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Effect of a Desensitizing Agent and an Adhesive System on Microleakage Associated with Cast Restorations Luted with a Resin-modified Glass Ionomer Cement

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A Thesis submitted in partial fulfillment of the requirements for the Master of Science Degree at Virginia Commonwealth University.

By

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Abstract

EFFECT OF A DESENSITZING AGENT AND AN ADHESIVE SYSTEM ON MICROLEAKAGE ASSOCIATED WITH CAST RESTORATIONS LUTED WITH A RESIN-MODIFIED GLASS IONOMER CEMENT

By Saleh Adulaziz Al-Rowaieh, D.D.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

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Purpose: This study evaluated microleakage associated with cast restorations that were luted with a resin-modified glass ionomer cement (RelyX) following obturation of the dentinal tubules with either a desensitizing agent (Gluma Desensitizer) or an adhesive system (Scotchbond Multipurpose Dental Adhesive). The effect of acid etching on the removal of the smear layer and its influence on the extent of microleakage associated with the adhesive system was also evaluated.

Materials and Methods: Extracted mandibular premolars (N = 48) were prepared for complete cast restorations and divided into 4 groups (N = 12). In group A (control), neither a desensitizing agent or a component of the adhesive system was applied prior to luting. In group B, Gluma Desensitizer was used to obturate the dentinal tubules. In group C, Scotchbond Multipurpose Dental Adhesive System was applied to tooth preparations according to the manufacturer's instructions. Tooth preparations in group D received the same dentin surface treatment as in group C, but no acid etching was performed. Cast restorations in all 4 groups were then luted with the resin-modified glass ionomer luting cement RelyX. All specimens were subjected to thermocycling between 8° and 55°C for 500 cycles in water baths, placed in a solution of 0.5% basic fuchsin dye for 24 hours, and then sectioned twice longitudinally, once mesiodistally and then buccolingually. All specimens were examined at X30 magnification with a stereo-microscope equipped with a digital camera. Photographs of all sections were made and the extent of microleakage along the tooth-cement interface was measured in millimeters using an image analysis software. Microleakage was perceived to have occurred along a segment of the tooth-luting cement interface when dye penetration from that segment into the dentinal tubules was detected. One-way analysis of variance ($\alpha = 0.05$) was performed to identify differences in mean microleakage among the luting groups, followed by Tukey's Honestly Significant Difference Test for pairwise comparisons.

Results: Large standard deviations were found in all 4 groups. No statistically significant difference was found among the control (0.64 ± 0.50 mm), Gluma Desensitizer (0.42 ± 0.24 mm), and Scotchbond Multipurpose without etching (0.67 ± 0.40 mm) groups.

However, a statistically significant difference was found between the Scotchbond Multipurpose with etching (1.51 ± 0.92 mm) group and each of the other groups. Conclusions: The large standard deviations obtained implied a marked amount of variability in microleakage within each group, which might be the result of the small sample size used. The increase in microleakage when 35% phosphoric acid was used prior to dentin bonding is difficult to explain. Within the limitations of the study, the results suggest that the use of a nonpolymerizing, resin-based (Gluma Desensitizer) material or a photopolymerizing, resin-based (Scotchbond Multipurpose) system without etching had no effect on microleakage under cast restorations luted with the resin-modified glass ionomer luting cement RelyX. The increase in microleakage when etching with 35% phosphoric acid was preformed might be explained by the phenomenon known as nanoleakage, but further investigation is recommended in this area.

Introduction

Microleakage is defined as the microscopic seepage of oral fluids between the interface of the tooth and a dental restoration (1). The importance of microleakage in clinical dentistry is well recognized. Although the exact level at which it becomes clinically significant remains undefined (2, 3) and although it does not directly correlate with clinical failure (4), microleakage has been associated with postoperative sensitivity (4, 5), recurrent caries (6), marginal staining (5), and pulpal pathology (7-13).

Researchers have studied microleakage of luting cements by attempting to simulate leakage of bacteria and/or their toxins. These in-vitro microleakage tests appear to be minimally influenced by the marginal adaptation of cast restorations (14), presumably because the weakest link involves the sealing influence of the luting cement (15). Possible causes of microleakage related to cast restorations include shrinkage of the cement on setting, cement dissolution, mechanical failure of the cement, and lack of adhesion of the luting cement to tooth structure (16).

A major advancement in dental materials technology has been the development of resin-modified glass ionomer (RMGI) luting cements (17). The introduction of this class of materials has expanded the choices of luting cements available to the clinician. The modification of the traditional glass ionomer chemistry by the addition of pendant methacrylate groups or polymerizable monomers has produced a material behaving in an intermediate manner between resin-composite and glass ionomer luting cements (18). The resulting product has some of the benefits of adhesive resin luting cements such as fracture toughness (19, 20) and very low solubility (15) along with some of the benefits of traditional glass ionomer luting cements such as limited fluoride release (21) and chemical adhesion to calcified tissue (22). In addition, microleakage of resin-modified glass ionomer luting cements has been shown to be less than that of zinc phosphate luting cement (23, 24) and comparable to that seen with adhesive resin luting cements (25). Compared with zinc phosphate and glass ionomer luting cements, the use of resin luting cements with dentin-bonding agents has generally resulted in less microleakage observed in in-vitro studies (16, 26-31).

