COMPOSITE REPLACEMENT OF AMALGAM RESTORATION VERSUS FRESHLY CUT DENTIN: AN IN VITRO MICROLEAKAGE COMPARISON.

A Thesis

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Hetaf Sameer Redwan B.D.S

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THESIS COMMITTEE

Thesis Advisor

David N. Bardwell, D.M.D.

Clinical Professor

Prosthodontics and Operative Dentistry

Tufts University School of Dental Medicine

Committee Members

Hans-Peter Weber, D.M.D.

Professor and Chair

Department of Prosthodontics & Operative Dentistry

Tufts University School of Dental Medicine

Samer Khayat DMD, CAGS

Assistant Clinical Professor

Department of Operative Dentistry and Prosthodontics

Tufts University School of Dental Medicine

Ala Ali BDS, MS

Clinical Instructor

Department of Operative Dentistry and Prosthodontics

Tufts University School of Dental Medicine

Matthew Finkelman PhD

Associate Professor

Department of Public Health and Community Service.

Tufts University School of Dental Medicine

ABSTRACT

Objective: To evaluate the microleakage of the composite restoration when bonded to a cavity previously restored with amalgam material compared to that of freshly cut dentin.

Materials and Methods: Thirty extracted intact human molars were mounted in autopolymerizing acrylic resin. Class II box preparations were prepared on occluso-proximal surfaces of each tooth (4 mm bucco-lingual width and 2 mm mesio-distal depth) with the gingival cavosurface margin 1 mm above the CEJ. Each cavity was then restored using high copper amalgam restoration (Disperalloy, Dentsply) then thermocycled for 10000 thermal cycles. The amalgam restorations were then carefully removed and replaced with Filtek Supreme Ultra Universal, 3MUniversal, ESPE except five of them, which were used for the SEM and EDS analysis. A cavity of the same dimensions was prepared on the other side of the tooth and restored with composite resin then they were thermocycled for 5000 thermal cycles. Twenty samples were randomly selected for dye penetration test using silver nitrate to detect the microleakage. The specimens were analyzed with a stereomicroscope at a magnification of 20x. All of the measurements were done in μm; two readings were taken for each cavity at occlusal and proximal margins. Two measurements were taken using 0 to 3 scale and the percentage measurement. **Results:** Corrosion products were not detected in both groups (fresh cut dentin and teeth previously restored with amalgam). No statistically significant difference between the microleakage of the two groups was found using a 0 to 3 scale at the occlusal margins (McNemar's test, $p= 0.727$) or proximal margins (Wilcoxon signed-rank test, $p = 0.174$). No significance difference was found between the two groups using the percentage measurements and Wilcoxon signed-rank test at either the occlusal

(p=0.675) or proximal (p=0.513) margins. However, marginal microleakage was statistically significant between the proximal and occlusal margins ($P < 0.001$). **Conclusion:** Within the limitations of this in-vitro study, no significant difference was found between the microleakage of non-discolored dentin in teeth that were previously restored with amalgam compared with freshly cut dentin. However, marginal microleakage in the proximal surface was higher than that in the occlusal surface.

DEDICATION

To my father, Dr.Sameer Redwan, who always encouraged me and motivated me to move forward. You are my biggest inspiration.

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Introduction

The foundation of adhesive dentistry was introduced in 1955 by Buonocore when he first prescribed the use of phosphoric acid and phosphomolybdate-oxalic acid to treat the enamel surface chemically by acid etching.[1] He concluded that a composite resin could be bonded to an enamel surface that was treated with 85% phosphoric acid for 3 seconds.[2] For almost fifty years, composite resin material has been used in dentistry; it is used in direct restorations of over 95% of anterior and in 50% of posterior teeth.[3]

The use of this material has increased recently and became the first choice for most carious lesions for several reasons. One of the main factors is that amalgam restorations do not adhere to the cavity wall chemically and there are differences in the coefficient of thermal expansion of amalgam and tooth structure, which opens and closes the gap on the amalgam-tooth interface when the intra-oral temperature changes.[3-5] These changes allow the transport of fluids, ions, molecules, and possibly bacteria and their toxins. The outcomes of these interactions are the release of mercury and corrosion products. Other factors include the consumer demand for highly esthetic restorations, as well as public concern about mercury in dental amalgam.[6-9]

Amalgam Alloy

Amalgam alloys were introduced into North America in the 1830s. Cast gold and amalgam have been the materials of choice for decades. However, the choice of dental materials has changed dramatically in the last twenty years. Dental amalgam is the most often used restorative material for posterior teeth due to its mechanical properties, easy placement technique, and low cost.[10-12]

Because patients are now more esthetically demanding, tooth-colored materials, such as resin-based composites, are used more often. Moreover, mercury used in amalgams has raised concerns about its biological toxicity and environmental hazards. People are exposed to mercury and other metals via vapor and corrosion products in swallowed saliva and by direct absorption through the oral mucosa. However, apart from the controversy regarding mercury toxicity, amalgam has other disadvantages, such as microleakage and lack of adhesion that makes undercuts necessary for their mechanical retention, thereby further weakening the remaining tooth structure. [10,12,13]

Composition of amalgam alloy

Amalgam is a metallic alloy formed by the reaction between mercury and an alloy in powder form that contains silver, tin, copper and zinc, among other metals, which could be classified into two groups: low copper content or traditional composition (5% or less copper) and high copper content (6-30% copper).

Silver is the main part of the alloy present in combination with tin as the inter-metallic compound Ag_3Sn, which is known as the γ phase. This γ phase reacts with mercury to form γ_1-Ag_2Hg_3 (the matrix phase in the microstructure) and γ_2-Sn_7Hg phases, leaving some unreacted γ -Ag_3Sn particles.

$$Ag_3Sn + Hg \rightarrow \quad Ag_3Sn + Ag_2Hg_3 + Sn_7Hg$$

After a short time, γ_2-Sn_7Hg that is newly formed around Ag-Cu eutectic will react with silver-copper particles, forming the η'-Cu6Sn5 phase of the copper-tin system along with some γ_1-Ag_2Hg_3 around the silver-copper particles. Each phase of the dental amalgam has a different corrosion potential and mechanism. [14,15]

Tarnish and Corrosion

Tarnish is a thin layer of corrosion that forms over copper, brass, silver, aluminum, and other similar metals as their outermost layer undergoes a chemical reaction. In the oral cavity, tarnish occurs by formation of hard and soft deposits on the restoration surface. Usually, it is a self-limiting reaction, in which only the top few layers of the metal react and the formed layer protects the underlying layers. Amalgam restorations often tarnish and corrode in the oral environment. The degree of tarnish and corrosion depends on several factors, including the individual's oral environment and the amalgam alloy used. [16-18]

Amalgam tarnish and corrosion is an oxidation-reduction reaction in which the metals in the amalgam alloy react by chemical or electrochemical reaction with nonmetallic elements in the environment to produce chemical compounds. Excessive corrosion can cause increased porosity, reduced marginal integrity and strength, as well as the release of metallic products into the oral cavity. [12]

Corrosion is often cited as an advantage because the corrosion products help to produce a good marginal seal. However, the space between the alloy and the tooth permits microleakage of electrolytes that cause crevice corrosion, which can lead to rapid deterioration of the amalgam's properties. The corrosion process is associated especially with the γ_2 phase because it is more electronegative than the γ and γ_1 phases. Corrosion is a process in which an anodic reaction occurs, resulting in a loss of electrons, while a cathodic reaction results in a capitation of electrons. In one single amalgam restoration that presents in an electrolytic environment such as saliva it shows areas that act as anode and others acting as cathode. [15,18,19] Corrosion of amalgam is a natural phenomenon in a metallic substance in

the oral environment and tends to discolor the tooth substance. This discoloration increases with age and is most prominent at the amalgam-tooth interface. [16]

Variables affecting corrosion

There are several factors that affect the corrosion processes of amalgam alloys, such as temperature fluctuations, movements of the medium surrounding and in contact with the metal surface, and solubility of corrosion products. Other factors that are related to the amalgam alloy include the particle size, mercury content, and quantity of the γ_2 phase, as well as the degree of surface finishing. Moreover, the presence of various acids, such as phosphoric, acetic and lactic acid, when present in the proper concentration and pH, can lead to corrosion. Various sulfides, such as hydrogen or ammonium sulfide, corrode silver, mercury and similar metals present in amalgam; water, oxygen and chloride that are present in saliva also contribute to corrosion attack. [12,19]

Corrosion causes structural changes inside the amalgam and is considered to be the main reason for marginal fractures at the outline of amalgam restorations. On the other hand, corrosion is regarded as beneficial, in that it is supposed to be the main factor in reducing marginal leakage over time by deposition of amalgam corrosion products in the marginal gap. In the fluid-filled marginal gap, amalgam constituents in ionic form might be valuable for deposition of complexes within the gap, as well as for diffusion into dentin and formation of salts within the dentinal structure. [20,21]

Conventional amalgam alloy shows a marked decrease in microleakage as restoration ages due to sealing by corrosion products formed by the gamma two phase, which is the weakest phase of set amalgam. [13] Jorgensen et al. [21] and Mateer and Reitz supported the

theory that the corrosion process releases tin ions from the γ_2-Sn_7Hg phase that react with nonmetallic ions in the saliva to produce tin salts.[22] The current status of amalgam research supports the hypothesis that corrosion is the primary factor associated with the breakdown phenomena that usually occur as either a bulk fracture or progressive breakdown at the marginal angles. Again, the quantity of corrosion mostly depends on alloy composition, manipulation, oral environment pH and surface roughness.[23,24]

Wei and Ingram have used the electron microprobe to analyze the tooth-amalgam interface of several clinical specimens. Their analyses showed high concentrations of tin in the corrosion products and migration of tin into dentin and enamel.[25] In 1970, Mateer and Reitz reported a layer-like corrosion product at the tooth-restoration interface, which suggested that the compounds form gradually by reaction of the amalgam and oral fluids.[22,23]

Access of metal ions into the tooth-tissue interface

Massler and Barber et al. concluded that the dentin discoloration at the tooth and restoration interface could show both black and brown discolorations and that they could appear simultaneously in the same tooth.[26] They reported that brown staining was caused by caries, and could be removed easily with hand instruments. Dark grayish-black dentin was referred to as the diffusion of amalgam constituents into dentin; it was not necessarily soft and was radio-opaque. Spectrographic analysis revealed large quantities of Hg and smaller amounts of Ag, Sn, Zn and Cu. Furthermore, an in vitro study showed that grayish-black staining consisted of metal sulfides. Typically, Sn was the element that was found in dentin in all dentin penetration studies.[26-28]

