QUANTITATIVE COMPARISON OF NANOLEAKAGE AMONG

FIVE RESIN LUTING AGENTS

AFTER AGING

by

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DEDICATION

This thesis is dedicated to my father, my mother, and my lovely husband
for their inspiration, love, and support
throughout my studies.

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INTRODUCTION

Conservative restorative procedures have been performed routinely. The use of adhesive resin composite for the luting of fixed prostheses allows for the design of conservative restorative procedures for ceramic restorations. Moreover, resin luting agents also provide enhanced mechanical and physical properties compared with traditional luting agents during the placement of some ceramic restorations. However, the technique sensitivity of traditional resin luting agents because of multiple application steps could affect the longevity of the properties of the luting agents.[1] Furthermore, dentin adhesion has proven to be less predictable and more difficult to ensure because of histological structure and composition.[2]

Resin luting agents have been divided into two subgroups used to prepare the tooth structure before luting. The first group uses etch-and-rinse adhesive systems. In the second group, self-etching (SE) primers are used to prepare the enamel and dentin. Treatment of the enamel and the dentin involves only the application of the self-etching primers.[3] The self-etching adhesive system partially removes the smear layer and has been anecdotally reported to cause reduced postoperative sensitivity compared with the etch-and-rinse system.[4] Although the SE systems result in less sensitivity for the patient, degradation of resin dentin bonds could occur due to the presence of hydrophilic monomers in self-etching adhesive systems.[5]

In 2002, a new subgroup of resin luting agents, self-adhesive luting agents, was introduced. Self-adhesive luting agents do not require pretreatment of the tooth structure prior to luting. The application procedure is very simple and can be accomplished in a single clinical step[3] to overcome the technique sensitivity of the traditional resin luting agents, because they have monomers with bonding and

demineralizing capacities.[6] Self-etching and self-adhesive luting agents are claimed to be less likely to cause postoperative sensitivity, because the smear layer and smear plugs are not removed; instead, they are incorporated into the bonding layer. In the self-adhesive luting agents, the chemical structure of the resin matrix results from multifunctional monomers with phosphoric acid groups that simultaneously partially demineralize and infiltrate enamel and dentin, which results in micromechanical retention or potentially chemical bonding.[7] The setting reaction is predominantly free radical polymerization that can be initiated by light exposure or through a self-polymerizing mechanism. This reaction results in extensive cross-linking and a high-molecular-weight polymer. Although these self-adhesive luting agents are very user-friendly and less technique sensitive, they show lower immediate bond strength values[8-10] and demonstrate a less stable interface under aging when compared with other adhesive groups.[11,12]

The presence of these materials in an aqueous environment might affect the behavior of the luting agents. Even though the resin luting agents are capable of providing an adequate immediate bond to enamel and dentin, the limited longevity of this bond remains an issue. Potential problems of one-step adhesives have been identified and include water-uptake and subsequent plasticization, water-and enzyme-induced nanoleakage, and the presence of voids due to phase-separation or osmosis.[13] Hydrolytic degradation of resin-dentin interfaces contributes to reduction in bond strengths over time.[14-18] Clinically, adhesive failures and marginal degradation present as retention loss, marginal discoloration, and secondary caries.[19]

However, the mechanisms of adhesive interface degradation of self-etching and self-bonding resin luting agents are still largely not understood.

AIM OF THE STUDY

The aim of this study was to investigate adhesive layer degradation of these materials by using a nanoleakage technique with five different resin luting agents, Panavia F2.0, RelyX Unicem, RelyX Unicem2, Maxcem, and Variolink II. Adhesive interface degradation was evaluated by observing the uptake of silver.

HYPOTHESES

The null hypotheses are as follows:

1.   There is no significant difference in silver uptake within the adhesive interface at the bottom of the hybrid layer among etch-and-rinse, self-etching, and self-adhesive luting agents after aging.

2.   There is no significant difference among the luting agents in silver uptake within the adhesive interface at the bottom of the hybrid layer either before or after aging.

The alternative hypotheses are as follows:

1.   There is a significant difference in silver uptake within the adhesive interface at the bottom of the hybrid layer among etch-and-rinse, self-etching, and self-adhesive luting agents after aging.

2.   There is a significant difference in silver uptake among the luting agents within the adhesive interface at the bottom of the hybrid layer before or after aging.

REVIEW OF LITERATURE

Currently, bonding to dentin is achieved by partially dissolving the dentin surface with etching agents and by resin infiltrating the exposed collagen mesh. The resulting layer, a resin-infiltrated collagen mesh, is commonly referred to as the hybrid layer.[20] Micromechanical retention with resin tags and a hybrid layer are considered the most important mechanisms of resin adhesives on tooth substrates.[21] Resin luting agents have been divided into two subgroups that follow the adhesive system used to prepare the tooth structure before luting: etch-and rinse and self-etch.

The first group utilizes etch-and-rinse adhesive systems (also referred to as total-etch adhesives). The procedure for tooth substrate treatment in this group is to apply an acid agent, often 37-percent phosphoric acid, to the prepared enamel and dentin for 15 seconds, then thoroughly rinse with copious amounts of water. Acid etching removes the smear layer formed on the tooth surface during cavity preparation and also exposes a scaffold of collagen fibrils (collagen-rich layer depleted of hydroxyapatite) and dentinal tubules on the treated dentin surface.[22] The dentin surface has to be moist before application of the bonding agent.[23] The use of priming components containing both hydrophilic and acidic resin monomers improves wetting of the demineralized collagen matrix. Then, the monomers of the resin adhesives infiltrate the collagen network and dentinal tubules of dentin and are polymerized there, forming micromechanical retention with many resin tags and a hybrid layer composed of collagen and resin polymers.

Acid-etching forms a collagen-rich layer of 3 um to 5 um. However, because of the technique sensitivity, the collagen network can shrink by air drying after water rinsing. The collapsed collagen layer can hinder the infiltration of monomers and

result in a portion of the collagen layer that is demineralized and left unfilled with sufficient resin. This layer is degraded with aging and hydrolyzed over time, destroying dentin bonding. Furthermore, the optimal amount of surface wetness necessary for wet bonding varies among marketed total-etch adhesive systems, which are acetone-based, ethanol-based, or water-based.[24] Also, it is impossible to simultaneously achieve uniform wetness on the axial, pulpal, and gingival walls because of differences in hydraulic conductance between superficial and deep dentin. Therefore, it is common to have over-wet regions and over-dry surfaces in the same preparation, which causes non-uniform resin bonding,[25] and thus the technique is a major factor in achieving successful dentin bonding. The wet -bonding technique was initiated to overcome this problem.[26,27] The wet-bonding technique keeps the collagen network swollen with water by blot-drying and helps the monomers to infiltrate the wet collagen network by using a hydrophilic bonding agent.

In the second group, self-etching adhesives use milder acids to etch the smear layer. Self-etch primer was demonstrated to overcome the technique sensitivity of etch-and rinse adhesives.[28-30] Self-etching adhesives are designed to bond to smear layer-covered dentin; a higher concentration of acidic resin monomers are often included, in the presence of water, to render these adhesives acidic enough to etch through thick smear layers and to bond to the underlying intact dentin.[31] Self-etching adhesives can come as one-step or two-step adhesives, depending on whether a self-etch primer and (mostly solvent-free) adhesive resin are separately provided or are combined into a single solution. One-step adhesives can be subdivided into one- and two-component adhesives. The single-component one-step adhesives are considered as the true one-bottle or all-in-one adhesives, because they do not require mixing, instead combining the conditioning, priming, and application of the adhesive resin.

Theoretically, by separating active ingredients, two-component self-etching adhesives possess a longer shelf life, but additional mixing of two components is needed.[32]

Contemporary dentin adhesives all contain hydrophilic resin monomers to improve their affinity for hydrophilic substrates such as dentin.[33] There are two types of hydrophilic monomers: neutral and acidic (ionic). The monomer 2-hydroxyethyl methacrylate (HEMA) is an example of a water-soluble, neutral hydrophilic monomer with a hydroxyl (-OH) functional group. Acidic monomer used in dental adhesives may be further divided into three categories: those with carboxylic acid (-COOH) functional groups (e.g., 4-methacryloxyethyl trimellitic acid anhydride [4-META]), those with phosphoric (-O-P-(OH)(OR)) functional groups (e.g., dipentaerythritol-pentaacrylate phosphate ester [PENTA]), and those with sulphonic ($-SO_3H$) functional groups (e.g., 2-acryloamido-2-methylpropane sulfonic acid [AMPS]).[34]

Self-etching adhesives are composed of an acidic monomer and water to etch, to prime, and to permeate the smear layer, and an organic solvent to assist in monomer penetration.[22] The self-etch primer does the etching and priming simultaneously, theoretically ensuring complete penetration of the adhesive.[13] The self-etch primer does not leave the collagen layer demineralized because of the simultaneous presence of acidic and resin monomers in the collagen layer.[22] In addition to micromechanical interlocking through hybridization, the mild self-etching adhesives will demineralize dentin only partially, leaving some hydroxyapatite crystals around the collagen in the submicron hybrid layer.[10] The residual hydroxyapatite may serve as a template for additional chemical and mechanical interaction with the functional monomer of the adhesive system. This template is important for the long-term stability of the bond.[10,35,36] However, self-etching

adhesives usually have bond strengths comparable to those obtained with etch-and-rinse systems.[10,37,38]

In an etch-and-rinse system, water is an important component for demineralization of dental hard tissue and is one of the factors of the technique sensitivity associated with bonding to a dehydrated collagen matrix.[39] Self-etching systems, on the other hand, eliminate this technique sensitivity and reportedly reduce postoperative sensitivity compared with the etch-and-rinse system by partially removing the smear layer without the need to use water for demineralization.[4,40] This reduction in technique sensitivity and postoperative sensitivity could be attributed to the components of SE systems being less aggressive than the phosphoric acid used in etch-and-rinse systems. Thus, a more superficial interaction with dentin occurs, leaving tubules largely obstructed with smear.[32] The degradation of resin dentin bonds may be expected due to the presence of higher amounts of hydrophilic monomers in self-etching adhesive systems.[5]

One of the major causes of the bond degradation in bond dentin is the presence of endogenous enzymes, mainly matrix metalloproteinase (MMPs). MMPs released and activated by adhesive procedures may provide dentin with low collagenolytic activity. The collagen fibrils can be hydrolyzed by these enzymes and thus compromise the long-term bonding effectiveness. Etch-and-rinse adhesives treat dentin aggressively and completely demineralize the surface up to a depth of 5 um. Mild self-etching adhesives demineralize dentin only partially and to a depth of less than 1 um, potentially releasing a smaller amount of enzymes and simultaneously exposing less collagen vulnerable to hydrolysis[41] For two-step etch-and-rinse adhesives, it has been shown that an MMP-inhibitor, chlorhexidine and galardine, can

be applied to dentin as an additional primer to enhance bond durability to some extent.[42-44]

ONE-STEP ADHESIVES

In 2001 the latest generation of the most simple-to-use one-step adhesives was introduced. Inoue et al. defined the one-bottle adhesive systems as having the three major steps found in the conventional adhesives, namely etching, priming, and bonding, combined into a single material that will prime and bond simultaneously.[45] The shortcomings of one-step materials have been documented by several researchers. Generally, the immediate bond strength is found to be reduced in comparison with the values found for multi-step adhesives. In 2009 Van Landuyt et al.[9] examined nine one-step self-etching adhesives with the following controls: one two-step self-etching adhesive, and one three-step etch-and-rinse adhesive. He found that for microtensile bond strength to both enamel and dentin, the control adhesives tended to perform better than one-step adhesives. However, no statistical difference could be found between the control adhesives and some of the one-step adhesives.

In addition, any kind of aging results in a decrease in bonding effectiveness of the one-step adhesives.[32] Moreover, numerous studies have demonstrated an increased interfacial nanoleakage.[40,46]

One-step self-etch adhesives have repeatedly been reported to exhibit high permeability resulting in water flow through the adhesive. This permeability can lead to a wide variety of seemingly unrelated problems, including incompatibility of chemically or dual-cured composites with simplified adhesives and expedited degradation of resin-dentin bonds.[25] The hydrophilic nature of the adhesive and the lack of a cured hydrophobic layer lining the adhesive have been listed as important reasons for such problems.

In 2003 Tay and Pashley[25] reported that one-step self-etch adhesives that were HEMA-rich showed enhanced water sorption from the host dentin, especially when the overlying composite is not immediately cured to block these osmotic effects.[47] In 2007 Van Landuyt et al. observed the presence of interfacial droplets in contemporary one-step self-etch adhesives. Two HEMA-rich adhesives and one HEMA-free adhesive were applied to enamel and dentin, with an overlying composite either immediately cured or cured after 20 minutes. They found that all one-step adhesives exhibited droplets at the adhesive resin/composite interface, and their number increased significantly when the composite cure was delayed. The droplets found in the HEMA-rich adhesives caused a significant drop in bond strength after delayed curing and resulted from water absorption from dentin through osmosis. On the other hand, in HEMA-free adhesives, droplets were located throughout the adhesive layer and were stable in number over time and were ascribed to phase separation.[47]

In 2002 Tay et al.[11,46] introduced the term "water tree" to describe the permeability of adhesives. Water-treeing is the reticular mode seen in the nanoleakage pattern – in particular, the silver deposits that were oriented perpendicular to the surface of the hybrid layer. The well-known water-treeing phenomenon in the dielectric insulation cable industry is responsible for water-induced deterioration of polymer insulation of electrical cables after aging.[11,46,48] Water trees in polyethylene-coated cables are submicroscopic, self-propagating, water-filled tracks formed electrochemically by the oxidation of the hydrophobic polymer into more hydrophilic moieties, resulting in the condensation of moisture from the hydrophobic polymer into the hydrophilic, electro-oxidized regions.[11,49] The increase in water conductivity results in self-propagation along these tracks and the growth of a microscopic tree-

like pattern off water channels. In dentin bonding, an electrochemical process is not required for water tree formation, because both hydrophilic resin monomers and water are present in a single-step, self-etch adhesive. Resin matrices with hydrophilic monomers have non-uniform chain mobility and are more permeable to water movement. This explains why most single step self-etch systems are permeable to fluid movement, even when resin-dentin interfaces are optimally sealed.[50] Water sorption by hydrophilic resin monomers within both the hybrid layer and the adhesive layer has been thought to contribute to the degradation of resin-dentin bond strengths over time.[51] This phenomenon is aggravated by the incorporation of increased concentrations of hydrophilic resin components into contemporary SE adhesives[52] because hydrophilicity and hydrolytic stability of resin monomer are generally antagonistic properties. Water-rich domains, as represented by the reticular mode of nanoleakage expression, manifest as self-propagating water trees along the adhesive-dentin junction and may result in a rapid deterioration of the mechanical properties of the adhesive along this region. In turn, this deterioration results in adhesive failure along the surface of the hybrid layer.[46]

Senares et al. demonstrated that bond strengths of one-bottle adhesives were inversely proportional to the acidity of the adhesives.[53] In 2010 Van Landuyt et al. also revealed that the bond strength of the one-step SE adhesives to dentin dropped significantly after 6 months of water storage; however, this difference was significant only for one of the two-step self-etch adhesives in the study. The difference was not significant in etch-and-rinse adhesives. Failure analysis by light microscopy and SEM revealed that the self-etch adhesive often failed just under the hybrid layer, whereas etch-and-rinse adhesive was observed to fail predominantly within the hybrid layer (in either the upper or lower part of the thick hybrid layer).[19]

STABILITY OF HYBRID LAYER

Dentin bonding is created by impregnation of the dentin substrate by blends of resin monomers. The stability of the bonded interface relies on the creation of a compact and homogenous hybrid layer. For the etch-and-rinse adhesive, after demineralizing the substrate with etching, bonding monomers impregnate the porous etched substrate.[21,54] Stable bonds can be achieved if the etched substrate is fully infiltrated by the adhesive to avoid different degrees of incomplete impregnation.[55,56] On the other hand, SE adhesives use acidic adhesive co-monomers that simultaneously demineralize and infiltrate dentin. Therefore, adhesive stability is related to the effective coupling of the co-monomers with the infiltrated substrate. In 2004 Yoshida et al. found that some two-step SE adhesives (with mild acidity, approximately pH2) may establish chemical bonds between specific carboxyl or phosphate groups of functional monomers and residual hydroxyapatite crystals presenting on the dentin collagen scaffold.[36] This additional interaction after infiltration of adhesive monomers into the decalcified substrate is claimed to enhance bond stability over time.[41]

The mechanism of adhesive interface degradation is still not entirely understood. Adhesive interface degradation can be categorized into two major phases: the hydrolytic degradation of the bonding resin within the hybrid layer and hydrolytic degradation of the unprotected collagen fibrils.[51,57-59]

DEGRADATION OF THE HYBRID LAYER

Clinical longevity of the hybrid layer involves both physical and chemical factors. Physical factors such as occlusal chewing forces, and the repetitive expansion and contraction stresses due to temperature changes within the oral cavity affect the

interface stability.[51,57-59] Acidic chemical agents in dentinal fluid, saliva, food and beverages, and bacterial products also affect the various pattern of degradation of unprotected collagen fibrils,[25,51,58,60] elution of resin monomer (probably from sub-optimal polymerization)[33,61] and degradation of resin components.[25,41,51] Water has been claimed to be one of the major causes of collagen degradation.