Clinical steps undertaken for the fabrication of a fixed prosthesis can be a source of potential insult to the pulp (32). As many as 1 to 2 million dentinal tubules become exposed during an average tooth preparation for a posterior cast restoration (33). Heat generation, pressure, and dentin desiccation resulting from this process may increase the likelihood of hypersensitivity (34). Dentinal hypersensitivity after the luting of cast restorations is therefore not uncommon (35). Also, a higher incidence of puplal necrosis has been associated with full crown preparations when compared with unrestored teeth (32,36).

Theories that explain the mechanism of dentinal hypersensitivity include: the direct nerve ending theory (37), the odontoblast-receptor theory (38), and the hydrodynamic theory (39). The latter theory, proposed by Brännström and Anstrom (39), is generally the most accepted. It states that dentinal tubule fluid movement stimulates the more peripheral branches of myelinated afferent nerves in the pulp, thereby eliciting sharp pain (39-41). It has been reported that occluding the dentinal tubules at their orifices can prevent fluid flow and hence reduce pain sensation (39, 42). Brännström et al. (43,44), Watanabe et al. (45), and Suda et al. (46) have presented techniques of resin impregnation for the desensitization of exposed dentin. Watanabe et al. (45) investigated the effects of dentin primers and a dentin-bonding agent on the sensitivity of dentin by Scanning Electron Microscopy (SEM). The observation showed that penetration of the dentin-bonding agent into the dentinal tubules was clearly promoted by the hydrophilic resin hydroxyethylmethacrylate (HEMA). The authors speculated that mechanical sealing of the exposed dentin surface by the dentin-bonding agent effectively prevents chemical and mechanical irritation. In a study evaluating the effect of sealing of the dentinal tubules on dentinal permeability, a significant correlation was found between the measurements of dentin sealing and dye penetration (47).

Several studies have evaluated the effect that desensitizing agents (i.e. dentin primers and/or dentin-bonding agents) may have on the retention of luted castings (48-51). These in-vitro tests have shown both an increase and decrease in retentive values, depending on the exact dentinal desensitizing agent/luting cement combination and methodology used in the test. Of these studies, only one utilized a method by which preparation surface area was controlled to reduce the variation in strength values and permit high discrimination among retention values related to these desensitizer/luting cement combinations (51). In that particular study, dentin treatment with a photopolymerizing dentin-bonding agent (All-Bond 2, BISCO Dental Products, Schaumburg, IL) prior to crown cementation with a resin-modified glass ionomer luting cement (Fuji Plus, GC Corporation, Tokyo, Japan) was shown to produce significantly higher crown retention values than without a dentinal desensitizing agent (control). The use of a nonpolymerizing, resin-based dentinal desensitizing agent (Gluma Desensitizer, Heraeus Kulzer, South Bend, IN) produced retentive values equivalent to the control group.

An increase in crown retention may be of great value in situations where less than optimal retentive and resistance forms to tooth preparation exist. However, it's equally valuable to be aware of the effect that dentinal desensitizing agents may have on microleakage of luted cast restorations. A 6-month in-vivo investigation by White et al. (23) revealed that the use of a RMGI luting cement (Infinity, Den-Mat Corp, Santa Maria, CA) with a dentin-bonding agent (Tenure, Dent-Mat Corp, Santa Maria, CA) tended to reduce microleakage at the tooth-luting cement (T-C) interface, compared with a group in which no dentin-bonding agent was used. This difference, however, was not statistically significant at the $p < 0.05$ level. It was suggested that it might become significant with larger sample sizes or over extended periods. No studies evaluating the effect of dentinal desensitizing agents on crown microleakage after crown insertion with a RMGI luting cement have been identified.

Gluma Desensitizer is a dentin primer that has been used successfully for obturating dentinal tubules and decreasing the potential for dentinal hypersensitivity (52-55). Another product that has been helpful in that respect is Scotchbond Multipurpose Dental Adhesive (SBMP)(3M Dental Products, St Paul, MN), which is a fourth generation dentin bonding system (55-57). The effect of these materials on microleakage associated with cast restorations luted with a resin-modified glass ionomer cement, such as Rely X (3M Dental Products, St Paul, MN), is unknown. A study designed to investigate this effort was therefore indicated.

Specific Aim and Hypothesis:

The purpose of this study was to investigate three desensitization methods' influence on microleakage associated with cast restorations luted with the RMGI cement RelyX. The hypothesis was that the desensitization methods would have different effects on microleakage as compared to cast restorations luted without dentinal desensitization.

Material and Methods

Forty-eight recently extracted, intact, dental caries-free human mandibular premolars were acquired for the study. Following disinfection in 0.5% solution of NaOCl (1:12 dilution of Ultra Clorox, Clorox Inc, Oakland, CA) for 8 hours, the teeth were stored in distilled water at room temperature. The teeth were then mounted within phenolic rings (Buehler Ltd, Lake Bluff, IL) using autopolymerizing acrylic resin (Trayresin Self Curing Resin, Dentsply Int Inc, York, PA). Tooth preparations for complete veneer cast restorations on all teeth were performed with a chamfer finish line using a diamond bur (No. 856.11.025, Brasseler USA, Savannah, GA) mounted in a straight handpiece (A-dec, Newberg, OR) with water irrigation. This process was carried out with the aid of a precision milling machine (Attachments International, San Mateo, CA) to help achieve consistent convergence of the axial walls and position the finish line at the same level circumferentially (Figure 1). The final occlusal-gingival dimension of the completed tooth preparations was 4 mm (Figure 2).