Mateer et al. detected low Hg and Ag content in blackened dentin, but the Sn content in dentin adjacent to amalgam was approximately as high as it was in the amalgam itself.[29] A number of authors also have demonstrated high concentrations of Sn and Zn in electron microprobe studies.[26,27,30,31] However, Kurosaki and Halse found that no metal ions had penetrated into non-discolored dentin; they found penetration of Zn and Sn only in dark discolored dentin. Because Sn produces black sulfides and Zn sulfides are white, they concluded that the black staining was caused by tin sulfides.[31,32] Moreover, in a very recent study, Sn in various amounts and traces of Zn and Cu were found in discolored dentin.[28] The release of mercury from restorations is time-dependent and proportional to the surface area of the restoration.[33]

Composite resin as an amalgam alternative: indication and limitation

The use of tooth-coloured restorative material rather than amalgam restorations requires the meticulous use of an adhesive technique.[34] This is due to the fact that dentin is more hydrophilic in nature compared to enamel, and therefore is more difficult to deal with it in terms of bonding to adhesive resin. However, enamel is known to have reliable bond strength when bonded to resin based composite, because its main components are inorganic, while dentin has a different composition and structure[35,36]

Although, composite resin is the material most commonly used for direct restorations, the behavior of the margin is one of the main factors that affect the longevity of the restoration.[34,37] Composite's polymerization shrinkage affects the marginal integrity because the restoration tends to shrink away from the margin. This factor, together with the

masticatory stresses and thermal changes, are considered to be the main causes of microleakage in class II restorations. [38, 41]

Dentin permeability is a significant phenomenon that affects the limit of the microleakage, which is determined by the presence of secondary, sclerotic, or tertiary dentin, as well as the presence of dead tracts and smear layer. [38] Moreover, polymerization shrinkage is influenced by material properties, such as resin composition and filler content, as well as by the technique used, such as the volume of cured material, velocity of polymerization, and direction of light application; in fact, that the larger the volume of composite to be polymerized, the larger the polymerization shrinkage. [34]

The magnitude of the polymerization stress depends on the e-modulus of the material and the configuration factor (c-factor), which is defined as the ratio of bonded to unbounded surfaces. It has been concluded that only the restorations with a c- factor less than one will withstand the stresses produced by the polymerization shrinkage of composite resin. However, bonding agents may resist the tensile force and maintain the marginal integrity, although this can cause cuspal movement and sensitivity. However, failure of the bone and micro-gap formation is still likely to occur in many clinical situations. [34,39]

Composite resin

The resin-based composite restorative materials are a physical mixture of materials. [40] They consist of three major components: an organic matrix, an inorganic matrix and a coupling agent. The organic matrix or resin matrix consists mostly of Bis-GMA (bisphenol-A- glycidyldimethacrylate). Because it is highly viscous, it is mixed with TEGDMA (triethylenglycol-dimethacrylate). [36] The dispersed inorganic particles consist of glass or

quartz.[36] These particles provide the mixture with mechanical reinforcement, as well as light transmission and light scattering that add enamel-like transmissions.[40] The coupling agent is silane that forms a bond between the organic and inorganic phases. Composites also contain an accelerator-initiator system that allows for self-curing, light curing and dual curing.[36]

With the improvments in adhesive composite materials and their mechanical properties, composite restorations are now the first choice for most carious lesions.[4] These materials have several advantages, including esthetics,[35] adhesive properties to the dental structures due to total etching techniques and conservative cavity preparation that is not possible with an amalgam restoration.[41] Thus, there has been a tremendous shift from using amalgam alloy restorations to adhesive composite resins for the restoration of posterior teeth.[42]

There are several factors that affect the clinical success of posterior composite resin restorations: (1) occlusion analysis; (2) complete caries excavation; (3) remaining tooth structure analysis; (4) use of appropriate layering and curing techniques to control the polymerization stresses; (5) equilibration for occlusal stresses, and (6) patient compliance in maintaining good oral hygiene.[42]

Although various generations of bonding agents have been developed to decrease polymerization shrinkage, microleakage remains a significant problem[43] and a major factor affecting the longevity of composite restorations.[44] This is a primary cause of marginal discoloration, secondary caries, pulpal inflammation, post-operative sensitivity, and restoration replacement.[45] Microleakage measurements usually provide an assessment of the sealing ability of adhesive materials, which is clinically relevant.[46]

Bonding/Adhesion

The adhesion of restorative materials to the enamel surface has become a reliable concept in modern restorative dentistry. However, the correlation between dentin permeability and dentin adhesion remains unclear.[2,47] The current challenge is to develop high bond strength in dentin similar to that obtained in acid-etched enamel.[48]

In the late 1960s, Buonocore proved that the etched enamel surface forms tag-like resin extensions that interlock micromechanically with enamel micro-porosities.[49] There was some variation in the phosphoric acid concentration and duration of etching; the current knowledge is that etching for 15 seconds with a 30 to 40% solution of phosphoric acid is clinically acceptable. However, dentin bonding is less predictable and reliable due to its complex histological structure and randomly arranged hydroxyapatite in an organic matrix. The dentinal tubules are the only pores available for micromechanical retention, and these tubules contain fluids that interfere with dentin bonding.[2,48,49]

Dentin bonding was further complicated due to the presence of a smear layer that can be defined as organic debris. This consists of collagen fiber and hydroxyapatite crystallites that are calcific in nature and produced by instrumentation of enamel, dentin or cementum. The smear layer interferes with the bonding by blocking the dentinal tubule. However, it was thought originally that the smear layer had an advantage in protecting the pulp by decreasing the permeability of dentin.[49,50]

With the improvements of dentin bonding, smear layer removal became necessary by using etch and rinse approach to allow establishment of effective interlocking bonding in which the adhesive penetrates the intratubular and intertubular dentin; this allows the resin to

penetrate into the conditioned dentin and results in the formation of intratubular resin tags and a hybrid layer. [49,51]

Bonding Adhesive Systems

Adhesive systems are divided into etch and rinse (total-etch), self-etch, and selective-etch, based on the adhesion mechanism and its composition. [52,53] Bonding to dental hard tissue can be achieved by using any of these adhesive systems. [54]

Efforts have been made to reduce the number of steps used in the adhesive systems and to reduce the technique sensitivity in bonding procedures. [54] The self-etch adhesive systems are less technique sensitive and also require a shorter clinical application time. This system does not require separate acid etch steps, as they condition and prime enamel and dentin simultaneously by infiltrating and partially dissolving the smear layer; thus, it does not require removal of the smear layer and smear plugs, as they are incorporated into the hybrid layer complex. [54,55]

According to Al-Ehaideb and Mohamed, the fifth-generation system has been shown to be effective in preventing microleakage. [56] In 2008, Waldman concluded that fifth-generation adhesive demonstrated superior marginal sealing compared with sixth-generation adhesive. [57] Other studies reported total-etch adhesives were more effective in reducing microleakage than self-etch adhesives. [58,59] De Silva et al. reported on the poor sealing ability of self-etching adhesives. They demonstrated that the self-etching primer adhesive system failed to generate sealed interfaces between the dentin and the composite resin. [60]

These findings are in agreement with studies that reported decreased leakage associated with total-etch, especially at the enamel margin, compared with the self-etch

system. However, other researchers have reported contradictory results. [58]

Etch and rinse approach

This is either a three-step etch/prime/adhesive (4th generation), or a two-step etch with phosphoric acid followed by prime/adhesive agent (5th generation; Figure 2). The fifth generation has been introduced to ease the clinical application by reducing the bonding steps. 35 to 37% phosphoric acid applied for 15 to 20 seconds is used for etching. [49] With this system, the enamel and dentin are treated with acid to remove the smear layer and demineralize the super-facial layer of hydroxyapatite crystals. This is then followed by a mixture of resin monomer dissolved in an organic solvent to infiltrate the etched dentin. This infiltration results in a hybrid tissue composed of collagen, resin, residual hydroxyapatite, and traces of water (Figure 3). This was first described in 1982 as the hybrid layer. [55,61] At the enamel surface, two types of resin tags usually form after the etch and rinse technique. Micro-tags fill the spaces surrounding the enamel prisms and several micro-tags result from resin infiltration and polymerization within the etched enamel prisms. Without a doubt, the micro-mechanical interlocking of tiny resin tags within the acid-etched enamel surface is still considered the best reliable bond to enamel. It also seals and protects the bond to dentin against degradation. [52,61]

Microleakage

Microleakage has been defined as the clinically undetectable passage of bacteria, fluids, molecules or ions between a cavity wall and the restorative material applied to it. [8] It

can cause hypersensitivity of the restored tooth, tooth discoloration, recurrent caries, and even pulpal injury and accelerated deterioration of the restoration material. [13]

Microleakage measurements provide an assessment of the sealing ability of adhesive materials, which is of clinical relevance. [62] According to Abo et al., an in vitro microleakage test combined with thermocycling is a useful method to assess sealing performance. In their study, they also demonstrated that the use of a large number of thermal cycles could simulate the conditions in the oral environment. [63] In a review performed by Heintze on the evaluation of in vitro sealing ability, microleakage tests are preferred over other methods, such as permeability assessment and SEM evaluation of marginal adaptation, as the latter are technique sensitive and time consuming. [64]

Today, the uses of composite resin restorations are becoming more and more common in replacing amalgam fillings. Specifically, Denmark, Sweden, and Norway have banned dental amalgam except when a specific exception is requested in individual cases, and several other countries (e.g., Canada, Italy, Australia) have taken steps to reduce amalgam use. However, their substitutes have not yet received systematic scrutiny with respect to their potential hazards. [65]

In many clinical situations, adhesive resin could bond to abnormal dentin as sclerotic dentin or discolored dentin, due to corrosion products that penetrated the dentinal tubules from the amalgam restorations. Several studies have shown that the bond strength was lower than or similar to that of normal dentin. [66-68] It is a very common observation that dentin underneath previous amalgam restorations shows extensive black discoloration. Even in the late nineteenth and early twentieth century, it had been suggested that dentin staining could be caused by the deposition of metallic sulfides and the penetration of silver and mercury

ions from the overlying amalgam.[28,68-73] Scholtanus et al. concluded that the discoloration of dentin underneath amalgam is an indicator of the presence of amalgam constituents, the effect of which on the adhesive restorative procedures is not known.[5] Harnirattisai et al. showed that bond strength to dark dentin after amalgam removal is lower than to normal dentin.[28] Data are lacking in the literature about microleakage of composite resins after amalgam removal.

Aim and Objective

To evaluate the microleakage of the composite restoration when bonded to a cavity previously restored with amalgam material compared to that of freshly cut dentin.

Hypothesis

There will be more microleakage of the composite restoration when bonded to a cavity previously restored with amalgam material compared to that of freshly cut dentin.

Clinical Significance

A large number of amalgam restorations have been replaced with composite resin over the years. Contemporary practice continues to recommend amalgam removal and replacement with tooth colored resins. The results of this study can help clinicians better serve their patients by addressing whether replacing amalgam restorations with composite resin affects the quality of the bond to dentin, and whether microleakage will be affected adversely.

