Microleakage Evaluation using Acetate Peel Technique

Abinash Mohapatra * / N Sivakumar **

Objectives*: To evaluate and measure the interfacial space or the restoration cavity integrity for probable microleakage using acetate film; acetate peel technique under different magnifications.* ***Study design****: Premolar tooth was obtained and standardized class V restoration with Z-350 done both buccally and lingually. The tooth was hemisectioned buccolingually and embedded in epoxy resin, polished, acid etched, washed and dried. The sectioned sample was flooded with acetone and a pre-cut acetate film placed over it. As the acetone evaporated, the film took the shape of the micro relief produced by etching and dried in 10-15mins. It was pulled off the tooth surface and mounted between two glass slides for examination under research microscope with different magnifications.* ***Results****: This enabled us to measure the interfacial space that might lead to leakage of restoration and also could detect the intricate details of tooth.* ***Conclusion****: The acetate peel technique is an efficient way to study the interfacial space/ restoration-cavity marginal integrity at a higher magnification and was a fast and reliable tool for assessing the microleakage of a restorative material.*

Keywords: *Acetate peel technique, Interfacial space, Microleakage, Acetate film*
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INTRODUCTION

Restorative dentistry has evolved by leaps and bounds from the age old amalgam restorations to the present day state of the art materials like nanocomposites. Nevertheless the primary requirement for the ideal restorative material is the perfect merging with the surrounding tooth structure without inherent flaws. Microleakage has been recognized as a major clinical problem with direct filled dental restoration.[1, 2] Microleakage is defined as the "passage of bacteria, fluids, chemical substances, molecules and ions between the tooth and its restoration." Studies have showed that the absence of a seal at the restoration margin promotes tooth discoloration/staining, an adverse pulpal response, postoperative sensitivity and recurrent caries.[3, 4]

The microleakage assessment may be more useful for comparative assessment of dental restorative materials. Many factors influence microleakage; the interfacial space, inadequate physical properties of the restorative materials and/or improper restorative technique or procedure.[4, 5]

It has long been thought that the longevity of dental restorations would be enhanced with a restoration-tooth interface that inhibits the movement of bacteria and/or its toxins. An ever present space the interfacial space exists between the tooth structure and the restorative material or base or liner. Leakage occurs because of the presence of marginal gaps between the restoration and the cavity wall. These gaps are caused by:

- Shrinkage stresses that causes localized failure at the tissue-restoration interface due to tendency for the restoration to pull away from the cavity wall as a result of polymerization shrinkage.
- Inadequate wetting and/ or placement of material along the cavity walls.

True adhesion has been the "holy grail" of dental restorative materials for many decades. To achieve this, three conditions must be satisfied viz; Sound tooth structure has to be conserved, optimal retention must be achieved and leakage must be prevented.[6]

A plethora's of methods were tried to evaluate microleakage or nanoleakage that is; the interfacial space. Retrospectively; visualization, diffusers, air, dyes, isotopes, bacteria, caries were used to evaluate leakage. The disadvantages with these methods were time consumption and need of special equipments. Moreover, the samples cannot be re-evaluated and measuring the gap or the interfacial space is not possible, which would help the manufacturers, researchers and clinicians to improve and predict the performance of restorative materials in the oral environment.[4]

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The acetate peel technique as shown by Fusun *et al* is a rapid way of preparing a large number of sequential replicas from an undecalcified but etched tooth surface effective to study the dental hard tissue.[7] A peel is the replica of an acid etched surface made on acetate film. This technique was developed by palaebotanists and carbonate petrologists to study the cellular patterns or structures of fossil plants for the texture and structure of carbonate rocks.[7]

Depending on the nature of substrate and the purpose, the peel can be prepared in different ways. In the present study the modified cellulose acetate peel procedure was used to predict microleakage and measure the interfacial space present between the restoration and the cavity margin.[7]

