The fluorescent dye cannot be used with zinc oxide eugenol cement since it is quenched by the cement.

Many criticisms have been levied against laboratory testing because of the absence of the effect of pulpal hydrostatic pressure on the dye. The in vivo and in vitro results, using fluorescent dye, have not been found to give identical results in hamsters(44). Loiselle et al(45) noted that mean microleakage scores obtained from in vivo testing were much lower than those from in vitro testing among human subjects. Stuever et al(47) performed endodontic treatment on teeth to be tested. Results obtained were closer to those of in vitro testing.

RADIOISOTOPES

There is general acceptance in the use of autoradiography, specifically using ^{45}Ca, in detecting microleakage(47). The principle involves the penetration of the radioisotope around the margins of the specimens such as that with the dye. A flat surface is necessary for good contact between the specimen and the radiographic film emulsion. The film is then developed and microleakage assessed by the radiolucency around the restoration. Minute amounts can be detected with the autoradiograph. This is due to the radioisotope's ability to penetrate deeper than the dyes that were used. The molecular size of the dye is 120 nm, while that of the radioisotope is 43.2 nm(48). The autoradiograph represents a permanent record of specimen leakage. Exposure time to the radioisotope is two hours in contrast to the dye, which is 24 hours or longer. The use of ^{45}Ca in this technique was standardized and refined by Swartz(49) in 1959. The special training needed in the handling of this radioactive material is one disadvantage of this microleakage test(50-52).

To determine if differences exist between in vivo and in vitro results, McCurdy et al (53) prepared Class V restorations in cats, to test five different restorative materials including acrylic resin, composite resin, silicate, gutta percha and amalgam. The cats were fed with animal food tagged with ^{45}Ca. Topical application of the radioisotope solution was also done on the restored teeth. The results were in agreement with those obtained from the in vitro tests. The difference existed with amalgam, where in vivo results showed faster decrease of leakage than with in vitro testing. The result of the study supported the use of the radioisotope in laboratory testing.

Interpretation of results from radioisotope studies is still qualitative. Menegale et al (54) presented a means of quantifying the results from their study on the effect of cavity wall texture on microleakage. They measured the area of penetration by the radioisotope and established a ratio between the area and the perimeter of the sectioned specimen. Vasudev et al (55) described a reverse radioactive absorption test that quantified microleakage around amalgam restoration.

SILVER NITRATE TECHNIQUE

The use of silver nitrate is second to the organic dyes in usage. Wu and Cobb(56) developed the silver staining technique to demonstrate microdefects in composite resins.

Silver was selected as the staining agent because of the strong optical contrast of silver paticles, and also, its penetration into the specimen can be easily detected by microprobe. This technique involves immersion of the specimens in a 50 percent solution of silver nitrate for two hours in the dark. The specimens are rinsed to remove silver ions on the surface, and then immersed in developing solution and exposed to fluorescent light for six hours. The silver ions absorbed in the specimens precipitate as silver particles at this stage. Specimens for microleakage studies are then sectioned. The degree of leakage may then be measured in the same manner as that used for organic dyes. The silver staining technique was also tested on amalgam restorations; however, the results were not consistent. It was reasoned that this occurred as a result of chemical reactions between components of the amalgam and the silver ions(57). The silver nitrate has been used to detect leakage at the hybrid layer created by the current dentin bonding systems(58,59).

CALCIUM HYDROXIDE TECHNIQUE

The calcium hydroxide technique was developed for possible use in vivo. As reported by Leinfelder(60), Borrows suggested the use of a suspension of calcium hydroxide in deionized water as a leakage detection agent. He demonstrated that, after thermocycling, the pH of the margin of acrylic restorations with pure calcium hydroxide base in a suspension of deionized water increased to 8.0. Leinfelder used this principle in an in vitro study using Class V preparations restored with either spherical, admixed or lathe-cut amalgam, or acrylic resin. Ice water (pH=7.00) was ejected on the surface of each restoration for one minute. The restorations were dried with an absorbent paper. A small piece of dampened pH paper was placed over each restoration. Light pressure was applied using a piece of rubber dam over the pH paper. After one minute, the pH paper was observed for a color change. A change in color from yellow to dark purple was recorded as a positive result. Rehfeld et al (61) tested different formulations of calcium hydroxide, including Dycal, VLC Dycal, Pulpdent liquid and reagent grade calcium hydroxide, to determine if the type of calcium hydroxide affected the results. The reagent grade of calcium hydroxide gave the most positive results for the longest period of time.

BACTERIA

The use of bacteria to study microleakage may be the most clinically relevant microleakage test. A bacterial study of the germicidal properties and the permeability of cements and filling materials by Fraser(62) was published in 1929. In his study of leakage around acrylic resin, Seltzer(63) used chromogenic microorganisms with extracted teeth that had Class V amalgam or acrylic resin restorations. They were immersed in a broth culture and incubated for seven to 60 days. At the end of the test period, shavings of the dentin under the restoration were cultured. The acrylic resin exhibited more bacterial penetration than amalgam restorations. The method requires a controlled sterile environment to avoid contamination with other bacteria.