Materials and Methods

Thirty extracted teeth were collected. Twenty teeth were used for the microleakage test and ten for the scanning electron microscope and energy dispersive x-ray analysis. All of the teeth were caries free, intact human molars and premolars. Teeth were collected from the general collection jar in the Oral Surgery Department at Tufts University School of Dental Medicine.

All of the samples were cleaned with an ultrasonic scaler (Cavitron GEN- 119, Sps^{TM}, Dentsply, PA) to remove saliva and debris. Thereafter, they were placed in distilled water at room temperature to prevent them from drying.

The teeth were placed in autopolymerizing acrylic resin (Technovit, Heraeus Kulzer, Germany) using a plastic mounting template (Ultradent, South Jordan, UT) with the experimental surface of the teeth exposed. The teeth were stored in distilled water at room temperature at all times.

Standardized Class II box preparations were prepared on the occluso-proximal surfaces of each tooth (4 mm bucco-lingual width and 2 mm mesio-distal depth) with the gingival cavosurface margin 1 mm above the CEJ and the cavosurface margins butt joint, [74] with #245 carbide burs (SS White, Lakewood, NJ) in an air/watercooled high-speed turbine. A new bur was used after each five preparations. [75]

The tofflemire matrix band was placed using a matrix retainer to mimic the clinical situation. The restoration procedure began with the application of two layers of varnish (Copalite, Cooley & Cooley, AL) (Table:1) according to the manufacture's instructions.

Each cavity was then restored using amalgam restoration (Disperalloy, Dentsply, DE)(Figure 4a.). All teeth were thermocycled for 10000 thermal cycles between water baths

at 5ºC and 55ºC with a 30 second dwell time. This procedure aged the material to simulate a year of clinical performance and the generation of amalgam corrosion products. [76]

Next, the amalgam restoration was carefully removed using #245 carbide burs (SS White, Lakewood, NJ) in an air/water cooled high-speed turbine (Figure 4b.). To prevent encroachment on the dentin, the final layer of amalgam was removed by means of an explorer. [77] The amalgam was removed from all of the samples except five of them, which were used for the SEM and EDS analysis.

A cavity of the same dimensions was prepared on the other side of the tooth using #245 carbide burs (SS White, Lakewood, NJ) in an air/water cooled high-speed turbine. The tofflemire matrix band was placed using a matrix retainer to ensure that the light curing of the restoration took place only from the occlusal side of the restoration so as to mimic a clinical situation. Both cavities were restored with composite material using a total-etch adhesive system.

The composite restoration began with the application of 35% phosphoric acid etching (Ultra-Etch, Ultradent, UT) for 20 seconds on enamel and 15 seconds on dentin. The teeth were rinsed with water for 10 seconds and blot dried with cotton pellets in order to attain a moist dentin surface and bonding with total-etch adhesive (ExciTE F DSC, ivoclar vivadent, NY) according to the manufacturer's recommendations.

The teeth were restored with composite resin material (Filtek Supreme Ultra Universal, 3MUniversal, ESPE, MN) via the Liebenberg technique. The first layer was very thin and was applied to a single dentinal surface without making contact with the opposing cavity walls. [78] The cavity was filled completely with wedge-shaped composite increments, and each cusp was built up separately. A blue light emitting diode (LED) was used with 800

mW/cm^2, followed by finishing and polishing (Figure 4c.). The light source was monitored every five curing cycles using a radiometer (Demetron L.E.D, Kerr Corporation , CA) to ensure that the light intensity was stable. [79]

All of the teeth were thermocycled for 5000 thermal cycles between water baths at 5ºC and 55ºC with a 30-second dwell time, which served to age the material to simulate the clinical performance.

For the microleakage test, all of the samples were coated with two layers of nail polish (Vinyl shine nail polish; Rimmel London, London, UK), except for a 2.0-mm rim around the restoration to allow contact of the leakage-tracing agent contact with the margins of the restoration. Thereafter, the teeth were immersed in a solution of 50 wt% ammoniacal silver nitrate (pH=9.5) (Fisher Scientific, NJ) for 24 hours followed by eight hours in a photo-developing solution (Eastman Kodak Co., Rochester, NY). [80]

The specimens were washed under running water for one minute. The nail polish was carefully removed using a #15 scalpel, after which the specimen was embdded in epoxy material (Epoxicure resin, Buehler, IL) (Figure 5). Each tooth was sectioned mesiodistally using a diamond saw and the blocks were positioned in a precision cutting machine (1000 Isomet, Buehler Ltd., Buehler, IL) (Figure 6 a.b.).

The specimens were analyzed with a stereomicroscope (Olympus America Inc., PA) at a magnification of 20x (Figure 7). All of the measurements were done in μm; two readings were taken for each cavity (occlusal and cervical = four readings per tooth). [81]

The specimens were scored according to the following degree of the dye penetration (Figures 8, 9):

Occlusal score:

0 – No dye penetration.

1 – Dye penetration into enamel.

2 – Dye penetration beyond the dentinoenamel junction.

3 – Dye penetration into the pulpal wall.

Proximal score:

0 – No dye penetration.

1 – Dye penetration into enamel.

2 – Dye penetration up to half extension of cervical wall.

3 – Dye penetration into more than half or complete extension of the cervical wall. [82]

The dye penetrations were also recorded in percentages measurements using the following formula: [81]

$$\text{Leakage number} = \frac{\text{Distance evidenced for dye}}{\text{Overall distance for margin}}$$

Scanning electron microscope and energy dispersive x-ray spectroscopy.

Five teeth were randomly selected from the groups described above.

All of the samples were embedded in epoxy resin material (Epoxicure resin, Buehler, IL). Each sample was sectioned mesiodistally by means of a diamond saw, and the blocks were positioned in a precision cutting machine (1000 Isomet, Buehler Ltd., Buehler, IL). Each section was mounted in a Hitachi table (Aluminum mount, Electron Microscopy Science, PA) with conductive adhesive (Graphite conductive adhesive, Electron Microscopy Science, PA). The samples were coated with a 4.5 μm-platinum layer using a coating machine (Sputter coater 208hr, Cressington, UK). Each section was analyzed with a scanning electron

microscope (Hitachi S-48000, Hitachi, TKY) and energy dispersive x-ray spectroscopy to analyze the metal penetration in the dentinal tubules below the interface between the restoration and the dentin as well as at the interface. All of the analysis was conducted using EDAX geniuses software operated at 20Kv .

Sample size calculation

A power calculation was performed using nQuery Advisor (Version 7.0). Assuming an 84% chance for the group with non-restored teeth to have lower microleakage than the group with previously restored teeth [83] , a sample size of n=20 teeth was adequate to obtain a Type I error rate of 5% and a power greater than 99%.

Statistical Analysis

For the 0-3 scale of microleakage at the cervical surface, counts and percentages were calculated, and statistical significance was assessed via the Wilcoxon signed-rank test. For the occlusal score, counts and percentages were calculated, and statistical significance was assessed via the McNemar's test. For the percentage scale of microleakage, median and interquartile ranges were calculated, and statistical significance was assessed via the Wilcoxon signed-rank test, because the assumption of normally distributed data was violated. P-values less than 0.05 were considered statistically significant. SPSS version 21 was used in the analysis.

Results

Microleakage test:

Dye penetration and consequently microleakage varied between the occlusal and proximal surfaces.

- *Occlusal marginal microleakage:*

The results of microleakage on a 0 to 3 scale are presented in (Table 2. Figure 10). For the group of teeth that were previously restored with amalgam, twelve teeth (60%) had a score of 0, and 8 (40%) teeth had a score of 1; for the fresh-cut dentin group, ten teeth (50%) had a score of 0, and ten teeth (50%) had a score of 1. McNemar's test revealed no significant difference in microleakage for the two groups at the occlusal surface ($p= 0.727$).

Percentage measurements data are presented (Table 3**)**. The median value for teeth that were previously restored with amalgam was 0%; for the fresh-cut dentin group, the median value was 13%. The minimum percentage of microleakage for the amalgam group was 0%, with a 16% maximum value. The minimum microleakage for the fresh-cut dentin was also 0%; the maximum value, however, was 33%. No significant difference was found between the groups ($p=0.675$).

- *Proximal marginal microleakage:*

The results of the microleakage on a 0 to 3 scale are presented in (Table 4.Figure 11) For the teeth that were previously restored with amalgam, eighteen teeth (90%) had a microleakage score of 2 or 3. For the fresh-cut dentin group, thirteen teeth (65%) had a

microleakage score of 2 or 3. The overall distribution of the microleakage score did not differ significantly between the two groups ($p = 0.174$).

Percentage measurements results are presented in (Table 5). The median value for teeth that were previously restored with amalgam was 78% and for the fresh cut dentin was 67%. The minimum percentage of the microleakage for the amalgam group was 39%, with a maximum value of 100%. While the maximum microleakage for the fresh-cut dentin group was also 100%, the minimum value was 0%. No significant difference was found between the groups ($p=0.513$). However, staitically significant difference was found between the occlusal versus the proximal margins ($p <0.001$) (Table 6).

Scanning electron microscope and energy dispersive x-ray spectroscopy:

The results of the SEM and EDX are presented in graphs and elemental analysis charts.

Teeth with amalgam restoration: Several areas were analyzed (amalgam restoration, varnish layer, dentin surface, and the dentinal tubules underneath the restoration). At the amalgam restoration (Figure 12 a.b), mercury, copper, silver, and tin were found as expected. Moreover, the varnish layer revealed chlorine to be associated with the chloroform in the varnish composition (Figure 13) and platinum metal from the coating. No metal was detected in the dentinal tubules below the amalgam restoration that was thermocycled for a 10000 or 15000 thermal cycle. (Figure 14,15).

Teeth that were previously restored with amalgam: No metal elements from the amalgam corrosion products were found in the dentinal tubules and the dentin surface beneath the composite restoration. However, silicon was detected and was found to be associated with dentin adhesive (Figure 16).

Teeth with fresh-cut dentin: Silicon was also detected to be associated with dentin adhesive. However, there were differences were found between the amount of silicon in the hybrid layer and the dentin underneath the restoration. (Figure 17a.b,18)

Discussion

In the present *in vitro* study, we compared the marginal microleakage by silver nitrate uptake at the proximal and occlusal margins. All of the samples were restored by high copper amalgam restoration and then thermocycled for 10000 thermal cycles in attempt to simulate aging of the restoration in the oral environment. Composite resin materials were then used to restore the teeth that previously had amalgam restorations, as well as the fresh-cut dentin, after which they were thermocycled for 5000 thermal cycles that served to simulate the clinical performance. The aim of this study was to evaluate the microleakage of the composite restoration when bonded to a cavity previously restored with amalgam material compared with that of freshly cut dentin. Based on the results of this study, no significant difference was found between the two groups.