MATERIALS AND METHOD

Acetate film and solvent

Acetate sheets or films are soluble in ethyl acetate, ethyl lactate, methyl acetate, diacetone alcohol and tetrachloroethane. Commercially produced acetate films/ sheets (Grafix Plastics; Cleveland; USA) were used for the present study. In order to choose the right type or thickness of the acetate film, the finger is wetted in acetone solvent and pressed gently on the film as mentioned by Fusun *et al*, if it leaves a finger print then that thickness of film is appropriate. So, considering the above criteria; 0.003" thickness cellulose acetate film and commercial grade acetone (CH_3COCH_3; *M.W.* 58.08; Rankem; Ranbaxy Chemicals Ltd; India) were used.[7]

Tooth/ Sample preparation

A human right first premolar tooth extracted for orthodontic reasons was selected. Standardized Class V cavities were marked on both buccal and palatal surfaces 1mm above the cementoenamel junction with the help of a kidney shaped template. The cavities were prepared using a high speed airotor hand piece with a straight fissure bur no. 9. The cavities were measured with a William's periodontal probe at 3mm length; 2mm width and 1.5mm depth according to Hall L.H.S and Swartz M.L criteria.[8] The prepared cavities were then etched with 37% orthophosphoric acid for 15–20 seconds and thoroughly washed with water for 15 seconds and blot dried. Two coats of Adper Single Bond 2 (3M ESPE) adhesive was applied to the cavity followed by gentle air blow to remove excess solvent and light cured for 10secs. The cavities were then restored with nanocomposite Z-350 (3M ESPE), condensed and light cured for 40 seconds. The restorations were polished using composite polishing kit (Soflex; 3M ESPE). The tooth was then embedded in a block of epoxy resin (M-Seal; Pidilite; India), such that one half of the proximal surface is inside the resin and the other half is outside. The tooth was then mounted in a hard tissue microtome and hemisectioned buccolingually.

Polishing and Etching

The surface of the sectioned teeth was thoroughly washed and wet-polished with silicon carbide papers of varying grits (P 600 – P 1000; 3M ESPE), as the polished surface must be flat and perfectly smooth. The sample was washed again and the tooth was etched for a time period of 1-2 mins. The etched surface was washed in distilled water and left to dry.[7]

Mounting and Peeling

The etched resin block was made fixed on molding clay so that the surface of the teeth was oriented face up and horizontal. Using a long shank dropper dispenser acetone was flooded onto the surface and a pre-cut acetate film was placed by bending the film on the drenched surface to avoid air entrapment. As the acetone starts evaporating the film gets pulled into the micro relief produced by etching and imprints the intricate details of the hemisectioned tooth surface. The film dried about 15-25 mins. The film was then removed gently by pulling/peeling from one corner and the excess film was trimmed using a curved scissors. The trimmed film was then placed between two thin glass slides and the edges of the slide were securely held by using adhesive tapes.[7]

Examination of the peel under research microscope

The prepared section was then observed under research microscope (*Olumpus BX 51*) with a mounted camera (ProgRes® C3; Jenoptik; Germany). For the measurement of interfacial space/ tooth restoration interface, ProgRes capture Pro 2.5 software was used. The peel was examined by transmitted and incident lights under different magnification (2X - 40X) for presence of any gap between the restoration and the cavity wall which might lead to possible microleakage.

RESULTS

Under 2X and 5X magnifications

As observed at these resolutions, the three components i.e. the enamel, dentin and the restoration were clearly visible. But the interfacial space could not be measured and a thick dark band was observed at a particular area of the restoration cavity wall/axial wall junction extending more than half way. (Figures 1 and 2)

Figure 1. Under 2X magnification: Showing enamel, dentin and cementum along with the composite restoration.