In 2003 Hashimoto et al. described two degradation patterns within the hybrid layer of a three-step etch-and-rinse adhesive system (Scotchbond Multi Purpose, 3M/ESPE, St. Paul, MN).[60] After the restorations were stored in the water for one year, the study found disorganization of collagen fibrils and hydrolysis of resin from interfibrillar space within the hybrid layer that caused weakening of the resin-dentin bond.

HYDROLYSIS OF RESIN FROM INTERFIBRILLAR
SPACES WITHIN THE HYBRID LAYER

Hydrolysis is a chemical process that breaks covalent bonds within the polymers by the addition of water to ester bonds, resulting in loss of resin mass through elution[25,62] and contributing to the reduction in bond strengths of dentin adhesives over time.[14-16,18,25] Given that hydrolytic degradation occurs only in the presence of water, adhesive hydrophilicity, water sorption, and subsequent hydrolytic degradation are generally correlated.[15,52,63-65] Dentin bonding systems that utilize separate nonsolvated hydrophobic bonding agents showed higher extents of polymerization and were correlated with less permeability to water.[61] Irrespective of the etch-and-rinse or the self-etch strategy, by combining hydrophilic and ionic resin monomer into the bonding material, such as in simplified adhesives, the bonded interface lacks a nonsolvated hydrophobic resin coating. Therefore, the hybrid layers

behave as semi-permeable membranes permitting water movements throughout the bonded interface, even after the adhesive is polymerized.[66]

In 2005 Cadenaro et al. revealed that regardless of bonding system and the number of steps required for its application, all systems exhibited variable degrees of incomplete polymerization that were correlated with their permeability to fluid movement. In simplified adhesives, either two-step etch-and-rinse or one-step self-etch presented more extensive incomplete polymerizations and adhesive permeability, due to the presence of more hydrophilic monomers.[61] Dentin bonding systems that utilize the separate nonsolvated hydrophobic bonding agents showed higher amounts of polymerization and were correlated with less permeability of water.[61]

DEGRADATION OF COLLAGEN FIBRILS

Several studies showed morphological evidences of resin elution and hydrolytic degradation of collagen matrices after long-term storage of dental adhesives. It has been proposed that the deterioration of collagen fibrils within the hybrid layer, detectable in both *in vitro* and *in vivo* tests, will result in many exposed collagen fibrils within the hybrid layer.[51,58-60] The integrity of the bonding interface depends on the creation of a compact and homogenous hybrid layer. In the etch-and-rinse adhesive system, the stable bonds can be achieved if the etched substrate is fully infiltrated by the adhesive.[55,56] A decreasing gradient of resin monomer diffusion within the demineralized dentin results in incompletely infiltrated zones along the bottom of the hybrid layer that contain denuded collagen fibrils in the demineralized dentin. One of the reasons is the insolubility of BisGMA in water-saturated dentin. By substituting ethanol for water, BisGMA/TEGDMA mixtures have been shown to infiltrate dentin and to produce high bond strengths.[67] Therefore, ethanol-wet bonding permits the use of hydrophobic resins that absorb little water for dentin bonding,

which probably results in more durable bonds. This has not yet been verified. The self-etch adhesive approach uses acidic adhesive co-monomers that simultaneously demineralize and infiltrate dentin, and adhesive stability is related to the effective coupling of the co-monomer with the infiltrated substrate.

NANOLEAKAGE

Several studies have determined that the penetration of bacterial products, various acids and bases, and water at the interfaces between the tooth structure walls and restoration affects the longevity of dental restorations.[60,68,69]

In 1994 Sano et al. found the leakage pathway through a porous zone in the hybrid layer-adhesive interface without the presence of a gap.[55] Silver nitrate has been used as a tracer of this pathway in many studies.[70-72] Sano's study in 1994 used scanning electron microscopy (SEM) to examine the samples and found that silver had penetrated into the submicron-sized space underneath the bonded dentin interface. The result revealed the submicron leakage pathway without gap formation, but it was not microleakage by definition. No specific term existed to describe this phenomenon at that time.

In 1995 Sano defined the nanoleakage phenomenon for the first time.[73,74] He described nanoleakage as "the diffusion of small ions or nanometer-sized molecules within either the hybrid layer in the absence of gap formation, partially or fully demineralized dentin, or the adhesive resin."[73] Silver nitrate was used as a tracer to disclose the open nanometer-sized pathways.[68,75] Nanoleakage cannot be seen with the naked eye or even with X10 to X20 magnification and requires the use of electron microscopy. Unlike microleakage that occurs through the gaps between composites and hybrid layers, nanoleakage occurs in the absence of interfacial gaps.[40] In spite of significant improvements gained in bonding, sealing, biocompatibility, and esthetics,

bonding to dentin remains challenging due to the dynamic structural characteristics of this hard tissue.[41,76] Other researchers have supported the idea that nanoleakage is an important factor contributing to degradation of the bond to dental tissue.[73,77]

It was first thought that nanoleakage would not occur in the dentin layer with self-etch adhesives because the etching and priming steps coincide. However, several researchers have demonstrated nanoleakage under the hybrid layer of self-etching adhesives.[13,78,79] Several studies regarding nanoleakage speculated that this was a manifestation of incomplete resin infiltration into the demineralized collagen network[13,79] or continued etching.[78] In addition, some authors have illustrated that polymerization can be inhibited by water in dentin, resulting in unpolymerized acidic monomers that continue to etch dentin.[18] This results in a weak area of exposed collagen under the hybrid layer. Unprotected collagen fibrils may constitute preferential pathways for degradation by oral and bacterial enzymes.[74,80] Yet another reasonable explanation could be that mild self-etch adhesives result in incomplete smear layer dissolution and encapsulation.[19]

In 2010 Van Landuyt et al. illustrated that the failures of SE adhesives were associated with filler debonding within the adhesive resin layer due to hydrolysis of the filler-matrix coupling. This debonding could be observed at the outer margins of the section that were in direct contact with the storage medium. The author suggested that water absorption results in hydrolysis of the coupling agent, resulting in filler detachment from the resin matrix. During TEM sectioning, these weakly attached filler particles have been detached from the adhesive resin. Furthermore, SE adhesive failed predominantly under the hybrid layer, and not at the dentin-adhesive interface, in spite of the presence of interfacial droplets and nanoleakage in the adhesive layer. This border was 0.5 um to 1 um thick and could be recognized as dentin or dentin

smear by the presence of hydroxyapatite crystal. The author suggested that the failures just under the hybrid layer may be attributed to insufficient encapsulation of the surface smear.[19]

MATERIALS AND METHODS

Five different resin luting systems, Variolink II (Ivoclar Vivadent, Schaan, Liechtenstein), Panavia F2.0 (Kuraray Medical Inc.), RelyX Unicem (3M ESPE, Seefeld, Germany), RelyX Unicem2 (3M ESPE, Seefeld, Germany), and Maxcem Elite (Kerr, Orange, CA), were evaluated in this study. The chemical compositions of all materials used in the experiment are listed in Table I.

SAMPLE PREPARATION

Twenty-five freshly extracted human molars that were collected and stored in 0.10-percent thymol were used (Indiana University/Clarian IRB NS1004-03). Flat dentin surfaces were created using a thin sectioning machine by removing the occlusal and the root portions of the tooth and leaving 2 mm of mid-coronal dentin. The dentin pieces were stored in artificial saliva at 37°C.

INDIRECT RESIN BLOCK PREPARATION

A template was used to fabricate indirect resin composite blocks of Radica (Dentsply, Ceramco, York, PA), a laboratory indirect resin composite (Figure 2). The base for the template was made of flat acrylic. Four pieces of acrylic with a height of 1.5 mm were glued at the four corners of a flat base. A putty matrix was made by thoroughly mixing the base and the catalyst of ExaFlex putty (GC, Alsip, IL) in a one-to-one ratio and applying it on the flat base. A clear flat acrylic plate was pressed on the surface of the mixed putty until it touched the four corners. After the putty matrix was set, square holes were cut in the matrix with a size of 8 mm x 8 mm x 1.5 mm as molds for fabricating the blocks demonstrated in Figure 1. The Radica resin was heated prior to application. The Radica Syringe Heater, preset to reach a temperature

of 60°C to 64°C, was allowed to warm-up for 30 minutes as shown in Figure 2. Ten

minutes prior to use, the Radica syringes were placed in the heater. An electric waxer

with a nickel-plated tip was used for manipulating the resin in the putty mold. Once

the mold was filled, a clear flat plastic plate was pressed across the surface of the

template until it touched the indicators at the four corners, and the assembly was

placed in an Enterra unit for 5 minutes of light polymerization (Figure 3). After

polymerizing, the resin was allowed to return to room temperature before the

polymerized block from the putty matrix was removed.[81]

LUTING PROCEDURE

      The 25 dentin specimens were randomly divided into five resin luting agent

groups. The dentin surfaces were luted with one of the luting agents by following the

manufacturer's instructions as described below.

      The first group was an etch-and-rinse adhesive system, Variolink II ([V],

Ivoclar Vivadent; Schaan, Lichtenstein). Thirty-seven-percent phosphoric acid was

applied to the prepared dentin for 15 seconds and then thoroughly rinsed with copious

amounts of water. A moist dentin surface was maintained by blotting excess moisture

from the dentin with a cotton pellet. Then, ExciTE F DSC bonding agent was applied

to the dentin and agitated on the prepared surface for 10 seconds.[23] A weak stream of

air was applied to disperse ExciTE F DSC into a thin layer and to remove any excess

adhesive. Then, the base and catalyst of Variolink II were mixed in a 1:1 ratio on the

mixing pad for 10 seconds and applied to the resin blocks. The resin blocks were

placed on the flattened dentin surface with a 1-kg load applied, and the excess luting

agent was removed with microdisposable applicator brushes. The light curing tip was

placed close and perpendicular to the interface between the dentin and the resin block

on each side and on the top surface of the resin blocks. The luting agent was

polymerized with visible light (DEMI LED light curing system, Kerr) and power density output of 1463 mW/cm^2 on each side for 40 seconds.

In the second group, Panavia F2.0 ([P], Kuraray Medical Inc, NY), a self-etching primer, ED PRIMER II, was used to prepare the dentin. Equal amounts of A paste and B paste were dispensed and mixed on a mixing pad for 20 seconds. The mixed luting agent was applied on the resin blocks within 3 minutes after mixing. The resin blocks were placed on the flattened tooth surface; a 1-kg load was applied and the excess luting agent was removed with microdisposable applicator brushes. The light polymerizing tip was placed close and perpendicular to the interface between the dentin and the resin block on each side and on the surface of the resin block. The luting agent was polymerized with visible light (DEMI LED light curing system, Kerr) at a power density output of 1463 mW/cm^2 on each side of the block for 20 seconds.[3]

The third group was RelyX Unicem ([R1], 3M ESPE, Seefeld, Germany), a pre-encapsulated self-adhesive luting agent. This type of material does not require any pretreatment of the tooth structure prior to luting. Each capsule was activated, inserted into an amalgamator, and mixed for 15 seconds at high speed. The capsule was removed from the mixing device and the luting agent dispensed onto the resin block.[7] The resin block was placed on the flattened tooth surface with a 1-kg load applied. The excess luting agent was light polymerized initially for 2 seconds in order to create an initial set. The excess luting agent was removed with a microdisposable applicator brush. Then, the light tip was placed close and perpendicular to the interface between the dentin and the resin block on each side and on the top surface of the resin block. The luting agent was polymerized with visible light (DEMI LED light curing system, Kerr) at a power density output of 1463 mW/cm^2 on each side for 20 seconds.

The fourth group was RelyX Unicem2, ([R2], 3M ESPE, Seefeld, Germany), a self-adhesive luting agent. For R2, the luting agent was dispensed directly from an automixing syringe and applied on the resin block.[7] The resin block was placed on the flattened tooth surface with a 1-kg load applied. The excess luting agent was light polymerized initially for 2 seconds in order to create an initial set. The excess luting agent was removed with microdisposable applicator brushes. Then, the light curing tip was placed close and perpendicular to the interface between the dentin and the resin block on each side and on the top surface of the resin block. The luting agent was polymerized with visible light (DEMI LED light curing system, Kerr) at a power density output of 1463 mW/cm^2 on each side for 20 seconds.

The fifth group was Maxcem Elite ([M], Kerr, Orange, CA) a self-adhesive luting agent. For Maxcem Elite, the luting agent was dispensed directly from an automixing syringe and applied on the resin block.[81] The resin block was placed on the flattened tooth surface; a 1-kg load was applied, and the excess luting agent was removed with microdisposable applicator brushes. Then, the light tip was placed close and perpendicular to the interface between the dentin and the resin block on each side and on the top surface of the resin block. The Maxcem Elite was polymerized with visible light (DEMI LED light curing system, Kerr) at a power density output of 1463 mW/cm^2 on each side for 20 seconds.

All the samples were immersed in artificial saliva at 37°C for 24 hours. Then, each sample was sectioned occluso-gingivally perpendicular to the luting interface from the midbuccal to midlingual surface of the tooth with three cuts, in order to create two 2-mm thick samples for each tooth with an ISOMET 1000 precision saw (Buehler, Lake Bluff, IL). The first half of the tooth was used in the control group, and the other half was used in the experimental group. The control group was

immersed in artificial saliva at 37°C and dye penetration was performed within 48 hours. In the experimental group, all specimens were immersed in artificial saliva at 37°C for 10 days and then thermocycled for 2500 cycles between 6°C and 48°C, with a dwell time of 30 seconds and a transfer time of 10 seconds. All surfaces of the specimens were coated with nail varnish (Beauty 21 Cosmetics, CA) to within 1 mm of the adhesive interface.

SILVER NITRATE EXAMINATION
DYE PENETRATION

After the appropriate storage period, all specimens were blot dried and immediately immersed in a 50-percent ammoniacal silver nitrate solution for 24 hours,[82] rinsed with distilled water for 1 minute, and placed in a photo-developing solution for 8 hours under a fluorescent light, to facilitate reduction of the silver ions into metallic silver particles. All specimens were cleaned in distilled water for 1 minute, air dried, mounted on aluminum stubs, and polished with 0.3 um of Gamma Micropolish II, deagglomerated alumina, (Buehler, IL) for 10 seconds and then with 0.05 um of polish for another 10 seconds. All specimens were rinsed with copious amounts of water for 60 seconds, placed in a desiccator for 24 hours,[83] and coated with gold prior to SEM examination.

SCANNING ELECTRON MICROSCOPE (SEM) AND
ENERGY DISPERSIVE SPECTROSCOPY (EDS) EXAMINATION

The SEM was used for observation of silver penetration within the specimens with secondary phase images. EDS is an analytical technique used for the elemental analysis or chemical characterization of the samples. The silver weight percent was analyzed with an EDS detector and software in a JSM-6310LV Scanning Electron Microscope (SEM) (JEOL Ltd, Tokyo, Japan). EDS can produce quantitative and

qualitative analysis of the distribution of various elements and is considered to be a sensitive and accurate chemical component detection method.[84] Three scan lines were selected; the first and last lines were 500 μm away from the dentinoenamel junction. The midpoint between the first and last lines was used as the middle line scan as demonstrated in Figure 2. Each line scan was comprised of 10 to 15 spot analyses dependent on the thickness of adhesive with a distance of 1 μm. The bottom of the fifth spot on each line was placed at the bottom of the hybrid layer as shown in Figure 3. There were three elements, Ca, Si and Ag, which were selected for elemental analysis. Spot analysis showed individual distribution of each element and possibly clarified the borders in composite, adhesive, and dentin layers. Silica represents the composite resin (considering that adhesives are less filled than composite). Calcium represents the element of dentin. At the point where the silica value drops close to zero and where the calcium element increases presented the area close to dentin. Silver element was used as a tracer to disclose the open nanometer-sized pathways. SEM secondary phase images were used for observation of silver penetration in the specimens at X500 to X1000 magnifications for each scan line.