Many techniques have been used for assessment of microleakage in dental restorations. In this study, dye penetration test was chosen because dye leakage is the most used method, to represent quantitative microleakage evaluation, which was developed by Douglas and Zakariasen et.al. [84] One of the goals of an ideal restoration is to prevent microleakage because it is an important aspect of the longevity of restorations. Moreover, it represents the passage of bacteria at the tooth restoration interface that may cause recurrent caries or pulpal irritation with subsequent pulpal inflammation. [13]

In this study, we used two measurements to evaluate the dye penetration at the proximal and occlusal margin to confirm whether or not different measuring techniques might affect the results. Hypothesis tests based on the two measurements resulted in the same conclusion. The 0 to 3 scale showed that all of the samples had microleakage with a score of 0 or 1 at the occlusal margin, meaning that none of them had dye penetration beyond the enamel-dentin junction. Moreover, all of the teeth that were previously restored with amalgam restoration had 90% microleakage at the proximal margin with score of 2 or 3 in comparison with the other group, which had 65% microleakage with the same score.

This may be due to the effect of the cavity preparation and application of varnish layer as the smear layer that formed could blocked the dentinal tubules with a smear plug, and even after the amalgam removal and the acid etching some of the dentinal tubules did not re-opened; further studies must confirm this idea. Dentin underneath amalgam restorations can be exposed to a variety of ions and molecules originating from amalgam, oral fluid. Amalgam ally does not adhere to cavity walls, which lead to formation of a marginal micro-gap between the amalgam and cavity wall facilitating transport of fluid, ions, molecules and possibly bacteria and toxins. The difference in coefficient of thermal expansion between amalgam and tooth structure result in intermitting opening and closing of the gap thus creating inward and outward transport of fluid along the amalgam- tooth interface. This way dentin is easily exposed to saliva, and products from bacterial metabolism. [5] All these factors explain the difference between the dentin of teeth that were previously restored with amalgam compare to fresh-cut dentin.

Even though the findings of this study showed no significant difference between the two measurements, the researcher should use both measurement techniques as a means of

eliminating the potential that the measuring technique could affects the results. The percentage measurements of dye penetration are in the form of exact numbers, rather than a range. Meanwhile, the other scale can specify the area of the dye penetration, assuming it only was found in the enamel surface or it extended into the dentin.

The findings of this study were in contrast with Ghavamnasiri's results, which found a difference in microleakage between the teeth that were previously restored with amalgam compared with freshly cut dentin. This difference may be the results of the fact that different varnish solutions were used, as Ghavamnasiri et al. used chloroform-free varnish. Other differences that may have had an impact include the dye tracer and the storage method used. According to Heintze et al. different tracers give different results, especially at the dentin margin.[85] However, comparison of the results from different studies is critical, since there are no generally accepted standards for experimental parameters, that includes type and concentration of the storage solution, time of storage, temperature during storage, type and duration of thermal cycling and/or mechanical cycling, and the scoring criteria. [86]

Different analytical methods have been used for detection of amalgam constitute. However, electron microprobe and electron dispersive spectroscopy (EDS) are commonly used for the elemental analysis. Both techniques are based on the detection of specific X-rays that are emitted by excitation of samples by bombardment with electron beams. In this study Energy Dispersive Spectroscopic method was used which produces more accurate data, EDS detects characteristic X-rays emitted by elements and produces graphs with peaks of specific energies of elements. This technique is suitable for determining the relative composition of solid materials. Limitations of this method are detector resolution and possible overlaps of peaks causing difficulties in interpretation.[5]

Scanning electron microscope images were obtained from the interface between the restoration and the tooth, as well as at the dentin surface just below the restoration. Energy dispersive x-ray analysis was then used to determine whether or not metal penetrated into the dentinal tubule and the dentin surface as a result of the amalgam corrosion. The results of this study agreed with those of Kurosaki and Fusayama et al. as well as Halse et al., who concluded that metal did not penetrate into the non-discolored dentin. They found metal penetration, such as of Zn and Sn, exclusively in the dark discolored dentine. They also demonstrated that Sn and Zn penetrated the softened dentin but never penetrated the normal dentin Moreover, it was concluded by Kurosaki and Fusayama et al. that the black staining was caused by Sn sulfides, as Sn produces black sulfides while Zn sulfides are white. [31,32] Scholtanus's review of the literature confirmed that penetration of metals from amalgam was only observed in discolored and demineralized dentin. [5,16,27-29,31]

The energy dispersive x-ray analysis of the present study revealed the presence of calcium and phosphorus, and these elements are correlated with the dentin composition. [87] EDX analysis showed that no metal elements penetrated from the amalgam restoration into the dentin. The findings of this study are in agreement with those of Wei and Ingram et al., Kurosaki and Fusayama et al., as well as Halse et al. [16,31,32] However, these results conflict with the results of Ghavamnasiri's research, as tin, silver, mercury and copper were found in the dentin adjacent to the composite restoration after the amalgam was replaced. However, high copper amalgam was used, and it has been reported in the literature that no mercury is released by the corrosion process. Corrosion of high copper amalgam in in vitro experiments revealed Ca-Sn-P-Cl complexes and crystalline products containing Sn or at amalgam-tooth interface. No Cu was found as Cu complexes were assumed to be leached out into the liquid

environment. [5,77]

The findings of this study conflict with those of Grossman's study, as several metal elements were found in the dentin and enamel after the amalgam restoration placement. Nevertheless, a conventional amalgam was used, and the samples were stored in 1%NaCl solution for one year, which may have affected the corrosion process or the corrosion products. [87] Moreover, Soremark et al. also found an increase in mercury and silver concentration in the dentin and enamel after the amalgam restoration, as well as a moderate increase in tin and zinc concentration. In this particular study, however, the research did not specify the amalgam type, which has a large effect on the corrosion products. [30]

The EDX analysis of the dentin below the composite restorations also revealed differing amounts of silicon, which related to the application of the adhesive. This finding is in contrast with that of Harnirattisai et al., who used single Bond (3M) and clearfil SE Bond (Kuraray) and did not find elements in the adjacent dentin that were related to the adhesive. [28] Our findings are in agreement with those of Ghavamnasiri's, who reported the presence of large amounts of tin, baruium, and silicon; the concentration of both tin and barium were related to the metal opaque included in the adhesive. [77]

Although there was no statistically significant difference between the two groups and metal did not penetrate the non-discolored dentin, the teeth that were previously restored with amalgam restoration had more microleakage (39%, to 100%) compared with the freshly cut dentin (0% to 100%). These findings may suggest that cavity preparation should be extended slightly beyond the removal of an amalgam restoration. Ghavamnasiri also recommended that approximately 0.5 mm of non-discolored dentin should be removed to improve the

gingival microleakage in order to achieve the same level that is obtained with an initial composite restoration. [77]

Since there were several different steps involved in our study, including storage, thermocycling, manipulation, and dye penetration, among others, the differing results may be the result of variation in any of the aforementioned procedures. [88] Research and data on the microleakage of composite resin restorations after replacing high copper amalgam adjacent to non-discolored dentin are lacking. Harnirattisai et al. concluded that the bond strength of discolored dentin after amalgam removal was less than that of the normal dentin surrounding it. However, microleakage was not tested in this particular study. [28] Ghavamnasiri et al. demonstrated that non-discolored dentin had more microleakage compared to freshly cut dentin, and that the amalgam corrosion products also penetrated the non-discolored dentin. [77]

Study Limitations and Further Research:

This is an in vitro study that has its limitations when compared with clinical trials. Clinical studies accurately replicate the performance of the restorations in the oral cavity and the corrosion process of the amalgam restoration will be. However, the microleakage test cannot be conducted clinically, but future studies might consider placement of the amalgam restoration in teeth that are planned for extraction.

In this study, we used the theromocycling method to simulate the clinical performance of the teeth in the oral cavity. The efficacy of the thermocycling method to simulate the aging has been a subject of controversy among researchers. [89,90] Although, it is the most frequently used method of aging for microleakage evaluation up to date, there is no consensus in the literature about a relevant regimen for aging. [91] Other aging methods

include water storage and thermomecahanical loading. Previous studies demonstrated thermocycling to be superior to water storage, and the difference was found to be statistically significant.[92] Furthermore, theromomecahanical loading has been shown to result in a better performance than thermocycling; the difference between the two is statistically significant.[93] Thermomechanical loading is thus recommended for use in future studies, as it is more analogous to the oral condition.

Due to time and resources limitations, the evaluation of dye penetration was conducted using a two-dimensional view, obtained from a single section through the center of the restoration. This procedure may have yielded an underestimate of the results. Future studies may employ multiple sectioning of the teeth to obtain a three-dimensional view, thereby achieving more accurate results. However, in this study the evaluation of dye penetration was conducted using a stereomicroscope at 20x magnification, which was sufficient to evaluate the dye penetration.

Conclusion

Within the limitations of the study, the following conclusions can be drawn:

- No significant difference was found between the microleakage of non-discolored dentin in teeth that were previously restored with amalgam compared with freshly cut dentin.
- Marginal microleakage in the proximal surface was higher than that in the occlusal surface.

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Appendix A: Tables

Table 1 Materials used in the study

Product, manufacturer	*Description*	*Composition*
Disperalloy,Dentsply	Dispersed phase admix amalgam, contains lathe cut particles and silver/copper eutectic spheres.	Silver 265 mg Tin 69 mg Copper 46 mg Zinc 4 mg Mercurey 400 mg
Copalite, Cooley & Cooley	Dental cavity varnish.	Diethyl ether Chloroform Phenolic resin
Ultra etch, Ultradent	35% phosphoric acid solution	Phosphoric acid, cobalt aluminate blue spinel, cobalt zinc aluminate blue spinel
ExciTE F DSC, Ivoclar vivadent	Dual-curing dental adhesive with fluoride release	Bis-GMA 25-50% Ethanol 10-25% 2-hydroxyethyl methacrylate 10-25% phosphoric acid acrylate10-25% potassium fluride <2.5%
Filtek Supreme Ultra Universal, 3M ESPE	Dental composite resin restoration	Bis-GMA, UDMA, TEGDMA, and bis-EMA(6) resins. The filler is a combination of silica filler and zirconia filler.