The tooth preparations were cleaned manually with a toothbrush, rinsed with water, and air-dried before impression making. The manual brushing was intended to remove any surface biofilm and contaminants that might have formed during storage of the specimens. Impressions of the prepared teeth were made in phenolic rings with polyvinyl siloxane (Aquasil LV and Aqausil Monophase, Dentsply Int Inc, York, PA).

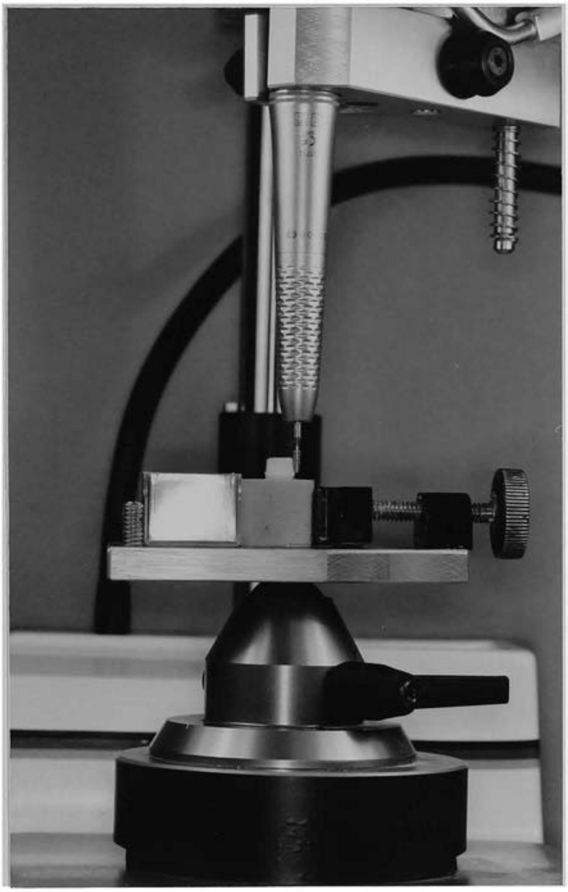

Figure 1. Standardization of tooth preparation was controlled using a precision milling machine

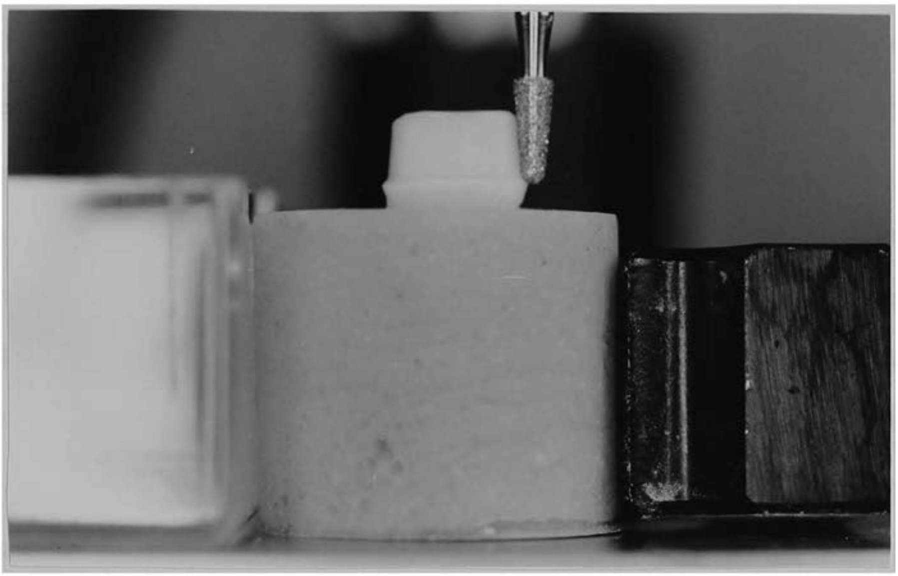

Figure 2. The completed tooth preparation with axial wall height of 4 mm

The teeth were stored in distilled water at room temperature without provisional restorations for approximately 2 months, during which time cast restorations were being fabricated.

Dies were fabricated with type IV gypsum (DieKeen, Heraeus Kulzer, Inc, Armonk, NY). The dies were trimmed and then each die was coated with 2 layers of die spacer (Cement Spacer, Blue, belle de st claire, Glendora, CA) to within 1 mm of the finish line. Patterns were formed in wax directly on the dies and margins were adapted. The wax patterns were invested in a carbon-free investment (Hi-Temp, Whip Mix Corp, Louisville, KY) and cast in a type III gold alloy (LDG 44, Jeneric/Pentron Inc, Wallingford, CT). The cast restorations were air abraded and ultrasonically cleaned to remove traces of investment material. Interferences with seating of the restorations onto their respective dies were identified by visual inspection and then eliminated using rotary instruments. The fit of the cast restorations was evaluated on the their respective tooth samples using a silicone disclosing medium (FitChecker, GC America Inc, Alsio, IL) (Figure 3). Adjustment to the intaglio surface of the restoration was made using rotary instruments until it was possible to see the entire restoration internal margin through the disclosing medium, which indicated the achievement of intimate fit. The adaptation of all the restorations was considered clinically acceptable. External surfaces of the restorations were finished and polished to achieve a smooth transition between the natural tooth structure and the cast restoration. All restorations were ultrasonically cleaned in 10% non-ionic ultrasonic solution (1:10 dilution of Multipurpose Non-Ionic Solution, Health Sonics Corp, Livermore, CA) to rid the intaglio surface of any debris that might have

