**The Relevance of Micro-leakage Studies**

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Abstract

Numerous laboratory studies have found that microleakage is usually unavoidable whilst clinical studies report substantially less pessimism about the sealing ability of dental restorations. This review of the literature is presented on various forms of leakage in dental restorations, distinguishing leakage, micro-leakage and nano-leakage. The phenomena, causes and methods to reduce leakage are described and the clinical relevance of in vitro and in vivo leakage studies evaluated. The authors’ laboratory and clinical experience on the determination of microleakage is discussed and put into perspective with clinical performance.

Aim of the review

The aim of this review was to question whether in vitro marginal integrity studies on dental restorative systems are a reliable indicator for clinical performance.

Introduction

One of the major objectives of tooth restoration is the protection of exposed dentine against bacteria and their toxins. The interface between the restoration and dental hard tissue is an area of clinical concern as insufficient sealing can result in marginal discoloration, secondary caries, and pulpite. For that reason, adequate sealing is essential for optimal clinical performance. However, the literature is not always consistent with the terminology of leakage. Different levels of leakage are discussed, such as clearly detectable leakage, and not clinically detectable but with absence of secure adaptation. This ‘hidden’ leakage is usually denoted by the term microleakage. Microleakage may be defined as the clinically undetectable passage of bacteria, fluids, molecules or ions between a cavity wall and the restorative material. Clinically, microleakage can lead to staining around the margins of restorations, post-operative sensitivity, secondary caries, restoration failure, pulpal pathology or pulpal death, partial or total loss of restoration (Figure 1). Microleakage is usually associated with invasion from the external environment through the margins of the restoration, but microleakage can also occur internally. In the latest studies, a new form of leakage, nanoleakage, has been described. The word nanoleakage is a specific type of leakage within the dentine margins of restorations, with fluid transport through some of resin bonding layers and is detectable only by electron microscopy techniques. Here the paths of transport are not related to bulk partition of material, but to hydrolytic degradation. Nanoleakage may be related to the acid etching procedure, by allowing the penetration of pulpal and oral fluids such as acids into porosities within or adjacent to the hybrid layer. Nanoleakage is independent from microleakage. The amount of such penetration depends on the type of bonding agent used, on the hydrophilic nature of the monomers within the adhesive and on different parameters of the application.
technique such as dentine moisture and etching time. Nanoleakage is less extensive than microleakage and has probably no immediate clinical relevance. However, the long-term stability of the adhesive bond between dentine and the restorative material may be adversely affected by the degradation phenomenon.

Regardless of the terminology used for leakage, the most desirable property that a restorative material should have is an adequate, complete and a lasting seal of the margins of the restoration. Most of the current literature on adhesive restorative materials and techniques focuses on the elimination of leakage, which is regarded as one of the major factors in determining the long term success of restorations.

Marginal staining can lead to marginal breakdown, poor aesthetics and consequently the need to replace the restoration. The penetration of bacteria and the presence of a gap can also cause sensitivity when chewing or when exposed to thermal stimuli and may lead to secondary caries (Figure 2). Every plaque retention site is a possible site for secondary caries to occur. Oral bacteria can multiply in the crevice around the restoration in a short period of time from the oral environment, tooth surface or smear layer. Subsequently the bacteria and their toxic products are able to diffuse through dentinal tubules and cause pulpal inflammation. Fluids along the interface may cause hydrolytic breakdown of the adhesive resin and collagen within hybrid layer thereby compromising the stability of resin-dentine adhesive interface. Microleakage of a restoration may vary over time. Resin-based composites placed in conjunction with certain dental adhesives are believed to lose their sealing ability over time, thus permitting microleakage (Figure 3). However, materials such as amalgam can seal the restoration margins through the formation of corrosion products over time (Figure 4). Furthermore, new marginal gaps may develop during the lifetime of restoration due to thermally or mechanically induced stresses. It has been demonstrated that modern dental adhesives have a positive influence on preventing microleakage that lasts only 6 months and then became influent after one year of storage.

**Causes for microleakage**

Microleakage is related to several factors, such as dimensional changes of materials due to polymerisation shrinkage, thermal contraction, absorption of water, mechanical stress and dimensional changes in tooth structure.

The polymerisation shrinkage of a composite resin can create contraction forces that may disrupt the bond to the cavity walls, leading to marginal failure and subsequent microleakage. Modern composite resins undergo volumetric contractions ranging between 2.6% to 4.8%. Even when modern dentine bonding agents exhibit bond strengths to dentine higher than 20 MPa, exceeding the contraction stress generated by polymerisation stress (13-17 MPa), the total contraction forces may be higher than the adhesive strength, leading to open margins.