Table 2 Counts and percentages for 0 to 3 Microleakage scale at occlusal margin

Tested groups At occlusal margins	*0 N (%)*	*1 N (%)*	*P value*
Previous amalgam	12 (60%)	8 (40%)	0.727
Fresh cut Dentin	10 (50%)	10 (50%)	

Table 3 Descriptive statistics for the % Microleakage scale at the occlusal margin

Tested Groups At occlusal margins	*N*	*Median*	*Interquartile range*	*Minimum*	*Maximum*	*P value*
Previous amalgam	20	0	8	0	16	0.675
Fresh cut dentin	20	13	7	0	33	

Table 4 Counts and percentages at the occlusal margin

Tested groups At proximal margins	*0 N (%)*	*1 N (%)*	*2 N (%)*	*3 N (%)*	*P value*
Previous amalgam	0 (0%)	2 (10%)	12 (60%)	6 (30%)	0.174
Fresh cut dentin	2 (10%)	5 (25%)	8 (40%)	5 (25%)	

Table 5 Descriptive statistics for the % Microleakage scale at the proximal margin

Tested groups At proximal margins	*N*	*Median*	*Interquartile range*	*Minimum*	*Maximum*	*P value*
Previous amalgam	20	78	38	39	100	0.513
Fresh cut dentin	20	67	52	0	100	

Table 6 % Microleakage scale at the occlusal margin versus the proximal margin

Tested groups Previous amalgam	*N*	*P value*
At occlusal margin	20	< 0.001
At proximal margin	20	

Appendix B: Figures

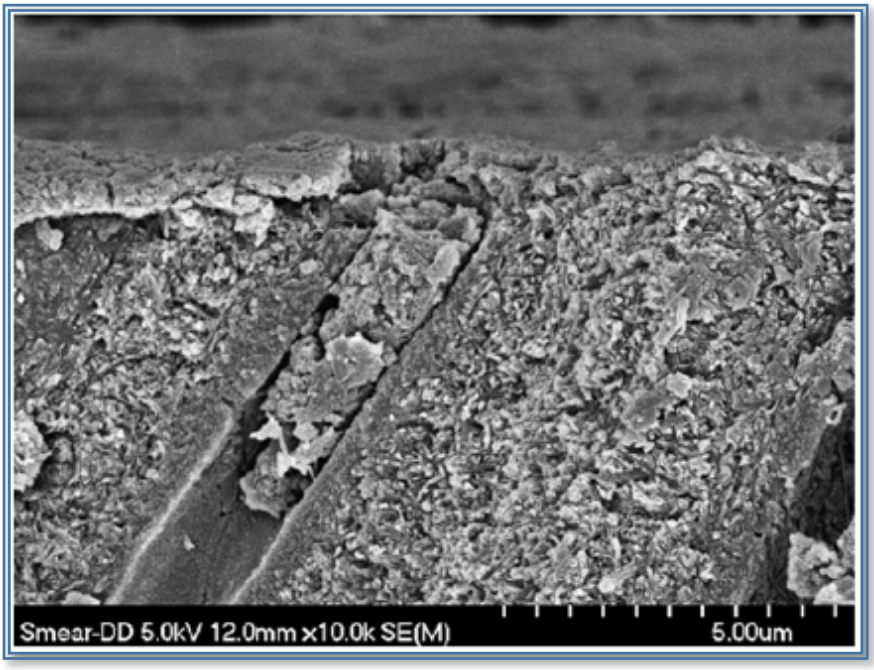

Figure 1 Field emission scanning electron micrograph of dentin smear layer and smear plug. [55]

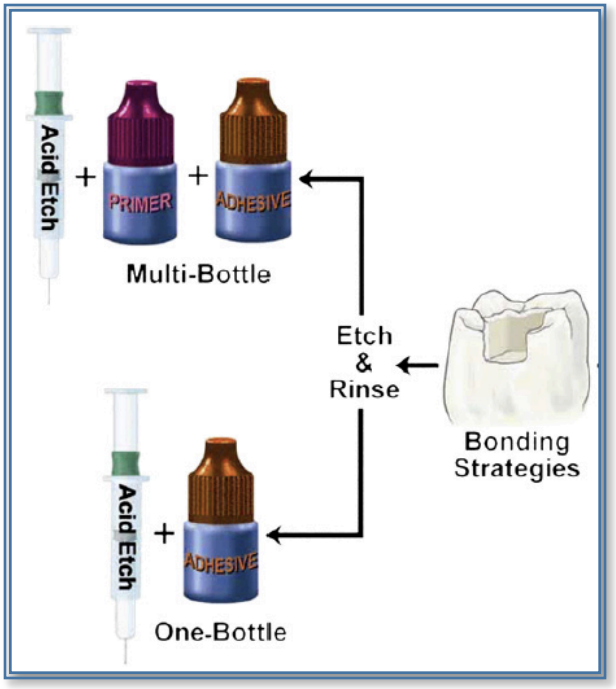

Figure 2 Etch and rinse classification. [55]

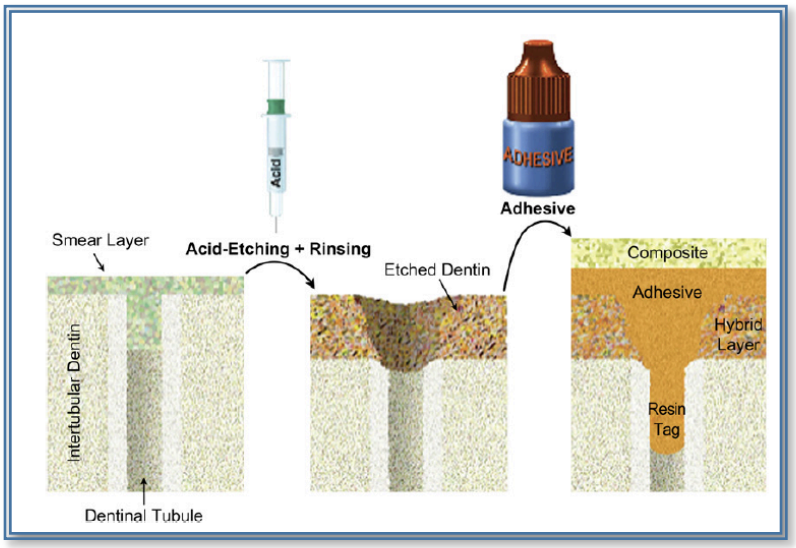

Figure 3 Interaction of one-bottle etch and rinse adhesive with dentin. [55]

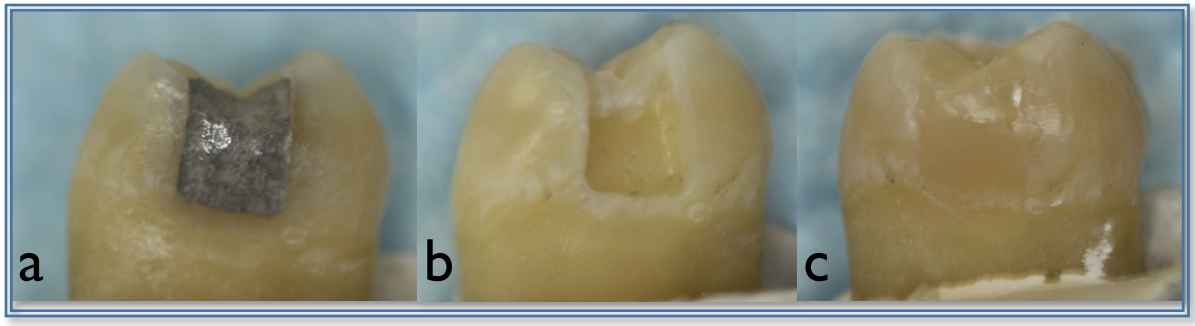

Figure 4 Restoration process a. amalgam restoration placement. b. cavity wall after removal of the amalgam. c. composite resin placement.

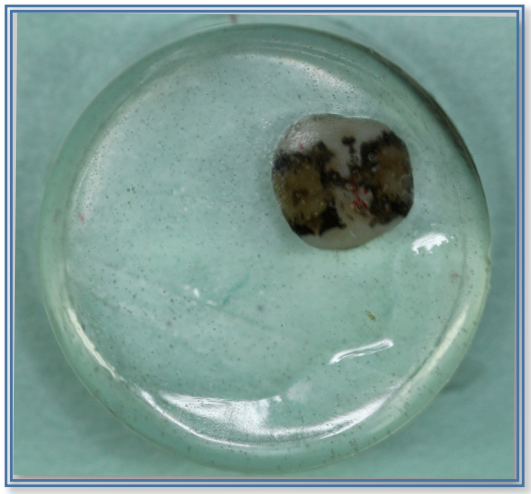

Figure 5 Mounting the sample in epoxy resin.

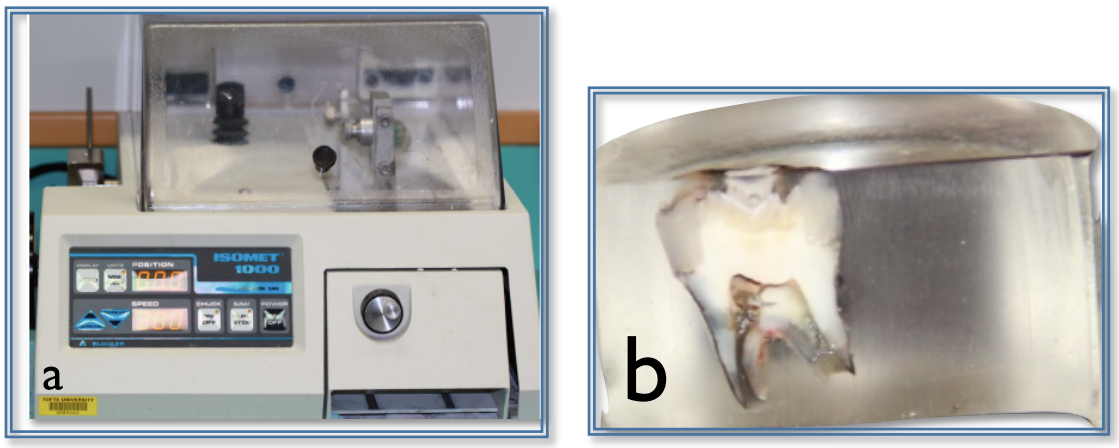

Figure 6 Sectioning the teeth a. ISOMET 1000 sectioning machine. b. sample of sectioned teeth.