STATISTICAL METHODS

Summary statistics (n, mean, standard deviation, minimum and maximum) were computed for the silver weight percent before and after aging. This was completed for each of the five resin luting systems and for each of the three lines and spot 3 through spot 6. Mixed-model analysis of variance (ANOVA) was performed to examine the effects of aging, resin luting system, and spot location on silver weight percent. The ANOVA included fixed effects for the aging, the resin luting system, the line and the spot, and the interactions among the aging, the resin luting system, and the spot location factors. The ANOVA also included random effects to correlate the

measurements taken at the three locations within each section and to correlate the two

sections per specimen. A 5-percent significance level was used for all comparisons.

RESULTS

SEM AND EDS ANALYSIS

The silver contents between luting agents are significantly different ($p < 0.0001$). Aging did not create a significant difference in silver contents ($p = 0.1019$). The interaction between aging and luting agents made a marginal difference in silver contents ($p = 0.0798$). The spot location among the third, fourth, fifth, and sixth spots made a significant difference in silver content ($p < 0.0001$). The interaction between aging and spot location, between luting agents and spot locations, and between aging, luting agents, and spot locations created significant differences in silver content ($p = 0.0004$; $p < 0.0001$, and $p = 0.0068$) presented in Table III.

After aging, Variolink II luting agent showed significant higher values in silver content compared with other types of looting agents, with Maxem Elite ($p = 0.00018$) and with Panavia F2.0, RelyX Unicem I and II ($p < 0.0001$) presented in Table IV. Line 1 showed significantly lower values in silver content compared with Line 2 ($p = 0.0292$). Line 3 showed marginally significant lower values for silver content than line 2 ($p = 0.0679$) presented in Table IV.

The comparison in each luting agent type is reported in Table V. At the fifth spot location, the bottom of the hybrid layer, there is no significant difference in silver uptake within the adhesive interface between luting agents ($p > 0.05$) presented in Figure 27. Moreover, there is no significant difference in silver uptake within the adhesive interface between specimens before and after thermocycling (aging) ($p > 0.05$), presented in Figure 28.

From EDS analysis, all resin luting agents exhibited nanoleakage both in the control and aging groups. The leakage patterns and failure analysis were revealed by

SEM secondary imagings of representative resin/dentin interfaces presented in Figure 16 to Figure 26. From the SEM evaluation, in the thermocycling (aging) group, there was relatively more separation in the adhesive interface observed compared with the control group. Variolink II presented a thicker adhesive resin layer and a higher amount of resin tags compared with the other adhesives.

For Variolink II and Panavia F2.0 control groups, the bonding interfaces were found intact in all samples presented in Figure 16, Figure 17, and Figure 19. However, after aging, in the Variolink II group, partial separation in the adhesive interface was found in two samples out of five samples presented in Figure 18, while in the Panavia F2.0 group, the adhesive interfaces were still intact in all five samples presented in Figure 20. In the one-step self-adhesive control group, the bonding interfaces of RelyX Unicem I, partial separation in adhesive interfaces was found in only one sample out of five samples presented in Figure 21, while in RelyX Unicem II, the partial separation was found in four out of five samples presented in Figure 23. After aging, in RelyX Unicem I group, partial separation in adhesive interfaces was found in two out of five samples presented in Figure 22, while in RelyX Unicem II, the partial separation was still found in four out of five samples presented in Figure 24. For Maxcem Elite group, both in control and aging groups, the partial separation was found in all samples presented in Figure 25 and Figure 26.

TABLES AND FIGURES

TABLE I

Materials used in this experiment

Material	Brand (Lot#)	Composition	Manufacturer
Indirect Resin composite block	Radica	Matrix: Urethane Dimethacrylate (UDMA) Filler: Barium fluoroaluminoborosilicate glass (silanated), Amorphous silica	Dentsply Ceramco
Etch-and-rinse luting agent	Variolink II (V)	ExciTE F DSC Phosphonic acid acrylate, dimethacrylates, Hydroxyethyl methacrylate, Highly dispersed silicon dioxide, ethanol, catalysts, stabilizers, fluoride, initiators Variolink II: Base (Monomer matrix): Bis-GMA, Urethane dimethacrylate (UDMA), Triethylene glycol dimethacrylate (TEGDMA) Inorganic fillers: Barium glass, ytterbium trifluoride, Ba-Al-fluorosilicate glass, and spheroid mixed oxide. Additional contents: catalysts, stabilizers, and pigments.	Ivoclar Vivadent, Schaan, Liechtenstein
Self-etching luting agent	Panavia F2.0 (P)	ED primer II Liquid A: 2-hydroxyethylmethacrylate (HEMA), 10-methacryloyloxydecyl dihydrogen phosphate (MDP), N-methacryloyl-5 aminosalicylic acid (5-NMSA), accelerator Liquid B: N-methacryloyl-5 aminosalicylic acid (5-NMSA), water, catalysts, accelerators. Panavia F 2.0 Paste A: 10-methacryloxyloxydecyl dihydrogen phosphate (MDP), hydrophobic aliphatic dimethacrylate, hydrophobic aliphatic dimethacrylate, hydrophilic aliphatic dimethacrylate, silanated silica, photoinitiator, benzoyl peroxide. Paste B: hydrophobic aromatic dimethacrylate, sodium aromatic sulfinate, accelerator, sodium fluoride, silanated barium glass (Continued)	Kuraray Medical Inc

TABLE I (cont.)

Materials used in this experiment

Material	Brand (Lot#)	Composition	Manufacturer
Self-adhesive luting agent (1)	RelyX Unicem (R1)	Resin matrix: multifunctional phosphoric acid modified methacrylate monomer, acetate, stabilizers, self-curing initiators. Inorganic fillers: silaned glass fillers, silica, calcium hydroxide, methacrylate monomers, self curing initiators.	3M ESPE, Seefeld, Germany
Self-adhesive luting agent (2)	RelyX UnicemII (R2)	Base: Methacrylate monomer containing phosphoric acid groups, TEGDMA, Methacrylate monomers, silanated fillers, initiator component, stabilizers, rheological additives Catalyst paste: Methacrylate monomers, Alkaline (basic) fillers, silanated fillers, initiator components, stabilizers, pigments, rheological additives	3M ESPE, Seefeld, Germany
Self-adhesive luting agent (3)	Maxcem Elite (M)	Hydroxy Ethyl Metha Acrylate (HEMA), 4 Methoxyphenol (MEHQ), Cumene HydroPeroxide (CHPO), Uncured Methacrylate Ester Monomer, Tinanium dioxide (TiO_2) and pigments, inert mineral fillers, ytterbium fluoride, activators, stabilizers and colorants	Kerr, Orange, CA, USA

TABLE II

Relative silver contents within the area of
hybrid layer (from spot 3 to spot 6)

Group		N	Min	Max	Mean	Std
All		555	0	100	64.1	30.4
Luting agent	V	120	0	100	87.6	18.5
	M	96	2.5	100	67.2	28.2
	P	120	3.0	100	52.1	33.1
	R1	107	2.8	97.4	54.7	26.9
	R2	112	6.1	99.3	58.1	28.4
Aging	Before	291	0	99.3	61.9	30.6
	After	264	0	100	66.5	30.0
Line	1	188	0	100	61.0	32.1
	2	179	3.0	100	69.0	29.2
	3	188	2.5	99.2	62.6	29.3
Spot	3	130	2.8	99.2	45.4	31.8
	4	132	7.2	99.3	53.0	29.7
	5	146	0	100	73.0	23.8
	6	147	0	100	81.8	20.8

TABLE III

Mixed model analysis

Effect	DF	F-value	P-value
Luting agent	4	11.48	<.0001
Aging	1	2.94	0.1019
Aging*Luting agent	4	2.10	0.0798
Line	2	2.42	0.0940
Spot	3	139.21	<.0001
Aging*Spot	3	6.19	0.0004
Luting agent*Spot	12	11.83	<.0001
Aging*luting agent*Spot	12	2.34	0.0068

TABLE IV

Pair-wise comparisons

Effect	Group1-Group2	Estimate	StdErr	T-value	P-value	Adj-Pvalue
Luting agent (after aging)	V-M	22.81	7.26	3.14	0.0018	0.0558
	V-P	28.20	7.06	3.99	<.0001	0.0031
	V-R1	34.73	7.36	4.72	<.0001	0.0001
	V-R2	30.88	7.16	4.31	<.0001	0.0008
	M-P	5.38	7.26	0.74	0.4583	0.9992
	M-R1	11.92	7.54	1.58	0.1148	0.8571
	M-R2	8.06	7.34	1.10	0.2725	0.9847
	P-R1	6.53	7.36	0.89	0.3752	0.9968
	P-R2	2.68	7.16	0.37	0.7084	1.0000
	R1-R2	-3.85	7.45	-0.52	0.6051	1.0000
	V-(R1+R2)	32.80	6.23	5.27	<.0001	
	M-(R1+R2)	9.99	6.44	1.55	0.1217	
	P-(R1+R2)	4.61	6.23	0.74	0.4602	
	(V+P+M)-(R1+R2)	15.80	4.74	3.33	0.0009	
Aging	Before-After	-3.34	2.42	-1.38	0.1830	0.1830
Line	1-2	-6.57	2.97	-2.21	0.0292	0.0738
	1-3	-1.09	2.93	-0.37	0.7105	0.9265
	2-3	5.48	2.97	1.85	0.0679	0.1602
Spot	3-4	-7.18	2.39	-3.00	0.0028	0.0150
	3-5	-27.74	2.35	-11.81	<.0001	<.0001
	3-6	-36.58	2.35	-15.58	<.0001	<.0001
	4-5	-20.56	2.34	-8.80	<.0001	<.0001
	4-6	-29.40	2.34	-12.59	<.0001	<.0001
	5-6	-8.84	2.26	-3.91	0.0001	0.0006

TABLE V

Comparison of luting agent types

Luting agent	Comparison	Estimate	StdErr	T-value	P-value	Adj-Pvalue
V	Before vs. After	-0.71	4.23	-0.17	0.8751	0.8751
	Line 1-2	-4.75	5.18	-0.92	0.3709	0.6364
	Line 1-3	-5.42	5.18	-1.05	0.3091	0.5581
	Line 2-3	-0.67	5.18	-0.13	0.8988	0.9909
	Spot 3-4	-2.60	4.42	-0.59	0.5579	0.9354
	Spot 3-5	-2.57	4.42	-0.58	0.5627	0.9375
	Spot 3-6	-4.92	4.42	-1.11	0.2691	0.6831
	Spot 4-5	0.03	4.42	0.01	0.9944	1.0000
	Spot 4-6	-2.32	4.42	-0.52	0.6016	0.9531
	Spot 5-6	-2.35	4.42	-0.53	0.5967	0.9513
M	Before vs. After	-4.24	7.46	-0.57	0.6003	0.6003
	Line 1-2	-8.86	8.44	-1.05	0.3073	0.5557
	Line 1-3	8.26	8.36	0.99	0.3366	0.5940
	Line 2-3	17.12	8.33	2.06	0.0546	0.1277
	Spot 3-4	-12.79	6.54	-1.96	0.0549	0.2156
	Spot 3-5	-27.26	6.22	-4.38	<.0001	0.0003
	Spot 3-6	-26.82	6.20	-4.32	<.0001	0.0003
	Spot 4-5	-14.47	5.87	-2.47	0.0164	0.0752
	Spot 4-6	-14.03	5.85	-2.40	0.0195	0.0881
	Spot 5-6	0.4461	5.13	0.09	0.9310	0.9998
P	Before vs. After	-15.31	5.64	-2.72	0.0532	0.0532
	Line 1-2	-4.70	6.90	-0.68	0.5045	0.7773
	Line 1-3	6.46	6.90	0.94	0.3620	0.6257
	Line 2-3	11.16	6.90	1.62	0.1234	0.2646
	Spot 3-4	-11.47	5.00	-2.29	0.0242	0.1072
	Spot 3-5	-34.89	5.00	-6.98	<.0001	<.0001
	Spot 3-6	-50.26	5.00	-10.06	<.0001	<.0001
	Spot 4-5	-23.42	5.00	-4.69	<.0001	<.0001
	Spot 4-6	-38.79	5.00	-7.76	<.0001	<.0001
	Spot 5-6	-15.37	5.00	-3.08	0.0028	0.0146
R1	Before vs. After	3.39	4.44	0.76	0.4878	0.4878
	Line 1-2	-7.44	5.43	-1.37	0.1910	0.3808
	Line 1-3	-6.75	5.20	-1.30	0.2139	0.4178
	Line 2-3	0.69	5.53	0.12	0.9024	0.9915

(continued)

TABLE V (cont.)

Comparison of luting agent types

Luting agent	Comparison	Estimate	StdErr	T-value	P-value	Adj-Pvalue
	Spot 3-4	-6.71	4.34	-1.55	0.1260	0.4151
	Spot 3-5	-35.58	4.29	-8.29	<.0001	<.0001
	Spot 3-6	-48.63	4.29	-11.34	<.0001	<.0001
	Spot 4-5	-28.87	4.34	-6.66	<.0001	<.0001
	Spot 4-6	-41.92	4.34	-9.66	<.0001	<.0001
	Spot 5-6	-13.05	4.29	-3.04	0.0032	0.0167
R2	Before vs. After	2.27	6.01	0.38	0.7250	0.7250
	Line 1-2	-3.23	7.36	-0.44	0.6662	0.9000
	Line 1-3	-6.15	7.34	-0.84	0.4126	0.6844
	Line 2-3	-2.93	7.36	-0.40	0.6954	0.9168
	Spot 3-4	-2.72	3.12	-0.87	0.3862	0.8196
	Spot 3-5	-39.11	3.06	-12.79	<.0001	<.0001
	Spot 3-6	-53.30	3.06	-17.44	<.0001	<.0001
	Spot 4-5	-36.39	3.06	-11.91	<.0001	<.0001
	Spot 4-6	-50.58	3.06	-16.55	<.0001	<.0001
	Spot 5-6	-14.19	2.90	-4.89	<.0001	<.0001

TABLE VI

Comparison of spot locations

Spot	group1	group2	Lut1	Lut2	Estimate	Std Err	T Value	Probt	Adj-p-value
03	Control	Control	V	M	50.74	9.44	5.37	0.0000	0.0001
03	Control	Control	V	P	65.51	8.82	7.43	0.0000	0.0000
03	Control	Control	V	r1	47.36	8.82	5.37	0.0000	0.0001
03	Control	Control	V	r2	50.41	8.82	5.72	0.0000	0.0000
03	Control	Control	m	P	14.77	9.44	1.56	0.1186	1.0000
03	Control	Control	m	r1	-3.38	9.44	-0.36	0.7208	1.0000
03	Control	Control	m	r2	-0.33	9.44	-0.03	0.9725	1.0000
03	Control	Control	p	r1	-18.15	8.82	-2.06	0.0402	0.9917
03	Control	Control	p	r2	-15.10	8.82	-1.71	0.0876	0.9998
03	Control	Control	r1	r2	3.05	8.82	0.35	0.7295	1.0000
03	Control	Aging	V	V	-7.36	7.48	-0.98	0.3256	1.0000
03	Control	Aging	m	M	-40.98	9.62	-4.26	0.0000	0.0145
03	Control	Aging	p	P	-24.11	7.48	-3.23	0.0014	0.3434
03	Control	Aging	r1	r1	4.11	7.97	0.52	0.6063	1.0000
03	Control	Aging	r2	r2	-4.42	8.02	-0.55	0.5819	1.0000
03	Aging	Aging	V	M	17.12	10.16	1.69	0.0928	0.9998
03	Aging	Aging	V	P	48.75	8.82	5.53	0.0000	0.0000
03	Aging	Aging	V	r1	58.83	9.24	6.37	0.0000	0.0000
03	Aging	Aging	V	r2	53.35	9.28	5.75	0.0000	0.0000
03	Aging	Aging	m	P	31.64	10.16	3.11	0.0020	0.4279
03	Aging	Aging	m	r1	41.71	10.53	3.96	0.0001	0.0434
03	Aging	Aging	m	r2	36.23	10.56	3.43	0.0007	0.2135
03	Aging	Aging	p	r1	10.08	9.24	1.09	0.2760	1.0000
03	Aging	Aging	p	r2	4.60	9.28	0.50	0.6204	1.0000
03	Aging	Aging	r1	r2	-5.48	9.68	-0.57	0.5717	1.0000
04	Control	Control	V	M	31.23	9.28	3.36	0.0008	0.2513
04	Control	Control	V	P	54.27	8.82	6.16	0.0000	0.0000
04	Control	Control	V	r1	41.75	8.82	4.74	0.0000	0.0020
04	Control	Control	V	r2	51.74	8.82	5.87	0.0000	0.0000
04	Control	Control	m	P	23.04	9.28	2.48	0.0135	0.8933
04	Control	Control	m	r1	10.52	9.28	1.13	0.2579	1.0000
04	Control	Control	m	r2	20.50	9.28	2.21	0.0278	0.9753
04	Control	Control	p	r1	-12.52	8.82	-1.42	0.1563	1.0000
04	Control	Control	p	r2	-2.54	8.82	-0.29	0.7737	1.0000
04	Control	Control	r1	r2	9.98	8.82	1.13	0.2581	1.0000
04	Control	Aging	V	V	-3.12	7.48	-0.42	0.6771	1.0000
04	Control	Aging	m	M	-12.76	8.90	-1.43	0.1526	1.0000
04	Control	Aging	p	P	-15.13	7.48	-2.02	0.0437	0.9937
04	Control	Aging	r1	r1	12.74	8.12	1.57	0.1177	1.0000

(continued)

TABLE VI (cont.)