The shape of the cavity can also challenge the adaptation of the restorative material to the margins. Indeed, the C-factor of cavities is closely related to the occurrence of microleakage, especially when restored with a composite resin and dental adhesive.

One of the weakest aspects of Class II composite resin restorations is microleakage at the gingival margin of proximal boxes. This is related to the absence of enamel at gingival margins, resulting in a less stable cementum-dentine substrate for bonding. This is sustained by Cagidiaco et al who demonstrated the presence of an outer layer formed partially by cementum located below the cemento-enamel junction that does not allow micromechanical retention by adhesive materials (Figure 5). In addition, the orientation of the dentinal tubules can negatively affect the quality of hybridization and thus favor leakage in resin-based restorations placed in deep interproximal boxes. It has also been reported that enamel micro-fractures can occur along the margins of

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**Figure 2:** Secondary caries.

**Figure 3:** Composite resin restorations.
restorations immediately after the polymerization of composite resin bonded to etched enamel and lead to microleakage in these areas26.

Another contributing factor may be the coefficient of thermal expansion 27. The coefficient of thermal expansion of composite resin (25 to 60 ppm°C-1) is several times larger than that of enamel (11.4 ppm°C-1) and dentin (8 ppm°C-1)28. This physical property is also reported to be responsible of microleakage in resin-based restorations29. In addition, micro-movements of the restoration along the cavity walls as a result of non-matching moduli of elasticity can contribute to the failure of the mechanical bond, leading to microleakage13.

Reducing microleakage

Methods to reduce microleakage during restorative procedures involve the application of combinations of materials, direct or indirect techniques and different curing strategies.

Various curing techniques to reduce or prevent microleakage remain controversial. Many authors claim that incremental placement and curing can generate less microleakage30, 31, while other researchers have found that both bulk and incremental techniques lead to the same amount of microleakage at the gingival margin32, 33.

The use of a relatively thick layer of a viscous bonding agent, resilient lining cements and low modulus of elasticity restorative materials have been advocated to absorb volumetric changes associated with polymerisation34. Flexible linings of flowable composite were proposed in 1996 to minimize polymerisation shrinkage in Class V cavities 35. These restorative materials are micro-hybrid resins, which are filled 60-70% by weight with filler particles ranging in size from 0.7-1.0 microns. Such composite resins exhibit a substantially lower modulus of elasticity that enables increased elastic deformation in which to flex and absorb polymerization shrinkage stresses36. In addition, flowable composites have a coefficient of thermal expansion similar to that of tooth structure37. This procedure is able to reduce microleakage38 and reduce stress by 18-50 % 34. However, this technique cannot prevent microleakage completely39.

Another approach to reduce microleakage in Class II restorations is the adaptation of a slow self-curing composite resin on the gingival margin located on cementum initially, followed by the layering of a light cured hybrid composite resin. Indeed, it has been demonstrated that light cured composite resins develop more polymerisation stresses than chemical cured composite resins40, 41. Again, this strategy does not seem to solve the problem completely42. Another approach is to apply indirect restorations (Figure 6), where bulk polymerization shrinkage may be partly overcome. The luting cement however, has to polymerize in situ and can compromise the marginal seal. The cement thickness can play a role in stress development43.

There is the need to obtain an adequate seal with the use of luting agent. In case of wider marginal gaps between inlays and cavities, a thick layer of viscous resin cement may create an optimal marginal seal44. Adhesively luted inlays have a small volume of resin that can reduce stress formation caused from polymerisation shrinkage 45, and show less marginal micro-fracture on enamel than direct restorations46, but conversely, the narrower the luting space, the more stress may occur47. In addition, this stress is increased by an unfavourable C-factor, which can be very high in cavities prepared for inlays48.

As the prevention of marginal gap formation is unlikely to be achieved, antibacterial effects of the restoration can be an important additional safeguard, because the inactivation of bacteria means a direct strategy to minimize the risk of secondary caries49. Most composite resins have little or no bacteriostatic or bactericidal effect against oral microorganisms. Silica-based filler and resin monomers such as TEGDMA, Bis-GMA and UDMA are not antibacterial against S. Mutans [52]. This lack of antibacterial properties means that no inhibitory effect against plaque accumulation can occur in these microleakage areas. Indeed, it has been demonstrated that more bacteria accumulation is seen on composite resins compared to other restorative materials 51. In addition, one study even demonstrated that composite resins could promote caries52. Glass-ionomer cements (Figure 8) exhibit a moderate antibacterial effect, in addition to the presence of fluoride-releasing components that can prevent premature demineralisation, and thus protection against secondary
The positive effect of zinc is still a neglected area in the literature. Furthermore, fluoride releasing materials, such as glass-ionomer and silicate cements, can affect bacterial metabolism via different mechanisms. In amalgam and other metallic restorations, the presence of metal ions such as silver or copper can have an antibacterial effect. Mercury has a long history as an antimicrobial agent effective against eukaryotic and prokaryotic organisms, even if the basis of this activity is not well established. In microleakage tests, fresh amalgam restorations usually show total involvement with the cavity wall. However, it has been reported that an initial poor seal of fresh amalgams improves with aging due to the deposition of corrosion products at the cavity-restoration interface. It is frequently reported that patients only complain about post-operative sensitivity during the first week after placement, whereafter the pain will disappear. Whether this effect can be attributed to improved sealing is questionable, as it has been documented that up to two years may be required to completely reduce microleakage around amalgam restorations.