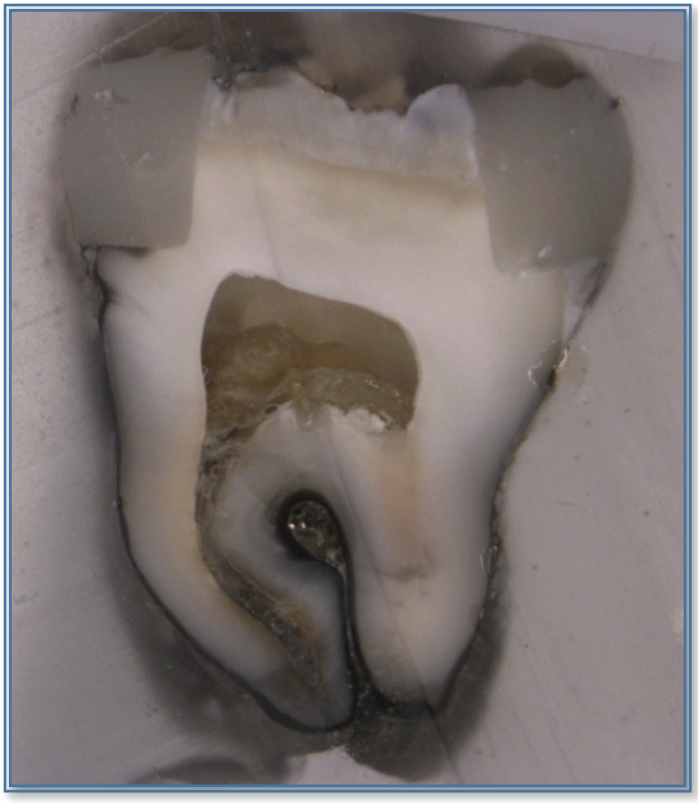

Figure 7 Microleakage sample in stereomicroscope (X8)

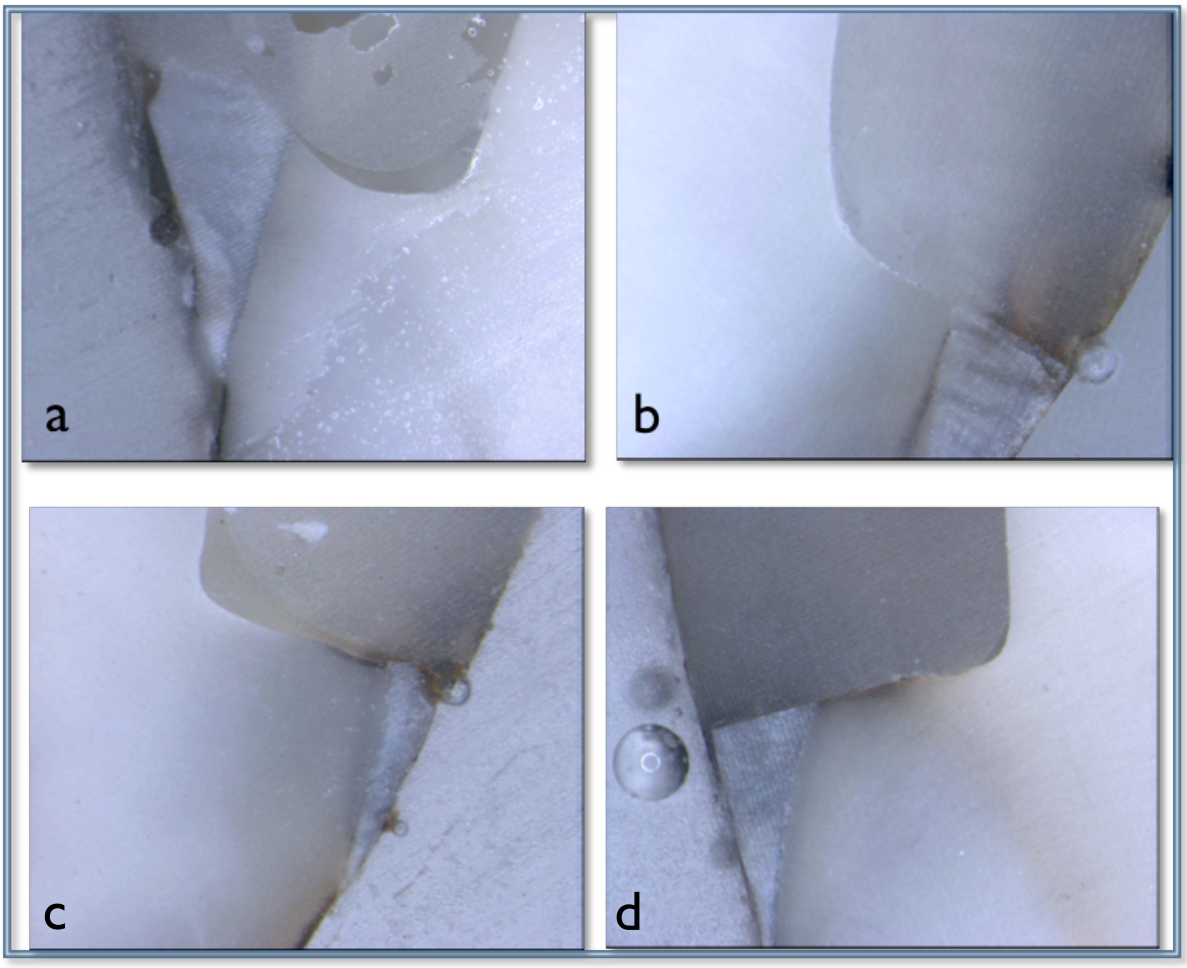

Figure 8 Microleakage at the proximal margin a. score 0 b. score 1 c.score 2 d.score 3

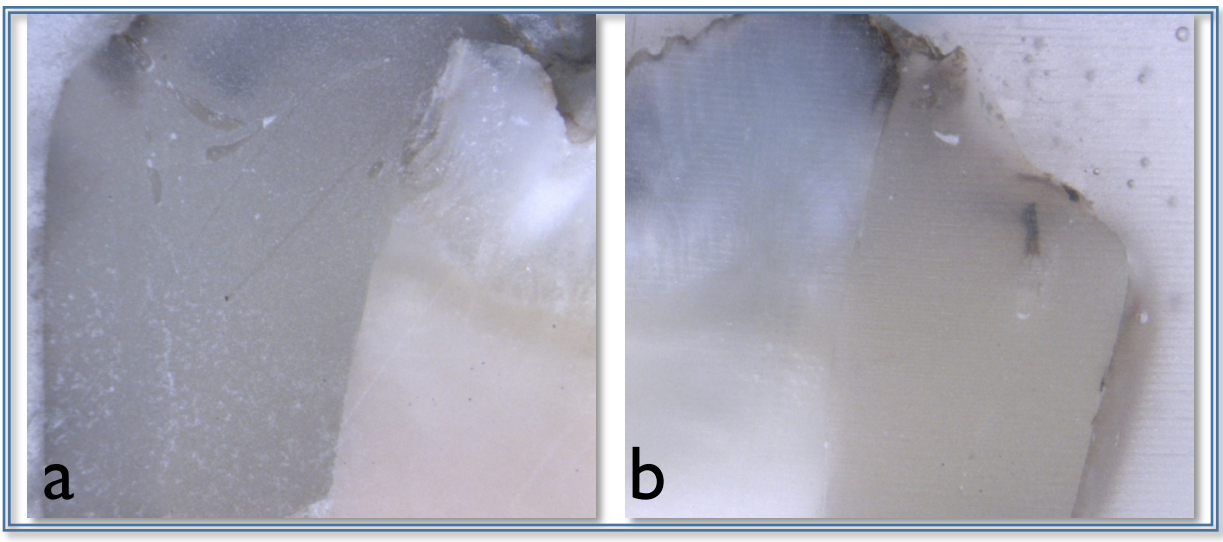

Figure 9 Microleakage at the occlusal margin a. score 0 b. score 1

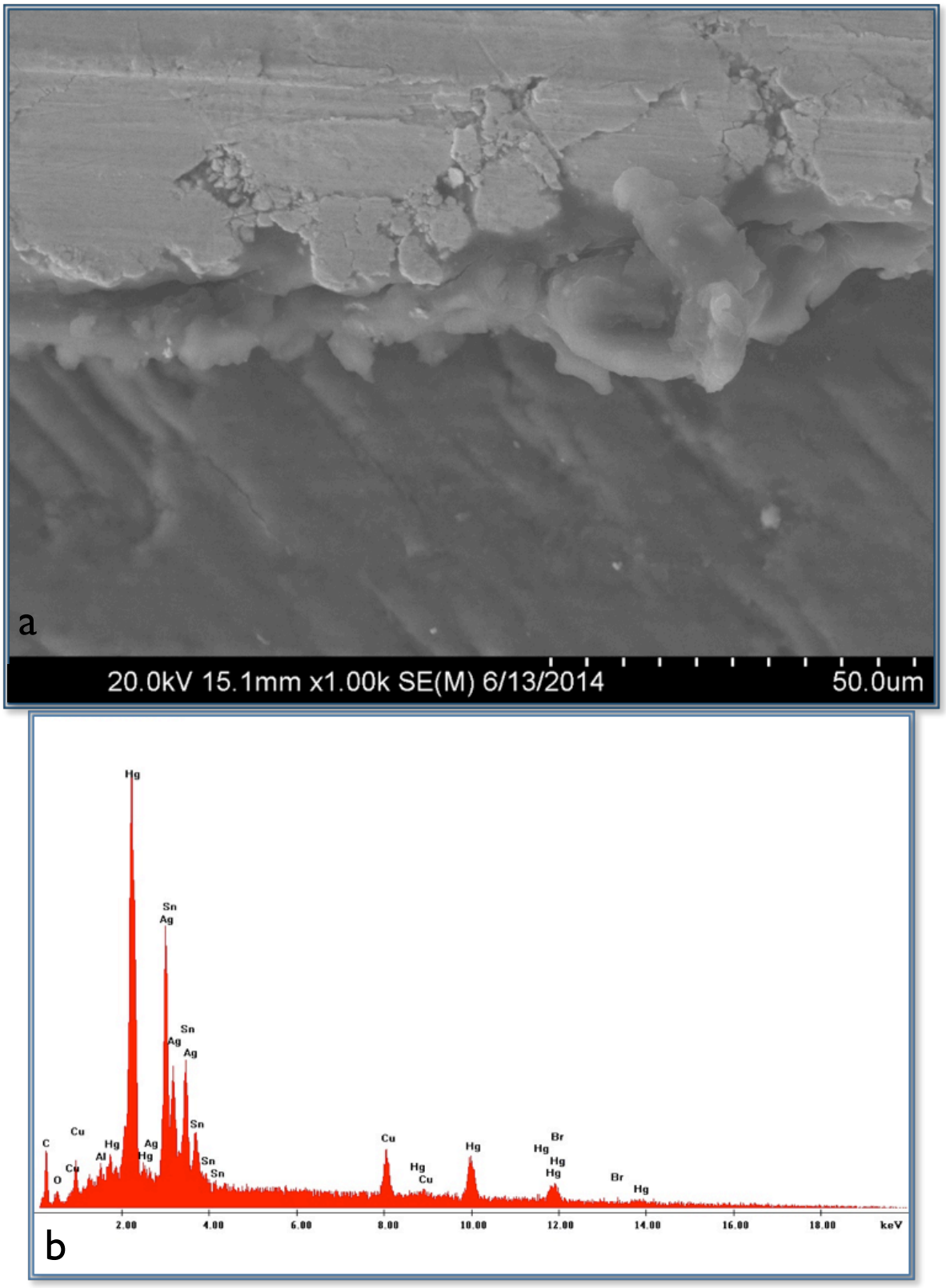

Figure 10 a. SEM of the dentin-amalgam interface showing the varnish layer. b. EDX analysis at amalgam restoration