Comparison of spot locations

Spot	group1	group2	Lut1	Lut2	Estimate	Std Err	T Value	Probt	Adj-p-value
04	Control	Aging	r2	r2	-2.81	8.02	-0.35	0.7259	1.0000
04	Aging	Aging	V	M	21.59	9.64	2.24	0.0258	0.9702
04	Aging	Aging	V	P	42.26	8.82	4.79	0.0000	0.0016
04	Aging	Aging	V	r1	57.60	9.37	6.15	0.0000	0.0000
04	Aging	Aging	V	r2	52.04	9.28	5.61	0.0000	0.0000
04	Aging	Aging	m	P	20.67	9.64	2.14	0.0327	0.9842
04	Aging	Aging	m	r1	36.02	10.15	3.55	0.0004	0.1569
04	Aging	Aging	m	r2	30.45	10.07	3.02	0.0027	0.5012
04	Aging	Aging	p	r1	15.35	9.37	1.64	0.1023	0.9999
04	Aging	Aging	p	r2	9.78	9.28	1.05	0.2924	1.0000
04	Aging	Aging	r1	r2	-5.57	9.80	-0.57	0.5706	1.0000
05	Control	Control	V	M	17.23	8.82	1.95	0.0513	0.9966
05	Control	Control	V	P	36.44	8.82	4.13	0.0000	0.0235
05	Control	Control	V	r1	27.60	8.82	3.13	0.0019	0.4144
05	Control	Control	V	r2	19.68	8.82	2.23	0.0262	0.9713
05	Control	Control	m	P	19.21	8.82	2.18	0.0299	0.9797
05	Control	Control	m	r1	10.37	8.82	1.18	0.2402	1.0000
05	Control	Control	m	r2	2.44	8.82	0.28	0.7818	1.0000
05	Control	Control	p	r1	-8.84	8.82	-1.00	0.3166	1.0000
05	Control	Control	p	r2	-16.77	8.82	-1.90	0.0579	0.9980
05	Control	Control	r1	r2	-7.93	8.82	-0.90	0.3692	1.0000
05	Control	Aging	V	V	12.06	7.48	1.61	0.1074	0.9999
05	Control	Aging	m	M	2.70	7.59	0.36	0.7228	1.0000
05	Control	Aging	p	P	-11.19	7.48	-1.50	0.1351	1.0000
05	Control	Aging	r1	r1	-3.86	7.97	-0.48	0.6281	1.0000
05	Control	Aging	r2	r2	5.59	7.48	0.75	0.4553	1.0000
05	Aging	Aging	V	M	7.86	8.92	0.88	0.3783	1.0000
05	Aging	Aging	V	P	13.18	8.82	1.50	0.1356	1.0000
05	Aging	Aging	V	r1	11.67	9.24	1.26	0.2072	1.0000
05	Aging	Aging	V	r2	13.20	8.82	1.50	0.1351	1.0000
05	Aging	Aging	m	P	5.32	8.92	0.60	0.5512	1.0000
05	Aging	Aging	m	r1	3.81	9.33	0.41	0.6834	1.0000
05	Aging	Aging	m	r2	5.34	8.92	0.60	0.5499	1.0000
05	Aging	Aging	P	r1	-1.51	9.24	-0.16	0.8703	1.0000
05	Aging	Aging	p	r2	0.02	8.82	0.00	0.9985	1.0000
05	Aging	Aging	r1	r2	1.53	9.24	0.17	0.8689	1.0000
06	Control	Control	V	M	7.91	8.82	0.90	0.3701	1.0000
06	Control	Control	V	P	14.97	8.82	1.70	0.0903	0.9998

(continued)

TABLE VI (cont.)

Comparison of spot locations

Spot	group1	group2	Lut1	Lut2	Estimate	Std Err	T Value	Probt	Adj-p-value
06	Control	Control	V	r1	6.48	8.82	0.73	0.4630	1.0000
06	Control	Control	V	r2	-0.91	8.82	-0.10	0.9180	1.0000
06	Control	Control	m	p	7.06	8.82	0.80	0.4236	1.0000
06	Control	Control	m	r1	-1.43	8.82	-0.16	0.8709	1.0000
06	Control	Control	m	r2	-8.82	8.82	-1.00	0.3178	1.0000
06	Control	Control	p	r1	-8.50	8.82	-0.96	0.3358	1.0000
06	Control	Control	p	r2	-15.88	8.82	-1.80	0.0724	0.9993
06	Control	Control	r1	r2	-7.38	8.82	-0.84	0.4027	1.0000
06	Control	Aging	V	V	-4.42	7.48	-0.59	0.5546	1.0000
06	Control	Aging	m	m	10.22	7.48	1.37	0.1724	1.0000
06	Control	Aging	p	p	-10.79	7.48	-1.44	0.1497	1.0000
06	Control	Aging	r1	r1	1.05	7.97	0.13	0.8951	1.0000
06	Control	Aging	r2	r2	6.58	7.48	0.88	0.3790	1.0000
06	Aging	Aging	V	m	22.55	8.82	2.56	0.0109	0.8543
06	Aging	Aging	V	p	8.60	8.82	0.98	0.3299	1.0000
06	Aging	Aging	V	r1	11.95	9.24	1.29	0.1967	1.0000
06	Aging	Aging	V	r2	10.10	8.82	1.15	0.2527	1.0000
06	Aging	Aging	m	p	-13.95	8.82	-1.58	0.1144	1.0000
06	Aging	Aging	m	r1	-10.60	9.24	-1.15	0.2519	1.0000
06	Aging	Aging	m	r2	-12.45	8.82	-1.41	0.1586	1.0000
06	Aging	Aging	p	r1	3.35	9.24	0.36	0.7172	1.0000
06	Aging	Aging	p	r2	1.50	8.82	0.17	0.8652	1.0000
06	Aging	Aging	r1	r2	-1.85	9.24	-0.20	0.8413	1.0000
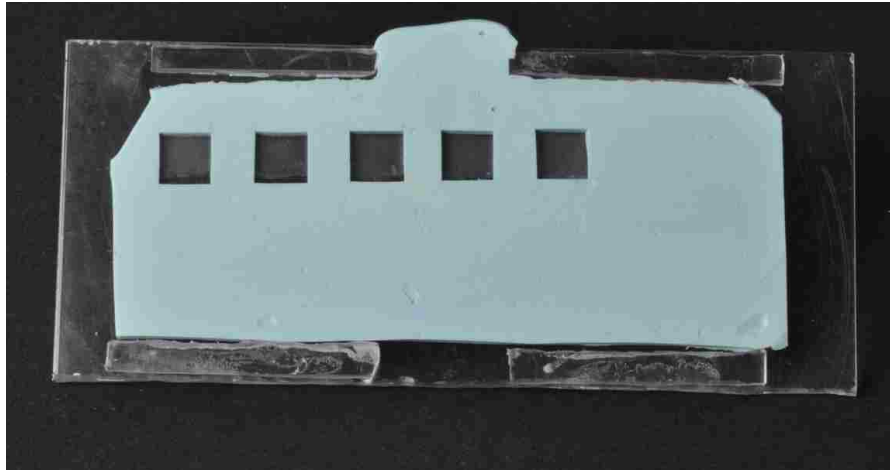
FIGURE 1.   A template used to fabricate indirect resin composite blocks of Radica®. The base for the template was made of flat acrylic. Four pieces of acrylic with a height of 1.5 mm were glued at the four corners of a flat base.

FIGURE 2.   Radica® (Dentsply, Ceramco, York, PA, USA), a laboratory indirect resin composite and Radica Syringe Heater.

FIGURE 3.   An Enterra unit® polymerization unit.