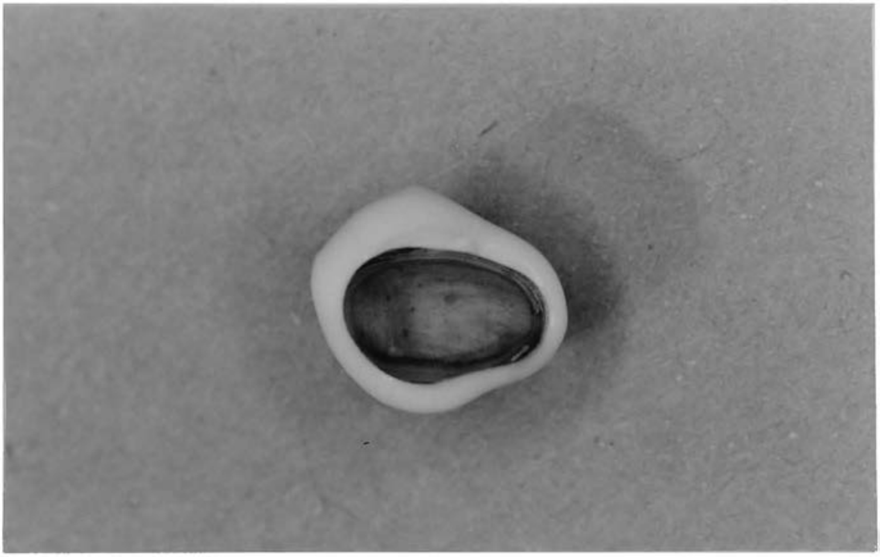

Figure 3. Fit of the cast restoration was evaluated using a silicone disclosing medium and adjustment was made until the entire internal margin was visible through the disclosing medium, indicating clinically acceptable adaptation.

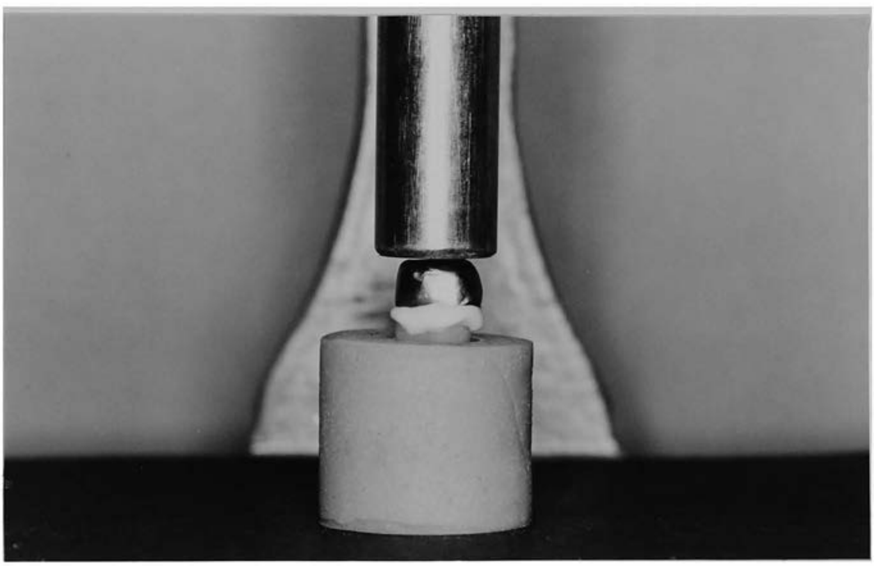

Figure 4. Cast restorations were luted under a static load of 5Kgs

formed during the fitting process. The restorations were then thoroughly cleaned with compressed air/water.

Prior to luting, the tooth preparations were carefully cleaned using the method described prior to impression making. The teeth were randomly divided into 4 equal groups of 12 specimens. Teeth in group A received no dentinal desensitization treatment (control). In group B, Gluma Desensitizer was applied to the surface of tooth preparations. In group C, the protocol recommended for using SBMP was followed, which included etching of the tooth preparation surface with 35% phosphoric acid. To observe the influence of leaving the smear layer intact on microleakage, the SBMP system was again used for group D, but without prior etching with 35% phosphoric acid. The RMGI luting cement RelyX was then used for luting all the restorations in the study. All materials were handled, proportioned, and applied according to the manufacturers' instructions except for in group D, where etching was not performed. Each restoration was seated on the tooth with digital pressure, and sustained under a static load of 5 Kgs for 10 minutes. All excess cement was removed after complete polymerization.

The specimens were recovered from the acrylic resin mountings by sectioning of the acrylic resin with a diamond disc on a lathe. All specimens were then artificially aged by thermocycling for 500 cycles of 24-second dwell time and 12-second travel time between water baths monitored at 8° and 55°C. The specimens were then dried with compressed air, and the apices were sealed with utility wax. Each entire specimen, except for the restoration and approximately 1-mm of tooth surface adjacent to the restoration margin, was sealed with two coats of fingernail varnish (L'oreal Fingernail Varnish, Cosmair, New York, NY). The specimens were then immersed in an aqueous solution of 0.5% basic fuchsin dye (ScyTek Laboratories, Inc, Logan, UT) for 24 hours, retrieved, rinsed, and allowed to air dry.