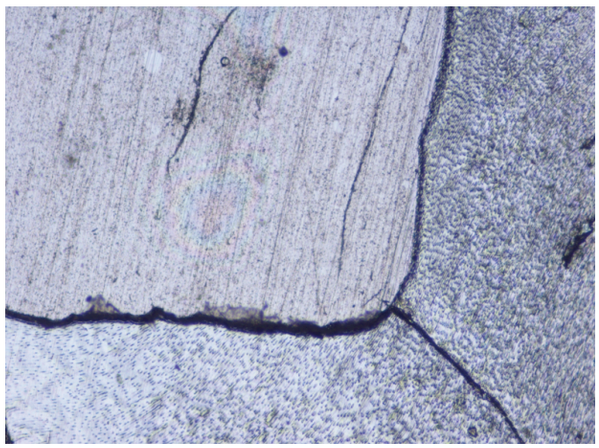

Figure 1. Under 2X magnification: Showing enamel, dentin and cementum along with the composite restoration.

Under 10X magnification

Here the merging of the restoration at the pulpal floor and the thick dark band was clearly evident and also the intricate details of enamel, dentin and cementum. The detailed structure of enamel, the dentino enamel and cemento enamel junction was seen. But the tooth restoration interface could not be measured. (Figures 3a, b)

Under 20X and 40X magnifications

Further, by increasing the magnification to 20X; resin tags were very evident, which denotes the formation of hybrid layer and the dark thick band was also seen (Figures 4a, b). These findings was very prominently observed by increasing the magnification to 40X.The hybrid layer or the merging of the resin to the tooth structure was seen and interestingly a gap at a particular area of the tooth restoration interface, which was dark thick band like structure under low magnification was clearly seen (Figures 5 a to c). So, considering these above findings the measurement of the interfacial space was performed under 40X magnification.

Measurement of interfacial space/tooth-restoration interface

Under 40X transmitted light resolution the restored tooth was critically examined for gap if present and to measure that if possible? The outcome was there was formation of resin tags which denotes absence of gap/ space between the

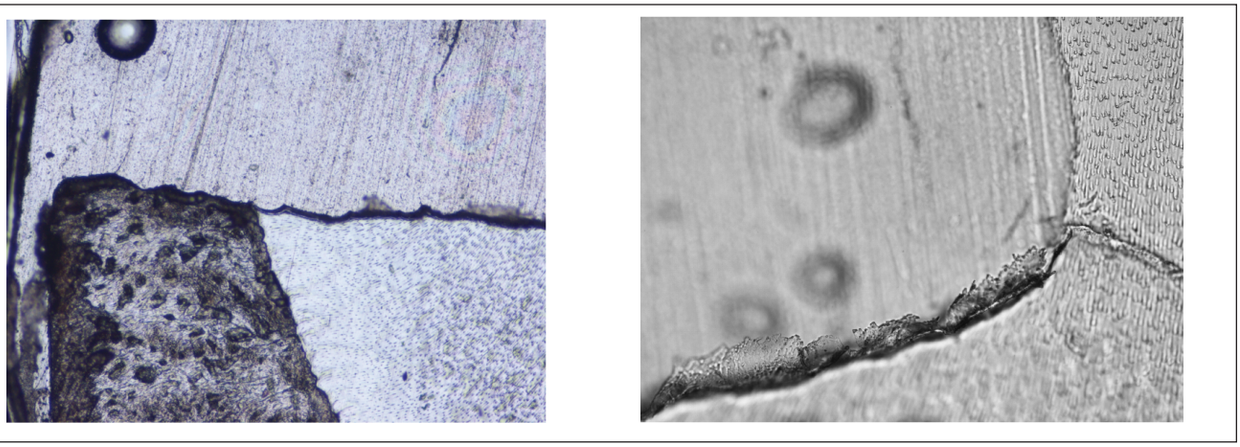

Figure 3 a, b. Under 10X magnification: Formation of resin tags and thick dark band was evident.