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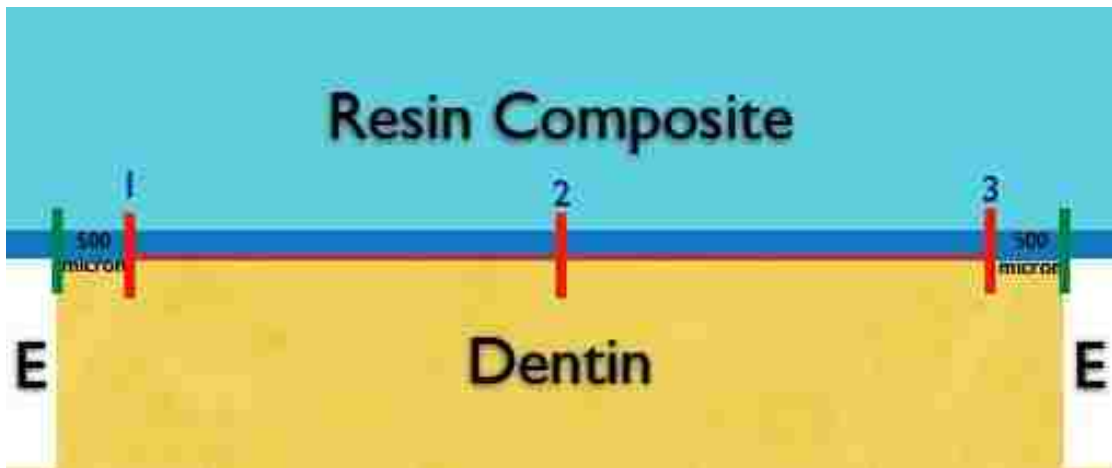
FIGURE 4. Scan line selection on each sample. Three scan lines were selected on each sample; the first and last lines were 500 μm away from the dentinoenamel junction. The midpoint between the first and last lines was used as the middle line scan.
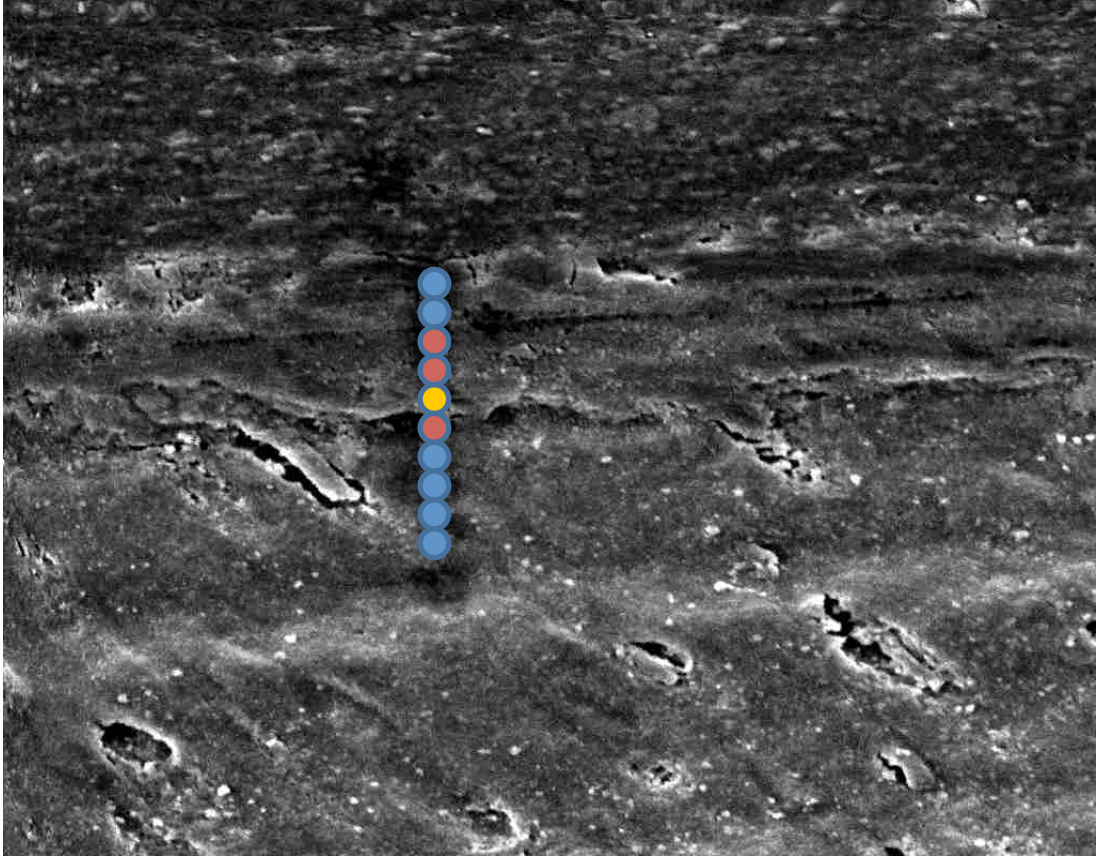
FIGURE 5.   The spot location. The bottom of the fifth spot on each line was located specifically at the bottom of the hybrid layer. The spot analysis was specifically focused at spot 3, 4, 5 and 6 to reveal the corresponding amounts of silver on each point, which was arranged into a single value.
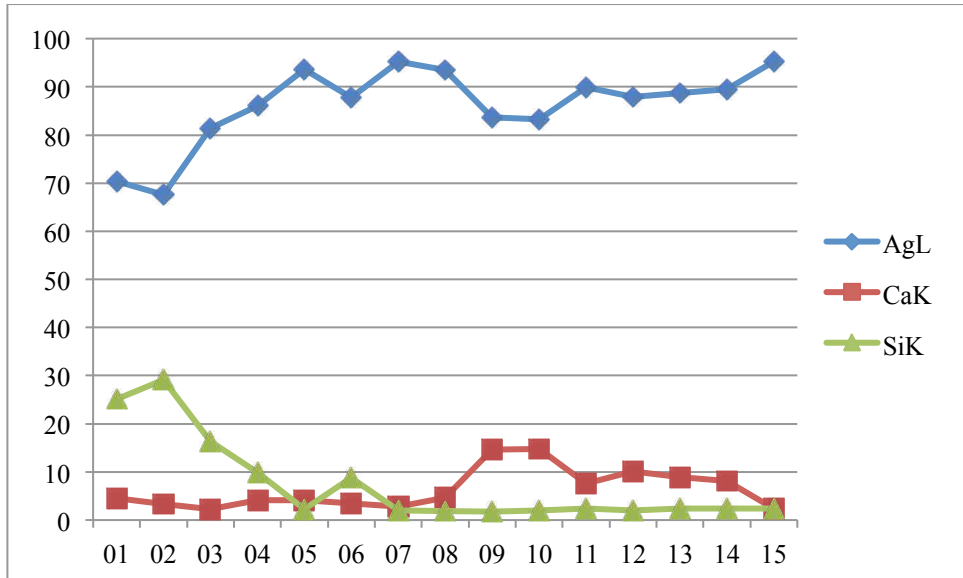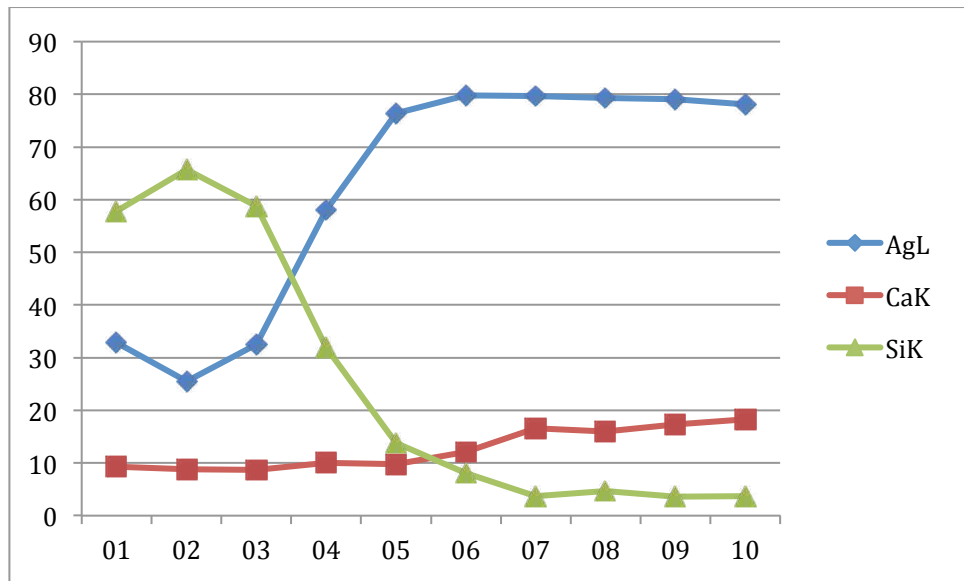
FIGURE 6.   Linear graphs of Ag, Ca, and Si evaluation in the control group of Variolink II.
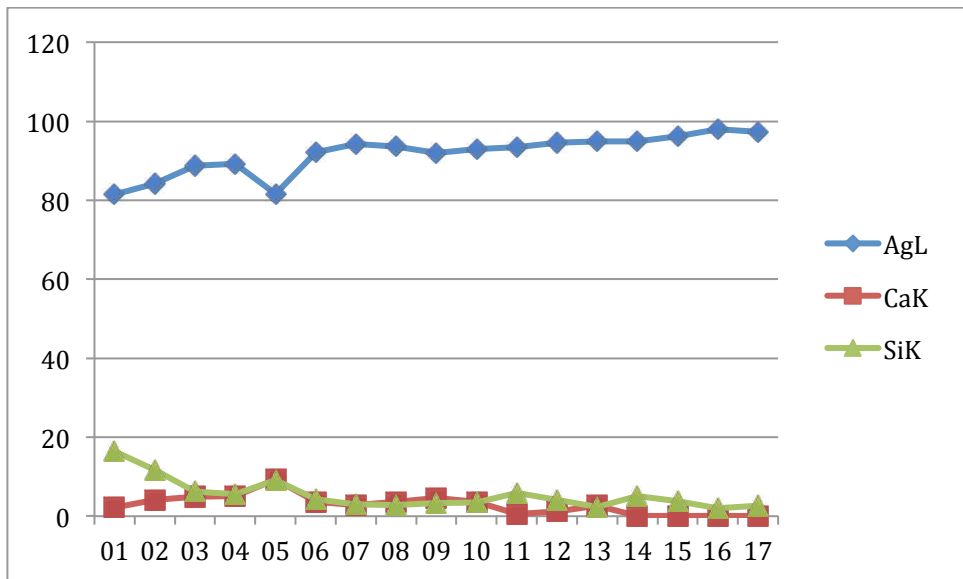
FIGURE 7.   Linear graphs of Ag, Ca, and Si evaluation in the aging group of Variolink II.
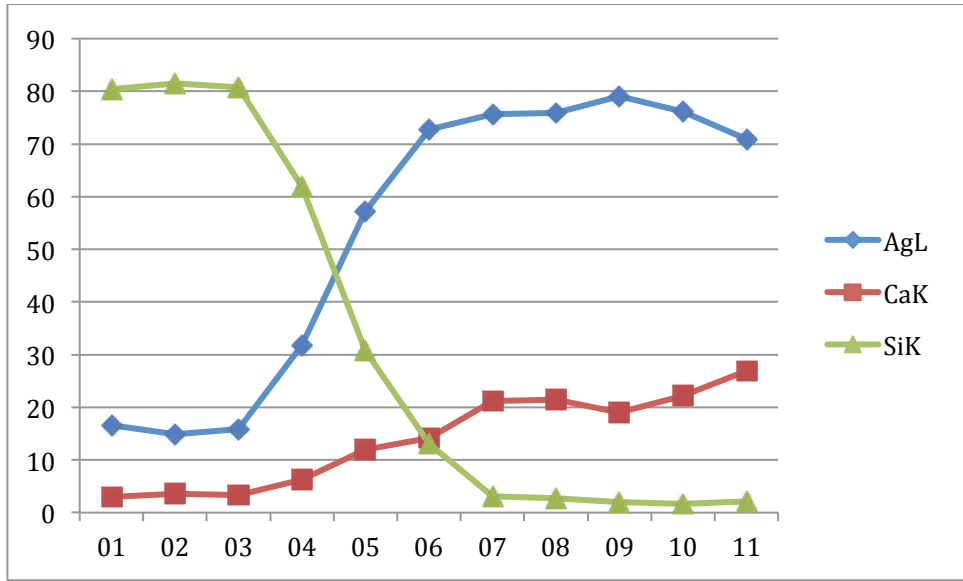
FIGURE 8.   Linear graphs of Ag, Ca, and Si evaluation in the control group of
Panavia F2.0.
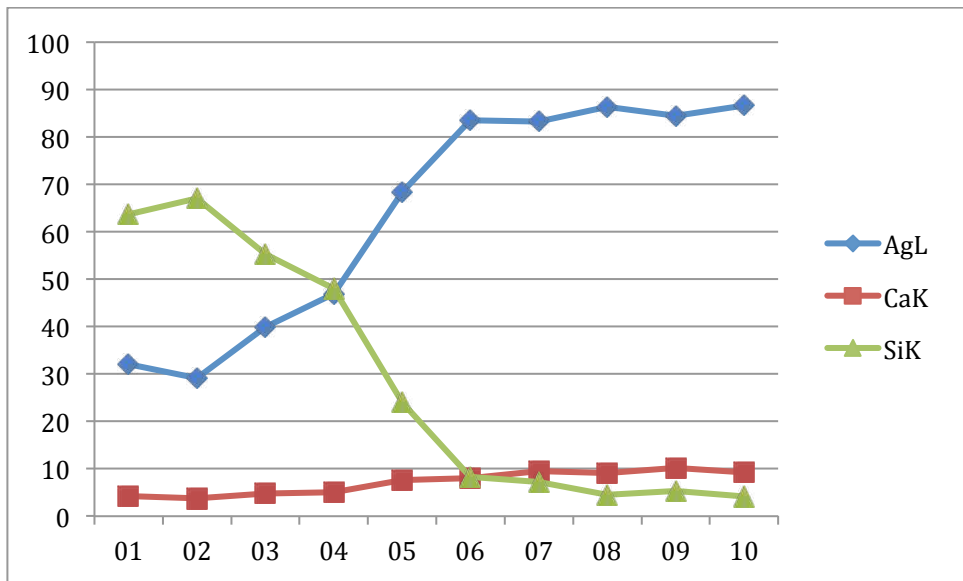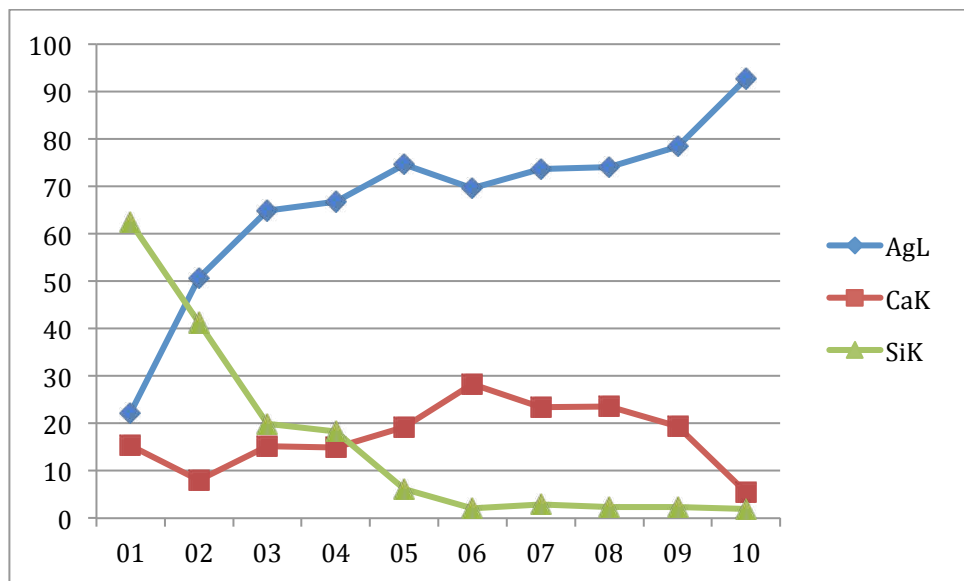

FIGURE 9.   Linear graphs of Ag, Ca, and Si evaluation in the aging group of
Panavia F2.0.
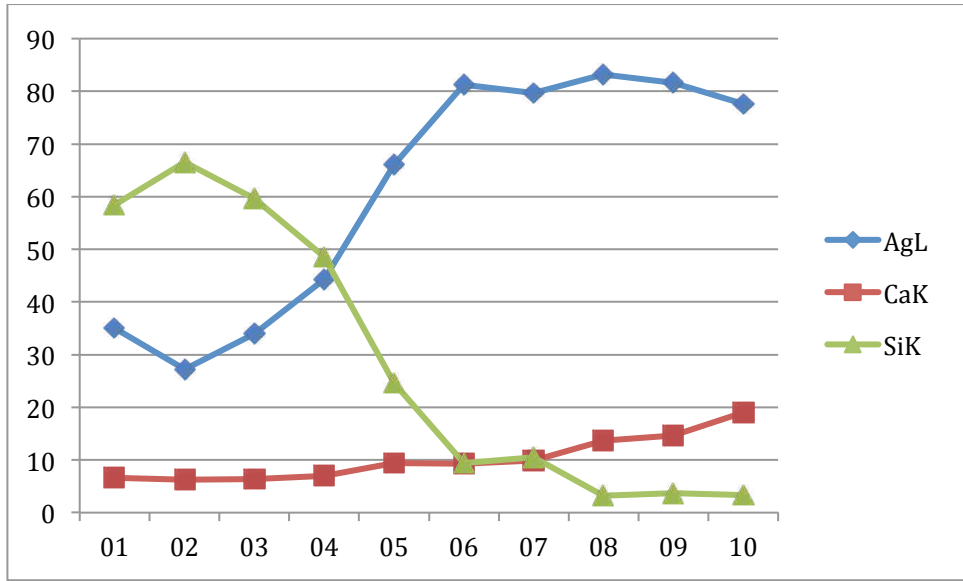
FIGURE 10.   Linear graphs of Ag, Ca, and Si evaluation in the control group of RelyX Unicem I.
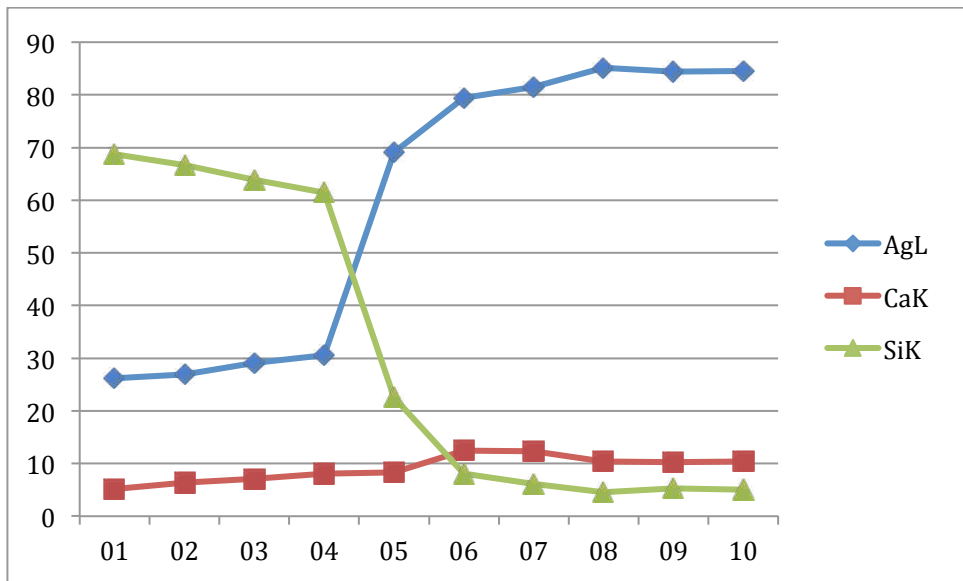
FIGURE 11   Linear graphs of Ag, Ca, and Si evaluation in the aging group of RelyX Unicem I.
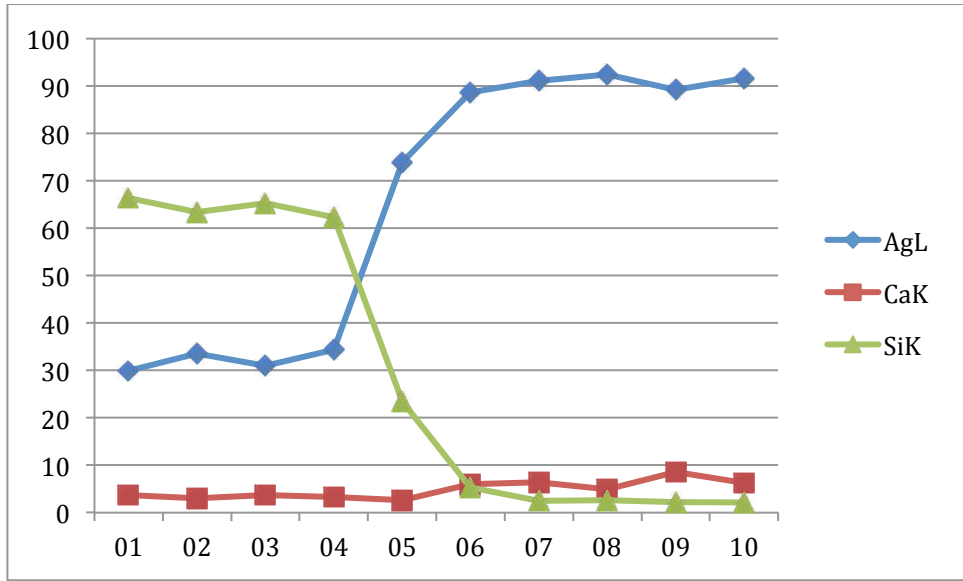
FIGURE 12.   Linear graphs of Ag, Ca, and Si evaluation in the control group of RelyX Unicem II.
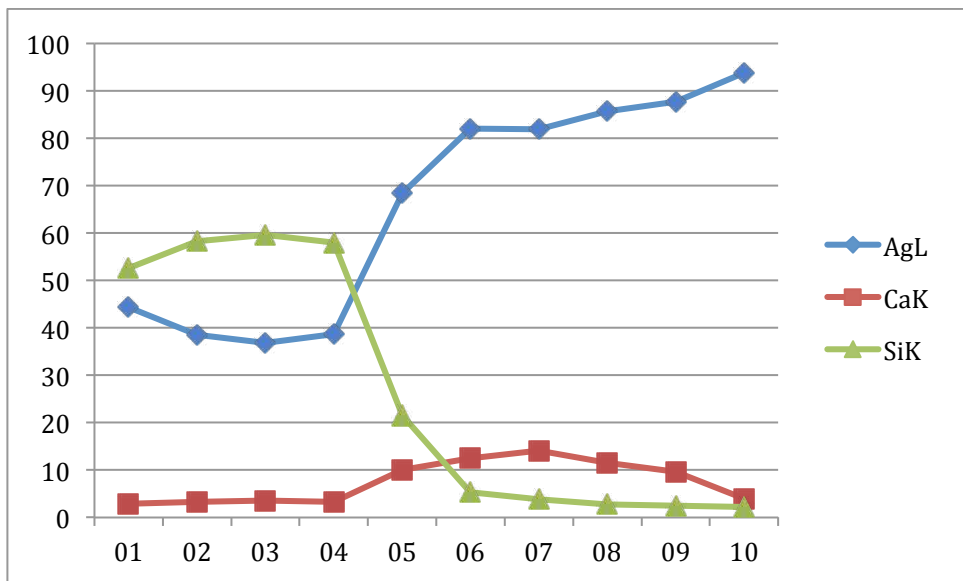
FIGURE 13.   Linear graphs of Ag, Ca, and Si evaluation in the aging group of RelyX Unicem II.