In preparation for sectioning, each tooth was bonded to a metal block using hot melt glue (Glue Sticks, Stanley, East Greenwich, RI). The metal block was then secured in a low speed saw (Isomet Saw, Buehler Ltd, Lake Bluff, IL) and the tooth was sectioned in half with a diamond blade (Isomet, Buehler Ltd, Lake Bluff, IL) longitudinally through the center, in a buccolingual direction (Figure 5). After recovery of the two halves of a tooth, they were reassembled, bonded to the metal block again with the hot glue, and samples were once again sectioned, longitudinally, but this time through the center in a mesiodistal direction. Water was constantly added to a trough below the diamond blade to maintain it clean. The two-stage sectioning method resulted in 8 interfaces for measuring microleakage. The majority of the cast restoration sections debonded as a result of the second sectioning stage.

The extent of microleakage was observed with a stereomicroscope at X30 magnification (Model DC2-456H, National Optical and Scientific Instruments Inc, San Antonio, TX). The stereomicroscope was supplied with an internal digital camera and an image analysis software (Motic Images 2000, Micro-Optic Industrial Group Co Ltd, BC Canada). Calibration of the stereomicroscope was performed according to the manufacturer's instructions before obtaining views of the sections via the camera.

Photographs of each section were made (Figure 6). The software provided a means by which each captured image could be transformed into its negative counterpart.

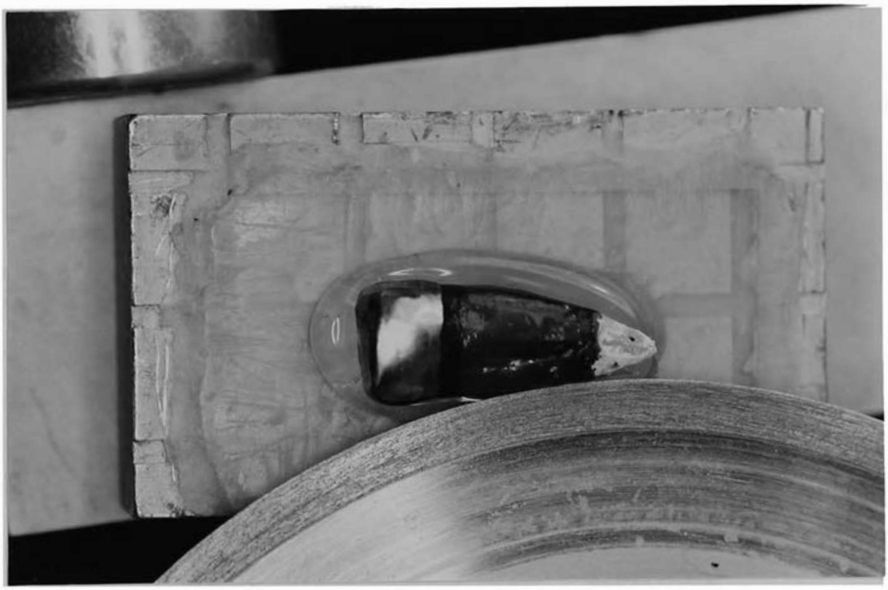

Figure 5. Sectioning of a specimen longitudinally using a low-speed saw

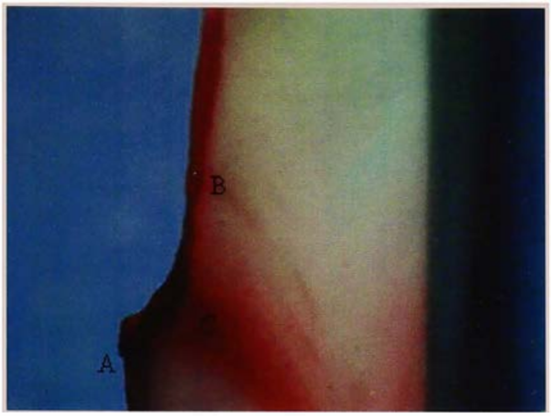

Figure 6. Microleakage observed at X30 magnification

Legend: A = Preparation margin where microleakage started; B = Suspected end point of microleakage; C = Dye penetration into the dentinal tubules

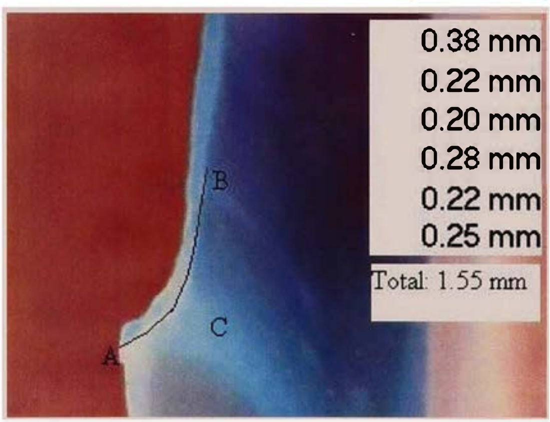

Figure 7. The image in Figure. 5 after transforming it into its negative counterpart to better assess the extent of microleakage. The total of 6 consecutive linear measurements made along the tooth-cement interface equals the total microleakage at that interface.

Legend: A = Preparation margin where microleakage started; B = Confirmed end point of microleakage; C = Dye penetration into the dentinal tubules.

This feature allowed the investigator to better visualize the distance the dye traveled along the T-C interface (Figure 7). Microleakage was perceived to have occurred along a segment of that interface when dye penetration from that segment into the dentinal tubules was detected. The software also made it possible to make measurements of microleakage along the interface observed on each section. However, only straight-line measurements were possible to be made using the software. Therefore, to determine the distance the dye traveled along curved interfaces, multiple consecutive short linear measurements were first made (Figure 7). Total microleakage at each interface was then calculated by adding up these measurements. Microleakage per specimen was defined as the mean microleakage of the 8 interfaces measured (27). Differences among means of experimental groups were determined using one-way analysis of variance (ANOVA)(α = 0.05). Tukey's Honestly Significant Difference Test (HSD) was then used for pairwise comparisons.