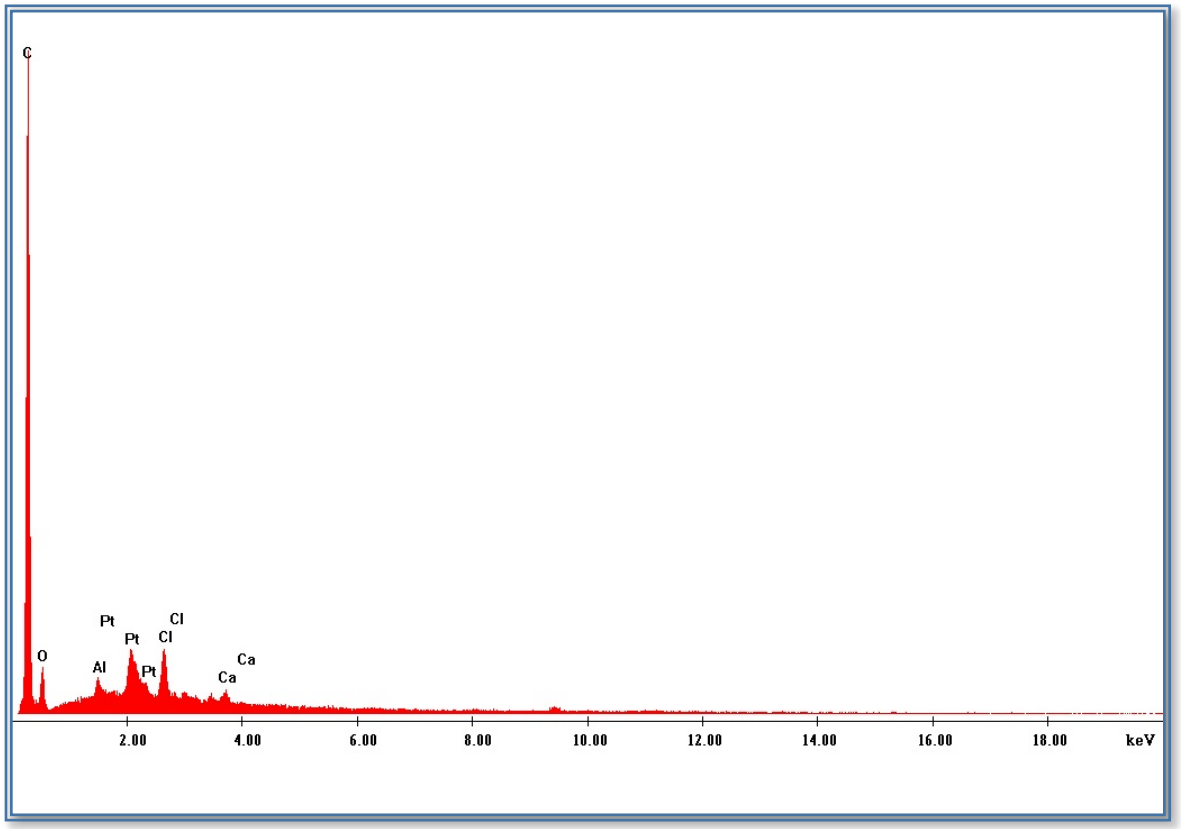

Figure 11 EDX analysis for varnish layer

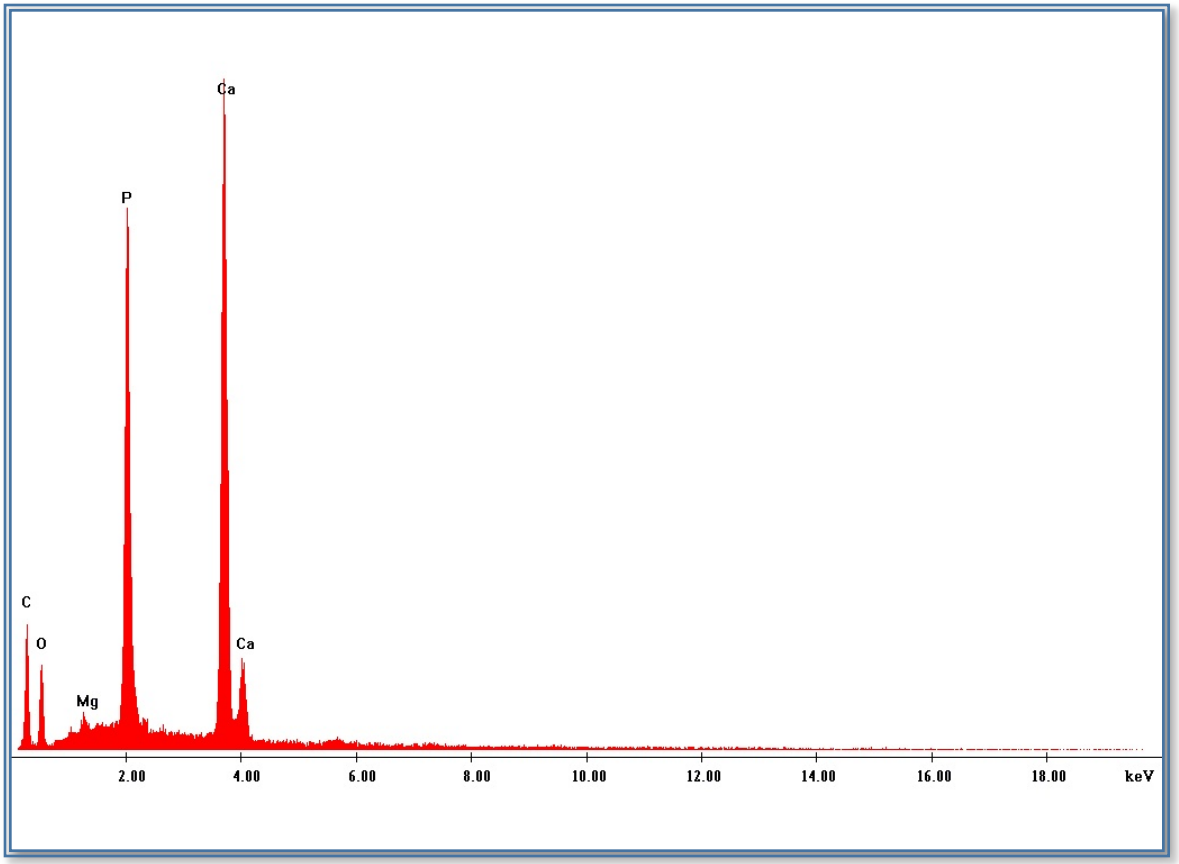

Figure 12 EDX analysis for dentinal tubules below the amalgam restoration (10000 thermal cycle)

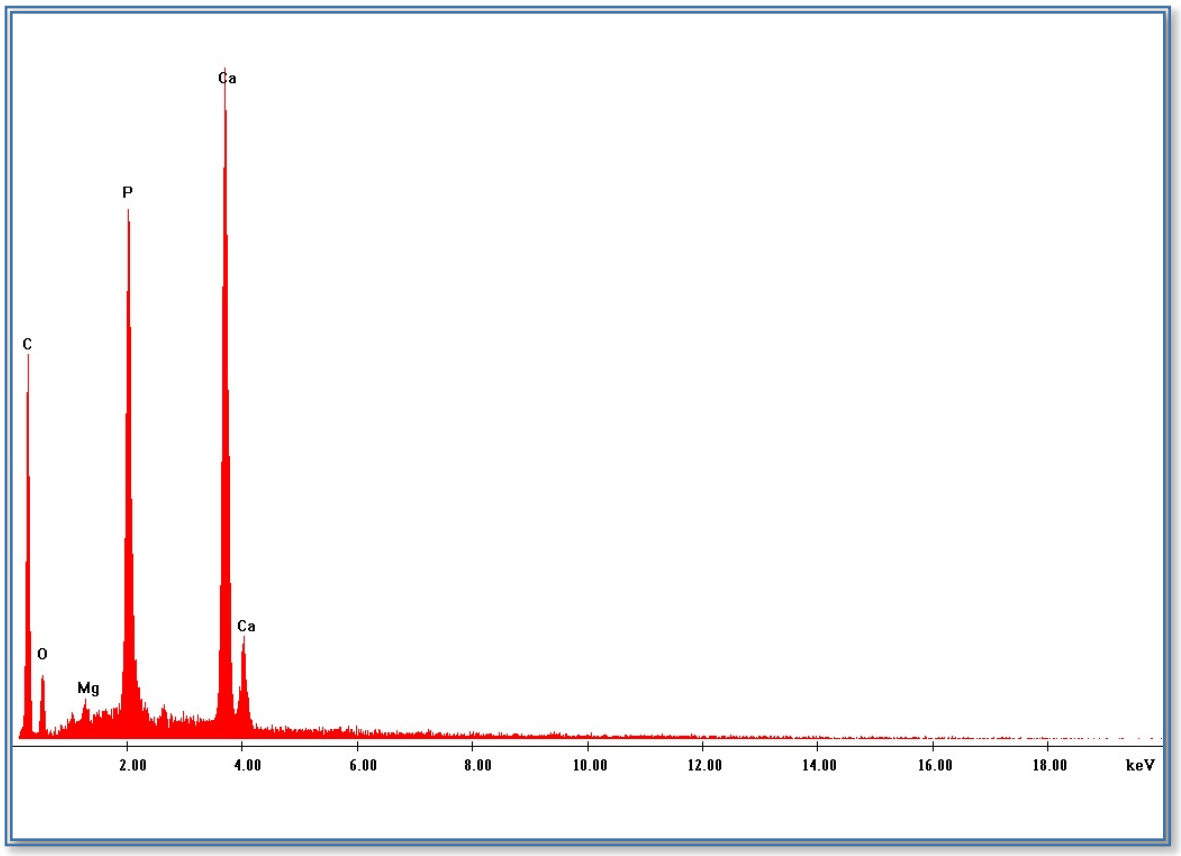

Figure 13 EDX analysis for dentinal tubules below the amalgam restoration (15000 thermal cycle)