FIGURE 14   Linear graphs of Ag, Ca, and Si evaluation in the control group of Maxcem Elite.

FIGURE 15. Linear graphs of Ag, Ca, and Si evaluation in the aging group of Maxcem Elite.

FIGURE 16.   The SEM image of the control group of Variolink II. The separation
was found between the adhesive layer and the resin composite (X500).
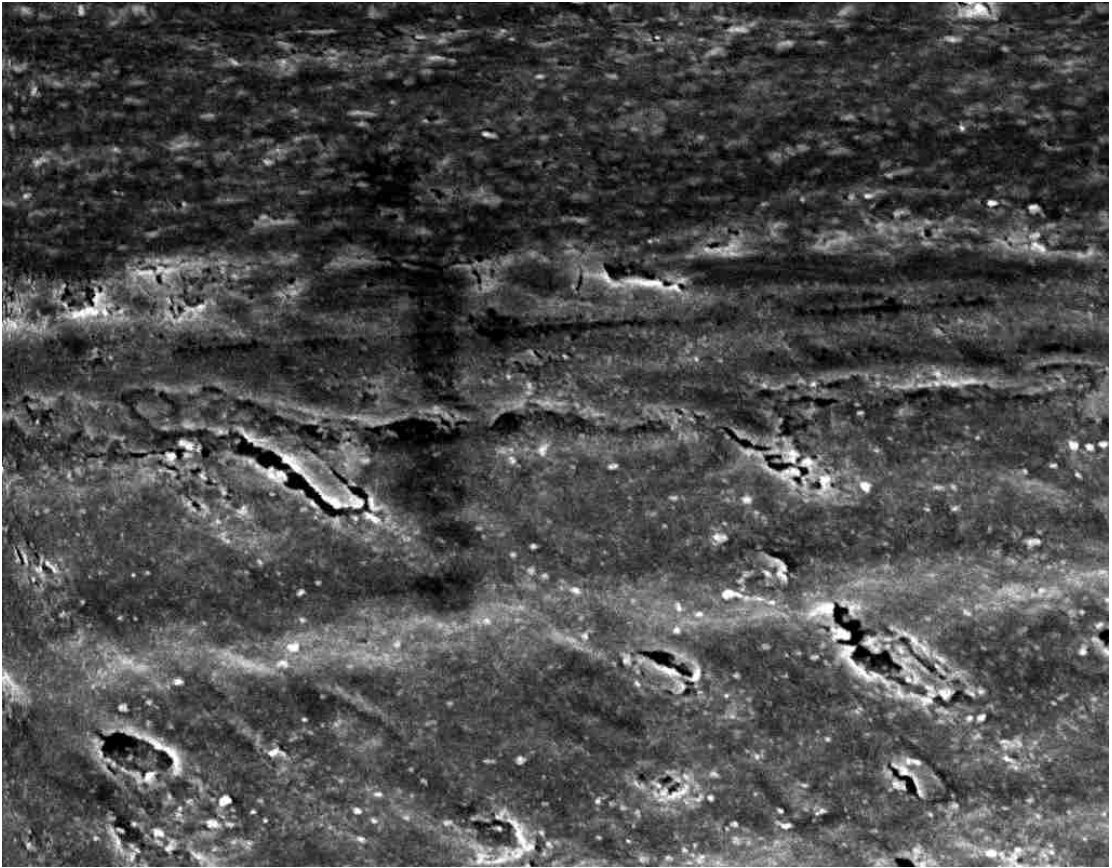
FIGURE 17.   The SEM image of the control group of Variolink II (X750).
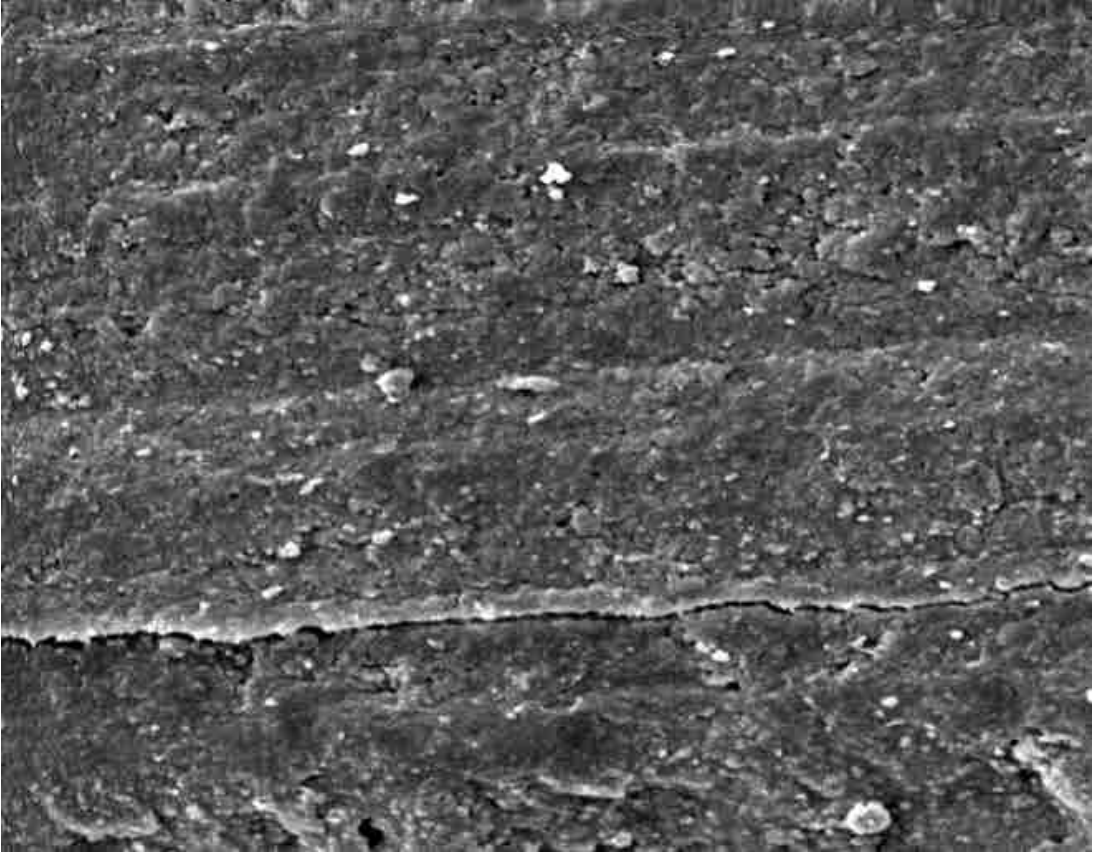
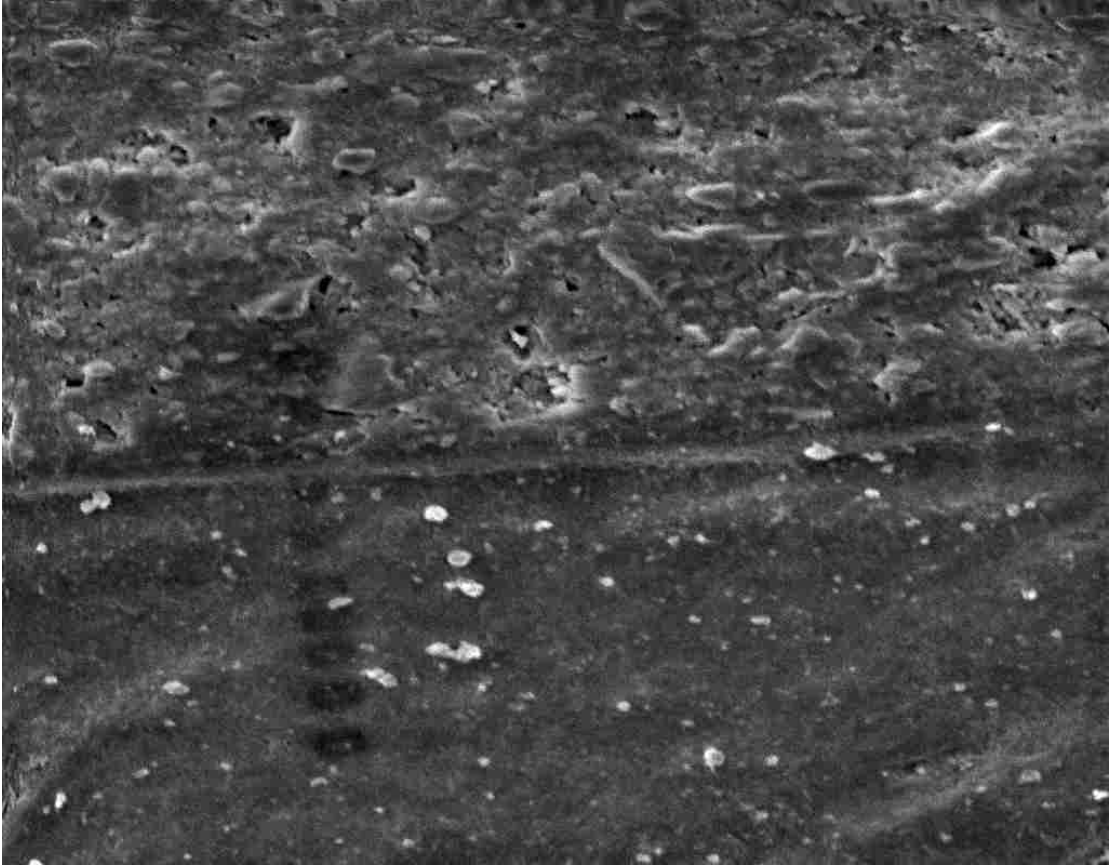FIGURE 18.   The SEM image of the aging group of Variolink II (X750).

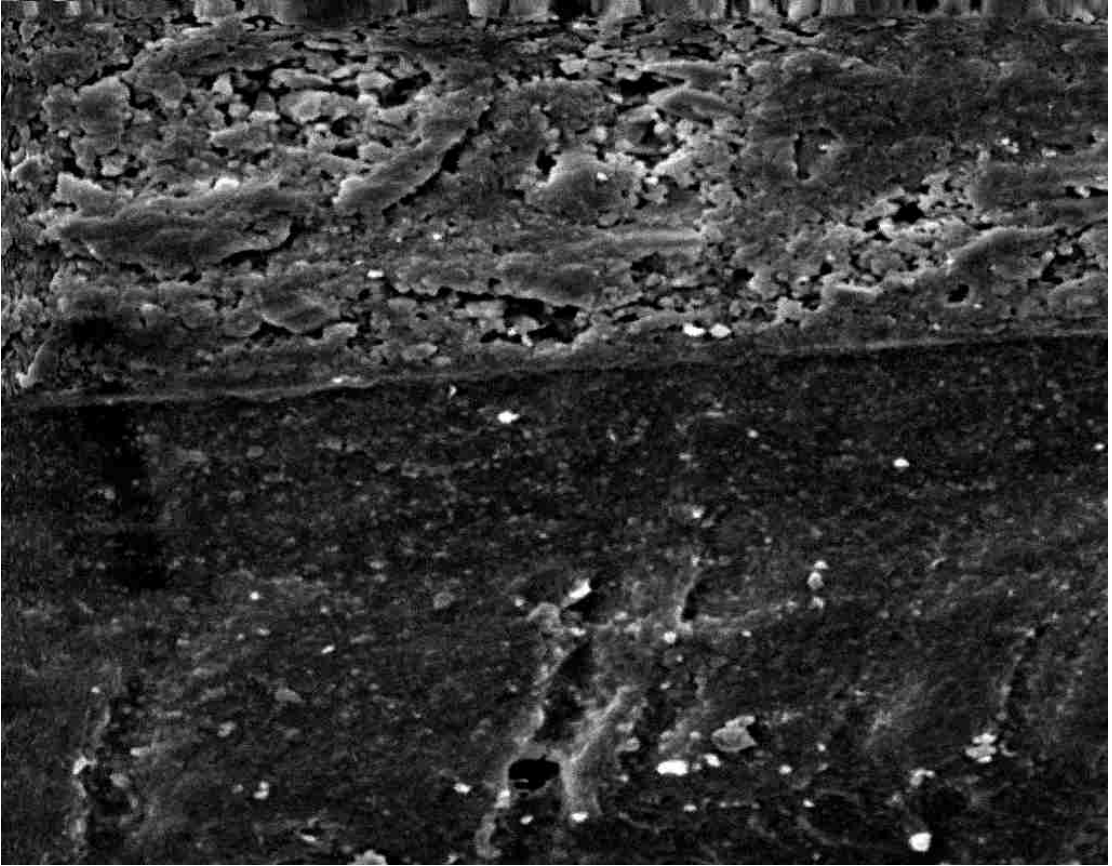FIGURE 19.   The SEM image of the control group of Panavia F2.0 (X750).

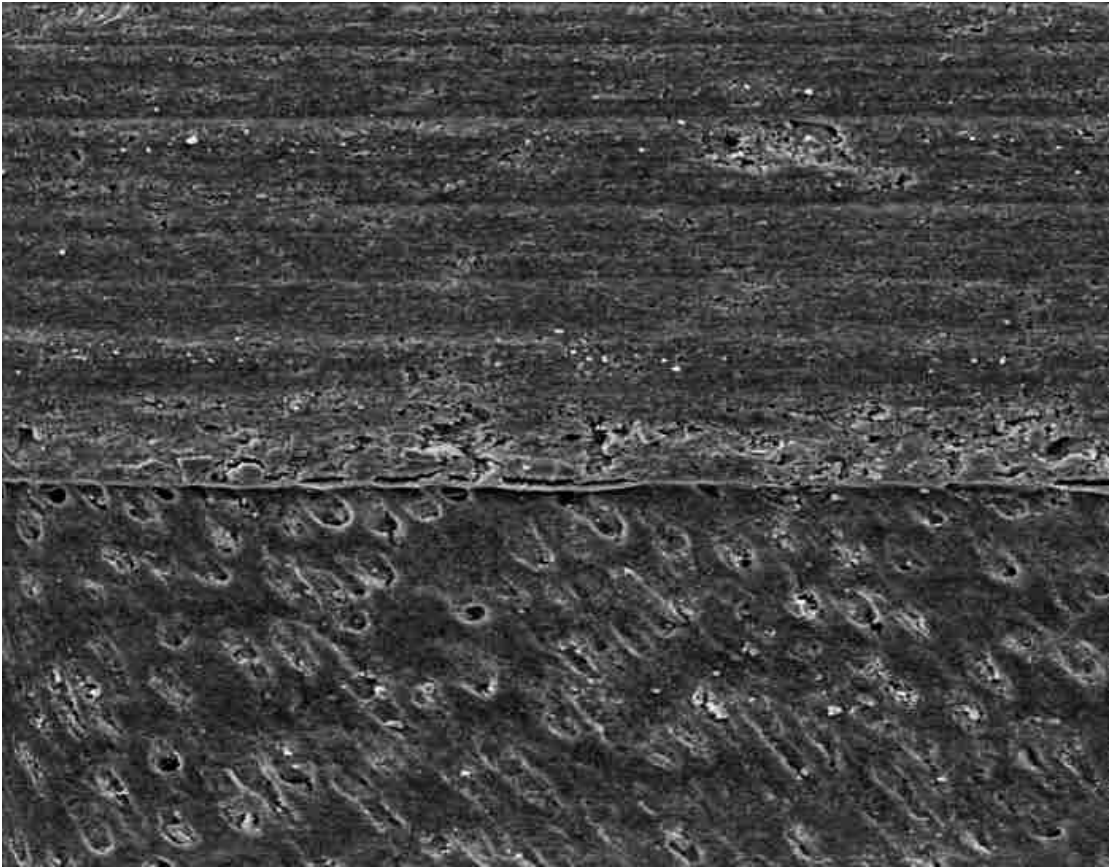FIGURE 20.   The SEM image of the aging group of Panavia F2.0 (X750).