Results

The mean microleakage for the specimens and the overall mean microleakage for each group are described in Table 1. (See Appendices A-D for complete data). Table 2. illustrates the results of the one-way AN●VA, which indicated a statistical difference in micro-leakage among the groups.

Table 1. Mean microleakage for specimens and overall mean microleakage for the groups (Measurements are in mm)

NO.	A	B	C	D
1	0.18	0.46	1.75	0.50
2	0.40	0.26	1.54	0.52
3	0.59	0.43	0.96	0.87
4	0.30	0.42	0.83	0.41
5	1.03	0.25	1.19	1.12
6	1.51	0.57	3.38	1.69
7	1.11	0.27	2.02	0.57
8	0.29	0.54	0.64	0.30
9	1.33	1.05	1.32	0.52
10	0.11	0.30	1.54	0.58
11	0.07	0.30	2.83	0.58
12	0.74	0.14	0.11	0.33
Mean	**0.64**	**0.42**	**1.51**	**0.67**
Standard Deviation	**0.50**	**0.24**	**0.92**	**0.40**

Table 2. Results of the one-way analysis of variance

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F Ratio	F*
Among groups	8.32	3	2.77	8.55	2.82
Error	14.27	44	0.32	F>F*(95%)	
Total	22.59	47			

Multiple pairwise comparisons of specific means revealed significant ($p < 0.05$) differences between group C and the other groups. None of the other differences between specific groups was statistically significant. (Table.3)

Table 3. Results of Tukey's HSD Test

	A	B	C	D
A	-	NS	S	NS
B		-	S	NS
C			-	S
D				-

S: Statistically significant difference, NS: Non-statistically significant difference

Discussion

In-vitro assessment of microleakage in the literature has been subject to different methods of evaluation and interpretation. However, microleakage is an intraoral microbiological process that is difficult to produce and measure in-vitro. Although it's possible to simulate the intraoral changes in temperature over time through artificial aging, it's rather difficult to simulate bacteria and their products. In this endeavor, certain technical factors might have resulted in misestimating dye leakage. As an example, the water used during sectioning could have diluted the dye and caused its intensity to decrease. Also, it is possible that the sectioning process itself could have caused smearing of the basic fuchsin dye across the specimen surfaces. The results would then show a greater deposition of the dye than actually occurred, thus giving misleading results. To decrease the amount of error involved in measuring dye leakage, it was decided to consider it to have occurred along a segment of the T-C interface only when it penetrated from that segment into the dentinal tubules. This provided a higher level of comfort in measuring microleakage as it had suggested that the stained area was not simply the result of smearing of the dye during sectioning.

Another possible limitation of the study was the ability to assure a good seal of the external aspect of the specimens prior to immersing them into the dye solution. Even though a strict regimen was followed in sealing the root apices and surfaces, dye leakage into the specimen could have started at the apical foramen or cementum, as opposed to preparation margin. Leakage pattern within some of the specimens did suggest that as their entire pulpal chamber was affected by the dye. However, once the dye had reached the pulpal chamber of a specimen and caused significant pulpal discoloration, it became difficult to identify the point at which the penetration had started as the leakage was also seen at the T-C interface and into the adjacent dentinal tubules.

All measurements were made using the image analysis software provided with the stereomicroscope. Although microleakage along the T-C interface could have occurred further along that path, it was not possible to appreciate in at the X30 magnification level, which was the highest available in this investigation. Although a higher power stereo-microscope could have been used, the field of view through which the measurements were to be made would have been narrower than desired for determining the full extent of observable microleakage across the coronal aspect of the section.

Sodium hypochlorite solution was used in this investigation for disinfection of tooth samples before testing to reduce the biohazard risk to the investigator. Studies are in support of this disinfection regimen during similar dentin bonding investigations and have shown no confounding influence on the bond strengths (58-59). Also, distilled water was the storage medium used throughout the investigation. Fritz et al. (60) demonstrated that long-term water storage did not have an adverse effect on the bonding of resin-modified glass ionomer cements to dentin and enamel.

Resin-modified glass ionomer cements are known for their capability of forming strong adhesive bonds with tooth surface, which is attributed to the glass ionomer component of the luting cement (22,61). This strong adhesive bond is assumed to cause less microleakage potential than would be found with traditional luting cements such as zinc phosphate. Studies have shown that microleakage of resin-modified glass ionomer luting cements is less than that of zinc phosphate luting cement (23, 24) and comparable to that seen with adhesive resin luting cements (25). In the in-vivo study by White et al. (23) the use of a dentin-bonding agent (Tenure) with a RMGI luting cement (Infinity) was not found to significantly decrease microleakage under cast restorations following six months of use. Currently there are no recommendations by manufacturers for use of a primer or adhesive agent prior to luting with a RMGI, as the adhesive bond to tooth structure achieved by the cement itself is considered clinically sufficient. However, the use of these agents might be desirable for obturating the exposed dentinal tubules after tooth preparation and before provisionalization to prevent or decrease dentinal hypersensitivity. The dentin primer Gluma, an aqueous solution of 2-HEMA and glutaraldehyde, has been shown to be effective in reducing dentinal hypersensitivity by occluding the dentinal tubules and by precipitating plasma proteins in the dentinal tubules (52-55). The use of a dental adhesive system such as SBMP, whose primer also contains HEMA, has helped achieve similar results (55-57). Whether use of a RMGI luting cement itself can reduce already existing postoperative sensitivity may merit more investigation.