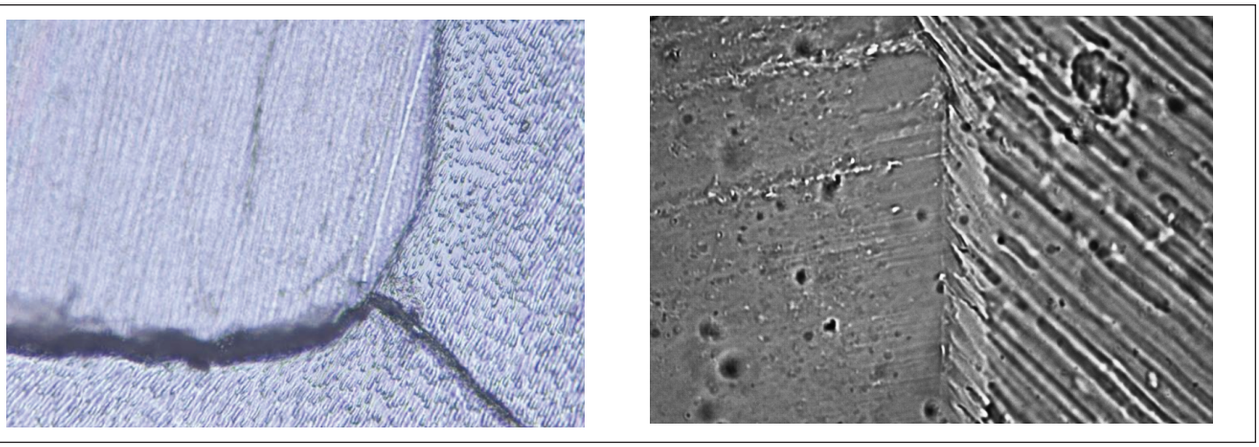

Figure 4 a, b. Under 20X magnification: Merging of the composite to the tooth structure and also the thick dark band seen very significantly, but the interface could not be measured.