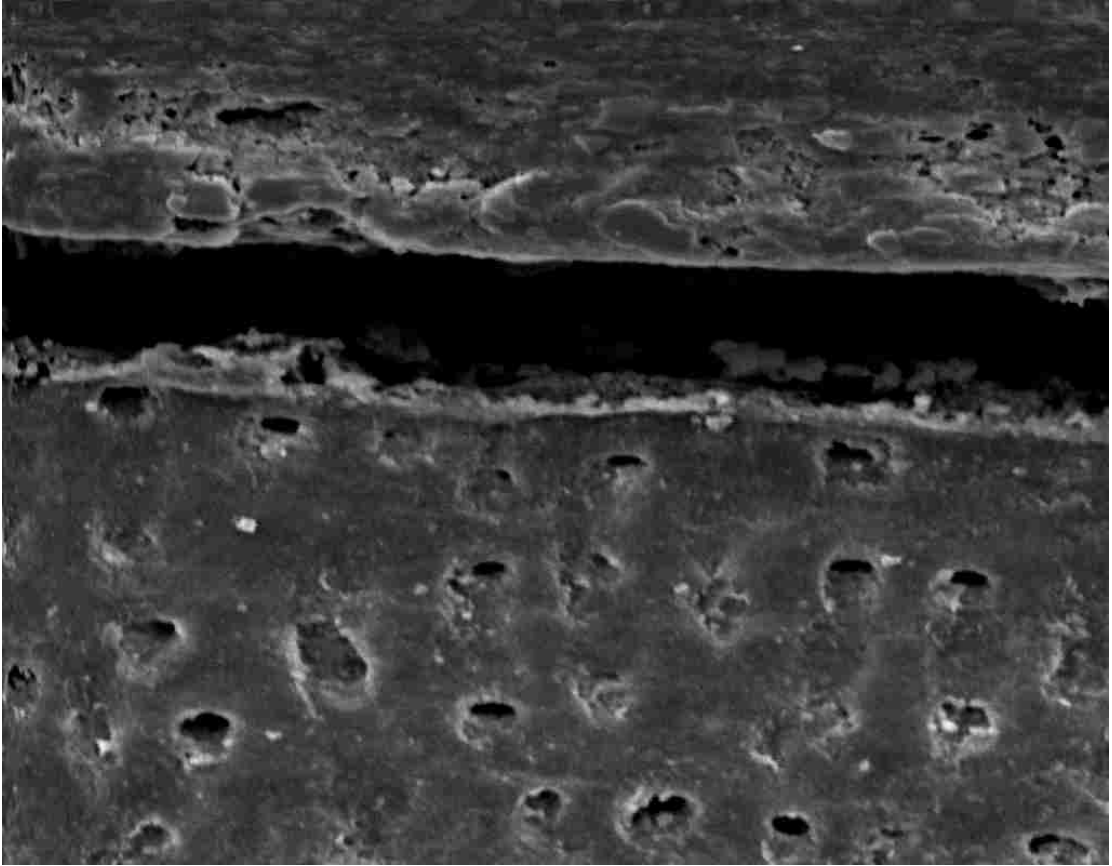FIGURE 21.   The SEM image of the control group of RelyX Unicem I (X750).

FIGURE 22.   Image of the separation in the adhesive layer of the aging group of
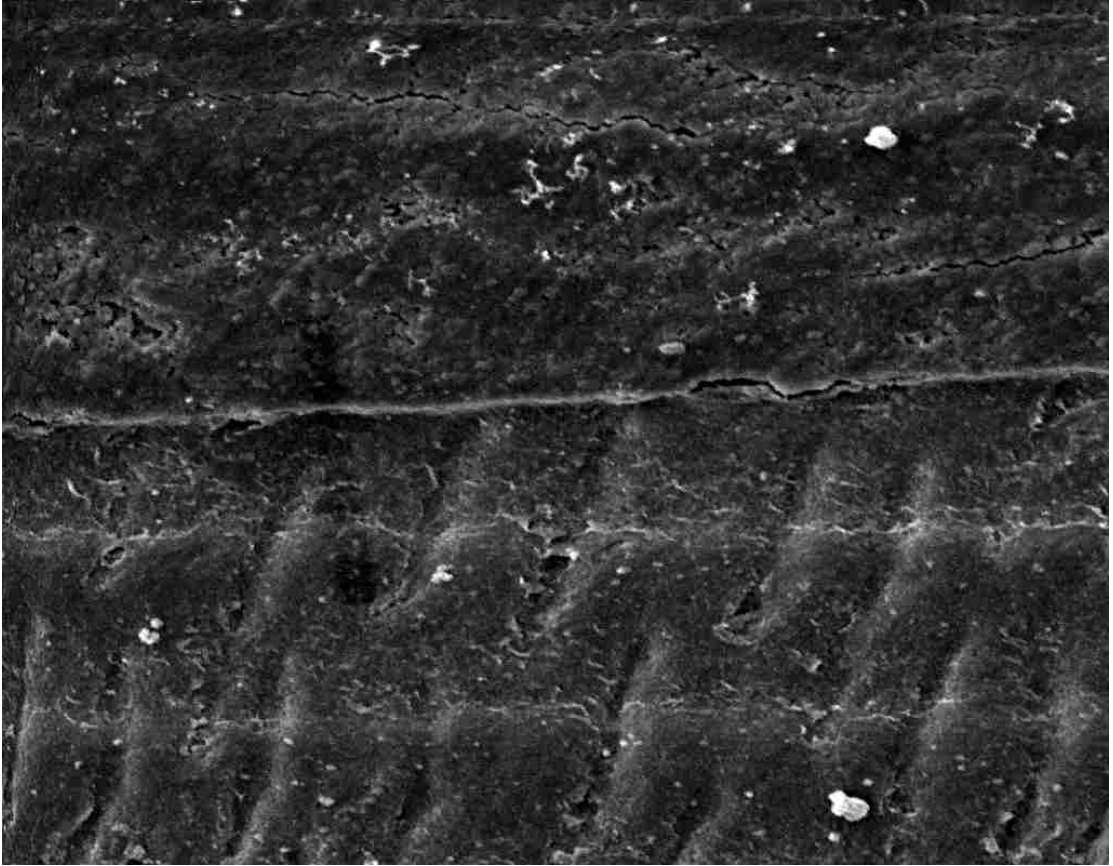RelyX Unicem I (X1000).

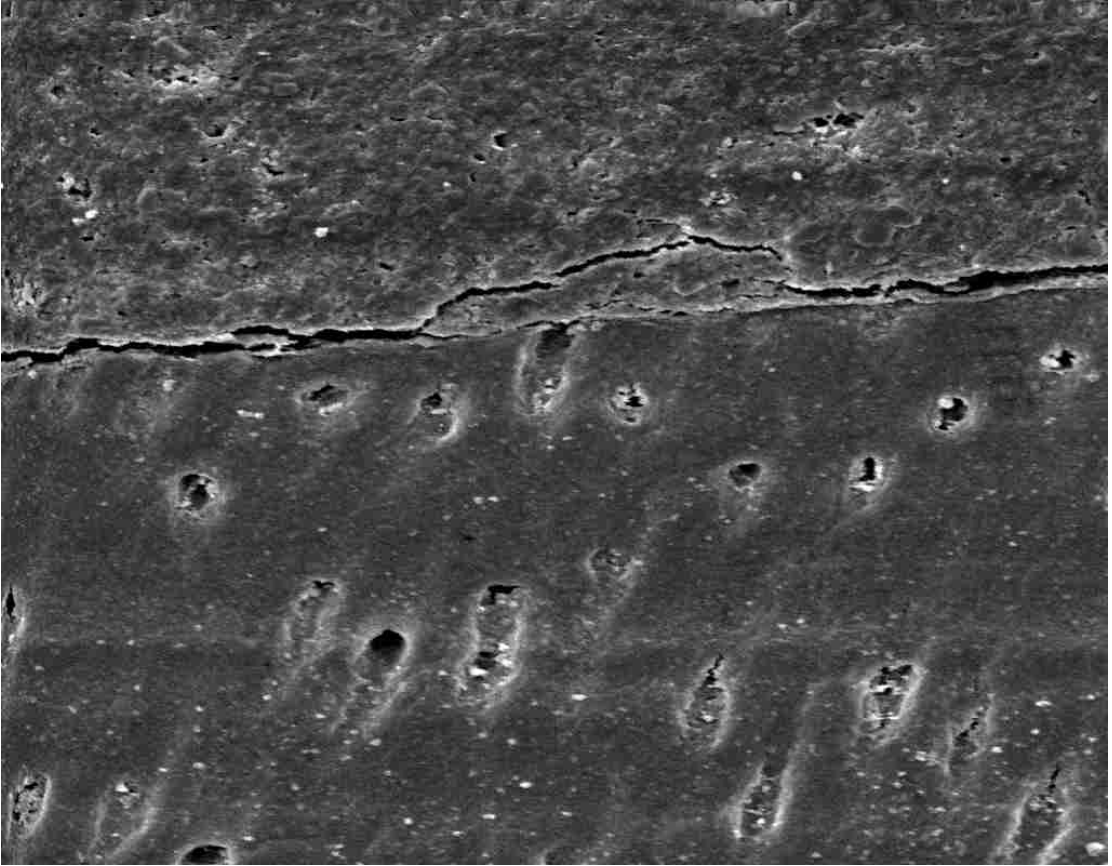FIGURE 23. The SEM image of control group of RelyX Unicem II (X750).

FIGURE 24.   Image of the separation in the adhesive layer of the aging
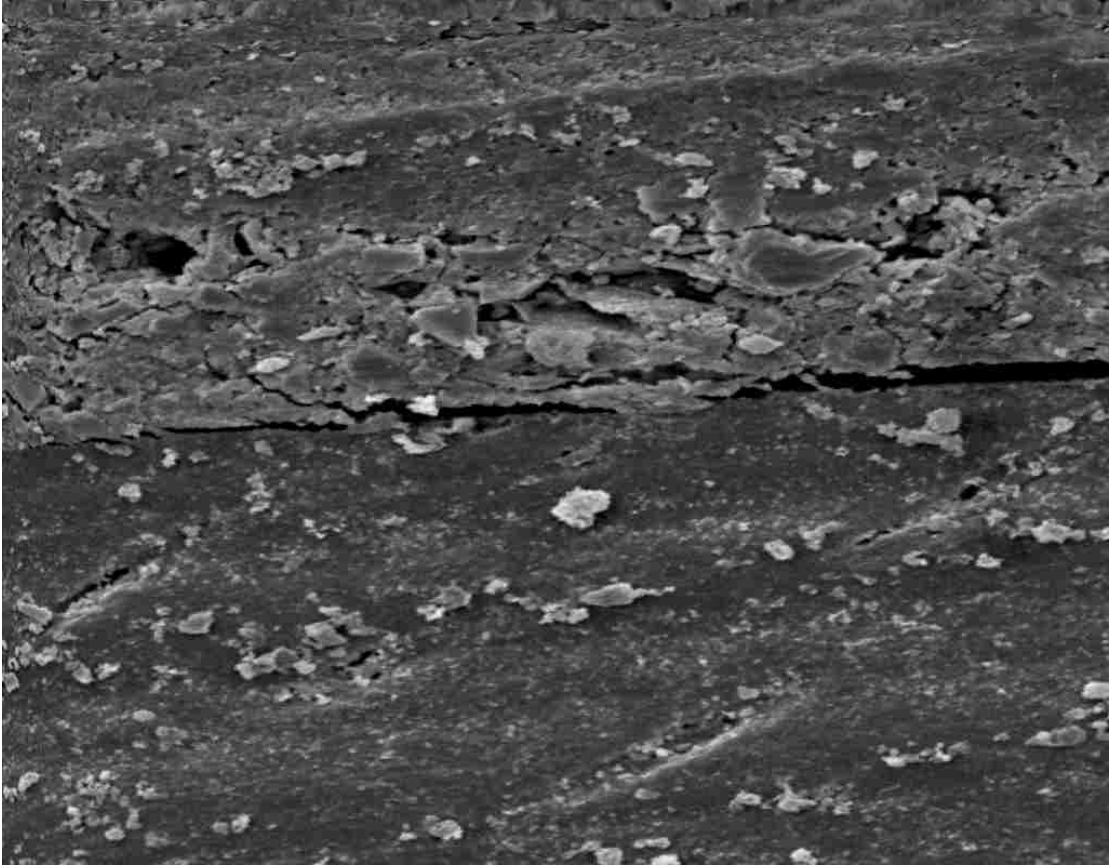   group of RelyX Unicem II (X750).

FIGURE 25.   The image of the control group of Maxcem Elite. The partial
separation in the adhesive interface was generally found in all samples
(X750).
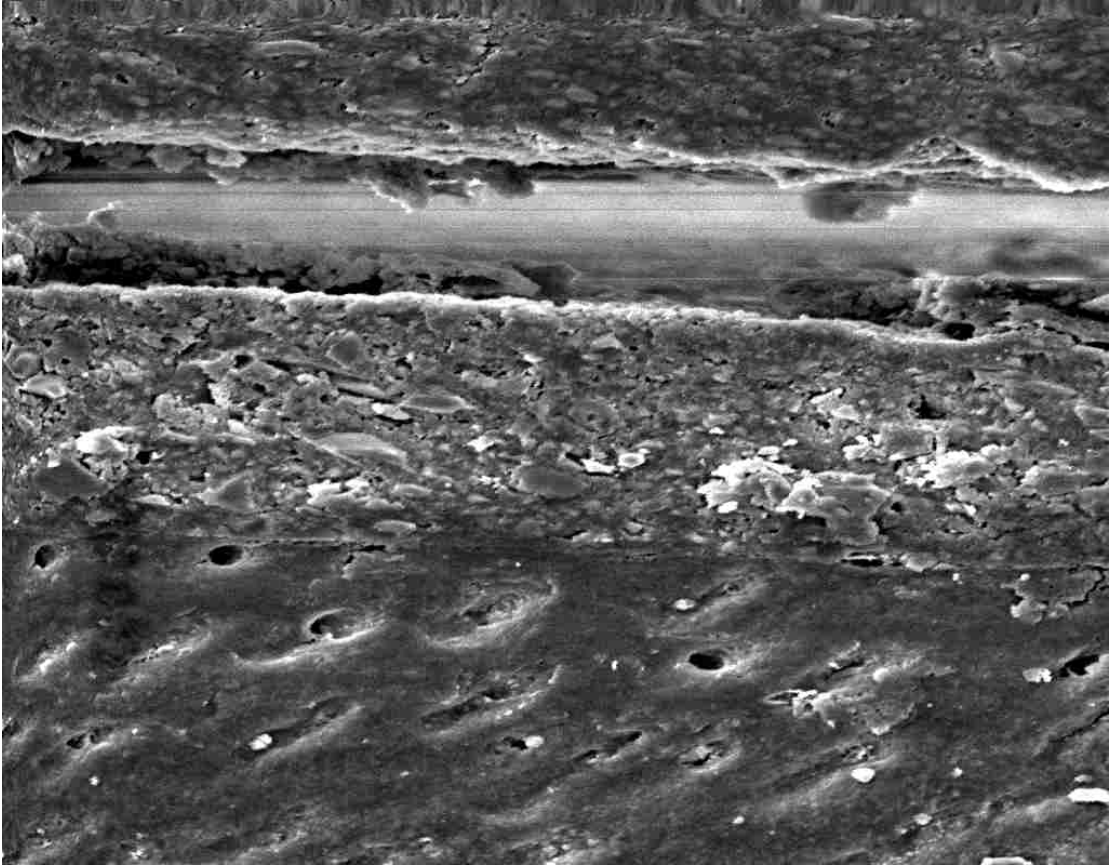
FIGURE 26.   The image of the aging group of Maxcem Elite. The partial separation
in the adhesive interface was generally found in all samples (X750).