A well-adapted extracoronal restoration that has been completed to exacting specifications with attention to detail has the best and most predictable prognosis (24). In this study, every effort was made to produce clinically acceptable cast restorations with intimately adapted margins. Microleakage, however, was noticed in all four groups.

Since the study did not include non-thermocycled groups, it would not be possible to attribute this finding to thermocycling. The number of cycles chosen for the study was 500. This was selected based on research by Crim and Garcia-Godoy (62), in which no difference was found in dye penetration around class V preparations restored with resin composite when the teeth were thermocycled for either 100 or 1500 cycles.

The results of this study suggest that when Gluma Desensitizer or SBMP system was used without etching, microleakage did not significantly differ from that in the control group. However, when phosphoric acid was used for etching the surface of the dentin, microleakage was significantly more than in the other 3 groups. The standard deviations obtained in each group were higher than ideally desired. This implies a marked amount of variability in microleakage within each group, which might be the result of the small sample size used.

The large standard deviation noticed within both SBMP groups might also reflect the technique-sensitivity of the system. The amount of moisture that should be present following the etching step can be crucial to the success of the dentin bond. Well-controlled application of the primer is also believed to be very important for the bond. Overdrying of the dentin surface following the application of both components could have resulted in weaker bonds. The photopolymerizing resin component of the system might have also been applied in thicker amounts than ideally desired, subsequently affecting the marginal seal by the restoration.

It is questionable if the variation was related to the age of the specimens used. In a study by Sidhu et al. (63), the effect of age changes in dentin on the effectiveness of two dentin-bonding agents in minimizing microleakage was investigated. The results showed that in general, the use of dentin-bonding agents significantly reduced microleakage along the tooth-restoration interface despite the effect of aging. In another study by Tagami et al. (64), similar dentin bond strengths were found to both young and old teeth for 4 different dentin bonding systems.

The increase in microleakage when 35% phosphoric acid was used prior to dentin bonding is difficult to explain. Sano et al. (65) reported a special kind of leakage in a porous zone of the hybrid basal portion, calling it nanoleakage. Most microleakage studies involve measuring the magnitude of movement of a tracer molecule through a gap between restorative materials and the walls of cavity preparations. The microscopic study by Sano et al. (65) examined the migration of silver nitrate into the interface between dentin and five different dentin bonding agents used to restore class V cavity preparations, in the absence of gap formation. Several different leakage patterns were seen; however, they all indicated leakage within the hybrid layer when viewed by SEM (65).

Nanoleakage appears as a consequence of the acid etching procedure allowing the penetration of oral and pulpal liquids into porosities within or adjacent to the hybrid layer (66). Scanning electron microscopy has shown that when etching was performed, nanoleakage gradually increased at the dentin interface (67). As a result, it was speculated that the bond strength of adhesive resin would gradually decrease over time. All studies on nanoleakage have been performed using a silver nitrate staining technique and SEM, which were not utilized in this investigation. Therefore, it remains unknown as to the influence of these mediums on the results. Further study in this regard is recommended.

Further research is also necessary to evaluate the effect of provisional restoration fabrication, provisional restoration duration, provisional cement removal, and restoration try-in procedures on the hybridized dentin layer. If the procedures required in fixed prosthodontics have no effect on the hybridized layer, an additional application of dentin bonding agent may not be indicated before luting of the definitive prosthesis (68). Because it has been demonstrated that dentin bonding agents can accumulate at the margins of preparations, applying multiple coats of bonding agent may cause increased microleakage at the margin (47).

Conclusions

Within the limits of this in-vitro study, the following conclusions could be drawn:

1. Microleakage observed with the use of Gluma Desensitizer or SBMP without etching for obturation of the dentinal tubules did not significantly increase microleakage associated with cast restorations luted with the RMGI cement RelyX.

2. Although a significant increase in microleakage was observed when phosphoric acid was used prior to use of the SBMP system, it would not be possible to discourage against etching of the dentin surface as this finding is yet to be explained and any clinical consequences determined.

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Appendices

Appendix A

Complete Data Table of Group A: No Dentinal Desensitization Treatment – Control*

1a	0.33	4a	0.14	7a	1.24	10a	0.16
1b	0.21	4b	0.12	7b	1.15	10b	0.52
1c	0.1	4c	0.13	7c	1.43	10c	0
1d	0.15	4d	0.08	7d	1.3	10d	0
1e	0.51	4e	0.67	7e	0	10e	0
1f	0.05	4f	0	7f	2	10f	0
1g	0.14	4g	0.66	7g	1.79	10g	0.179
1h	0	4h	0.56	7h	0	10h	0
2a	0.37	5a	0.85	8a	0.62	11a	0
2b	0	5b	1.86	8b	0.25	11b	0
2c	0.57	5c	0.4	8c	0	11c	0
2d	0.69	5d	1.35	8d	0.31	11d	0
2e	0.29	5e	0.45	8e	0.35	11e	0
2f	0.33	5f	1.4	8f	0	11f	0
2g	0.6	5g	0.69	8g	0.36	11g	0.53
2h	0.35	5h	1.24	8h	0.45	11h	0
3a	0.35	6a	1.73	9a	1.3	12a	0.22
3b	1.99	6b	1.83	9b	1.26	12b	1.4
3c	0	6c	2.04	9c	1.26	12c	0.29
3d	0.23	6d	0.85	9d	1.42	12d	0.71
3e	1.53	6e	1.61	9e	1.16	12e	0
3f	0.35	6f	1.64	9f	1.31	12f	1.32
3g	0.28	6g	0.36	9g	1.41	12g	0.77
3h	0	6h	1.98	9h	1.49	12h	1.17