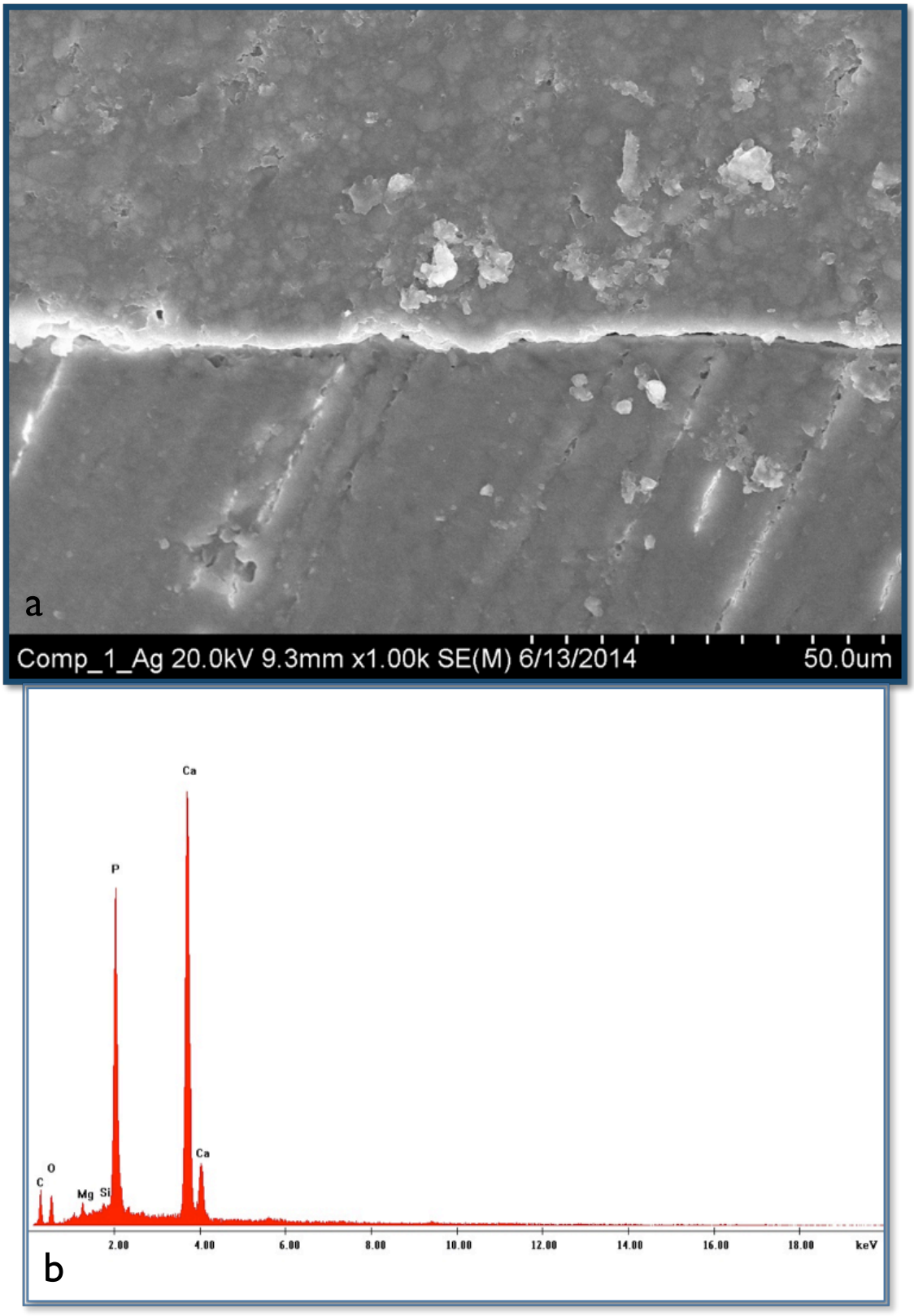

Figure 14 a. SEM of the dentin-composite interface that was previously restored with amalgam b. EDX analysis for dentinal tubules below composite restoration.

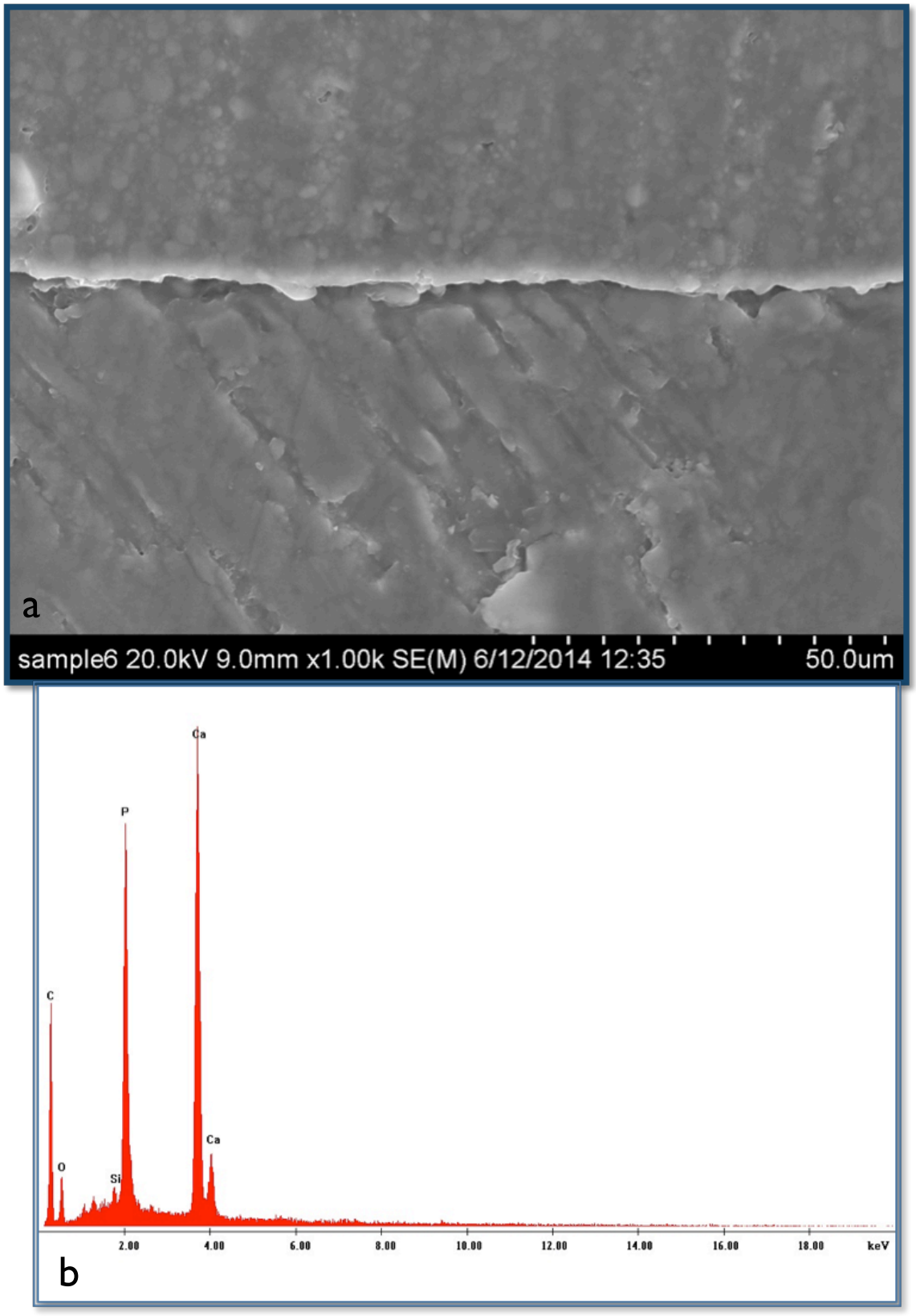

Figure 15 a. SEM of the dentin-composite interface for fresh –cut dentin b. EDX analysis for dentinal tubules below the composite restoration.

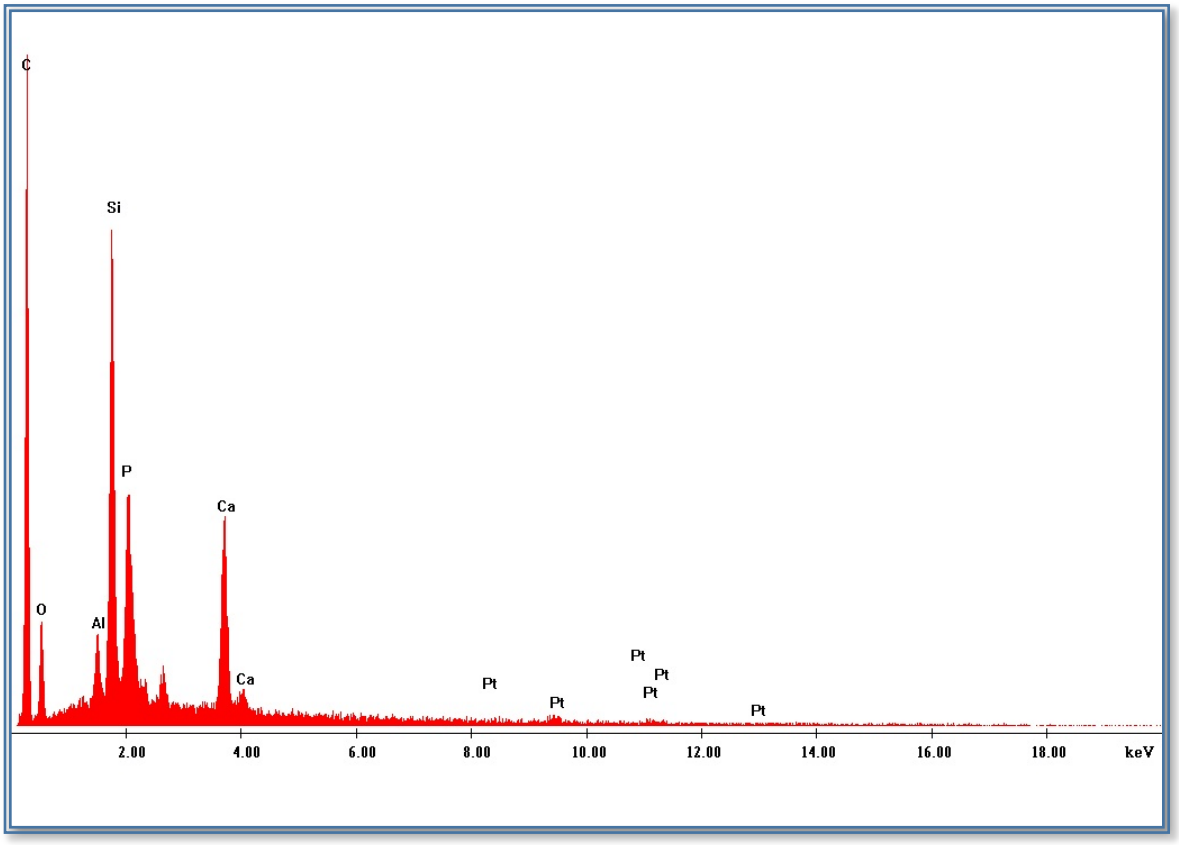

Figure 16 EDX for hybrid layer of fresh cut dentin