Mean
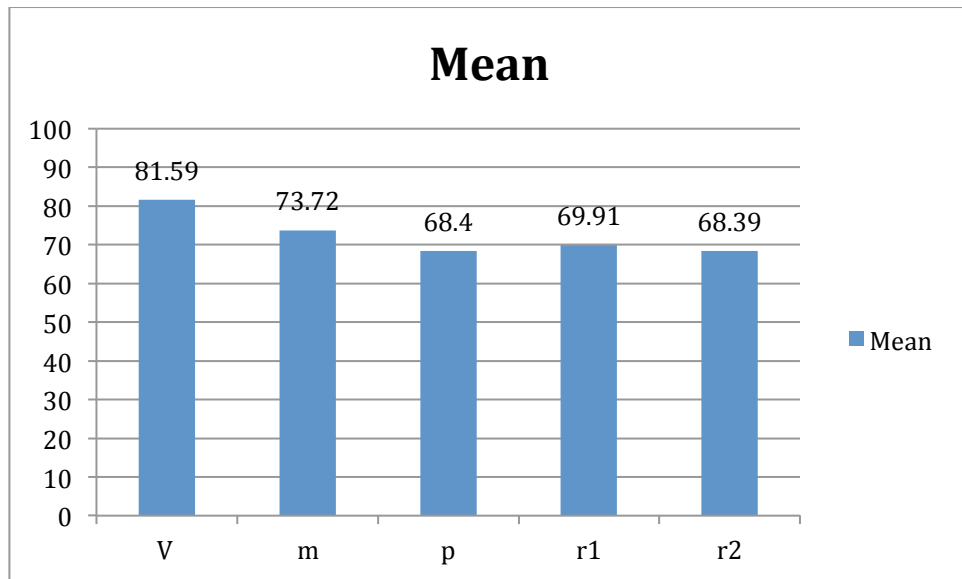
Spot = 05
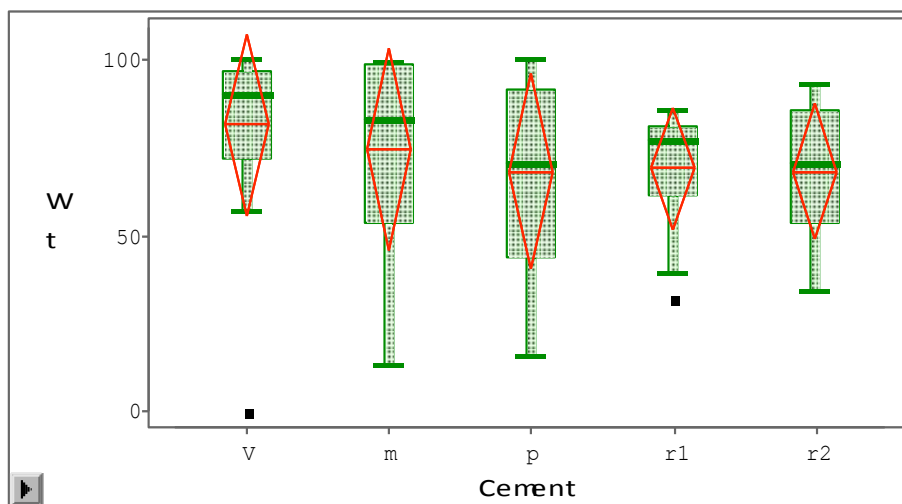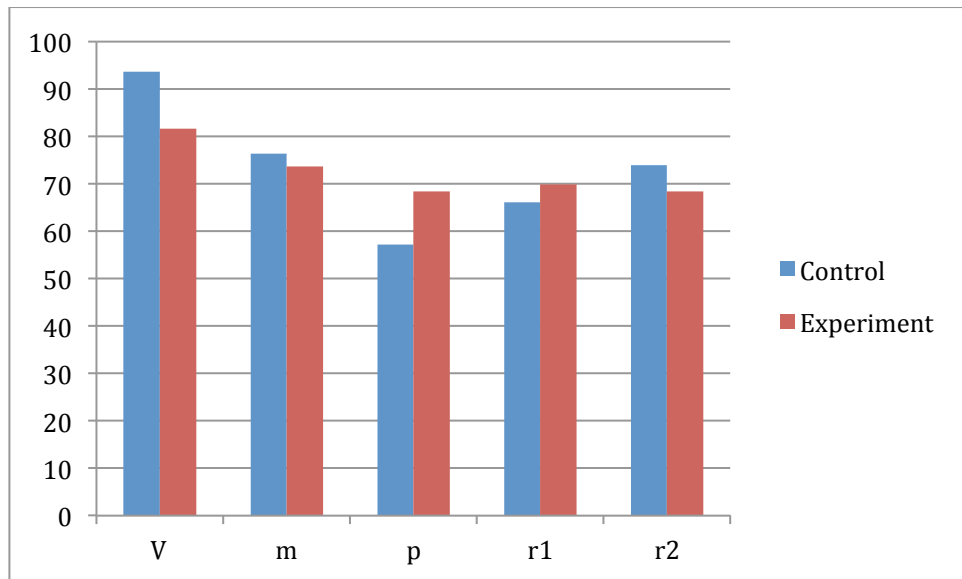

FIGURE 27. No significant difference in silver uptake within the adhesive interface between luting agents after aging (p > 0.05).
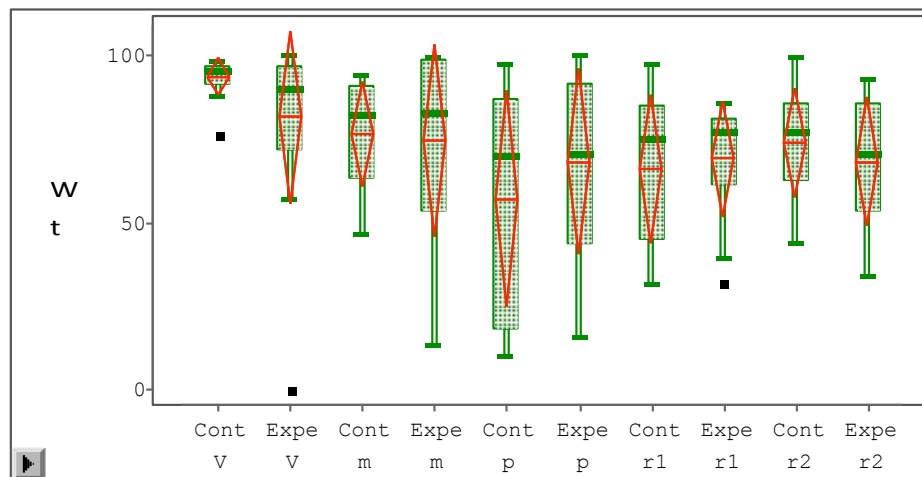
FIGURE 28. No significant difference in silver uptake within the adhesive interface between luting agents before and after aging ($p > 0.05$).

DISCUSSION

This study applied a dye penetration technique and elemental analysis to evaluate the silver uptake among five resin luting agents before and after aging. The quantitative silver uptake was analyzed by EDS software in the SEM microscope. The study was performed under *in vitro* conditions with restricted environments.[85] Under *in vivo* conditions, normal functioning, habitual bruxism, thermal changing, and malocclusion impose stresses throughout the tooth and the restoration, which may affect the longevity of the luting agents. In this study, the oral condition was simulated by thermocycling and storing in the artificial saliva at $37^{o}C$ in the laboratory. However, the mechanical loading simulation was not included in the study due to the limitations of the machine of the laboratory.

The dentin structure is extremely porous and spongy and thus very permeable. Besides, dentinal tubule structure, intertubular dentin is indeed constructed of a mineralized collagen network that is full of nanometer-sized pores.[86] The silver penetration could be found generally in dentin surfaces. In our study, the specific areas for evaluation were focused in the area at the bottom of the hybrid layer (spot 5) and from 3 um above (spot 3 through 5) and 1 um below the bottom of the hybrid layer (spot 6).

In 2002 Li reported a possibility that minerals such as amorphous calcium phosphates that are re-precipitated in the bonded interfaces of the nonrinsing adhesives may be dissolved in an acidic silver nitrate solution.[87] That may lead to a false positive result.[88] Therefore, in our study, basic ammoniacal silver nitrate solution was used to avoid the possibility of residual mineral dissolution within partially demineralized hybrid layers by mildly acidic $AgNO_3$. Presumably, silver

deposits should be found in the area of discrepancy between the depth of demineralization and the extent of resin infiltration. However, the silver deposits were still found generally in all the hybrid layers, adhesive layer, and just underneath the hybrid layer.

Quantitative evaluation of nanoleakage from EDS analysis provided information about conventional etch-and-rinse adhesives, Variolink II luting agents had significantly higher silver uptake from spot 3 through spot 6 both before and after aging compared with the other luting agents. One of the possible reasons for the higher silver deposition in both the control and aging groups is the increased hydrophilicity of adhesive during the preparation of the luting procedure. Due to the technique sensitivity of the etch-and-rinse adhesives related to wetness of dentin before luting, a greater degree of wetness was allowed in this study to achieve successful bonding in this adhesive system. Regarding the higher hydrophilicity of the adhesive layer, the amount of quantitative nanoleakage of etch-and-rinse luting agent, Variolink II, was found to be higher than the self-etch luting agents in this study.

Numerous studies have recommended the use of acidic solutions to remove the smear layer and the follow-up use of primer application to gain the proper resin infiltration and better adaptation into the dentinal wall.[73] However, due to the technique sensitivity, the multiple-steps application may compromise the expected ideal adhesive performance. From Sana et al.'s study in 1995, it was found that nearly all adhesive systems, including the so-called gold-standard three-step etch-and-rinse adhesives, have repeatedly been documented with a certain degree of silver deposition within, underneath, and above the hybrid layer.[86]

Another possible explanation of the highest silver uptake in Variolink II was the misplacement of spot 5 at lower than the actual bottom of the hybrid layer. As

seen in Figure 6 and Figure 7, the plotted graphs from spots 1 through 10 of Variolink II resembled the other luting agents from the sixth through the 10th spots on graphs where fewer silica contents were found underneath dentin.

In 2008 Breschi et al.[89] described that the major degradation mechanism of etch-and-rinse adhesive has been thought to be based on both hydrolysis and enzymatic breakdown of collagen fibrils and polymerized resin matrix in the hybrid layer. The hybrid layer is vulnerable to enzymatic degradation especially in the insufficient resin infiltration/encapsulation of the collagen fibrils situation.[90] Since hydrolytic degradation occurs only in the presence of water, adhesive hydrophilicity, water sorption, and subsequent hydrolytic degradation are generally correlated.[15,52,63-65] In many studies, the one-step self-etching adhesives normally present incomplete polymerizations and more extensive permeability than etch-and-rinse adhesives, probably due to the presence of higher concentrations of hydrophilic monomers.[19,85] Regarding the combination of hydrophilic and ionic resin monomers into the bonding, the hybrid layers of one-step self-etch adhesives behave as semi-permeable membranes permitting water movements throughout the bonded interface even after the adhesive is polymerized.[66]

The SEM evaluation done under the significant amount of vacuum caused dehydration stress and created propagation cracks through the adhesive interface. But these artifactual gaps created during specimen preparation can be easily differentiated from the true nanoleakage gaps by the absence of silver particles as shown in Li et al.'s study.[87] Besides, the sectioning of the specimen after luting might create the separation in the adhesive layer. These unwanted artifacts are indicated in the weak area in the adhesive interfaces. In the present study, partial separation through bonding interfaces was found most often in the self-adhesive luting agents in both the

control and aging groups, especially RelyX Unicem II (four out of five samples) and Maxcem Elite (all samples) that represents less integrity of this self-adhesive luting agents. Interestingly, in Variolink II, which presented the highest amount of silver penetration, there are only two partial separations in the adhesive interfaces after aging.

Panavia F2.0 is a self-etching luting agent comprised of three amphiphilic monomers (HEMA, MDP and 5-NM-SA) with intense and stabilized hydroxyapatite.[91] It has been reported that the high concentration of the hydrophilic and ionic resin monomer in ED primer creates a highly permeable layer.[85] An incomplete penetration of the resin monomers into the demineralized collagen layer was also reported causing nanospace formation.[74] In 2003 Tay et al. reported that when the water is not completely removed from the primed dentin, the porous anionic hydrogels are formed through copolymerization of HEMA and acidic resin monomers.[40] Moreover, the water may occur in the area of incomplete polymerization in the resin matrix.[40]

For RelyX unicem, the bonding reaction occurs though interaction of dentin with the ionized phosphoric acid-methacrylate monomers in the mixture. The bonding mechanism can be considered similar to that of glass ionomers with an intermediate interfacial layer incorporating partially dissolved smear particles.[77] The ionization may occur from the water either from the dentinal tubules or from the neutralization reaction of phosphate monomers with basic fillers.[7] However, from the study of Holderegger et al. in 2008, this luting agent was the least influenced by thermocycling and showed less sensitivity to variations in handling and aging.[92] In Gerth et al.'s study, the increased chemical reaction with calcium from hydroxyapatite was found that may explain the bonding performance of this material to dentin and better results

in the nanoleakage evaluation.[93] Given that the high viscosity characteristics of the material may limit the penetration of resin through the demineralizing components, the application technique with constant pressure has been recommended to prevent the gap formation at the resin/dentin interface.

The durability of the adhesives depends on the quality of the hybrid layer (i.e. the adequate impregnation of the dentin substrate) to reduce collagen degradation or reduce the rate of water sorption. Standard clinical protocols that showed improved bonding quality.[89] For example, the use of an additional layer of hydrophobic resin agent,[94] multiple layer applications,[64,82,84] enhanced solvent evaporation,[95] prolonged curing time,[61] the use of MMP inhibitors[44,96,97] and the use of electric current to improve monomer impregnation.[98,99]

SUMMARY AND CONCLUSIONS

The results can be summarized as follows:

1.	There is no significant difference in silver uptake within the adhesive interface between etch-and-rinse, self-etching, and self-adhesive luting agents after aging at the bottom of hybrid layer ($p > 0.05$)

2.	There is no significant difference in silver uptake within the adhesive interface before or after aging in each luting agent at the bottom of hybrid layer ($p > 0.05$).

3.	All resin luting agents exhibited nanoleakage, after both 24-hour storage and 10-day storage with thermocycling.

From the results, it can be concluded that all resin luting agents exhibited nanoleakage, after both 24-hour storage and 10-day storage with thermocycling. The durability of resin luting agents relies on the quality of the hybrid layer, which is involved in technique sensitivity, and on the composition of the material, composition, and aging. The decrease in bond integrity to dentin in the one-step self-etch luting agents may become problematic as seen from failure analysis revealed by SEM images in this study. Future research should focus on the exact location of these failures and possibly increase the storage time to improve understanding the degradation of resin luting agents.

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ABSTRACT

QUANTITATIVE COMPARISON OF NANOLEAKAGE AMONG

FIVE RESIN LUTING AGENTS

AFTER AGING

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Potential problems of one-step adhesives have been identified, including

water uptake and subsequent plasticization, water-and enzyme-induced nanoleakage,

and the presence of voids due to phase-separation or osmosis. Clinically, adhesive

failures due to marginal degradation present as retention loss, marginal discoloration,

and secondary caries. However, the mechanisms of adhesive interface degradation of

self-etching and self-bonding resin luting agents are not fully understood. The

objective of the study was to investigate adhesive layer degradation by using a

nanoleakage technique with five different resin luting agents.

Materials and Methods: Five different resin luting systems, Variolink II,

Panavia F2.0, RelyX Unicem, RelyX Unicem2, and Maxcem Elite were evaluated in this study. The 25 dentin specimens were randomly divided into five resin luting agent groups. Flat dentin surfaces were created mid-coronally and were luted with luting agents. Then, each tooth was sectioned occluso-gingivally. The first half of each tooth was used as a control group and the other half was used as the experimental group. The control group was immersed in artificial saliva at 37°C and SEM examination with chemical analysis was performed within 48 hours. In the tested group, all specimens were immersed in artificial saliva at 37°C for 10 days and thermocycled. For the SEM examination, the specimens were immersed in a 50-percent ammoniacal silver nitrate solution for 24 hours.[22] SEM was used for observation of silver penetration of the specimens. Three scan lines were selected. For elemental analysis, natural apatite, olivine minerals, and pure silver metal were chosen as standards for Ca, Si and Ag. Data were analyzed using ANOVA with a 5-percent significance level.

Results: At the bottom of the hybrid layer, there was no significant difference in silver uptake within the adhesive interface between luting agents ($p > 0.05$) and there was no significant change in silver uptake within the adhesive interface after thermocycling (aging) ($p > 0.05$).

Conclusion: All resin luting agents exhibited nanoleakage after both 24-hour storage and 10-day storage with thermocycling.

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