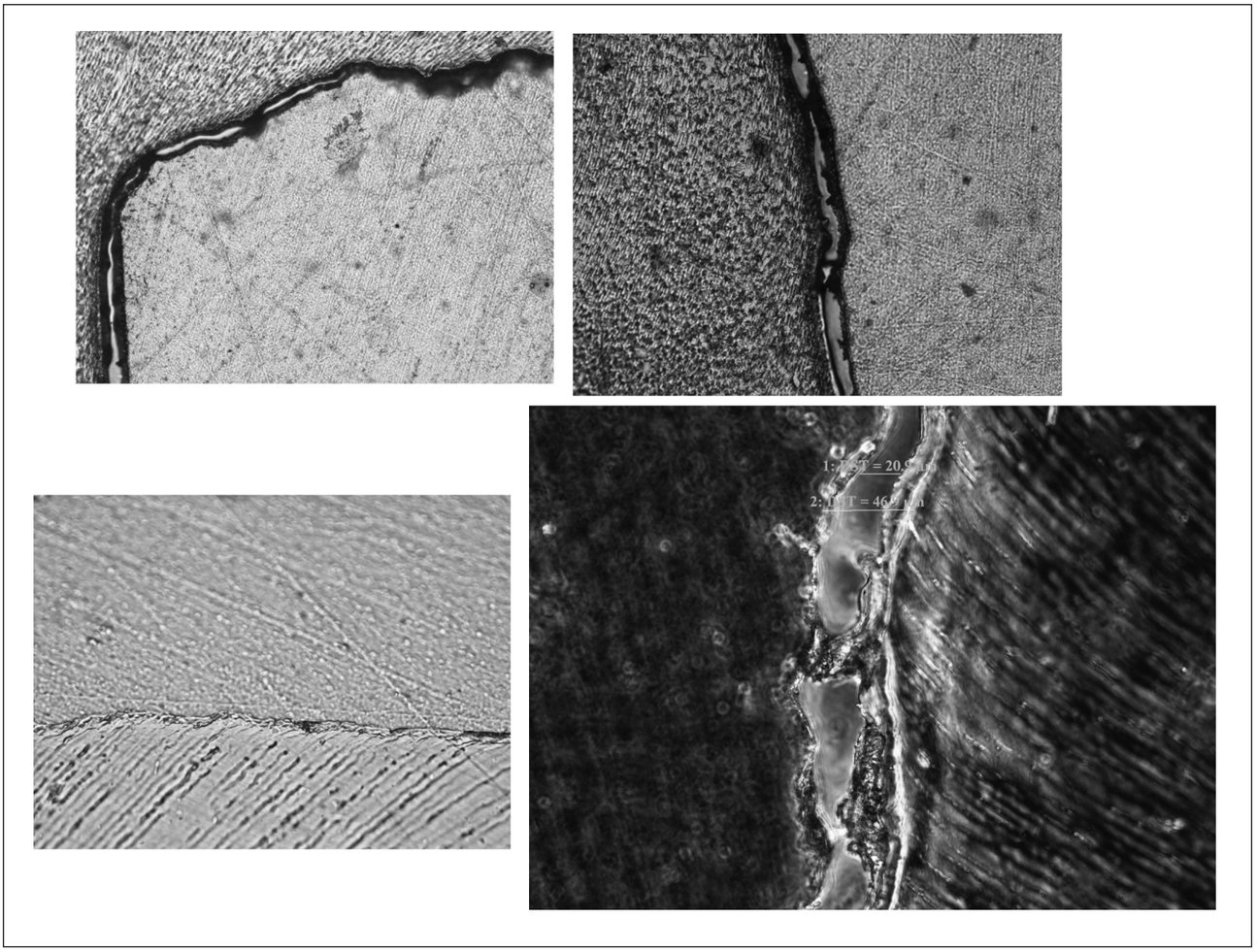

Figure 5; a, b, c, and d. Under 40 X magnification: The interfacial gap was clearly visible and also the resin tags in the dentin. The gap was measured and two readings were noted.

restoration and the cavity wall, but in a particular area as observed in low resolution as the dark thick bands, which was actually the gap/ space present which might lead to leakage and this could be measured to perfection. The gap was measured using, ProgRes capture Pro 2.5 software tool and noted in µm. Two readings were noted as two distinct borders were visible; one denoting the acetate film that has curled into this gap and other reading from the actual restoration margin and the cavity outline; 20.9 µm and 46.9 µm respectively. (Figure 5d)

DISCUSSION

One of the inevitable characteristics of dental composites is shrinkage during free radical polymerization, as monomer molecules are converted into a polymer network, reducing intermolecular spaces. Volumetric shrinkage can range from 2.6–7.1% with light cured composites; this represents a tensile strength of 13 MPa, which tends to pull the restoration away from the cavity wall.[9,10] When this shrinkage occurs in a confined structure such as a tooth cavity, contraction stresses are produced leading to failure of bonding to the tooth structure, thereby compromising the longevity of the restoration.

Variables having strong influence on marginal adaptation of composite restoration:

- Preparation of the cavity
- Technique of etching and use of bonding agent
- Technique of placement of composite and its curing mechanism
- Finishing and polishing of the restoration.[11,12]

In our study, cavities were prepared on both the buccal and lingual surfaces of the tooth specimen; 1mm above the cementoenamel junction, as studies have revealed that the butt joint margins placed less than 1mm from cementoenamel junction exhibited the most cervical leakage.[8] The adhesive and the composite obtained for the study was from the same manufacturer and was applied according to the manufacturer's instructions. This ensured compatibility between adhesive and the restorative material.

For primary evaluation of a new restorative material,

researchers and manufacturer evaluate the physical properties of it and microleakage is one such criteria. However, the wide variety of evaluation techniques used for in-vitro studies leads to confusion because they lack common parameters for comparison. Moreover, the samples once exposed to different dye penetration methods cannot be re-evaluated as the depth of dye flow varies, thus varying results.