SECONDARY CARIES FORMATION

The secondary caries technique uses either bacterial culture or a chemical system. This method has the advantage of linking the development of artificial caries with microleakage. Ellis and Brown(64) used L arabinosus in Class I amalgam restorations, with or without varnish. A niacin deficient medium was placed on the coronal side, and a niacin solution was placed on the pulp side of the tooth. The coronal side was inoculated with the test bacteria. Selected specimens were stained using ammonium purpurate to better define the carious lesion.

The acidified gelatin gel technique has been shown to produce lesions of histologic features identical to early caries(65). Grieve(66) used the technique in his study of liners and varnish under Class V amalgam restorations. Acid resistant varnish was used around the tooth terminating 0.5 ml short of the restoration margin. Twenty-four hours after restoration, the specimens were placed in a 20 percent solution of gel adjusted to pH 4.0 by the addition of 30 percent lactic acid for 10 weeks. Thymol was added to the gelatin to inhibit bacterial growth. Ground sections of the teeth were examined under polarized light microscopy.

Grieve(67) used acidified agar in various concentrations to produce secondary caries-like lesions. He was able to produce experimental lesions around amalgam restorations at concentrations of 0.6 percent, 0.9 percent, and 1.2 percent. Grieve and Glyn Jones(68) suggested that the lesions produced by this technique may not be the most appropriate in use for specimens where the cavity preparation is treated by etching. There was a similarity in the microscopic appearance between the unetched specimens subjected to the leakage test, and the controls that were etched with 30 percent phosphoric acid but not subjected to the leakage test, which made interpretation of results difficult.

AIR PRESSURE

Quantitation of microleakage has been a drawback of most of the tests. Quantitation of results was made possible by other tests that were developed. Air pressure was used by Harper(19) in 1912 to penetrate the interface between amalgam restorations and walls of Class II preparations in steel dies. A tube that could deliver three to 30 psi of air pressure was attached to a hole at the pulpal floor of these dies. Emergence of bubbles from the margins confirmed the presence of leakage. Results are quantified by the amount of air pressure needed to demonstrate leakage, and the method was nondestructive. Pickard and Gaylord(71) presented a means of utilizing the air pressure in a longitudinal study of Class I amalgam restorations. Behavior of some individual leak paths over a period of time showed abnormalities. Possible reasons for this was attributed to movement of small fragments of enamel and dentin, or small fragments of amalgam or corrosion products. Examination of these specimens are done under water, thus, the area of leakage cannot be determined, and photographic records are difficult to obtain. Other authors have used this technique with modifications(72,73).

Derkson et al (74) made use of a liquid pressure method

that was similar to the air pressure technique. Extracted human unerupted third molars were sectioned at the cementoenamel junction. The coronal pulp was removed. Pieces of plexiglass, with center holes to accept an 18-gauge stainless steel tube, were used. The tube was flush with the surface of the plexiglass. The metal tube was sealed in place with cyanoacrylate and this was attached to a filtration apparatus, which operated with nitrogen gas at a pressure of 5 to 15 psi, applied to a pressure reservoir with a plastic beaker of phosphate buffered saline containing 0.2 percent fluorescein dye. Movement of a small bubble from a micropipette permitted fluid movement to be quantified. The value of the test using air or liquid pressure, and the area where the pressure is applied that is considered unnatural, has been questioned by other researchers.

NEUTRON ACTIVATION ANALYSIS

Neutron activation analysis is a quantitative means of measuring microleakage. Going et al (73) used the technique in determination of microleakage in vivo and in vitro. Neutron activation of ^{55}Mn was used. In vivo specimens were soaked in an aqueous solution of a nonradioactive ^{55}Mn salt using a latex isolator. The teeth were then extracted, and placed in a nuclear reactor where the ^{55}Mn was activated to ^{56}Mn. The gamma-ray emission of ^{56}Mn was measured with a scintillation detector and a germanium crystal linked to a gamma-ray spectrometer. The in vivo uptake was found to be generally greater than the in vitro uptake. Improvement of the method was done by Meyer et al (74) by selecting a tracer which gave more consistent results than ^{55}Mn. The variability of the results was said to be caused partially by the presence of manganese in either the tooth or in the restorative material. Dysprosium was found to be the most suitable tracer since it provided the least variation in the results and allowed the fastest activation and counting procedure.

ELECTROCHEMICAL METHOD

The electrochemical method was adapted for use in restorative research from endodontic research. Jacobson and von Fraunhofer(75) described the technique as one that permits accurate detection of the onset of leakage and provides quantitative results over a period of time. The principle of the technique involves insertion of an electrode into the root of an extracted tooth, so that it makes contact with the base of the restoration. The restored tooth is sealed to prevent electrical leakage through the normal structure, and immersed in an electrolyte bath. A potential is then applied between the tooth and the bath and leakage is assessed by measuring the current flowing across a serial resistor.