It has been reported that over time, water sorption can cause gap reduction by hygroscopic expansion of resin-based composites. However, this mechanism cannot be relied upon to solve the problem of microleakage.

**Measuring microleakage**

Microleakage is usually evaluated with in vitro models. A number of techniques including bacterial, chemical or radioactive tracer molecules infiltration are available. Colour dye penetration studies are the most commonly employed techniques. Since new materials are constantly being introduced on to the market, short-term laboratory assessments are required because clinical evaluations are expensive, time consuming and require ethical approval. In contrast, in vitro studies such as microleakage tests can provide important information on possible clinical performance of new restorative materials. These are methods of screening dental materials and determining the presence of microleakage, with the theoretical ability to transfer the findings to the clinical environment.

Microleakage tests are very common research methods, even if these studies have often proved contradictory and were performed with different procedures and without standardization. Nonetheless, it is reported that microleakage tests may be reliable parameters to predict in vivo performance.

Data based on the aetiology of caries leads to the conclusion that every plaque retentive site has the potential for secondary caries. A common problem with in vitro studies is that frequently the sample size is very limited. In the literature, studies are often based on only ten to twelve specimens per group, which may be insufficient for correct statistical analysis.

To some extent, the oral environment can be replicated by water storage and thermocycling of samples. The use of thermocycling as a simulation of clinical aging is a common artificial aging technique. There are disagreeing opinions about the influence of thermocycling on microleakage. Some authors reported the absence of any influence of thermocycling on microleakage, while others show an increase of microleakage at the cementum-dentin-restoration interface after thermal stressing. In these studies, methylene blue was employed as a tracer to evaluate the degree of infiltration. The small particle size and the permeability of dentinal tubules may lead to an overestimate of the relevance of this infiltration. The area of methylene blue is calculated to be approximately 0.52 nm², smaller than the average size of bacteria. As bacteria have a diameter of 0.3-1.5 microns, this technique cannot distinguish between too narrow and sufficiently wide gaps to allow bacteria passage. An interesting finding was that the use of the methylene blue tracer led to higher microleakage scores than other microscopic evaluations. Little data is available on crevice dimensions. Cooley and Barkmeier found gaps of 10 microns around Vitrebond restorations. In addition, the dwelling time of the specimen in methylene blue seems to have no influence on microleakage scores.

Often the evaluation of penetration scores is performed on one or more specimen cuts and by optical microscopic observation. This evaluation method may be less sensitive than three-dimensional evaluation. However, it is reported that the use of several (for example, three) sections of one tooth may avoid under-estimation of in vitro microleakage. This technique is mainly qualitative and to some extend, a quantitative method of evaluation is a useful tool to show the pattern of dye penetration and can indicate where the penetration occurs. Based on above
methodologies, it was concluded that so far, no adhesive restorative technique is available that guarantees a reliable marginal adaptation when margins are located in cementum-dentin. 

Discussion and Conclusions
Although the contribution of microleakage to restoration failure remains controversial, microleakage studies are still the most popular test method employed to obtain a preliminary idea about the quality of a new material or combination of materials. Many studies using identical laboratory techniques on the same material are often contradictory, probably due to different manipulation and handling of the materials. Studies using different methods are frequently more conflicting. There is a need for an improved laboratory technique for determining marginal adaptation and interpreting the results. An in-vitro study that shows no microleakage for one material and significant microleakage for another might be indicative for a satisfactory clinical performance of the first material. However, the second material may perform adequately in a clinical situation as well. Furthermore, the correlation between clinical studies and laboratory results is rejected in a recent article by Heintze, which found that a minimal threshold level for the acceptance of microleakage tests is unachievable. Microleakage studies are often used to assess the bonding quality and questions remain on the validity of such comparisons. From this review, it may be concluded that microleakage studies are relatively easy to perform and might differentiate the quality of various materials. However, the real clinical significance of these tests remains vague. In reality, it is the clinical assessment of materials that reveals good clinical performance, while in vitro microleakage studies may predict incorrect results.

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