* Measurements are in millimeters

Appendix B

Complete Data Table of Group B: Gluma Desensitizer*

1a	1.15	4a	0	7a	0.45	10a	0
1b	0.56	4b	0	7b	0	10b	0.38
1c	0.46	4c	0.75	7c	0	10c	0.14
1d	0.73	4d	0.39	7d	0	10d	0.57
1e	0.78	4e	0	7e	0.41	10e	0.67
1f	0	4f	0.64	7f	0.5	10f	0
1g	0	4g	1.6	7g	0.4	10g	0
1h	0	4h	0	7h	0.38	10h	0.64
2a	0	5a	0.65	8a	0.59	11a	0.77
2b	0	5b	0.66	8b	0.93	11b	0.1
2c	0	5c	0.38	8c	0.36	11c	0
2d	0.89	5d	0	8d	0.39	11d	0.26
2e	0.49	5e	0	8e	0.71	11e	0.15
2f	0	5f	0	8f	0.32	11f	0
2g	0.68	5g	0.34	8g	0.62	11g	0.56
2h	0	5h	0	9h	0.44	11h	0.54
3a	1.23	6a	1.02	9a	1.09	12a	0.29
3b	0.44	6b	1.04	9b	0.91	12b	0.56
3c	0	6c	0.41	9c	0.78	12c	0.29
3d	0.65	6d	0	9d	1.11	12d	0
3e	0.28	6e	0.34	9e	1.08	12e	0
3f	0	6f	0.77	9f	1.01	12f	0
3g	0.81	6g	0.56	9g	1.05	12g	0
3h	0	6h	0.44	9h	1.39	12h	0

* Measurements are in millimeters

Appendix C

Complete Data Table of Group C: SBMP*

1a	1.37	4a	0.69	7a	0.61	10a	3.59
1b	1.46	4b	1.25	7b	3.49	10b	1.1
1c	1.45	4c	0.38	7c	4.06	10c	0.87
1d	2.62	4d	0.98	7d	2.23	10d	2.34
1e	1.75	4e	0	7e	1.54	10e	0
1f	1.65	4f	0.69	7f	2.64	10f	0
1g	2.01	4g	1.42	7g	0.21	10g	1.3
1h	1.7	4h	1.22	7h	1.4	10h	3.12
2a	2.25	5a	0.69	8a	0.51	11a	3.02
2b	0.74	5b	1.85	8b	0.98	11b	4
2c	1.89	5c	2.25	8c	1.11	11c	0.89
2d	2.57	5d	1.38	8d	0.51	11d	4
2e	1.93	5e	1.78	8e	1.09	11e	4
2f	0.86	5f	0.16	8f	0.88	11f	1.64
2g	1.34	5g	0.68	8g	0	11g	4
2h	0.73	5h	0.72	8h	0	11h	1.09
3a	1.24	6a	2.41	9a	0	12a	0
3b	1.04	6b	4.01	9b	0.64	12b	0
3c	0.74	6c	3.82	9c	0.92	12c	0
3d	0.9	6d	4.04	9d	0.76	12d	0
3e	0.31	6e	1.74	9e	1.36	12e	0
3f	0.93	6f	3.68	9f	1.7	12f	0
3g	1.29	6g	3.69	9g	1.5	12g	0.85
3h	1.22	6h	3.64	9h	3.66	12h	0

* Measurements are in millimeters

Appendix D

Complete Data Table of Group D: SBMP without Etching*

1a	0.74	4a	0.51	7a	0.91	10a	0.68
1b	0	4b	0	7b	0.32	10b	1.56
1c	0.97	4c	0	7c	0	10c	0.85
1d	0	4d	0.36	7d	0.57	10d	0.39
1e	0.68	4e	0.85	7e	1.24	10e	0.43
1f	1	4f	0	7f	0.84	10f	0.18
1g	0.63	4g	1.07	7g	0.68	10g	0.56
1h	0	4h	0.49	7h	0	10h	0
2a	0	5a	0.87	8a	0	11a	0.77
2b	0	5b	1.59	8b	0.32	11b	0.53
2c	1.01	5c	0.98	8c	0.05	11c	0.83
2d	0.39	5d	0.63	8d	0.48	11d	0.43
2e	0.88	5e	0.85	8e	0	11e	0.12
2f	1.17	5f	1.14	8f	0.38	11f	0.57
2g	0.69	5g	1.62	8g	0.51	11g	0.65
2h	0	5h	1.31	8h	0.67	11h	0.71
3a	0.59	6a	1.87	9a	1.19	12a	0
3b	0.86	6b	1.46	9b	0.54	12b	0
3c	0.64	6c	1.64	9c	0.96	12c	0
3d	1.9	6d	0.45	9d	0.34	12d	0
3e	0.85	6e	2.95	9e	0	12e	1.36
3f	0.72	6f	2.22	9f	0.1	12f	0.5
3g	0.84	6g	0.61	9g	0.63	12g	0
3h	0.59	6h	2.35	9h	0.36	12h	0.78

* Measurements are in millimeters

Vita

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