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Review Article

A NEW APPROACH TO MICROLEAKAGE ASSESEMENT: REVIEW

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A B S T R A C T

Microleakage continues to be a topic of great interest, despite of advances and various modifications in terms of technique and placement of material in both restorative and endodontics clinical failures still exist. Most of the failure are probably attributed to poor marginal adaptability, proliferation of microorganisms that remain viable after chemical and mechanical preparation and cause periapical tissue irritation. There are various methods used for assessment of microleakage, some of them lack clinical relevance, while others are technique sensitive however all the methods are two dimensional and hence they definitely cannot correlate to the exact clinical leakage occurring in at the tooth restoration interface or apical leakage. Recently a three dimensional microleakage assessment method has be introduced known as the micro computed tomography which enables a three dimensional evaluation of microleakage both coronal and apically. This review describes three dimensional methods for leakage assessment and its advantages over other methods used for microleakage testing along with the predictability of the most popularly used microleakage testing methods.

INTRODUCTION

Microleakage is the major factor influencing the longevity of dental restorations as it results in many severe biological effects on the teeth including pulpal pathology, hypersensitivity and recurrence of caries. A variety of in vitro methods have been used over decades to study leakage which include dye penetration, neutron activation, electrochemical, fluid filtration, bacterial penetration and dye extraction[1,2]. In addition to this various technique like scanning electron microscopy, transmission electron microscopy and electron probe microscopy analysis have been used to image and measure microleakage. All the above mentioned techniques are two dimensional in nature and does not consider the entire tooth-restoration interface. Over the last few years, efforts have been made to investigate microleakage three dimensionally. One of the most advanced technique is the advent of Micro computed tomography introduced in dentistry as MCT Skyscan 1072. This review describes three dimensional methods for leakage assessment and its advantages over other method used for microleakage testing along with the predictability of the most popularly used microleakage testing methods.

The different methods of microleakage testing along with the drawbacks are discussed

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Fluid filtration method

Fluid filtration method was developed by Pashley *et al* based on the principles of air- pressurisation technique where instead of air, pressurized liquid was applied to the tooth. The main advantage of the method is that the it is non-destructive, therefore the samples can be reinvestigated, and another advantage is that it provides some level of quantitative as well as qualitative analysis. However the drawbacks included high technique sensitive and is a two dimensional method for evaluation of leakage.

Electro chemical method

In an attempt to develop a technique that can assess restorative leakage longitudinally the electro chemical method was developed, in which the tooth was incorporated into an electrochemical unit under electric transmission. However this technique is apparently not used for conductive materials and is extremely sensitive as this is related to electric transmission.

Neutron activation method

This technique basically used a non radioactive manganese salt as a chemical marker and was allowed to leak around the margins of restorations. These specimens were then placed in the core nuclear reactor and exposed to pulsed neutrons. This method is quite complicating and more over does not hold significance in clinical point of view.

Bacterial penetration

This method was introduced by Fraser (1929), who examined the presence of bacteria after immersion of glass tubing with cultured broth. This method used bacteria and evaluated the penetration of those after sectioning the tooth. Bacterial method continued to be used till today and upgraded as they have clinical relevance (Mathrau 2001, Britto 2003, De Deus 2006). Advanced technology such as SEM has been used to investigate the presence of bacteria at the tooth-restoration interface. The most important advantage of the bacterial penetration method is capacity of replicating and simulating the clinical conditions to bacterial leakage. However there aroused many disadvantages such as the method is difficult in terms of cultivating and controlling the bacterial population and secondly, the methodology lacks standardization and reproducibility.

Radio isotope method

Radio isotope method has been widely applied in many of the studies, this method involves use of a broad range of substances including ^{45}Ca, ^{131}I, ^{3} S,^{22} Na, ^{32}P and ^{14}C which have been described as markers, the methodology involves penetration of isotopes through gaps as small as 40nm. However the method is two dimensional and the tracers has affinity to restorative materials leading to increased measurements.