CAVITY PREPARATION

Going et al (76) chose to use Class V preparation for their study, using radioisotopes to reduce the variables inherent in an occlusal preparation due to the presence of pits and fissures. In their preliminary study, they found that the penetration of the isotope into dentin was dependent on the underlying dentin. The general absence of sclerotic and secondary dentin in the labial and lingual surface reduced variability in the study.

THERMOCYCLING

Nelsen et al (77) observed extrusion of fluid from margins of acrylic restorations when they were immersed in ice and then warmed with the fingers. They concluded that marginal percolation is caused, in part, by the difference in the coefficient of thermal expansion of the tooth and the restoration, and by the thermal expansion of fluids occupying the tooth/restoration interface. Brown et al.(78) reported that it is not unusual for incisors to be subjected to 50oC cycles several thousand times a year from taking in food or liquid at varying temperatures.

In 1978 Kidd et al (79) suggested that thermocycling may not be of clinical importance in relation to composite resin. Using a gelatin gel technique, they found that teeth subjected to thermocycling exhibited a reduction or no change in leakage pattern, compared to those which were not thermocycled. Wendt et al (80) determined the effect of thermocycling on dye penetration in the in vitro assessment of microleakage composite resins. There was no increase of microleakage in restorations when thermocycling was used. Rossomando and Wendt(81) determined that the need for thermocycling is dependent on the restorative material's ability to conduct heat in relation to it's mass. They also concluded that the dwell time should be clinically relevant e.g .10 seconds

Cycling temperatures are based on the average upper and lower thermal tolerances of individuals. Nelsen et al (77) found these thermal tolerances to be 4°C for the lower thermal tolerance and 60°C for the upper thermal tolerance, among five test subjects. The temperature within the Class III acrylic restoration placed was determined using a thermocouple. The temperature recorded was 9°C at the lower thermal tolerance and 52°C at the upper thermal tolerance, giving a temperature differential of 43°C. Plant et al (82) recorded upper thermal tolerance temperatures in six subjects and found them to be in the range of 50°C to 55°C. Palmer et al (83) recorded the mean upper and lower temperatures for 13 test subjects for the mandibular posterior area to be 53.1°C ± 4oC and 1.0°C ± 1oC, respectively. The maxillary teeth had a mean upper limit of 58.5°C ± 3.3°C.

SPECIMEN EVALUATION

Evaluation of specimens, in most microleakage studies involving tracer penetration, uses a two-surface scoring method. The specimen is sectioned longitudinally through the center of the restoration(28,84,85). Christen and Mitchell(41) developed a system to evaluate the total marginal interface of the restoration. They scored multiple surfaces of the restoration and presented this as a more realistic evaluation of the leakage pattern. Wenner et al (86) conducted a pilot study scoring six surfaces of three sections of a tooth through the restoration. They found that the probability of finding a false negative was 33 percent if only a single section was evaluated. Mixson et al (87) compared the

two-surface and multiple-surface scoring methodology in comparing Class V preparations of different types using two different dentin bonding agents. Results suggest that microleakage at the proximal corners of the restoration may be more severe.

The scoring of the specimen is based on a standard criteria developed by the researcher assigning numerical value to represent the extent of dye penetration(1,13). With ^{45}Ca, standard radiographs have been used to guide the evaluator on what particular rating to assign(88). The interpretation of radiographs and specimens has been criticized as relying on qualitative and subjective judgement in evaluation(1,13,17).

LEAKAGE PATTERNS

The cervical margins of restorations have generally shown a greater degree of microleakage than the occlusal margins, even in restorations with enamel margins(30, ,89,90). Liberman et al (90) attributed this to fractures on enamel, to the permeability of dentin, and to the difference in the prismatic pattern of enamel on the occlusal and cervical margins as reported by Gwinnett(91). Leinfelder et al (31) suggested that when using composite filling material, this may be due to the surface area of enamel being much greater along the occlusal margin than the gingival margin. The polymerizing filling material tends to pull away from the gingival margin, toward the occlusal margin. Charlton et al (84) reported a deviant pattern of leakage on the axial wall of Class V amalgam restorations, with no sign of the leakage path on both the gingival and occlusal margins. No further report on this deviant or nonuniform pattern of leakage has been documented. Gale et al (92) presented a three-dimensional model of the microleakage pattern using cross-sectional surfaces of the test specimen where silver nitrate was used. The sectional images were taken by a computer image analyzer which was later assembled into a three-dimensional image. They believed that testing with dye tracers should be done under vacuum to eliminate air entrapment which hinders penetration.

CONCLUSION

Different microleakage testing methodologies are available to researchers. These testing methodologies have their advantages and disadvantages. While efforts are being made to make the testing clinically relevant, the in vitro results do not necessarily reflect the clinical performance. Results of different microleakage studies have not been in agreement and the reason for the difference has been attributed to different testing methodologies. Testing for microleakage of restorative materials should use the same test methodology to reduce variability in the results.

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