Our aim of the study was to find a technique which can measure the tooth restoration interface and if possible can the sample be re-evaluated and stored? To achieve this, cellulose acetate peel technique as shown by *A. Fusun et al* was applied. A peel is a simple replica or mirror image of an acid etched surface. This technique is used by palaebotanists and carbonate petrologists to study different cellular patterns of fossil plants for texture and structure of carbonate rocks.[7,13]

The thickness of acetate film selected was 0.003inches because it could reproduce or imprint the acetone dipped finger accurately when pressed over it. *A Fusun et al* in their study mentioned that the thickness of cellulose acetate film for dental hard tissues should be 0.1mm or less.[7]

It has long been thought that the longevity of dental restorations would be enhanced, with a restoration-tooth interface or interfacial space that inhibits the movement of bacteria and/ or its toxins. However, it is size of this space and the bacterial activity occurring within, that contributes to leakage leading to recurrent caries. Although the size of an offending bacterium is 2 µm, a space ranging from 2–20 µm is necessary for bacterial penetration and deposition of a bacterial film. The incidence of caries requires a space not less than 50 µm; fortunately this space can be detected with a dental instrument but is at the lower limit of visual acuity which makes the clinician difficulty to appreciate.[3,4,14] This space can be measured with the present technique. When examined under transmitted light with different magnifications ranging from 2X–40X.

When observed in 2X magnification we could appreciate the tooth and the restoration, but the interfacial gap could not be measured. This gap was very well appreciated under 40X and moreover could be measured to perfection, thus 40X was standardized for this technique, as this view was of optimum clarity. The property of cellulose acetate film when laid on acetone solvent is that it softens as it reacts and curls/ flows into the gap between the restoration and cavity margin if present. As the film curls into this gap/ space, it creates two distinct margins on either side of the tooth-restoration interface that is the actual cavity margin and the restoration. This was measured using the ProgRes capture Pro 2.5 software tool; ProgRes® C3; Jenoptik; Germany Company and which was 46.9 µm, which means that microleakage has occurred. Perfect union between the tooth structure and the restoration is the primary requirement for an ideal restorative material. Morphological in vitro microleakage studies have indicated that both resin and collagen matrices may degrade upon storage. Catastrophic failure of the resin-dentin bonds can lead to leakage that is very difficult to measure and interpret. Acetate peel can give us overall view of the tooth structure and the interface if present that can cause microleakage and can be predicted and interpreted in µm as there are different dental restorative materials claiming have superior properties than the other.

Merits of this technique:

- Microleakage is one of the most common factors for failure of a restoration leading to secondary caries or pulp pathosis in primary dentition.
- As preventive dentistry is a major tool and the procedures are technique sensitive, a material of better adhesion and superior adaptation is necessary to prevent leakage.
- By using acetate film peel technique we were able to prepare numerous replicas of the same tooth-restoration margin/ interface.
- It proved to be a simple, inexpensive and faster way of measuring the interface
- Peels are stable and can be preserved for further evaluation if required.
- This peel technique can open a new foresight where clinicians can choose and apply the best material to its optimum effect for a child.
- Can supplement other microleakage techniques with more accuracy.

Demerits of this technique:

- The technique uses acetone as the solvent and the inflammable nature of acetate film, has to be kept in mind.
- The peel curls in different forms, so focusing in one plane was difficult.
- Artifacts were observed which could be misinterpreted.
- Inability to visualize all the areas as film curls.
- Air entrapment between the tooth and acetate film was common (technique sensitive).

CONCLUSION

As the study was to determine if modified cellulose acetate technique can be used to measure the interface that can lead to microleakage, proved to be a reliable tool, only at higher magnification. This technique is simple, inexpensive and needs lesser tools for leakage evaluation.

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