Dye penetration / dye extraction method

This methods involves assessment of leakage using dye such as 0.5% Blue Fuchsin, 2%methylene blue, 50% silver nitrate. Dye leakage and the other leakage methods have a questionable scientific significance.

Reliability of Leakage Studies

Studying sealing ability in endodontics in the beginning of the 90s, Wu *et al*. (1993, 1994a) adapted the fluid transport method to evaluate root filling. Since its introduction, this fluid transport experimental set-up has been employed by several research groups (Wu *et al*. 1994b, Abramovitz *et al*. 2001, Pommel & Camps 2001, De-Deus *et al*. 2008), and as a consequence, its advantages are well known. Today, the fluid transport model still represents a reliable method to study sealing ability in endodontics as well as restorative dentistry. However, the fact remains is that the clinical significance of the results of fluid transport studies are unknown.

In 2005, a new and innovative method to evaluate sealing ability was developed and referred to as the glucose leakage model (GLM) (Xu *et al*. 2005). In the GLM, glucose solution is used as a tracer and this methodology was accepted by an established research group as an improvement over the fluid transport method (Shemesh *et al*. 2006). The GLM represents an advance in methodology and has the potential to add value to the conclusions of laboratory leakage studies, particularly as glucose entering the root canal from the oral cavity could lead to multiplication of bacteria that might survive root canal preparation and filling and potentially lead to periapical inflammation (Xu *et al*. 2005). Therefore, the use of the GLM is thought to be more relevant than other tests (Xu *et al*. 2005, Shemesh *et al*. 2006), but again, its clinical significance remains to be determined.

Using an innovative approach, Oliver & Abbott (2001) were the first to attempt to determine the clinical relevance of laboratory leakage studies. These authors employed extracted root filled teeth to determine the correlation between dye leakage and the clinical outcome of treatment. They concluded that the results of dye leakage studies were not relevant from a clinical standpoint. However, it must be noted that the authors employed a linear dye leakage model and more reliable and sophisticated leakage models remain to be tested in this way. Almost 5 years passed until another research group investigated extracted root filled teeth. Susini *et al*. (2006) used dye extraction as the laboratory model; the advantages of this model over linear dye leakage are well documented in the literature (Hashem & Hassanien 2008). Yet again, that study did not find a positive correlation between dye leakage and the presence of periapical pathosis as determined from radiographs. Nevertheless, the results of the dye penetration did correlate with the technical quality of the root filling as judged radiographically. Many reasons can explain these findings; but it is important to remember that an association was established between the in vivo status of the root filling and the results of a laboratory test.

Three dimensional methods

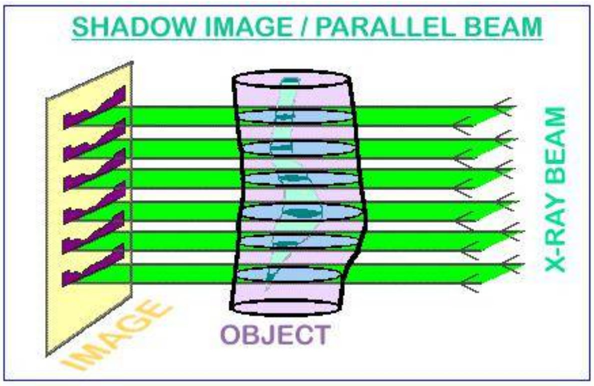
The 3 dimensional analysis was pioneered by Youngson in 1992, he introduced the technique of producing serial sections using water cooled wire saw. Each section was approximately 200µm thick. 3D models were then created by hand tracing projected transparencies and reconstructed by computer aided tools. Computer image analyser was then applied to count the surface areas of dye leakage but the volume was calculated manually .However the methodology was destructive.

This model as upgraded by Gale 2010, who developed a reconstructed model with high resolution in which the surface separation was approximately 100-200µm compared to 280µm. The images of consecutive surfaces, which were created by sequential grinding were photographed by computer with image resolution about 93µm per pixel. For this method instead of water soluble eosin, a high contrast water fast 50%silver nitrate was used.

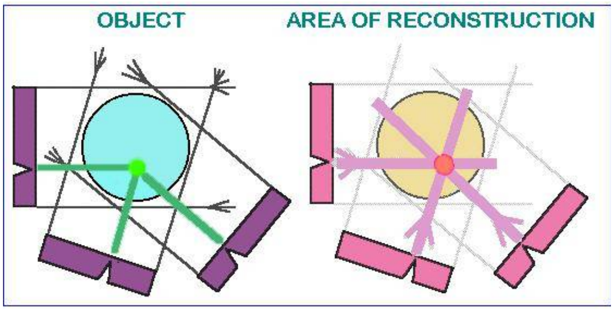
Micro computed tomography was introduced by De Santis 2013, this is a computer aided design where the samples are not destructed, the methodology is similar to as mentioned previous, but this method involves a computer software which analyses the tooth in sections giving a 3D image of different locations of leakage more over it also measures the volume of leakage. X-ray micro-CT offers high resolution imaging of small samples: up to 5-20 micrometres of spatial resolution on samples with a maximal size of 3 cm in length. Analysis is non-destructive and radiographs and CT scans give 2-D and 3-D microscopic images of the internal structure.

Basic principles of microtomogaphy

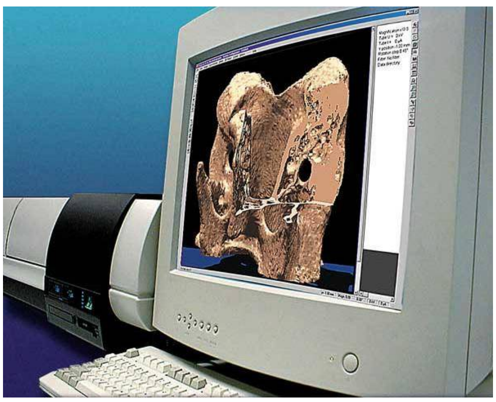
Any x-ray shadow image is corresponding to a two-dimensional projection from the three-dimensional object. In the simplest case we can describe it as a parallel x-ray illumination. In this approximation each point on the image contains the integration of absorption information inside the three-dimensional object in the corresponding partial x-ray beam.



For parallel geometry one can divide the problem of a three-dimensional reconstruction from two-dimensional projections into the serial reconstruction of two-dimensional object slices from one-dimensional shadow lines. A possibility of this reconstruction on a simple example, an object with only one point with significant absorption in an unknown place. In the one-dimensional shadow line we will have a decreasing of intensity of the shadow of absorption in the object area, we can initialise in the computer memory an empty array of pixels (picture elements) corresponding to possible object displacement. Of course one must be sure that all parts of the reconstructed object will be inside the field of view. Because we have the position of the shadow from the absorption points of the object, we can mark on the area of reconstruction in the computer memory all possible positions of absorption points inside the object as lines.



The "SkyScan-1072" is a compact desktop system for x-ray microscopy and microtomography. It consists of the combination of an x-ray shadow microscopic system and a computer with tomographic reconstruction software. This system allows making a non-destructive three-dimensional reconstruction of the object's inner structure from two-dimensional X-ray shadow projections.



The equipment contains an x-ray microfocus tube with high-voltage power supply, a specimen stage with precision manipulator, two-dimensional x-ray CCD-camera connected to the frame-grabber and a Dual Pentium computer with colour monitor. However the 50% silver nitrate solution can cause erosion at margins giving confounding results.

CONCLUSION

Microleakage is definitely an important issue in modern dentistry, particularly when new adhesive materials are constantly introduced. Various methodologies have been developed but all present with drawbacks. It is reasonable to conclude that research should focus on new more reliable microleakage method

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